The Reproductive Ecology of the Scallop, *Chlamys bifrons*, in South Australia

by

Craig Andrew Styan

Department of Zoology

University of Adelaide

August 1998

Chlamys bifrons

Table of Contents

Chapter 3 continued

List of Tables, Plates and Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 5 continued

Chapter 6

Chapter 7

Abstract

A model predicting fertilisation success rates in natural populations was developed and fitted for the scallop, *Chlamys bifrons*, in shallow subtidal areas in southern Australia. Parameterising with field collected data, I modelled fertilisation success within and between populations, also examining how population structure and scallops' spatial distribution patterns might affect fertilisation. I also then made predictions about the genetic consequences of the low and variable rates of fertilisation success that scallops were likely to experience.

A reworked version of the Vogel-Czhiak-Chang-Wolf fertilisation kinetics model (that now accounts for the likelihood of polyspermy) was developed to describe the chance of successful sperm-egg collision. Incubation of eggs in serially diluted sperm solutions in the laboratory fitted this reworked model and allowed prediction of the effects, in terms of fertilisation success, of sperm dilution in the field.

Monitoring of reproductive activity of *C.bifrons* at two sites and in another species, *C.asperrima*, suggested that both spawned several times each year, but *C.bifrons* spawned during summer and *C.asperrima* in winter. Fine scale reproductive patterns of *C.bifrons* at Largs Bay suggested some asynchrony in spawning there, as did laboratory measurements of male spawning rates. A new model (the "spawning fecundity" model) was proposed, which would institute a new, important role for population age/size structure in the dynamics of external fertilisation

Two field experiments showed that *C.bifrons* fertilisation success is greatly affected by small changes in the distance between spawners, but not much by where spawning was takes place. Fertilisation dropped away very rapidly with inter-spawner distance; females > 100cm from a spawning male had negligible rates of fertilisation. Based on the field experiments, models of spawning within randomly dispersed populations and a real population at Largs Bay (which incorporated information about spatial distribution patterns of individuals from surveys conducted on a range of spatio-temporal scales) suggested that unless scallop densities are very high, fertilisation success of *C.bifrons* was predicted to be low and, equally importantly, there would also be a high variance in success between individuals within populations. This may have important consequences for our understanding of the ecology and evolution of free-spawners such as *C.bifrons*

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institution and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Craig Styan

15 August 1998

Acknowledgments

This thesis has taken a long time and to produce and it's not possible to properly thank all who have helped along the way. But to all who contributed - THANK YOU! Some special mentions do, however, need to be made.

Underwater work is labour intensive and in a field-based study such as this it was necessary to press-gang many individuals into helping at some stage, either as diving buddies or as "boat bimbos". My sincere thanks are extended to all who helped in the field - not only did we manage to get a lot of work done, but, I at least, usually had fun as well. Amongst those who regularly helped me explore the underwater delights (!) of Largs Bay and Edithburgh Jetty were Ian Magraith, Andrew Melville, Jo Strzlecki, Simon Bryars, Stuart Blanch, Emma Cronin, Becky Fisher. On a (hollow) promise of a feed of scallops, Alan Butler even known to get wet once! I am sure that all involved are pleased that we now know a little more about the sex life of scallops!

An extra special thanks needs to be extended to Ian Magraith who made sure all the boats and field gear worked every time and helped out on many a weekend. Andrew Melville's enthusiasm for getting wet is surely abnormal, but also meant that he was a regular assistant when most sensible people would be doing something warm. His enthusiasm was infectious and his help invaluable.

Piers Brissenden sourced various, but important, bits and pieces for me and coped with my continual requests at awkward times. He and all the other support staff of the Department of Zoology are sincerely thanked.

The Uni bar on Friday nights, drinking Coopers Ale, and the cricket at Adelaide Oval will remain among highlights of my time as a post-graduate. Fran Sheldon, Steve Milne, Helen Vanderwoude, Paul Shultz-Motel, Sam Wade and Stuart Blanche similarly believed in such things and I very much enjoyed their company whilst being distracted by these uniquely South Australian activities.

Jon Havenhand of Flinders University was in many ways a "pseudo-supervisor", providing advice throughout. Particularly at the start of things, Jon's encouragement, ideas and constructive criticism were important. Similarly, Rod Connolly also provided a sounding board for many ideas in the formative stages.

Joanna Strzlecki helped at all stages of this project. For a start, she assisted me on many occasions in the field and also provided a critical, incisive ear to many ideas (hare-brained and otherwise) and proofed manuscripts. Jo constituted the rest of the "marine group" for much of my

time as a graduate student in the Department of Zoology and so was the first person I turned to for help, advice or general encouragement (particularly when our supervisor did a runner!).

Dr Alan Butler is just an "all-round nice-guy" and I have learnt many things from him. I believe (or at least hope) his hands-off approach to supervision worked - Alan largely let me have my head as far as the project went, but he was always there to provide advice and encouragement when needed. Regularly, his ability to be able to provide critical advice, after just a few minutes of jumbled update from me, was amazing. Alan turned out to be the perfect choice as supervisor for my doctoral studies and he has greatly influenced my development as a scientist (and as a person).

Finally and most importantly, I need to thank my parents, Barry and Brenda. Their love, support and encouragement was immeasurable. Without this I would not have succeeded at all. This thesis is dedicated to them.

Chapter 1

General Introduction : Variable fertilisation success and the population dynamics of the scallop, *Chlamys bifrons,* and other free-spawning marine invertebrates.

Over the last 12 years, and since the publication of Pennington's (1985) paper, there has been an accumulation of evidence that for many free-spawning organisms (those that simply shed eggs and sperm into the water column where fertilisation may take place), fertilisation is not always a completely efficient or successful process (reviewed in Levitan 1995, Levitan and Petersen 1995). In essence, this reproductive failure can occur because external fertilisation is a spatially dependent process, though there are also a range of other reasons why fertilisation can fail, such as parental genetic incompatibility or inviability of gametes (see Levitan 1995). Rapid dilution of gametes into a three-dimensional body of water can mean that where a female is releasing eggs sperm concentration is low and consequently each egg will have very little chance of successfully colliding and fusing with a sperm. The recent demonstration of the potential for sperm limitation (despite male and female spawners being separated by only small distances) in a range of field experiments (e.g. Pennington 1985, Grosberg 1987, Yund 1990, Levitan 1991, Babcock and Mundy 1992, Brazeau and Lasker 1992, Levitan et al. 1992, Babcock et al. 1994, Levitan and Young 1995, Lasker et al. 1996, Levitan 1996a, Coma and Lasker 1997a, 1997b, Styan 1997) and modelling work (Denny 1988, Denny and Shibata 1989, Denny et al. 1992, Young et al. 1992, Morris 1994, Andre and Lindegarth 1995, Levitan and Young 1995) and observations of incomplete fertilisation success in a number of natural spawnings across a range of taxa and conditions (see table 1.1) suggests that sperm limitation can and does occur in natural populations of free-spawning invertebrates.

For populations of free-spawners that we wish to harvest or otherwise manage, it is easy to envisage why incorporating fertilisation limitation into models of population dynamics might be important - reductions in densities to low levels (such that inter-spawner distances are large) might reduce the number of viable larvae that a population produces. Given the high fecundities of many invertebrates, there is at least the potential for small changes in fertilisation success to magnify into large changes in subsequent numbers of recruits and adults. Quinn et al. (1993)

modelled the impact of harvesting on the red sea urchin, *Strongylocentrotus franciscanus*, incorporating an Allee Effect. This refers to a decreased *per capita* reproductive success due to decreased fertilisation success at lower adult densities and also, in this case, larval recruitment facilitated by the presence of adults. They found that the dynamics of the system were altered by the inclusion of Allee Effects and that this impacted significantly on the optimal harvesting strategy. Particularly worrying was the finding that for populations displaying strong Allee Effects, slight increases in mortality through fishing or natural causes led to catastrophic population crashes. Concern about this type of effect has lead to many suggestions that sperm limitation may be an important component of the population dynamics of a range of free-spawners (Levitan 1995 and references therein, Levitan and Young 1995, Lasker and Brazeau 1996, Styan 1997) and especially commercially important mollusc species such as scallops (Oresanz et al. 1991, Peterson and Summerson 1992, Stokesbury and Himmelman 1993, Peterson et al. 1996), abalone (Shepherd and Brown 1993, Keesing and Babcock 1996), and fanshells (*Pinna spp.*) (Butler et al. 1993).

Given the obvious potential importance of sperm limitation, a logical next step is to develop an understanding of when, where and how often sperm limitation actually occurs in field populations of free-spawners and moreover what effects this might have for the population dynamics and evolutionary ecology of these animals. This thesis attempts to do this by developing a predictive model of the dynamics of free-spawning for a population of the queen scallop, *Chlamys* (*Equichlamys*) *bifrons*, at Largs Bay (see figure 1.1) in South Australia and to assess whether, and under what conditions, sperm limitation is likely for this species. Where appropriate, and for comparative purposes, I will also examine aspects of the fertilisation ecology of *C.bifrons* at a second location across Gulf St.Vincent, Edithburgh Jetty, and in another species commonly found there and at other places in southern Australia, *Chlamys* (*Mimachlamys*) *asperrima* (see also plate 1.4). *A priori*, there is also no reason to assume that fertilisation success will be uniform throughout a population and, as will be explained below, documenting the distribution and understanding the causes of variability in fertilisation success across a range of scales may be more important than just obtaining estimates of average fertilisation success within a population. The potential for fertilisation to vary amongst individuals, patches (groups of individuals) and

populations can easily be postulated for *Chlamys bifrons* by considering known features of its ecology and life history (see below). A particular aim of this thesis, then, will be to propose and test models of how variability (at a range of scales) in fertilisation success may be created and, in the process, predict the level, distribution and consequences of this variation in natural populations of *C.bifrons*.

Population dynamics and the functional importance of understanding variation in fertilisation success

It is well recognised that for many, if not all, marine invertebrates that have a planktonic dispersal stage, there is usually a large discrepancy between the larval potential (or numbers of eggs produced by adults) and the numbers of larvae that subsequently return to a population as settlers or recruits (reviewed in Rumrill 1990). Understanding the causes of larval loss (and its variability) has been the subject of much inquiry and is at the core of much of the renewed interest in larval biology and "supply-side ecology" (Underwood and Fairweather 1989). Consequently, "closing the loop" by developing predictive models of population dynamics of marine invertebrates that incorporate both larval and adult stages of the life cycle has become a major goal for marine ecology (Grosberg and Levitan 1992, Eckman 1996). There has been speculation (e.g. Pennington 1985, Rumrill 1990) that for free-spawners at least, a large part of what appears to be larval loss may be directly attributable to fertilisation limitation rather than other sources of planktonic mortality. However, despite the potential for a reduction in population wide fertilisation to be important, it should be remembered that other stages of a marine invertebrate's life history might also have a large influence on its population dynamics (reviewed in Rumrill 1990, Eckman 1996). Several authors (Rumrill 1990, Levitan 1995, Eckman 1996) have emphasised this and suggested that the dynamic importance of fertilisation failure will depend upon whether populations are limited by larval supply and the extent to which mortality in these other planktonic stages is density-independent: if either of these conditions is not met, the effects of a decrease in average fertilisation success may be swamped by effects occurring at other life stages. Indeed, in his recent review of the population dynamics of marine invertebrates Eckman (1996) suggests that it might be possible to assume fertilisation rates of 70-80% for moderately dense populations

of spawners and rates of ~20% for less dense populations and that empirical data suggests that such estimates are in error by no more than a multiple of 2-4. Further, he adds that "Given the typically high rates of mortality of larvae in the plankton, and the influence of advection on the fate of planktonic larvae", "this degree of uncertainty may not be especially important". Whilst accepting (and strongly agreeing) that factors affecting other stages of aspects of an animals life history may also be important, I believe that assertions such as Eckman's perhaps under-emphasise the potential population dynamic consequences of external fertilisation and the practical importance of understanding this process.

For a start, empirical evidence does NOT suggest that fertilisation is normally (or uniformly) high in field populations (Levitan 1995, 1996b). Consequently, it is unjustified to assume that high levels of fertilisation are always achieved (e.g. Eckman 1996). It would also be a mistake to assume that fertilisation within populations does not vary. The evidence so far collected suggests precisely the opposite - observations of natural spawning events show that fertilisation rates can be both high and very low and vary amongst individuals within populations (see table 1.1). An important point to remember about these often fortuitously, opportunistically obtained estimates is that there is little indication of how representative or biased they might be. For example, it is possible that larger, more synchronous spawning events (with higher ensuing fertilisation success) are more likely to be witnessed and so more likely to be represented in empirical observations, despite being in fact less frequent than less successful spawnings. Similarly, we usually have little idea about the effect potential sampling biases may have had on estimates of fertilisation (Levitan 1995, Styan 1997, but see Marconato et al. 1997).

There is also some evidence of discernible relationships between adult stock and subsequent recruitment levels for at least some scallop species (e.g. Dredge 1988, Caddy 1988, McGarvey et al. 1993, Peterson et al. 1996), though in most of these cases, there is still a deal of variability associated with these relationships. In itself this does not suggest that fertilisation success will be important, but it suggests the conditions in which reduced average fertilisation success may become important are at least sometimes met. There are also several cases where fertilisation failure has been directly implicated in a significant reduction in population sizes. Sadly, densities

of the once common white abalone (*Haliotis sorenseni*) in California have been reduced to such low levels that the few remaining individuals are unlikely to be able to successfully reproduce, and indeed no recruits are now found so the species may be on the verge of extinction (Malakoff 1997). It was suggested by Levitan (1991) that the observed continued low recruitment of *Diadema antillarum* following a wide-spread mass mortality throughout the Caribbean was likely a result of population densities being reduced to a point where sperm limitation effects could significantly reduce larval production. Further, regardless of the relationship between stocks and recruits at higher densities or the relative importance of differing sources of larval mortality once some eggs become fertilised, there is at least the logical possibility that population densities could be reduced to such a level that fertilisation completely fails. Thus, there might be a "critical minimum population density" required for any reproduction to occur; this would represent a lower bound for the viability of a population. Clearly, understanding when (or if) this might occur should be an important goal for scientifically based management of free-spawners.

Fertilisation failure is relevant to the development of any model of egg production within populations in that the number of larvae an animal produces may be decoupled from simple estimates of its fecundity (egg production). Management approaches that are primarily concerned with egg production such as egg-per-recruit analyses may be especially impacted by this. In a practical sense, this decoupling will only become important if the average fertilisation success varies across population densities or under differing management options. If it does not, then the average egg production will simply (and perhaps unimportantly) be scaled by a factor commensurate with the reduction in fertilisation success. However, previous work shows that such decoupling can occur and that the way it occurs in different conditions or densities can vary in surprising ways. For example, Levitan (1991) showed that for the sea urchin (*Diadema antillarum*) decreases in individual fecundity at higher densities (caused by increased intra-specific competition for food) were offset by increases in fertilisation success associated with decreased inter-spawner distance. Understanding the dynamics of fertilisation success may make it possible to identify those management strategies likely to maintain high levels of fertilisation and future models of egg production might incorporate such information. In a similar vein, there may also be a practical value in understanding variation in fertilisation success at smaller scales - there are

numerous ways in which this sort of small scale reproductive variation could be generated (some of these are outlined below for *C.bifrons* populations). For example, variable fertilisation success at the patch scale might create a "source/sink" (Pulliam 1988) multipartite population structure, with areas of high and low fertilisation success. Determining whether such structure occurs in populations of *C.bifrons* is one aim of this thesis. Management might seek to identify and differentially conserve individuals or groups of individuals (patches) of high and low fertilisation success. Of course other factors such as fecundity will also affect the reproductive contribution made by an individual, and larger scale oceanographic transport may also play an important role in determining whether a local population or patch is ultimately a source or a sink (e.g. Shanks 1995), so these might also need to be incorporated into models of the reproductive potential of individuals or patches.

Finally, a potentially very important, though unexplored, consequence of variance in fertilisation success amongst individuals within a population is that it may affect the genetic structure of populations and, in particular, effective genetic population sizes (Nunney 1994a, 1994b). If an animal has high fertilisation success, then its offspring may be disproportionately represented in subsequent recruitment, skewing the genetic composition of future generations. If large enough, this skewing can reduce the effective genetic size of a population (Nunney 1996 derives equations for determining how this reduction will occur) and most importantly, this effect may occur even in populations which are not limited by larval supply, i.e. populations for which reduced fertilisation success might otherwise be relatively unimportant.

The only study that has compared effective genetic population size to adult population size (N_e / N) for a free-spawning marine invertebrate is that of Hedgecock et al. (1992) for a population of the oyster, *Crassostrea gigas*, in Dabob Bay, Washington. Hedgecock (1994a, 1994b) later suggested that the very low value (N_e / N $<10^{-6}$) that was found may have resulted from variable reproductive success amongst individuals caused through either variable fertilisation success or oceanographic transport of larvae. The magnitude of this estimate has been questioned on several grounds (Nunney 1996) and it should be noted that generally there are numerous difficulties and biases involved in both direct and indirect estimation of N_e / N (Nunney and Elam

1994, Nunney 1995). Nunney and Elam (1994) found that N_e / N is not usually below 0.5 and in most situations is fairly close to 1.0 though their review was based on studies of terrestrial animals and so did not consider the effects of the dynamics of free-spawning. Nunney (1996) showed clearly that it is not the absolute magnitude of individual fecundities that leads to a reduction in N_e (as was initially suggested by Hedgecock et al. 1992 as an explanation of $N_e / N < 10^{-6}$), but rather the degree of variation (in excess of random variation) in lifetime reproductive success amongst individuals within a population (N_e is inversely proportional to variation). For most terrestrial animals, this variation in lifetime success is usually fairly low (Nunney and Elam 1994) which explains their findings that N_e is usually fairly close to 1.0. However, as noted by Nunney (1994a, 1994b) variance in lifetime reproductive success may be much greater for marine invertebrates than terrestrial animals. As illustrated in many observational studies (see table 1.1) and experimental studies (Pennington 1985, Grosberg 1987, Yund 1990, Levitan 1991, Babcock and Mundy 1992, Brazeau and Lasker 1992, Levitan et al. 1992, Babcock et al. 1994, Levitan and Young 1995, Levitan 1996a, Coma and Lasker 1997a, 1997b, Styan 1997), free-spawning has at least the potential to create large variation in individual reproductive success and thus to affect N_e. Whether sufficient variation in individual fertilisation occurs in natural populations to alter effective genetic population sizes significantly has not previously been assessed; to do this for *C.bifrons* will be a particular goal of this thesis.

Clearly then, it may be important to understand and predict variation in fertilisation success at a range of scales rather than just to estimate population wide average rates. Therefore, in developing a model of fertilisation success of *C.bifrons* populations, I will determine the distribution of success within a natural population at three scales (individuals, patches or groups of individuals and entire populations) and then also assess the effects of this in terms of whether (and if so, by how much) effective genetic population sizes are affected by this variation.

The scallop, Chlamys bifrons*, at Largs Bay, South Australia - a model species*

Chlamys bifrons is found in depths of 2 to 50 meters right along the southern Australian coast, where it is a common inhabitant of subtidal soft sediment communities (Ludbrook and Gowlett-Holmes 1989). It grows to a size of up to 130 mm in some places with a maximum longevity of about 12-14 years (Wolf and White 1995), though at Largs Bay individuals larger than 95 mm are rarely seen (pers. obs., and see chapter 7). *C.bifrons* has separate sexes and, like all other scallops, reproduces by free-spawning. Prior to this study it was not known when this occurs. It is not the target of a large commercial dredging operation, but does support a small diver based fishery in South Australia and it is important to recreational divers, with daily bag limits placed in several states (Kailola et al. 1993).

Scallops (Pectinidae) and bivalves in general have long been thought to be organisms likely subject to sperm limitation in wild populations (e.g. Belding 1910, Oresanz et al. 1991, Peterson and Summerson 1992, Stokesbury and Himmelman 1993, Peterson et al. 1996), yet fertilisation success has not directly been examined in any species of scallop. There is also no clear indication of the scale (of population densities) over which sperm limitation might occur for these animals, nor how fertilisation success might decline with increasing distance between spawners. This is because large difference in fertilisation success have been noted between previous fertilisation ecology studies conducted over comparable inter-spawner distances. Pennington (1985) found that whilst fertilisation of *Strongylocentrotus droebachiensis* was near 100% for females spawning adjacent to a simulated spawning male, when the female was moved 100cm downstream, fertilisation decreased to less than 5%. He also found fertilisation decreased even more rapidly with female position downstream when experiments were conducted in times of faster current flow. Apparently fertilisation success declines with inter-spawner distance in a similar way for the urchins *Diadema antillarum* and *Strongylocentrotus franciscanus* (Levitan 1991,1992) and the brooding coral *Briareum asbestinum* (Brazeau and Lasker 1992). Fertilisation decreased more slowly as females were moved downstream away from males for the coral *Plexaura kuna* (Coma and Lasker 1997a) and was still greater than 10% up to 500 cm downstream for the urchins *Heliocidaris erythrogramma* and *Evechinus chloriticus* (Styan 1997, S.Mead, R.C. Babcock, C.A.

Styan unpublished manuscript). Yund (1990) found a pattern of relatively high rates of fertilisation (though still decreasing with inter-spawner distance) for the hydroid *Hydractinia echinata* several metres downstream of a male. Even more strikingly, Young et al.'s (1992) model predicted nearly 100% success of female *Stylocidaris lineata* 5 m downstream of a male and in field experiments Babcock et al. (1994) found female fertilisation was 10% or greater up to 30 metres downstream of a spawning male for the crown of thorns starfish, *Acanthaster planci*. There have been few studies of the fertilisation ecology of bivalves (most studies have focussed on echinoderms or coelenterates) and again, the divergent results from these provide few clues as to how fertilisation works in scallops. Andre and Lindegarth (1995) used laboratory spawning experiments and modelling to predict the scale over which fertilisation declines with inter-spawner distance for the intertidal cockle, *Cerastoderma edule*. Moderately high rates of fertilisation (~20 %) were predicted for *Cerastoderma* even up to 1m downstream of a male (though this estimate was strongly dependent upon predicted flow speeds when spawning is occurring) and the authors constructed a heuristic model that suggested fertilisation will be high (>50%) in even low to moderate population densities (1 m^{-2}), if spawning occurs in large patches of individuals in shallow water. Downing et al. (1993) measured reproductive success as a function of local density in a natural population of the brooding freshwater mussel *Elliptio complanata* and found fertilisation was high when mussels were at very high local densities (>20 m^{-2}), but reduced to zero when local density was still reasonably high (10 m^{-2}). Differences noted between studies may simply reflect methodological biases associated with differing sampling apparatus and methodologies (Levitan 1995, Styan 1997, S.Mead, R.C. Babcock, C.A. Styan unpublished manuscript). For example, all of these studies involving echinoderms have used the presence of fertilisation membranes as a criterion of fertilisation, possibly biasing upwards estimates at higher ambient sperm concentrations (see chapter 2). The differences may also be the result of varying species-specific attributes of the animals in question or differing environmental influences present during experimentation. Given the range of factors listed in table 1.2 that all interact to affect fertilisation (Levitan 1995), many of which are likely to vary between species or spawning locations, it would not be particularly surprising there were differences in the dynamics of external fertilisation between species!

The first aim of this study of the fertilisation ecology of *C.bifrons* then, is to determine the distance that females can be away from males and still have eggs fertilised and to examine the way fertilisation changes with increasing distance from a male. In itself, this would provide some indication of the population densities over which fertilisation might fail, and allow for comparison with previous studies. Perhaps more importantly, I will also attempt to scale up models of spawning between individuals to models of spawning within populations and then assess how fertilisation varies with changing population density. This is because, although models of spawning between pairs of individuals give an indication of the likely relationship between population density and fertilisation success, it is not easy intuitively to account for the effect that coincident multiple spawning individuals on fertilisation success rates. As outlined above, this will involve predicting fertilisation success of whole populations and also documenting the distribution of success amongst individuals within populations. There are however, three further features that I believe need to be considered in developing these models of *C.bifrons* fertilisation dynamics. These are firstly, to determine how scallop size and fecundity directly affects the process of fertilisation, secondly, to understand the direct effects of the environment where a scallop is spawning on the process of fertilisation, and thirdly to understand the indirect effects of the spatial structure of scallop populations on fertilisation dynamics.

At Largs Bay , *C.bifrons* appear reproductively active (judged by the visual appearance of gonads and ability to be induced into spawning - see also chapter 4) by about a shell length of 55 mm, at which point they are probably 1.5 -2 years old based on Wolf and White's (1995) work. Between this and their maximum size there of 95 mm there is a very large difference in the mass of the gonads of males and females (a nearly 5-fold difference in wet gonad weight - see chapter 4). Intuitively, it would also be expected that if scallops release synchronously (and this will be addressed in chapter 4), then larger males would release sperm at a higher rate than small males simply because they have more sperm to release. Models of sperm dispersal in turbulent near-bed flows (e.g. Denny 1988, Denny and Shibata 1989, Babcock et al. 1994) and experimental manipulation of the number of males spawning at a focal point (Pennington 1985) all indicate that the distance downstream that fertilisation works is dependent upon sperm release rate. Consequently, it would be expected that population structure should be an important factor

affecting the dynamics of spawning because male size (or fecundity) should directly influence fertilisation. However, Levitan (1991) found that in small experimental arrays of urchins both population density and size significantly affected female fertilisation, but found little effect of male spawner size. Perhaps the study of Levitan (1991) simply did not have enough power to detect a small but biologically significant male size effect, but how large an effect Levitan's study was able to statistically detect was not addressed. In any case, I will test the hypothesis that (male) size structure has an effect on the dynamics of spawning between male/female pairs and within populations of *C.bifrons*. Importantly, if size/fecundity does affect the dynamics of external fertilisation, an additional goal will be to determine how it does so.

Small scale hydrodynamics of near-bed flows can obviously have a large influence on the dynamics of external fertilisation, simply because the dilution and dispersal of relatively immotile gametes is expected to be largely controlled by the way and rate at which water moves (Denny 1988). In some instances, this influence can be very large with the relative fertilisation success achieved by animals in differing habitats or conditions varying greatly (e.g. Denny and Shibata 1989, Denny et al. 1992). At Largs Bay (and other places, pers. obs.) *C.bifrons* are found inhabiting patches (areas of seafloor 10-10000+ m^2) of a range of obviously different habitat types, which I have classified here as "Sand", "Seagrass" and "Silt" (see also chapter 7 for detail of how much of each habitat type was present). Sand areas (plate 1.1) are just that, areas of bare coarse sand whilst seagrass patches (plate 1.2) are areas of *Posidonia spp.* meadows of varying density, typically in the range of 500 to 5000 shoots m^{-2}. Silt areas (plate 1.3) are apparently areas of degenerated seagrass (dead rhizomes are sometimes present just below the sediment surface) covered by very fine sediments, and often inhabited by the large bivalve *Pinna bicolor* and abundant filamentous algae. These patches are arranged in an interspersed mosaic pattern at Largs Bay, with usually fairly distinct boundaries between patches of different habitat types. Tidal currents at Largs Bay are quite slow - maximum flow measured there was ~8 cm sec^{-1} , but at another location, Edithburgh Jetty, maximum currents are faster (~14 cm sec^{-1}). These observations suggest a possible mechanism that might act to generate variation in fertilisation success of *C.bifrons* : differing physical characteristics (principally near-bed flow) of the patches or locations might affect the way gametes disperse and so directly affect the dynamics of

fertilisation. Clearly it will be necessary to test whether spawning in different areas/habitats directly affects *C.bifrons* fertilisation dynamics rather than assume fertilisation works the same way in all areas/habitats.

External fertilisation is a spatially-dependent process and, because of this, the variation in the spatial distribution of scallops may result in variation in fertilisation success. Of course, this will depend on the interaction of spatial scale over which fertilisation decreases (which in turn may be affected by factors such as scallop size/fecundity and spawning conditions) and the spatial dispersion patterns of scallops. Hence, variation in scallop density that might exist between habitat types, between patches within habitats and within patches might all lead to variation in individual fertilisation success within the Largs Bay population. Preliminary observations were that scallops are quite abundant on silt patches, moderately common in seagrass and rare on areas of bare sand. Consequently, in addition to producing a model of how fertilisation might work in hypothetical, randomly dispersed *C.bifrons* populations, I will also measure the patterns of scallop density at a range of scales (between habitats, amongst patches within habitats, within patches, all over 2.5. years) and incorporate this into a model of fertilisation success in a real scallop population.

Measuring fertilisation success and outline of the model of scallop fertilisation success

A range of factors have been identified, all of which can influence the dynamics of fertilisation (Levitan 1995) - these are listed in table 1.2. These factors act on a range of scales, from effects on the level of gamete interaction, to effects dependent upon attributes of individuals or populations of spawners, and physical features about the environment where spawning takes place. This is a long list, and I will not go into detail here about the way in which each of these can affect the dynamics of fertilisation - Levitan's (1995) review has already done this and I have highlighted the particular areas that will be investigated in this thesis. This list is also reproduced here to emphasise the necessity of considering a range of scales and perspective in considering what factors are likely to be important in developing a model of scallop fertilisation, particularly when developing a mechanistic, bottom-up model like the one that will be developed here.

Ideally, an investigation of fertilisation success would be able to directly measure fertilisation, experimentally manipulating only whatever factor is of interest (such as population density and/or spawning environment) and observing what happens to rates of fertilisation. However, to measure fertilisation success in (manipulated) natural populations, spawning must be observed, workers must be equipped with the right sort of sampling equipment (Levitan 1995, Styan 1997) and sampling must be done in an unbiased, representative way (i.e. replicated sampling conducted randomly throughout all spawning events). Unfortunately, our understanding of reproductive patterns on the fine time scale necessary to forecast when spawning events occur (and so make these observations) is generally very poor. Indeed, recorded observations of spawning events by any marine invertebrate (see table 1.1) are extremely rare and most have been opportunistically collected (Levitan 1995). There is also usually no indication of how representative (or unbiased) these observations are. There are, however, some notable exceptions to this generalisation, where predictable spawning patterns have allowed observation and measurement of fertilisation within a population, across a spawning period: Oliver and Babcock (1992) measured fertilisation of mass spawning corals, *Montipora digitata* across several nights of spawning. Lasker and colleagues (Brazeau and Lasker 1992, Lasker et al. 1996, Coma and Lasker 1997a, 1997b) were able to measure variation in fertilisation success over a range of scales in the Caribbean octocorals *Briareum absetinum*, *Plexaura kuna* and *Pseudoplexaura porosa*. Williams et al. (1997) have also utilised the predictable nature and brooding habit of the intertidal polychaete *Arenicola marina* to set out worms in experimental burrows at the correct times to measure fertilisation in field populations. The long-term retention of eggs in the mantle cavity of the freshwater mussel, *Elliptio complanata*, allowed Downing et al. (1993) to directly assay fertilisation of individual females and relate this to the density of neighbouring mussels. The studies of Downing et al. (1993), Lasker et al. (1996) and Coma and Lasker (1997a, 1997b) are exceptional in that they provide estimates of the level and the distribution of variation in fertilisation success that occur amongst individuals within spawning populations. As argued above, this information might be the most important to gather.

Unfortunately, the prospects for being able to make similar direct measurements of fertilisation during natural spawning of *C.bifrons* are not good at all. *A priori*, it is not even known during what periods of the year the scallops spawn and so expecting to witness spawning events is unrealistic, much less set up replicated manipulative field experiments prior to spawning. The focus of the questions asked in this thesis are also large in spatial scale - determining how fertilisation varies within and across a range population densities. Even if spawning times could be predicted, to manipulate and monitor populations or even patches of individuals on this scale and across a range of spawning conditions and male size combinations would be prohibitive. Instead, I will use computer simulations to scale up models of the way fertilisation varies with distance between an individual pair of male and female spawners to a model of fertilisation within a population, an approach successfully employed by Morris (1994) and Levitan and Young (1995).

Although methods to induce scallops to spawn in a reasonably natural way have been developed (see chapters 3,4) and so direct manipulative experiments involving spawning scallops are possible (see chapter 5) these are extremely difficult. Not least this is because of the limited times when scallops are ready to spawn (and a previous lack of knowledge of when these times occur) and an intensely frustrating variability in the time lag between induction of spawning via serotonin injection and the actual onset of spawning. This latter feature in particular, means that getting scallops to spawn coincidently underwater in an experimental setting is very difficult (nonetheless, this was done for a limited set of experimental conditions in chapter 5). Given this difficulty, to examine adequately all of the factors outlined above (distance between spawners, habitat, flow speed, male size) would be prohibitively time consuming. Consequently, the way in which the model of spawning between pairs of individuals will be developed will also involve deconstructing this into component processes. This involves measuring the rate at which scallops release sperm (chapter 4), then determining how this sperm disperses in varying field conditions (chapter 5) and to what concentration it has diluted at varying distances from the male. This dilution of sperm is then interpreted in terms of likely fertilisation success by determining the relationship between ambient sperm concentration and the likelihood that an egg becomes fertilised (i.e. fertilisation kinetics, based on a model developed in chapter 2 that is fitted in chapter 3). A general schema for the development of this model of fertilisation for *C.bifrons* is outlined in figure 1.2.

Thesis Outline

In Chapter 2, as a prelude to the development of the model for the mechanics of free-spawning, I rework an established mathematical description of fertilisation kinetics of free-spawned gametes. That is, I make adjustments to an established model (Vogel et al. 1982) that mathematically describes how the likelihood of egg fertilisation varies with ambient sperm concentration and gamete characteristics. These adjustments (principally taking into account the possibility of multiple coincident sperm-egg collisions and ensuing polyspermy) may have important consequences for our understanding of the dynamics and evolution of free-spawning. In Chapter 3 I use a series of laboratory experiments to fit this fertilisation kinetics model for both *C.bifrons* and *C.asperrima*. Specifically, I ask how ambient sperm concentration affect the likelihood that a scallop egg will become fertilised. In part, this work will also test the model developed in chapter 2. This relationship is used in later chapters to relate ambient sperm concentration and the effects of sperm dispersal and dilution to the likelihood of fertilisation.

Chapter 4 describes spawning patterns of two species of scallops (*C.bifrons* and *C.asperrima*) on a variety of scales. Broad scale reproductive cycles are described for *C.bifrons* from Largs Bay and Edithburgh Jetty and, for comparison, a second species, *C.asperrima,* from Edithburgh Jetty. Size-fecundity relationships are described for ripe scallops and sperm release rates measured in the laboratory. Using both of these, the likely pattern of spawning synchrony within a population of *C.bifrons* is assessed.

Next, in chapter 5, I develop the model of spawning between pairs of scallops and then extend this to a model of fertilisation within populations. Sperm dispersal away from a single male in field conditions is estimated using dye release experiments in three different habitats that scallops inhabit and at two locations, each characterised by differing tidal current regimes. Using these data, sperm release rates from chapter 4 and the fertilisation kinetics model from chapter 3, an indirect model is constructed of how sperm disperses away from a male, and then what effect this dispersal has in terms of the chance that an egg released at varying positions around a male will become fertilised. Predictions of fertilisation are compared for scallops spawning in a range of

habitats and flow speeds. A field experiment then tests for differences between this indirect model and fertilisation measured directly when scallops were induced to spawn in the field in a limited subset of spawning conditions. Then, using models of sperm dispersal away from a single male, a series of computer simulations of larger numbers of (randomly dispersed) simultaneously spawning scallops are run, from which I estimate average fertilisation success across local populations (patches) of varying densities in a range of habitats and current flows.

In chapter 6, I focus on the individual success rates of both female and male scallops within the simulated populations from chapter 5 and on the variation in fertilisation success amongst individuals within populations. Large skews in individual reproductive success potentially can reduce effective genetic population size, so I also determine the effects of the predicted variability in individual spawning success upon estimates of this parameter.

Temporally repeated, spatially replicated measures of the dispersion patterns within and between different habitat types were made for a population of scallops at Largs Bay and these are described in chapter 7. Of some interest in their own right, these spatial data are then used in conjunction with the model of *C.bifrons* fertilisation developed in earlier chapters, to predict fertilisation success in a real population at Largs Bay. The effect of spatial variation in scallop dispersion patterns, and that of a large die-off that occurred during this study are examined in terms of population-wide average fertilisation success, the distribution of individual fertilisation success, and the effective genetic population size at this location.

In the final chapter I outline the general features of the model that is developed in this thesis, in particular the level and the importance of variable fertilisation success of *C.bifrons*. I review the consequences of the patterns uncovered for our understanding of the population dynamics of the scallops and free-spawners in general and look to ways in which the predictions of this model may be tested in the future. Remaining areas of uncertainty are discussed as a pointer to future research, which might improve the model and our understanding of the dynamics and consequences of free-spawning.

Chapter 2 has been accepted for publication and is about to appear as a research note in *The American Naturalist* (152: 290-297, August 1998). Other chapters are yet to be submitted for publication. Appended to this thesis is a recently published paper (*Marine Ecology Progress Series* 150 : 293-296.) which describes a device I designed to collect eggs from free-spawners and presents results from a field experiment in which I assayed the fertilisation success of urchins (*Heliocidaris erythrogramma*) when spawners were spaced at various distances apart from each other. This device had been developed with a view to using it in field experiments to assay the fertilisation success of *C.bifrons*. However, though the air-displacement sampler may prove a useful device in some circumstances (e.g. with urchins), the very small eggs of *C.bifrons* and the extreme difficulty encountered in inducing scallops to release eggs in the field meant that alternative sampling methods had to be used in the field experiments on scallops described in this thesis.

Thus, this thesis will build up a detailed model of the way fertilisation works from the level of gamete interaction upwards, to predict fertilisation success between individuals, then patches of randomly dispersed individuals and finally to examine the level and consequences of variation between individuals at a range of scales. The way this model is developed - by decomposing the model into smaller submodels that can be then reconstructed into a model of population success - is strongly reductionist. Such an approach should not be a goal in itself as it can lead to the development of increasingly complex models of poor predictive power (due to the magnification of errors from within submodels); and it can cause a change of focus to a less predictive, but more explanatory goal (Peters 1991). Of course, I hope to have avoided such a loss of focus. The approach I have used here had been chosen primarily for practical reasons, but I hope that it will lead to two outcomes that may not have otherwise been achieved. The first is the development of a model of scallop fertilisation that is predictive across a wider range of conditions than could be developed by a strict manipulative/mensurative approach. The second is that it will allow insight into the mechanisms generating variability in fertilisation success; this, in turn, may lead to a better understanding of the dynamics of free-spawning in general and facilitate extrapolation to, and a focus for further studies of, other free-spawning species.

Table 1.1. Field surveys of the range of individual fertilisation success measured in natural populations of free-spawning algae, invertebrates and fish (after Lasker et al. 1996).

Taxon	Range	Reference
Algae		
Fucus cerranoides (brown alga)	88-100%	Brawley (1992)
Fucus vesiculosus (brown alga)	78-100%	Pearson and Brawley (1996)
Fucus distichus (brown alga)	95-100%	Serrao et al. (1996)
Polysiphonia lanosa (red alga)	24-90 %	Kaczmarska and Dowe (1997)
Invertebrates		
Briareum asbestinum (gorgonian)	<0.01-6.5%	Brazeau and Lasker (1992)
Plexaura kuna (gorgonian)	0-95%	Lasker et al. (1996), Coma and Lasker (1997b)
Pseudoplexaura porosa (gorgonian)	0-80%	Lasker et al. (1996), Coma and Lasker (1997a)
Montipora digitata (coral)	0.2-75% †	Oliver and Babcock (1992)
Campularia everta (hydroid)	77-100%	Coma et al. (1996)
Arenicola marina (polychaete)	0-90%	Williams et al. (1997)
Elliptio complanata (freshwater bivalve)	0-100%	Downing et al. (1993)
Acanthaster planci (asteroid)	23-83%	Babcock and Mundy (1992)
Actinopyga lecanora (holuthurian)	67-78%	Babcock et al. (1992)
Bohadschia argus (holuthurian)	0-96%	Babcock et al. (1992)
Holothuria coluber (holuthurian)	9-83%	Babcock et al. (1992)
Cucumaria miniata (holuthurian)	1-100%	Sewell and Levitan (1992)
Alcyonidium sp. (bryozoan)	63-100% *	Temkin (1996)
Bowerbankia gracilis (bryozoan)	0-83% *	Temkin (1996)
Cribrilina corbicula (bryozoan)	0-100% *	Temkin (1996)
Dendrobeania lichenoides (bryozoan)	13-100% *	Temkin (1996)
Electra pilosa (bryozoan)	87-100% *	Temkin (1996)
Hippodiplosia insculpta (bryozoan)	100% *	Temkin (1996)
Schizoporella serialis (bryozoan)	89% *	Temkin (1996)
Tricellaria gracilis (bryozoan)	50-100% *	Temkin (1996)
Watersipora arcuata (bryozoan)	89-100% *	Temkin (1996)
Fish		
Halichoeres bivattatus (wrasse)	20-100%	Petersen (1991a)
Thalassoma bifasciatum (wrasse)	0-100%	Petersen et al (1992)
Xryichthys novcula (wrasse)	70-95%	Marconato et al. (1995)
Sparisoma radians (parrotfish)	20-100%	Marconato and Shapiro (1996)

† Variation in (population wide) average fertilisation success between nights of mass spawning.

* Temkin's (1996) estimates of the fertilisation of brooding Bryozoan species indicate variation between eggs of differing development stages rather than variation between individuals. Thus, additional variation for these species may have resulted from sampling immature eggs. Additionally, it is not stated how many parent colonies each of these samples were taken from.

Table 1.2 Factors influencing fertilisation, from Levitan (1995). Underlined factors are those addressed directly or indirectly in this thesis.

Gamete	Individual	Population	Environmental
Sperm	Behaviour	Density	Topographical complexity
Morphology	*Aggregation*	Size	Flow
Behaviour	*Synchrony*	Spatial distribution	*Advective velocity*
Velocity	*Spawning posture*	Size Structure	*Turbulence*
Longevity	*Spawning rate*	Age structure	Water depth
Egg	Morphology	Sex ratio	Water quality
Size	*Size*		*Temperature*
Jelly Coat	*Reproductive output*		*Salinity*
Chemotaxis	Age		*pH*
Sperm receptors	Energy allocation		
General			
Age			
Compatibility			

Plate 1.1. Sand habitat at Largs Bay, South Australia. A marked *C.bifrons* (75 mm shell height) is centre of the picture. 4m depth.

Plate 1.2. Seagrass habitat at Largs Bay, South Australia. Again, a marked *C.bifrons* (75 mm shell height) is centre of the picture, showing a typically dense *Posidonia sp.* meadow. 4m depth.

Plate 1.3. Silt habitat at Largs Bay, South Australia. Present are the large shells of *Pinna bicolor* and filamentous algae and a marked *C.bifrons* (75 mm shell height) is centre of the picture. 4m depth.

Plate 1.4. Numerous *Chlamys asperrima* bysally attached to a fallen pier piling at Edithburgh Jetty, South Australia. 6m depth.

Figure 1.1. Map of field locations.

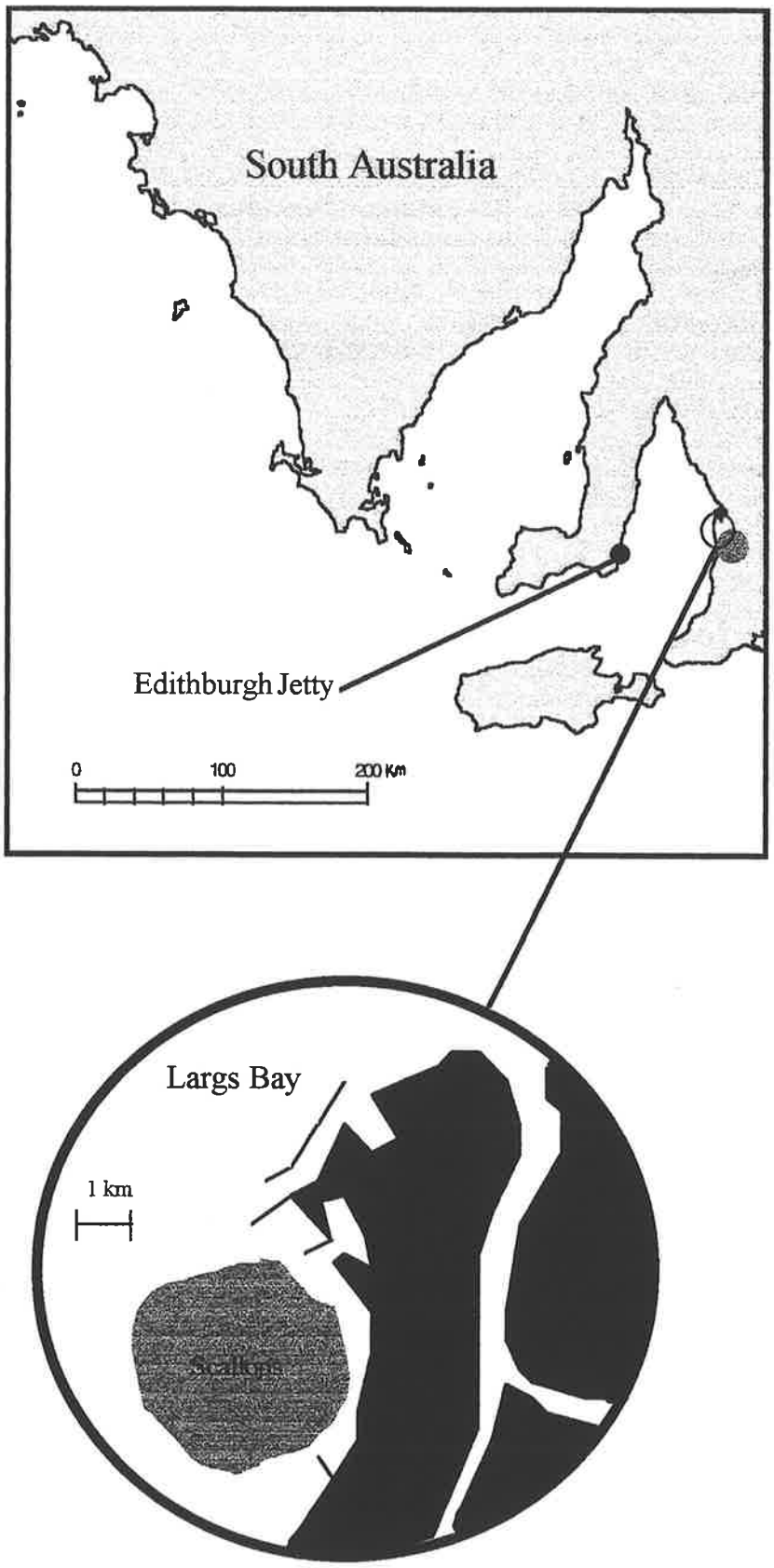
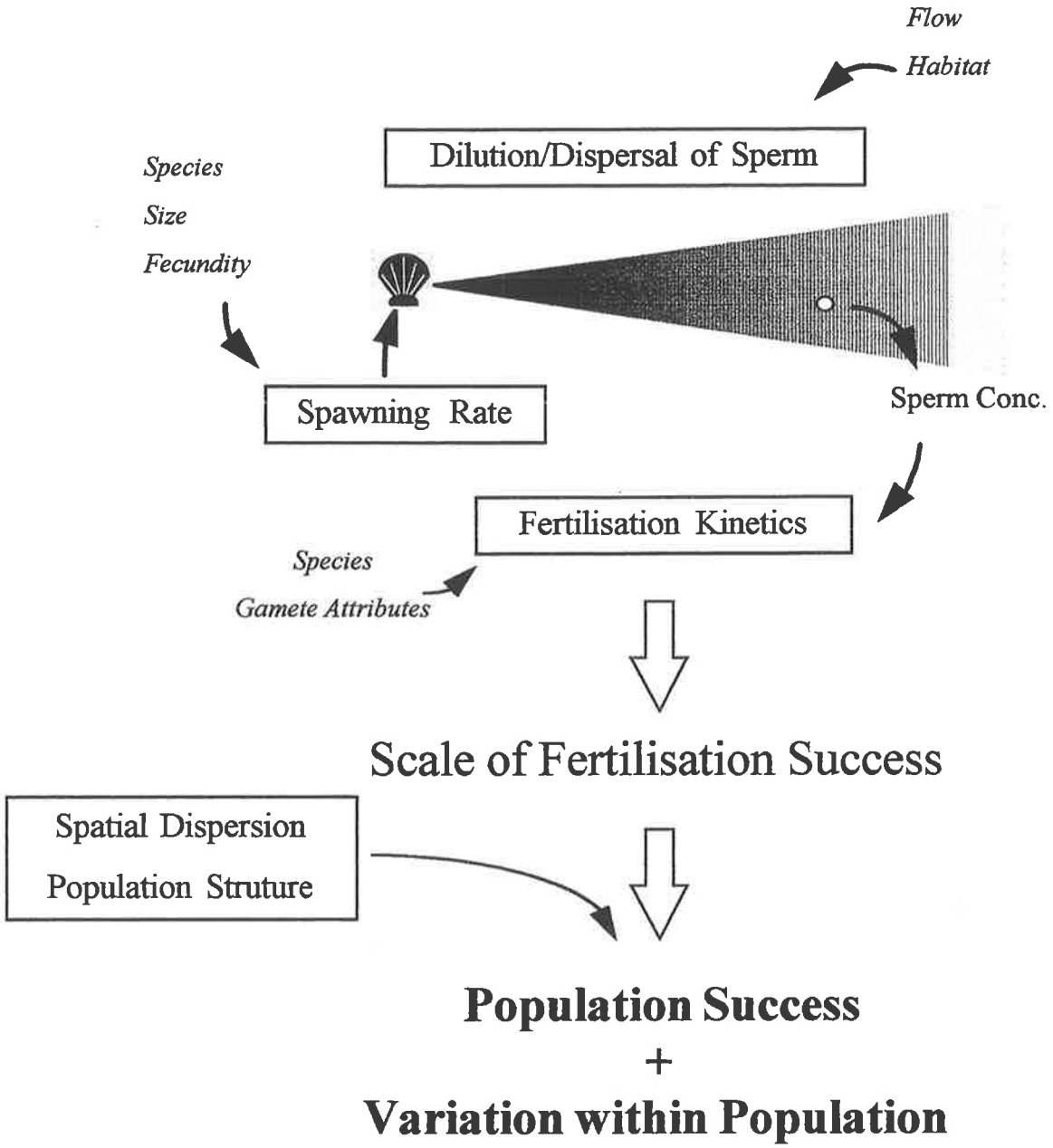


Figure 1.2. Outline of *C.bifrons* fertilisation success model.



Chapter 2

Reworking of a Fertilisation Kinetics Model

Recent recognition of sperm limitation effects, and quantification of resultant variation in fertilization success, has had important consequences for the understanding of the ecology and life-history of marine organisms that reproduce by free-spawning (reviewed in Levitan 1995, Levitan and Petersen 1995). Central to this developing theory is the idea that the likelihood of fertilization is a function of ambient sperm concentration. Vogel et al. (1982) produced a mathematical model of this relationship, based on a model of bimolecular kinetics and a random collision of gametes. This model (hereafter referred to as the VCCW model) is the basis of several analyses of individual and population fertilization success of free-spawning invertebrates (e.g. Denny 1988, Denny and Shibata 1989, Denny et al. 1992, Levitan and Young 1995, Morris 1995, Babcock et al. 1994), reproductive strategies in hermaphroditic fish (Petersen 1991b) and gamete provisioning in invertebrates (Levitan 1993, 1996a, 1996b; Podolsky and Strathmann 1996). The VCCW model, however, examines only the consequences of sperm limitation and does not consider the possibility that contact with more than one sperm may lead to polyspermy.

The VCCW model assumes that all eggs that are contacted and fertilized by at least one sperm develop into normal embryos when, in fact, we would ordinarily expect that fertilization by two or more sperm will result in polyspermy and either mortality or abnormal larval development. Presumably, implied in the VCCW model's "fertilization by at least one sperm" criterion is the assumption that eggs possess an intrinsic block that prevents polyspermy. Such blocks are typically effective in avoiding the consequences of high sperm concentrations and are common to many taxa (reviewed in Jaffe and Gould 1985), and although there is debate about whether all species possess fast acting or complete blocks (e.g. Byrd and Collins 1975, Dufresne-Dube et al. 1983), at least two types of block may be involved: a fast electrical block occurring within seconds of sperm contact, and a slower cortical reaction (Schuel 1984). However, in the VCCW model, polyspermy blocks are implicitly "perfect" in that once an initial sperm fertilizes an egg, there is (instantaneously) no possibility whatsoever that other sperm egg can also fertilize. In reality, there

will be a finite time period between the initial fertilization and polyspermy block activation whilst cellular changes necessary to prevent further fertilizations take place. During this time additional fertilizations (hence polyspermy) might occur. At least one recent study (Brawley 1992) found that up to 7% of eggs were polyspermic in a natural population of free-spawning (fucoid) algae. A more realistic model of fertilization kinetics would take into account the finite time delay in the activation of polyspermy blocks and be able to predict the likelihood of polyspermy.

A more complete model of fertilization kinetics might also address one concern of Podolsky and Strathmann (1996) regarding Levitan's (1993, 1996a) incorporation of sperm limitation effects to account for intermediate egg sizes of at least free-spawning marine invertebrates. Using the VCCW model, Levitan (1993) suggested that because, in sperm limited conditions, larger eggs provide larger targets for sperm (and increase the rate of sperm-egg collisions), they should be selected for, counter-balancing other forces that favor small eggs. Podolsky and Strathmann (1996) reasoned that if eggs were sperm limited, why would they not have evolved to become better targets in a manner that does not require an increase in investment in egg size? For example, exudation of chemoattractants could increase the effective surface area of eggs without a large increase in energy expenditure per egg. Similarly, energetically inexpensive external structures such as jelly coats and accessory cells can increase fertilization rates at a given sperm concentration (Bolton and Havenhand, unpublished manuscript). Simply increasing the number of sperm receptor sites in an egg membrane (i.e. increasing β / β_0 - Vogel et al. 1982) would also reduce sperm limitation effects at low ambient concentrations. Podolsky and Strathmann (1996) also point that most eggs are spherical, which minimizes surface area available for sperm contacts. In this chapter, I suggest one possible answer to Podolsky and Strathmann's (1996) question "Given the potential for increasing egg size non-organically, why do many free-spawners maintain small eggs?" (p168) is that larger eggs (and increased rate of sperm/egg collisions) can result in increased rates of polyspermy.

In this chapter, I first outline the VCCW model. Then I modify parameters of the model and incorporate a polyspermy block that takes a finite period of time to act. In doing so, the new

model developed accounts for effects of egg target size on the potential for polyspermic fertilization. I will then argue that care needs to be taken when assaying fertilization success in the laboratory and field experiments and that understanding the selective forces associated with fertilization is not a simple matter of "bigger eggs are better". In natural spawning circumstances where eggs may be exposed to a wide range of ambient sperm concentrations, avoiding polyspermy may be as important as increasing the chance of sperm/egg collisions

The Vogel - Czihak - Chang - Wolf (VCCW) Model

Vogel et al. (1982) developed three mathematical models to describe the collision (and fertilization) of sperm with eggs. Their model of greatest interest (the "Don Ottavio model") makes the assumption that sperm swim randomly and attach permanently to the first egg they encounter, whether or not fertilization takes place. Note that the labeling of equations "(VCCW n)" below refers to the original numbering of terms (n) in the paper by Vogel et al. (1982).

Based on analogy with bimolecular kinetics, the number of sperm, S_e , (sperm μl^{-1}) that collide with an egg in a time period (t) is

$$S_e = S_o(1 - e^{-\beta_0 E_0 \tau}) \qquad \text{.....(1) (VCCW 9)}$$

where S_o is the starting ambient sperm concentration (sperm μl^{-1})

β_o is the bimolecular collision rate constant ($mm^3\ sec^{-1}$)

τ is the sperm half-life (sec) - which is substituted with t, the time (sec) eggs are exposed to sperm, if $t < \tau$

Here the collision rate constant (β_o) is estimated by the product of the total cross-sectional area of the egg, σ (mm^2), and sperm swimming speed, υ ($mm\ sec^{-1}$) :

$$\beta_o = \sigma \upsilon \qquad \text{.....(2) (VCCW 7)}$$

Therefore the average number of sperm sampled by one egg from a solution of eggs of concentration E_o (eggs μl^{-1}) is :

$$\frac{Se}{Eo} = \frac{So}{Eo}(1 - e^{-\beta_o E_o \tau}) \qquad \text{.....(3) (VCCW 10)}$$

Vogel et al. (1982) suggest that not every sperm that hits an egg is necessarily a potential fertilizer, a feature of other models of sperm/egg kinetics (e.g. Rothschild and Schwann 1951). Only a fraction of the egg surface where sperm have attached provide sites that are penetrable to the sperm (Vogel at al. 1982). Vogel et al. (1982) define β as the rate collision constant of sperm contacts with penetrable egg surface (receptor sites). The fertilizable fraction of the egg surface can then be estimated as the ratio of β and β_o (the rate collision constant of sperm hits with the total egg surface). Thus, the average number of potential fertilizing sperm (x) an egg will sample is

$$x = \frac{\beta}{\beta_o}\frac{S_o}{E_o}(1 - e^{-\beta_o E_o \tau}) \qquad \text{.....(4) (VCCW 11)}$$

In practice, β/β_o is fitted from experimental data using non-linear regression methods (Vogel et al. 1982, Levitan et al. 1991, Levitan 1993 and Levitan and Young 1995) because, although it is possible independently to estimate the rate collision constant β_o (see VCCW 7), it is difficult to independently measure the fertilizable fraction of the egg surface to predict β. Fitted estimates for β/β_o have at least two other (not mutually exclusive) interpretations. First, some fraction of the sperm population may be unable to penetrate and fuse on contact with a receptor on the egg surface (Vogel et al. 1982), reflecting some characteristic of the quality of the sperm rather than the egg. Second, it may be that only a fraction of the potential sperm/egg contact results in fertilization because of some form of incompatibility between particular egg/sperm combinations. Such incompatibility in particular male/female crosses has been noted in ascidians by Grosberg

(1987) and Havenhand (1991). For the purposes of this paper, it is not necessary to identify which mechanisms render not every sperm/egg contact a potential fertilization, but it is important to make clear that in later analyses the ratio (β/β_o) is varied independently of β_o . To emphasize this, I suggest that the fraction of sperm-egg contacts that are potentially fertilizing contacts should be expressed as a term, F_e , termed the "fertilization efficiency" of a particular egg and sperm suspension.

Thus, rewriting VCCW (11), the average number of potential fertilizers per egg is

$$x = F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o \tau}) \qquad \text{.....(5)}$$

If sperm contacts are randomly distributed throughout the egg population and so can be modeled as a Poisson process, then the probability of an egg not being contacted at least once is

$$e^{-x} = \exp(-F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o \tau})) \qquad \text{.....(6) (VCCW 12)}$$

Vogel et al. (1982) suggest that all other eggs contacted by one, two or more potential fertilizers will be fertilized. Thus, the proportion of eggs fertilized becomes

$$\varphi_\infty = 1 - e^{-x} = 1 - \exp(-F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o \tau}))$$

.....(7) (VCCW 13)

To calculate the fertilization ratio after a time t which is smaller than τ, the above argument is altered simply by replacing τ with t

$$\varphi_{\infty} = 1 - e^{-x} = 1 - \exp(-F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o t}))$$

.....(8) (VCCW 14)

Fertilization = Only One Hit Model

The VCCW model describes the proportion of eggs that will be contacted by <u>at least one</u> potential fertilizing sperm. Here, I model successful fertilization as occurring when <u>only one</u> fertilizing sperm contacts. Eggs are assumed not to have any means of blocking polyspermy, so that eggs receiving two or more fertilizing sperm become polyspermic and do not develop normally. Using equation 5 and modeling sperm contacts as a Poisson process, the proportion of eggs being contacted only once by a fertilizing sperm, is

$$\varphi_1(t) = xe^{-x}$$
$$= (F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o t}))\exp(-F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o t}))$$

.....(9)

Which has a maximum of $1/e$ (=0.37) when $x = 1$ which occurs when

$$S_o = \frac{E_o}{F_e(1 - e^{-\beta_o E_o t})}$$ .....(10)

Accounting For Polyspermy Blocks

Many species appear to have efficient (perhaps absolute) blocks to polyspermy that are activated shortly after an egg has been contacted by a fertilizing sperm for the first time (e.g. Rothschild and Schwann 1951, Jaffe 1976, Brawley 1987). It is in this time period (between the first contact of a fertilizing sperm and the activation of a block) that extra contacts by fertilizing sperm can lead to polyspermy. If an egg avoids further fertilizing sperm during this period, then even an egg that (eventually) gets contacted multiple times will avoid polyspermy. Thus, the proportion of monospermic zygotes will be

$$\varphi_{\text{mono}}(t) = 1 - e^{-x} - (\text{eggs that suffer polyspermy before block activates})$$

.....(11)

To determine the proportion of eggs that become "polyspermic", we need to consider only eggs that are (eventually) contacted by more than one potential fertilizer. The proportion (found by subtracting equation 9 from VCCW 13) is

$$\varphi_{>1}(t) = (\text{those contacted by at least 1}) - (\text{those contacted by only 1})$$
$$= 1 - e^{-x} - xe^{-x}$$

where x is defined in equation 5

.....(12)

Once these eggs have been fertilized once, let the time for a block to polyspermy to established be t_b. It is during this time that if one of these eggs is contacted and fertilized again at least once, the egg will become polyspermic. In order to estimate polyspermy, the same bimolecular kinetic argument used by Vogel at al. (1982) can be used to predict that the mean number of extra

fertilizing sperm that will contact an egg in a time period t_b in the population of eggs already contacted once as

$$b = F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o t_b}) \qquad \text{.....(13)}$$

Again, according the Poisson distribution, the number of these eggs that get contacted at least once in the window of opportunity (t_b) during which polyspermy can occur is

$$\varphi_{poly}(t_b) = 1 - e^{-b} = 1 - exp(-F_e \frac{S_o}{E_o}(1 - e^{-\beta_o E_o t_b})) \qquad \text{.....(14)}$$

Thus the proportion of polyspermic eggs will be

$$\varphi_{poly}(t, t_b) = (1 - e^{-x} - xe^{-x})(1 - e^{-b}) \qquad \text{.....(15)}$$

And so the proportion of monospermic zygotes will be

$$\varphi_{mono}(t, t_b) = 1 - e^{-x} - (1 - e^{-x} - xe^{-x})(1 - e^{-b})$$

where x and b are defined in equations (5) and (13)

.....(16)

The general form of the original VCCW model, the one-hit-only model and the polyspermy-adjusted version of the VCCW are shown in figure 2.1, for the sea urchin Strongylocentrotus franciscanus using parameters estimated by Levitan (1993). All models shown in this figure are special cases of the more general (polyspermy-adjusted) model; assuming a perfect polyspermy

block (t_b =0), equation 10 reduces to equation VCCW (14). On the other hand if an egg has no polyspermy block at all (t_b =∞), equation (10) reduces to equation 3 above with a maximum monospermic fertilization probability of e^{-1}. Graphically, the proportion of eggs that become polyspermic can be visualized here as the difference between the VCCW (t_b = 0) plot and the polyspermy-adjusted models (or the one-contact-only model if it is considered that eggs have no polyspermy blocks). The actual positions and differences between models are quite sensitive to parameters used.

Some consequences of the new model

Scoring fertilization in free-spawned eggs

In laboratory incubations of eggs in serially diluted sperm solutions, previous workers (Vogel et al. 1982, Levitan et al. 1991, Levitan 1993, Levitan and Young 1995) have recorded exceptionally good fits to the original (no polyspermy) VCCW model. If polyspermy block inducement times for the eggs used were very short, then there might be little difference between the original and polyspermy-adjusted VCCW models (see figure 2.1) and so close fits would be expected using either model. These studies, however, scored eggs as fertilized if a fertilization membrane appeared or egg cleavage was noticed. Given the rapid formation of fertilization membranes (within 1 minute, Schuel 1984), membranes may form after an initial sperm contact regardless of whether further polyspermy-inducing hits also occur. If so, then this would provide an alternative explanation for the very good fit of echinoderm data to the original VCCW model because then what was being measured was "at least one contact" by a fertilizing sperm (i.e. membranes present) rather than "after only one fertilizing sperm contact" (successfully fertilized). Potentially this would lead to an overestimation of zygote production because some eggs scored as fertilized may have been polyspermic. The amount of overestimation will depend upon how quickly the polyspermy block establishes after the first fertilizing sperm contact but will be greatest at high sperm concentrations (see figure 2.1). This speculation about scoring bias needs testing.

Notably, Oliver and Babcock (1992), scoring only cleaved eggs, recorded decreasing fertilization rates at high sperm concentrations in sperm dilution experiments with corals, presumably as a result of polyspermy. Other fertilisation kinetics curves where cleaved eggs were scored (e.g. mussels Sprung and Bayne 1984, scallops Desrosiers et al. 1996, Styan unpublished data, ormer/abalone Clavier 1992, Keesing and Babcock 1996) also display similar patterns that are characteristic of the adjusted VCCW model. Clotteau and Dube (1993) using nuclear staining methods with bivalve (*Spisula solidissima*) eggs, also record proportions of polyspermy which appear from their paper (their figures 3, 5) to be in accordance with the general shape of equation 15 above. All of these data are in better accordance with the polyspermy adjusted model than the original VCCW model, and therefore support the use of the former. Wherever possible, either eggs should be allowed sufficient time to develop cleavages before scoring or nuclear staining methods (e.g. Clotteau and Dube 1983, Brawley 1987) used to assay fertilization, rather than score the presence of fertilization membranes. This would avoid potential problems of overestimation of zygote production due to inclusion of polyspermic eggs.

Estimation of polyspermy block activation times

Estimates of polyspermy block activation times vary across taxa (eg ascidians ~21 sec, Lambert and Lambert 1981; urchins probably less than a few seconds, Spinelli and Albanese 1990) and there has been some debate about whether fast blocks exist at all for some taxa e.g. Byrd and Collins (1975), Jaffe and Gould (1985). Quite clearly in figure 2.1, the predicted way fertilization success decreases with increasing sperm concentration is dependent on the time it takes a polyspermy block to activate. Fertilization kinetics curves of eggs with slowly activating blocks peak at values much less than 1, with fertilization then decreasing rapidly with increasing sperm concentration. In contrast, the curves of eggs with fast blocks show higher peak fertilization and past this peak fertilization decreases much more slowly with increasing sperm concentration. Given reliable estimates of other parameters such as β_0 and t or τ, it may be possible to fit the modified VCCW fertilization kinetics model to kinetics curve data and estimate polyspermy block activation time.

Optimal egg sizes for fertilization

A general feature of the polyspermy-adjusted model is an optimal sperm concentration where fertilization is maximized (figure 2.1). At lower sperm concentrations sperm are limiting and at higher concentrations excess sperm lead to increased polyspermy. The actual sperm concentration at which this maximum occurs and the maximum proportion of eggs that can become fertilized depend on egg target size (β_o), fertilization efficiency (F_e) and the time taken for a polyspermy block to activate (t_b). Thus, at a given sperm concentration, there is also an optimum egg size that maximizes zygote production (figure 2.2). Note, again, the strong dependence of optima on model parameters. Figure 2.2 also highlights an important difference between the original (non-polyspermy) VCCW model and the polyspermy-adjusted version. In the VCCW model, larger eggs always result in an increase in fertilization success but in the polyspermy-adjusted model, larger eggs can result in a decrease in zygote production i.e. they become "too fertilizable". Levitan (1996b) found empirically that in sperm limited situations, larger sea urchin eggs within batches were more likely to develop raised fertilization membranes. It would be interesting to determine whether these larger eggs were also more likely to suffer polyspermy at higher sperm concentrations, as predicted by the polyspermy-adjusted model. When figure 2.2 is recast, holding egg size constant and varying F_e (figure 2.3), a similar pattern results - at high sperm concentrations higher fertilization efficiency can lead to decreased zygote production.

In the field, the eggs of free-spawning individuals are likely to experience a vast range of ambient sperm concentrations. Although exact concentrations depend upon hydrodynamic conditions and the species involved, ambient sperm concentration near a releasing male typically can vary by at least several orders of magnitude. In some circumstances sperm is viscous and remains in isolated streams of high concentration for some time/distance downstream after release (Thomas 1994a, 1994b). In other instances sperm diffusion is rapid over small distances/times (e.g. Denny 1988, Babcock et al.1994, Levitan and Young 1995, see chapter 5). Sperm concentration at a single position can also vary markedly over short time intervals as a result of small scale

hydrodynamic heterogeneities (Lasker et al. 1996). Fertilization success of *Strongylocentrotus franciscanus* eggs with varying degrees of fertilization efficiency (F_e) in a range of ambient sperm concentrations are shown in figure 2.4 Here, egg size is fixed (135μm diameter) and fertilization success is expressed as the number of zygotes produced per ml of reproductive tissue (using egg size and kinetics parameters from Levitan 1993, 1996a). In figure 2.4.A, the original VCCW model was used and more efficiently fertilized eggs always result in a higher fertilization success, regardless of ambient sperm concentration. However, when the influence of polyspermy is taken into account as in figure 2.4.B where the polyspermy adjusted model is used, the more efficiently fertilized eggs do not always result in a higher fertilization success. Instead, the egg that does best depends on the ambient sperm concentration - at low sperm concentrations more efficiently fertilized eggs do best, but at higher concentrations, this relationship reverses with the less efficiently fertilized eggs doing better.

Thus, the risk of polyspermy, may at least in part be an explanation for the retention of effectively small egg sizes in free-spawners. An extreme example of this strategy may be the micropyles of some fish eggs (Ginzburg 1972) or restriction of fertilization to only the small area around the site of polar-body formation in hydrozoan eggs (Freeman and Miller 1982). It is possible to speculate that certain, otherwise puzzling, observed adult behaviors might also be strategies to avoid polyspermy. An example is the absence of aggregation on small-scales in partially mobile organisms such as the scallop *Chlamys bifrons* . These scallops are somewhat evenly spaced apart on small spatial scales (see chapter 7) - a behavior which conventional wisdom suggests should decrease fertilization success (e.g. Babcock et al.1992, Stokesbury and Himmelman 1993) but which instead might be an adaptation to avoiding areas of high sperm concentration found immediately around releasing males (see chapter 5).

Fertilization may be a process under strong selection pressure as suggested by Levitan (1993, 1996b), but understanding how this selection acts will not be straightforward. There may be a trade-off between sperm limitation and sperm avoidance, in turn depending upon the frequency of exposure of eggs to differing sperm concentrations. Work addressing the effects of sperm

limitation on the ecology and evolution of free-spawning marine invertebrates has effectively just begun. Future such work should also consider the importance of excess sperm and polyspermy. A next step in this process will be to test the polyspermy adjusted version of the VCCW model empirically, determining whether it can be used to describe and predict the fertilization kinetics of free-spawners and then estimate important parameters such as F_e and t_b. This is done in chapter 3.

Figure 2.1 Predicted fertilisation kinetics curves for *Strongylocentrotus franciscanus* using the polyspermy-adjusted model. Model parameters (egg size, collision rate constants and egg concentrations, Levitan 1993) are common, but polyspermy block inducement times (t_b) vary.

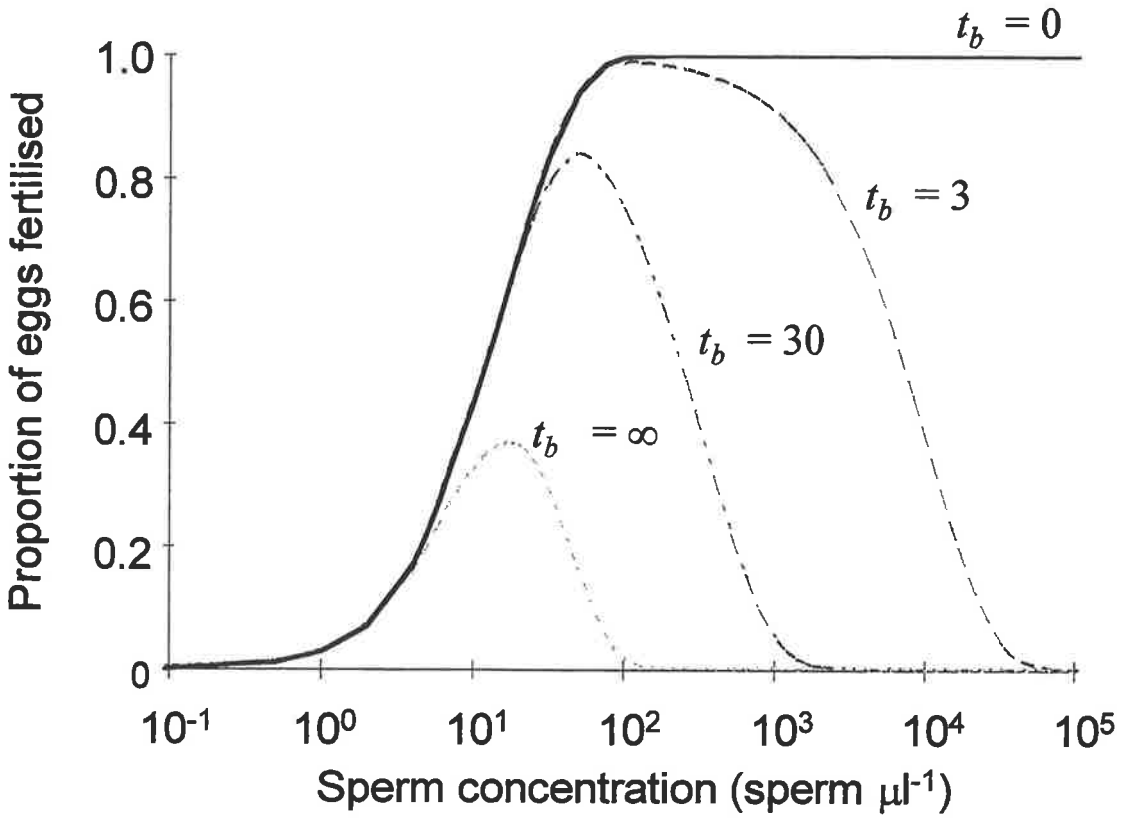


Figure 2.2 Relationship between egg size and fertilisation success for *Strongylocentrotus franciscanus* using the polyspermy-adjusted fertilisation kinetics model. Four polyspermy block inducement times are shown (t_b =0 s, solid; t_b =3 s, dashed; t_b =30 s, dot-dashed; $t_b = \infty$ s, dotted) in two ambient sperm concentrations (A. 10 sperm μl^{-1} ; B. 100 sperm μl^{-1})

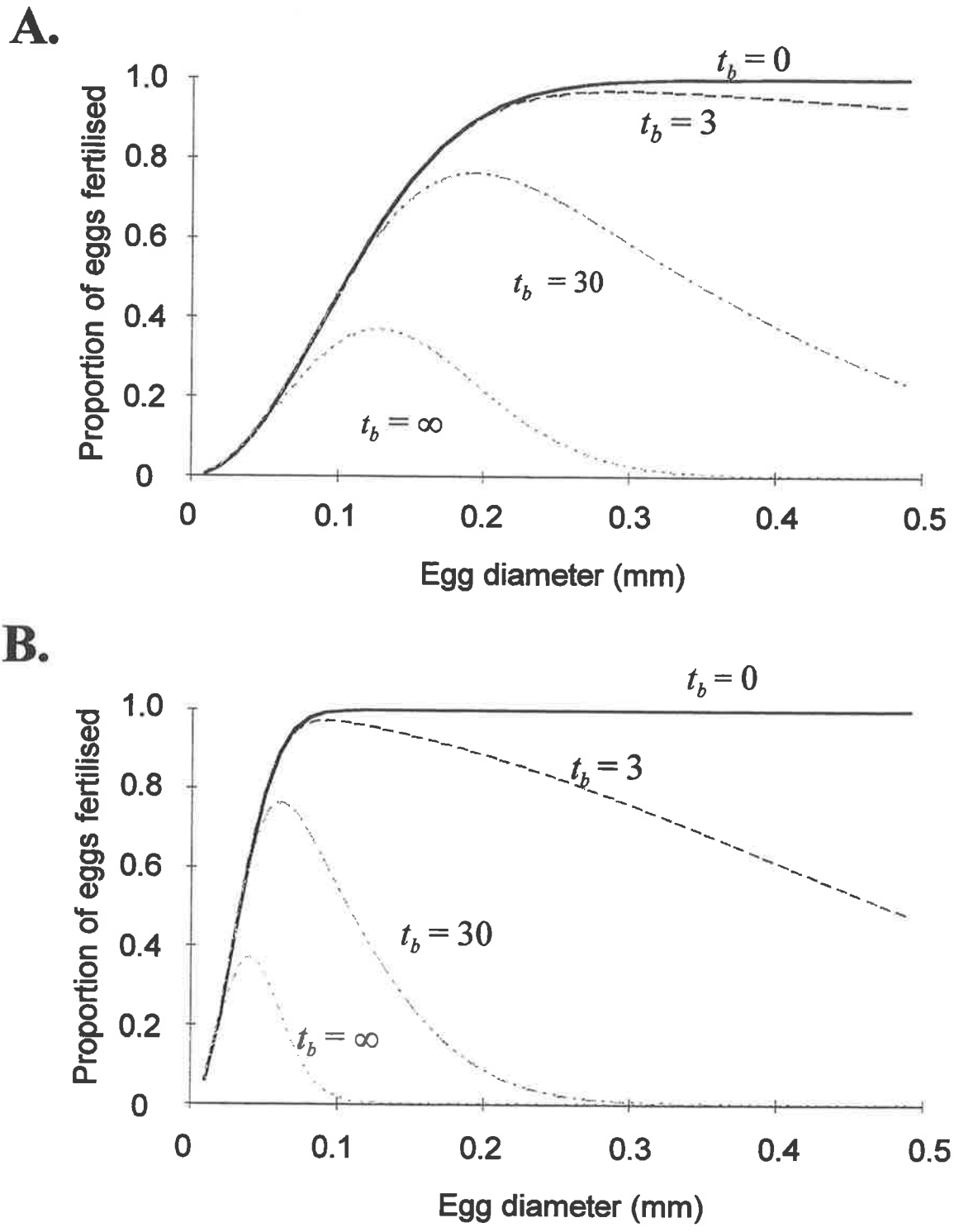


Figure 2.3 Relationship between fertilisation efficiency (*Fe*) and fertilisation success for *Strongylocentrotus franciscanus*. Egg diameter and sperm-egg collision rate (βo) (from Levitan 1993) are held constant. Success is shown for eggs with varying polyspermy block inducement times (t_b=0 s, solid; t_b =3 s, dashed; t_b =30 s, dot-dashed; $t_b = \infty$ s, dotted) in two ambient sperm concentrations. (A. 10 sperm μl^{-1} ; B. 100 sperm μl^{-1})

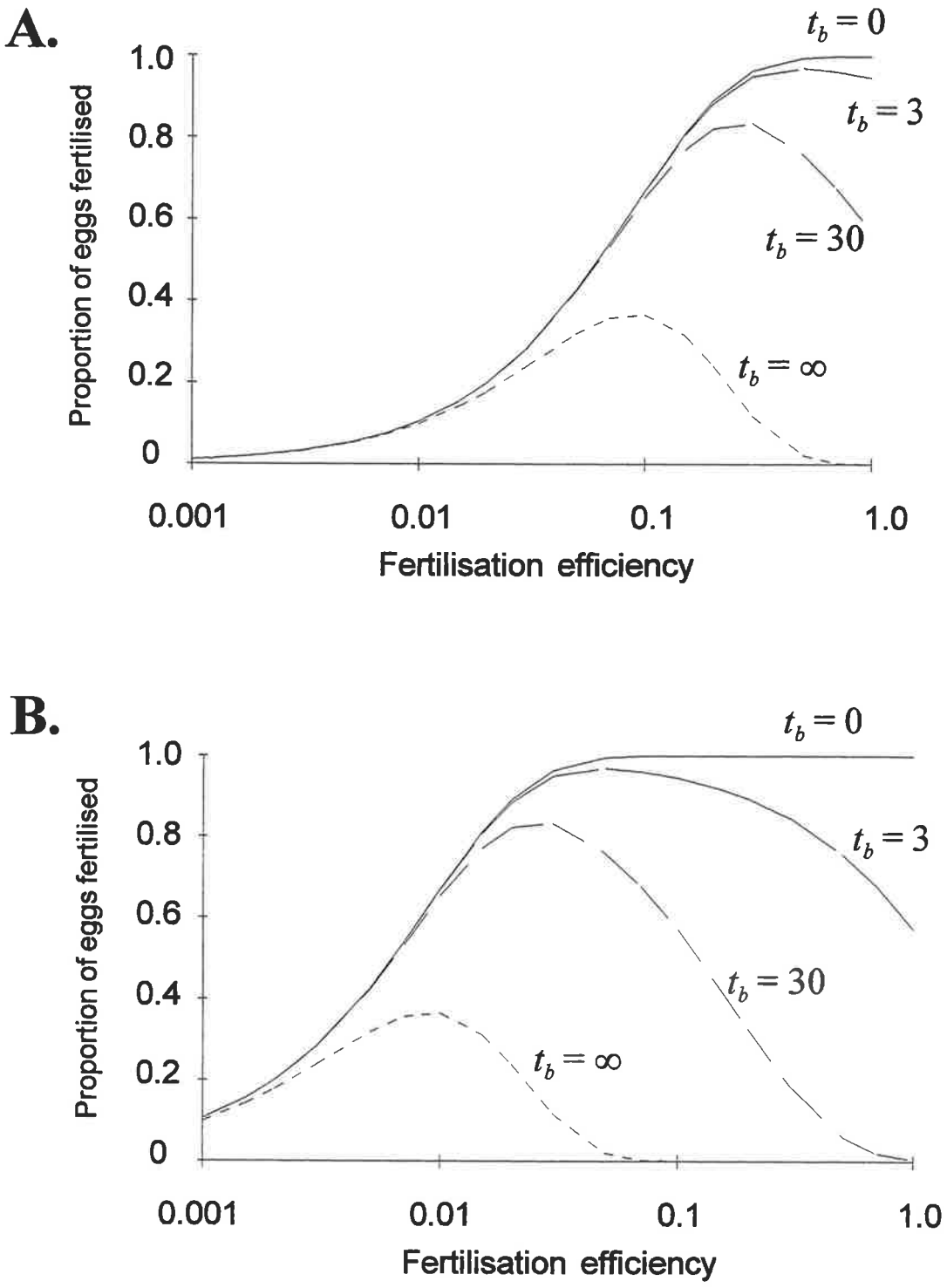
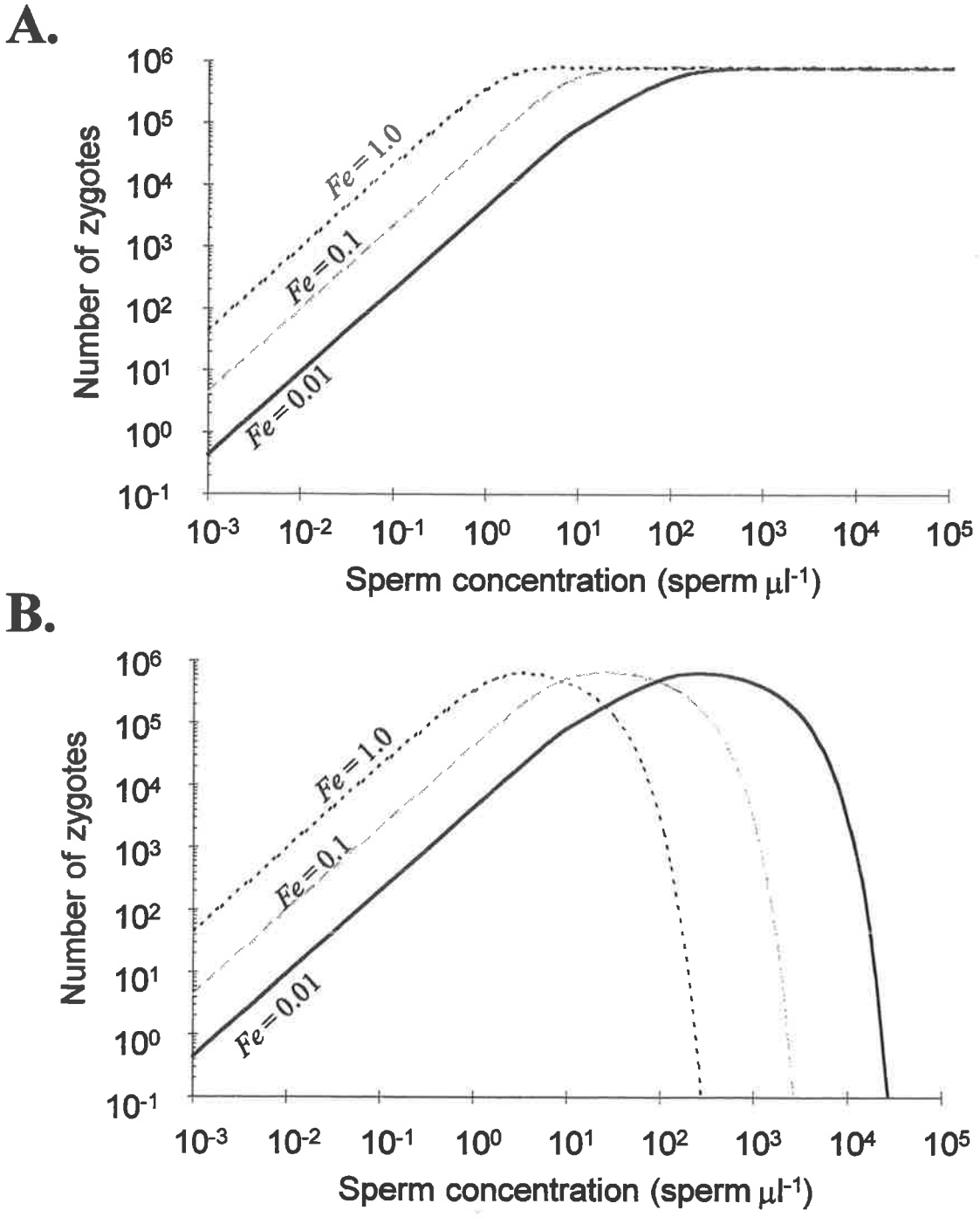


Figure 2.4 The effect of egg hittability and polyspermy on fertilisation success in varying sperm conditions. The number of *Strongylocentrotus franciscanus* zygotes produced from 1ml of egg material are modelled when fertilisation efficiency (*Fe*) is set at 1.0 (dotted line), 0.1 (dot-dashed) and 0.01 (solid line). Egg size and collision rate constant from Levitan (1993). A. Using the original (non-polyspermy) VCCW model ($t_b = 0$ sec). B. Using the polyspermy-adjusted model. Byrd and Collins (1975) failed to detect a fast polyspermy block in the congener *S. purpuratus* so t_b is set at 30 sec. Use of other finite (non-zero) values for t_b shifts these curves left or right along the sperm concentration axis and affects the maximum number of zygotes produced but does not alter the general shape nor relationships between curves.



Chapter 3

Fertilisation Kinetics of *Chlamys bifrons* and *Chlamys asperrima*

Introduction

This chapter examines the dynamics of fertilisation of the scallops *Chlamys bifrons* and *Chlamys asperrima* at the smallest scale, that is, at the level of sperm-egg interaction. In it, I determine how ambient sperm concentration affects the likelihood that a scallop egg will be fertilised. These data were not previously available for either *C.bifrons* or *C.asperrima.*

Previous work has shown that the relationship between sperm concentration and the likelihood an egg will be fertilised can differ markedly between species (e.g. Oliver and Babcock 1992, Levitan 1993) and also differ within species when measured at different times (Oliver and Babcock 1992, Benzie and Dixon 1994). There is also strong evidence that such variation is dynamically important and can be manifest as real variation in field fertilisation rates. In field experiments, variation in gamete traits translated to variation in the distance downstream sea urchin eggs were fertilised, both within species (Levitan 1996a) and between species (Levitan 1997). High fertilisation rates found at large distances downstream (30m+) for *Acanthaster planci* in natural spawning and experiments (Babcock and Mundy 1992, Babcock et al. 1994), which are much greater than those recorded in equivalent experiments with urchins (e.g. Pennington, 1985, Levitan 1991, Levitan et al. 1992, Styan 1997, S.Mead, R.C. Babcock, C.A. Styan unpublished manuscript) have been attributed to *Acanthaster*'s possession of extremely fertilisable eggs (Benzie and Dixon 1994 - though Babcock et al. 1994 suggest that the cause is the high fecundity of *Acanthaster*). Consequently, it is important to attain a reliable measure, for each scallop species, of how sperm concentration affects the likelihood of fertilisation of eggs in order to construct an adequate model of fertilisation success in natural populations.

Most simply, the relationship between ambient sperm concentration and fertilisation chance can be measured empirically by incubating unfertilised eggs in variously diluted sperm solutions in test tubes in the laboratory (e.g. Levitan et al. 1991, Oliver and Babcock 1992, Levitan 1993, Benzie

and Dixon 1994). This allows construction of a fertilisation kinetics curve where the likelihood that an egg will become fertilised is plotted against ambient sperm concentration and, provided this curve is constructed over a sufficient range of sperm concentrations, visual estimation of values such as F_{10} (concentration at which 10% of eggs exposed will be fertilised), F_{50} (50% eggs fertilised) and F_{max} (concentration where a maximum proportion of eggs are fertilised). These $F_{critcial}$ values can then be used as criteria in models of individual or population fertilisation success (see chapters 5,6,7).

Additionally, it is useful to express the relationship between ambient sperm concentration and fertilisation likelihood as a continuous function such as the (polyspermy-adjusted) fertilisation kinetics model developed in chapter 2. Such continuous functions allow for easier incorporation into simulation models and are (albeit without accounting for polyspermy) at the core of previous models of fertilisation success (e.g. Denny 1988, Denny and Shibata 1989, Denny et al. 1992, Babcock et al. 1994, Levitan and Young 1995, Morris 1994). Fitting of this model requires measurement of several additional gamete attributes: egg size and sperm swimming speed to calculate the collision rate constant β_0 and sperm half-life t (and how sperm longevity varies with sperm concentration) (Vogel et al. 1982 , Levitan et al. 1991, Levitan 1993). Note that this attempted fitting will also constitute at least a partial test of the polyspermy-adjusted fertilisation kinetics model developed in chapter 2, in that a poor fit of the model to data would lead to a rejection of the model as a description of the kinetics of fertilisation.

Having a well-fitted mechanistic model of fertilisation kinetics might also allow fertilisation to be predicted in circumstances outside of the still conditions eggs experience in test tubes in the laboratory. For example, by having a measure of F_e, t_b and egg size, it might be possible to predict the likely fertilisation effects of highly turbulent waters found on wave-exposed rocky shores, where it has been suggested that sperm-egg collision rates are related to shear velocities rather than sperm swimming speed (e.g. Denny and Shibata 1989). Analyses of the adaptive consequences of varying life-history traits such as egg size (Levitan 1993, 1996b, Podolsky and Strathmann 1996, chapter 2) area also reliant on having a fitted model of fertilisation kinetics.

This chapter, then, has two main aims: 1) to empirically measure how fertilisation chance for both *C.bifrons* and *C.asperrima* varies with ambient sperm concentration; and 2) to fit the polyspermy-adjusted mathematical model of fertilisation (equation 16), developed in the last chapter, to this empirical data.

Materials and Methods

Collection of ripe scallops

Chlamys bifrons were collected from Largs Bay on 22 March 1995 and returned to the laboratory where they were kept in 40 L tanks at 21°C for up to 3 weeks. They were fed a mixture of *Isochrysis galbani* and *Tetraselmis selecia* during this time. *Chlamys asperrima* were collected from Edithburgh Jetty (16 June 1994 and 2 August 1994) and similarly maintained in the laboratory until use, but with water temperature held at 14°C. All scallops used were visually ripe and collected at times when scallops are naturally spawning in the field (see chapter 4 for a more detailed definition of "ripe" and confirmation that these scallops were collected during the time when field populations were about to spawn). Each set of gametes was checked for visible signs of viability before use (motility of sperm and a solid, round appearance of eggs).

Collection and maintenance of gametes

Ripe scallops were injected in the gonad and the adductor muscle with 0.5 ml of 10^{-4} M 5-hydroxytryptamine (serotonin) solution and placed in separate, clean containers filled with seawater. This induced spawning (on average) within 10-60 minutes for males and 120-240 minutes for females, though there was considerable variation between individuals in the time delay between injection and onset of spawning. Serotonin (and other monoamines) have been identified as key components in the physiology of natural spawning in bivalves (Croll et al. 1995, Martinez et al. 1996) and, in laboratory conditions, exposure to exogenous serotonin has been

shown to produce normally fertilising gametes in a range of bivalves (Gibbons and Castanga 1984) including *C.asperrima* (O'Connor and Heasman 1995).

Fresh gametes were collected in glass 2 litre beakers as released by the scallops. Egg and sperm solutions were held at room temperature (=21°C or 14°C for *C.bifrons* and *C.asperrima* trials respectively) for as short a period of time as possible, except where time delays were required (see sperm half life methods). In all cases eggs were used within 15 minutes of collection and within 5 minutes for sperm solutions. Egg solutions were gently filtered through a 45 μm (*C.asperrima*) or 80 μm (*C.bifrons*) nitex mesh to remove any damaged eggs and debris such as scallop pseudofaeces that might contaminate gamete experiments. Sperm solutions were kept as concentrated as possible (to minimise ageing effects - see sperm longevity section below) until dilutions were made.

Fertilisation kinetics

Freshly collected, concentrated (approx 10^4 sperm μL^{-1}) sperm solutions collected from serotonin induced scallops and were used to create a series of 10-fold dilutions that were 10^0, 10^1, 10^2, 10^3, 10^4, 10^5 , 10^6, 10^7 , 10^8 dilutions of the original. For the experiments involving *C.bifrons* gametes, an extra set of intermediate dilutions were also created that were $0.3x10^1$, $0.3x10^2$, $0.3x10^3$, $0.3x10^4$, $0.3x10^5$, $0.3x10^6$ dilutions of the original. A 1 ml subsample of the 10^0 or 10^1 dilutions was taken for later enumeration of absolute sperm concentrations (5 replicate counts using a haemocytometer).

9 ml of each sperm dilution were pipetted into a series of 15ml glass test-tubes, and as a control for errant fertilisations caused by the physical treatment of eggs or sperm contamination of the stock seawater used for dilutions, 9 ml of (sperm-free) seawater was pipetted into an extra test-tube. 1 ml of a solution containing freshly spawned eggs (0.8 egg μl^{-1}) was immediately added to each test tube, which was swirled and eggs then allowed to settle to the tube bottom. After 3.5 hours, the eggs were transferred into plastic vials, fixed with a few drops of formalin and stored for subsequent counting. Later, 100-200 undamaged eggs from each sample were assayed under

200x magnification and scored as either fertilised (eggs showing normal cleavage patterns of cell division) or unfertilised (eggs showing no such cleavage or abnormal cleavage patterns - see plates 3.1 and 3.2).

Sperm half-life

As before, a series of 10-fold sperm dilutions (10^0, 10^1, 10^2, 10^3, 10^4) were created and pipetted into test-tubes. I ml of egg solution eggs (0.8 egg μl^{-1}) was also added to these, but after sperm had aged for a specific time interval (1, 30, 60 or 120 minutes). This was repeated on 4 occasions, resulting in 20 sperm concentrations, each aged for 4 lengths of time. After addition, eggs were left for 3.5 hrs, fixed and scored as above. Sperm half-life was deduced as either the slope of the decrease in fertilisation rate or exponential term in a decaying exponential at each sperm concentration, depending on which model produced the best fit by eye. These half-lives were then plotted against the sperm concentration in which they were derived to provide a general relationship between sperm longevity and concentration.

Sperm swimming speed

A drop of 10^1 dilution (approximately 10^3 sperm μl^{-1}) sperm solution was placed under a mounted coverslip on an albumin coated slide. Fresh sperm were always collected and used within 5 minutes of male scallop first spawning. Sperm were video taped under 200x magnification using a phase-contrast compound microscope fitted with a video head. The presence of slide walls can severely affect sperm swimming speeds (Winet 1973) so focus was set mid-plane in an attempt to minimise this (Levitan et al. 1991, Levitan 1993). Several fields of view were recorded for each individual to ensure that sufficient numbers of sperm could be tracked. Laboratory (and so hopefully slide) temperatures were held at field water temperatures when spawning occurs (14° C, 21° C for *C.asperrima* , *C.bifrons* respectively). Slides were not held under illumination for long, to minimise heating taking place during filming and wherever possible, the first sperm that were filmed (those that were likely to have been least affected by changes in slide temperature) were measured.

Sperm trajectories were tracked using a motion analysis machine (Motion Analysis VP110, Motion Analysis Corporation, Santa Rosa, CA, USA). The speed of 9 haphazardly selected sperm were measured from each individual scallop. Care was taken to ensure individual sperm were not measured twice, particularly in fields of view with multiple trajectories, and sperm that were obviously impeded by slide walls, debris or swimming out of the field of view were excluded.

Egg size

Fresh eggs (10 haphazardly chosen per individual) were measured using a calibrated ocular micrometer. Samples from 9 *Chlamys bifrons* and 7 *Chlamys asperrima* individuals were measured.

Fitting of experimental data to theoretical models

Least squares, non-linear regressions using SYSTAT for Windows (version 5) were performed using the quasi-newton and simplex (Systat 1992) iteration algorithms. I tested a range of starting parameter values and the sensitivity of parameter estimations to variation in β_o given there may be error in measurement of parameters such as egg size and, in particular, sperm swimming speed. β_o values were first estimated as the product of the measured sperm swimming speed and egg cross-sectional area (see also last chapter). Next, I repeated the model fitting using two other estimates of β_o, one of which could be considered an uppermost estimate (="High β_o") and the second a lower estimate (="Low β_o"). "High β_o " was calculated using a sperm swimming speed that was 50% greater than measured and to account for a possible influence of chemoattractants (whose effect might increase the effective diameter of the egg) + 50 μm was added onto the egg diameter. "Low β_o" was calculated with egg size as measured, but with a sperm swimming speed 50% smaller than measured. Finally the model was also fit without fixing β_o at all, using two sets of starting parameters (0.001, 0.1, 100) and (0.1, 0.01, 10) for β_o, F_e and t_b respectively. Both raw and the standardised *C.bifrons* data and the *C.asperrima* data were fitted and in each case, the best fit estimates of F_e and t_b were recorded along with the Pearson-cross correlation product (r^2).

Results

The proportions of normally cleaved eggs after 3.5 hours are plotted against ambient sperm concentration in figure 3.1 and 3.2 (*C.bifrons*) and in figure 3.3 (*C.asperrima*). All control samples (no sperm added) showed no signs of fertilisation. Clearly, fertilisation rates were related to ambient sperm concentration. Low fertilisation levels were found at low sperm concentrations, increasing with increasing sperm concentration up to a point after which higher sperm concentrations lead to a decreased fertilisation rate. Presumably, this later decrease was because of increasing occurrences of polyspermy. The presence of polyspermy was not tested for directly using nuclear staining methods (e.g. Brawley 1992), but at higher sperm concentrations, many sperm were usually visible around the perimeter of uncleaved eggs (see for example plate 3.2), and some trials appeared to have lysed eggs.

The maximum proportion of eggs fertilised in the *C.bifrons* experiments was substantially less than 1.0, and this was the case for each set of dilutions. In the *C.asperrima* experiments maximum fertilisation rates approached much nearer to 1.0. One hypothesis for approximately 30% of the C.bifrons eggs remaining unfertilised might be that this proportion were simply inviable eggs and never fertilisable in the first place, perhaps related to artificial spawning, laboratory conditions etc. To account for this I also standardised the fertilisation data for *C.bifrons* so that maximum fertilisation was 0.99, these are shown in figure 3.2.

Visual estimates of F_{10}, F_{50}, $F_{65} = F_{max}$ (sperm concentrations in which eggs have a 10%, 50% and 65% chance respectively of becoming fertilised) from figures 3.1 are 4, 20 and 100 sperm μl^{-1} respectively for *C.bifrons* and from figure 3.3, $F_{10} = 0.2$, $F_{50} = 20$ and $F_{99} = F_{max} = 1000$ sperm μl^{-1} for *C.asperrima*. F_{10}, F_{50}, and F_{99} (= F_{max}) using the standardised *C.bifrons* curve shown in figure 3.2 are 2, 20 and 100 sperm μl^{-1} respectively.

Both egg size and measured sperm swimming speeds differed between species and nested ANOVAs indicated there was substantial variation in each between individuals within a species.

These nested ANOVA tables are shown in Table 3.1. Using the average size of eggs from each individual sampled, average (± S.E.) egg diameter across individuals was 116.5 (± 1.11) μm for *C.bifrons* and 71.2 (± 1.65) μm for *C.asperrima*. Average sperm swimming speeds across individuals were 209.8 (± 11.11) μm s^{-1} and 166 (± 23.17) μm s^{-1} for *C.bifrons* and *C.asperrima* respectively.

Sperm longevity at varying concentrations is plotted in figure 3.4 and was positively correlated with sperm concentration in both scallop species. Such a relationship between sperm longevity and concentration appears to be common in other invertebrates and is known as "the respiratory dilution effect " (Chia and Bickell 1983, Levitan et al. 1991, Levitan 1993).

The polyspermy fertilisation kinetics model fitted well to all sets of data, and both the standardised and unaltered *C.bifrons* data. Estimates of F_e and t_b along with r^2 values of the fit of the model are shown in table 3.2. Visually, the predicted values of these models are shown coincident with experimental data in figures 3.5, 3.6 and 3.7 for *C.bifrons*, standardised *C.bifrons* and *C.asperrima* respectively. Of particular note is that within a set of data, a nearly identical fit of models was obtained using different starting β_o values and that each of these starting values also provided quite different estimates of F_e and t_b. Graphically, the similarity of each model with different parameter values can be seen in the tight overlapping of the predicted curves for the *C.bifrons* data in figure 3.5.B.

Discussion

Fitting of the polyspermy-adjusted fertilisation kinetics model and the limitations of simple sperm dilution experiments

The polyspermy-adjusted fertilisation model could be parameterised to provide an very good fit to the observed dilution experiment data collected for *C.bifrons* and to a lesser extent, *C.asperrima* (at low sperm concentrations *C.asperrima* fertilisation was slightly underestimated). In particular,

at high sperm concentrations fertilisation success declined which is a feature of the polyspermy-adjusted fertilisation kinetics model developed in chapter 2 (and not a feature of the original Vogel et al. 1982 model). Presumably this decrease was a result of increased rates of polyspermy occurring - though this was not measured directly by assessing the ploidy levels of each egg as has been done in other studies (e.g. Brawley 1992, Clotteau and Dube 1993). Future efforts might directly test this, but these data do support the use of the polyspermy adjusted version of the VCCW model over the original (Vogel et al. 1982) in that a predicted decrease in fertilisation at high sperm concentrations was observed for both scallop species.

In the *C.bifrons* experiments, maximum fertilisation never exceeded 0.70 in any replicate. In studies with other groups where fertilisation has been measured other than by counting fertilisation membranes (e.g. corals, Oliver and Babcock 1992, abalone , Keesing and Babcock 1996, fucoid algae, Brawley 1992), maximum fertilisation rates of much less than 1.0 (~0.8 - 0.93) were also observed. The polyspermy-adjusted version of the VCCW model developed in chapter 2 predicts maximum fertilisation rates less than 1.0, particularly if polyspermy blocks are slow to activate, and so provides a possible explanation for this. However, an alternative explanation is that for some reason the large proportion of the *C.bifrons* eggs (0.30) that remain unfertilised were never fertilisable in the first place - perhaps the method of spawning inducement or when eggs were collected induced unripe, unfertilisable eggs to be released (and presumably natural spawning would not do so). However, there are several lines of reasoning that would suggest there is no reason to suspect eggs were unfertilisable. First, additional sperm dilution experiments conducted at other times and out of the laboratory with *C.bifrons* (to act as controls for egg viability in field experiments described in chapter 5) also all failed to record maximum fertilisation proportions greater than 0.80. Second, serotonin is naturally involved in the spawning process (Croll et al. 1995, Martinez et al. 1996) and O'Connor and Heasman (1995) report that serotonin inducement had no deleterious effects on *C.asperrima* fertilisation or subsequent larval development. Nor is it reported that serotonin has any deleterious effects on gamete quality in other bivalves where it has been used to induce spawning (e.g. Matsutani and Nomura 1982, Gibbons and Castanga 1984). In the trials involving *C.asperrima*, in which I used the same spawning and incubation methods as with *C.bifrons*, much higher maximum fertilisation rates were recorded in all trials.

Reproductive cycle data presented in the next chapter also suggest that *C.bifrons* were collected at times when spawning was likely in field populations, so it is unlikely that unripe scallops accounted for the unfertilised 0.30. Finally, the fit of the model to the standardised *C.bifrons* data is not especially better - although a higher r^2 value is recorded, the fitted curve still has a maximum significantly lower than 0.99. By eye the model does not fit the standardised data points significantly better than the model fitted to the unstandardised data in figure 3.1. Again, directly testing the ploidy levels of eggs would have helped to resolve whether the "missing 0.3 eggs" at optimal concentrations are suffering polyspermy or were unfertilisable in the first place.

Quite clearly, the fitted values that were resolved for F_e and t_b depend not only on the data from the sperm dilution experiment, but are also highly dependent on the estimated value of β_o used to fit the polyspermy-adjusted fertilisation kinetics model. Within each experimental curve, no one set of parameters was clearly best and fitted F_e values in this study ranged across two orders of magnitude from 0.31 to 0.0014 and t_b from 2 to 165 secs. Also clearly evident was a negative correlation between β_o and t_b . In hindsight, this makes sense as β_o and F_e are logically highly correlated - at least mathematically, an egg's hittability increases with increasing size (β_o) and/or an increasing proportion of successful sperm contacts (F_e). It is not surprising then that for a given set of data, when β_o was set low, the fitted F_e value was correspondingly high and vice versa. Thus, it would also have been possible to generate an even larger range of estimates of F_e and t_b by fitting the model with more extreme β_o values. This presents somewhat of a problem in estimating a true value of F_e and t_b if there is error in estimating β_o.

In this study and others (Levitan et al. 1991, Levitan 1993), it was assumed that β_o can accurately be estimated by measuring egg diameter and sperm swimming speed. However, it is easy to imagine where potentially large errors could be introduced into this estimate. For a start, effective egg target size may larger than that based on measured egg diameter. Egg produced sperm chemoattractants have been found in many phyla (Miller 1966, 1975, 1977, 1985) including bivalves (Miller et al. 1994) and may increase the effective diameter of target eggs. Similarly, measured sperm swimming speeds may have been biased (and so also affect β_o estimates) as a

result of the slide/video set up in which it was measured. I measured only free swimming, fast moving sperm - very slowly moving sperm were assumed to be (unnaturally) impeded by flotsam and/or slide walls and so disregarded. Other factors such as egg produced chemoattractants have been shown to increase sperm speed and decrease longevity (Bolton and Havenhand 1996) and temperature associated changes in water viscosity could also have an effect on speed and longevity (Bolton and Havenhand, in prep). What was measured under the video was also straight line speed, yet the sperm clearly were travelling in a circular path under the coverslip and in a larger volume may well have been travelling in a helical path. Possibly, this then overestimates actual nett sperm displacement which may be more important for estimating collision rates than simple linear speeds.

The model also assumes that β_o does not vary with sperm concentration. This too, is probably unreasonable. At high concentrations it would be expected that there would be many more sperm/sperm collisions than at low concentrations, thereby reducing the average sperm velocity (Rothschild and Swann 1951). Given a fixed amount of energy to be expended within a lifetime, sperm experiencing lower numbers of interrupting collisions would be more likely to travel at high speeds, use limited energy reserves and expire quickly. This would be in accord with the observation of a respiratory dilution effect (Chia and Bickell 1983) observed here in both scallop species. A higher rate of sperm/egg collisions at low than at high sperm concentrations might explain why the polyspermy adjusted VCCW model appears to underestimate *C.asperrima* fertilisation at low concentrations (10^{-2} - 10^{1} sperm μl^{-1} ; see figure 3.7). Again, this needs further testing.

Attaining precise, true estimates of β_o, F_e and t_b is not of great importance if the conditions experienced by gametes in the test-tubes closely resemble those they would experience in field conditions : all that matters then is a description of the relationship between sperm concentration and fertilisation likelihood and, as in this study, this can easily be measured experimentally. However, if the fertilisation kinetics model is to be used to extrapolate how fertilisation chance varies in other conditions (which possibly cannot be easily mimicked in the laboratory) then knowing what proportion of hits on an egg surface are likely to be successful (i.e. F_e) is important.

For example, Denny and Shibata (1989) predicted very low rates of fertilisation (~ 0.001) for urchins living on wave-swept rocky sea shores. This low figure is, at least in part, a result of a low sperm-egg collision efficiency (F_e = 0.01) used in their model - if F_e was raised, then considerable increases in the predicted rates of fertilisation were expected (Denny and Shibata 1989). In these situations, independent information about sperm-egg contact rates and times for induction of any polyspermy block will be needed in addition to the simple sperm dilution experiments like the ones in this study and Vogel et al (1982), Levitan et al. (1991), Levitan (1993) and Levitan and Young (1995). This will probably necessitate (time-consuming) approaches such as careful staining and enumeration of sperm/egg fusions (e.g. Baker and Presley 1966, Vacquier and Payne 1973, Brawley 1992) and physiological measurements of polyspermy block induction times (reviewed in Jaffe and Gould 1985). The work in this chapter clearly shows that only if precise (and ideally, independent) estimates of parameters such as β_0 and t_b are in hand, will it be possible to fit parameters such as F_e from sperm dilution experiments.

It must also be remembered that there are many other potential differences between fertilisation occurring in a test tube and in field conditions. For example, Mead and Denny (1995) have shown in laboratory studies that high sheer stress can lead to a reduction in fertilisation (as opposed to an expected increase through increased egg-sperm contacts) presumably because of an inability of sperm to bind to egg coats when rotating at high speeds. Environmental variables such as pH, salinity and temperature (Rupp 1973, Greenwood and Bennett 1981, Mita et al. 1984, Christen et al. 1986, Fong et al. 1995, Serrao et al. 1996) and pollution (Riveros et al. 1996) have also been shown to directly affect fertilisation. Putative genetic incompatibilities between particular parental crosses have also been shown to affect fertilisation (Grosberg 1987, Havenhand 1991, J.N. Havenhand pers. comm.).

Adaptive significance of egg size, sperm limitation and polyspermy

These scallop data allow for a comparison with data collected by Levitan (1993) in constructing his egg size/sperm limitation hypothesis. He found that, at least for three species of stongylocentrotid urchins, the species with the larger eggs were more likely to become fertilised

(develop fertilisation membranes) in low ambient sperm concentrations. He also found the same effect in laboratory experiments for eggs within two of these urchin species (Levitan 1996b). The two species of scallop investigated here also have quite different sized eggs : *C.bifrons* ~117 μm and *C.asperrima* ~71 μm. This seven-fold difference in egg volume between scallops is larger than that between the three *Strongylocentrotus* species studied by Levitan (1993) (their diameters were 84, 135 and 145 μm). This study did not find any strong evidence that, at least between species, larger eggs (*C.bifrons*) were more likely to be fertilised at low sperm concentrations than smaller eggs (*C.asperrima*). In fact, it seems the opposite was true. If parameters such as sperm swimming speed, F_e and t_b are held constant, the polyspermy-adjusted VCCW model suggests that larger eggs will be more fertilisable at low sperm concentrations, but these parameters appeared to differ markedly between the scallop species.

C.bifrons appears to be especially sensitive to polyspermy, with a rapid drop in fertilisation as ambient sperm concentrations increases beyond F_{max} . *C.asperrima* does not seem to be as sensitive as *C.bifrons*, but does also suffer polyspermy at high sperm concentrations. Like *C.bifrons*, some other scallops such as *Pecten maximus* also appear to be extremely sensitive to polyspermy in laboratory incubations (Gruffydd and Beaumont 1972) and this was also found for the abalone *Haliotis tuberculata* (Clavier 1992). However, other species such as the giant scallop *Placopecten magellanicus* (Desrosiers et al. 1996), the clam *Spisula solidissima* (Clotteau and Dube 1993), blue mussel *Mytilus edulis* (Sprung and Bayne 1984), greenlip abalone *Haliotis laevigata* (Keesing and Babcock 1996) and corals *Favites pentagona*, *Monitpora digitata* and *Platygyra sinensis* (Oliver and Babcock 1992) are less sensitive in that polyspermy rates increase (or, strictly, fertilisation decreases at high sperm concentrations) more slowly with increasing ambient sperm concentration.

It is only possible to speculate as to what causes the apparent difference in sensitivity to polyspermy between the *Chlamys* species in this study. I reasoned in the last chapter (with the use of a theoretical mathematical model) that increasing fertilisation likelihood of free-spawned eggs may not be a simple case of "bigger is better" and that it may be important to also take into

account the increased potential of larger, more hittable, eggs to suffer polyspermy. *C.bifrons* has abnormally large eggs for a pectinid (diameter = 117μm), as most other scallops' eggs (including *C.asperrima*) are between 50 and 75 μm in diameter (Cragg and Crisp 1991) - perhaps these large eggs, in combination with faster sperm swimming speeds, result in a higher number of sperm/egg collisions before blocks can take action, making the blocks less effective in *C.bifrons* than *C.asperrima*. Additionally, perhaps the larger eggs somehow make block induction (or complete blockage) physiologically slower. Unfortunately, it is very difficult to determine between these without confident estimates of the parameters β_o, F_e and t_b .

Choice of fertilisation kinetic models for scallops

For the purposes of developing the model of field fertilisation success in this thesis it will be assumed that a) the parameters (β_o, F_e and t_b) measured here are accurate estimates; and b) fertilisation conditions measured in the test-tubes in the laboratory are similar to those which would be experienced in spawning events in the field (see also below). In this sense, the modified VCCW model appears to be a suitable predictive tool, relating ambient sperm concentration to fertilisation likelihood.

The fitted relationship between ambient sperm concentration and fertilisation success for *C.bifrons* (albeit with the reservations about extrapolating from the test tube experiments noted above) will allow the dilution of sperm plumes in field conditions to be interpreted in terms the likelihood of fertilisation for eggs released into various places around and away from a male releasing sperm. However, to do this certain further assumptions need to be made about the way in which fertilisation occurs in the field and, in particular, how eggs sample water containing sperm. Two models of this could be entertained. The first is that eggs drift once spawned, thus remain in a "piece" of water (such as an kolmogorov eddy) and after a time the sperm within this will expire and so not be potential fertilisers (given the short half-lives measured above, this may be quite a short time if concentrations are low). This is equivalent to containment in a test tube - the effect of sperm expiration was taken into account in fitting the experimental data by replacing t (contact

time) with τ (sperm half-life) in the fertilisation kinetics model. Alternatively, spawned eggs may sink and be stationary, and so if a male is nearby, will continually sample fresh (unexpired) sperm as it drifts past the eggs. In this case it is not appropriate to replace t with τ. The effect of doing this for scallop eggs is shown in figure 3.8.

I will assume the latter of the two models (stationary eggs) for several reasons. First, *C.bifrons* eggs are negatively buoyant in laboratory conditions - but at what rate they would sink and how far (or if) they would first drift before settling out is unknown in field conditions. Secondly, this also makes incorporation into spawning population models easier (see chapter 6). Finally, this is also the more conservative assumption in terms of rejecting a null model that scallops' reproduction is sperm limited - (see chapter 8)

The second assumption that needs to be made is the appropriate sperm-egg contact time likely to occur in the field. As shown in figure 3.9, contact time can have a significant effect, particularly over the first few minutes on the relationship between sperm concentration and fertilisation (or polyspermy) chance. At longer contact times (> 3600 seconds), this effect becomes less strong. Levitan (1996) used a contact time of t = 600 seconds for urchins and sand dollars (Levitan and Young 1995), based on his observations of their natural spawning behaviour. I have chosen to use t = 3600 seconds as my observations are that when induced to spawn (see chapter 5), male scallops typically do so with reasonable vigour for at least an hour. Thus, in the field, eggs released at the start of a spawning event may be exposed to sperm for about an hour. Again, longer sperm-egg contact time is conservative in terms of rejecting a null model of no fertilisation limitation.

Table 3.1.A Nested analysis of variance of differences in sperm swimming speeds (mm sec^{-1}) - between species and individuals nested within species. *Chlamys bifrons* (8 sperm speed measurements for 9 indivduals) and *C. asperrima* (8 sperm speed measurements for 6 individuals)

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SPECIES	31613.310	1	31613.310	37.338	<0.001
INDIV {SPECIES}	116615.701	13	8970.439	10.595	<0.001
ERROR	101602.197	120	846.685		

Table 3.1.B Nested analysis of variance of differences in egg diameter (mm) - between and individuals nested within species. *Chlamys bifrons* (9 individuals, 10 diameters each) and *C. asperrima* (6 individuals, 10 diameters each)

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
SPECIES	73730.184	1	73730.184	3849.270	<0.001
INDIV {SPECIES}	1832.462	13	140.959	7.359	<0.001
ERROR	2509.217	131	19.154		

Table 3.2.A Model parameters after fitting polyspermy-adjusted fertilisation kinetics model developed in chapter 2 to *Chlamys bifrons* sperm dilution experiment data.

	Low β_o	Measured β_o	High β_o	Fitted (a) β_o	Fitted (b) β_o
β_o	*0.00109*	*0.00218*	*0.00632*	0.23688	0.23688
F_e	0.01703	0.00879	0.00346	0.00137	0.00137
t_b	165.01	160.09	141.60	10.090	10.090
r^2	0.906	0.907	0.912	0.936	0.936

Table 3.2.B Model parameters after fitting polyspermy-adjusted fertilisation kinetics model developed in chapter 2 to *Chlamys bifrons* sperm dilution experiment data that had been standardised (see text for details).

	Low β_o	Measured β_o	High β_o	Fitted (a) β_o	Fitted (b) β_o
β_o	*0.00109*	*0.00218*	*0.00632*	0.00014	0.07883
F_e	0.04149	0.02152	0.00849	0.31475	0.00293
t_b	32.33	31.19	27.33	33.37	6.34
r^2	0.961	0.962	0.962	0.961	0.965

Table 3.2.C Model parameters after fitting polyspermy-adjusted fertilsation kinetics model developed in chapter 2 to *Chlamys asperrima* sperm dilution experiment data.

	Low β_o	Measured β_o	High β_o
β_o	*0.00036*	*0.00071*	*0.00283*
F_e	0.12867	0.06525	0.01793
t_b	2.25	2.22	2.03
r^2	0.927	0.928	0.930

Plate 3.1. Normally fertilised (2 cell stage) *C.bifrons* egg. The egg is 117 μm in diameter.

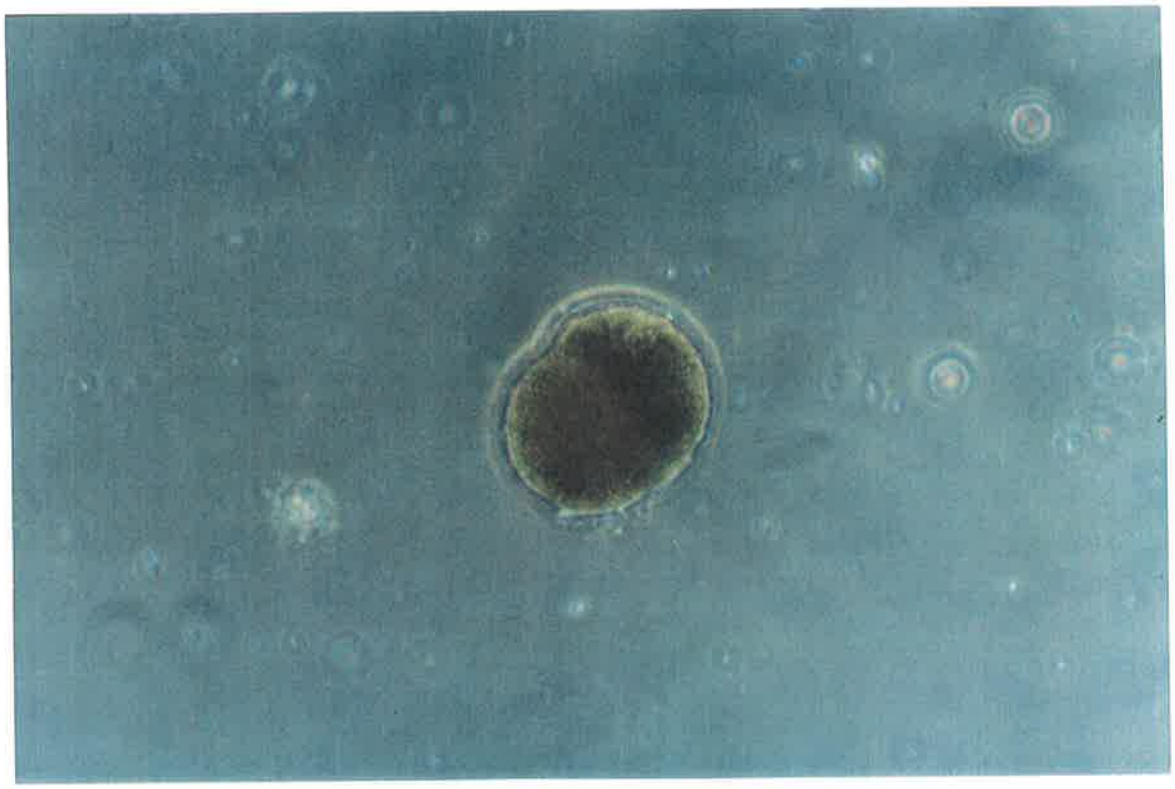


Plate 3.2. Putatively poyspermic *C.bifrons* eggs after exposure to high ambient sperm concentration - an abnormally dividing (4 cell) egg and an egg showing no division after 3 hours despite many sperm at the egg surface. Both eggs are approximately 120 μm in diameter.

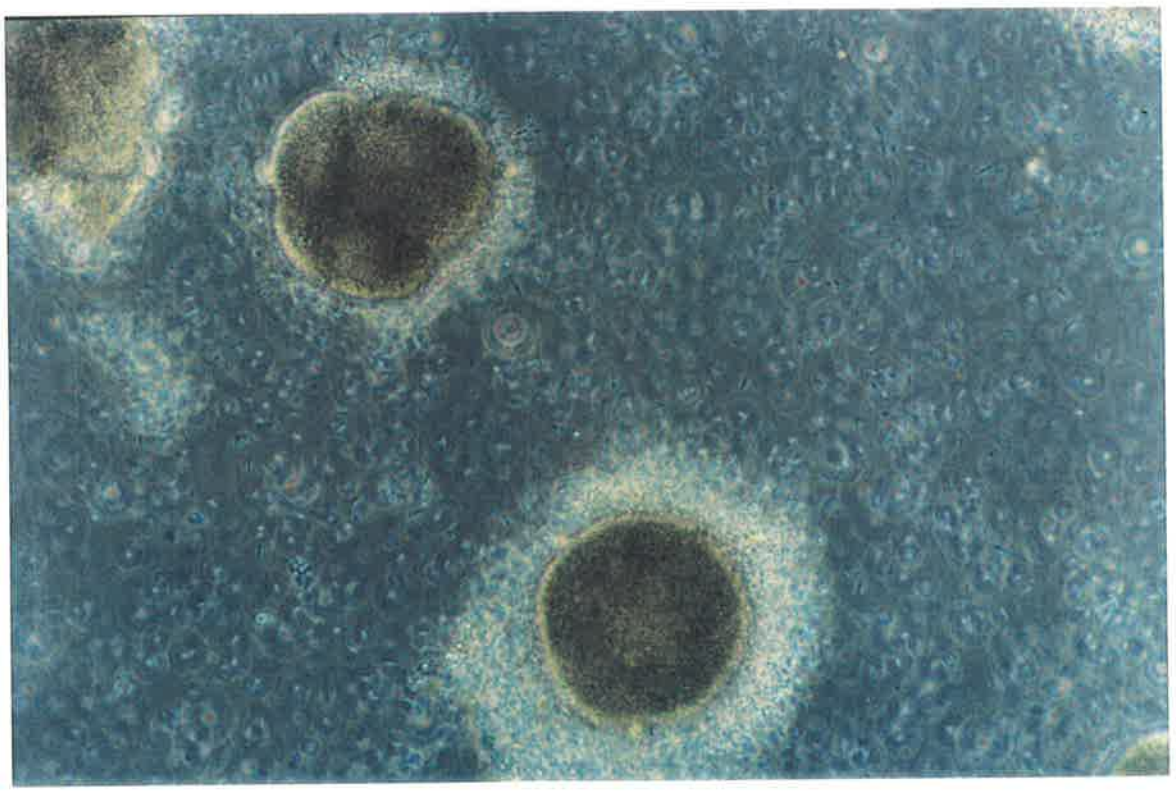


Figure 3.1. *Chlamys bifrons* fertilisation kinetics curve. Proportion of eggs fertilised (φ) after 3hrs in varying sperm concentrations. Pooled data from 4 independent male/female crosses.

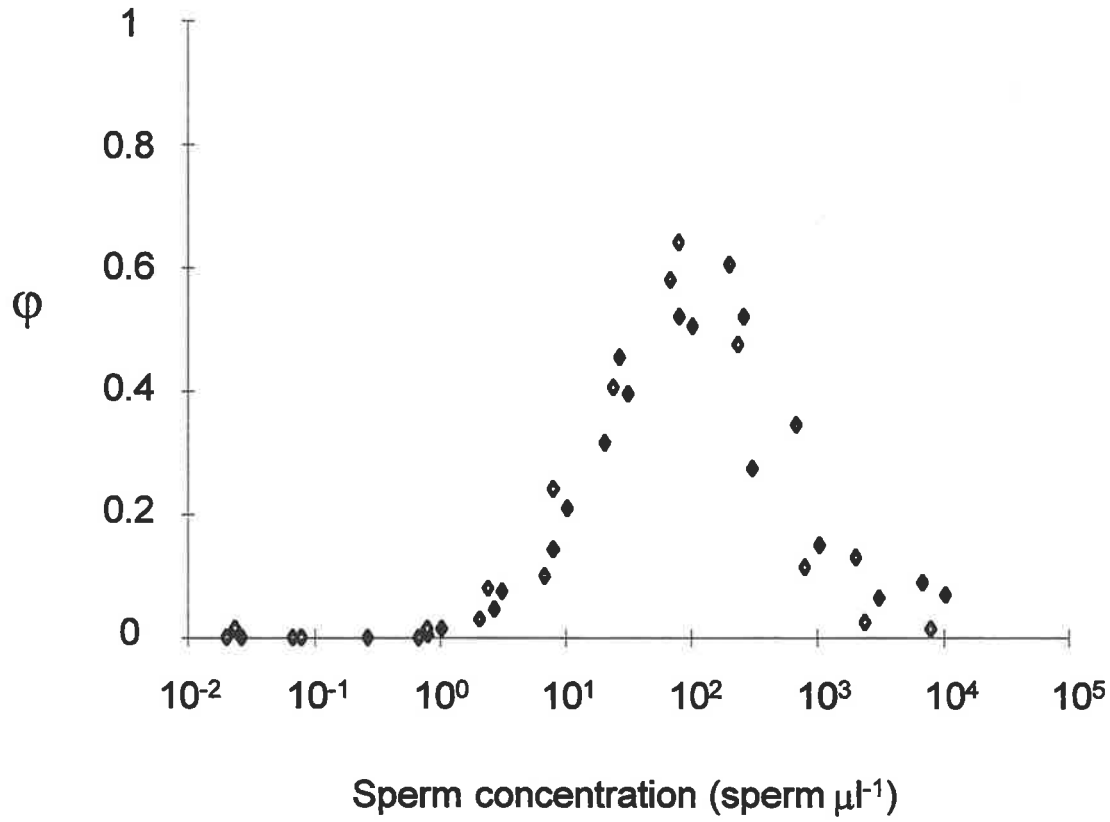


Figure 3.2. Standardised *Chlamys bifrons* fertilisation kinetics curve. Data from figure 3.1 were adjusted by a factor of 1.43 to achieve 0.99 maximum fertilisation.

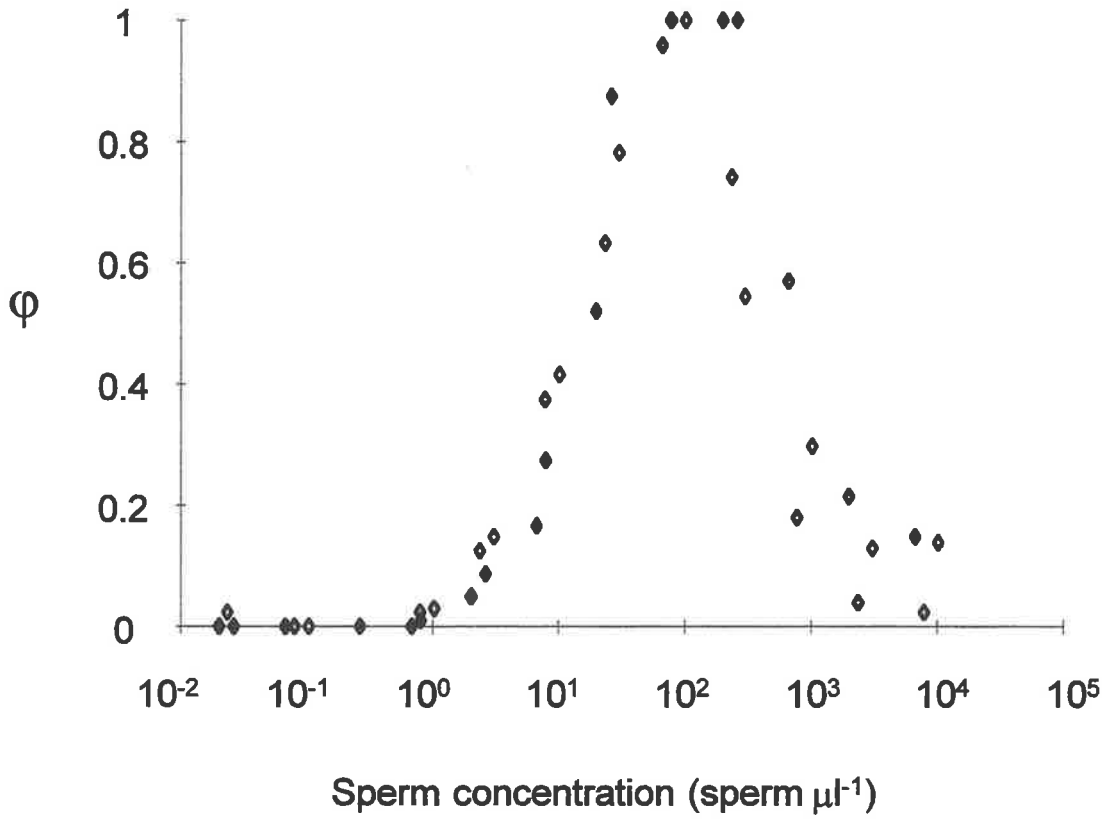


Figure 3.3 *Chlamys asperrima* fertilisation kinetics curve. Proportion of eggs fertilised (φ) after 3hrs in varying sperm concentrations. Pooled data from 6 independent male/female crosses.

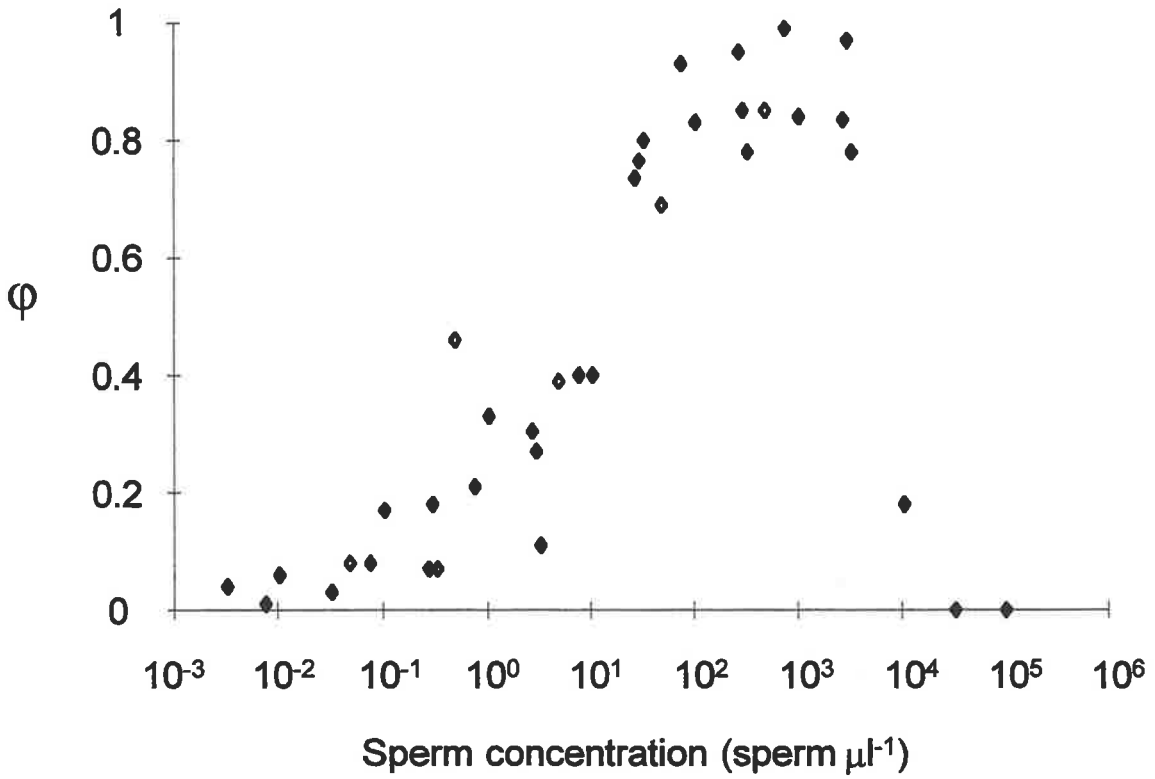


Figure 3.4 Sperm half-lives of *C.bifrons* (circles) and *C.asperrima* (triangles) at varying sperm concentrations.

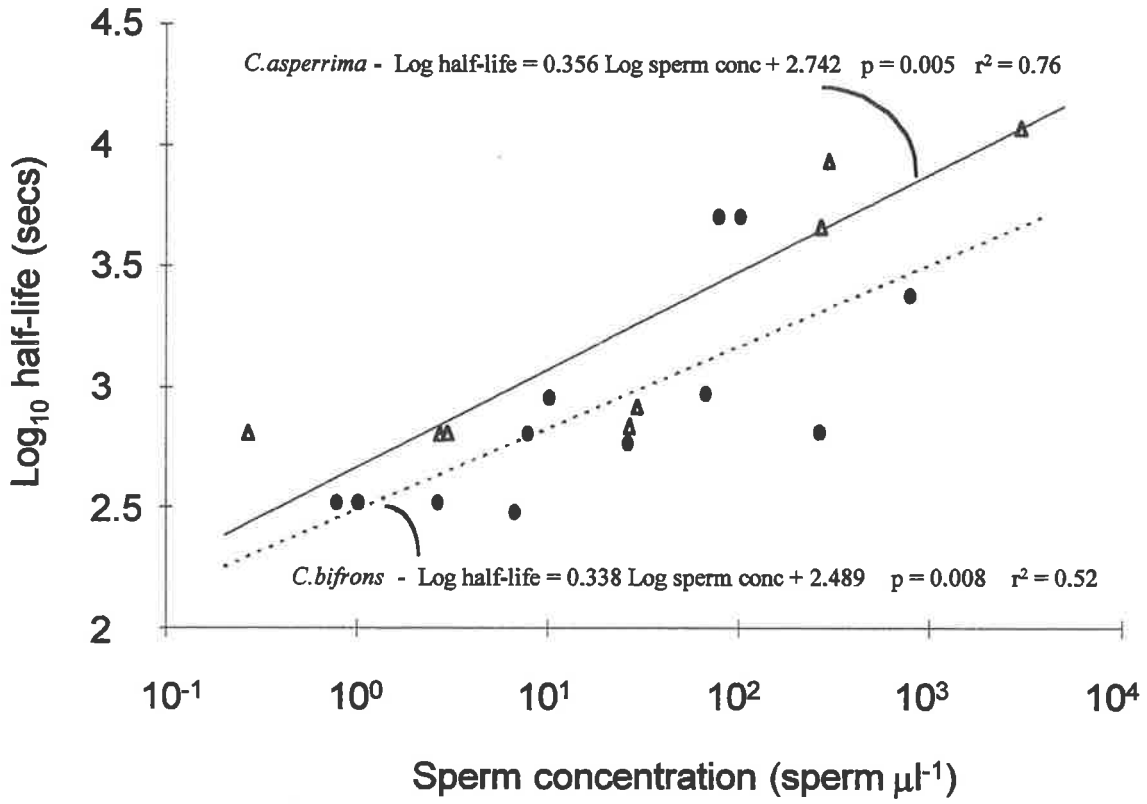


Figure 3.5 Fit of theoretical (polyspermy-adjusted) fertilisation kinetics model to experimental data for *Chlamys bifrons* **A**. Using measured β_0. **B**. Using low estimate of measured β_0(dotted), high estimate of measured β_0 (dashed) and extremely high β_0(solid). Model parameters used are given in table 3.2..

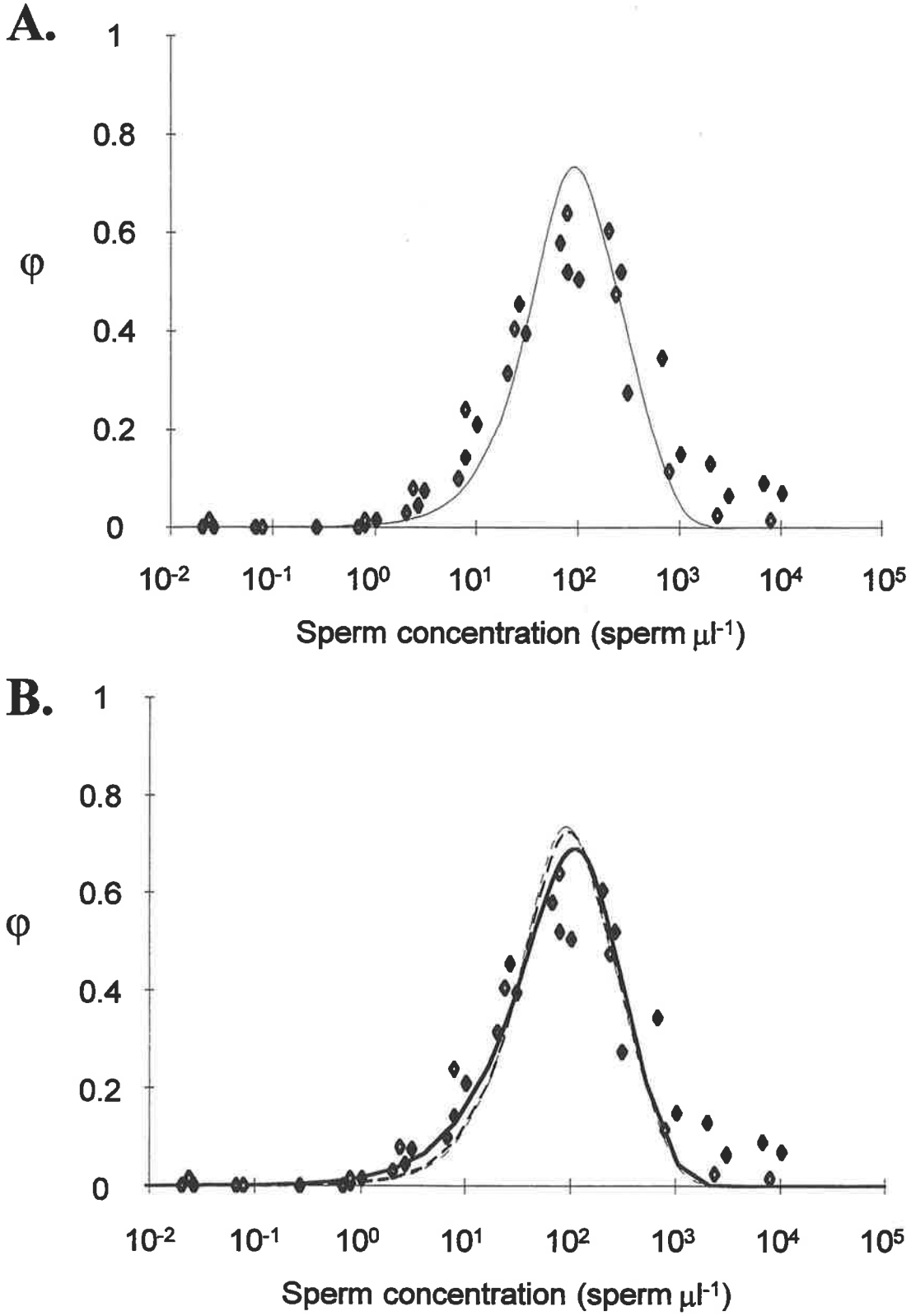


Figure 3.6 Fit of theoretical (polyspermy-adjusted) fertilisation kinetics model to standardised experimental data for *Chlamys bifrons*. Measured β_o and model parameters given in table 3.2.B were used.

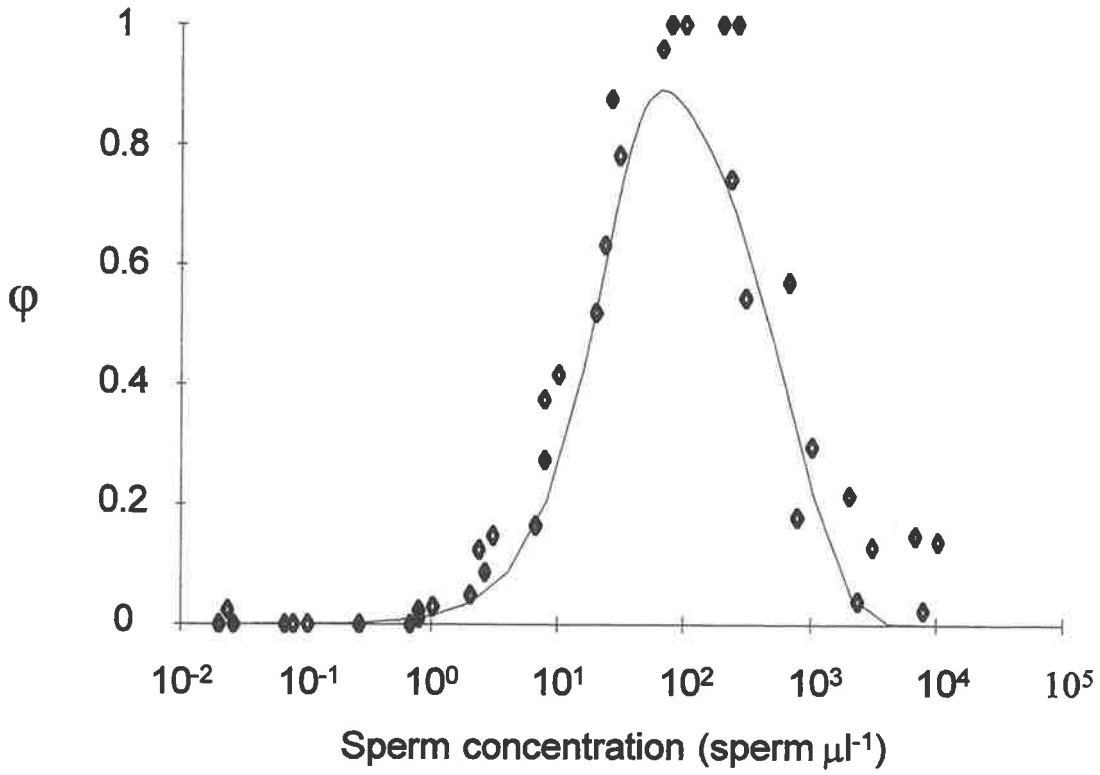


Figure 3.7 Fit of theoretical (polyspermy-adjusted) fertilisation kinetics model to experimental data for *Chlamys asperrima*. Measured β_o and model parameters given in table 3.2.C. were used.

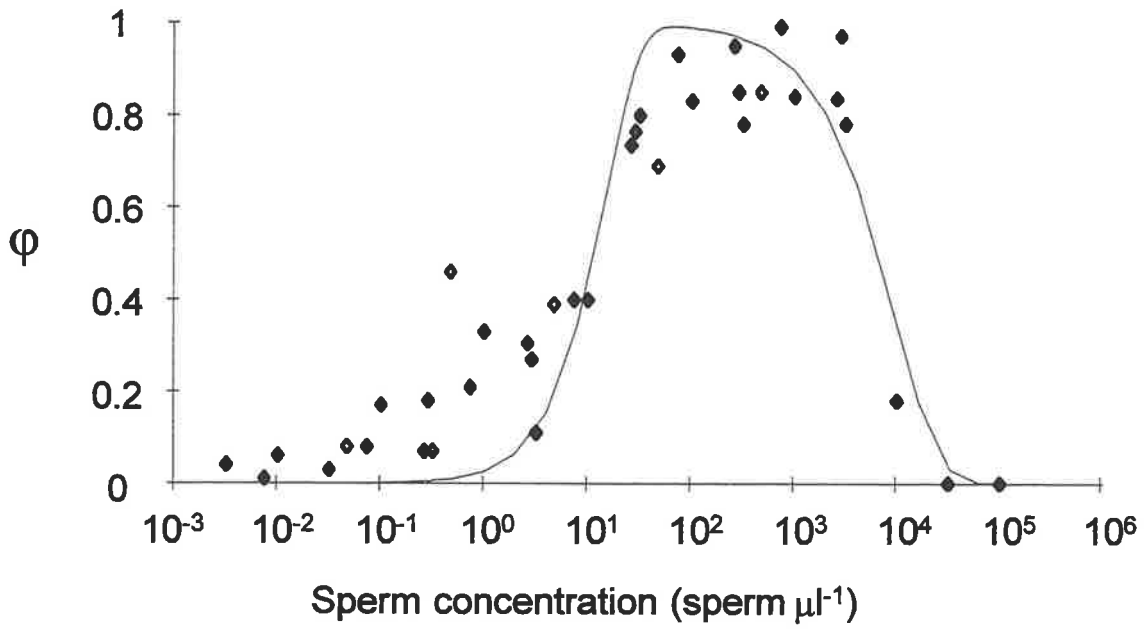


Figure 3.8 Alternative fertilisation kinetics curves for *C.bifrons*, dependent upon assumptions of gamete behaviour in field conditions. Eggs can either be continuously exposed to fresh sperm thus t is the appropriate term in the polyspermy adjusted VCCW model (dashed line, here $t = 3600$ secs) or eggs exposed to expiring sperm, in which case the approriate term is the sperm half-life τ (solid line).

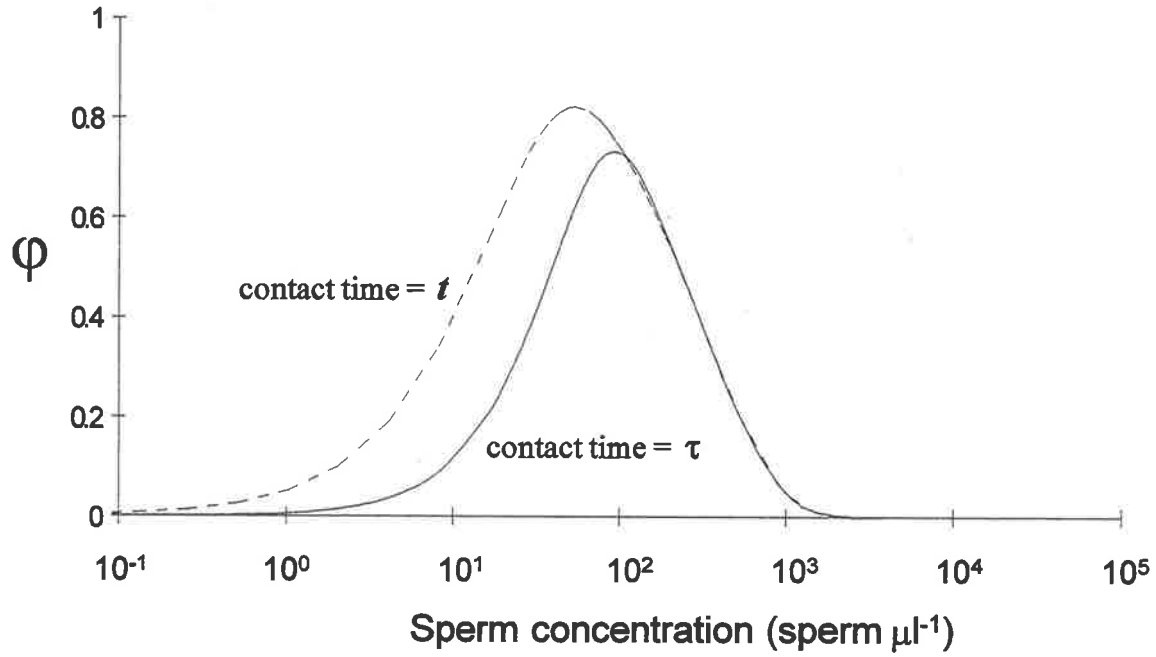
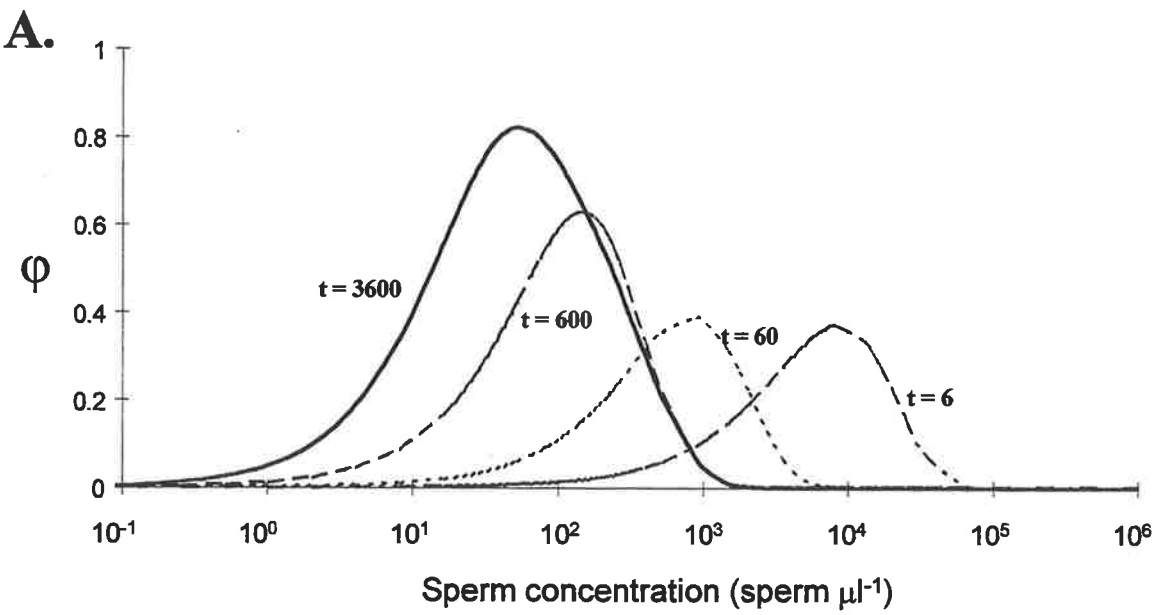
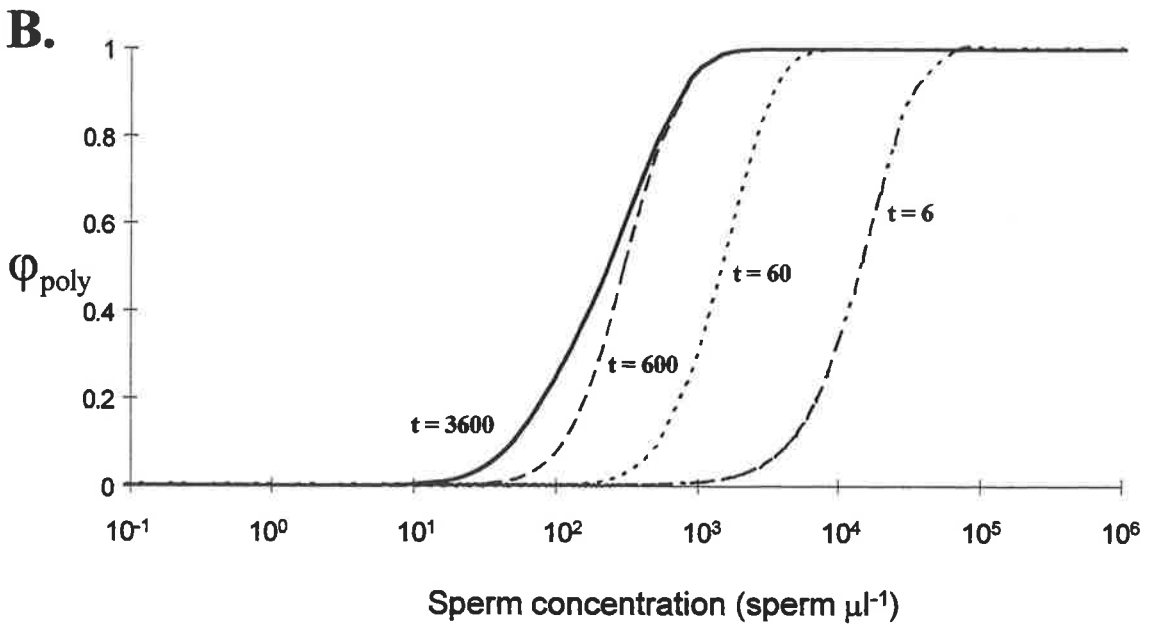


Figure 3.9. Effects of sperm-egg contact time (t) on *C.bifrons* fertilisation kinetics. A. Effects on proportion of eggs normally fertilised. B. Effects on proportion of eggs suffering polyspermy.





Chapter 4

Patterns of reproductive activity and spawning rates of *Chlamys bifrons* and *Chlamys asperrima*

Introduction

In developing a model of the fertilisation ecology of *Chlamys bifrons*, it is important to have an understanding, at a range of levels, of the way scallops spawn. Determining when scallops spawn is important to ensure use of ripe scallops in fertilisation experiments and to site other field experiments and surveys at appropriate times of the year (e.g. work conducted in chapters 3, 5, 7). Similarly, a knowledge of the rate at which males release sperm can be used in conjunction with models of near-bed water movements and fertilisation kinetics (e.g. Denny and Shibata 1989, Young et al. 1992, Babcock et al. 1994, Morris 1994, Andre and Lindegarth 1995, Levitan and Young 1995), to predict gamete dilution (and resultant effects on fertilisation success) away from spawners. Finally, at an intermediate (within population) scale, assessing how synchronously spawning occurs between individuals may also be important because this determines the effective population density of spawners. Unfortunately, virtually no information has been available about spawning on all of these scales for any free-spawning invertebrate, nor on any of these scales for either *Chlamys* species in South Australia. This chapter seeks to redress this.

Broad scale patterns of reproduction

Reproductive cycles of many animals are usually determined through temporally repeated measurements of gonad weight or some form of body component ratio such as a gonad somatic index, with rises and subsequent falls in either usually taken as an indication of spawning. The discrete nature of scallop gonads (which make them easy to dissect) and apparently large differences in gonad weight between ripe and unripe scallops has resulted in a high degree of success using this approach (Barber and Blake 1991), in contrast to mixed results in other groups such as fishes (West 1990).

Worldwide, there is a plethora of studies documenting the reproductive cycles of scallops (Barber and Blake 1991) and a great deal of work has already been completed for Australian scallops. Broad scale patterns of reproductive activity have been documented for *Amusium balloti* (Dredge 1981, Joll and Caputi 1995), *Pecten fumatus* in Port Philip Bay (Sause et al. 1987) and Jervis Bay (Fuentes 1994). Yet, despite its widespread distribution across southern Australia and its importance as a recreational and commercially harvested species (Kailola et al. 1993), no studies have documented the reproductive cycles of *C.bifrons*. Reproductive cycles have recently been described for *Chlamys asperrima* in Tasmania (Zacharin 1994) and Jervis Bay (O'Connor and Heasman 1996), but to date no direct data exists for those occurring in South Australia and those from elsewhere may not necessarily be applicable at other sites. Studies on a range of scallop species have found large differences in the timing of putative spawning peaks across geographically dispersed populations (e.g. Bricelj et al. 1987, Joll and Caputi 1995) and differences between sites (but within species) in size-fecundity relationships (e.g. MacDonald and Thompson 1988, Barber et al. 1988). Peak gonad weights of *C.asperrima* occur in late September and early October in Tasmania (Zacharin 1994) and Jervis Bay (O'Connor and Heasman 1996) but Chernoff (1987) reports settlement of (putative) *C.asperrima* larvae in April in experimental studies at one location (Edithburgh Jetty) in South Australia. Given a larval period of about 20 days, Dix (1976), this would indicate spawning in about March there. Yet at this time in Tasmanian populations (Zacharin 1994), gonads are extremely regressed and scallops incapable of producing larvae. O'Connor and Heasman (1996) did, however, note that despite the appearance of a clear annual pattern, in most collections throughout the year there were some individual scallops macroscopically classified as "ripe" .

The first aim of this chapter, then, is to determine (on the broad scale) at what times of the year *C.bifrons* and *C.asperrima* spawn at two sites within South Australia, Largs Bay and Edithburgh Jetty. To achieve this, scallop populations were repeatedly sampled over 2.5 years at both sites to document temporal changes in the weight and appearance of gonads.

Sperm release rates

At the individual scale, it is important to know at what rate scallops release sperm, and how this release rate might vary with individual species, size or individual fecundity. Using models of sperm dilution in turbulent near-bottom flows based on turbulent diffusion models of Csanady (1973), Denny (1988), Young et al. (1992), Morris (1994), Babcock et al. (1994), and Andre and Lindegarth (1995) have all demonstrated that there should be a strong correlation between sperm release rates and the distance downstream over which fertilisation occurs - with high release rates (Q) resulting in higher fertilisation at a given point downstream. In field experiments, Pennington (1985) also found that increasing the sperm release rate (by trebling the numbers of male urchins used as a sperm source) reduces the rate at which fertilisation success decreases downstream. Despite its importance, there are very few direct measures of sperm release rates. Indeed, the high degree of uncertainty in this important parameter was recognised by most of the above modellers, hence they examined the sensitivity of fertilisation models to variation in sperm release rates. Consequently, to develop a successful model of scallop fertilisation dynamics it will be necessary to have precise measures of sperm release rates and, importantly, how these might vary within a species.

In many marine invertebrates such as scallops (Bornadelli and Himmelmann 1995), gonad weight changes allometrically with animal size - so we might expect sperm release rates to do the same. Consequently, factors such as the size- or age-structure of a population may have important influences on the dynamics of free-spawning. Surprisingly however, Levitan (1991) found in experimentally induced spawnings of the urchin, *Diadema antillarum*, that male size had little effect on fertilisation within small arrays of individuals. Unfortunately, there is very little information as to how sperm release rates might vary within a species and almost no data where spawning rate, and in particular sperm release rate, has been measured as a function of animal size. Levitan et al. (1992) failed to detect a significant relationship between the amount of sperm released by *Strongylocentrotus franciscanus* and urchin size, and Keesing and Babcock (1996) also failed to find a relationship between size and the amount of sperm released in experimentally induced spawnings of the greenlip abalone, *Haliotis laevigata*. In contrast, Levitan (1988) did find

a positive relationship for the urchin *Diadema antillarum* (sperm volume $\propto$ test diameter $^{2.30}$) but, to date, no study has tested whether the allometry of spawning rates differs from the allometry of reproductive investment (gonad weight).

Some estimates of sperm release rates have relied upon measuring the difference in gonad weights between ripe and spawned animals and equating this weight difference with a measure of sperm density in testicular material to estimate the number of sperm released in a given time period - a fairly indirect measure (e.g. Pennington 1985, Young et al. 1992 Babcock et al. 1994). Others (e.g. Levitan 1988 ,1991, Lindegarth and Andre 1995, Keesing and Babcock 1996) have more directly measured spawn rates by enumerating numbers of sperm released into containers in which animals had been spawning over a given time period. This latter approach will also be used in this chapter. A further problem with estimates of urchin spawning rates (Levitan 1988, 1991), and also of field experiments in which urchins have been induced to spawn (e.g. Pennington 1985, Levitan 1991, Levitan et al. 1992, Styan 1997), is that spawning is usually induced with KCl injection. This causes a mechanical contraction of body wall muscles and expulsion of gametes through the gonophore, possibly in an unnatural way or at a rate that is different from (greater than) natural spawnings - though this proposition has not been tested. Hence, direct measurements of natural spawning rates are especially lacking.

Intra-gonadal injection with serotonin (5-hydroxytryptamine) has been shown to induce spawning readily in bivalves (Gibbons and Castanga 1984, O'Connor and Heasman 1995) and because it has been implicated as a natural product in the physiology/biochemistry associated with spawning in scallops (Croll et al. 1995, Martinez et al. 1996) it can be assumed that reasonably natural spawning behaviours are induced. Utilising this, I measured the spawning rates of ripe individuals of both scallop species in the laboratory. A particular goal of this work will be to document the variability in spawning rates that might exist within a population. Further, I will also test whether this variability in spawning rates is allometrically related to scallop size in the same way that gonad investment is.

Finer scale patterns of reproductive activity

One model that has intuitive appeal (it should be "good" for spawning success) is that spawning will be synchronous (or epidemic) within a population - obviously this should decrease inter-spawner distance (and gamete dilution) to a minimum. Indeed, there are reports of direct observations of such mass epidemic spawning (even amongst unrelated species), for example: corals (Kinzie 1993), sponges (Hoppe and Reichert 1987), the spectacularly large numbers of scleratinian coral species on the Great Barrier Reef (Babcock et al. 1986), multiple species in a temperate assemblage (Minchin 1992) plus anecdotal accounts of divers' observations of spawning events (Pennington 1985).

There are also accounts, however, of observations of asynchronous spawning within natural populations. For example, Levitan (1988) in prolonged, systematic observations of *Diadema antillarum* found a high level of asynchrony between individuals with only 5 % of individual urchins spawning at any one time. Similarly, Babcock et al. (1992) made numerous observations of single or limited numbers of individuals (almost invariably males) spawning amongst a range of invertebrate groups on coral reefs. There is also indirect evidence to suggest that spawning may not always occur synchronously, or at least occurs in multiple events rather in a single epidemic event. Measuring reproductive patterns of the scallop *Placopecten magellanicus* on fine temporal scales (where samples are taken days or weeks apart rather than at monthly intervals) revealed gradual reductions in gonad indices rather than sudden drops that were detected by less intensive sampling programmes (Parsons et al. 1992, Bomadelli et al. 1996). Indirect measurement of reproductive condition of intertidal limpets (Lasiak 1994) suggested spawning occurred across an extended time period and at any given time a high level of asynchrony in reproductive condition amongst individuals. O'Connor and Heasman (1996) also noticed that throughout the year, some individual *C.asperrima* within a collection were in a condition ready to spawn.

It cannot be simply assumed that scallops will spawn synchronously - instead this should be treated as a working hypothesis and wherever possible, tested. Unfortunately, this is extremely difficult to do directly. The best, and most definitive way of doing this would be to observe

scallops spawning in the field. Then it would be a matter of determining the proportion of scallops within the population that were spawning, and ideally the variability of this over time (if there were any variability at all). Unfortunately, the likelihood of being able to make these measurements is very small as natural spawning events are very rarely witnessed in nature (Levitan 1995) - see table 1.1 which is an exhaustive list of all published measures of fertilisation success made during observations of natural spawnings. Added to this, the almost complete lack of information about broad scale patterns of reproductive activity for both scallop species in South Australia, means planning even when to start looking is impossible and the likelihood of witnessing field spawning something akin to searching for a needle in a temporal haystack! Instead, an alternative test may be to try to measure synchrony indirectly through observation of changes in gonad weight and visual estimates of ripeness. If spawning was completely synchronous and occurred in a single event, it would be expected that a single large, sudden drop in gonad weight would be detected (i.e. a uniform decrease). If sufficient samples are taken, and importantly, over appropriately small time and spatial scales when spawning is occurring in field populations, it should be possible to determine whether this occurs or if instead spawning occurs in a series of repeated events. Similarly, by examining the proportion of scallops considered ripe, within and between samples, it should be possible to get some idea of the degree of synchrony of reproductive patterns amongst individuals and how this might change through time. Total synchronous spawning should be associated with the presence followed by a complete absence of individuals in a ripe state. Of course, it is synchrony on very small time scales that will be important to the dynamics of fertilisation (i.e. whether scallops spawn at the same instant) and so the inferences that can be made from these observations may be limited if sampling does not also occur at similarly small time intervals during the period when spawning is likely. Consequently, sampling at one site, Largs Bay, was planned to occur as frequently as possible (samples taken days or up to a week apart) when it was considered from cumulatively collected samples that spawning was imminent.

Methods

Reproductive cycles - sample collection and processing

Samples of scallops were haphazardly collected by divers on scuba from early November 1993 (though only from March 1994 onwards from Largs Bay until June 1996) and returned to the laboratory. Within 24 hrs of collection (scallops not processed immediately were kept in a refrigerated room at 5°C - no evidence was found to suggest that scallops spawned during this short holding period) the size, sex and gonad weights were measured and a visual (macroscopic) estimate of scallop 'ripeness' made for each scallop. Each scallop was also assayed visually for the presence and abundance of a suite of macroparasites and scallops parasitised by larval bucephalid trematodes excluded from further consideration. For both *C.bifrons* and *C.asperrima*, this was a low proportion of the population (~3 %) (see chapter 7).

Height, as a measure of scallop size (Barber and Blake 1991) was measured to the nearest mm as the distance from the hinge of the scallop to the furthermost point along the leading edge of the shell. The sex of both species of scallop is usually easily determined - female gonads are purple and orange/peach coloured in *C.bifrons* and *C.asperrima* respectively, whilst male gonads are a creamy/white in both species (see plates 4.1 - 4.4). Even during times where scallops exhibited extremely regressed gonads, it was usually still possible to visually sex animals, though in some cases it was necessary to examine gonads microscopically for ovarian or testicular material. Gonads, with an attached siphon and included gut loop, were dissected out, blotted on absorbent paper and weighed (as wet weights) to the nearest 0.01g. Each scallop was also scored on a qualitative scale that was independent of sex or species, based on the macroscopic appearance of the gonad in terms of fullness, thickness and colour intensity. This scale was similar to others such as those utilised by Dredge (1981) and O'Connor and Heasman (1996). Each animal was scored as: 0 - gonads small and regressed completely and water filled, almost no gametic material visible macroscopically and the gut loop within the gonad clearly visible; 1 - gonads small and largely regressed with very large water filled spaces between gametic material, but able to detect colour of material and the gut loop within the gonad reasonably visible; 2 - gonads

moderate size and thickness and fairly solidly packed with gametic material though with some obvious spaces, gonad not particularly full looking and colour very distinct though still fairly dull, only tip of the gut loop visible near the end of the gonad; 3 - gonads very firmly packed and looking full, no gaps between gametic follicles and colour intense and often brighter, gut loop not visible; 4 - as for 3, gonad extremely large, colour very bright and looking very full, almost ready to burst, gut loop not visible. It is hoped that this scale represents the likelihood that a scallop was ready to spawn, with a score of 0 or 1 indicating that a scallop was unable to spawn, 2 - that it had developing eggs or sperm, but was not quite ready to spawn or had just released mature gametes, and 3 or 4 indicating a readiness to spawn mature gametes. This scale is obviously very subjective, and care was taken to try to ensure reputability between sampling times over 2.5 years.

Reproductive cycles - sample sizes and design

Chlamys bifrons were routinely collected from two sites, Largs Bay and Edithburgh Jetty. *C.asperrima* was routinely collected from Edithburgh Jetty but not from Largs Bay as the species was relatively uncommon there, meaning that sufficient numbers for regular sampling could not be found. The temporal frequency of sampling varied somewhat between locations and seasons. In part, this reflected site accessibility and the stochasticity of prevailing weather conditions, but also systematic seasonal increases, in attempts to survey with higher resolution during periods of spawning activity. Broadly, the time interval between samples at Edithburgh Jetty varied somewhere between fortnightly and monthly, whilst samples were collected from Largs Bay more frequently - approximately monthly during winter months when gonads were regressed, increasing to nearly daily when *C.bifrons* gonads were large and spawning imminent. In each collection, about 16 (range = 5 to 35) scallops were sampled, wherever possible from a range of sexually mature sizes (assumed to be greater than 55mm for *C.bifrons* and greater than 35mm for *C.asperrima*). All scallops in a sample were collected on one dive. At Edithburgh Jetty, samples were repeatedly taken from the same location (in the immediate vicinity of the jetty) and haphazardly from across the whole site. However, at the much larger Largs Bay site, scallops were collected from only the area that could be covered by a diver in one dive (and often much

less than this). Thus, all scallops in a Largs Bay sample were taken from the same part of the site (ie a patch ~100-1000 m^2) rather than being sampled from the whole population at a particular time. The location of these sampling patches within Largs Bay changed and was haphazardly chosen each time.

Relationship between size and fecundity

For scallops (and many other marine invertebrates) wet gonad weight is usually allometrically related to size :

$$\textit{wet gonad weight} = a\ (\textit{shell height})^b$$

(e.g. Peterson 1983, Bomadelli and Himmelmann 1995). Thus, to determine the relationship between scallop size and gonad mass $\log_{10}$ wet gonad weight was regressed against $\log_{10}$ shell height. Separate regressions were calculated for scallops of different sexes, species and locations and in each case only scallops that were considered ripe (visual gonad index = 3 or 4) were analysed.

Reproductive cycles - data analyses

There are a multitude of gonad indices that can be constructed from measurements of the ratio of various body components such as gonad, somatic and adductor muscle weights, and which have been used to document putative temporal changes in scallop reproductive activity (reviewed in Barber and Blake 1991). However, Bomadelli and Himmelmann (1995) recommend measuring wet gonad weight and the shell height, as a largely (seasonally) invariant measure of scallop mass, then standardising gonad weights using an allometric relationship between shell height and gonad mass. Note that because the samples here contained variously sized individuals, allometric standardisation was necessary to equally weight each individual. A standardised gonad weight was calculated for each scallop collected (using the allometric relationships described above) and the mean and sample standard deviation calculated for each collection, again treating each group

(species, sex, location) of scallops separately. Sizes to which gonads were standardised were the average over all collections (i.e. over the 2.5 years) of a particular sex/species/location combination.

Measurement and comparison of spawning rates

Male scallops in ripe condition were collected from the field during periods when gonad indices indicated population wide readiness to spawn. Ripe *C.bifrons* were collected from Largs Bay on 27/10/94 and 7/10/95. *C.asperrima* were collected from Edithburgh Jetty on 13/6/94, 2/8/94 and 11/7/95. Animals were returned to the laboratory and maintained for up to 10 days in 40L aquaria, held at field temperatures and fed a mixture of *Isochysysis galbani* and *Tetraselmis selecia* until used. Note that before use scallops were also checked for ripeness by visually scoring macroscopic gonad condition (criteria: score >3) as live animals exhibited a swimming/escape response (opening and shutting of valves) when picked from aquaria. Each scallop was injected in the gonad and the adductor muscle with 0.5 ml of a 10^{-4} M 5-hydroxytryptamine (serotonin) solution to induce spawning and then placed in a plastic ice-cream container filled with 1L (*C.bifrons*) or take-away food container filled with 500 ml (*C.asperrima*) of fresh, sperm-free seawater.

Scallops were carefully watched for signs of initiation of spawning activity, which usually occurred 10-240 minutes after injection. Once a scallop had started to release sperm, it was allowed to do so undisturbed in the container for a period of time, after which the (still-spawning) scallop was moved into a new container of sperm-free seawater. Scallops were usually left spawning in containers for 15 minutes before being moved to the next, but this time was varied if they were releasing very large or small quantities of sperm. Scallops were continually moved to new containers every 15 minutes or so until it could be seen that they had stopped spawning (no sperm visibly being released from the siphon for 15 minutes). Sperm samples from each container were counted (5 counts) using a haemocytometer on the phase-contrast microscope to determine the average rate at which scallops were releasing sperm during the time that scallop was spawning into the container. The maximum of these rates and their average over the first

hour was calculated as well as the total fecundity (number of sperm) released over the entire spawning event by each scallop. Log_{10} -transformed, these were regressed against log_{10} scallop height.

Results

Size - fecundity relationships

Allometric relationships between wet gonad weight and shell height for *C.bifrons* at Largs Bay and both *Chlamys* species at Edithburgh Jetty are illustrated on the log-log plots in figures 4.1.A, 4.1.B and 4.1.C for males and 4.2.A, 4.2.B and 4.2.C for female scallops respectively. Treated as separate groups (sex/species/location), significant regression lines ($p < 0.05$) were found for all groups of scallops considered to be visually ripe (visual index = 3 or 4).

Patterns of reproductive activity

Patterns of reproductive activity are presented in three ways : First, mean (+/- standard deviation) standardised gonad weights for each collection are plotted through time, treating each sex separately. Standardised gonad data for *C.bifrons* at Largs Bay, the site with the most frequent sampling, are shown in figure 4.3.A-F. Similarly, standardised gonad data at Edithburgh Jetty are shown in figure 4.4.A and 4.4.B for *C.bifrons* and *C.asperrima* respectively. Secondly, mean (+/- standard error) visual ripeness scores for each site/species combination are shown in figure 4.5. Note here, that the scores of both sexes in a sample were combined to determine means and standard error. Finally, the proportion of each sample in each of three states ("ripe" = visual index score of 3 or 4; "intermediate" = visual index score of 2; "unripe" = visual index of 0 or 1) are shown in figure 4.6.A for *C.bifrons* at Largs Bay, 4.6.B for *C.bifrons* at Edithburgh Jetty and 4.6.C for *C.asperrima* at Edithburgh Jetty.

On the broadest scale, reproductive activity in *C.bifrons* at both sites (indicated by highest mean standardised gonad weights and visual indices) appeared to occur over warmer months

(September to April), with gonads visually regressing and losing weight in the winter months (May-August). In contrast, *C.asperrima* at Edithburgh generally had low standardised gonad weights and visual indices during summer, and highest values for both of these measures during the winter months (June - August). These patterns were consistent over the 2.5 years of sampling and in all cases there appears to be a high degree of synchrony between sexes of the same species/at the same location. Within years, *Chlamys bifrons* at Largs Bay displayed a semi-annual pattern of reproductive activity. Three broad peaks in standardised gonad weight (fig. 4.3.A) and mean visual index (fig. 4.5.A) are clearly present in the first full summer of sampling there (1994/95). This pattern is also suggested in figure 4.6.A. Presumably, this indicates 3 spawning events for this species there. The first two of these peaks (October and early January) show a rapid drop in mean standardised gonad weights and visual index as might be expected with a reasonably synchronous spawning event. However, the third peak (around March) is followed by a more gradual decline in these indices, possibly indicating spawning, but also is likely to represent resorption of energy from gametic material as gonads start to regress over the winter period. In the second summer (1995/96) the pattern for *C.bifrons* at Largs Bay is repeated, although it is not as clear, and possibly only two spawning peaks occur. A first peak in standardised gonad weight and visual ripeness appears to be present in late September / early October. Mean gonad weights again build up after this until January, after which the weight and visual indices decline, but only gradually, much like the putative third spawning/ gonad regression in 1994/95, but that decline started later, in March.

The data for *C.bifrons* at Edithburgh, shown in figures 4.4.A and 4.5.B do not present strong evidence of multiple putative spawning events during the active reproductive period over summer. However, sampling was much less frequent at this site and it can be seen, particularly by comparing the plots of mean visual ripeness in figures 4.5.A and 4.5.B, that samples were not taken at Edithburgh Jetty at times where peaks and putative spawning events were occurring at Largs Bay. Also noteworthy is that lower peak (mean) values were recorded for the visual estimate of gonad ripeness index at Edithburgh.

There appears to be some evidence of two peaks per year in the reproductive data for *C.asperrima* at Edithburgh Jetty. In both 1994 and 1995 there appears to be a first peak in both the standardised gonad weight and visual indices that occurred in early June. This is then followed by a small drop in the indices, followed by another rise to a higher peak in both and then a large drop occurring in August/September. This pattern is also evident in the proportion of ripe scallops data shown in figure 4.6.C. One interpretation of this is a partial spawning occurring in June, followed by a major spawning at the end of winter. Visually ripe *C.asperrima* were in greatest abundance during winter months, but in the first summer of sampling there was a small proportion of ripe scallops in samples.

Considering the *C.bifrons* at Largs Bay data on a fine time scale, it can easily be seen in figure 4.3.B-F that there was a large degree of variability between samples that were taken often just days apart. This is true for both the mean standardised gonad weight index and, to an extent, visually estimated ripeness index. Of note is that some groups of samples often showed dips or peaks in both indices, despite there being obvious upwards or downwards trends in the time series data. Data were not collected at Edithburgh Jetty at frequent enough intervals to make appropriate comparisons.

Spawning rates

A total of 38 scallops (18 *C.bifrons*, 20 *C.asperrima*) that were injected with serotonin spawned within 4 hours and were used in the analyses below. The time between injection and the onset of spawning was quite variable and ranged from 10 to 240 minutes, though most spawned within 40 minutes. Scallops generally released a constant stream of sperm from the siphon, though in some instances clouds of sperm were released in puffs as scallops "flapped" their valves quite violently. It appeared as though there had been a build up of sperm within the mantle cavity before this flapping occurred - whether this buildup or release in clouds would have happened outside the still conditions of the spawning containers (i.e. in the field) is unknown. Once spawning, most scallops showed a typical pattern of vigorous spawning for the first few sampling periods (until 30-60 minutes), followed by a slowing of release. Some scallops continued to release small amounts of

sperm up to 4 hours after the initiation of spawning, but most had stopped releasing significant amounts of sperm after the first hour. Rather than plot the release of sperm through time for each individual, these data were summarised by determining the maximum rate recorded, the average rate of release over the first hour and the total amount of sperm released by each scallop. These various measures of sperm release are shown in figure 4.7, plotted against scallop size (log_{10} height). For both species, data from scallops collected at separate times were pooled.

The highest (maximum) rate measured for any *C.bifrons* was 3.7×10^7 sperm sec^{-1} and 4.8×10^7 sperm sec^{-1} for *C.asperrima*. There was no detectable relationship between maximum rates and height for either species - slopes of linear regressions were not significantly different from 0 ($p<0.05$) for both species (see figure 4.7.A). Analysis of covariance (table 4.4.1.A) failed to detect a difference between the two species in the allometric relationships between maximum release rate and size. Linear regressions were also non-significant when data for species were pooled (ie treating all data, irrespective of species, as just "scallops"). Average maximum rates were 5.4×10^6 sperm sec^{-1} for *C.bifrons* and 4.0×10^6 sperm sec^{-1} for *C.asperrima* (log-transformed before averaging, then back transformed).

Non-significant regressions also suggest there was no relationship between average spawning rates over the first hour of spawning and height (see figure 4.7.B). Again this was true whether treating the *C.bifrons* and *C.asperrima* data separately or pooling and again ANCOVA failed to detect a difference between the allometric relationships of the two species (table 4.1.B). Average sperm release rates over the first hour were 1.5×10^6 for *C.bifrons* and 5.7×10^5 for *C.asperrima*. Similarly, there appeared to be no relationship between the total amount of sperm released by a scallop after serotonin spawning and the size or species of a scallop (see figure 4.8.C, table 4.1.C). In contrast, significant allometric relationships (gonad weight with shell height) were found for scallops collected in the above reproductive cycles data for ripe (Visual Index = 3 or 4) male scallops for both species (shown in figure 4.8.D), though no difference could be detected between the species in this (table 4.1.D)

The interaction term in an ANCOVA was used a test of whether the slope of regression of log gonad weight against log shell height differed from the slope of a spawning measure (log maximum rate, log average rate, log total sperm released) against log shell height. For *C.bifrons* this term was non-significant when comparing gonad weight with maximum rate ($p = 0.293$), and gonad weight with total sperm released ($p = 0.055$). The comparison of gonad weight with average rate was significant ($p = 0.002$); $n = 181$ in each comparison. For *C.asperrima*, two comparisons were non-significant : gonad weight with average rate ($p = 0.197$), and gonad weight with total sperm released ($p = 0.174$). The comparison of gonad weight with maximum rate was highly significant ($p < 0.001$); $n = 93$ in each comparison. The above analysis was then repeated but the data from both species were pooled. When treated as simply "scallops", a highly significant interaction term ($p < 0.001$) was found for all three comparisons (gonad weight with maximum rate, gonad weight with average rate, gonad weight with total sperm released; $n = 274$).

Discussion

Patterns of reproductive activity

The results of this study indicate that there is quite a difference between *C.bifrons* and *C.asperrima* in the broad scale patterns of reproductive activity. At both sites, *C.bifrons* are reproductively active over a large part of the year, from spring (September) to near the end of autumn (April), principally when water temperatures in Gulf St. Vincent are warmer (~18-25°C at Largs Bay, pers. obs). In contrast, *C.asperrima* appears to spawn in winter and spring, when water temperatures are much cooler (~10-14°C, pers. obs.). In laboratory conditions, these species have a fairly similar larval biology in terms of time spent as dispersive larvae, settlement requirements and their size at settlement (see Dix 1976, Rose and Dix 1984), so these differences in reproductive patterns could lead to many questions about what evolutionary pressures may have led to the timing differences. Similarly, the differences in reproductive timing might stimulate future studies of how temporal variation in factors like water temperature and food availability affects larval biology or other recruitment processes.

These reproductive patterns place into question one aspect of the work of Chernoff (1987), relating to the timing of an experimental field investigation of the mechanisms underlying the frequent association between sponges living on the shells of *C.asperrima* and the scallop host. Testing whether this is facilitated by scallops (*C.asperrima*) preferentially settling near suitable sponges, Chernoff (1987) conducted a field experiment to determine if the presence or absence of sponges affected the numbers of scallop recruits settling onto standard settlement monitoring substrata (plastic mesh/orange bags). Her results indicated no effect of sponges on scallop settlement. However, these experiments were conducted at Edithburgh Jetty during March to May, a time when, according to the reproductive data collected here (and from other places too - Zacharin 1994), *C.asperrima* larvae are unlikely to have been present in the water column. The population of *C.asperrima* at Edithburgh Jetty should probably be considered to be open in that larvae settling there are likely to originate from another source some distance away, so perhaps *C.asperrima* adults at another (source) site may have been producing larvae at this time. On the other hand, *C.bifrons* are common at this site and are in reproductive condition shortly before this time, so possibly these were settling on experimental collectors rather than *C.asperrima*. *C.bifrons* does not have an association with sponges and there would be no reason to expect sponges to influence their settlement. Again, this is in line with the experimental findings and supports the idea that Chernoff may have been measuring *C.bifrons* rather than *C.asperrima* settlement, but clearly the identity of the settlers needs to be addressed before any conclusions about the settling behaviour of *C.asperrima* are reached.

The pattern of repeated spawning within a year displayed by *C.bifrons* at Largs Bay was unusual (cf. studies reviewed in Barber and Blake 1991) with three distinct peaks clearly evident from both the gonad weight and visual ripeness indices in 1994/95, though possibly there are only two peaks/spawning events occurring in 1995/96. This suggests this population produces several cohorts of larvae per year. Again, this might lead to questions about the relative success and population dynamic importance of these separate larval inputs, and the effect that differing environmental conditions experienced during the year have on their survival and recruitment. Reproduction over a large part of the year may introduce difficulties to the interpretation of size frequency data - particularly if growth is slow or variable over the first 6 months. Similarly, the use

of size and (putatively) annual growth checks in the shell to age individuals and determine age at first reproduction (as was done for Tasmanian *C.bifrons* by Wolf and White 1995, assuming a single cohort of annual recruitment), may be somewhat complicated for the South Australian scallops.

The build up and loss of gonad weight for both sexes of *C.bifrons* at Largs Bay was rapid and it is easy to see the value of the fine time scale over which sampling occurred at this site. At Edithburgh Jetty, sampling was much less frequent and somewhat more regular in temporal spacing i.e. samples were taken approximately "monthly" for *C.bifrons* and only slightly more frequently than this for *C.asperrima* during winter months when they were approaching spawning. As a result of sampling only at a broad temporal scale, it is not possible to determine the fine scale reproductive patterns for the scallops at Edithburgh and impossible to determine if similar multiple spawning peaks also occurred there. Instead, only a broad pattern (that *C.bifrons* were found in ripe condition across the spring-autumn period) can be distinguished there.

Generally, there was a gradual reduction in gonad weight after most gonad weight peaks found for *C.bifrons* at Largs Bay rather than the very sharp, distinct drop over two samples that would be expected if spawning had occurred synchronously in one event. This might indicate repeated spawning over at least several days, and possibly over much longer at the end of the summer, but gradual resorption of gametes might also explain the very gradual loss of gonad weight in March/April in both sampling years. As noted earlier, this is a feature of scallop reproductive patterns where samples have been taken over finer temporal scales (e.g. Parsons et al. 1992, Bornadelli et al. 1996). However, another feature of the pattern of standardised gonad weights of *C.bifrons* at Largs Bay measured on a fine time scale was a high degree of variability between subsequent samples taken around the time of gonad weight peaks, where, despite a trend over several samples for a relatively gradual decrease or increase in average standardised gonad weight, pairs of subsequent samples within this trend/series would often vary by large amounts and in reverse to longer term trends. This is also a feature of some time series collected by Parsons et al. (1992) and Bornadelli et al. (1996) though it was not addressed in either of these studies. Possibly, these small scale rises and falls over the period of days represents the buildup

and release of gametes, but I think this unlikely as this would rely on a very rapid regrowth of gametes. Rather, this apparently aberrant variation may be representative of real variation amongst patches within the Largs Bay population. Samples were taken within patches and subsequent patches which were sampled changed from one sampling time to another. Thus, one interpretation of the observed small scale variability through time is that there might be some difference between patches of *C.bifrons* across the population at Largs Bay in terms of when spawning starts to occur: one sample might measure scallops after scallops had spawned within that patch, then a subsequent sample measure scallops at another patch where they had not yet spawned. Another (non-exclusive) interpretation is that, perhaps because of patch differences in flow or food availability, scallops from different patches differ in their size/fecundity relationships. If such fecundity differences occurred, then differences in average gonad weights that were detected through time could reflect spatial differences in size/fecundity relationships rather than just temporal (spawning activity) changes. The variation that was detected could also reflect an interaction between these two potential sources of variation between patches, i.e. differences in the size/fecundity relationships of scallops and a difference in when spawning occurs.

This study initially focussed on a larger (population) scale on the assumption that spawning would be synchronous and epidemic throughout the population (though entertaining the idea that spawning might be prolonged over a time period). Thus, I assumed that any sample taken from within that population would be representative of the entire population at Largs Bay, regardless of whether scallops were taken from across the entire population or all from within the same patch. Clearly, this may not have been a reasonable assumption and unfortunately the sampling strategy that was used here (and in other studies) does not allow for discriminating whether observed variation is simply temporal variation associated with spawning that is displayed across the entire population, or an interaction of this with differences that occur between patches (i.e. spatial variation). Conducted again, sampling might aim to collect scallops from across the entire population at every time, rather than collect all scallops in a particular sample from a single patch. This would avoid the problem of small scale (patch) spatially autocorrelated differences. However, what is of most importance is what happens on the patch scale - because it is at this (smallest) scale that spawning synchrony counts and determines the effective density of spawners.

Measuring across the entire population might overlook the small scale synchrony that counts and instead measure larger scale asynchrony that is functionally less important in the dynamics of free-spawning. If there are differences between patches within the Largs Bay in either size/fecundity or timing of spawning, then to detect these would require that replicate samples be taken from within multiple patches through time. An hierarchical sampling design involving measuring changes within patches and between patches through time will be necessary in future studies but, of course, a major difficulty is that the sampling involved here is destructive and given the relatively low densities of scallops that are found at Largs Bay, patches might soon become exhausted. Further work will be needed to determine better sampling designs to indirectly measure spawning synchrony on small scales.

Future sampling programmes should also consider that spawning activity is measured indirectly by sampling gonad states, and address the untested nature of these indirect methods. One obvious problem of these indirect methods is the assumption that a reduction in gonad weight represents gamete release - the loss could be resorption of genetic material. Obviously, the use of the visual estimate of gonad ripeness is a very subjective and relies on the ability of the observer to accurately assess gonad condition. To a large degree, this depends upon the experience of the assessor and on their having a good knowledge of just what "ripe" scallops look like. In this study, I am fairly confident that I quickly determined this over the first few months of sampling and pilot studies when *C.bifrons* was building up gonad condition and *C.asperrima* losing condition. Other visual estimates of ripeness involving (labour intensive) histological sectioning are also subjective in this regard and also rely on the untested ability of workers to identify the reproductive status of individuals. Long term temporal consistency could also be especially difficult to control for if samples are immediately processed, and though efforts were made to help ensure this consistency of the visual index, there was no obvious way of testing whether this was achieved in this study.

Statistical testing is frequently used to determine if mean gonad weights or GSI values of subsequent samples are different from one another, with a statistical difference interpreted as an indication that spawning has indeed occurred within the population (e.g. Parsons et al. 1992,

Bomadelli and Himmelmann 1995, Bomadelli et al. 1996) even though it is not known *a priori* how much reduction in gonad weight is associated with spawning i.e. when it is not known what a spawning will look like. This cannot, however, provide any more information than simply documenting temporal changes (and variability) in reproductive indices, which is the reason why I have not tested statistically for differences between samples. It is an inappropriate use of statistical inference to infer that the failure to detect a change also implies that a change in reproductive condition (or spawning) has not occurred - it may simply mean that one has failed to detect this. Without an expectation of what a change associated with spawning should be, non-significant tests cannot be assessed for their power to have rejected a null hypothesis of no change. Using the significance of such tests to determine what a spawning event looks like (and therefore whether one has occurred) mistakes statistical significance for biological significance. Use of these tests is also flawed (like this study) in that the sampling designs used (e.g. Parsons et al. 1992, Bomadelli et al. 1996) are not adequate for detecting small temporal scale changes and cannot control for the potential presence of small scale spatial variability in the timing or fecundity of scallops within a population.

Future sampling programmes might first develop and test an independent, predictive model of what ripe and unripe scallops look like and/or how much gonad weights change during spawning - this might involve measuring groups of scallops before and after serotonin induced spawning. Importantly, this could then be used to independently determine the reproductive status of individuals subsequently collected from the field. The accuracy and consistency of visual ripeness estimates could also be assessed and adequate planning done to ensure sampling with statistical power necessary to detect relevant biological changes in field populations on small temporal and spatial scales. However, determining what sort of change will be expected in a spawning event may be complex if spawning is not synchronous (and this should be tested, rather than simply assumed). The expectation may not simply be a uniform change in gonad weight across a population, but some complex function of gonad weight loss, male size and spawning synchrony (see below).

Fairly strong conclusions can be reached from the reproductive cycle data about the broad scale patterns of reproductive activity of both species of scallops. To a similar extent, some finer scale patterns (of multiple spawning events across the summer) for *C.bifrons* at Largs Bay were also fairly clear. However, the indirect sampling did not provide any definitive indications as to how synchronously scallops spawn on very fine spatial and temporal scales except the observation that spawning apparently does not involve one event occurring synchronously throughout the entire population and that possibly there are differences between patches within the Largs Bay population. Principally, this lack of inferential power relates to the problem that the spatial (within patches) and temporal (within days) scales which are of interest are much smaller than can easily be measured. The faults of this study appear to be common with many other studies of reproductive patterns that have tried to infer small scale patterns of spawning synchrony; work will need to be done to improve the designs of indirect sampling methods. Presently, perhaps more important inferences about the level of spawning synchrony within populations can be obtained from the measures of spawning rates made in the laboratory.

Spawning synchrony, population structure and the dynamics of fertilisation

There appeared to be little evidence of a relationship between the size of a scallop and the amount of sperm or the rate at which this was produced when induced to spawn. Though the lack of a statistically significant slope on the plots of spawning rates and sperm released against scallop size is fairly striking, the failure to detect a significant relationship is not very informative in itself - one explanation could simply be the regressions were not very powerful, given the variability observed between individuals. This is clearly reflected in the large confidence intervals calculated for the slope of the regression lines. Other studies (Levitan 1988, 1991, Levitan et al. 1992, Keesing and Babcock 1996) have also noted considerable variability in spawning measures between individuals. Of more importance are the comparisons of allometry of gonad weight with the allometry of the spawning measures; at least one significant difference occurred between the allometry of spawning rate and gonad weight for each species (average rate in *C.bifrons* and maximum rate in *C.asperrima*) and the decision to classify two other comparisons (total sperm released, *C.bifrons* and average release over the first hour, *C.asperrima*) as non-significant were

marginal (p = 0.055 and 0.073 respectively). That any of the comparisons within species were significant provides evidence to suggest that scallops do not release sperm at a rate directly proportional to their investment in gonad size. Of course, the reverse is not true - failing to detect a difference does not indicate that a difference does not occur. The weight of evidence becomes even more strong if the data from the two scallop species are combined. When the spawning measure and gonad weight data from both species were pooled as just "scallops", highly significant differences were found between the allometry of all three spawning measures and gonad weight. These are somewhat artificial comparisons, but both *Chlamys spp.* live sympatrically in similar habitats, and have relatively similar fertilisation kinetics so that when they spawn, the dynamics of the way sperm is dispersed and the effects this has on fertilisation are expected be fairly similar (although countering this, it should be noted that there are also some obvious differences between the species such as the timing of reproduction within the year and spatial distribution patterns - see also chapter 7).

If scallops do release an amount of sperm in proportion to their investment in gonad weight, then they do not appear to do this in one completely synchronous event. An alternative model of spawning behaviour can be constructed (albeit *post hoc*) that better fits the spawning patterns observed here and that also makes intuitive sense. In this model ("the spawning frequency model") all male scallops within a species spawn at a fairly fixed rate, but larger and more fecund individuals can do this more often. In this way, scallop spawning could be allometrically related to size, but it will be the length of time over which scallops spawn that is related to size, rather than instantaneous rates. The data presented here suggest that scallops release a fixed amount of sperm in a laboratory serotonin-induced spawning event, so it is expected that larger scallops will spawn more frequently (probably on the scale of days), rather than for longer within a single spawning event. This model of spawning behaviour also means that within a spawning event, (male) population size- or age-structure will have very little effect on the dynamics of fertilisation. In later chapters this simplifies the modelling of some aspects of fertilisation within a population. Multiple spawnings over a period of days to weeks is also consistent with the observed reproductive patterns of both male and female *C.bifrons* at Largs Bay that suggest gradual release of gametes rather than total release in one completely synchronous event.

If correct, the spawning frequency model would institute a novel, important role for population (particularly male) size structure in the dynamics of fertilisation. Larger individuals will have the capacity to spawn more often during a period ("window of spawning opportunity") when spawning can occur. Providing male spawning occurs at random within this time window with respect to other males (female spawning behaviour may also be important, but is discussed below), few of the small males will be spawning at any given time, whilst a greater proportion of the large males will be. Consequently, average size of individuals will affect the effective population density of spawning males.

In terms of understanding the evolutionary ecology of spawning, the spawning frequency model suggests a different mechanism of sperm competition amongst free-spawning marine invertebrates - rather than individual males trying to "outspawn" each other within single spawning events, males compete with each other through time. It suggests there may also be a conflict in terms of optimal spawning strategies of male and females. A male's success is greatest if he metes out sperm for as long a period of time as possible, yet female success should be greatest when spawning is as fast as possible (and epidemic) simply because there is the greatest amount of sperm present at this time (but too much sperm can also be a bad thing and may result in high rates of polyspermy - see chapter 2). A similar conflict occurs in the free-spawning wrasse, *Thalassoma bifasciatum*, where males that release lower amounts of sperm over multiple matings (and consequently achieve the greatest individual reproductive success) convey less reproductive success to females than less successful males who release higher amounts of sperm less often (Warner et al. 1995).

The spawning frequency model can also be argued from a consideration of how sperm is likely to disperse in field conditions (see also chapter 5) and how males might optimise their spawning rates: first it is assumed that an instantaneous index of a male's (relative) fertilisation likelihood can be measured as an integral of the area of spread of a sperm plume over the sea floor and the fertilisation success consequences of variation in sperm concentration within this plume (this depends strongly on the assumption that eggs sink - see chapters 3,5). Second, we recognise that there is a temporal aspect to the dynamics of fertilisation and accept that a male's (relative)

chance of fertilisation is the product of the instantaneous index and the length of time he spawns. Logically, the rate at which a predicted instantaneous index of sperm plume area increases with increasing sperm release rate is always less than one, essentially because released sperm dilutes into a 3-dimensional volume of water, but sperm plume area only increases as an area (square) function. We also know from the fertilisation kinetics model in chapter 2 that fertilisation likelihood always increases with increasing ambient sperm concentration at a rate less than one (though sometimes it is close to one) and at very high concentrations where polyspermy becomes increasingly likely the rate of change is negative. So, in a randomly distributed population, males do not double their fertilisation chance for a doubling of sperm release rate. This effect is also demonstrated in the field study of Levitan (1991), where he found that even though there was a significant relationship between urchin size and sperm release when injected with KCl, this did not translate into a significant effect on fertilisation levels across an experimentally spawned population. Thus, given that the rate of increased returns diminishes with extra gonad investment, why would larger scallops expend energy attaining a larger gonad size? The answer is that a male with twice as much sperm (gonad investment) can get twice the return in terms of fertilisation chance, but by measuring sperm release over time, i.e. only if he releases at a given rate for twice the length of time. In terms of paying off for investment in gonad weight, spawning for longer (or more often) would seem the optimum strategy for a large male to adopt - it retains the competitive value for each sperm produced. Of course, this argument also assumes that it is not simply much easier for large scallops to produce extra sperm, but there is evidence to suggest that isn't the case and that sperm production is energetically expensive in scallops (e.g. Vahl 1981). The observation of fairly high sperm release rates (up to 3.7×10^7 sperm sec^{-1}) and the above logical argument also suggests that "windows of opportunity" when spawning can take place will be relatively short and that some level of synchrony is to be expected - if spawning was completely at random through time, the optimal sperm release strategy would be to release as slowly as possible for as long as possible, resulting in a constant dribble of sperm from scallops !

Though the sexes of both of the *Chlamys* species are separate, hermaphroditism is widespread in the Pectinidae (Benniger and Le Pennec 1991) and this too is consistent with the spawning frequency model and some degree of asynchrony in spawning. Hermaphroditic scallops such as

Pecten fumatus do not release both type of gametes simultaneously (pers.obs.) - they are at least partially self-fertile and simultaneous release would result in high levels of self-fertilisation that may be deleterious (Beaumont and Budd 1983). Rather, in laboratory induced spawnings they consistently release sperm first, later followed by eggs (as an aside it is not known whether the time delay involved is enough to prevent selfing in field conditions). Obviously a population of hermaphrodites spawning completely synchronously would all first release sperm then all release eggs and consequently have very low fertilisation success! Instead, for hermaphroditism to work, some level of asynchrony is required.

It should be remembered that the logical integrity of the spawning frequency model in no way proves this occurs in real populations as deductive reasoning about the nature of unseen mechanisms is fraught with danger. The logic of the argument is strongly dependent upon there being a temporal component to spawning and assessing male fitness.

There is a range of reasons why spawning may occur over a relatively short time period, thus ensuring synchrony. As noted earlier, there have been some observations of highly predictable, synchronous free-spawning behaviours (e.g. Babcock et al. 1986, Hoppe and Reichert 1987, Kinzie 1993). For a start, there is an obviously large selective advantage for females to be involved in synchronous spawning, particularly at low population densities when such a strategy would minimise sperm limitation, and males also need some degree of synchrony in order to spawn when at least some eggs are present in the water. Avoidance of times when egg predation is high or spawning to coincide with favourable larval conditions such as high food availability, water temperatures and oceanographic circulation would all tend to select for spawning occurring at the same time (reviews in Olive 1992, Morgan 1995). Recently Serrao et al. (1996) found that algae on exposed rocky shores restricted their gamete release to periods of calm water, avoiding times of high water movement which is when fertilisation is likely become limited by turbulent diffusion (Denny and Shibata 1989). Clearly, logically deducing how scallops should spawn is difficult and, ideally, models of spawning patterns should be based on observations of field populations.

In the spawning frequency model, the timing of spawning by a male should be independent of other males spawning and, whilst not completely random, should be induced by some external extrinsic factor or possibly female-produced pheromones or eggs. There is some evidence to both support and reject this. Physical changes in the physical conditions that scallops experience in the laboratory such as rises in temperature (e.g. O'Connor and Heasman 1995, pers. obs), increased water flow (Desrosiers and Dube 1993) or high phytoplankton concentrations (Starr et al. 1990, pers. obs.) have all been shown to induce spawning of isolated males. Lunar period has also been shown to be important in a range of other groups (e.g. Hoppe and Reichert 1987, Levitan 1988, Brazeau and Lasker 1990, Johnson 1992). These observations all suggest male spawning can be initiated independently of other individuals. However, numerous studies (Beach et al. 1975, Coll et al. 1994, Zeeck et al. 1996) have chemically isolated pheromones released by spawning invertebrates and suggested that these might be important in cueing spawning (as opposed to being non-functional by-products). Recently, Hamel and Mercier (1996) found in laboratory conditions that the synchrony of gametogenesis amongst laboratory held holothurians, *Cucumaria frondosa*, was greatest when individuals were kept in contact with each other. Water borne spawning pheromones are likely to dilute rapidly in field conditions (cf. Moore and Atema 1991, Weissburg and Zimmer-Faust 1993, Zimmer-Faust et al. 1995), probably just as quickly as gametes (see chapter 5), and so may have limited utility as a synchrony cueing mechanism in real populations. This, however, has not been experimentally addressed. Note, however, that there are anecdotal and uncontrolled "experiments" to suggest otherwise. Pennington (1985) cites two observations where the presence of crushed urchins (which releases gametes) was associated with the initiation of spawning of other urchins. The addition of sperm to water, often in combination with physical changes such as temperature rises, is a well known aquacultural technique for inducing both males and females to spawn in the laboratory (e.g. O'Connor and Heasman 1995, pers. obs.). I often witnessed whole aquaria (males and females) "accidentally" spawning when keeping ripe scallops in laboratory conditions (notably, I also witnessed isolated spawning males and females within aquaria). However, it is not known whether spawning scallops in these aquaria were responding directly to other spawning scallops or were all simply responding in unison to changes in environmental conditions or stresses associated with the aquaria; carefully controlled experiments would be needed to determine this. Laboratory conditions are often

stressful for animals, and it might simply be this stress (or an interaction of this with other conditions) that induces spawning. Manipulative experiments and observations are needed to test whether factors found to be important in the laboratory also induce spawning in field conditions before we can assess whether these observations have bearing on models of spawning synchrony.

The spawning frequency model makes some further testable predictions that might be addressed in future studies. One important and simply tested prediction is that, within a population, larger or more fecund males will have the capacity to be induced into spawning more often over a short time period, and the increased capacity should be allometrically related to scallop size in the same way that gonad weight is. Similarly, across populations with differing peak fecundities, spawning rates and amount of sperm released per spawning should be independent of population, but the frequency of spawning should be dependent upon population fecundity. It is unfortunate that suitable data was not collected for *C.bifrons* from Edithburgh Jetty during the course of this study to allow comparison with the more fecund Largs Bay population. Another unique prediction that is made by the spawning frequency model is that (relative) variance (e.g., coefficient of variation) in measures such as gonad weights and visual ripeness should increase within this spawning window, as some individuals release gametes before others and variance will decrease again once all animals finish spawning. There should also be greater (relative) variability of indices amongst smaller or less fecund individuals than amongst larger individuals. In contrast, the synchronous spawning model predicts that (relative) within sample variance will be the same for groups of both large and small individuals and should not vary during spawning. Unfortunately these models cannot easily be distinguished from the data collected here. In future studies I would recommend that small numbers of scallops are not collected from a wide range of size classes (in this study this was done, naively, to ensure a "representative" sample from the population), but instead that scallops be collected from a limited range of divergent size classes to allow for a test of differences in fine scale reproductive patterns between them.

The data and some arguments presented in this chapter support the model that size structure may directly affect the effective population density of a spawning population and suggest that total

synchrony is unlikely, though this is awaits further testing. Unfortunately, there is no clear indication of exactly how fertilisation might be affected by size structure, except a vague prediction that populations of larger or more fecund males will spawn more synchronously. Female spawning behaviour is also likely to be critically important in that it is unknown whether they also spawn at random within limited time windows, or instead only in response to the presence of sperm from spawning males (which would increase synchrony). Given this uncertainty about how synchronously spawning occurs, models of fertilisation within scallop populations (chapters 5, 6 and 7) will also have some degree of uncertainty associated with them. Presently, the only way to visualise the fertilisation effects of variation in population size or fecundity under the spawning frequency model is to model populations as spawning synchronously, and then also to consider what happens if modelled effective spawner densities are reduced by some arbitrary amount. In any case, I now have estimates of scallop spawning rates which allows for the next step which is to measure the dispersal of sperm in various field conditions and (also using work presented in chapter 3) to assess how fertilisation success varies between pairs of individual spawners. This is done in the next chapter.

Tables 4.1.(A-D) Analyses of covariance testing for differences between species in allometric relationships.

A. Log maximum spawning rate measured as dependent variable

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	p
Log Ht	0.092	1	0.092	0.347	0.560
Species	0.145	1	0.145	0.546	0.465
Species x Log Ht	0.155	1	0.155	0.584	0.450
Error	9.017	34	0.265		

B. Log average spawning rate over the first hour as dependent variable

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	p
Log Ht	0.017	1	0.017	0.051	0.823
Species	0.021	1	0.021	0.063	0.803
Species x Log Ht	0.035	1	0.035	0.103	0.750
Error	11.441	34	0.336		

C. Log total sperm released as dependent variable

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	p
Log Ht	0.166	1	0.166	0.680	0.415
Species	0.009	1	0.009	0.038	0.847
Species x Log Ht	0.010	1	0.010	0.042	0.838
Error	8.327	34	0.245		

D. Log gonad weight as dependent variable

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	p
Log Ht	2.019	1	2.019	216.087	<0.001
Species	0.007	1	0.007	0.708	0.401
Species x Log Ht	0.010	1	0.010	1.090	0.297
Error	2.168	232	0.009		

Plate 4.1. Male *Chlamys asperrima* (45mm shell height) opened to show a white/cream gonad (visually estimated reproductive state of 2 - see text).

Plate 4.2. Female *Chlamys asperrima* (45mm shell height) opened to show a orange/peach coloured gonad (visually estimated reproductive state of 2).

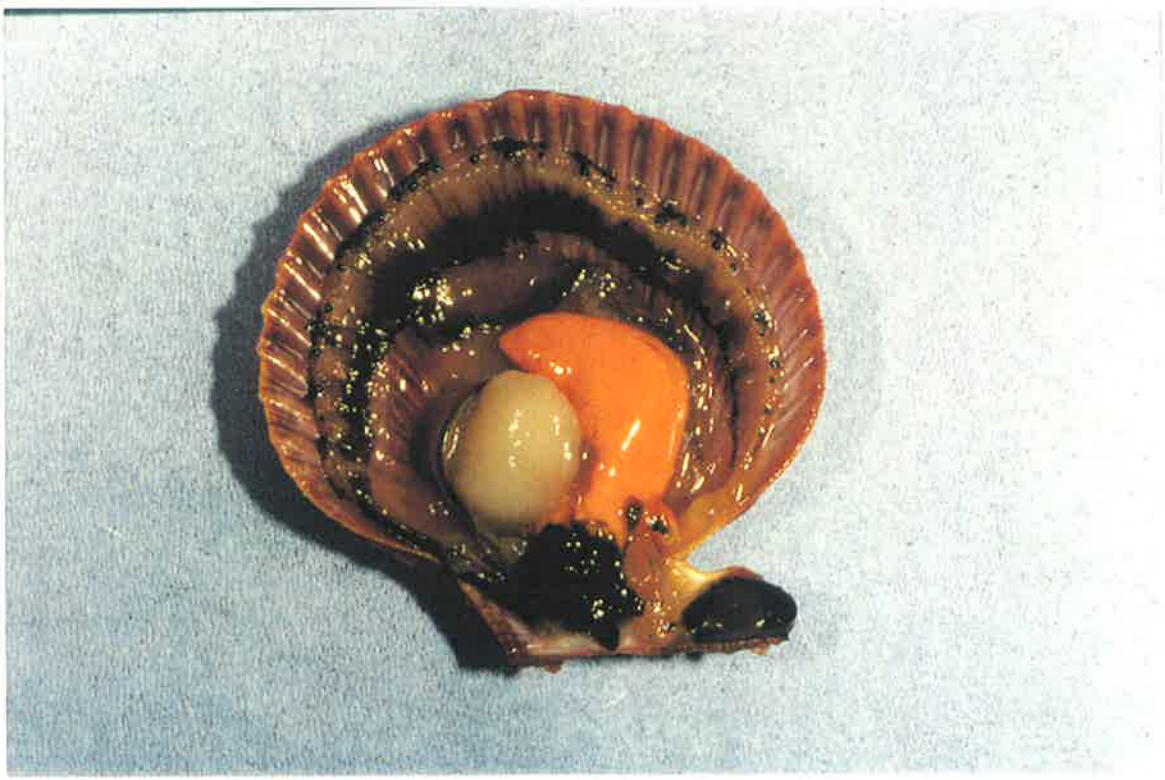


Plate 4.3. Male *Chlamys bifrons* (70mm shell height) opened to show a white/cream gonad (visually estimated reproductive state of 2).

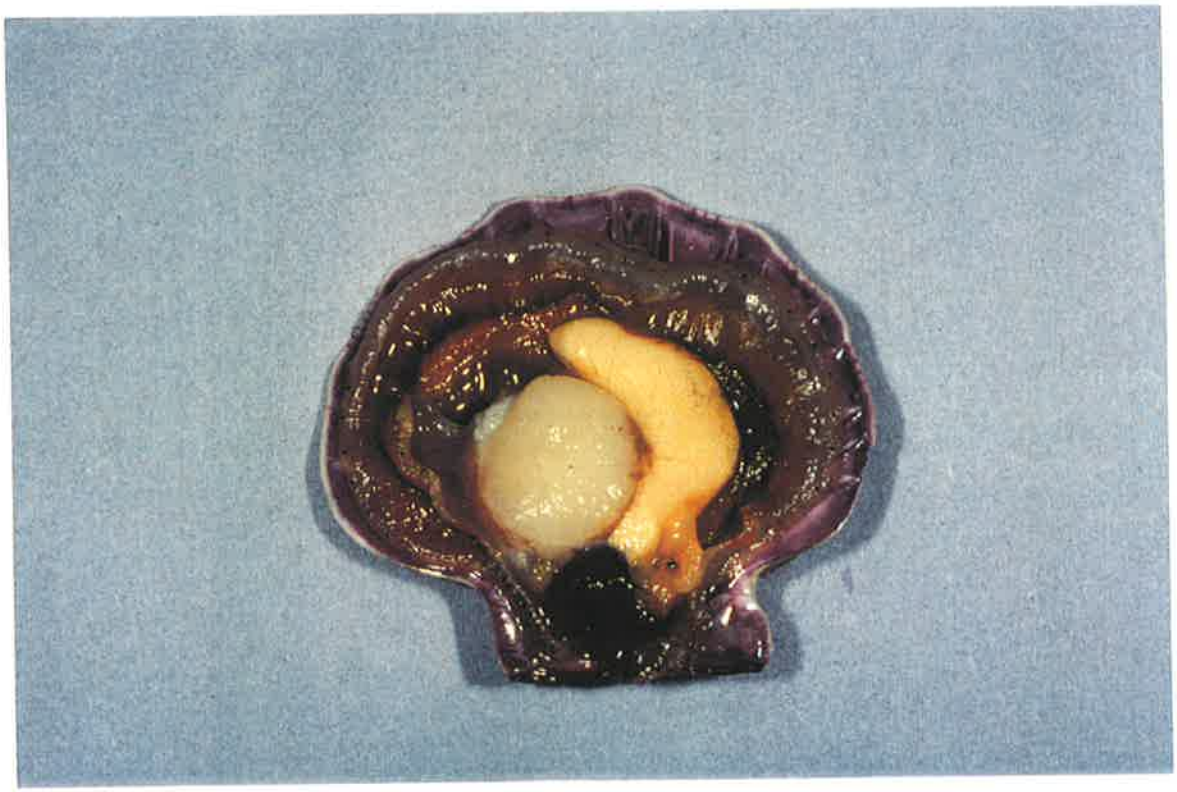


Plate 4.4. Female *Chlamys bifrons* (75mm shell height) opened to show a purple coloured gonad (visually estimated reproductive state of 2).

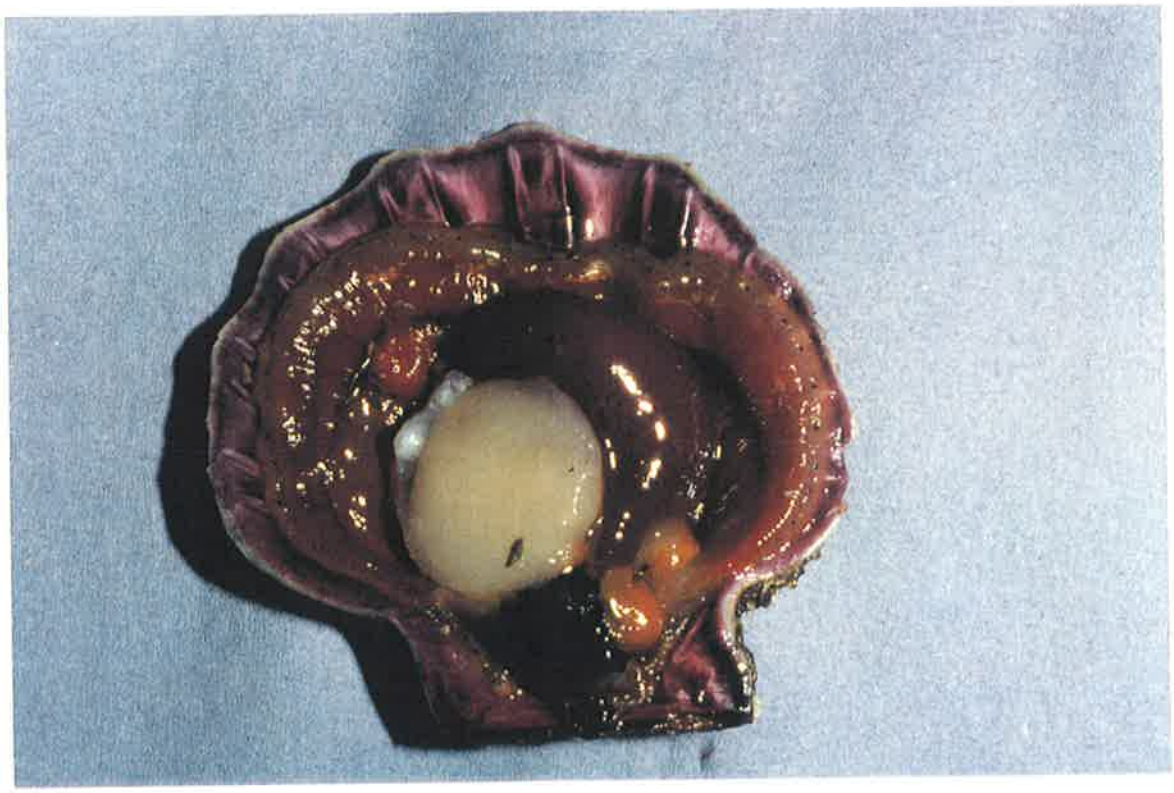


Figure 4.1 Relationship between male size and wet gonad mass for ripe (visual index = 3 or 4) scallops. A. *Chlamys bifrons* at Largs Bay; B. *C.bifrons* at Edithburgh Jetty; and C. *Chlamys asperrima* at Edithburgh Jetty. Least squares regressions are shown and 95% confidence intervals of slope estimates are reported in brackets.

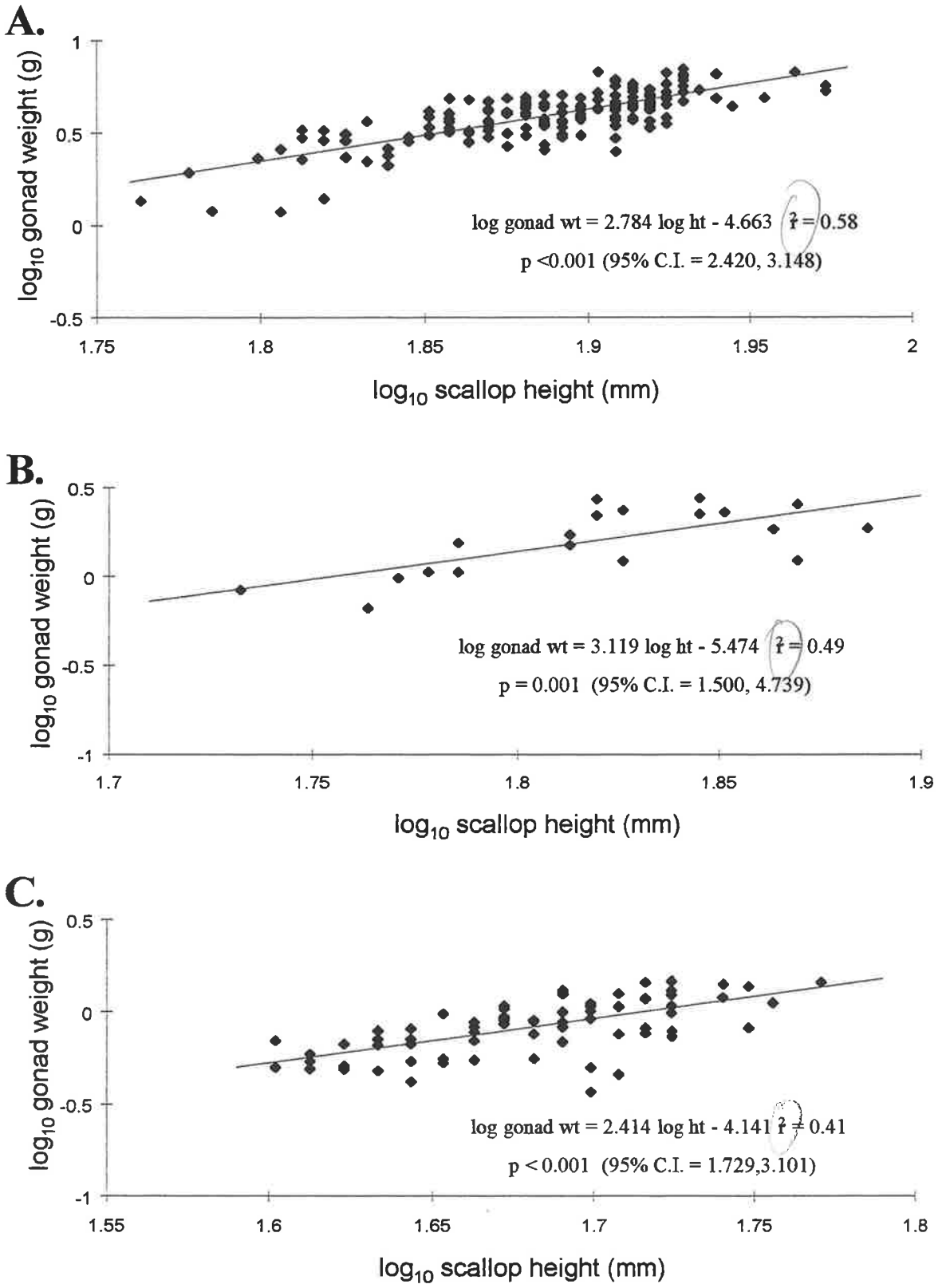


Figure 4.2 Relationship between female size and wet gonad mass for ripe (visual index = 3 or 4) scallops. A. *Chlamys bifrons* at Largs Bay; B. *C.bifrons* at Edithburgh Jetty; and C. *Chlamys asperrima* at Edithburgh Jetty. Least squares regressions are shown and 95% confidence intervals of slope estimates are reported in brackets.

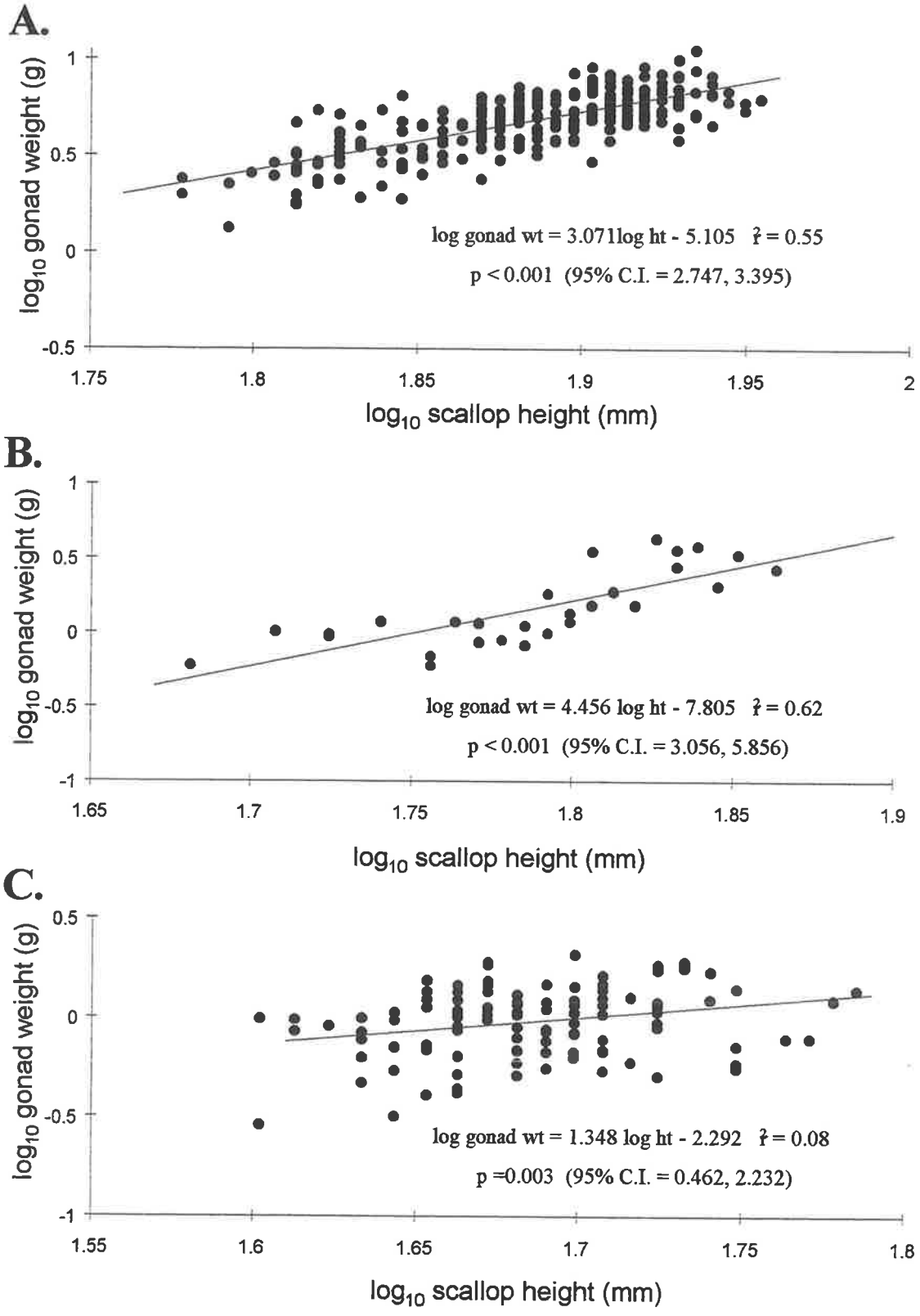
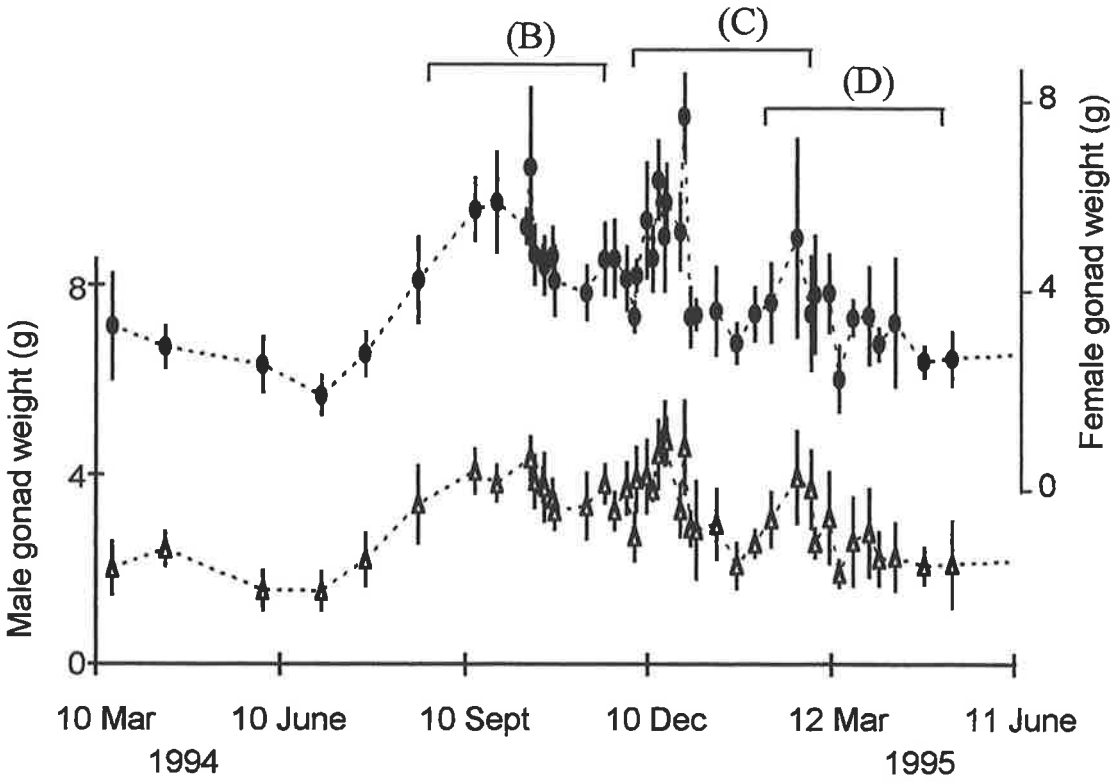


Figure 4.3.A. Reproductive cycle of *Chlamys bifrons* at Largs Bay. Standardised gonad weights (+/- St.Dev.) are shown separately for male (hollow triangles) and females (solid circles). Average sizes, to which gonad weights were standardised, were 77.66 mm (females) and 77.39 mm (males). Higher resolution patterns during the periods B-F are shown in figures 4.3.B-F.



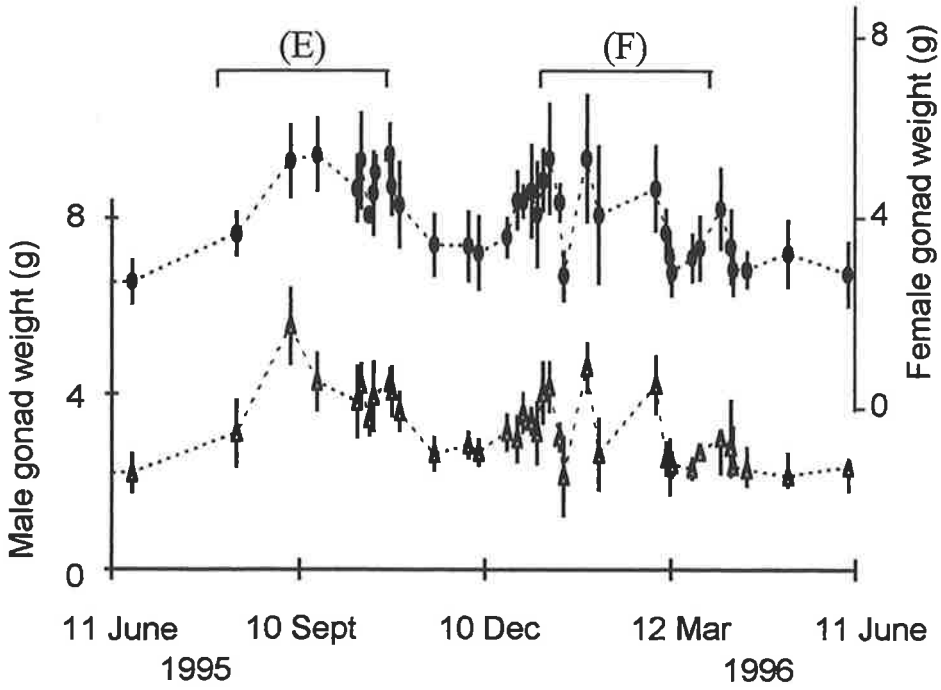


Figure 4.3.B-F. Fine scale visualisation of the reproductive cycle of *Chlamys bifrons* at Largs Bay. Mean standardised gonad weights (with 95% C.I.) are shown separately for male (hollow triangles) and females (solid circles). Each of the 5 putative spawning peaks are shown here separately - plot letters correspond to time periods outlined in figure 4.3.A.

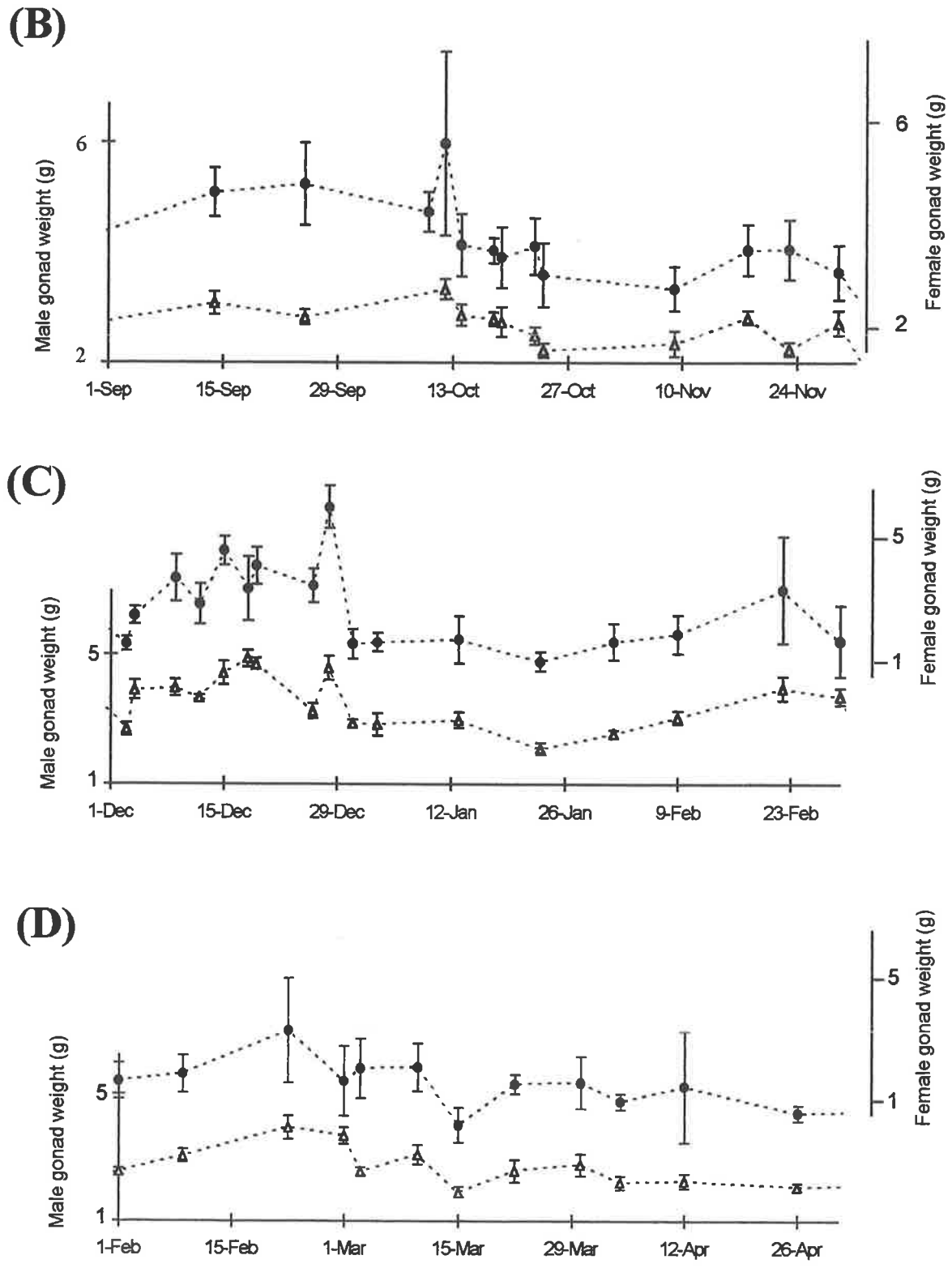


Figure 4.3.B-F continued. Fine scale visualisation of the reproductive cycle of *Chlamys bifrons* at Largs Bay. Mean standardised gonad weights (with 95% C.I.) are shown separately for male (hollow triangles) and females (solid circles). Each of the 5 putative spawning peaks are shown here separately - plot letters correspond to time periods outlined in figure 4.3.A.

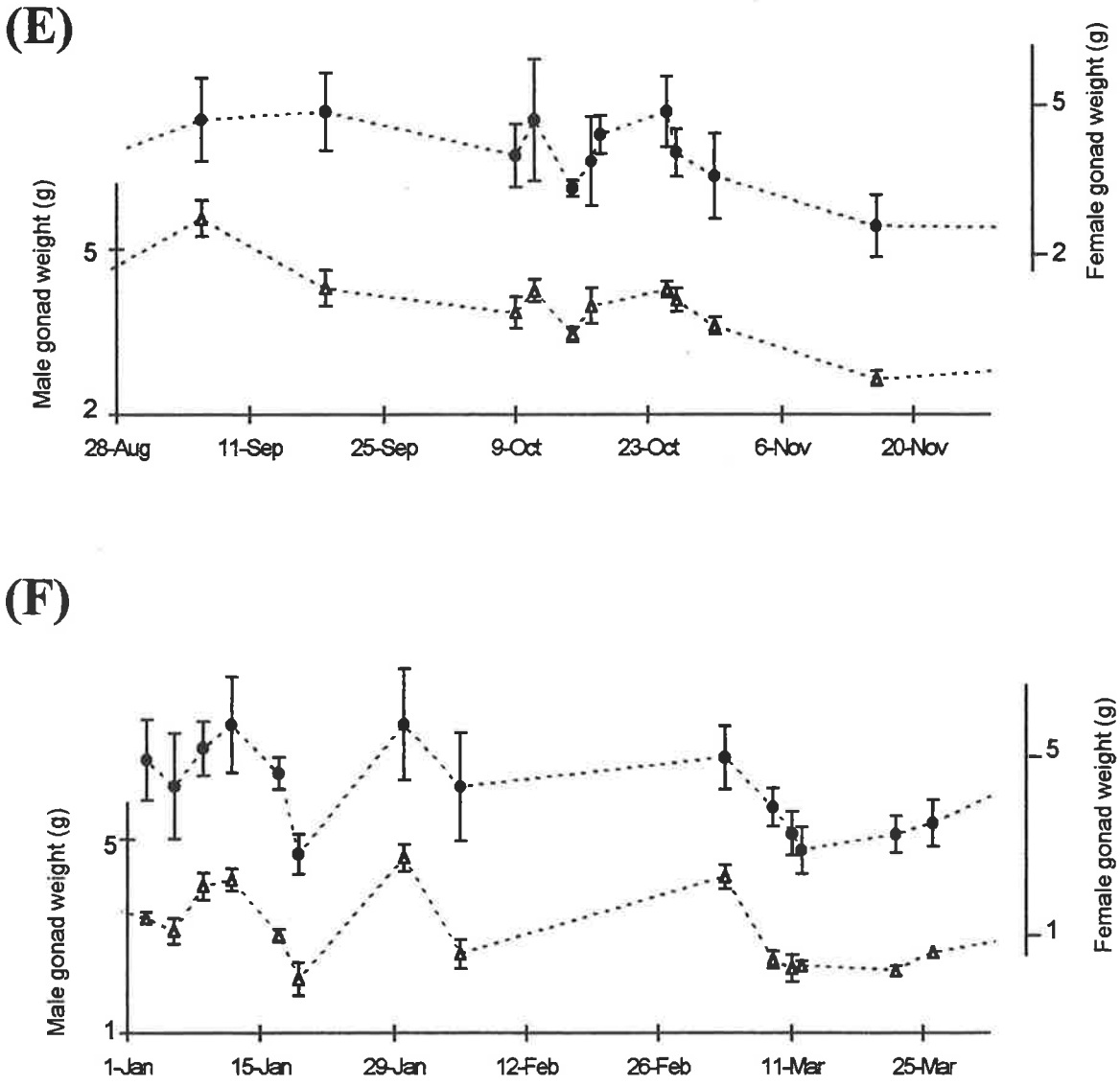
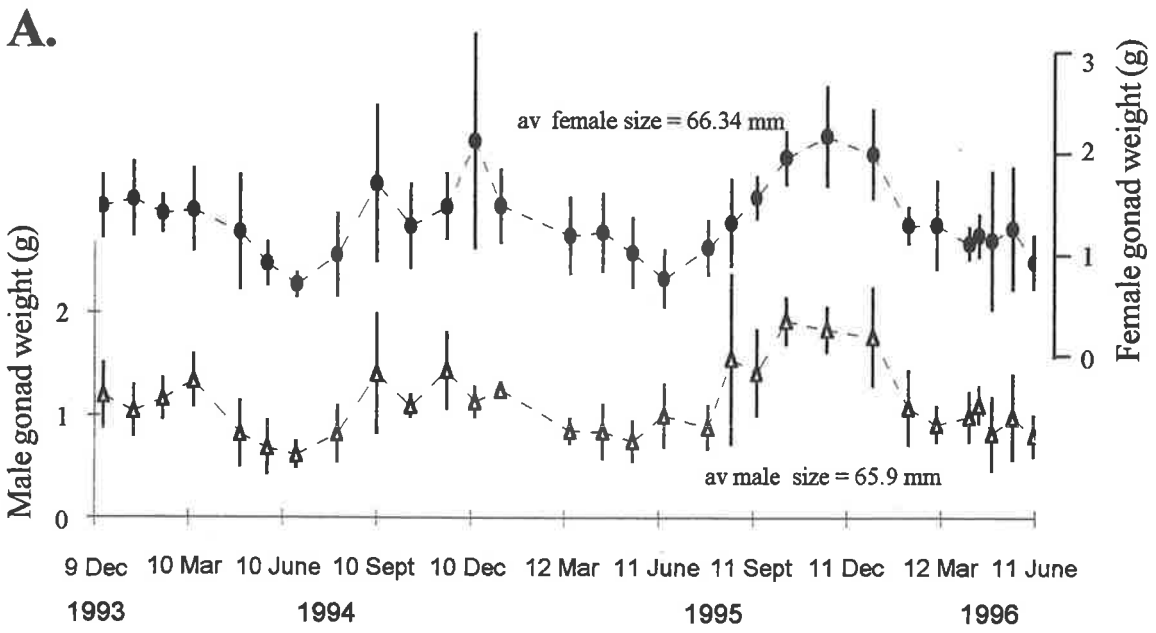


Figure 4.4. Reproductive cycles of scallops at Edithburgh Jetty. In each plot mean (+/- St.Dev.) standardised scallop gonad weights are plottd separately for males (hollow triangles) and females (solid circles).. A. *Chlamys bifrons* ; B. *Chlamys asperrima*.



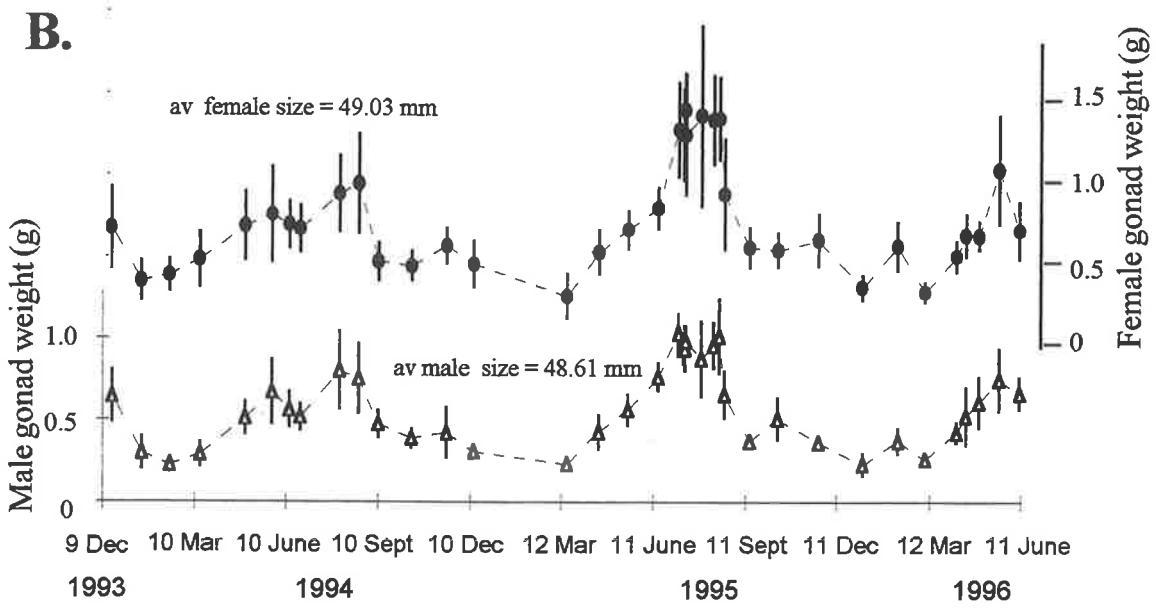


Figure 4.5. Temporal patterns of mean (+/- S.E.) visually estimated gonad ripenes. A. *C.bifrons* at Largs Bay; B. *C.bifrons* at Edithburgh Jetty; C. *C.asperrima* at Edithburgh Jetty.

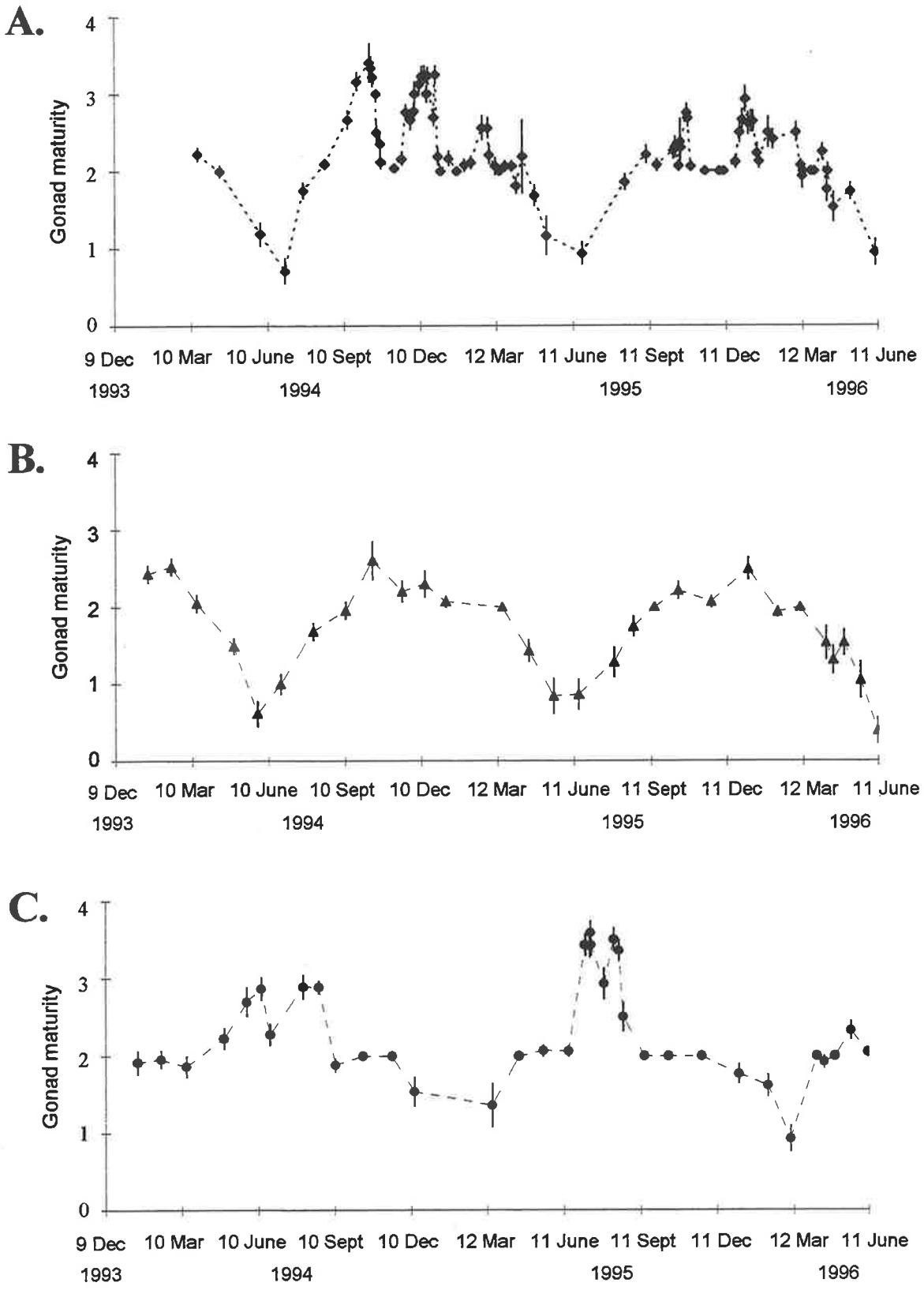


Figure 4.6. Seasonal patterns of the proportion of scallops in three classes of visually estimated gonad ripeness. The proportion of the population (combined sexes) considered "ripe" (visual index = 3 or 4) represented in black, the proportion "intermediate" (V.I.=2) in white and the proportion "resting or regressed" (V.I. = 0 or 1) in grey. (A. *Chlamys bifrons* at Largs Bay ; B. *C.bifrons* at Edithburgh Jetty ; C. *Chlamys asperrima* at Edithburgh Jetty).

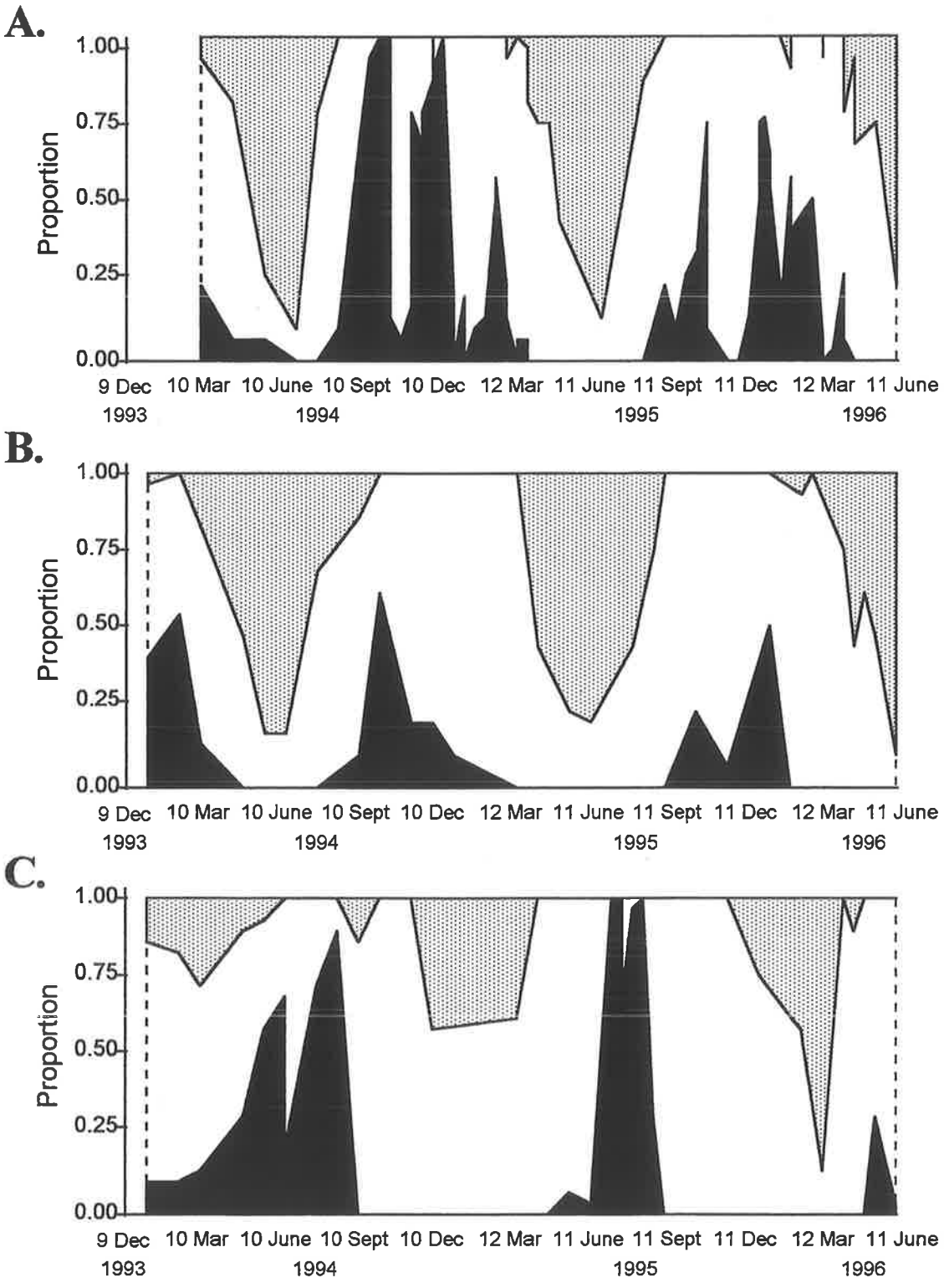
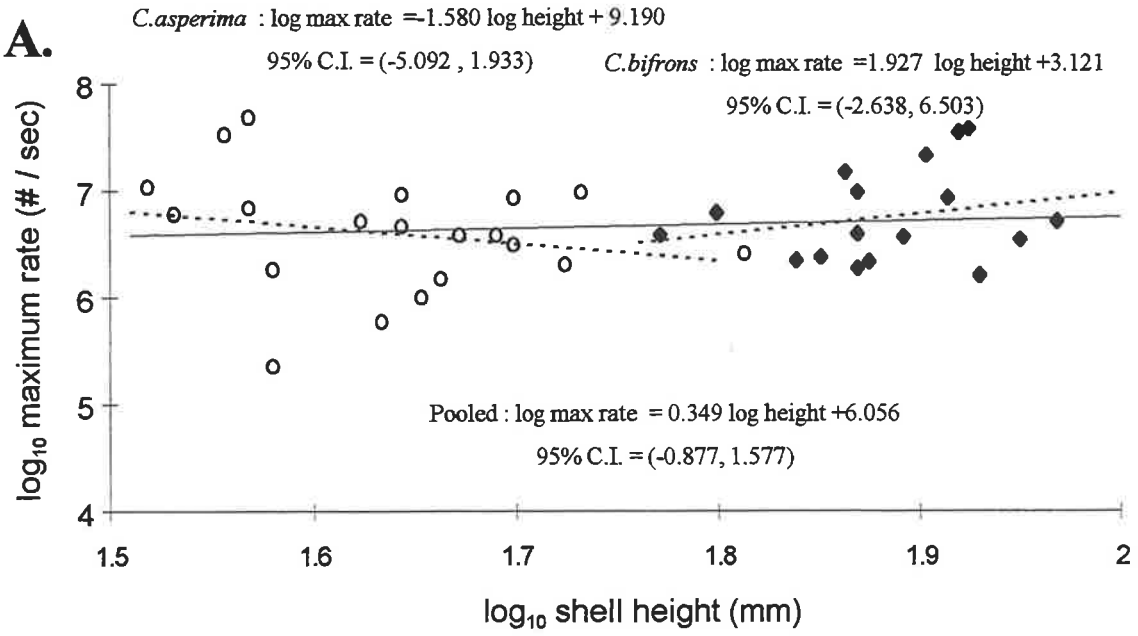


Figure 4.7. Relationship between scallop species and size and amount of sperm released during seratonin induced spawnings. Hollow circles *C.asperrima*, solid triangles *C.bifrons*. A. Maximum spawning rate. B. Average spawning rate over first hour. Solid lines are regression lines for pooled data of both species, regression lines for each species treated separately are shown as dashed lines. 95 % confidence intervals of the slope of each regression line are also shown. (Hollow circles *C.asperrima*, solid triangles *C.bifrons*.)



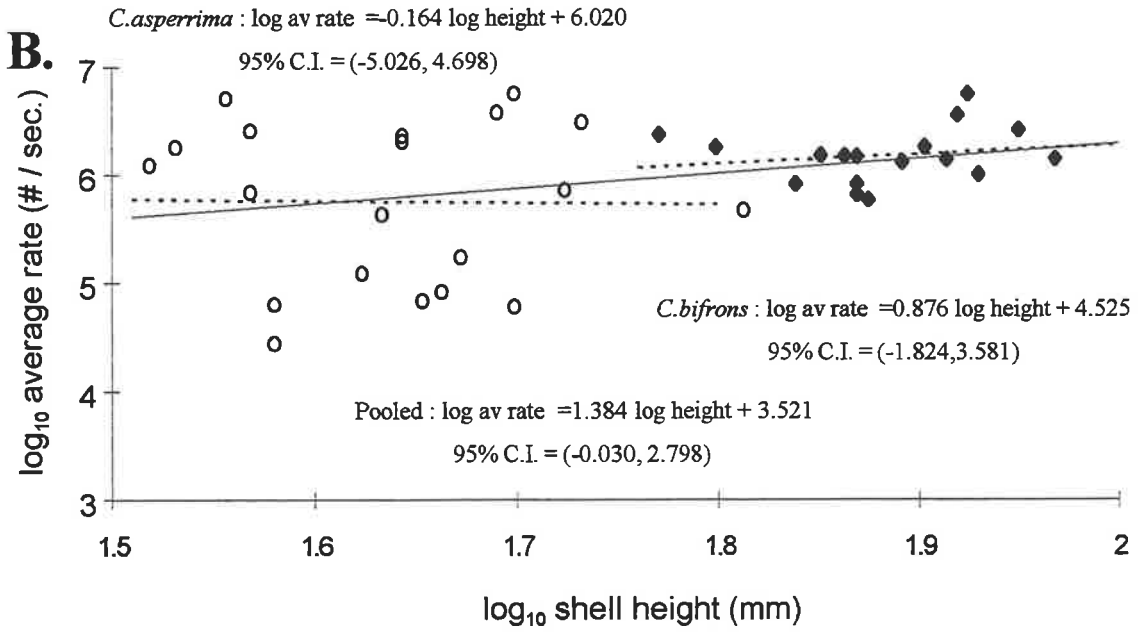
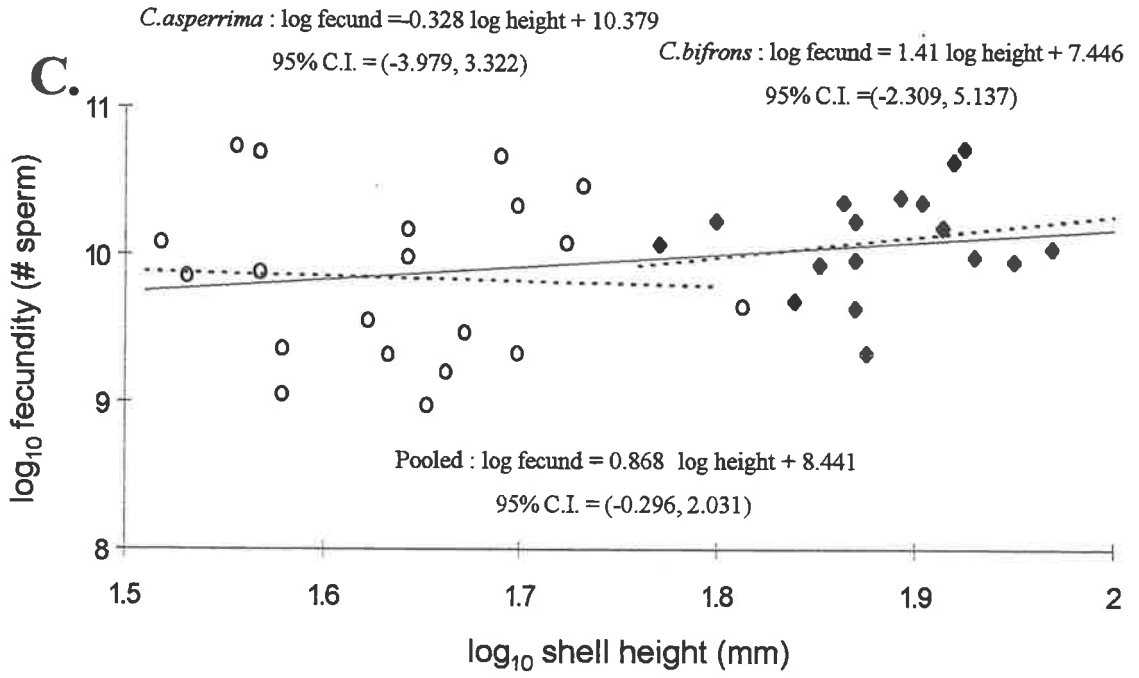
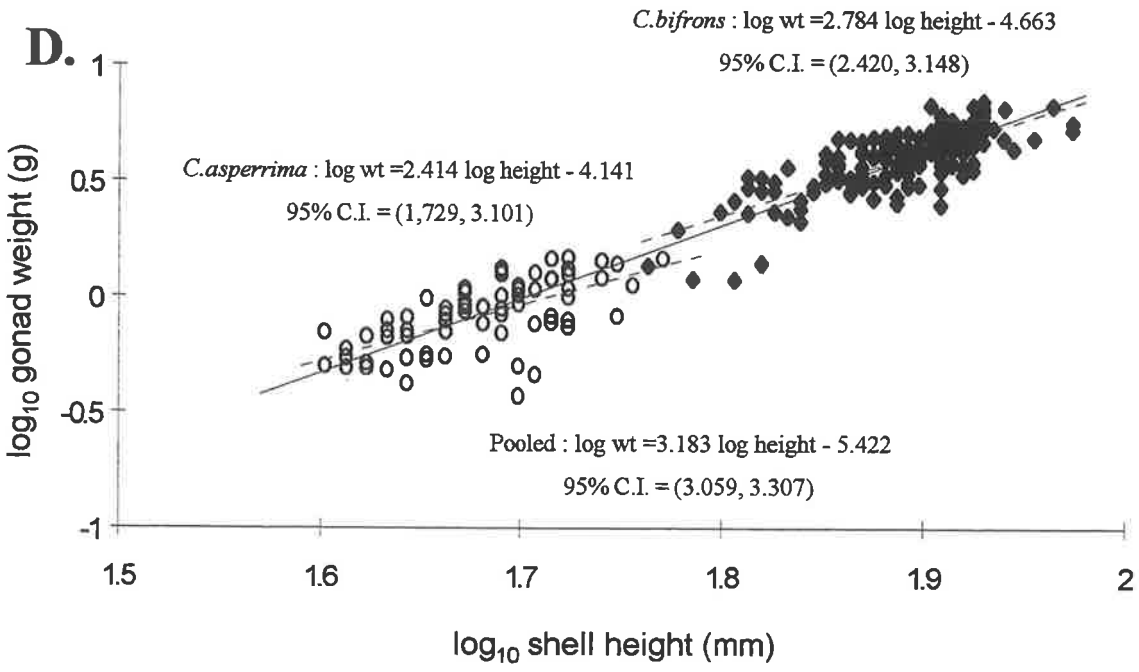


Figure 4.7. continued. C. Total amount of sperm released during induced spawnings. D. Relationship between male scallop size and species and wet gonad weight. Solid lines are regression lines for pooled data of both species, regression lines for each species treated separately are shown as dashed lines. 95 % confidence intervals of the slope of each regression line are also shown. (Hollow circles *C.asperrima*, solid triangles *C.bifrons*.)





Chapter 5

A model of external fertilisation for *Chlamys bifrons* in a range of habitats and locations

Introduction

In this chapter I develop, and calibrate with field data from Largs Bay, a model showing how fertilisation success varies as the distance between individual male and female *Chlamys bifrons* increases and how this relationship varies in a range of conditions in which scallops may spawn. I then scale up the model of fertilisation between individuals to simulate spawning within populations of randomly dispersed scallops in a range of habitat types. These models form the basis of a later examination of the potential genetic effects associated with free-spawning (chapter 6) and further simulations of fertilisation success within real *C. bifrons* populations such as the one at Largs Bay (chapter 7).

The influence of habitat and inter-spawner distance on scallop fertilisation

As discussed in chapter 1, though few models of the process of fertilisation exist for bivalves (and no specific model exists for any species of scallop), such models may provide important information necessary for conservation or management purposes of these ecologically and economically important animals (Oresanz et al. 1991, Peterson and Summerson 1992, Stokesbury and Himmelman 1993, Peterson et al. 1996). Field experimental studies in which pairs of spawners have been progressively moved apart (or towards each other) and ensuing fertilisation success measured have now been conducted for a range of free-spawning animals (Levitan 1995, see also chapter 1). The emerging pattern from these accumulating descriptions of how fertilisation varies with inter-spawner position and distance is that this relationship differs considerably among taxa and/or conditions in which experiments have been conducted (reviewed in Levitan 1995, Levitan and Peterson 1995, see also chapter 1). The first aim of this chapter then, is to describe the relationship between inter-spawner position and distance specifically for pairs of male and female *C.bifrons* and to determine if (and how) fertilisation in scallops might differ from other free-spawners.

Describing the "relationship between inter-spawner position/distance and fertilisation success" may not, however, be a simple task because where spawning takes places may have important effects on the dynamics of external fertilisation. Using hydrodynamic models and field tests of the rate of dye dilution, Denny et al. (1992) predicted that urchins living in different habitats on a wave-exposed rocky shore were likely to exhibit vastly different rates of fertilisation success: those on open exposed intertidal areas were predicted to have very low rates of fertilisation success (<1%), primarily as a result of turbulent mixing and resultant rapid dilution of gametes (sperm). In contrast, urchins (at the same densities) in surge channels were more likely to experience high fertilisation success because, although their sperm was diluted just as quickly by turbulent wave action, there was very little mixing of water between surge channels and the open ocean, so limiting the volume of water into which sperm could be diluted. Petersen (1991a) and Petersen et al. (1992) found that the fertilisation success of wrasse that free-spawned into the open water column was reduced on days of highest water movement (wind-induced wave-action) and numerical models (e.g. Denny and Shibata 1989) and field experiments (Pennington 1985. Levitan et al. 1992, Coma and Lasker 1997b) suggest that female fertilisation decreases more quickly with distance downstream of a male spawner when current flows are faster - though Levitan (1991) and Coma and Lasker (1997a) did not find a strong influence of flow speed over a range of slow flows.

In Gulf St. Vincent, *C.bifrons* are found across locations that have varying tidal-induced current strengths (see also chapter 1) and so, at least potentially, differences in flow speeds associated with these locations could have a large influence on dynamics of external fertilisation. An additional factor to consider for *C.bifrons* is that at some locations individuals can inhabit a range of distinct habitat types (e.g. silt, seagrass and sand patches), each of which might also uniquely influence near-bed water movement, so affecting gamete dispersal and, consequently, the process of fertilisation. For example, obvious differences in sediment composition between habitats (such as very fine silts on silty habitats and much larger, coarse sand particles found in sand habitats - see plates 1.1, 1.3) might be indicative of differences in hydrodynamic conditions that exist between habitats. Flume studies and field observations of numerous species of seagrasses that, like *Posidonia sp.* at Largs Bay, form meadows indicate that flow underneath

canopies (where scallops are likely to release gametes) is quite different and often reduced relative to flow over open, unvegetated areas like bare, sandy patches (e.g. Fonseca et a. 1982, Gambi et al. 1990, Nowell and Jumars 1984, Ackerman and Okubo 1993, Worcester 1995). The large rigid shells of *Pinna bicolor* (see plate 1.3) that commonly protrude from the seafloor in silty habitats might also significantly disrupt near-bed flows and consequently have effects on water movement and gamete dispersal in a way unique to silt habitats.

More specifically then, the first aim of this chapter is to determine how water flow varies across habitats or locations where scallops spawn and to determine whether any differences are likely to result in measurable, direct effects in the way gametes disperse and so in the way scallop fertilisation varies with inter-spawner distance. This is done at two locations and in a range of habitats that *C.bifrons* are likely to occupy in South Australia (see also chapters 1 and 7). How fertilisation success changes with increasing inter-spawner distance is compared when scallops are spawning in silt, seagrass and sand habitats at Largs Bay (where currents are slow) and in sand and seagrass habitats in faster currents at Edithburgh Jetty.

The main methods used in this chapter to describe how scallop fertilisation success varies with inter-spawner distance and habitat are indirect, reconstructing the dynamics of fertilisation between pairs of spawners from component processes (see also figure 1.2). Fertilisation chance at various positions away from a male releasing sperm is determined by incorporating information about how fast sperm is released; how that sperm disperses; and the relationship between ambient sperm concentration and the likelihood that an egg will become fertilised. Those processes were measured in chapters 4 and 3 respectively and the remaining component (measuring how sperm disperses in field conditions) is quantified in this chapter.

Estimating how sperm disperses in the field is also addressed in a rather indirect way, by measuring the dilution of a sperm mimic (fluorescein dye) released from a mock scallop. A more direct approach to measuring fertilisation dynamics would involve actual (naturally or induced) spawning animals releasing gametes in a series of field experiments (e.g. Pennington 1985, Levitan 1991, Levitan et al. 1992, Babcock et al. 1994, Styan 1997), conducted in each of the

habitat/location combinations of interest. Such a direct approach would most closely approximate natural spawning behaviours and conditions, hopefully minimising experimenter induced artifacts. However, because all but very small such experiments are likely to be very difficult for scallops (see chapter 1), the indirect method was employed here for practical reasons. As a broad test of the validity of the indirect (dye as a sperm mimic) method adopted, a small number of scallops were also directly induced to spawn in a limited set of field conditions (on silt at Largs Bay only) and fertilisation assayed in eggs released at varying distances away from a spawning male in the field; the results of these trials were compared with those predicted using the indirect model in the same spawning conditions. Large differences in the pattern of variation of fertilisation success with increasing inter-spawner distance would be taken as an indication that the indirect methods used were invalid.

Modelling fertilisation success in populations

Rather than just an understanding of how fertilisation between pairs of individuals varies with inter-spawner distance, an ability to predict average fertilisation success across a population may be of more use in management. This is because, although pair-spawner experiments and models of the inter-spawner distance / fertilisation success relationship can provide some indication of the likely population densities that are required for high levels of population wide fertilisation success (density can be expressed in terms average inter-spawner distances and then related to the pair-spawner experiment results e.g. Styan 1997), these predictions may not take into account the superimposition of multiple sperm plumes which might occur when more than one male spawns at the same time. Particularly at higher population densities, coincident sperm plumes might result in large areas of significantly higher ambient sperm concentration than predicted when only considering single males. Illustrating this, Levitan and co-workers (Levitan et al. 1992) found that fertilisation success in small experimental populations of urchins (arrays of up to 16 individuals) varied with population size (as well as density). Not all individuals will be eqi-distant within a population and it may not be easy to envisage the effects of this spatial variation on fertilisation success within a population.

Ideally, making predictions of fertilisation across/within populations should also be addressed with a series of (large scale) field experiments which integrate both the effects of coincident sperm plumes present in a population of spawners and other factors of interest such as the different ways sperm may disperse in various conditions. Again, however, replicated experiments at an appropriate (large, population) scale would be virtually impossible for scallops (see also chapter 1). An alternative approach to predicting fertilisation success within populations is to scale up empirically derived models of fertilisation between pairs of spawners (such as those outlined above) in a series of computer simulations of multiple individuals spawning within populations (e.g. Morris 1994, Levitan and Young 1995). The obvious advantage of this approach is that it can be easily repeated for a range of population densities and across a range of spawning conditions.

Therefore, in this chapter I also model fertilisation success in (hypothetical) populations of randomly dispersed scallops, across a range of population densities and in each of the habitats and locations noted above. Although populations of *C.bifrons* in which individuals are randomly dispersed at all spatial scales are probably quite unrealistic (see chapter 7), modelling such populations here allows me to compare scallop fertilisation success within populations across different places or conditions without the confounding effects of differential spatial dispersion patterns within habitats. Thus, any differences that are observed in these comparisons (between habitats at a given population density) will be related strictly to direct physical (flow) effects that particular habitats/locations have on the process of fertilisation at the population scale. Variability in fertilisation success related to spatial variation in density within real populations is dealt with later in chapter 7.

Methods

Given the necessity of using an indirect approach to developing a model of scallop fertilisation, two approaches could have been used to indirectly predict near-bed hydrodynamics and the dispersal of sperm in field conditions. A commonly used method (e.g. Denny and Shibata 1989, Denny et al. 1992, Young et al. 1992, Babcock et al. 1994, Benzie et al. 1994, Andre and

Lindegarth 1995, Levitan and Young 1995) is to apply a numerical model of turbulent near-bed flow (e.g. Denny 1988, or Babcock et al.'s 1994 modification of this) to predict average steady state concentrations within a sperm plume. A disadvantage of this approach is that it requires parameterisation of diffusion constants in field conditions, which can be problematic - for example, Denny and Shibata (1989) possibly overestimated fertilisation of urchins on rocky shores with their use of inaccurate diffusion constants (Levitan 1995). It should, however, be noted that the numerical model has shown considerable success in some studies where its predictions have been tested with field experiments (e.g. Babcock et al. 1994, Benzie et al. 1994, Levitan and Young 1995). Nonetheless, the Denny (1988) model was designed for turbulent flow over relatively flat habitats. It was not really designed to predict dispersal in complex habitats such as underneath the canopy of a seagrass meadows or around *Pinna* shells. In any case, estimating diffusion constants within these sorts of habitats would be especially difficult. Instead, the method used in this study was to release a sperm mimic (fluorescein dye) in the field and directly measure both quantitatively and qualitatively (visually) how it disperses. In this way the effects of heterogeneities in flow caused by features such as seagrass blades, large *Pinna bicolor* shells and other bed irregularities (that are real and potentially important features of the environments in which scallops live and spawn) can be accounted for directly.

Dye release from a mock scallop

In order to estimate sperm dispersal in field conditions, dye was released at a constant rate from a mock scallop (see plate 5.1). This mock scallop consisted of a pair of plaster filled scallop valves connected by two metres of thin plastic tubing (internal bore = 2 mm) to a 2 L plastic blood transfusion bag filled with fluorescein dye (concentration : 30 g L^{-1} seawater). The open end of the tube from the bag and release point for dye was fixed in the exhalant position of a real scallop (approximately 1.5 cm above the substratum). Along a tape measure laid-out in line with the prevailing current, the mock scallop was placed at a zero position with the fluorescein bag positioned as far away from this (to the side and upstream) as possible to minimise any effects it may have on water flow. During a "spawning", dye was released from the bag at a constant rate (~120 ml min^{-1}) - the dye concentrations and rate of dye release were determined after a

preliminary experiment on silt habitat at Largs Bay and set so that enough dye would be present 4 m downstream of the release point to be detected spectrophotometrically (see below). Flow of dye out of the bag was created by placing 2 x three-pound diver's lead weights on a slate on top of the bag (which caused a constant, positive force on the bag) and unstoppering the connecting tube. Dye was released from the bag for 10 minutes to allow a steady-state dye plume to form (preliminary observations were that this was more than sufficient time for this to develop, even in slow flows). The exact rate of dye release during runs was quantified by measuring the volume of dye filled into the reservoir bag and that remaining after a timed run. After the 10 minutes of dye flow, samples of water/fluorescein were collected into 10 ml syringes, ~2 cm (scallop exhalant position height) above the substratum. Working progressively upstream, and carefully from above so as to minimally disturb the dye plume, 3 replicate samples of the dye plume (near the tape measure and collected over about 30 seconds at each point) were taken at 400, 200, 100, 50, 20, 0 cm downstream of the release point and 20 cm and 100 cm upstream. A series of 3 samples were also taken at least 800 cm upstream to allow measurement of the absorbance of dye free sea water. Plumes formed fairly distinct boundaries where dye concentration dramatically decreased (see plate 5.1), and by marking the edges of the plume with small stakes as water samples were taken, a visual estimate of plume width was made at each sampling position. Dye flow was stopped after all sampling had occurred. Mean flow speed 1 m above the substratum was measured on each occasion by timing a small, discrete amount (a "blob") of fluorescein dye across 1 m (repeated 4 times on each occasion).

Dye was released and plume characteristics measured in three different habitats (silt, seagrass and sand) at Largs Bay during summer 1995/96. Only low currents speeds (2 - 8.14 cm sec^{-1}) were experienced here, such low maximal current flow speeds are characteristic of this location (pers. obs.). Four replicate plumes were measured in each habitat, each conducted in the middle of a unique, haphazardly chosen patch, with the order of trials haphazardly determined. Depth at this location was between 4 and 6 m. In March 1996 dye release was repeated at a second location (Edithburgh Jetty), which experiences faster maximal current speeds. Here, dye was released during times of highest tidal flow (9-14 cm sec^{-1}), about one hour into the flooding tide. At Edithburgh only seagrass and sand habitats are found, and again four replicate plumes were

measured in both habitat types, each time in a unique haphazardly chosen patch. Depth at this location was between 3 m and 5 m.

Modelling sperm dispersal upstream and downstream

Absolute concentrations of fluorescein in water samples were measured in the laboratory using a spectrophotometer set at an absorbance of 490 nm. These values were then expressed as a concentration relative to the rate of dye being released from the mock scallop when the sample was taken. Treating each replicate plume separately, averages of the 3 replicate (log_{10} transformed) proportions of dye release rate at each sampling point up- and downstream were calculated to produce a curve describing how dye concentration changed along the X-axis (parallel to current flow, in line with the scallop) in each plume in each habitat and location.

To model how sperm would have diluted if it had been released instead of the dye, I substituted a range of (log) sperm release rates for (log) dye release rate. Thus, sperm concentration at any point is proportional to dye concentration with the same proportionality constant as the relationship of sperm release rate to dye release rate (see also figure 5.4). Three male sperm release rates were modelled (substituted) in acknowledgment of the variability of laboratory spawning rates measured in chapter 4 and uncertainty of how fast sperm would be released in the field. Estimates of *C.bifrons* sperm release rates that were used were 1.5×10^6, 5.4×10^6 and 3.7×10^7 sperm sec^{-1} and were, respectively, the average rates after serotonin induction measured over the first hour of spawning, the average of maximum spawning rates for individual males, and the absolute maximum rate at which a male had been recorded spawning (see chapter 4).

Here, an assumption has been made that sperm dispersal would be accurately mimicked by the dispersal of passive particles such as fluorescein dye. Thomas (1994a, 1994b) showed that this assumption is plainly not true in some cases and that, particularly at low currents speeds, gametes of some marine invertebrates disperse in viscous strings or clumps rather than as plumes of passively dispersing free particles. However, my observations of sperm of both species of scallop in the laboratory and in the field (in low flows) suggest that scallop sperm is not very viscous and

does appear to disperse much like fluorescein. Notably, sperm solutions in still conditions in the laboratory did not "settle out" even after long time periods (2 + hrs), suggesting that even small convection currents present in test-tube and flasks were sufficient to maintain a homogenous mix of sperm within beakers.

The distances up and downstream that modelled sperm concentration exceeded various critical ambient sperm concentrations, corresponding to particular fertilisation chances, were calculated from plots of sperm concentration with increasing distance form the sperm source (each plume within a habitat was treated separately as an independent replicate). The particular (but arbitrarily chosen) fertilisation chances examined were F_1, F_{10} and F_{50}, which corresponded to 1%, 10% and 50% likelihood that eggs will be fertilised (see chapter 3). As noted in chapter 3, Mead and Denny (1995) found that increasing turbulence/higher shear velocities can increase rates of sperm/egg contact (and so increase fertilisation likelihood at a given sperm concentration) and also that, when high enough, turbulence in itself can reduce fertilisation likelihood by directly disrupting the process of sperm/egg binding. However, I considered that the relatively low flow speeds at both locations would be adequately mimicked by the still, non-turbulent conditions present in test-tubes in the laboratory.

Interpolation of sperm concentration at any point along the x-axis in this plot was made by making the assumption that (log) sperm concentration changed in linearly between two adjacent sampling points where dye concentration had been measured. Note that here a sperm half-life was not included in the fertilisation kinetics model, as it was assumed that eggs would sit where released and continually sample fresh sperm as it was released and transported away from the male (see chapter 3). This should conservatively lead to an overestimation of fertilisation at all but very high sperm concentrations.

Directly measuring fertilisation success in the field - using real scallops !

Field trials in which real *C.bifrons* males were induced to spawn and release sperm, and female fertilisation success assayed at varying positions downstream used, conducted on silt habitat patches at Largs Bay in January/February 1995 and January-March 1996. Ripe female scallops (maintained in condition in the laboratory) were induced to spawn by injecting 0.5 ml of serotonin solution into the gonad and the adductor muscle as described in chapter 3. Once spawned, 1 ml samples of concentrated egg solution (approximately 2000 eggs) were loaded into otherwise unfilled plastic 12 ml syringes. Trials were conducted as quickly as possible after release of eggs and though there were inevitable delays in the setting up of experiments, eggs were kept in cooled foam containers and used within one hour of spawning. Once eggs were available, a single (ripe) spawning male (serotonin induced to spawn and transported underwater in a sealable plastic bag) was placed out in the field adjacent to a tape measure laid in line with the prevailing current direction. The male was left to spawn for 10 minutes and was periodically checked from above for continued spawning during this time (and carefully ensuring minimal sperm plume disturbance). Then, working upstream, water samples were drawn into the egg-filled syringes at 400, 200, 100, 50, 20 cm downstream, right next to the spawning male, and 20 and 100 cm upstream of it. Two forms of controls were necessary in these trials. The first was a pair of (procedural) controls for errant fertilisations caused by sperm contamination or treatment induced artifacts associated with collecting and keeping eggs in syringes. One egg-filled syringe was filled with (sperm-free) water sampled at least 10 m upstream of the spawning male and a second syringe had no water added to it at all but was carried underwater with the others. The second set of controls were designed to test that gametes used in trials were viable, and so ensure that any non-fertilisation of eggs used in field syringes was due to sperm concentration effects alone. For these, concentrated sperm solution was recovered from the sealable plastic bag used to transport the spawning male and a series of three 10-fold dilutions made from this. Egg-filed syringes sampled water from the bag, from the dilutions and water from a sperm-free water container. Eggs in all syringes were incubated for three hours, then fixed in formalin. Later, a sample of 200 from each syringe were scored with a phase-contrast microscope at 200X as fertilised (showing normal cleavage lines, cell division) or non-fertilised (showing abnormal or no cellular divisions).

Numerous (8) attempts to run the field fertilisation success experiment using real scallops were unsuccessful because serotonin induced scallops failed to produce viable eggs and sperm simultaneously. This was despite injecting multiple (10-15) individuals with serotonin on each attempt. Unfortunately, only this number of individuals could be used each time because a relatively small laboratory maintained collection of scallops was used as a source of experimental animals - this ensured that trials using fully ripe animals (originally from Largs Bay) could be conducted for some time after the brief spawning peaks each season and when water conditions were suitable for underwater work. The main difficulty was that, though some females eventually released eggs on most occasions, the time taken to release eggs after serotonin injection was extremely variable - ranging between 15 minutes and 5 hours. Similarly, although males released gametes more quickly after injections than females, again the time interval before gamete release was variable - from 20 minutes to 3 hours - and often took longer than 1 hour. Consequently, getting males and females to spawn at appropriate times so as to ensure fresh gametes (i.e. eggs less than 1 hr old) and actively spawning males for use in trials took considerable judgement (and luck)! Nonetheless, despite the logistical nightmare, this trial was successfully repeated three times in 1995 and four times in 1996, each time in a unique haphazardly chosen silt patch. For analysis, data from both years was pooled.

A two way ANOVA (fixed factors - distance from male and method of predicting fertilisation success) was used to compare results from trials involving real scallops with predictions of fertilisation (made at similar distances away from a male) made using the indirect dye substitution method as above. Four (indirect) estimates of fertilisation success were made at each sampling point using each of the 4 independent dye plumes measured on silt to predict how sperm dispersed away for the male. Here sperm was modelled being released at the rate I considered most likely (namely, the average maximum *C.bifrons* rate = 5.4×10^6 sperm sec^{-1} - see chapter 4) and I assumed an half-life in the model of fertilisation kinetics (as described in chapter 3) because eggs in the syringes will be exposed only to expiring sperm.

Simulating fertilisation within randomly dispersed populations

Visual Basic™ macros for Microsoft Excel™ were written to simulate a small population of spawning *C.bifrons* in a range of habitats and calculate individual female success within populations. In the simulation model, a single female scallop was placed in the centre of a 20 x 20 m patch - she released her eggs at this point (and they remained there) and each female released one unit of eggs. Also placed in the patch were a number of male spawners - the number of these varied according to the population density simulated (which ranged from 0.01 to 40 scallops m^{-2}) and their exact position within the patch was determined by two independent random numbers. Each of these males released sperm and produced a steady state plume around himself - the dimensions of these plumes varied according to which habitat spawning was taking place in and the modelled sperm release rate and were based on empirical measurements of dye dispersal in the above field experiments.

For each habitat/location (silt, sand and seagrass at Largs Bay, sand and seagrass at Edithburgh Jetty), I calculated what I considered an "average" dye plume - the dimensions of these were based on average dye concentration downstream at each sampling point and average plume width across 4 replicate dye plumes measured in the field in each habitat/location. Note that inside seagrass canopies visual estimates of plume widths were not made because it was not possible to see clearly what was happening at the lower levels in the canopy without moving seagrass aside and disrupting the plume. So in seagrass habitats, I assumed that the rate of dye diffusion perpendicular to the current was the same as the rate of upstream diffusion. Thus, at the point where dye concentration was measured highest, the rate of change of dye concentration with distance sideways was set at the rate of change of concentration upstream. In the case of no net bulk flow movement of dye (ie only simple diffusion acting), this assumption leads to the reasonable model of a equal rates of decrease in concentration in all directions around the release point (i.e. diffusion from a point source) and circular plumes. Examination of the one-dimensional dye dispersal curves (see later) suggest that little bulk flow occurred in any single direction in the seagrass habitats and that this assumption was probably quite reasonable.

For modelling purposes, it was assumed that the dye (sperm) plumes around the releasing male at the level just above the sea bed (where eggs would be released) could be considered as a composite of increasingly large, overlapping elliptical areas where specific ambient sperm concentrations occurred (see figure 5.1). Such elliptical shapes for areas of equal concentration within sperm plumes are predicted by an idealised hydrodynamic model (Denny and Shibata 1989) and are illustrated graphically in Babcock et al. (1994). This also seemed to be a reasonable approximation of what I saw in the field (see plate 5.1). Elliptical areas were determined using a combination of the longitudinal dye concentration/dispersal measures and visual estimates made of the width of dye plumes in the field (this is illustrated in figure 5.1): the solid line in the top part of figure 5.1 represents the curve of average sperm concentration against distance. Upstream and downstream points along the X-axis in this "average" plume were first determined (where sperm concentration dropped below chosen critical sperm concentration sperm was substituted for dye as above). These points defined the axis of the ellipse parallel to flow direction. The second axis of the ellipse (perpendicular to flow) was positioned half-way between these points and was assumed to be as long as the visual estimate of width of the dye plume at that point (see bottom part of figure 5.1).

Decomposing the (average) sperm plumes released by males in these simulations into a series of ellipses where increasing ambient sperm concentrations occurred (see figure 5.1 and below) made it relatively easy to determine how much sperm each randomly positioned male contributed to ambient concentration at the central (egg release) point, because of a key geometric property of ellipses, viz. that the distance from one focus of the ellipse to a point on the perimeter and back to the other focus is equal to the length of the long axis of the ellipse (this defines the shape of an ellipse). Thus, if the Cartesian positions of the foci of an ellipse are known (these were determined from the lengths of the long and short ellipse axis, and the position of the male within the patch) and the length of its long axis is also known, then this information can be used to determine if a point (the central position with eggs) is inside or outside the plume (ellipse): if the central eggs lie within a plume then the distance from one focus to the central (eggs) point and on to the other focus will be shorter than the length of the long axis of the plume. For each male, this property was sequentially tested for ellipses of increasing sperm concentration (and decreasing ellipse

size) and the sperm concentration contributed by a male was equal to the highest concentration found within an ellipse for which this test held. Obviously, the resolution in this model in terms of the amount of sperm contributed to the central point depends largely on the number of ellipses the male's plume is decomposed into: if very few ellipses are used, then the resolution is low. If, on the other hand, the plume is decomposed into very many ellipses (that all differ slightly in concentration) then resolution will be much higher and smaller differences between males' contributions can be modelled. Of course, there was a large trade-off between resolution and computational time requirements, so I decomposed the average plumes into approximately 10-15 (depending on the plume) ellipses ranging from 0.001 to 150 sperm μl^{-1}, including 2-3 ellipses within each order of magnitude of sperm concentration in this range. This enabled reasonable resolution, whilst constraining of repeated runs of high population density simulations to reasonable computational times on the 166 Mhz computers available (<48 hrs for a run!).

Having summed the total amount of sperm present where the eggs were released (from all males in the patch), the fertilisation kinetics model developed in chapter 3 was used to determine the fertilisation success of eggs bathed in that ambient sperm concentration. Fertilisation kinetics parameters used were as determined in chapter 3 and noted above: β_0 = 0.00218, F_e = 0.00879, t_b = 160.9 and an egg/sperm contact time of 3600 secs. This whole process was then repeated 1000 times, each time simulating a new female and a new group of randomly positioned males within the patch. In this way, the process mimicked sampling 1000 randomly chosen females within a very large randomly distributed population of males and females, allowing determination of the average success across the population wide (the distribution of female fertilisation success amongst individuals is dealt with in the next chapter). This whole simulation process (1000 runs) was repeated for a range of population densities (0.01 to 40 scallops m^{-2}) and all of this repeated in each of the habitat/locations for which there were empirical dye dispersal models (silt, seagrass, sand habitats at Largs Bay and seagrass and sand at Edithburgh Jetty) for males releasing sperm at rate of 5.4×10^6 sperm sec^{-1} (the average maximum rate from chapter 4). In addition, a limited investigation of variation in sperm release rates was done by running simulations across a range of population densities with males modelled spawning at the rates of 1.5×10^6 and 3.7×10^7 sperm sec^{-1}, but only on silt habitat at Largs Bay.

Results

Dye / sperm plumes around males

ANOVA of dye concentrations in the field experiments clearly indicated not only that there were detectable differences between habitats in the way in which dye concentration changed with increasing distance from the mock male, but also that there were detectable (significant at p = 0.001) differences in this relationship between replicate plumes within habitats. This was reflected in significant habitat x distance and distance x plume{habitat} interaction terms in the ANOVA in table 5.1.A, respectively.

Figure 5.2 illustrates the large differences in the pattern of dye concentration between trials in seagrass meadows and those in open habitats (on silt, sand): at both locations higher concentrations of dye (averaged across plumes) were sampled further downstream in the open habitats (silt, sand) than in the seagrass, whilst there was substantial dye concentrations recorded upstream in the seagrass habitats, but not in the open habitats. This pattern was also immediately quite obvious when conducting the trials in the field - generally, dye formed a distinct plume spreading out downstream in the open habitats (see plate 5.1), but within the seagrass canopy dye appeared to diffuse out fairly equally in all directions.

Considering just seagrass habitats at the two sites, significant ANOVA interaction terms (distance x habitat and distance x plume{habitat} - see table 5.1.B) again indicated that differences in the way dye dispersed in seagrass habitats occurred between locations and between individual plumes within these. Movement of dye within the seagrass canopy is complex and bulk flow is not always simply in the same direction as midstream currents - in several individual plumes in seagrass at Largs Bay, concentrations of dye actually decreased much more slowly upstream of the scallop than downstream. This is also reflected in figure 5.2 which shows that (on average) at the slow flow location (Largs Bay) there appeared to be greater amounts of upstream movement of dye than at Edithburgh Jetty. At both locations some dye dispersed upwards to the top of the

seagrass canopy, where it met faster (and more turbulent) flows and was moved away. In the faster currents at Edithburgh Jetty, there seemed to be greater upwards diffusion of dye and then faster currents moved this dye away quite quickly, forming a plume in a similar shape to that on open habitats, but at the level of the top of the seagrass canopy. Possibly, there was also diffusion from this plume back downwards into the seagrass canopy.

Within open habitats, ANOVA interaction terms (distance x habitat and distance x plume{habitat}) were again significant - see table 5.1.C. Figure 5.2 clearly shows dye concentrations (averaged across plumes) decreased much more quickly upstream in sand habitat in the faster currents at Edithburgh Jetty than in the slower currents (sand, silt) at Largs Bay. There was essentially no upstream dispersal of dye on sand in the faster currents at Edithburgh Jetty - this could easily be seen when trials were being run. Another obvious, large difference that occurred between plumes in open habitats was in the width of the plumes that formed. Figure 5.3 shows that the visual estimates of plume width were much greater on both sand and silt in the slow flows at Largs Bay than on sand in the faster flows at Edithburgh Jetty. An additional qualitative difference between plumes was that in some trials on silt habitat, there seemed to be pooling of dye around the point of release. In particular, there often appeared to be an accumulation in seabed depressions and eddies in the lee of large *Pinna bicolor* shells. This occasional pooling manifests in the slower initial drop in concentration found at 20 cm downstream in the silt trials than was found in the two sand trials, shown in figure 5.2.C. Such pooling of dye did not seem to occur in flat sand habitats at either of the sites.

Figure 5.4 shows how the dye dispersal measures was used in conjunction with the estimates of sperm release rates gathered in the last chapter and fertilisation kinetics models developed in chapter 3 and 4 to predict fertilisation at varying points up and downstream of a spawning male. For this the scallops were assumed to have spawning rates of 5.4×10^6 sperm sec^{-1} (considered the most likely spawning rate) or 3.7×10^7 sperm sec^{-1} (considered a high estimate of real spawning rates) and the fertilisation kinetics model from chapter 3 (as also used above) was used.

Differences that were observed in dye concentration between habitats were not always reflected in the predicted distance away from a male where fertilisation (to a certain level) can occur (see figure 5.5). One-way ANOVA indicates significant differences between habitats when measuring fertilisation upstream, regardless of the sperm release rate or level of fertilisation success used a criterion. However, fertilisation downstream did not always differ significantly between habitats. Rather, it depended upon what sperm release rate was modelled and what level of fertilisation was used as a measure. Significant differences were detected when high sperm release rates were modelled and low fertilisation success rates were used as a criterion, but not detected when other combinations of parameters/measures were used.

Measuring fertilisation success with real scallops

Sperm-free control eggs in all trials failed to show fertilisation, but gamete quality controls in one trial in 1996 indicated that no eggs became fertilised even when exposed to sperm. Fertilisation was also not observed at any distances downstream in this trial and also in two of the gamete-quality control free trials conducted in 1995. For those two 1995 trials it was assumed that inviable eggs had been used and data from these were disregarded from further consideration. It is unclear whether this putative egg inviability was due to immature eggs being used (perhaps as a result of being released through serotonin induction or unripe scallops being induced) or handling factors such as eggs becoming too hot on the boat before/after experiments (sampling was conducted during the warmer summer weather). Consequently, 4 successful, independent trials were completed.

The pattern of rapidly decreasing fertilisation success with distance from the spawning male was similar for both methods - highest at distances close to the sperm source and decreasing rapidly to low levels even 50 cm downstream. A two-way ANOVA (table 5.2) failed to detect a significant distance x method interaction effect nor was an effect of method detected. Of course, this non-significance is not surprising given the large amount of variability in fertilisation observed but note that large amounts of variability at a sampling point are both predicted by the indirect model and

observed in the trials with real spawners, at least at sampling points near the spawning male (see figure 5.6).

Fertilisation success across populations

The proportion of all eggs fertilised in the simulated population (or population average) of spawners on silt habitat at Largs Bay and the way this varies with changing male density is shown in figure 5.7. Low levels of fertilisation were found at even moderate densities of male spawners (e.g. < 20% at 0.5 males m^{-2}) with fertilisation increasing with increasing male density. At very high population densities (> 10 males m^{-2}) fertilisation started to decrease because of increasing areas of very high sperm concentration and ensuing polyspermy. At least in randomly dispersed populations, reduced spawning synchrony amongst adults reduces the effective population density of spawners. The effect modelling only 0.2 of the male population spawning at a time is also illustrated in figure 5.7, with a right shifting of the average fertilisation curve along the density axis. Assuming spawning takes place randomly within the population, other levels of (incomplete) spawning synchrony can likewise be visualised by converting (by multiplying by an appropriate factor) actual population density into effective density and reading fertilisation success from figure 5.7 (or 5.8 or 5.9) at this effective density.

The modelled rate at which simulated males (still on silt at Largs Bay) were releasing sperm also had a large effect on average fertilisation. This can be seen in figure 5.8 where higher rates of sperm release resulted in higher rates of fertilisation at lower population densities and peak fertilisation also occurring at a lower density. In contrast, figure 5.9 illustrates that when sperm release rates were held constant (at 5.4×10^6 sperm sec^{-1}) the habitat in which modelled spawning took place had little effect on fertilisation rates and that population density (over the range simulated here) had a much larger effect on rates of fertilisation.

Discussion

Physical influences of habitat on the dispersal of dye

There were clear, detectable differences in the way in which dye dispersed across the range of habitats in which *C.bifrons* might spawn: within seagrass canopies dye diffused upstream and downstream at approximately equal rates, whereas in open sand and silt areas there was little upstream diffusion of dye. The unique dispersal pattern within seagrass habitats might have been expected *a priori* because the presence of seagrass blades have previously been shown to be able to physically dampen or reduce flow within canopies to negligible levels (at least at lower levels within the canopy where scallops sit and release gametes) (Fonseca et al. 1982, Nowell and Jumars 1984, Gambi et al. 1990, Ackerman and Okubo 1993, Worcester 1995). Hence, if this dampening of flow happened, then in the negligible flows present within the canopy dye should diffuse slowly at equal rates in all directions away from the release point. Conversely, in open areas where there should not be such reductions in flow, dye would be expected to be transported downstream away from the release point by this directional flow, resulting in a clearly visible plume forming downstream.

The dampening/alteration of flow and hence dye dispersal within seagrass canopies did not seem to be greatly affected by flow speed (at least at the flow speeds tested here) as there were only small (although statistically detectable) differences between locations (Edithburgh and Largs Bay) in terms of dispersal within seagrass canopies. Certainly, the patterns of dispersal in seagrass habitats at both locations were clearly more similar to each other than to patterns of dispersal in any of the open areas. In contrast, location (and maybe flow speed - see below) had more obvious, easily described effects on dispersal patterns in the open habitats. In the faster flows of Edithburgh the plumes on sand were narrower at given distances downstream than on silt or sand in the slow flows at Largs Bay. It also appeared that the higher flow rates reduced the amount of upstream diffusion on sand at Edithburgh Jetty relative to that in slower flows at Largs Bay. Again, these patterns are fairly much what would have been expected - e.g. a smoke plume from a

chimney on a windy in contrast to a plume on a calm day. Formal hydrodynamic models of near-bed flow in open habitats (Denny 1988) also predict that dye/sperm plumes in open areas will be narrower and form more quickly in the faster flows than in the slow flows.

Of course, with the sampling design that was used here, any differences in dye dispersal patterns within habitats that were observed between locations cannot really be attributed to just current speed differences; many other location specific factors may be confounded with this. For example, seagrass blade density, differences in the number of "sand ripples" on sand patches or wind-induced wave action may all also differ between locations (though the last is unlikely to have had a large effect because all trials were only conducted on calm days and low seas). Differences in these sorts of factors between sites within habitats, as well as numerous other differences such as variation in mean flow speed between trials or the abundance of *Pinna* shells and/or small filamentous algae amongst sites within silt habitats probably account for some part of the small, but detectable differences that occurred between replicate plumes within habitats.

Though making such a comparison was not really an aim of this study, it is worth noting that some of the average dye concentration curves produced here - those in sand habitats - (see figure 5.2), at least qualitatively, look very much like previous models of (averaged) sperm dispersal in turbulent flows made using the hydrodynamic model of Denny (1988) or Babcock et al's (1994) modification of this.

Models of fertilisation success

Assumptions about fertilisation kinetics and spawning rate parameters can have a large bearing on predicted patterns of fertilisation success- as clearly illustrated in figures 5.4, 5.5 and 5.8. However, when realistic estimates of these parameters (i.e. fertilisation kinetic parameters, average of maximum sperm release rates - obtained in chapters 3 and 4) were used in the models, fertilisation decreased in a very similar way (at least on silt habitat at Largs Bay) as found in the experiments in which real spawning scallops were used. Even when high parameter estimates are used (for example substituting a sperm release rate of 3.7×10^7 sperm sec^{-1} - the

maximum rate recorded in chapter 4), the general pattern is fairly constant - fertilisation still decreases fairly rapidly with inter-spawner distance (though obviously less rapidly than with lower sperm release rates) and, correspondingly, average fertilisation success is still relatively low (though again greater than predicted with lower sperm release rates) at even moderate population densities (< 1 scallop m^{-2}).

It was reassuring to be unable to detect a difference between predicted results for the indirect model (when using the spawning rate and fertilisation kinetic parameters chosen *a priori* to be most likely) and those obtained directly with real spawning scallops but, of course, with the large (observed and predicted) variability between trials at each sampling point, the power of this test to detect subtle differences between methods would have been very low. This is not to say, however, that this comparison was a trivial test. For example, if the indirect method had been seriously in error for whatever reason, and fertilisation using real animals had decreased much more slowly with increasing inter-spawner distance (perhaps like some sea urchins , Styan 1997 or Crown-of Thorns Starfish, Babcock et al. 1994), then I would have expected this to have shown up in this test with a large difference between predicted and measured fertilisation particularly at large distances downstream. That two independent methods predict similar patterns of fertilisation (reducing fairly dramatically with very small increases in inter-spawner distances) means that it is possible to be reasonably confident that the dynamics of *C.bifrons* fertilisation between pairs of spawners have been adequately captured in these models. Given this, the most striking feature of the model of *C.bifrons* fertilisation developed here is the very small distances between male and female that are nevertheless enough to cause reproduction to fail to substantially. The rapid rate of decrease in *C.bifrons* fertilisation with inter-spawner distance is similar to patterns found by Pennington (1985), and Levitan (1991, 1992) for urchins, though perhaps the decrease with distance is even slightly more rapid than in those studies. Whilst some studies with other spawners have shown fertilisation to decrease much more slowly over considerably greater distances (eg crown of thorns starfish, Babcock et al. 1994; urchins Styan 1997 & S.Mead, R.C. Babcock, C.A. Styan unpublished manuscript; abalone Keesing and Babcock 1996), at least some other studies involving small bivalves (e.g. freshwater mussels, Downing et al. 1993) suggest that fertilisation success can drop away over even shorter inter-spawner distances than in *C.bifrons*.

Whether differences in dye dispersal occurring in various habitats or conditions translated into detectable differences (in terms of how far up or downstream fertilisation is successful) depended on the specifics of fertilisation kinetics and spawning rate parameters used in models of fertilisation as well as how fertilisation is assessed (i.e. what fertilisation criteria were used). Perhaps surprisingly, the clear differences in dye concentrations at points up/downstream (and consequent differences between habitats that could be detected in terms of how far up/downstream fertilisation occurred) were not manifest in the results from the models of spawning within randomly dispersed populations. In part this conclusion came about because I compared fertilisation across a wide range of population densities - the effects of density appeared far greater than any potential, subtle differences among habitats. The range of densities examined here (0.01 - 10 m^{-2}) is not, however, extravagantly large - such densities reflect natural densities of a number of (fished) scallop species (Oresanz et al. 1991; and see chapter 7). The second part of the explanation comes from an examination of the 2-dimensional plumes (sets of ellipses) used in the simulation models that were generated from the field measures and a consideration of the way fertilisation within populations was modelled. It turns out, that in terms of area of seafloor covered by dye (sperm), there were only very marginal differences between habitats/locations. In a sense, the effect of habitat and currents was just to "bend" the shape of the plumes and their direction relative to the spawning male - plumes were either long and narrow or short and wide, or circular, but their overall areas and the total amount of dye (sperm) just above the seafloor remained fairly constant. If this was the only effect that habitat/location had, then the lack of difference between population averaged fertilisation success rates amongst scallops in a range of habitats/locations is not surprising.

As noted earlier (chapters 2,3), fertilisation chance is directly proportional to sperm concentration, so in a <u>population</u> of randomly dispersed individuals the position/shape of sperm plumes relative to a spawning male scallop is largely irrelevant once multiple individuals are sampled. Instead, what is important is the total <u>amount</u> of sperm present in a given area. So for a noticeable difference in fertilisation rates to have arisen between habitats (across a range of population densities) in the population simulations, there would have had to have been a difference in the total amount of sperm within plumes between habitats/locations just above the seafloor. For this

to have happened there would have had to be a difference between habitats/locations in terms of the rate of diffusion of sperm away from the sea-floor, which was not detectable in the relatively slow flows encountered in both locations. This does not mean that large differences could not occur elsewhere or that small differences did not occur).

Whilst distance up/downstream at which various fertilisation criteria were met provides a relatively easy means of assessing the scale of inter-spawner distances over which external fertilisation is successful (e.g. Pennington 1985, Levitan 1991, Levitan and Young 1995, Coma and Lasker 1997a, 1997b, Styan 1997), those measures do not incorporate the sidewards, cross-current diffusion of gametes clearly illustrated in previous numerical models (Denny and Shibata 1989), field experiments (e.g. Babcock et al. 1994) and here. Clearly, care needs to be taken when extrapolating patterns derived from linear array experiments such as those conducted in the first part of this chapter (e.g. Styan 1997); a greater distance downstream that fertilisation occurs downstream in one habitat or set of conditions would not necessarily mean that, at a given population density, average fertilisation success would be detectably greater in that habitat/conditions than in others. If differences between habitats are of interest, future studies should probably endeavour to gather more detailed information about the width of sperm plumes and not just how quickly sperm concentration along the centre of plumes decreases downstream. This might involve visual estimate of dye plume width as in this study, or its sampling in two dimensions and spectrophotometric analysis; but a more desirable, direct approach would be to sample fertilisation success at places in a range of directions other than directly up or downstream from the spawning male. This means, of course, an increase in the size and complexity of experiments.

One of the reasons I sought to contrast gamete dispersal in the (relatively) strong and slow flows of the two locations was to determine if fertilisation worked more effectively over longer distances or over larger areas in particular tidal flows (i.e. during tidal flows or at the turn of tides). Intuition suggests that it might be optimal for spawning to occur during slower flows which should reduce the amount of turbulent mixing and gamete dilution. Given that the flows at Largs Bay and Edithburgh are unlikely ever to be much stronger than those sampled here (tidal amplitudes even

during spring tides are not much greater than when sampling occurred) the evidence presented here suggests that variation in the relatively slow flow speeds will have very little effect on the process of sperm dilution and fertilisation. This suggests, then, that the timing of spawning events would not be a very important determinant of average fertilisation success. This is unfortunate, for if I had found evidence for a strong selective force that might favour spawning at particular times, this might have help to narrow down future searching efforts to observe real spawning events in the field. As it stands, there is little reason (in respect of fertilisation success) to expect that *C.bifrons* should spawn at any particular time of the lunar month or during any particular phase of the diurnal tidal cycle.

In addition to the numerous assumptions discussed above (and in previous chapters) four additional important assumptions were made when scaling up the pair-spawner models to models of fertilisation within populations. Firstly, I assumed that scallops released sperm in a constant stream and that measured laboratory spawning behaviours after inducement with serotonin (see chapter 3, 4) were natural. It is easy to imagine that this may not be true - serotonin injection and holding animals in laboratory conditions must be stressful to the scallops to some degree. In natural spawning events scallops might instead release in a series of bursts of high concentration "puffs" of sperm. Indeed, occasionally some serotonin induced scallops in the laboratory did release sperm as a series of high concentration "puffs" rather than in a constant stream (though this was fairly rare). Such spawning behaviour ("puffs") has been reported by others for other bivalves such as the giant clam *Tridacna gigas* (Braley 1984) and abalone (Clavier 1992); I have also witnessed this type of sperm release behaviour (puffs) in abalone (*Haliotis laevigata*) spawning in relatively natural conditions (R.C. Babcock, C.A. Styan *et al.* unpublished data). How *C.bifrons* releases sperm during a real, natural spawning event would be of great interest - this, however, requires witnessing natural spawning events, an improbable event (see chapter 4).

Secondly, past studies (particularly the careful field studies of Lasker et al. 1996, Coma and Lasker 1997a, 1997b) have revealed the high level of variability in fertilisation success that can occur between eggs released at a common point but at different times. It is easy to imagine how this variation might be generated, with both temporal variation in gamete release rates and

temporal variation in small scale hydrodynamic conditions potentially contributing to variation in small scale, localised sperm concentrations that in turn manifests as variable fertilisation success (Lasker et al. 1996, Coma and Lasker 1997a, 1997b). However, if the goal of sampling is to measure average across populations or variation in fertilisation success amongst individuals then much this variation at a particular point is essentially noise and not of much functional importance (see next chapter). Of course, sampling programmes must be careful to take account of this variation and adequate replication within a range of heirachical levels (i.e. within individuals, amongst individuals, amongst habitats, times etc) is essential in order not to attribute variation falsely to the wrong level. What I measured with the dye models was a time-averaged (and across plume averaged) concentration of dye at various sampling points away from a releasing source (this is also what is produced in the steady state models of Denny 1988, Babcock et al. 1994). After sampling many individuals, such "averaging out" of the variation in dye concentration at sampling points should not affect average rates of fertilisation of eggs released at those points. But on a smaller scale, because individuals release limited numbers of eggs, it may lead to an underestimation of variation amongst individuals who, by chance, might release most of the eggs at times of high or low passing sperm concentration (see also next chapter).

Thirdly, the model of egg behaviour I have used in the simulations is also an important assumption that warrants some consideration. I assumed that eggs simply sink where they are released and sample water (for sperm) as this flows past/over them through time. In this way, it is largely only males upstream (or close upstream) of the female that could fertilise her eggs. This assumption was much the same as taken by Denny and Shibata (1989), who in their models only considered the effect of sperm from upstream males for urchins living on a wave-expose shore. In contrast, when simulating fertilisation within populations analogous to those here, Levitan and Young (1995) and Morris (1994) made a different assumption about egg behaviour. In both of those simulation models, eggs drifted across the landscape, though only for a certain (limited) distance. As a consequence of this, not only was the density of simulated individuals an important determinant of average fertilisation success, but also the size of populations and average fertilisation success tended asymptotically towards 100% as population density and size increased (polyspermy was not accounted for in this model) (Levitan and Young 1995). Less than 100%

fertilisation success was often predicted, however, because in small, finite populations, there was some chance that unfertilised eggs would drift out of an area where sperm were being released and would not drift back into an area where spawners were present. Under an extreme version of the drifting model of egg behaviour, if populations are large enough (and eggs drift far enough before losing viability) then all eggs will eventually become fertilised, regardless of the density or dispersion patterns of individuals (Levitan and Young 1995).

I modelled *C.bifrons* eggs as sinking where released because the eggs were negatively buoyant in laboratory conditions (see chapter 3) and because the low current speeds experienced in the field locations would seem unlikely to keep eggs suspended for very long, particularly given the many obstructions such as *Pinna* shells or seagrass blades that may enhance settlement of eggs out of the water column. This is clearly an untested assumption and better models of the behaviour of eggs in field conditions are needed to improve the reliability of simulations of fertilisation within populations; ideally, these will be based on measurements of real egg dispersal made in field conditions (e.g. Benzie et al. 1994) or possibly of the dispersal of egg mimics (e.g. Koehl and Powell 1994).

Fourthly and importantly, fertilisation success here has been modelled in hypothetical (and probably unrealistic) populations of randomly dispersed individuals. In chapter 7, that particular shortcoming is overcome as I describe how *C.bifrons* are dispersed and then use this information in similar models of fertilisation within real populations. But, before moving on to that, in the next chapter I re-examine the outcomes of the models produced here and illustrate the substantial variation in fertilisation success that might occur amongst individuals within populations.

Tables 5.1.(A-C) Analyses of variance of the effects of distance and habitat on dilution of dye released from a "mock" scallop. 3 estimates of dye concentrations were made at 7 positions away from the scallop. This was repeated in 4 (haphazardly located) independent plumes in each of 5 chosen habitats. Position downstream and habitat type were treated as fixed factors and plumes treated as a random factor nested within habitats. Data were log(x+1) prior to analysis.

A. Comparing across all distances downstream and habitats

Source	DF	Sum of Squares	Mean Square	F-Ratio	p
Distance	6	10.636	2.659	1.891	0.164
Habitat	4	610.023	101.671	147.138	<0.001
Distance x Habitat	24	240.514	10.021	14.503	<0.001
Plume (Habitat)	15	21.095	1.406	7.793	<0.001
Distance x Plume (Habitat)	90	62.190	0.691	3.829	<0.001
Error	278	50.168	0.180		

B. Same analysis but only comparing seagrass habitats (Largs Bay with Edithburgh Jetty).

Source	DF	Sum of Squares	Mean Square	F-Ratio	p
Distance	6	345.026	57.504	9.190	<0.001
Habitat	1	2.124	2.124	2.533	0.163
Distance x Habitat	6	10.985	1.831	3.158	0.014
Plume (Habitat)	6	5.031	0.839	8.834	<0.001
Distance x Plume (Habitat)	36	20.871	0.580	6.108	<0.001
Error	111	10.536	0.095		

C. Comparing only open sand habitats (Largs Bay with Edithburgh Jetty)

Source	DF	Sum-of-Squares	Mean Square	F-Ratio	p
Distance	6	330.834	55.139	85.212	<0.001
Habitat	1	8.670	8.670	5.302	0.061
Distance x Habitat	6	37.176	6.196	9.575	<0.001
Plume (Habitat)	6	9.811	1.635	7.911	<0.001
Distance x Plume (Habitat)	36	23.295	0.647	3.131	<0.001
Error	111	22.943	0.207		

Table 5.2. Analysis of variance of the effects of distance away from a male and the method used to predict female *C. bifrons* fertilisation success in field trials on silt habitat at Largs Bay. Proportion of eggs fertilised at 7 positions along a linear transect away from a serotonin induced spawning male were compared with predicted fertilisation success along a similar transect by the indirect (dye dispersal/ fertilisation kinetics) model. Fertilisation data were arcsin transformed before analysis.

Source	DF	Sum of Squares	Mean-Square	F-Ratio	p
Distance	6	1.580	0.263	14.597	<0.001
Method	1	0.012	0.012	0.692	0.410
Distance x Method	6	0.160	0.027	1.479	0.209
Error	42	0.758	0.018		

Plate 5.1. Fluorescein dye release from the "mock scallop" on sand habitat, Largs Bay, 4m depth. On the left side of the picture is the dye reservoir bag on which is a sheet of white perspex board and two 3lb diver's lead weights. The weight of the lead forces dye from the bag through the thin connecting tubing to the dye release point on the mantle of the mock scallop. The position of the mock scallop (and release point for dye) is indicated by the white arrow.

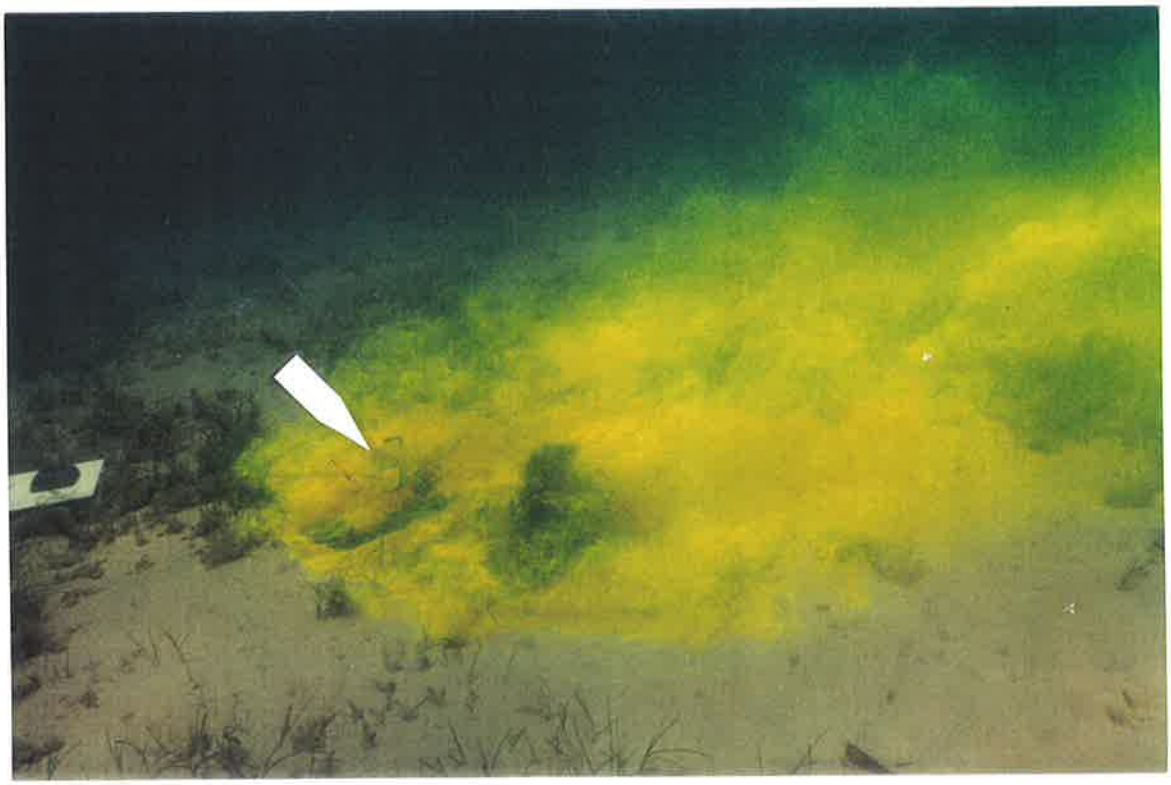


Figure 5.1. Outline of the calculation of sperm plume area. The solid line in the top half of the diagram shows modelled sperm concentration up and downstream from the spawning scallop. End points where sperm concentration drops below F_{crit} are determined from this dye/sperm (field) dispersion model. This forms one (long) axis of an ellipse where sperm concentration is at least F_{crit}. The lower half of the diagram is a plan view of sperm dispersal in the field, based on visual estimates of dye plume width. The midpoint of the long axis of the ellipse is assumed to be its widest point. The width of this is estimated from the visual (field) estimates of dye plume width at that point point downstream. This forms the second axis of an ellipse where sperm concentration is at least F_{crit}.

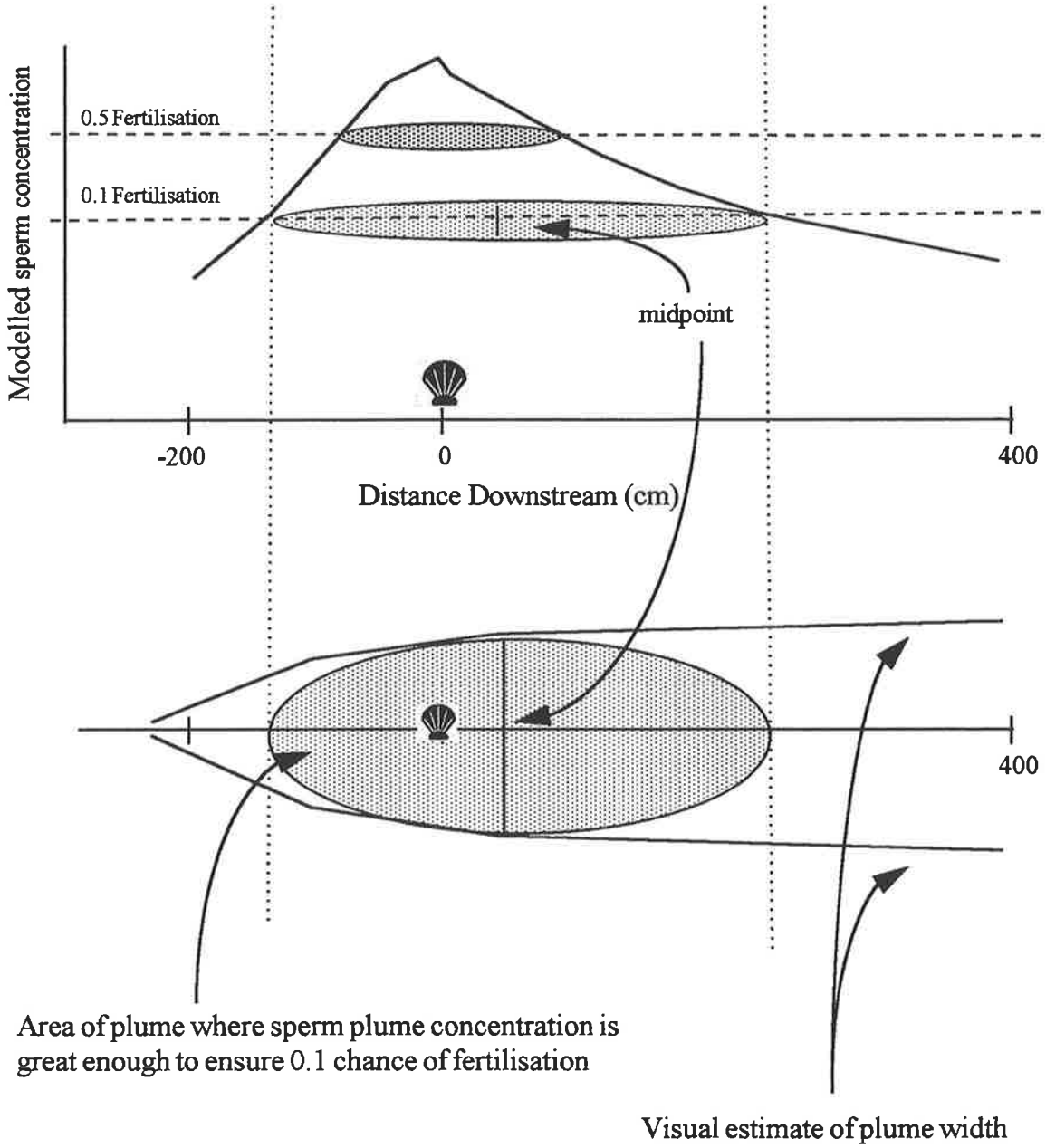


Figure 5.2. Fluorescein dye dilution as a function of distance up and downstream from a release point in different habitats and tidal current speeds. Average (+/- S.E.) dye concentration from four replicate plumes in each habitat/current regime are plotted as a $\log_{10}$ proportion of stock dye release rate from mock scallop (release rate standardised to 1 dye unit sec^{-1} in each replicate). A. Sand habitat, Largs Bay (slow currents). B. Sand habitat, Edithburgh Jetty (fast currents). C. Silt habitat, Largs Bay (slow currents).

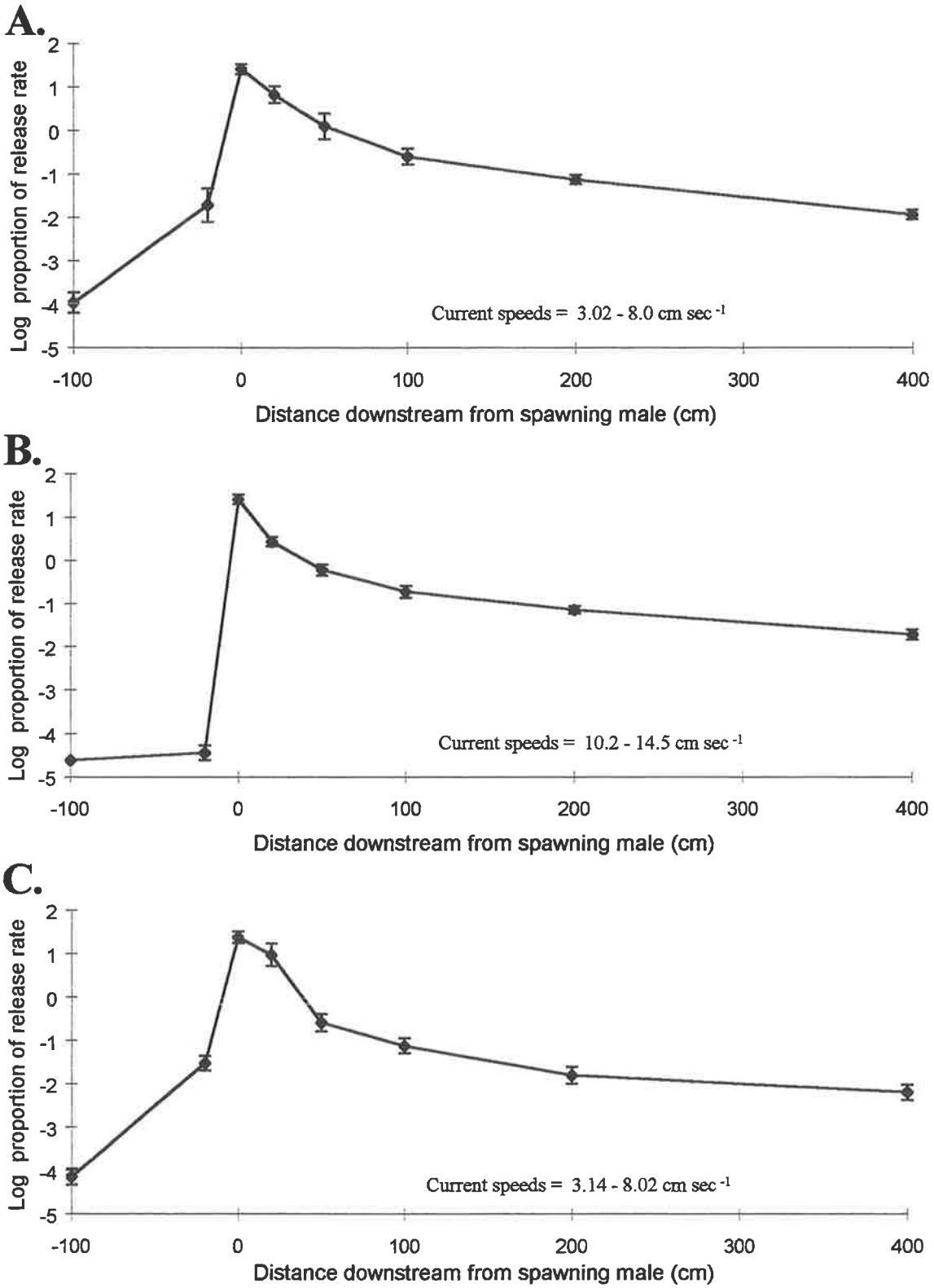
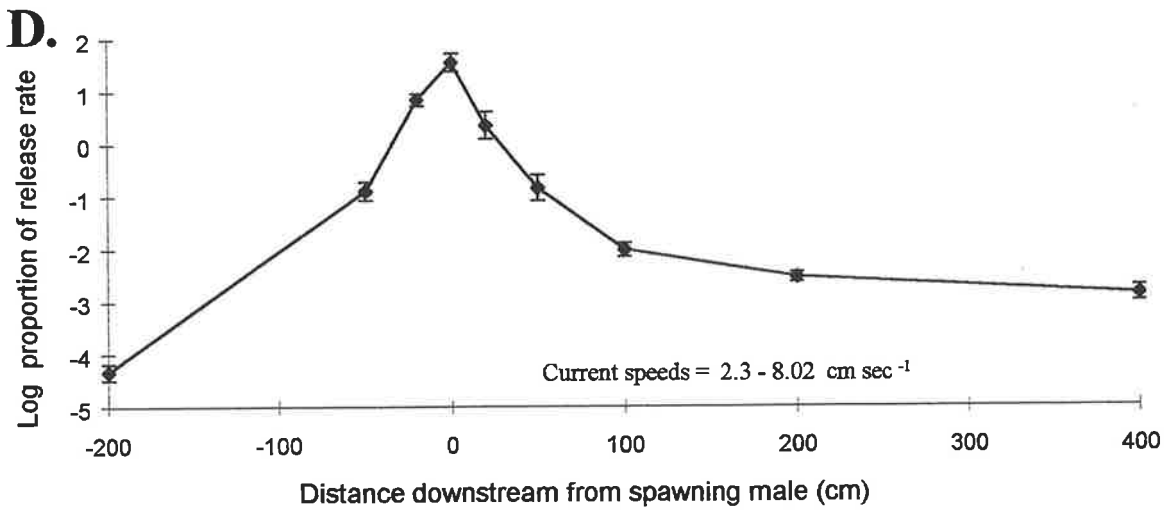


Figure 5.2. continued Fluorescein dye dilution as a function of distance up and downstream from a release point in different habitats and tidal current speeds. D. Seagrass habitat, Largs Bay (slow currents). E. Seagrass habitat, Edithburgh Jetty (fast currents).



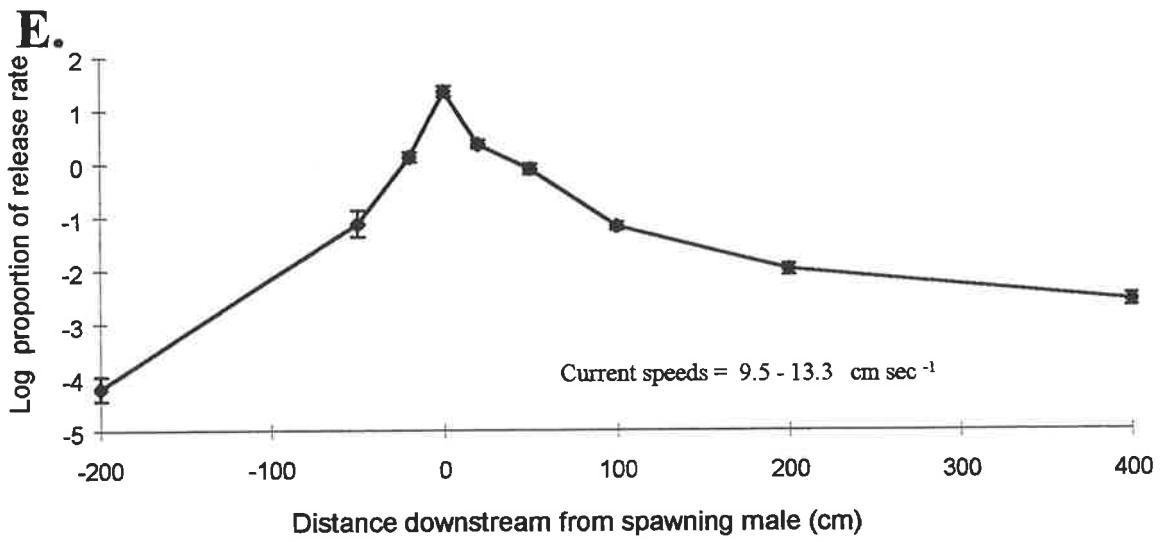


Figure 5.3. Mean (+/- S.E.) dye plume width in open habitats. Plume widths from 4 replicate areas were estimated visually in each habitat/current regime. Dye release point was at the 0cm point along the x-axis. Slow currents on silt habitat at Largs Bay (solid circles), slow currents on sand habitat at Largs Bay (hollow diamonds) and faster currents on sand habitat at Edithburgh Jetty (hollow triangles).

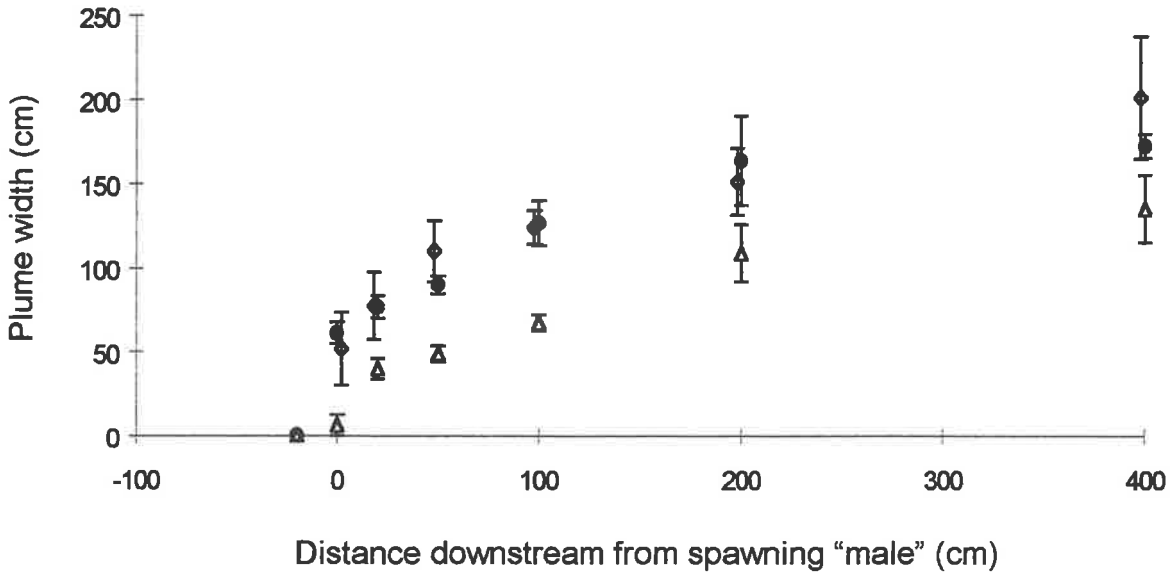


Figure 5.4. Combination of field dye dispersion and laboratory-based fertilisation kinetic models to predict female fertilisation chance at varying distances up and downstream from a single spawning male *C.bifrons* on silt habitat at Largs Bay. Two sperm release rates are modelled here - mean maximum rate (5.4 x 10 ^{6} sperm sec^{-1}, solid diamonds, solid line) and absolute maximum rate measured (3.7 x 10 ^{7} sperm sec^{-1}; hollow triangles, dashed line), based on work presented in chapter 4. Dotted horizontal lines indicate fertilisation chance based on polyspermy-adjusted VCCW model described in chapters 2,3 ($F_e = 0.00081$, $\beta_o = 0.0218$, $t = 3600$, $t_b = 160.09$).

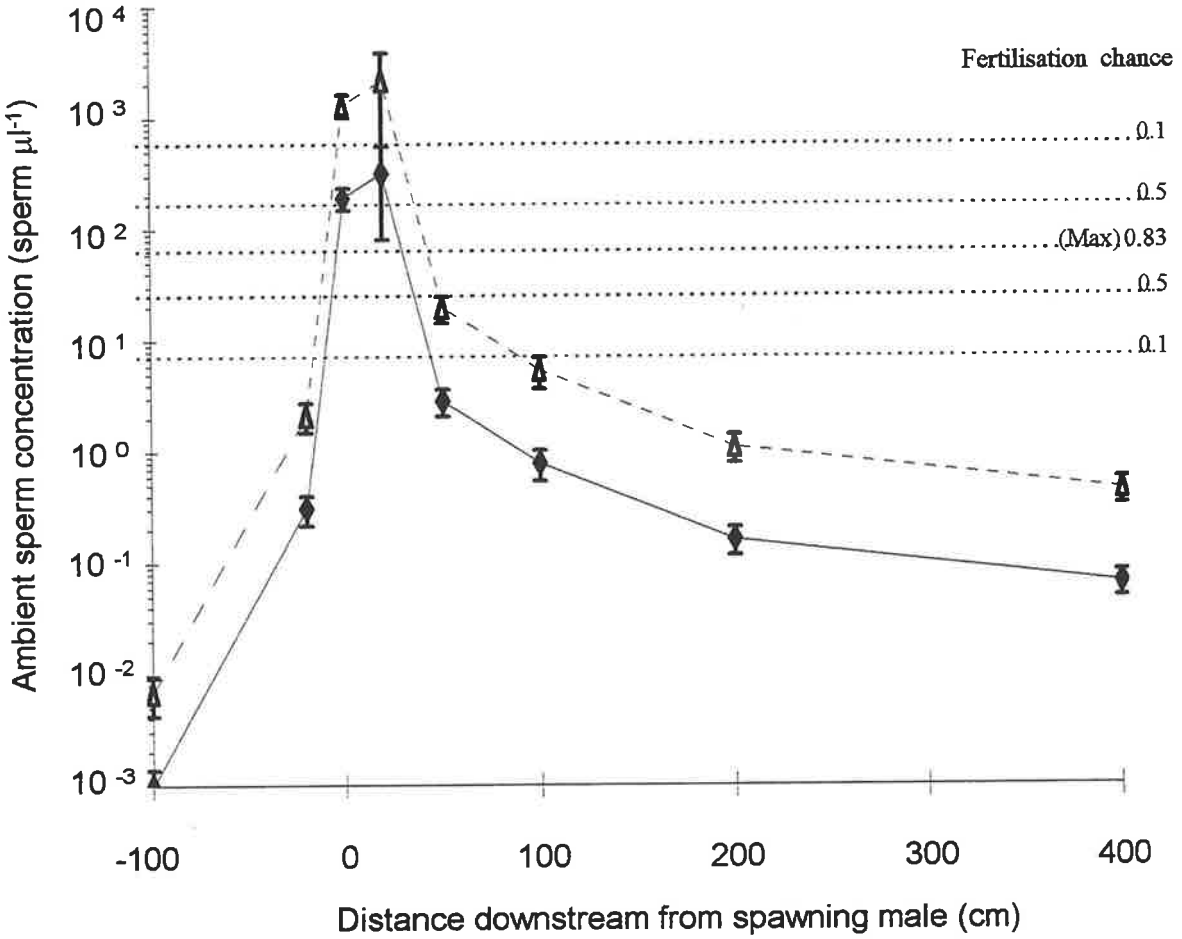


Figure 5.5. Predicted fertilisation success as a function of distance away from a single spawning *C.bifrons* male in differing spawning environments. Mean (+/- S.E.) distance upstream (below x-axis) and downstream (above x-axis) where sperm concentration is greater than F_{crit} (A. 0.1515 sperm $\mu l^{-1} = F_{0.01}$; B. 1.588 sperm $\mu l^{-1} = F_{0.10}$; C. 10.6 sperm $\mu l^{-1} = F_{0.50}$) are modelled in differing habitats and current regimes (black - silt habitat, Largs Bay; shaded - sand habitat, Largs Bay; diagonal stripes - seagrass habitat, Largs Bay; white - sand habitat, Edithburgh Jetty; vertical stripes - seagrass habitat, Edithburgh Jetty). In each plot three sperm release rates are modelled : 1.5 x 10^6 sperm sec^{-1} , 5.4 x 10^6 sperm sec^{-1} and 3.7 x 10^7 sperm sec^{-1}. p-values are results of one-way ANOVA (habitat as a fixed factor) - results below plots testing plume distance upstream and above plots, testing plume distance downstream.

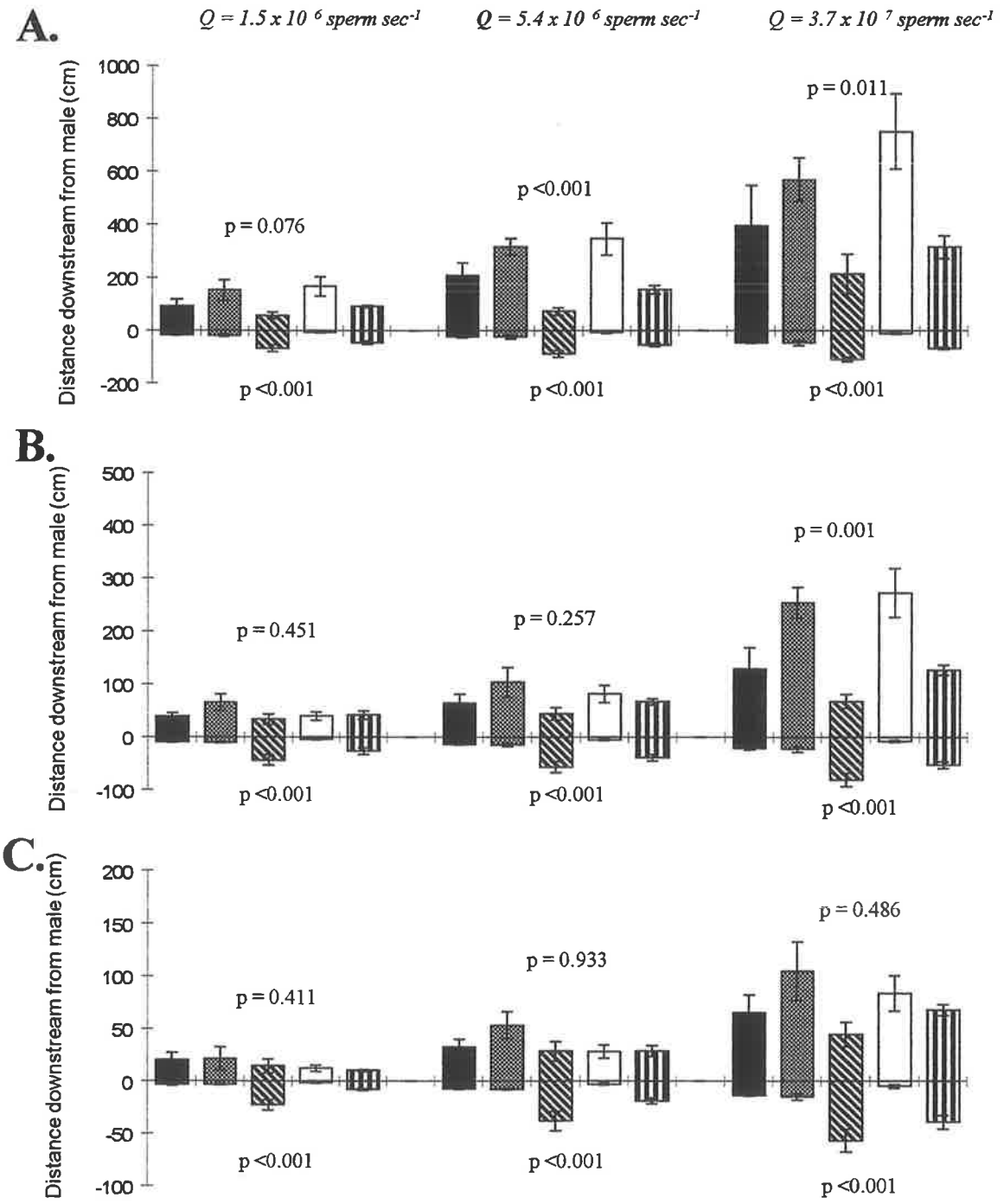


Figure 5.6. A comparison of two methods of predicting field fertilisation success of *C.bifrons* as a function of distance away from a spawning male scallop on silt habitat at Largs Bay. Solid circles are mean (+/- St.Dev.) success of eggs, held in syringes, exposed to water at positions downstream of a real male scallop that had been induced to spawn with serotonin (n = 5 independent replicates). Hollow triangles are mean (+/- St.Dev.) success predicted in the same conditions using replicate dye plumes to estimate sperm dilution and fertilisation kinetics models fitted in chapter 3 (n = 4 independent replicates; male spawning rate modelled at 5.4×10^{6} sperm sec^{-1}).

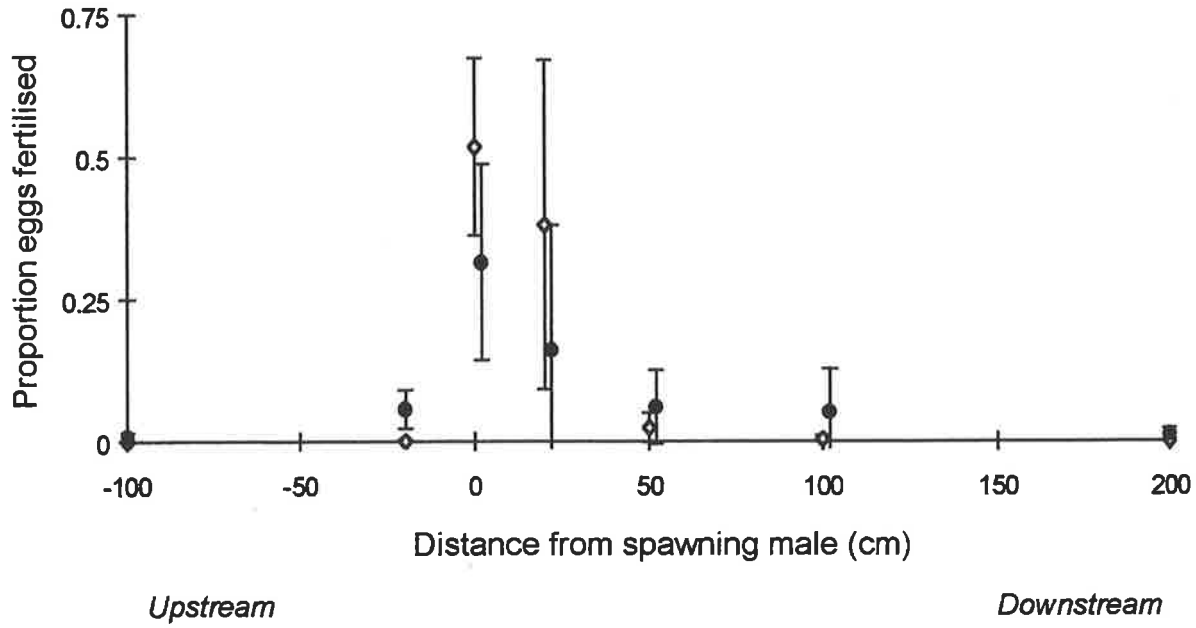


Figure 5.7. Influence of male *C.bifrons* density and reduced spawning synchrony on population fertilisation success of females. Plotted points are total proportion of eggs fertilised from 1000 simulated female *C.bifrons* spawning in slow currents on silt habitat at Largs Bay - solid diamonds (solid line) all scallops spawning synchronously, hollow diamonds (dashed line) 0.2 of the population spawning synchronously. Randomly dispersed male scallops were modelled spawning at 5.4×10^{6} sperm μl^{-1}.

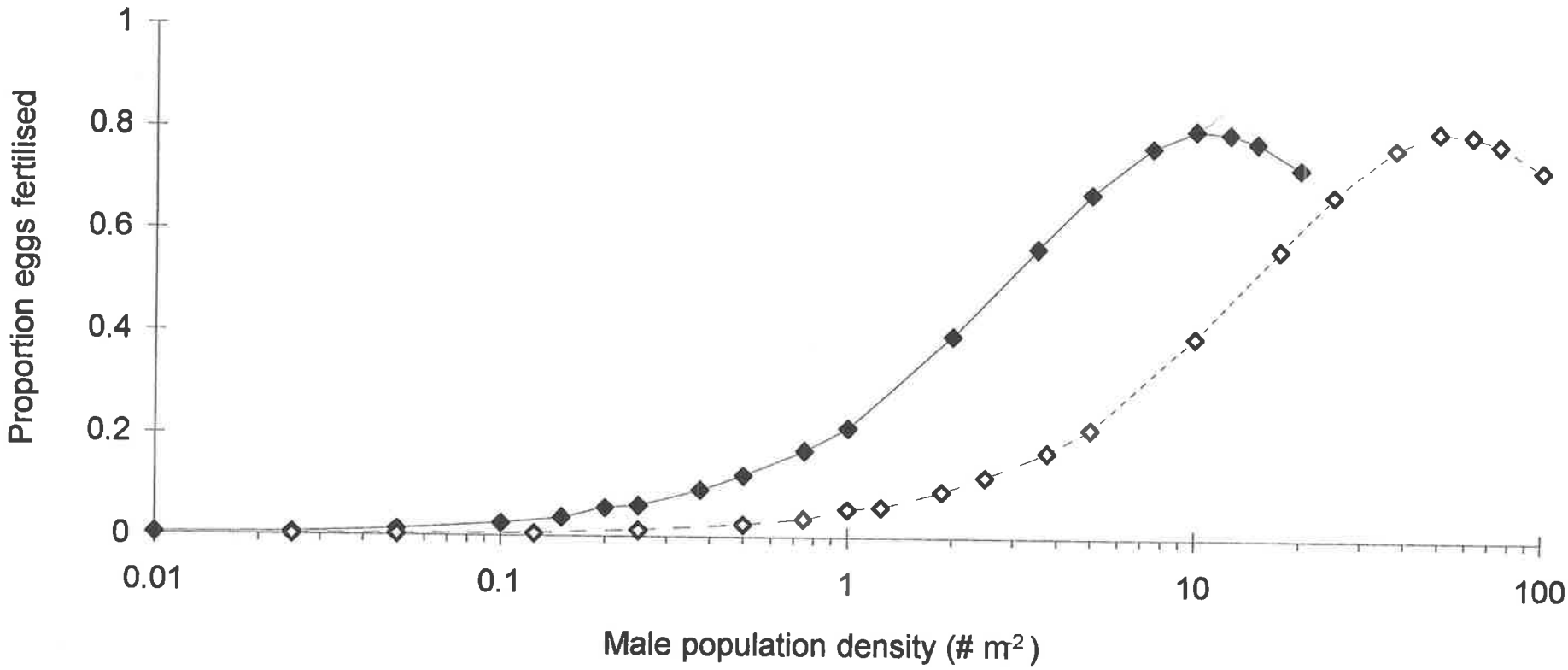


Figure 5.8. Influence of male spawning rate and density on population fertilisation success of female *C.bifrons*. Plotted points are total proportion of eggs fertilised from 1000 simulated female *C.bifrons* spawning in slow currents on silt habitat at Largs Bay - hollow squares (dashed line) males spawning at 1.5×10^6 sperm μl^{-1} ; solid diamonds (solid line) at 5.4×10^6 sperm μl^{-1}; hollow triangles (dotted line) at 3.7×10^7 sperm μl^{-1}. All randomly dispersed male scallops were modelled spawning synchronously.

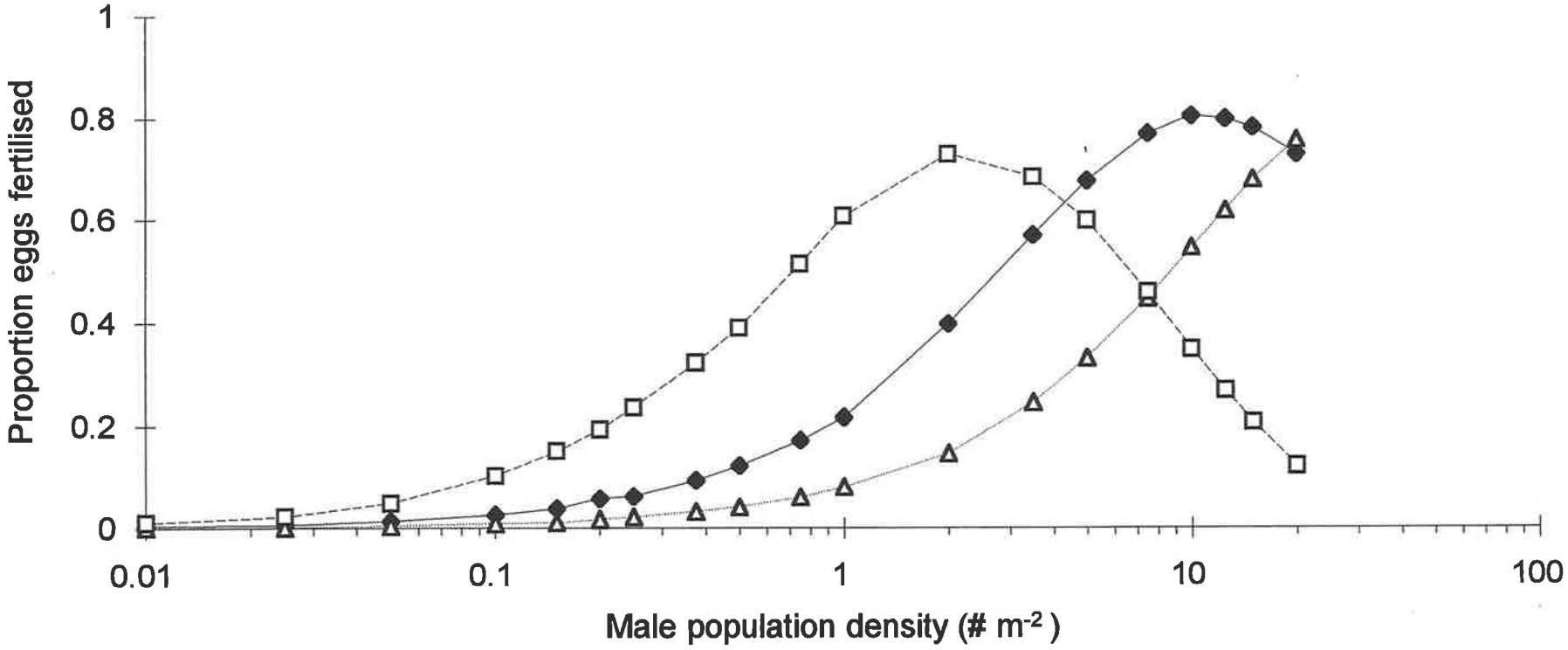
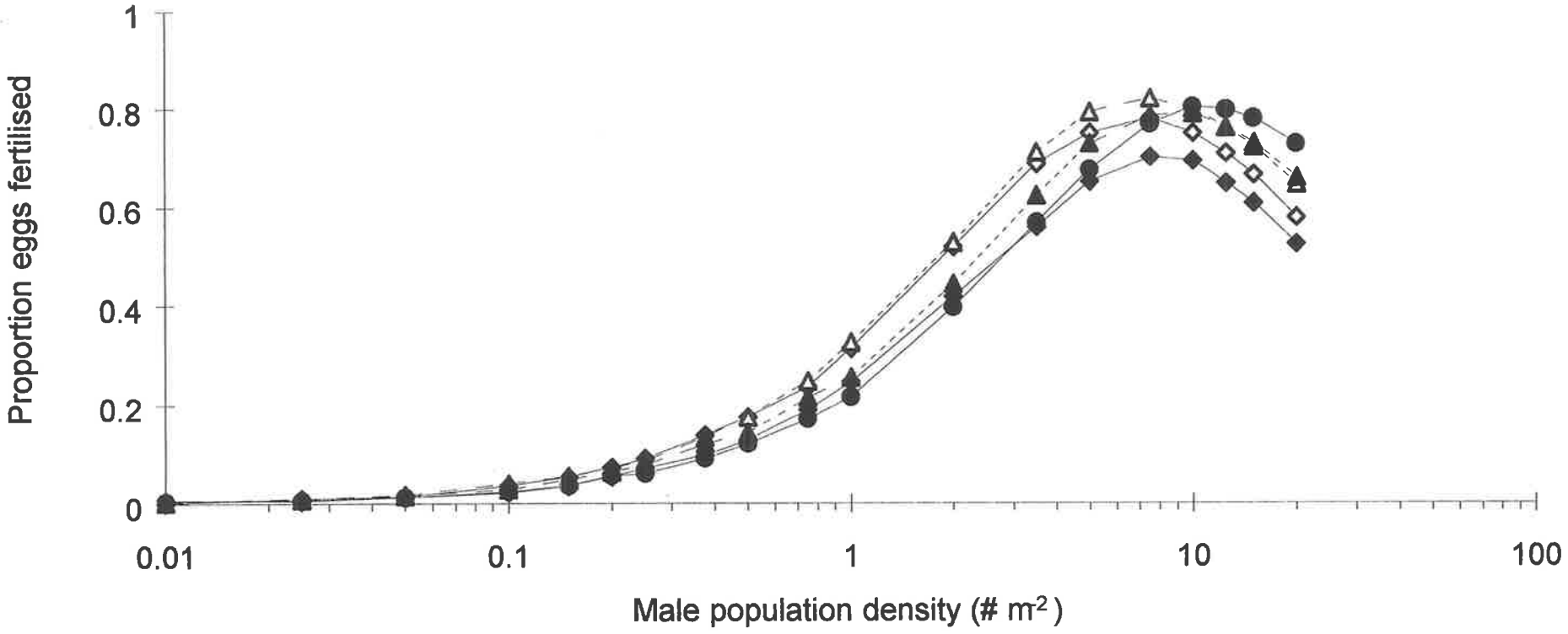


Figure 5.9. Influence of male density and spawning environment on population fertilisation success of female *C.bifrons*. Plotted points are total proportion of eggs fertilised from 1000 simulated female *C.bifrons* in a range of spawning environments (silt habitat Largs Bay - solid circles, solid line; sand habitat Largs Bay - hollow diamond, dotted line; seagrass habitat Largs Bay - solid diamond, solid line ; sand habitat Edithburgh Jetty - hollow triangle, dotted line; seagrass habitat Edithburgh Jetty - solid triangle, dotted line). All randomly dispersed male scallops were modelled spawning synchronously at 5.4 x 10^{6} sperm μl^{-1}.



Chapter 6

Fertilisation success of *Chlamys bifrons*: variation amongst individuals within randomly dispersed populations and the effect this has on the effective genetic size of populations.

Introduction

In the last chapter, the models of fertilisation success averaged across populations clearly illustrated an "Allee Effect" (Allee 1931). There was a marked reduction in (average) per-capita reproduction at low population densities relative to reproduction at higher densities and, though the exact details of the change in average fertilisation success with population density did depend on the rate of sperm release and perhaps to a very small extent on where spawning was taking place, the general pattern was the same across all the populations simulated. In all of these populations, sperm limitation (or, at very high densities, excess sperm) caused reductions in average reproductive success. In this chapter I re-examine data from the same (randomly dispersed) populations of *C.bifrons* modelled in the last chapter but, just as the success of multiple simulated individuals was used to estimate average fertilisation success, here I use this data to estimate the variation in fertilisation success that occurs amongst individuals within populations.

It is at least as interesting to understand how much variation in individual fertilisation success occurs within free-spawning populations and how this varies between populations as it is to understand variation at other scales (e.g. comparing mean success rates amongst populations), not least because of the potential genetic effects. At least potentially, any change in a breeding system that leads to a skewing in the distribution of reproductive success amongst individuals could alter the effective genetic size of a population, which, by definition, affects the rate of loss of heterozygosity within a population through time (Wright 1938). A particular goal of this chapter, then, is to translate variation in individual fertilisation success amongst individuals within populations into a form which has some functional meaning, namely, the effective genetic sizes of those populations. As well as simply estimating the level of variation likely to be present within populations, I test for two other patterns in this variation within a range of populations - whether

the level of variation within a population changes with population density (and fertilisation success), and whether the amount of variation is influenced by the habitat or location in which spawning takes place.

Methods

Individual fertilisation success within randomly dispersed populations

In the last chapter fertilisation success was modelled within large (hypothetical, randomly dispersed) populations of scallops spawning in either seagrass, sand or silt habitats at Largs Bay and sand and seagrass habitats at Edithburgh Jetty. In each of these habitats, males were modelled spawning at 5.4×10^6 sperm sec^{-1} and fertilisation was simulated across a range of population densities. In addition, populations (also across a range of densities) were also modelled on silt habitat at Largs Bay, but with males releasing sperm at 1.5×10^6 sperm sec^{-1} or 3.4×10^7 sperm sec^{-1}. Each sampled "population" consisted of 1000 independent runs in which a female's fertilisation success within a patch (surrounded by randomly placed males) was estimated - each of these runs simulated an individual scallop within a large population. I re-examined the data created in the last chapter and used the 1000 runs in each simulation run as independent estimates of fertilisation success within that population -each run was treated as a separate individual.

A model of male success was also run in which a male placed in the center of a 15m x 15m patch was the focus. Appropriate numbers of females and male were randomly placed within the plot according to the desired density of the trial. The mechanics of this male model were similar to the female success model of the last chapter in the way in which male plumes were deconstructed into component ellipses, sperm summed where eggs had been released and the fertilisation kinetics model used to determine fertilisation success. For those females lying within the plume of the central male, the contribution of sperm from the central male and then from each other male in the plot was calculated. This was then converted to fertilisation success and the central male

was apportioned a fraction of this, proportionate to his contribution to the ambient sperm concentration experienced by the eggs. This was done for each female in turn and the central male's fertilisation success was the sum of his success with each female. Again, this process was repeated multiple times, each time randomly locating the non-central males and females within the patch, and leading to a frequency distribution of male success. At most (lower) population densities, I conducted 500 runs of this, but because of the very high computational time required for runs at high densities (500 runs would have taken > 3 weeks running on 166 MHz machines in some cases!), only 135 and 35 runs were conducted for population densities of 10 and 20 scallops m^{-2} respectively. Male success using this simulation model was only determined for *C.bifrons* populations spawning at 5.4×10^6 sperm sec^{-1} on silt habitat at Largs Bay.

Relating variation in individual fertilisation success to effective genetic population size

As an approximation (see later), Nunney's (1996) equation 7 was used to relate variation in individual fertilisation success to a reduction in N_e:

$$N_e = \frac{N}{1 + \frac{\sum I_{si}}{2}}$$

Where I_{si} is the standardised lifetime variance in reproductive success of sex *i*. Here, I have assumed that variation in fertilisation success predicted in the simulation models is equivalent to variation in lifetime reproductive success (but also see below and next chapter). Nunney (1996) provides derivations for this from the Hill equation (Hill 1972) which in turn generalised the work of Wright (1938). Amongst the numerous assumptions associated with this equation is that generations do not overlap.

Results

Fertilisation within randomly dispersed populations

The proportions of all eggs fertilised in the simulated population (or population average) of spawners are shown in the last chapter. Though estimates of individual variation were deliberately left off those figures (for clarity), substantial variation in individual fertilisation success of females occurred within some populations. Importantly, the amount and the way in which this variation was distributed amongst individuals also changed with population density and to an extent spawning conditions. As a way of visualising variation in individual reproductive success and how this changed with population density, for each set of conditions (habitat/location/sperm release rate) and at 3 population densities (0.1, 1, 10 scallops m^{-2}), I ranked all individual females within a population in order of increasing fertilisation success. I then plotted cumulative fertilisation as a proportion of the total fertilisation within that population against the rank of individuals (see figure 6.1). I did the same to construct figure 6.2, ranking male's according to their relative fertilisation success and plotting cumulative reproductive success against rank. One pattern in all of these plots is striking - at high population densities (10 males m^{-2}) the cumulative curve is a fairly straight line, indicating little variation between the least successful and most successful individuals in terms of reproductive success - each individual contributes approximately equal amounts to the total reproductive effort of the population. However, at low densities, reproduction within the population became highly skewed, with often only a small fraction (the few most successful scallops) accounting for a large proportion of the larvae produced in that population. For example, an extreme example of this skewing of reproduction is that in low density (0.1 males m^{-2}) populations in seagrass, less than 10% of the females produced the bulk (>90%) of the larvae. Comparing lines of cumulative fertilisation in figure 6.1 indicates that the amount of skew in individual success within a population (and the way skewing changed with population density) varied according to habitat and according to rate of sperm release.

Reproductive skewing amongst males spawning on silt at Largs Bay was just as pronounced as for females in those conditions. Indeed, slightly higher rates of variation amongst male than female individuals were noted, particularly at higher densities (and high average success rates). This pattern was expected given that in the simulation model (and presumably in nature) a male can potentially fertilise multiple females - so individual male success could vary between 0 and n = number of females in the simulated population, whereas an individual female's success could only range between 0 and 1 (i.e. between none and all of her eggs being fertilised).

The patterns of variation in reproductive success amongst individuals within populations, and how this varies with population density and spawning conditions, are presented in a different way in figure 6.3, where I have plotted standardised variation ($var/mean^2$) in fertilisation success within populations across a wider range of population densities. A general pattern is that standardised variation in success is (very) high at low population densities and that standardised variation reduces as population densities increase. As before, this reflects an increase in the skewing of reproductive success amongst individuals as population densities (and average success) decrease. This general pattern is demonstrated for all of the randomly distributed populations - regardless of where spawning took place or at what rate sperm was released. Actual values of standardised variation at particular population densities did depend on sperm release rate (figure 6.3 B) and to a small extent on spawning habitat (figure 6.3 C). Slight increases at extremely high population densities reflect instances where areas of very high concentrations of sperm occurred and caused some scallops to have reduced success through polyspermic fertilisations.

Effective genetic population size

Making the important assumption that the variation in reproductive success modelled above can be considered as lifetime reproductive variation, I have plotted in figure 6.4 how the ratio of effective genetic population size to actual population size (N_e/N) changes with population density for a population of scallops spawning on silt habitat at Largs Bay. Two sets of points are plotted here - the first (hollow circles) incorporates only variation in female fertilisation success into equation 7 of Nunney (1995) and represents N_e/N when only considering the female population

(which might be relevant when considering mtDNA diversity). The second set of points include both male and female standardised variation into the equation and represents N_e/N that includes both males and females. Low levels of N_e/N (<0.10) are found at population densities below about 0.5 scallops m^{-2} when considering the whole population, and about 0.15 scallops m^{-2} when considering only the female population.

Discussion

The models revealed that, at least in some hypothetical populations of *C.bifrons*, there can be a large amount of variation in reproductive success amongst individuals. Others have also suggested that there is (perhaps characteristically) likely to be substantial variation in reproductive success amongst individuals within marine populations, though generated by alternative (but not mutually exclusive) mechanisms. For example Sinclair's (1988) "member -vagrant hypothesis" emphasised the potential for reproductive variance amongst individuals within a population, and also stressed the constraints that sexual reproduction places on individuals if they are contribute to future generations. However, Sinclair's model largely concentrated on the physical forcing of large scale oceanographic processes, rather than external fertilisation (and its interplay with small scale variation in density and small scale hydrographic processes) as a mechanism that could generate reproductive variation. In contrast to variation that might be caused through oceanographic processes, the defining feature of the variable fertilisation models here was a strong covariance between decreasing average success (or equivalently, population density) and increasing variation in success amongst individuals.

In hindsight, this general pattern of variation amongst individuals and how it changes with density are just what would be expected given the way I have modelled the process of external fertilisation. Essentially because external fertilisation is a spatially dependent process (see chapters 1, 5), then variation in the spatial distribution of spawners can, at least potentially, propagate into variation in fertilisation success. In all populations where animals are not uniformly spaced apart (which, in reality, will be most populations), there will be some variation in inter-

spawner distances which may in turn may generate variation in fertilisation success amongst individuals. Obviously, whether (and how much) individual variation is generated will be a function of population density, the dispersion patterns of individuals and the relationship between inter-spawner distance and fertilisation success. In the case of *C.bifrons*, the very small inter-spawner distances over which fertilisation changes (see last chapter) lead to a fairly dramatic illustration of this, with large variation occurring, even at moderately high population densities, because of the rapid drop-off in success with increasing spawner distance. In these randomly dispersed *C.bifrons* populations fertilisation success was essentially "all or nothing"; scallops had either fairly high success (>50%) or very little (<1%). Notably, Downing et al. (1993) recorded similar "all or nothing" fertilisation success within a population of freshwater (brooding) mussels. Similarly, Coma and Lasker (1997a) described the distribution of fertilisation success amongst egg samples collected for a gorgonian species, *Pseudoplexaura porosa*, across two years at a reef in Panama (their figure 2). Though the variation they described does not necessarily equate to variation amongst individual colonies, there appeared to be a bimodal distribution of fertilisation success amongst samples, with a large number collections demonstrating high fertilisation success (>70%) and most of the rest with much less than this (< 30%).

There is little other comparative data available yet to determine whether changing, high levels of individual variation occur in other populations of free-spawners, particularly in those species for which fertilisation success does not drop away with inter-spawner distance as quickly as in *C.bifrons*. The required manipulative field studies, in which individual fertilisation success is measured after alteration of the density of natural populations, have not yet been conducted (other than Babcock et al. 1992), but several studies have reported substantial variation in fertilisation success amongst individuals within naturally spawning populations (e.g. Sewell and Levitan 1992, Downing et al. 1993, Lasker et al. 1996; see chapter 1) in line with the predictions of the *C.bifrons* model. Unfortunately, previous simulation models of fertilisation success in (randomly dispersed) populations of other free-spawners (e.g. Morris 1994, Levitan and Young 1995) reported only population wide average rates of fertilisation (although in multiple monte carlo runs, both studies did account in their mean estimates for the variation in proximity to co-spawners between individuals within random populations). However, logically, a broad prediction

would have to be that there should be less variation amongst individuals (at a given density) for free-spawners whose fertilisation works more effectively over large spatial scales. This should be tested, and future studies of reproduction in free-spawners should ask, not only whether average fertilisation success changes with population density, but also whether there is an associated change in the variation of success amongst individuals.

One important consequence of the likely high levels of variation amongst individuals present within populations, will be that generally very high numbers of individuals will need to be sampled in order to generate precise estimates of (or conduct statistically powerful tests about) average fertilisation success within populations. A very important consideration in future field sampling programmes (particularly of natural spawning events) will be to replicate sampling adequately through time and space so that observed variability in fertilisation success can be properly partitioned into short time scale variability associated with eddies, release rate variability etc. and the inter-individual variation in success rates associated with density and spatial pattern, which is likely to be of more interest. Lasker and colleagues (Lasker et al. 1996, Coma and Lasker 1997a, 1997b) have also stressed the need in field sampling for adequate replication at a range of scales.

It should also be pointed out that there are several details of the way in which these *C.bifrons* simulations were conducted that may have minimised estimates of variation in fertilisation success in populations. Simulated sperm released from males was modelled to form "average plumes" within habitat/locations (these were constructed by taking averages of empirical measures of plumes in the field - see last chapter) so consequently any variation amongst sperm plume dimensions within habitats was ignored. Similarly, potential variation in the size/shape of individual plumes stemming from variation in individual sperm release rates (which was quite substantial, but not related to individual size - see chapter 4) was also ignored in the models that utilised average plumes. Additionally, because steady state plumes were simulated, any variation through time which occurs in sperm concentration within a plume was also ignored. Field evidence (Lasker et al. 1996, Coma and Lasker 1997a, 1997b) has shown that sperm concentration at fixed points within plumes can vary substantially through time - if only a small number of eggs are released at set times, then the "sampling error" within plumes/times might

also translate into real variation in the reproductive success amongst individuals. A further assumption was that no genetic incompatibilities occurred amongst specific matings - again, there is some evidence that such mating incompatibilities do exist (Grosberg 1987, Havenhand 1991) and this, too could generate additional reproductive variation in real populations (Levitan 1995).

The simulation models also assumed populations spawned completely synchronously. Here, as in the last chapter, because individuals were randomly dispersed within simulated populations, reduced levels of synchrony can be visualised by simply "right-shifting" the curves along the x-(density)-axis (i.e. reducing synchrony is equivalent to reducing the effective density of spawners). Thus, reducing spawning synchrony not only reduced average rates of fertilisation success (see last chapter) but also increased the amount of individual variation in reproductive success within populations. As suggested in chapter 4, the evidence that completely synchronous (on short time scales that count) really happens in nature is equivocal, and there are at least some intuitive reasons to believe that synchrony may instead be a function of population age/size structure.

Clearly, the amount of variation in individual fertilisation success that occurs within real populations could be much greater than that predicted in the models here, although the variation could also be much lower than predicted - as noted in the last chapter, if eggs drift for very long distances across populations then all eggs may achieve uniformly high success (and see below and next chapter for another counter-claim to high variance).

As noted in chapter 1, changes in average rates of fertilisation success may or may not be dynamically important (eg Levitan 1995, Eckman 1996), particularly if there is little evidence that populations are "supply limited" by the availability of settling larvae. However, the possibility of a high degree of reproductive skewing suggests that, for free-spawning marine invertebrates, there is some potential for the effective genetic population size of a population to be much less than the number of adults present (Hedgecock 1994a, 1994b). At least for scallops, there is considerable scope for N_e /N to be less than 0.1 (but see below). Indeed, recorded estimates of scallop population density in a range of species (Oresanz et al. 1991) are often as low as those which in these simulations would lead to N_e /N being less than 0.10. Additionally, because the models here

suggest that reproductive variance is inversely related to population density, the genetic effect associated with external fertilisation is a "double whammy", whereby reducing population densities reduces N_e as a simple consequence of a reduction in numbers (N) but then also further reduces N_e as a result of increasing variation in individual reproductive success. This double effect might be of major concern to managers trying to conserve populations of free-spawners under pressures such as fishing.

Whether effective genetic population sizes can actually be reduced to levels that may be important in a management sense is, however, unclear. This is particularly so for scallops which, although sometimes existing at fairly low densities, are typically found in geographically large populations (beds) (Brand 1991, Oresanz et al. 1991). Although N_e/N might be very low, by virtue of very large population sizes (N), effective population size (N_e) in many natural scallop populations may still be large, perhaps above levels where the negative risks associated with low N_e (such as the risks of inbreeding and consequent loss of heterozygosity, fixation of deleterious recessives etc) are of much concern.

Extrapolating, it is also possible to use the general patterns produced here to speculate about the potential contribution that variable fertilisation success may have made to a controversial and excessively low (Nunney 1996) estimate of N_e/N ($<10^{-6}$) derived by Hedgecock et al. (1992) for oysters in Dabob Bay in the Pacific Northwest. In discussing this extremely low estimate (and possible explanations for it) Nunney (1996) pointed out that it is not the absolute magnitude of individual fecundities that leads to a reduction in N_e / N (as incorrectly suggested by Hedgecock et al. 1992), but rather the degree of variation (in excess of random variation) in lifetime reproductive success amongst individuals within a population. There were particular methodological concerns such as that the estimate was based on temporal changes in allozyme frequencies, this may have been biased by the introduction into later samples of hatchery bred oysters (Nunney 1996) and, generally, there are a range of difficulties and controversies associated with direct and indirect methodologies of estimating effective population size (e.g. Husband and Barrett 1995, Nunney 1995, 1996). Aside from these issues, Nunney (1996) suggested that N_e / N will not normally reduce below 0.5 and in most cases will be nearer 1.0

because estimates of standardised variance in lifetime reproductive success published for a range of terrestrial animals vary from 0.08 to 1.35 (mean ± S.D of 16 estimates = 0.441 ± 0.322). However, as clearly illustrated here, the suggestion that N_e/N will not normally drop below 0.5 may not stand for free-spawning marine invertebrates because standardised variation in individual reproductive success for some populations of free-spawners can potentially be very much larger than for terrestrial animals, particularly at low population densities. Even so, a N_e/N of $< 10^{-6}$ still seems an excessively low estimate, but proper assessment of this really requires more information about how oyster fertilisation varies with distance and about the population densities and dispersion patterns of adult spawners.

As a cautionary brake to further wild speculation, it should be noted here that my use of Nunney's equation (7) to estimate N_e/N must also be considered only a fairly rough approximation for a number of reasons. For a start, for simplicity, and as outlined in much more detail in Nunney (1996), there are several other components of lifetime reproduction variation such as fixed genetic variation and variation in individual fecundity which were not estimated here, but which might be incorporated into more accurate estimates.

Critically, I assumed that any variation in fertilisation success within a simulated spawning event equated to variation in lifetime reproductive success. This means that I have assumed that the scallops either spawn once where they are and die, or continue spawning in exactly the same conditions throughout their lives (the distinction is unimportant here). This would be applicable only if scallops were sessile and/or semelparous; *C.bifrons*, like most scallops, is neither. *C.bifrons* can move by swimming and whilst movement experiments indicate that they probably do not swim very often (< 0.2 swims day^{-1}), nor move very far in any one swim (usually < 40 cm; Styan, unpublished data), given enough time, changes in the positions of individual scallops are likely to be quite large. Additionally, at shallow locations like Largs Bay, wave action can move scallops large distances during winter storms, even resulting in some animals being washed ashore (A. Melville pers. comm.). *C.bifrons* also live on average for at least several years (age can be up to 12-14 years, Wolff and White 1996) with good evidence that they spawn more than once per year (at least twice, and possibly three times annually - see chapter 4).

Given the small spatial scales over which fertilisation changes (see last chapter) even small amounts of individual movement will be probably be enough to change the distribution of neighbours such that the fertilisation success of particular individuals is radically affected. If so, a scallop's low fertilisation success in one spawning may not be repeated in at least some of its next spawnings - it and its neighbours may redistribute themselves (even by random walk processes) at some stage such that it now attains high fertilisation success. The central limit theorem would suggest that, regardless of how skewed reproduction may be within single spawning events, if an individual scallop's success is randomly assigned each time from a distribution of successes likely to occur within each spawning, then as individuals participate in more spawning events, each individual's lifetime success will be more closely approximate to the average of the population - i.e. the variance in reproductive success amongst should decrease with multiple redistribution and spawning events. Possibly, this might generate a temporal component to the sort of "chaotic genetic patchiness" that has been observed in some marine populations (Johnson and Black 1984, Watts et al. 1990, Johnson et al. 1993). The effect of multiple spawning events on individual lifetime variance in reproductive success is dealt with more thoroughly in the next chapter where I will further develop the model to predict variation in fertilisation success within a real, natural populations of *C.bifrons* such as that at Largs Bay.

Figure 6.1. Modelled distribution of female fertilisation success within a randomly dispersed *C.bifrons* population (n = 1000 females). In each plot, for 3 population densities (10, 1, 0.1 scallops m^{-2}), the cumulative contribution of females to the larval pool was plotted against their rank order in terms of fertilisation success within the population. Values in brackets next to curve labels are average population fertilisation success rates. A. Silt habitat, Largs Bay, male spawning rate 1.5×10^{6} sperm sec^{-1} ;B. Silt habitat, Largs Bay, male spawning rate 5.4×10^{6} sperm sec^{-1}; C. Silt habitat, Largs Bay, male spawning rate 3.7×10^{7} sperm sec^{-1}.

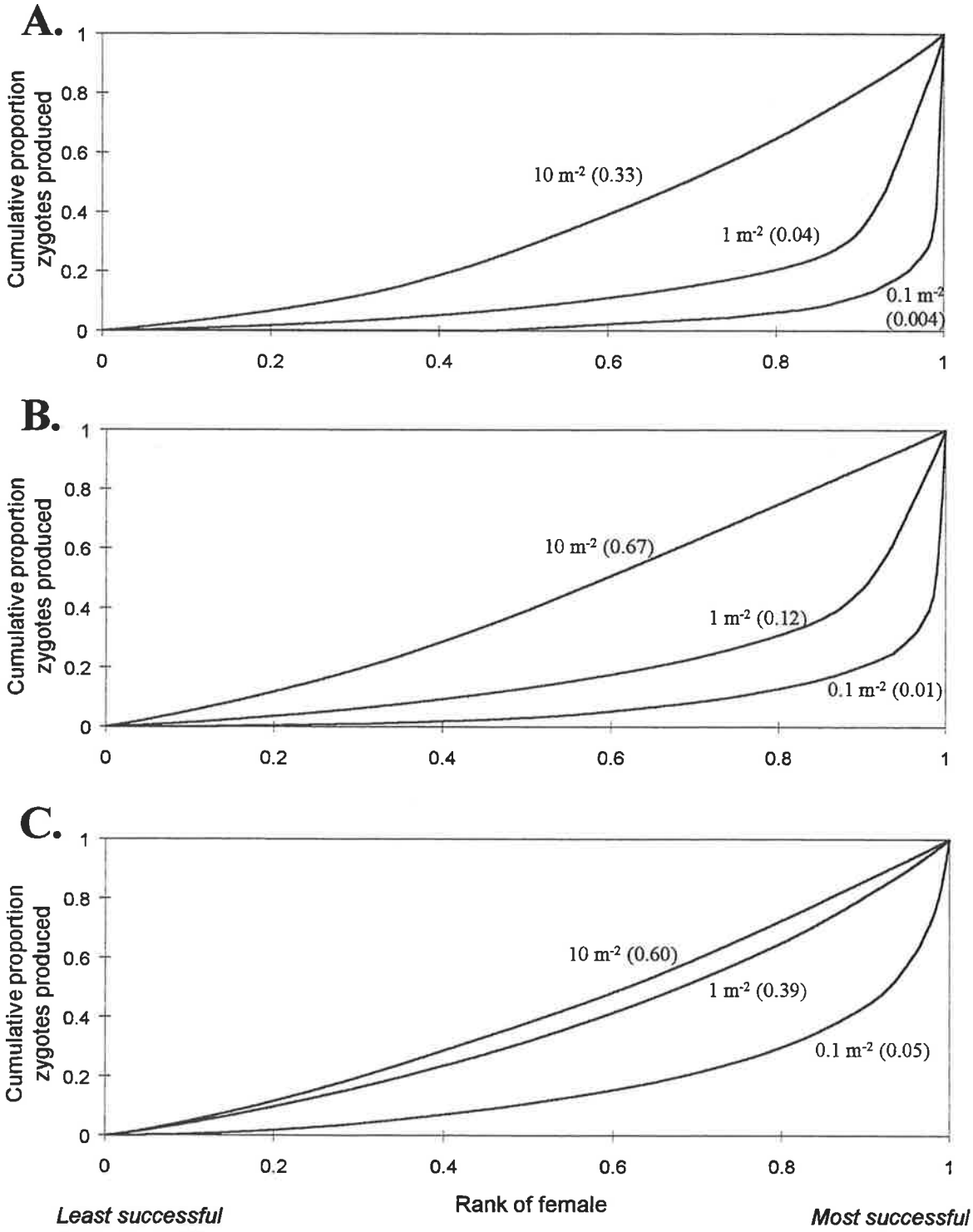


Figure 6.1. cont. D. Sand habitat (slow currents), Largs Bay , male spawning rate 5.4 x 10^6 sperm sec^{-1} ; E. Sand habitat (faster currents), Edithburgh Jetty , male spawning rate 5.4 x 10^6 sperm sec^{-1} ; F. Seagrass habitat (slow currents), Largs Bay; male spawning rate 5.4 x 10^6 sperm sec^{-1} G. Sseagrass habitat (faster currents), Edithburgh Jetty , male spawning rate 5.4 x 10^6 sperm sec^{-1}.

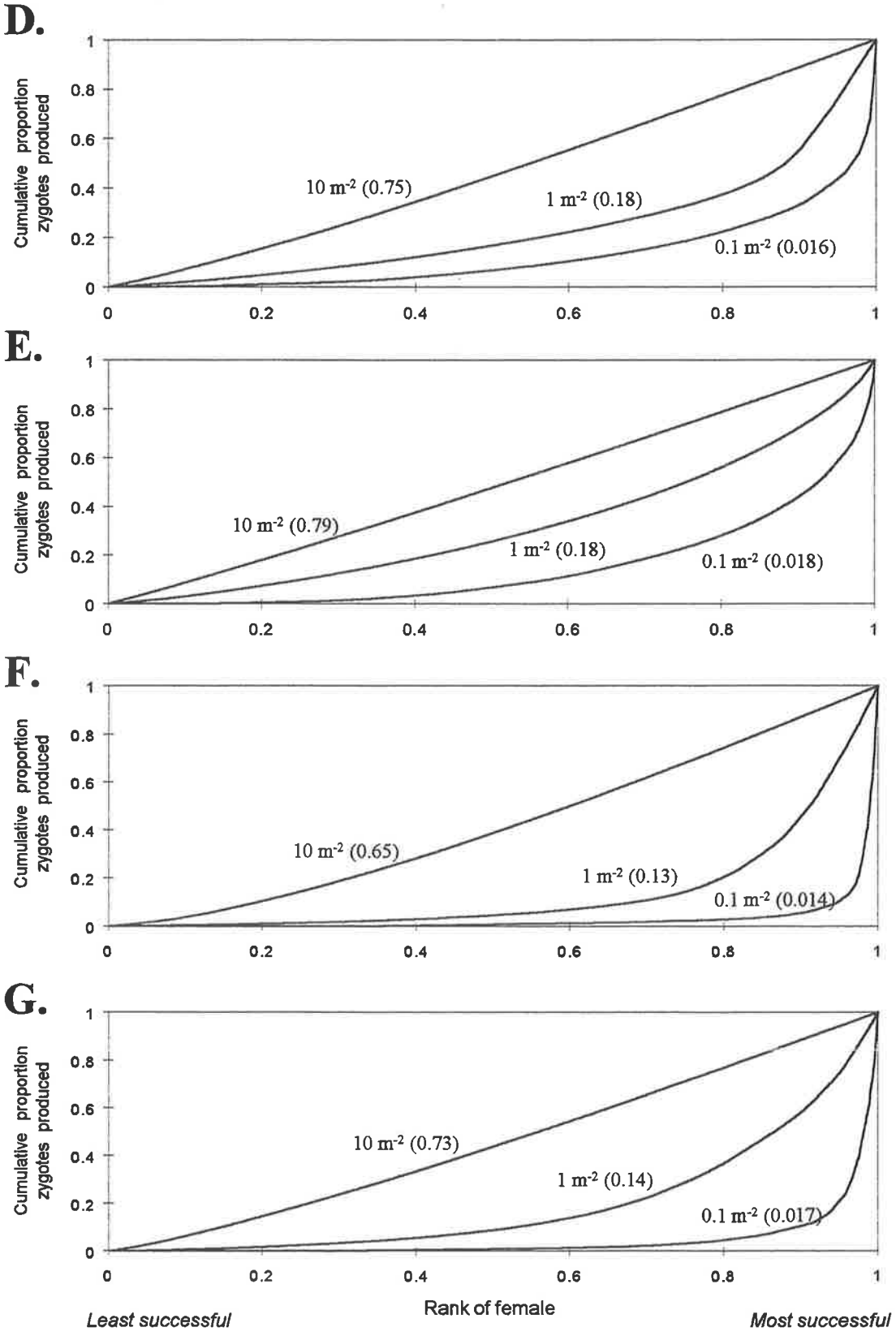


Figure 6.2. Modelled distribution of male fertilisation success within a randomly dispersed *C.bifrons* population (n = 1000 males). For 3 population densities (10, 1.5, 0.1 scallops m^{-2}), the cumulative contribution of males to the larval pool was plotted against their rank order in terms of fertilisation success within the population. Values in brackets next to curve labels are average population fertilisation success rates. Scallops were simulated spawning on silt habitat, Largs Bay, male spawning rate 5.4 x 10^6 sperm sec^{-1}.

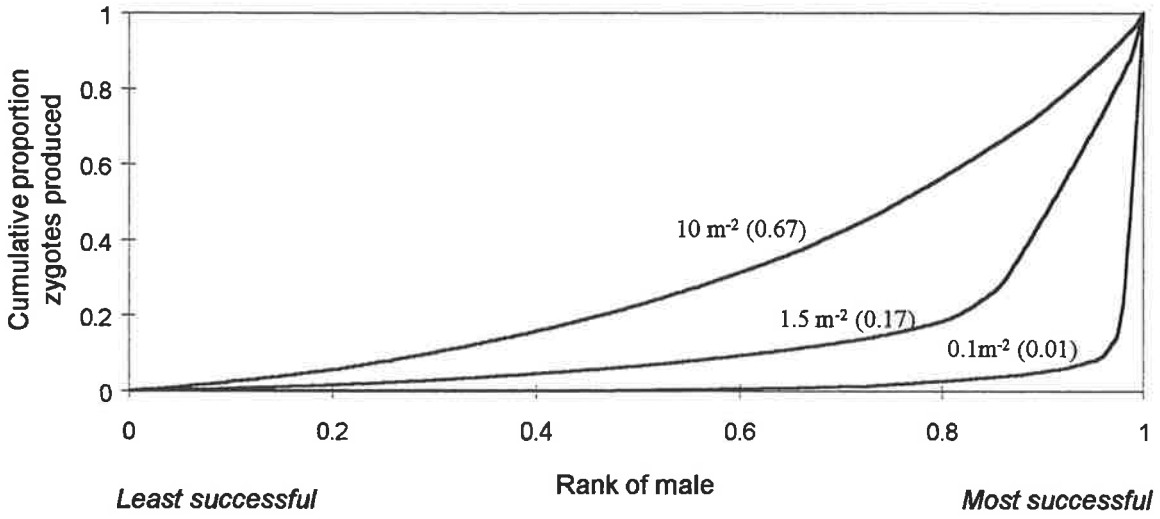


Figure 6.3. The effect of sex , male spawning rate and spawning environment on modelled levels of standardised variation of individual fertilisation success within populations (n = 1000 females) of randomly dispersed populations of *C.bifrons*. A. Female variation (solid circles) and male variation (hollow triangles) on silt habitat at Largs Bay, male spawning rate = 5.4 x 10^6 sperm sec^{-1} ; B. Female variation on silt habitat at Largs Bay with male spawning rate = 1.5 x 10^6 sperm sec^{-1} (hollow diamonds), 5.4 x 10^6 sperm sec^{-1} (solid circles), and 3.7 x 10^7 sperm sec^{-1} ; C. Female variation (male spawning rate = 5.4 x 10^6 sperm sec^{-1}) in seagrass, Largs Bay (solid diamonds), on sand, Largs Bay (hollow diamonds), in seagrass, Edithburgh Jetty (solid triangles), and on sand, Edithburgh Jetty (hollow triangles).

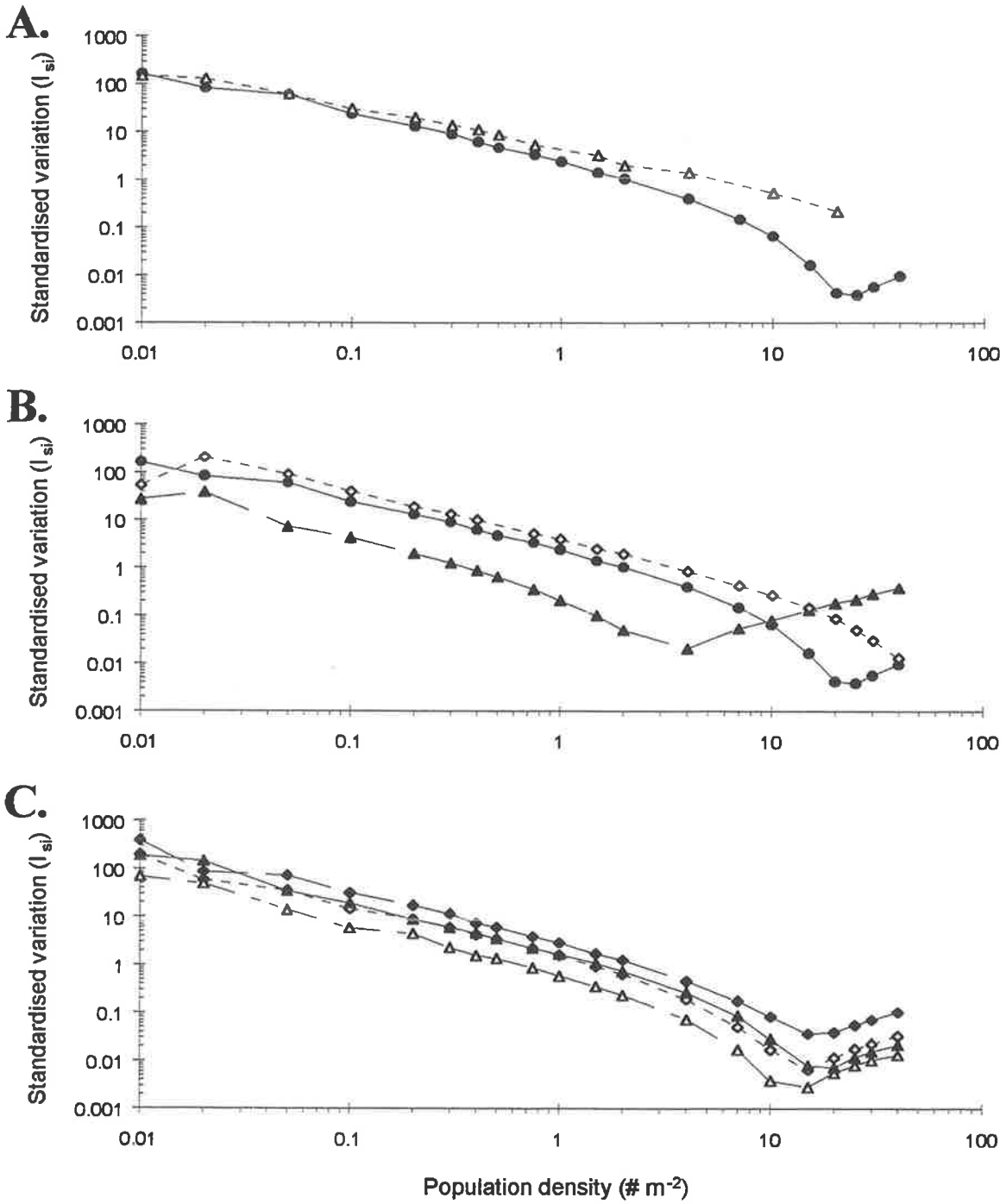
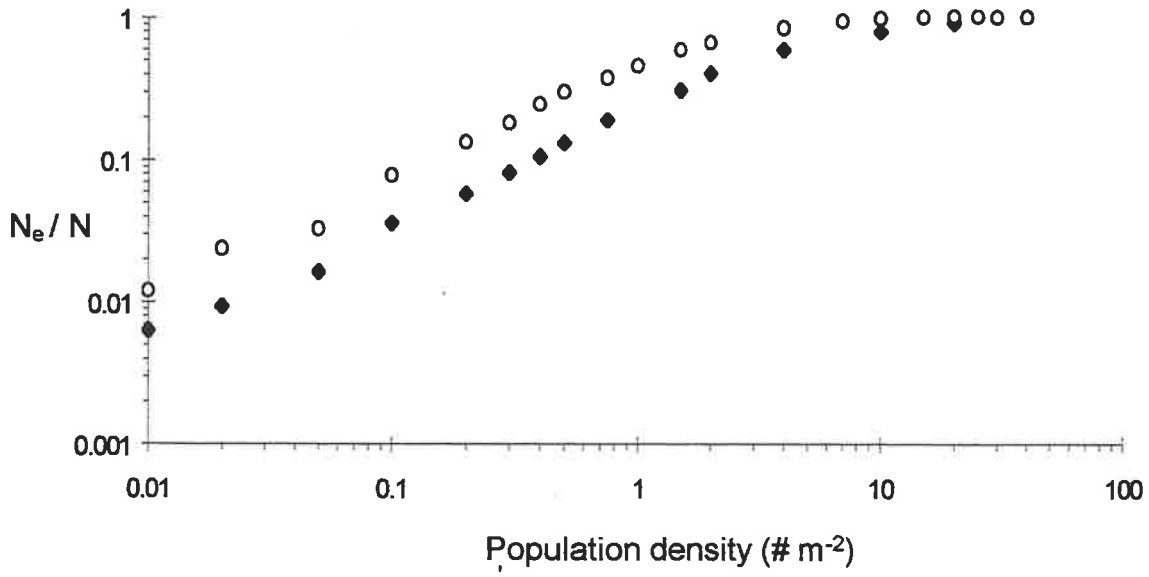


Figure 6.4. Modelled effect of population density on ratio of effective population size (Ne) to actual population size (N). Randomly dispersed *C.bifrons* were modelled spawning on silt habitat at Largs Bay. Effective population size was calculated from modelled estimates of female only variation in fertilisation success (hollow circles) and combined male and female variation (solid diamonds), using equation 7 of Nunney (1996).



Chapter 7

Spatial distribution patterns, multiple spawning events and how these might affect fertilisation success and effective genetic population size of *Chlamys bifrons* at Largs Bay, South Australia.

Introduction

In this cumulative chapter I again model fertilisation success of *Chlamys bifrons*, but this time I estimate fertilisation success within real scallop populations, such as those at Largs Bay. To do this, I sample and incorporate information about the spatial distribution of individuals in a real population into a model which I then use to predict variation in fertilisation success in the Largs Bay population through time and at several spatial scales. Thus, these simulations take into account variation in fertilisation success that results from both the small direct (habitat induced) effects of the place a scallop is spawning, and the natural variation in the localised density of scallops that occurs within the Largs Bay population.

Fertilisation in Real Populations

Natural spatial variation (on a range of scales) in the abundance scallops is of key interest in this chapter because, as illustrated in the last two chapters, such variation in abundance might propagate into variation in fertilisation success amongst groups or individual scallops. In the last chapter I explored how much inter-individual variation in reproductive success potentially might occur within hypothetical populations of randomly dispersed scallops and how this variation might differ between these specific types of populations. However, *a priori*, truly random dispersion patterns at all spatial scales within a *C.bifrons* population must be considered fairly unlikely. In Chapter 1, I noted initial field observations that, at Largs Bay, there appeared to be substantial variation in scallop density amongst habitats (silt, seagrass and sand). I also noted the possibility that there may be significant variation in scallop density within each of these habitats at two smaller spatial scales - amongst patches within habitats and/or within patches. Given that small changes in inter-spawner distance can dramatically affect the fertilisation success of *C.bifrons* (see chapter 5), even subtle differences in the dispersion patterns of real populations (relative to

randomly dispersed populations) might mean that the predictions of fertilisation success in the last two chapters are inappropriate for the Largs Bay population. An obvious step, then, in developing a model of fertilisation in a real population such as that at Largs Bay is to describe how scallops are distributed within it, with a view to then incorporating this information into a simulation model.

Thus, in this chapter I document patterns of *C.bifrons* abundance at Largs Bay, uncovered from field surveys of dispersion patterns made at a range of spatial scales and over time (at 6 monthly intervals over 2.5 years). Specifically, I document how scallop abundance varies across 3 spatial scales - across habitats, across patches within habitat types, and on very small spatial scales (such as within patches) - and how all of these might vary though time. Then, I run further simulations of fertilisation within *C.bifrons* populations, now incorporating information about the precise position of scallops in real field populations. A particular goal was to gain an understanding of how spatial variation in abundance contributes to variation in fertilisation success within natural scallop populations.

The impact of multiple spawning events

As noted in the last chapter, like most scallops, *C.bifrons* can move. Given the small spatial scales over which the success of *C.bifrons* external fertilisation changes (see chapter 5) even small amounts of individual movement will be probably be enough to change the distribution of neighbours such that the fertilisation success of individuals may radically affected. *C.bifrons* also live on average for at least several years (age can be up to 14 years, Wolff and White 1995) and there is now good evidence that they spawn more than once per year (at least twice, and possibly three times - see chapter 4). So, an individual scallop's low fertilisation success in one spawning may not be repeated in at least some of its subsequent spawnings - it and its neighbours may redistribute themselves (even by random walk processes) such that it now attains high fertilisation success. If that were the case, then the variance amongst individuals observed in one spawning event might overestimate lifetime variance in fertilisation success. Thus, to determine by how much lifetime variance might be overestimated, and generally to illustrate the effects of repeated

spawning and individual longevity on lifetime variance in fertilisation, I also run another set of simulations following individuals' fertilisation success over numerous spawning events.

Methods

Measuring scallop dispersion patterns at Largs Bay

Scallop dispersion patterns at Largs Bay were sampled 5 times (at approximately half-yearly intervals) from March 1994 until April 1996. These times (March 1994, October 1994, April 1995, October 1995, April 1996) corresponded with the approximate start and end of each summer's spawning season (see chapter 4). At each time, 4 (replicate) patches of each habitat type (silt, seagrass, sand) were each located by dropping anchor in a haphazardly chosen site and divers swimming in a straight line from this until a distinct, required habitat patch was encountered and sampled. Anchoring sites were interspersed across the whole of the Largs Bay site at each time and chosen by driving at full speed in a straight line from the edge of the sampled population, in a haphazardly determined direction, for a randomly determined time. When searching underwater for habitat patches, divers swam several metres above the bottom, which, because *C.bifrons* are relatively cryptic when viewed from this distance in the often low visibility conditions of Largs Bay (see plates 1.1- 1.3), ensured that sampled patches were chosen without regard to scallop abundance. Habitat types are quite distinct at Largs Bay (see plates 1.1-1.3) and can be assessed whilst swimming several metres above the bottom. The only other criterion for chosen patches was that they were at least as large as 8m x 8m in order to accommodate the rope grid used for sampling.

At each haphazardly chosen patch, an 8 m x 8 m plot was laid out as a series of crisscrossed weighted ropes, each 2 m apart. Pre-measured rings on the rope and use of a measured length of rope as an hypotenuse ensured that the grid was square. Following marking out of the grid, two divers spent up to an initial 5 minutes searching for all scallops (principally *C.bifrons*, but also *C.asperrima* and *P.fumatus* when found) within the grid, labelling each scallop by placing a

numbered rubber band around it and replacing it where it was found. Rubber bands prevented scallops from swimming and divers were able consistently to locate and label scallops before scallops moved. One diver then conducted a focussed search, sequentially moving throughout the entire grid searching within a 1m x 1m quadrat which was lined up against two sides of the 2m x 2m squares throughout the rope grid. The $1m^2$ quadrat was further divided into 10 cm x 10 cm squares which allowed the diver to collect and record the exact position of each labelled scallop at a fine spatial resolution. Later in the laboratory, the size (shell height) of each labelled scallop was measured and its sex determined through examination of its gonads (see also chapter 4). A second diver followed the first through the grid, searching for any scallops inadvertently missed by the collecting diver, and finally both spent an additional 5 minutes searching across the grid for missed scallops. In this way, though *C.bifrons* can be relatively cryptic within seagrass meadows or partly recessed into the seabed on silty areas, all scallops in the grid were recorded. Indeed, though the focus here was to search for adult scallops (> 55 mm shell length; i.e. those that could reproduce), careful searching within 1 m^2 quadrats meant that even recruits and small juveniles (down to less than 10 mm shell length) were recorded (Styan, unpublished data).

Abundance of habitat patches

In order to determine the relative abundance at Largs Bay of the 3 habitat types (silt, sand, seagrass), during April 1996 I ran out a series of 8 x 200 m line transects, each haphazardly placed within the Largs Bay area that had previously been sampled for scallops (see figure 1.1). Again, each transect started at a haphazardly located anchoring site, and proceeded in a straight line in a pre-determined random direction underwater. At 1m intervals, I classified the habitat type (silt, sand or seagrass) directly underneath the tape measure used to run out the transect. Where points lay on transitional areas (i.e. those areas where habitats were merging and/or indistinct e.g. seagrass and silt habitats), I classified according to the dominant habitat type in the area immediately surrounding the point. In most situations, however, habitat changes were abrupt and obvious, making discrimination easy. The number of patches of each habitat type were also calculated from these data, with a patch defined as a continuous run of points classified as the same habitat type.

Analysis of spatial patterns

Rather than simply display results of the surveying of scallops as a lengthy series of maps, two approaches were used to describe how scallop abundance varied on a range of scales - Morisita's Index and nearest neighbour measures.

Morisita's Index (Im) can be defined as

$$\mathrm{Im} = \left(\frac{X}{X-1}\right)\left(\frac{1}{\mu}\right)\left(\frac{\sigma^2}{\mu} + \mu + 1\right)$$

(Morisita 1971)

Where X is the number of individuals in a population, μ is the mean density of individuals and σ^2 is the variance of these amongst sampling units. Statistical testing (based on a chi-square distribution - see Hurlburt 1990) can be done to determine whether Im values differ significantly from 1.0, but it is worth noting here that, because Morisita's Index (Im) is essentially a variance to mean ratio, like other such indices, tests of whether Im =1.0 should NOT be used to determine whether a population is "random", "aggregated" or "more even than random" (Hurlburt 1990). Although many authors continue inappropriately to interpret Im (and related indices such as variance to mean ratios) in just this way (e.g. MacDonald and Badjik 1992, Stokesbury and Himmelman 1993, Goshima and Fujiwara 1994), there are serious flaws in their logic. For a start, failure to detect differences in various indices of spatial pattern (such as Im or variance to mean ratios) from what would be expected in randomly dispersed populations (e.g Thouzeau et al. 1991, MacDonald and Badjik 1992, Goshima and Fujiwara 1994) does not mean that those populations were randomly dispersed at the scales examined (Hurlburt 1990). This may be, firstly, the test may simply not be powerful enough to detect differences from the null hypothesis (e.g., Im = 1.0). Secondly and more importantly, as imaginatively illustrated by Hurlburt (1990), Im values of exactly 1.0 are quite possible under (an infinite number of) non-random dispersion patterns. For them to have some meaning, terms such as "aggregated" or "more even than random" require *a priori* (non-circular) definition (Hurlburt 1990).

As a means of describing spatial dispersion patterns, however, Hurlburt (1990) suggests that Im values (calculated at a range of spatial scales) are informative. Hurlburt (1990) points out that Im values can literally be interpreted as a measure of how much more or less likely it is that two animals (sampled at random) will have occurred in the same sampling unit than if they had been randomly dispersed within a population of X individuals. For example, if an Im value of 2.0 is attained, then this literally means that the probability that two animals drawn at random will have come from the same sampling unit is 2 times the probability of this occurring if the distribution of individuals had been random. Thus, if animal abundance is measured at a range of spatial scales, then Morisita's Index values can be used as a way to describe how animals are dispersed on a range of spatial scales relative to a randomly dispersed population. Here, Im values were compared across a range of scales - across populations (sampled at different times), between habitat types and at several spatial scales within survey plots. Because they are the small spatial scales in which *C.bifrons* fertilisation operates, of key interest were Im values at small scales (0.5 x 0.5 m quadrats, 1 x 1 m quadrats and 2 x 2 m quadrats).

Nearest neighbour distances were also calculated for scallops in each survey plot. Nearest-neighbour measures can be particularly sensitive to edge effects (because scallops near the edge of plots are constrained to having nearest neighbours within plots, whereas in reality the nearest neighbour may be an unmeasured animal just outside the plot) and so these measures are open to some biases (Pielou 1977). To avoid this potential bias, and rather than constraining nearest neighbour measures to a subset of scallops only near the center of plots (e.g. Krebs 1989, Stokesbury and Himmelman 1993), I treated plots as being toroidal (i.e. nearest neighbour measurements can "loop" around plot borders, continuing on opposite sides). Thus, this treatment assumes that the 8 adjacent 8m x 8m plots around a central (mapped) plot (which were not mapped but could have been) all contain exactly the same distribution of scallops as the central mapped plot (i.e. the mapped plot was imagined to be the central plot of a 3 x 3 array of identical plots). This is equivalent to making the assumption that the distribution of scallops within the central plot was repeated regularly across the whole patch in which the plot was situated. Note that in this treatment, the nearest neighbour of a scallop at the edge of a plot may in some cases be a scallop near the edge on the opposite side of the plot. The primary reason for this approach

(and not constraining NN estimates to a subset of scallops near the centre of plots) was so not to preclude estimation of NN-distances for the large number of individuals (particularly those in low density patches) whose nearest-neighbours would otherwise have been further away than plot borders.

As a method of testing for randomness in the pattern of scallop distribution on a small (within patch) scale, I conducted a series of randomisation tests based on (toroidal) nearest neighbour distances. In essence, these tests compared the distribution of scallops within patches (measured by nearest neighbour distances) in the Largs Bay population with hypothetical, simulated populations where scallops were randomly dispersed on the patch scale. For each individual scallop in the surveyed real populations I determined the (toroidal) distance to its nearest and second and third nearest neighbours. Next, to determine what (toroidal) nearest neighbour distances would be if scallops had simply been dispersed at random within patches, a series of simulations were run in which scallops were randomly placed (using a random numbers process) within an 8m x 8m plot at a density equal to that which a real scallop experienced within the surveyed plot. Within the simulated plot, a scallop was randomly chosen, and the (toroidal) distance to its 1st, 2nd and 3rd nearest neighbours measured. This whole process was repeated 1000 times at each plot density, thus creating a frequency distribution of nearest neighbour distances across a range of patch densities. I then determined where the measured nearest neighbour(s) distances of each of the real (surveyed) scallops would occur within the frequency distribution of NN-distances found in the simulations of randomly dispersed scallops at the same plot density as the real scallops. Using a 5% significance criterion, a NN distance was considered significantly less or greater than those in randomly dispersed population if it occurred within the upper or lower 2.5th percentiles of the 1000 simulation runs.

Incorporating dispersion patterns into models of fertilisation at Largs Bay

Dispersion patterns of real scallop populations (at Largs Bay) were then incorporated into a fertilisation model that worked in a similar way to that constructed for the (hypothetical) randomly dispersed scallop populations in chapters 5 and 6. In this series of simulations however, each 8m

x 8m mapped plot from the surveys was used as a patch where spawning took place. Note that when maps of scallop dispersion were being made (see above) the direction of tidally driven currents was taken into account when setting up the grids, so that grids were lying square with the direction of flow. Each survey plot was used twice in the simulations - once with the flood tide moving across the survey plot and once on the ebb tide; each of these was treated as separate, independent plot. Thus, there were 8 maps of each habitat in each survey period, though not all maps contained scallops for which fertilisation could be estimated.

In each survey plot, males were simulated releasing sperm at 5.4×10^6 sperm sec^{-1} (the average maximum release rate measured in chapter 4). How this sperm then dispersed depended on where spawning was taking place - which was either in a seagrass, silt or sand area. Average sperm plume dispersion models (based on empirical measures of average dye concentrations and plume width estimates at Largs Bay - see last chapter) were used to determine how this happened and, as in the randomly dispersed population model, eggs were assumed to stay where they were released by females. The amount of sperm present (from all males' plumes in the plot) where each female released eggs was summed and the fertilisation kinetics model developed in chapter 3 used to determine the eggs' fertilisation success (as in chapter 5). Again, male fertilisation success was calculated by first determining the fertilisation success of each female and the contribution each male made to this female's fertilisation success (this was proportionate to the amount of sperm he contributed to the total sperm pool there), and then summing across females for each male.

I considered that the fertilisation success of scallops that were inside, but near the edges of plots could not be reliably determined because they may have had neighbours outside of the plot that might contribute significantly to their fertilisation and which (because they were unmapped) I would not know about. So, to avoid this potential underestimation, I ignored those scallops near the edges of plots (though of course they still could affect the fertilisation of other scallops within their plots) and estimated fertilisation success only for individuals for which I could be sure that all relevant neighbours had been mapped. Thus, only scallops within a central bordered region had their fertilisation success estimated. The bounds of this central region in each habitat were set

such that the maximum sperm that could be contributed by an unmapped male, just outside the plot, to a point at the edge of the border was just 0.001 sperm μL^{-1} (equivalent to less than 0.1% fertilisation chance).

Variation in fertilisation success

As well as simply estimating the variation in fertilisation success amongst individuals, I also attempted to estimate how much of this variation was generated by the variation in spatial abundance of spawners at several spatial scales: variation in local, very small scale densities amongst individuals within patches; variation in density amongst patches within habitat types; and variation in density amongst habitats. This was done in a way similar to standard methods of partitioning variance amongst heirachical levels in ANOVA (e.g. Sokal and Rohlf 1995); variation amongst individuals was considered to be the result of small scale variation in localised density within patches, plus variation in density amongst patches, plus variation in density amongst habitats. To estimate variance due to variation amongst patches and habitats, all individuals within a patch were assumed to achieve the average success of measured individuals within that patch (i.e. zero variance within patches). Similarly, to estimate variance due to density at the habitat scale, all individuals within a habitat were assumed to achieve average success of measured individuals within their habitat (i.e. zero variance amongst individuals or patches within a habitat). The proportion of variance at each level was then calculated as the difference between subsequent levels divided by the total variance observed (amongst individuals).

Estimates of standardised variation were again used with equation (7) of Nunney (1996; see chapter 6) to estimate the ratio of effective genetic to actual population size (N_e / N).

To examine how multiple spawning events (with intervening redistributions of scallops within a population) affects the lifetime variance in fertilisation success amongst individuals, I ran another set of simulations (again, Visual Basic™ macros were written for Excel™). First, I made the assumption that the frequency distributions of sampled individuals (predicted in the model above) were representative of the way fertilisation success is distributed amongst individuals within a

population during a single spawning event. Note that male and female distributions from the pre and post die-off populations (see below) were treated separately. Then, over a number of sequential spawning events, individual scallops were randomly assigned their fertilisation success in each event from the base frequency distributions. This was repeated for 2000 individual scallops of each sex -in each simulation, after which the standardised variances and then N_e/N were calculated (as above). This whole process was repeated for a range (1 to 21) of spawning events occurring during a scallop's lifetime.

Results

The spatial distribution of scallops at Largs Bay

The area surveyed at Largs Bay consisted of nearly equal proportions of (interspersed) silt, seagrass and sand patches. The overall ratio of the habitat types in the 8 x 200m transects was 0.34 : 0.37 : 0.29 (seagrass : silt : sand). There were more sand patches (n = 35) recorded in the area surveyed than either silt or seagrass patches (n = 28, 26 respectively). Accordingly, measured dimensions (mean ± std.dev.) of sand patches were much smaller (12.7 ± 9.1) than both silt and seagrass patches (20.2 ± 23.6, 22.7 ± 20.8 respectively).

Two broad scale patterns of scallop abundance through time in the three habitat types are illustrated in figure 7.1. As also indicated in the ANOVAs in table 7.1, scallop abundance varied considerably amongst habitats and the overall abundance of scallops also varied through time. Scallops were essentially absent in sand, but present in silt and seagrass and the abundance in silt patches was about twice that in seagrass at each time sampled. Overall density of adult scallops was low and changed through time, with the largest change (overall abundance dropped by ~60%) occurring between the October 1994 and April 1995 surveys. The patterns in figure 7.1 and the non-significance of the habitat x time ANOVA interaction terms in table 7.1 suggest that the change in density occurred in fairly a uniform way across the silt and seagrass habitats.

The large change in abundance between the October 1994 and April 1995 surveys coincided with an apparent die-off of scallops that I observed in March 1995. During several dives over the period of 1-3 March, a seemingly large proportion of scallops were observed to be moribund and large numbers of scallops were being preyed upon by the large whelk *Pleuroploca australis*. On one dive during this period, I observed 34 scallops being attacked by *Pleuroploca*. This is an extraordinarily high number; normally, when dives covering similar areas were conducted for this work and that in chapter 4, a total of only one or two scallops would be observed being attacked in the numerous dives (>10) made each month. Though the exact causes of this die-off are unknown, it appeared that the scallops were sick and /or stressed, which in turn increased their susceptibility to attack by whelks, rather than whelk predation *per se* causing the mortality. Indeed, during this period the mantle tissue of many scallops was retracted and their valves did not close rapidly when approached by a diver (as is the normal behaviour of healthy scallops). It is strongly suspected that this brief period of mortality accounted for the change in overall abundance that was observed between October 1994 and April 1995

Population size (age) structure did not appear to change across the times surveyed here, as illustrated in figure 7.2. Mean size of adult scallops (those greater than 55 mm) was remarkably constant, even between the October 1994 and April 1995 surveys, suggesting that mortality was relatively independent of individual size during the putative die-off event.

A fortuitous (for me, not the scallop population !) by-product of this large change in scallop numbers is that it allowed me to compare two quite different scallop populations at Largs Bay - before and after the die-off. Consequently, I have treated samples taken in March and October 1994 as representative of a "pre die-off " population and samples taken in April and October 1995 and April 1996 as representative of a "post die-off" population. Shown in table 7.2, mean adult scallop densities for these were 0.133 and 0.059 scallops m^{-2}. Mean (± std. dev.) scallop densities in silt and seagrass habitats were 0.270 (± 0.124) and 0.124(±0.049) in the pre die-off population and 0.127 (± 0.054) and 0.046 (± 0.050) in the post-die-off population. The maximum density recorded in any one 64 m^2 survey plot was 0.512 scallops m^{-2}, in a silt patch in March 1994.

There was evidence that, within populations, scallops were much more likely to be found in some patches than would be expected if they were simply distributed amongst patches in a population in a random manner. Morisita's indices at this scale (i.e. treating 64 m^2 survey plots as sampling units) were (detectably) greater than 1.0 (see table 7.2). Im values calculated for a range of smaller quadrat sizes are also shown in table 7.2 - note that these Im values also take into account the distribution of scallops amongst survey plots. Calculated at a range of sampling scales, in the pre-die-off population scallops were 1.9 to 3.7 times more likely to be found together than they would be in a random spatial distribution. In the post die-off population, Im values ranged from 2.09 to 0.86. In both populations, at the smallest sampling scale (0.25 m^2 quadrat), Im values were lower and not detectably different from 1.0.

Considering scallops in silt and seagrass habitats separately, Im_{64} (i.e. treating survey plots as replicate samples) values significantly greater than 1.0 were found for scallops in silt in the pre die-off population (Im_{64} = 1.31), in seagrass in the pre die-off population (Im_{64} = 1.22), and in seagrass in the post-die-off population (Im_{64} =1.96). A non-significant Im_{64} (1.18). was found for scallops in silt in the post die-off population.

Considering spatial patterns on a smaller, within plot scale (i.e. treating each plot separately), figure 7.3 shows Im values measured across an hierarchical range of smaller spatial scales, indicating how estimates of Im varied with plot density. The dotted lines on each of these plots indicate the 95 % confidence limits of an Im = 1.0, based on mean scallop density and the number of quadrats within a plot that could be used to estimate this (in a 64 m^2 plot there were 256 x 0.25 m^2 quadrats, 64 x 1 m^2 quadrats and 16 x 4 m^2 quadrats). These confidence intervals were used to assess the significance of each Im estimate. $Im_{0.25}$ was below 1.0 for most plots but estimates of $Im_{0.25}$ were only detectable as significantly less than 1.0 once plot density was greater than about 0.18 scallops m^{-2}; this occurred for 9 plots. There were also 4 plots in which the estimate of $Im_{0.25}$ was greater than 1.0 and which were outside the upper 95 % confidence intervals of $Im_{0.25}$ = 1.0. Estimates of Im_1 were mainly (though not uniformly) lower than 1.0 at low densities (< 0.2 scallops m^{-2}). At higher plot densities (which were mainly silt plots) Im_1 estimates were greater than 1.0, however, none of the Im_1 estimates (except one on seagrass) were outside

the 95 % confidence intervals of $Im_1 = 1.0$. At the largest scale within plots examined (2 x 2 m quadrats), Im_4 estimates were evenly spread above and below 1.0 and all well within the 95 % confidence limits of $Im_4 = 1.0$.

The frequency distributions of (first) nearest neighbour distances within seagrass and silt habitats in the pre and post die-off populations are illustrated in figure 7.4. Mean nearest-neighbour distances were broadly correlated with mean densities in each of the habitat/populations (i.e. silt - pre die-off < silt - post die-off ≅ seagrass - pre die-off < seagrass -post die-off).

The number of instances where (1st, 2nd and 3rd) nearest neighbour distances of sampled scallops were detected as either greater or lower than those of a randomly dispersed population (using a 5% significance criterion) are presented in table 7.3. There was little evidence that NN_1 distances differed from those expected in a population of scallops in which individuals were randomly dispersed within plots. However, there were more NN_2 and NN_3 distances detected as significantly less (at the 5% level) than would be expected if scallops were randomly dispersed within plots. Most strikingly, of the pre die-off scallops in silt habitat, 11 out of 145 sampled individuals had second nearest-neighbour distances detected as significantly less than what would have been expected (i.e. using a 5% significance criterion, 3.625 tests would have been expected to be significant if scallops were randomly dispersed). Similarly, 8 out of 145 tests of NN_3 were significant for scallops on silt in the pre die-off population. The remaining comparisons also produced slightly more significant results for NN_2 and NN_3 distances than expected if scallops were simply randomly distributed within plots.

The sex ratio of all scallops collected during sampling (i.e., pooling times and habitats) was approximately one, although there were also a small number of scallops whose sex was indeterminate as they had been castrated by a larval (bucephalid) trematode that infests scallop gonads (see also Sanders 1966, Sanders and Lester 1981). The ratio of male : female : castrated was 0.490 : 0.465 : 0.035 (n = 353 individuals). That ratio was more or less repeated in both the pre and post die-off populations when these were treated separately - (0.531 : 0.441 : 0.028 , n = 211 individuals) and (0.451 : 0.50 : 0.049 , n = 142 individuals) respectively. Pooling across

populations (times), the sex ratio of scallops in silt habitats (0.488 : 0.467 : 0.045 , n = 244 individuals) was similar to that of scallops in seagrass (0.52 : 0.449 : 0.02 , n = 98 individuals). At a smaller scale, however, significant skews in sex ratios occurred in some patches which represented something more than just sampling error imposed by taking samples of relatively low numbers of individuals. Considering only patches in which there were > 6 scallops, 5 of 23 patches had a ratio of male : (female and castrated) that was outside the 95 % confidence intervals predicted by simple binomial sampling of individuals from a population with the same sex ratios as the overall Largs Bay population. Four significant results more than would have been expected (1) with an alpha level of 0.05, lead to a rejection of the null hypothesis that a process of non-selective binomial sampling could be used to predict the sex of scallops within patches.

There was also some evidence that the distribution of (trematode) castrated individuals was non-random amongst habitats and patches. Seven patches each contained 1 castrated individual; 5 were silt patches and 2 were seagrass. Additionally, two other silt patches (each with 16 individual scallops) contained 3 castrated individuals. The frequency of castration was greater for scallops in silt (11/244 = 0.0451) than for those in seagrass (2/102 = 0.0196) and sand (0/7 = 0), although not significantly so (Chi-square test; p=0.465). Similarly, a Chi-square test failed to detect a difference in castration rates amongst the 20 silt patches surveyed (p =0.254). However, the reliability of both of these tests is cast into some doubt given the low castration rates (meaning that only very large differences would have had been able to be detected) and the unreliability of chi-square analyses when expected frequencies are as low as here (Zar 1984). Nevertheless, given that the frequency of trematode castration within silt inhabiting scallops was 0.0451, then the (binomial) probability that a silt patch of 16 scallops would contain 3 castrated individuals simply by chance is very low (p = 0.005). This happened twice in a small sample of patches, suggesting that scallops within some silt patches were much more likely to have been castrated than those in other silt patches.

Fertilisation at Largs Bay

Overall, average fertilisation success within both populations was low (see table 7.4). Average fertilisation success of both males and females differed between the two populations, with significantly higher average fertilisation success (both female and male success) occurring in the pre die-off population than in the post die-off population.

Within populations, there was some evidence that fertilisation varied between scallops in different habitats. Only a few individuals were found on sand habitats, and being essentially isolated, they all failed to reproduce successfully (5 females, 2 males). The average rate of fertilisation of females on silt habitat in the pre die-off population was 0.085 (0.049, 0.121), n= 73 and for males 0.074 (0.037, 0.112), n= 84 (where values in brackets are 95 % confidence intervals and n is the number of scallops for which fertilisation was predicted). In the post die-off population on silt both female and male success was lower, with average female success at 0.013 (0.005, 0.020), n= 67 and male success similarly 0.014 (0.006, 0.021), n=62. In contrast, average rates for females and males in seagrass were consistently low in both the pre and post die-off populations : females' average fertilisation rate was 0.016 (0.002, 0.029), n= 33 and males' 0.011(0.003, 0.019), n=51 in the pre die-off population, and in the post die-off population these rates were 0.045 (0, 0.099), n=18 and 0.031 (0, 0.064), n=29 for females and males respectively.

Average fertilisation rates within patches (shown in figure 7.5) were generally low and varied with patch density, although this relationship was not very strong as several seagrass patches showed abnormally high rates compared to other patches at the same density. Note that in many instances (particularly for lower density plots) few individuals were sampled per plot so standard errors are quite large (but left off this figure for clarity).

Variation in fertilisation between scallops pooled across habitats accounted for 27% and 29% of total female and male variation in the pre die-off population and 6% and 29% (female, male) in the post-die -off population, whilst variation in fertilisation between patches of scallops accounted for 11% and 17% of total female and male variation in the pre die-off population and 68% and 9%

(female, male) in the post-die -off population. The remaining variation (61% and 53% in the pre die-off population and 26% and 63% in the post-die -off population) was attributed to variation in success that occurs amongst individuals within patches.

As indicated by the high estimates of standardised variation (shown in table 7.4), reproduction was highly skewed within both pre and post die-off populations, with a small number of individuals achieving high success and accounting for most of the (predicted) larval production of the scallops sampled. The large degree to which reproduction was skewed amongst all individuals within both pre and post die-off populations is illustrated in figure 7.6. Estimates of standardised variation increased with a decrease in density. Based on these estimates of standardised variation, N_e/N was substantially lower than 1.0 in both populations and, corresponding to changes in standardised variation, the reduction in population density after he die-off resulted in a reduction in N_e/N, as illustrated in figure 7.7.

Figure 7.7 also clearly illustrates the large effect that repeatedly sampling from even highly skewed distributions can have on estimates of the lifetime variation in fertilisation success amongst individuals (in figure 7.7 this variance is represented as the ratio of N_e/N, but standardised variance is effectively just the reciprocal of this). Much as would be expected, the simulations predicted a large decrease in the lifetime variation in fertilisation success amongst individuals as these individuals spawn (and redistribute themselves) repeatedly. The effect this has on the effective population size is greatest over the first few spawning/redistribution events.

Discussion

The spatial distribution of C.bifrons at Largs Bay

On a broad spatial scale, scallops are usually found in distinct areas of higher abundance (i.e. beds) relative to abundance on surrounding areas of seafloor (see reviews in Brand 1991 and Oresanz et al. 1991). This is true also of *C.bifrons* and, though no specific attempts were made in

this study to precisely delimit boundaries, experience diving around the area suggested that most scallops in the Largs Bay population occurred in the general area outlined in figure 1.1. Given that external fertilisation is a process operating on fine spatial scales (see chapter 5), it was scallop dispersion patterns on similarly finer scales that were of most interest here.

At larger, within population, spatial scales, both amongst habitats and amongst larger areas (patches) within habitats, *C.bifrons* were (on average) much more likely to be found in some places than would be expected if they were simply distributed amongst areas in a population or within habitats in a random manner. *C.bifrons* were found predominantly in silt areas and though they were also found in seagrass (*Posidonia sp.)* meadows, they were only very rarely found in sand patches. These broad-scale patterns persisted even when a an abrupt, heavy mortality occurred, which suggests that either the die-off affected individuals within the whole population equally (i.e. there were no density-dependent or habitat-dependent effects) and /or scallops redistributed themselves rapidly to reform patterns after the change in density. There was little evidence to suggest that, during this die-off, mortality was either size or density dependent; the frequency distributions of adult size did not change greatly over this period (figure 7.2), nor did nearest neighbour distances (figure 7.4) in silt and seagrass habitats appear to be affected unequally by the reduction in density, despite there being large differences in the density of scallops in these habitats.

The broad-scale dispersion patterns uncovered here are also in line with the results of Wolf and White (1997) who found that, at a location in Tasmania, *C.bifrons* was more common in seagrass habitats than adjacent sand habitats ("silt" or muddy habitats were not present in his study area). Elsewhere in the world, other scallop species have been similarly reported to be more common in some habitat types than others, though the specific patterns of abundance across habitat types varies considerably with different species (e.g. Caddy 1970, Schneider et al. 1987, Thouzeau et al. 1991, Goshima and Fujiwara 1994, Stokesbury and Himmelman 1995). Indeed, a stark contrast also occurs amongst the two *Chlamys sp.* found in South Australia. *C.bifrons* is found on soft sediment habitats (in ways outlined above and discussed below), whereas *C.asperrima* is

largely restricted to hard substrata on reef habitats and is usually found in large, dense aggregations of individuals (Pitcher and Butler 1987, Chernoff 1987; see also plate 1.4).

Numerous studies (e.g.Caddy 1970, MacDonald and Badjik 1992, Stokesbury and Himmelman 1993, Goshima and Fujiwara 1994) have presented evidence that, within beds, various scallop species are distributed in ways other than randomly dispersed. For example, Stokesbury and Himmelman (1993) describe the giant scallop, *Placopecten magellanicus*, as being randomly dispersed within one 16 x16 m plot at one higher density site, and aggregated within another lower density plot at a site in the Baie deChaleurs, Canada (but see earlier cautions about such classifications). Within a well defined *P.magellanicus* bed at another location, and using different sampling and analytical methods, MacDonald and Badjik (1992) detected "clumping" (their interpretation of significant Im values) at moderately small spatial scales (1.1 to 4.5 m^2). Here, using Morisita's indices calculated over a range of spatial scales I found evidence that *C.bifrons* at Largs Bay also was non-randomly distributed on several spatial scales. Similarly, the randomisation test involving NN measures lead to the rejection of the null hypothesis that on small scales (i.e. within 64 m^2 plots) scallops were distributed in ways similar to randomly dispersed populations.

There were also significant skews in the sex ratios of adults within survey plots (both male and female biased) and parasitic trematodes appeared to be found more frequently in some groups of scallops than would be expected if the likelihood of infection was uniform across the scallop population. These last patterns, in particular, appear to be novel and, because of their obvious implications for external fertilisation, may warrant further examination in the future. To my knowledge, similar fine scale patterns have not been described for any other scallops. In the only comparative study which I could find, MacDonald and Badjik (1993) found no evidence to suggest that on small spatial scales sex ratios in *P.magellanicus* were other than 1:1.

At most spatial scales, scallops were between 2 and 3 times more likely to be found in association with another scallop, than if they had been dispersed at random across the Largs Bay area. Significantly high Im values also indicated that scallops were grouped into habitats (mainly silt),

and within these, more commonly found in some patches than others. This would seem to make sense in terms of a scallop wishing to maximises its fertilisation success -being near other scallops should increase the chance of successful fertilisation and traits that promote this should be selected for. However, the neat story changes somewhat when very small scales are considered (i.e. sampling units of 0.25 and 1 m^2).

Although there was strong evidence that scallops were aggregated at larger spatial scales, at the smallest spatial scale examined (0.25 m^2) there was no evidence that scallops were found together more often than if they had been randomly dispersed across the entire Largs Bay location. When dispersion patterns within each plot are examined on these fine scales, if anything, scallops appear to be less likely than random to be found in close association with each other. Unfortunately, given the low densities of scallops in many plots, the 95 % confidence intervals about Im estimates were very wide and hence the power of tests to detect deviations from $Im_{0.25}$ =1.0 was very low. Nonetheless, values of $Im_{0.25}$ were found to be both significantly greater and less than 1.0 in separate, replicate, plots and, if nothing else, this clearly illustrates that natural dispersion patterns for scallops can vary within a population and that, to describe such patterns adequately, sampling must be replicated across the population. The patterns of most significance are those in the higher density plots, for these contain most of the scallops in the sample population. $Im_{0.25}$ were generally lower than 1.0 for these plots, but, in contrast, Im_1 were greater than 1.0.

Given the way *C.bifrons* fertilisation varied with very small inter-spawner distances (outlined in chapter 5), it will be the frequency of co-occurence of scallops on the smallest scales examined here that should largely determine population rates of fertilisation success. It seems strange then, that scallops should not be grouped together on these scales and even avoid each other on this scale -a strategy which might be expected to decrease fertilisation success! It may be that scallops have very little say in the matter - perhaps dispersion patterns are enforced on scallops by some extrinsic factor. For example, in silt habitats, I often noticed *C.bifrons* individuals recessed in the lee of large *Pinna* shells (see plate 1.3). This might be because the large shells obstruct the movement capabilities of scallops (i.e. the scallops get stuck there), or perhaps

because scallops actively chose to stay there (maybe feeding is enhanced in the turbulent wake of the large shells). In either of these cases, it might be the dispersion patterns of *Pinna* that are being reflected in the dispersion patterns of the scallops.

As noted by Allee (1931) and reiterated by Levitan and Young (1995), there also may be costs for *C.bifrons* that are associated with aggregating on small scales - for example, decreased growth rates through competition, increased pathogen spread (or trematode castration rates), higher predation rates, or maybe simply the energetic cost of moving often enough to find and maintain contact with other scallops in low density populations is too great. In chapters 2, 3 and 5 I also highlighted another potential cost that scallops might suffer when they are too close to other spawners, namely, the potential for polyspermy. Models of sperm dispersal in chapter 5, indicated that if a female scallop were very close (i.e. adjacent) to male spawners, then her eggs would likely suffer high levels of polyspermy. Such costs will be traded-off against the benefits of being close (such as high fertilisation success) and it may be that, in the case of *C.bifrons,* the costs of being close to other scallops on very small scales simply outweigh any benefits in fertilisation success. Obviously, further work would be required to develop an understanding of how (and why) such small scale patterns are generated.

A description of the nearest neighbour distances and, in particular, the frequency of NN distances within a population is probably more intuitively useful as a way to describe the spatial pattern of individuals within a population than using Morisita's Indices, at least in the context of predicting fertilisation success. Given information about how inter-spawner distance affects fertilisation (chapter 5), this information can easily be interpreted in terms of how this might functionally affect fertilisation success (although see cautions about such interpretations in chapter 5). For example, the low average success and large skews in the reproductive success of individuals both before and after the die-off would be expected from an examination of the distribution of nearest neighbours shown in figure 7.4. Those plots indicated that only a very small proportion of the scallop population (as sampled - see below) were likely to be within the very short critical distances of each other necessary to achieve a high fertilisation chance. Of course, nearest neighbour estimates here (using toroidal plots) were not unbiased measures, simply because of

the way in which the distances were calculated (and may not have been representative - see below). Ideally, nearest neighbour measures should be measured in very much larger plots than in this study, negating the need for modifications such as assumptions about a toroidal nature for plots and/or minimising the "edge effects" that might be imposed if nearest neighbours within a (non-toroidal) plot are measured. The field effort involved in this, however, would be very great.

The focus of this chapter was simply to describe these sorts of patterns and then to use this information in a model of fertilisation success within real *C.bifrons* populations, but understanding how these patterns are generated may be of some future interest. Perhaps with an understanding of the underlying mechanisms, it would be possible more precisely to describe the spatial dispersion patterns of *C.bifrons* and/or to estimate sampling errors involved in measuring these distributions (see below).

In terms of trying to explain how patterns of spatial distribution are generated, the mobility of scallops is obviously one factor that needs to be considered. Indeed, numerous studies have examined scallop movement and its potential role in generating dispersion patterns (e.g. Hartnoll 1967, Peterson et al. 1982, Winter and Hamilton 1985, Minchin 1989, Carson et al. 1995, Carson et al. 1996, Stokesbury and Himmelman 1996, Arsenault and Himmelman 1996a, Hamilton and Koch 1996). Wolf and White (1997) recently suggested that differential movement and predation of adults in different habitats might account for the differences in abundance of *C.bifrons* in different habitats at a location in Tasmania (though the field experiments on which they based these conclusions were severely limited by poor replication and a lack of appropriate controls). Focus should not, however, be exclusively on movement, as dispersion patterns at a range of scales are unlikely to be controlled by just one factor. A large amount of work on numerous scallop species in soft-sediment/seagrass systems has illustrated that differential predation in habitats either as adults or juveniles and settlement processes can all also be important determinants of adult distribution patterns (see reviews in Brand 1991 and Oresanz et al. 1991, Pohle et al. 1991, Garcia-Esquivel and Bricelj 1993, Barbeau et al. 1994, Arsenault and Himmelman 1996b, Hatcher et al. 1996).

Whatever the proximate causes of the spatial distribution of *C.bifrons*, it seems likely that the mechanisms involved will be complex and will require a great deal of work to elucidate them. Indeed, some patterns of scallop dispersion uncovered here are just baffling. For example, it is very difficult indeed even to propose plausible models to explain patterns such as the localised sex ratio skews that occurred in some patches.

Models of fertilisation success

As in models in the previous two chapters, numerous assumptions were made in the construction of the models of fertilisation here. Again, potential sources of additional variation in fertilisation (such as variation in the dimensions of individual sperm plumes, genetic incompatibilities, etc) have all been ignored and average sperm dispersal plumes used in the models. I have, again, also assumed that all individuals spawn synchronously (and of course in chapter 4 I suggested that complete synchrony was quite unlikely!) - this should lead to a conservative overestimation of effective population density and consequent fertilisation success. Here, I will not re-discuss the implications of these assumptions for estimates of fertilisation success, except to stress again that the only way to validate (or invalidate) predictions made here is to assess fertilisation in the field during real spawning events. This remains an unfulfilled challenge.

It is very important to recognise that the dispersion patterns described here and used in the simulation models constitute sample distributions of how the scallops at Largs Bay were dispersed. If these samples are used to make inferences about the entire population there, then there will be some degree of error in these inferences. It was impossible to estimate those errors because it is not known how scallops are distributed (except that they are not distributed randomly). Instead, I had to assume that the dispersion patterns sampled here were representative of how scallops at Largs Bay were distributed, ignoring any error associated with sampling. If this assumption was incorrect, then it will constitute a major source of error in the predictions made here about fertilisation success within the Largs Bay population.

In very large randomly dispersed populations (such as those modelled in chapters 5 and 6), there are by chance always some individuals that will be very close to each other (exactly how many depends on the density of the population). Particularly in low density populations, it was these rare, close individuals that generally had high fertilisation success rates (except when polyspermy took effect) and these individuals disproportionately contributed to the overall fertilisation success of a population. Although scallops in the (real) Largs Bay populations were more frequently found in some patches than would be expected, on the very small scales that are likely to be the most important determinants of fertilisation success (particularly 0.25 m^2), pairs of individuals in the same area appeared to be relatively uncommon, as indicated by low $Im_{0.25}$ values. This, of course, may simply have been sampling error (indeed, I often could not reject the null hypotheses that $Im_{0.25}$ values were equal to 1.0), but in the models of fertilisation this will be treated as a real absence of close, successfully fertilising individuals.

In terms of fertilisation success across a population, what really counted was fertilisation in the few highest density patches of scallops (which were mainly found in silt habitats), obviously because this is where most scallops were. Indeed, a large part of the variability in fertilisation success was generated amongst individuals within plots, despite there being large differences in density amongst survey plots. Again, sampling error may have meant that rare higher density patches (perhaps even greater than the highest recorded here ~ 0.5 scallops m^{-2}) were not included in the surveys. Should such (unsampled) high density patches have existed, then it is likely that predicted fertilisation across Largs Bay would have been much higher. Not only would those (unsampled) high density patches contain a large number of scallops, but, based on the positive relationship between plot density and average fertilisation success illustrated in figure 7.5 (and models in chapter 5), then (on average) the fertilisation success of each of these more numerous individuals in the higher density patches would be likely to be relatively higher than in the lower density (surveyed) patches included here.

The problem of potentially misrepresenting fertilisation in the real populations by missing important, closely spaced individuals is largely unavoidable. By definition, rare individuals (or rare, high density patches) are difficult to detect. That particular problem is, however, avoided in

models where spatial distribution patterns of individuals are known precisely (as in the models of hypothetical randomly dispersed populations in chapters 5 and 6), and because in those models thousand of virtual individuals can be simulated, thus ensuring rare but important events are captured in the models. One approach to circumvent this problem may have been to attempt to fit the data I collected to various distributions (other than a Poisson) such as the binomial with various exponents (e.g. Stokesbury and Himmelman 1993). Then, given a known distribution of scallops, the probability of not sampling various scallops (or patches of scallops) could have been determined. However, this would use the same flawed logic cautioned against earlier (Hurlburt 1990). The distribution of scallops within Largs Bay could be any of an infinite number of distinct distributions (binomial or otherwise) - simply failing to reject a null hypothesis that the distribution of scallops was not different from a particular, nominated distribution would not mean that scallops were distributed in that way.

My own observations from many dives at Largs Bay during the course of the work in this and other chapters (especially chapter 4), is that the survey plots mapped in this chapter were representative of the real population in the field. Certainly, I did not come across any exceedingly high density patches of scallops which were outside the range of densities recorded here.

Regardless of whether I missed (by chance) some scallops that may have achieved high fertilisation success, it is clear that at least some scallops within the Largs Bay population (i.e. most of those that were sampled here) would achieve very low fertilisation success, whilst others would achieve relatively high fertilisation success. Variation in fertilisation success amongst individuals within a population appears, then, to be a fairly fundamental aspect of the process of external fertilisation in *C.bifrons*. Fertilisation success can vary markedly amongst individuals in different habitats, amongst individuals in different patches within habitats, and amongst individuals within the same patches. Again, all this highlights the need for highly replicated, spatially interspersed field measures of fertilisation success in naturally spawning populations. As indicated above, it will not be a trivial task to ensure that unbiased, representative samples of a population are taken.

Notwithstanding the numerous caveats noted above, given the predictions of low average fertilisation success in the real populations at Largs Bay, it is tempting to suggest that, at present densities, that population is unlikely to be self-sustaining. More appropriately (because the larvae of *C.bifrons* take 17-20 days from fertilisation to settlement; Dix 1976), it would seem unlikely that the Largs Bay population would be an important source of larvae for other local populations. Thus, the Largs Bay population might be considered to be a "sink" population in the sense of Pulliam (1988).

Of course, as discussed in chapter 1, it is quite possible that life-stages other than fertilisation may be much more important in population dynamics (i.e. in determining subsequent population numbers). Perhaps populations are sustained even with the low rates of fertilisation success and larval production that were predicted here. In this case, predicted decreases in average success rates such as that which accompanied the die-off seen here may be relatively unimportant. Resolving this will require further studies that "close the loop" and estimate the importance of the full range of other life-stages such as planktonic losses and mortality, mortality during settlement, recruitment and post-recruitment events (Eckman 1995). Such studies will be difficult and for animals like scallops that have widely dispersing larvae will need to be conducted on very large spatial scales indeed (cf. the "basin-scale" studies of Peterson and Summerson 1992, Peterson et al. 1996).

The variance-reducing effects of multiple spawning events

Broadly, in both pre and post die-off Largs Bay populations there was a prediction of reasonably high degree of variation within populations with a few individuals in each population reproducing exceptionally well (>50% of eggs fertilised), most reproducing at rates much lower than this and some completely unsuccessful individuals (which had no eggs fertilised). Assuming the distributions of fertilisation that were predicted in the model were representative of the distribution of individual success within a real spawning event (see cautions about this above), I simulated what would happen to variation in individual reproductive success if individuals spawn more than once in their lifetime (and each time their success is randomly assigned from these distributions).

As expected (from the central limit theorem), provided scallops redistribute themselves between spawning events and their subsequent fertilisation rates are independent of their previous success, then increasing the number of times an individual spawns during its lifetime quickly reduces the amount of predicted variation amongst individuals in lifetime reproductive success.

As discussed in the last chapter, little is known about how thoroughly scallops redistribute themselves within a population between spawning events - they may just move within patches, or between patches within habitats, or scallops may be completely redistributed over the total area of Largs Bay. Sampling of scallop dispersion patterns occurred over a reasonably long time period, at the start and finish of spawning seasons and during this time there was no evidence to suggest that that overall patterns of scallop dispersion changed, except for an overall decrease in density associated with a large die-off event (which appeared to be evenly spread across the population). Unfortunately, I do not have any information about how dynamic these spatial patterns were: whether the positions of high density patches of scallops changed, nor how the composition of individuals within patches may have changed (i.e. did scallops which I recorded here in a high density patch remain in that area, and in a high density patch for their entire lives ?).

It is unlikely, however, that a lack of a detailed knowledge about the dynamics of dispersion patterns is critical here, at least in terms of predicting individual variation in lifetime fertilisation success. In a given spawning event, a large part of the variation in fertilisation success amongst *C.bifrons* individuals occurs amongst individuals within patches. Thus, if scallops redistribute themselves within patches (which will require very little movement on each individual's part), their subsequent fertilisation success should be largely independent of their previous success rates. Consequently, it will functionally matter little if scallops redisperse over the entire population or over smaller, patch scales. Of course, this argument is largely dependent on the specifics of the model developed here and may only be applicable to animals like *C.bifrons* whose fertilisation varies dramatically over very small inter-spawner distances (see chapters 5, 6). For other species whose fertilisation varies less quickly, then larger distances would need to be covered between spawnings for subsequent success rates to be independent of previous success and so for

repeated spawnings to reduce inter-individual variation in lifetime fertilisation (and reproductive) success.

I have suggested here another way in which the size- or age-structure may influence the genetic structure of a population (particularly if population densities are low). Multiple spawning opportunities (interspersed with opportunities for movement and re-dispersion), provide a buffer for individuals to short-term, chance failures in fertilisation success. Thus, the longevity of individuals may directly influence variation in individual reproductive success and, consequently, the effective genetic size of a population. This proposed mechanism is genetically analogous to the demographic importance of individual longevity for population persistence in light of highly variable recruitment success i.e. the "storage effect " (Chesson 1984). This genetic effect may be important to consider in a management sense (see below), but whether these effects actually occur in real populations, again, needs further consideration and field testing.

"Chaotic genetic patchiness", that is, small scale heterogeneities, but larger scale homogeneity in allele frequencies, has been observed in several detailed studies of the spatial and temporal patterns of allozyme variation in several marine invertebrate species (Johnson and Black 1984, Watt et al. 1990, Johnson et al. 1993). Amongst several explanations for these observations (which in some cases were for internally fertilising organisms), Johnson et al. (1993) suggest that such patterns may be created if only a small number of individuals within a population produce larvae and these remain together as a fairly coherent unit until recruitment. In a very similar way, variable fertilisation success might be a mechanism that generates chaotic (temporal) patterns of allele frequencies (Hedgecock 1994a, 1994b). In small, low density populations, chance disproportionate representation of some individuals' offspring in particular spawning events (through sperm limitation effects) might lead to a high variance in allelic frequencies over short time periods. Given changes to dispersion patterns, these particular skews would be unlikely to be consistently repeated in each spawning event. So, whilst there might be large differences in genetic composition of recruits in each separate recruitment pulse, as illustrated above, over numerous spawning events we might expect a lower variance in allele frequencies. Again, whether such a mechanism actually operates remains to be assessed in future field studies.

Allee effects in populations of free-spawners

In models of fertilisation success within populations of a sea biscuit (*Clypeaster rosaecus*), and crown of thorns starfish (*Acanthaster planci*), Levitan and Young (1995) and Morris (1994) found that average fertilisation success rates decreased with both population density and population size. That is, there was a reduced per-capita reproductive effort with reducing population density/size, i.e. an "Allee effect" (Allee 1931), in these circumstances, caused through sperm limitation. A key finding from both sets of simulations was that Allee effects could occur across a wide range of (even high) population sizes and densities (Morris 1994, Levitan and Young 1995). Moreover, fertilisation success increased asymptotically towards 100% with population size/density and, contrary to common expectation, there was not a threshold population size/density above which Allee Effects did not occur (Levitan and Young 1995). As suggested by Levitan and Young (1995), this later finding was an important one for our understanding of the ecological and evolutionary dynamics of free-spawning as a reproductive strategy.

In chapter 5, I constructed models of fertilisation in randomly dispersed populations of scallops that were, at least broadly, similar to those of Levitan and Young (1995) and Morris (1994). One key differences between those simulations and those in this thesis were that in Morris' (1994) and Levitan and Young's (1995) simulations, eggs drifted across populations of randomly dispersed spawners of various sizes and densities (in contrast, *C.bifrons* eggs were modelled to stay where released and essentially were only fertilised by upstream males, more like the models of Denny and Shibata 1989). Nonetheless, the *C.bifrons* simulations produced essentially the same general patterns as the echinoderm population simulations - Allee effects occurred across a wide range of population densities (in the *C.bifrons* simulations population size was irrelevant). A unique difference in the *C.bifrons* simulations was that maximum fertilisation was not 100% and at higher densities average success rates decreased - this was related to increasing occurrences of polyspermy, which was not accommodated in the fertilisation kinetic models of either echinoderm species.

In chapter 6, I also illustrated that, at least within randomly dispersed populations, an Allee effect in free-spawners constitutes not only a decrease in average fertilisation success with population density, but also an associated increase in inter-individual variation in success rates. Again, in the randomly dispersed populations, this aspect of the Allee effect covaried with population density and there did not appear to be a critical, threshold density above which variation did not occur. Unfortunately, neither Morris (1994) nor Levitan and Young (1995) specifically estimated variation in success amongst individuals (though in monte carlo runs they did account for such variation, created by differences in the exact positions of spawners within randomly dispersed populations), but I would expect that covariation in rates of inter-individual variation with population density/size would also have been found in their simulations had they specifically measured this (see chapter 6).

In the simulations in this chapter, *C.bifrons* were clearly not randomly dispersed at numerous spatial scales, including some very small scales which we might expect to be critical to determining fertilisation success. Even with these unique, non-random spatial dispersion patterns, predicted fertilisation success was low and there was still substantial variation amongst individuals within a population. Further, when a reduction in overall density occurred at Largs Bay, average fertilisation success decreased, and there was also some indication that the relative (standardised) variance amongst individuals increased.

As noted in earlier chapters and by Levitan (1995), both low average success and significant variation amongst individuals has been noted at least in some surveys of fertilisation during natural spawning events. Levitan (1995) also pointed out that, generally, lower rates and higher inter-individual variation appear more common when population densities are low and/or there is a low degree of spawning synchrony. It might be tempting then, to suggest that Allee Effects in free-spawning marine invertebrates will be characterised by both a decrease in average fertilisation success AND an increase in the relative (standardised) variation in fertilisation success amongst individuals with a decrease in population density. These features, however, are probably only true where reductions in density occur as randomly chosen individuals removed from across an entire population. Such reductions were obviously the case in the simulations of randomly dispersed

individuals in chapters 5 and 6, and in the simulations of Morris (1994) and Levitan and Young (1995). The scallops that died during the large die-off in March 1995 also appeared to occur throughout the Largs Bay population, probably in a fairly random way. It is, however, easy to imagine other sorts of reductions in density that could occur which might result in very different changes to average fertilisation success and the variability of success amongst individuals to those outlined above and in earlier chapters.

The relationship between local density and average fertilisation success within a patch at Largs Bay was very much like the relationships developed for the randomly dispersed populations in chapter 5, even though there was strong evidence that scallops were not randomly dispersed within plots. Broadly, the average fertilisation success of *C.bifrons* individuals within plots at Largs Bay was positively correlated with density (figure 7.5) but here was, of course, some variation in the relationship caused by localised sex ratio skews, the varying abundance of non-reproducing castrated individuals and the specific dispersion patterns of scallops within surveyed plots. High rates of fertilisation occurred in the few high density patches of scallops that were found and it was essentially the numerous scallops in these patches that were responsible for most of a population's predicted reproduction. Obviously, removing a given number of scallops from the high density silt patches where fertilisation was generally successful would have a very different effect from removing the same number of largely unsuccessful scallops from sand patches or very low density silt and seagrass areas. In the former case there would be a reduction in average success and an increase in the relative variation amongst individuals. In the latter case, there would be an increase in the average success and a decrease in the relative variation amongst individuals. Clearly, the form that Allee effects may take will depend on how reductions in density occur (and the specifics of the process of external fertilisation).

Table 7.1.A. Analysis of variance of density of adult *C.bifrons* at Largs Bay in three habitats, with surveys being conducted at 5 times (both factors fixed). Densities of scallops were $\log_{10}(x+1)$ transformed prior to analysis.

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	p
Habitat	0.04734	2	0.02367	48.36	<0.001
Time	0.01172	4	0.00293	5.99	<0.001
Habitat x Time	0.00747	8	0.00093	1.9089	0.082
Error	0.02202	45	0.00049		

Table 7.1.B. Analysis of variance of density of adult *C.bifrons* at Largs Bay as above, but only considering scallops on silt and seagrass habitats (ie excluding the few scallops on sand). Again, scallop densities were $\log_{10}(x+1)$ transformed prior to analysis.

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	p
Habitat	0.01521	1	0.01521	20.97	<0.001
Time	0.01790	4	0.00448	6.17	0.001
Habitat x Time	0.00126	4	0.00031	0.43	0.784
Error	0.02177	30	0.00073		

Table 7.2. Morisita's index of mean crowding of *C. bifrons* at Largs Bay, calculated at a range of spatial scales, before and after a large die-off event in March 1995.
* indicates significantly different (at p= 0.05 level) from Im=1.0, the expectation on a random spatial dispersion of scallops

	Before Die-Off	*After Die-Off*
No. 64 m^2 survey plots	24	36
Mean density (scallops m^{-2})	0.133	0.059
Standard Deviation	0.134	0.063
Im_{64}	2.40*	1.90*
Im_{16}	2.52*	2.09*
Im_{4}	2.71*	1.57*
Im_{1}	3.68*	1.30
$Im_{0.25}$	1.90	0.86

Table 7.3. Randomisation test based on nearest neighbour distances in a population of *C.bifrons* at Largs Bay compared with those in randomly dispersed populations. Shown are observed numbers of nearest neighbour distances that were in the upper and lower 2.5th percentiles of distributions of nearest neighbour distances which were predicted in simulations of randomly dispersed individuals at patch densities equivalent to those in the field. Expected are numbers of scallops that would be expected to be found in these percentiles if scallops were randomly dispersed. NN_1 represents first nearest neighbour distance, NN_2 second nearest neighbour distance, NN_3, third nearest neighbour distance.

Silt *(pre die-off)*	NN_1		NN_2		NN_3	
	observed	*expected*	*observed*	*expected*	*observed*	*expected*
No. Scallops	145		145		145	
<0.025	2	*3.625*	11	*3.625*	8	*3.625*
>0.975	1	*3.625*	4	*3.625*	3	*3.625*

Silt *(post die-off)*	NN_1		NN_2		NN_3	
	observed	*expected*	*observed*	*expected*	*observed*	*expected*
No. Scallops	100		100		100	
<0.025	2	*2.5*	4	*2.5*	4	*2.5*
>0.975	3	*2.5*	2	*2.5*	3	*2.5*

Seagrass *(pre die-off)*	NN_1		NN_2		NN_3	
	observed	*expected*	*observed*	*expected*	*observed*	*expected*
No. Scallops	64		64		64	
<0.025	2	*1.65*	3	*1.65*	1	*1.65*
>0.975	3	*1.65*	1	*1.65*	3	*1.65*

Seagrass *(post die-off)*	NN_1		NN_2		NN_3	
	observed	*expected*	*observed*	*expected*	*observed*	*expected*
No. Scallops	31		27		27	
<0.025	0	*0.775*	2	*0.675*	3	*0.675*
>0.975	0	*0.775*	1	*0.675*	0	*0.675*

Table 7.4. Estimates of fertilisation success for *C.bifrons* in the pre and post die-off populations at Largs Bay, and the effect this has on effective genetic population size (N_e). Fertilisation success is measured in arbitrary units - 1.0 represents all eggs released by a female being successfully fertilised (see text). Values in brackets are lower and upper 95 % confidence estimates.

Pre Die-Off	Female	Male	N_e / N
No. Sampled Individuals	106	138	
Mean	0.063 *(0.040 , 0.086)*	0.049 *(0.030 , 0.068)*	
Variance	0.0184	0.0159	
Standardised Var	4.61	6.56	0.152
Post Die-Off			
No. Sampled Individuals	81	91	
Mean	0.020 *(0.008 , 0.031)*	0.018 *(0.008 , 0.028)*	
Variance	0.0035	0.0030	
Standardised Var	9.12	9.39	0.098

Figure 7.1. Density of *Chlamys bifrons* in three habitat types over 2.5 years at Largs Bay. Mean (+/- S.E.) density of adult scallops (>55mm shell height) in silt (grey), seagrass (black) and sand (unfilled) habitats. Four (64 m ^{2}) plots were haphazardly placed within each habitat type at each time. The arrow indicates the approximate time of an apparent die-off of scallops in March 95.

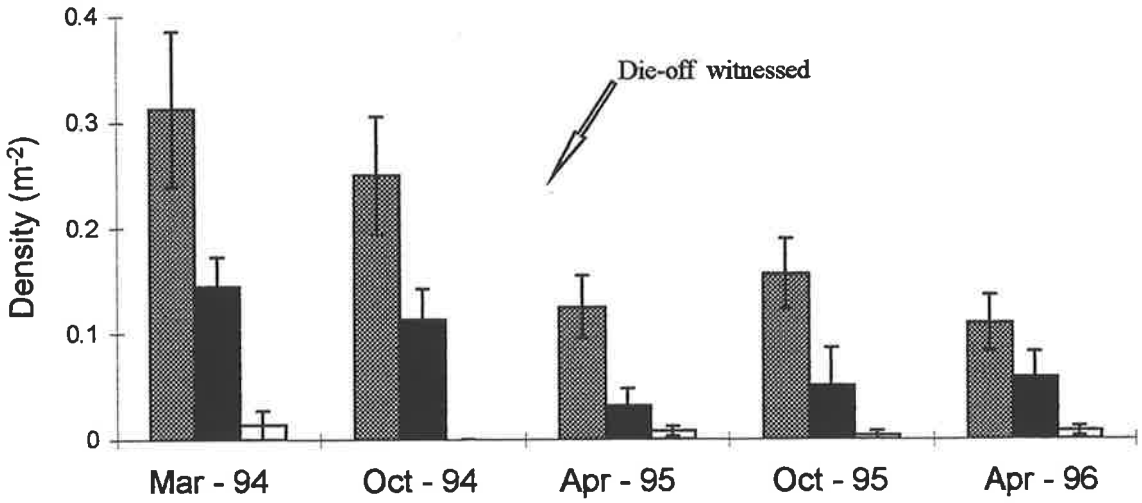


Figure 7.2 Population structure of *C.bifrons* at Largs Bay. Frequency distributions of adults (solid bars), sub-adults (grey bars) and recruits (hollow bars) are shown over 2.5 years. Mean size of adult scallops (>56mm) within a collection are shown on the right of each figure and population density and total number of scallops sampled (n) on the left.

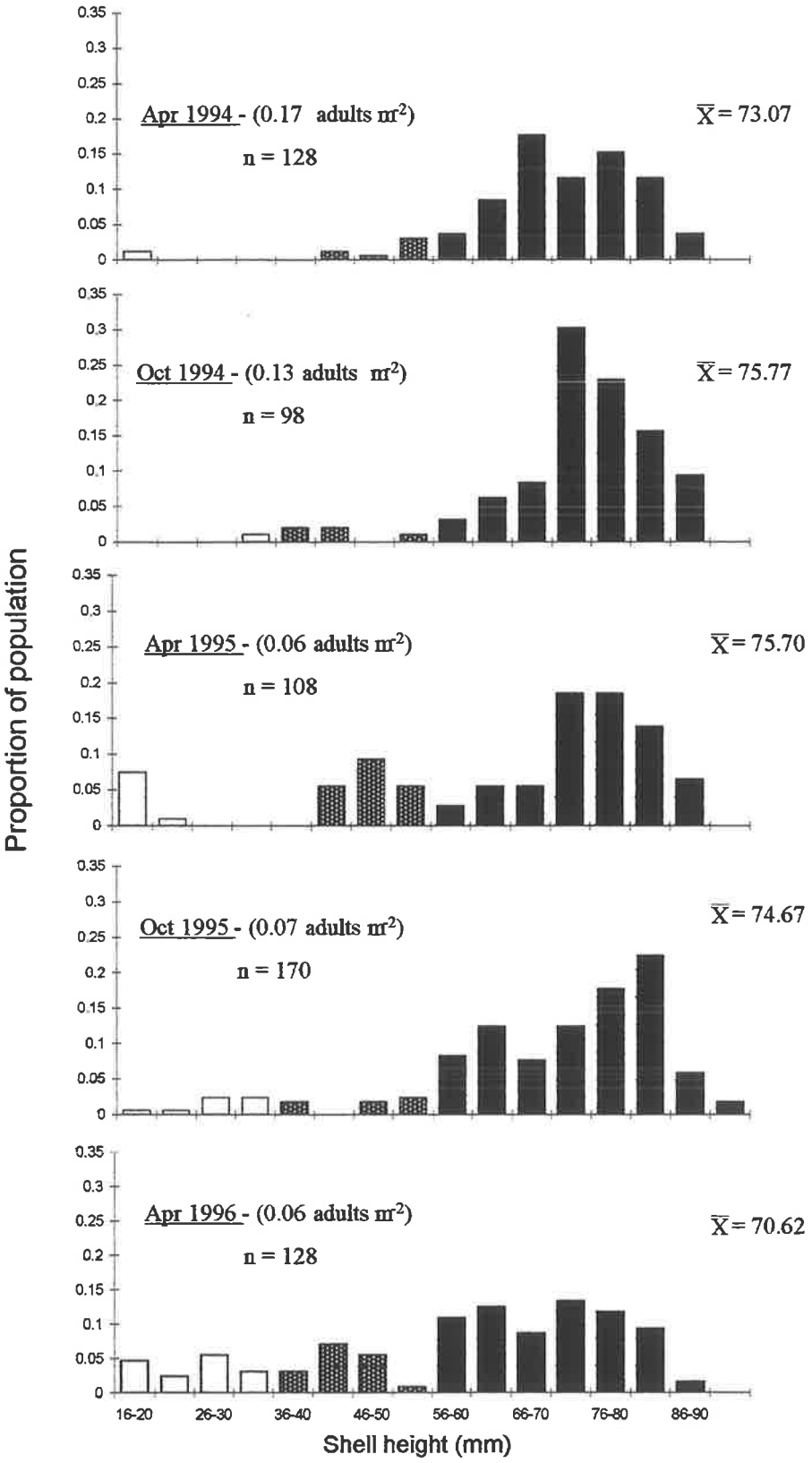


Figure 7.3 Dispersion of adult *C.bifrons* within survey plots at Largs Bay. Im (Morisita's index of mean crowding) values were calculated at 3 spatial scales (0.25 m ^{2} 1 m^2 & 4 m^2) and are shown for seagrass (Hollow circles) and silt (solid diamonds) plots. Dashed lines indicate upper and lower 95 % confidence intervals about Im = 1.0

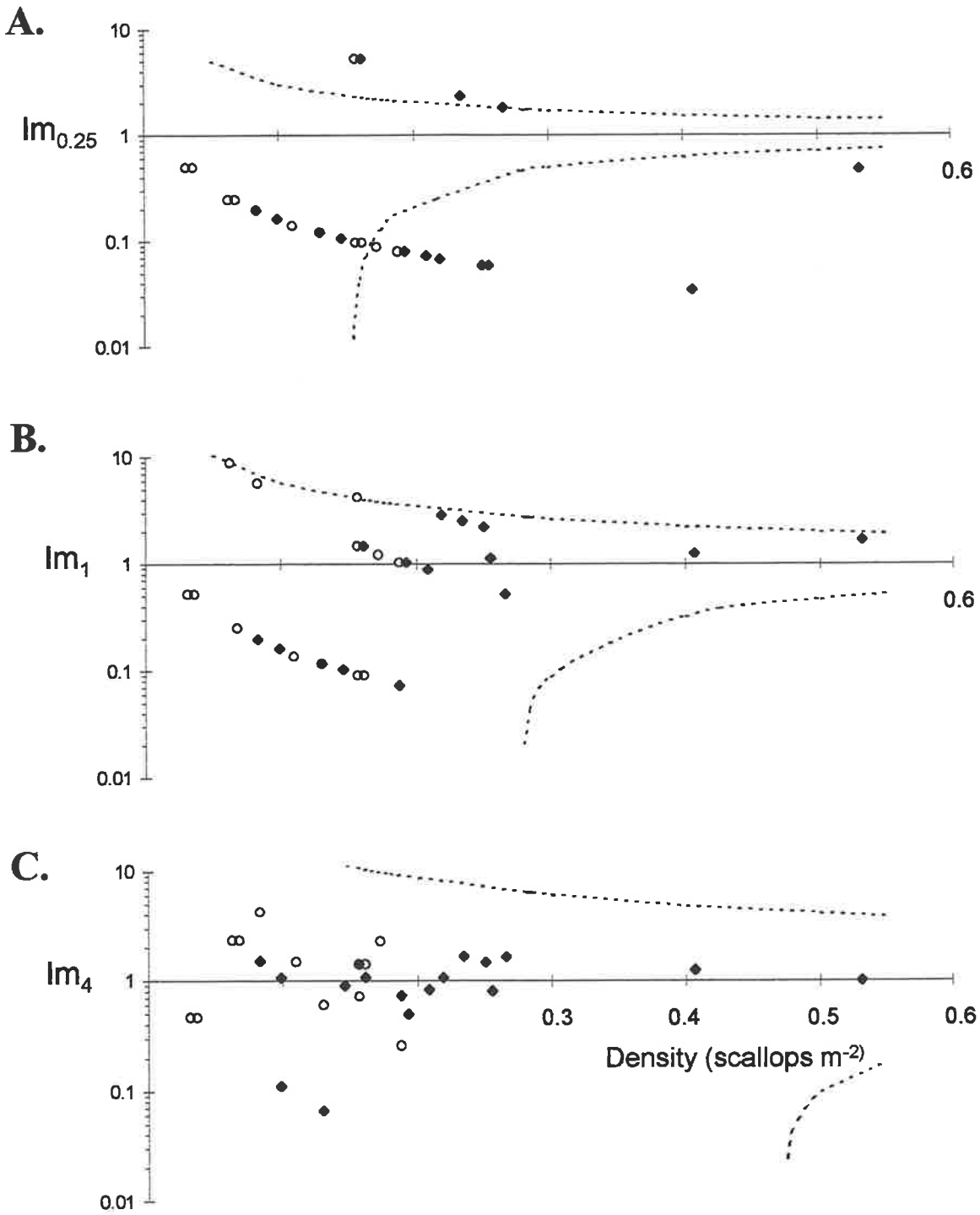


Figure 7.4. Distribution frequencies of *C.bifrons* nearest neighbour distances in different habitat types. A. Silt habitat; B. Seagrass habitat. In each, figures on the left are data from pre-March 1995 (before a die-off occurred) and right figures are after March 1995 (post die-off). Mean nearest neighbour distances in each group is noted on each plot.

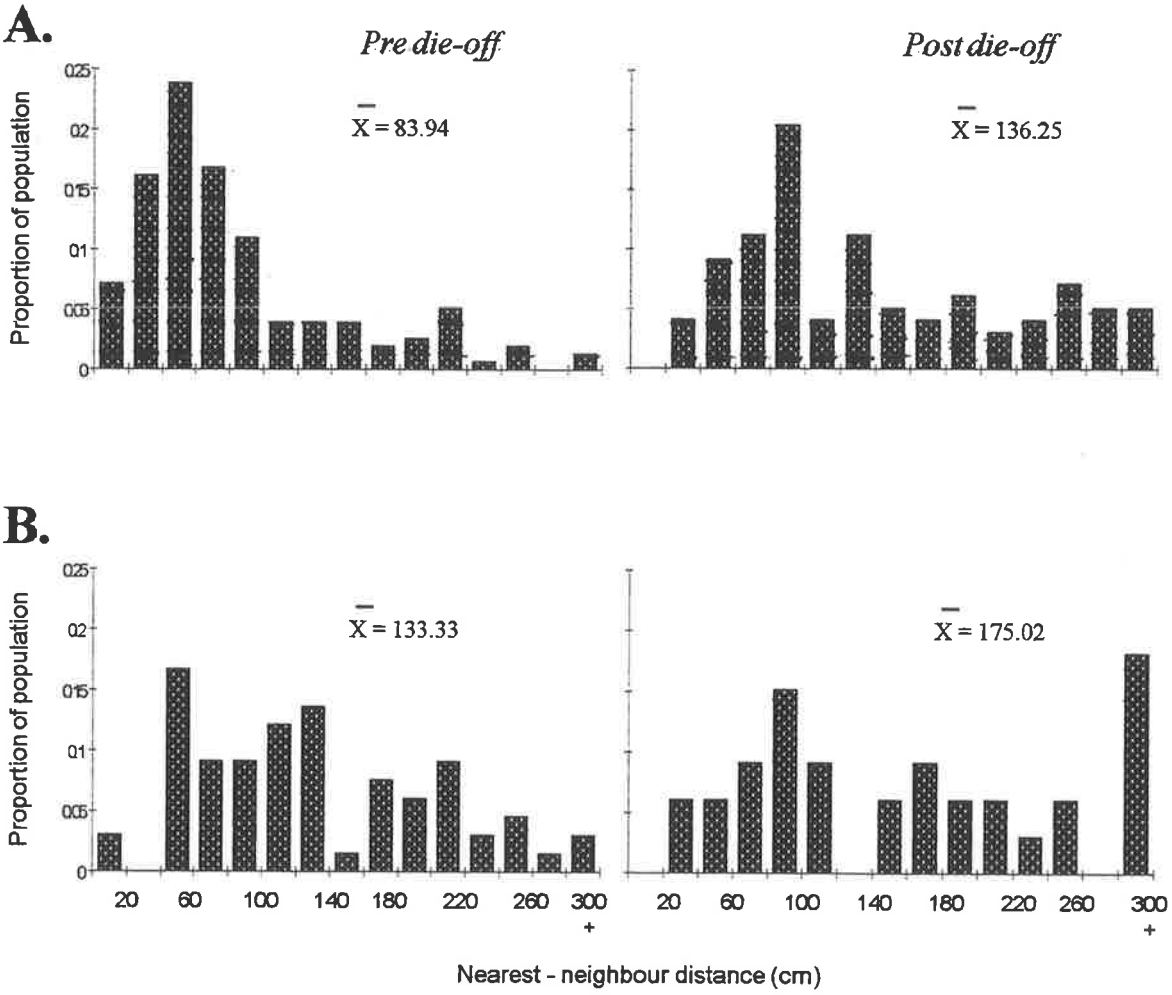
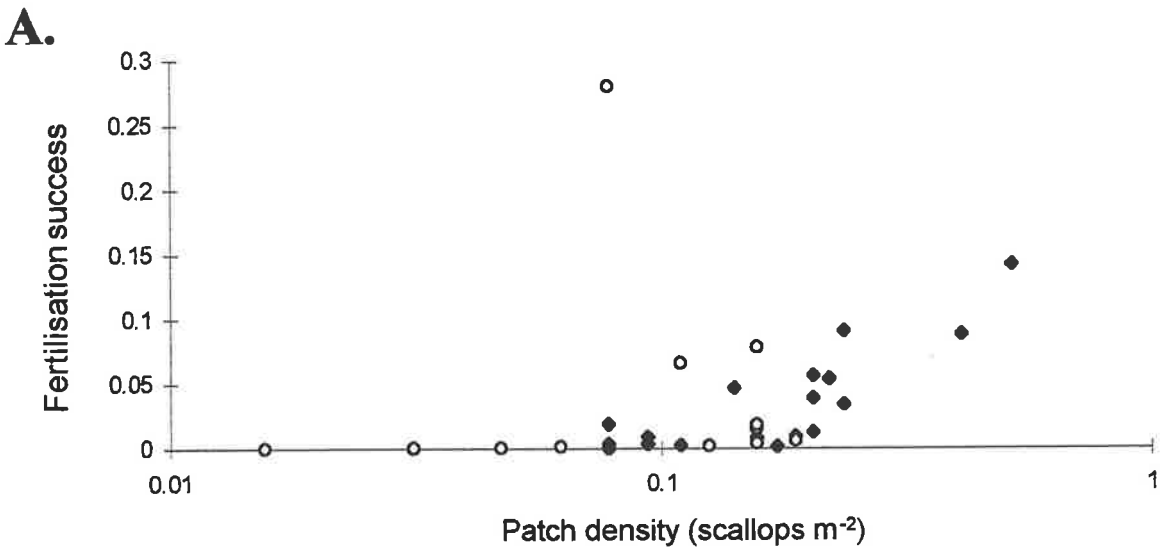


Figure 7.5. Average fertilisation success within survey plots. Fertilisation success is measured in arbitrary units - 1.0 is representsall eggs released by a single female successfully fertilised (see text). Silt patches are represented by solid diamonds, seagrass patches as hollow circles. A. Female success; B. Male successs.



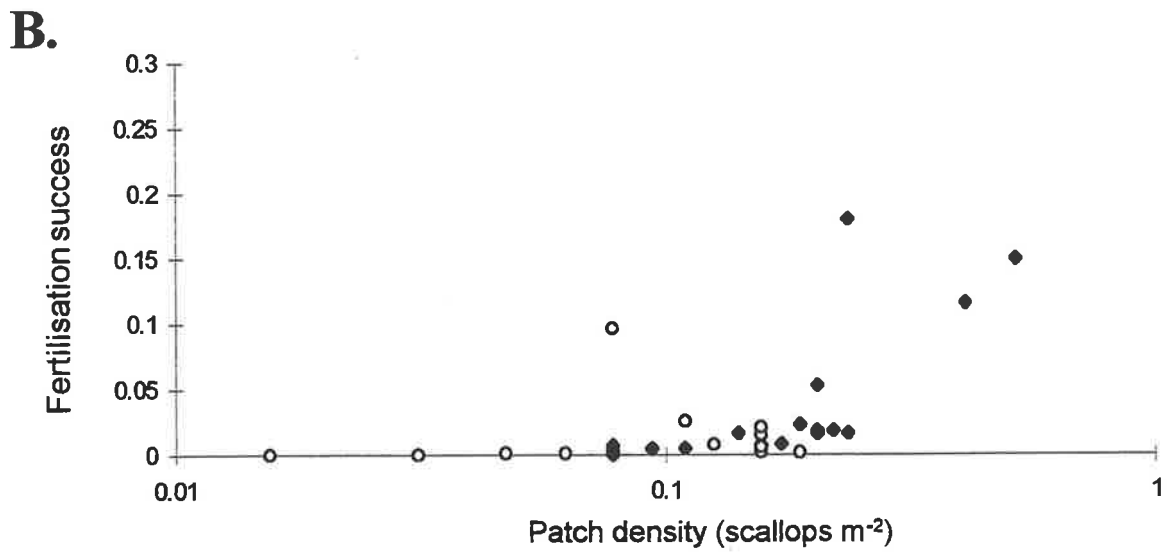
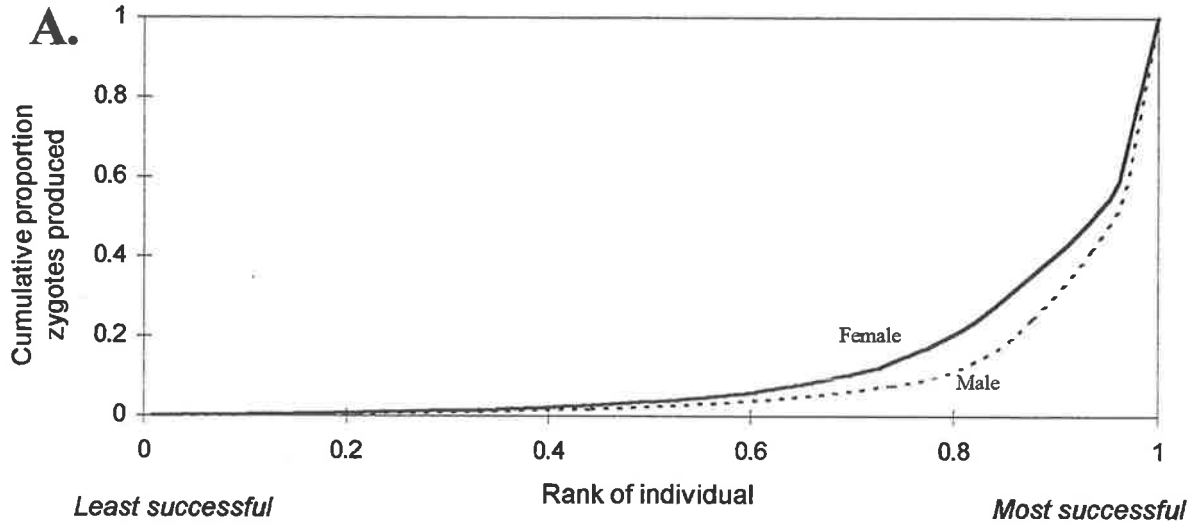


Figure 7.6. Predicted distribution of individual fertilisation success within *C.bifrons* populations at Largs Bay, South Australia. Cumulative reproductive success as a proportion of total reproduction within the population is plotted for scallops ranked in order of reproductive success. A. Pre die-off population (Mar -Oct. 1994); B. Post die-off population (April 1995 - April 1996).



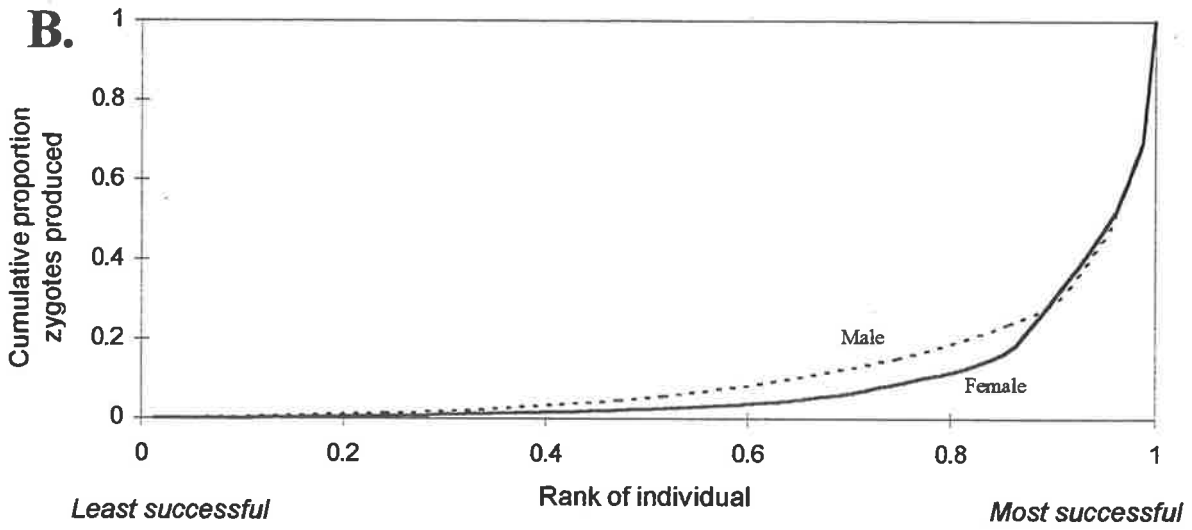
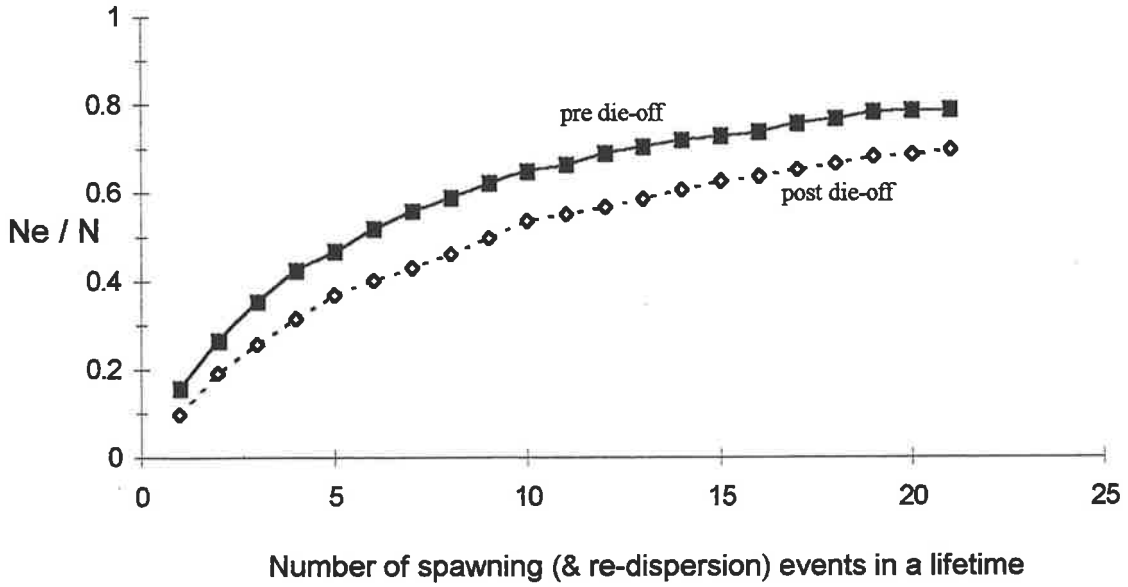


Figure 7.7. Modelled effects of repeated spawning events within individuals' lifetimes on Ne/N for pre and post die-off populations of *C.bifrons* at Largs Bay.



Chapter 8

General summary and conclusions

In this thesis I set out to develop a model of how successful the process of external fertilisation is likely to be for the scallop *C.bifrons*. Given that it was extremely unlikely that I would be able to witness real spawning events, an indirect approach was adopted, building up a model of external fertilisation in scallops from measurable component processes. In adopting a mechanistic, highly reductionist way of addressing external fertilisation in scallops, I hope not only have I been able to determine when external fertilisation is likely to fail or succeed, but also that I have helped to develop an understanding of the mechanisms generating variation in fertilisation and what the consequences of this variation may be. Throughout this thesis, I have tried to stress the assumptions that had to be made in order to construct the models. Below I summarise the main findings of this thesis and, as the story is by no means complete, highlight future directions of research that may prove productive

This thesis started with a general introduction in which I set out the general questions that I sought to address and the directions that this thesis would take. I argued that fertilisation success may play an important part in the population dynamics of free-spawners such as scallops and so should be examined in more detail. I stressed that an understanding of how variation in fertilisation success is generated may be much more important than gaining simple estimates of the average level of success within a population.

In chapter 2 I revised a pre-existing mathematical description of the collision of gametes and the process of fertilisation. A fairly simple re-working of the Vogel et al. (1982) model attended to one biologically unrealistic assumption it contained (the new model now includes the possibility of polyspermy) and produced a model of fertilisation kinetics that now appears to be much closer to what is measured in real experimental situations. If nothing else, the new model reinforced the need to use care when assaying fertilisation success, suggesting that the common practice of scoring raised fertilisation membranes (e.g. Pennigton 1985, Levitan 1993, Styan 1997) may sometimes lead to an overestimation of fertilisation rates. An argument was also developed from

Levitan's (1993, 1996a) hypothesis that large eggs may be a life-history trait that will be selected for in sperm limited conditions and which might in part explain intermediate-sized eggs in marine invertebrates - I showed that because in high sperm conditions rates of polyspermy can be quite high (unless blocks to polyspermy are super-efficient), then large, more fertilisable eggs may not necessarily be advantageous, especially if eggs are likely to be exposed to a wide range of ambient sperm concentrations in the field. In future, workers should not just consider the effects of sperm limitation, but also consider the potential effects that excess sperm (and ensuing polyspermy) may have on the process of external fertilisation.

In chapter 3, I conducted a series of laboratory experiments with the gametes of *C.bifrons* and *C.asperrima* and, for both of these species, quantified the relationships between ambient sperm concentration and the likelihood that an egg will become fertilised. The fertilisation kinetic curves of both these species could be fitted well to the mathematical model that was developed in the preceding chapter, but deriving unique, precise estimates of some model parameters was problematic without reliable independent estimates of gamete collision rates. Despite differences in egg diameter, at low sperm concentrations there appeared to be little difference between the two species in the relationship between sperm concentration and fertilisation chance. However, maximum fertilisation rates were much lower than 100% for *C.bifrons* (in contrast to *C.asperrima*) and at higher concentrations *C.bifrons* success decreased very rapidly with increasing sperm concentration, suggesting that *C.bifrons* was particularly sensitive to the effects of polyspermy, perhaps a result of its abnormally large eggs compared with other pectinids (Cragg and Crisp 1991).

In chapter 4 I documented temporal patterns of gonad development across 2.5 years for *C.bifrons* at Largs Bay and Edithburgh Jetty and *C.asperrima* at Edithburgh Jetty; from these I inferred spawning patterns of both *C.bifrons* and *C.asperrima* at a range of scales. The indirect evidence suggested that the two species of scallops spawned at opposite times of the year to each other and that *C.asperrima* spawned in late winter and early spring in South Australia (as elsewhere), whilst *C.bifrons* spawned across the warmer summer months (October - April). Apparently *C.bifrons* spawned three times during the summer of 1994/95 and twice in 1995/96, whereas there

was some evidence that*C.asperrima* had a minor spawning each winter (June), followed by a major spawning event in September. Small scale spatial variation in the exact date and time of spawning might exist within populations, so finer scale patterns of spawning were largely unresolvable, even for *C.bifrons* at Largs Bay for which samples were taken frequently. Future studies must ensure that small scale temporal and spatial factors can be unconfounded. Sperm release rates of male scallops were not allometrically related to the size of individuals as might have been expected. Instead, release rates appeared relatively invariant across individuals of varying size and also across scallop species. Building on this, an alternative model of spawning synchrony was postulated, in which the size- or age-structure of a population directly influences the likelihood that individuals will be spawning in synchrony and so directly influences the effective density of spawners.

In the next chapter (5), I incorporated spawning rate data and fertilisation kinetic models with empirically derived estimates of sperm dispersal in a range of field conditions to produce a model of the variation in *C.bifrons* fertilisation success with increasing inter-spawner distance. Using dye release to simulate sperm dispersal, I found some quite stark differences in the way gametes might disperse in different habitats. In open areas such as silt or sand patches distinct plumes were formed in a downstream direction whereas in seagrass patches dye just appeared to diffuse out equally in all directions, probably owing to a lack of flow within seagrass canopies. In the open areas, differences could be detected in the width of plumes between locations associated with different current speeds, with higher current speeds leading to narrower plumes. Some differences in the concentration of dye at points directly up or downstream of the dye release point could be detected amongst habitats/locations and also amongst replicate plumes within habitats. Consequently, differences in the relationship between inter-spawner distance and fertilisation success could also be detected amongst habitats using some fertilisation criteria. However, these differences were probably not very important in a population sense, for simulations of spawning within randomly dispersed populations suggested that average success is affected much more strongly by population density than by the habitat or location in which spawning is taking place.

Perhaps the most striking result in chapter 5, however, was the very small inter-spawner distances over which *C.bifrons* fertilisation decreases. Using reasonable parameter estimates, the model predicted that fertilisation would be essentially negligible if a female was greater than 100 cm downstream of a spawning male. The results of a field spawning experiment confirmed this prediction. Corresponding with the very small spatial scales over which *C.bifrons* fertilisation can vary, simulations of success within randomly dispersed populations indicated that quite high densities of scallops (>0.5 m^{-2}) are necessary in order for there to be even moderately high (> 20%) average rates of fertilisation within a population. Maximum rates of fertilisation required even higher population densities (~2 m^{-2}).

I estimated the potential variation in fertilisation success that could occur amongst individuals within a population in chapter 6. By examining the data that were produced in the models of spawning within randomly dispersed populations in chapter 5, I showed that in some circumstances very large skews in the distribution of reproduction success amongst individuals could occur within populations. Moreover, as population densities decreased, the skewness of reproduction increased markedly, with fewer and fewer individuals achieving high rates of fertilisation success and so contributing disproportionately to the overall reproduction within a population. In some situations, this skewing may become large enough to affect the effective genetic size of a population.

In chapter 7, I described the spatial dispersion patterns of *C.bifrons* within the Largs Bay population over the period from early 1994 to 1996. During this time there was a large, sudden mortality of adult scallops that reduced overall densities by about 60% but, despite this, patterns of scallop distribution remained largely unchanged. Although there were approximately equal amounts of each habitat type within the survey area, scallops were about 3 times more likely to be found in silt habitats than in seagrass and only very few were ever found in sand patches. Within habitats too, there was evidence in the form of Morisita's indices and a randomisation test based on nearest neighbour distances that on some (intermediate) spatial scales scallops were more likely to be found in association with other scallops than would be expected if they were simply distributed randomly. In contrast, at very small spatial scales, it appeared that scallops were less

likely to be very close to each other than in a randomly dispersed population. Overall, sex ratios were approximately even, with slightly fewer females being found than males. A small proportion (~ 3%) of the animals found were parasitically castrated by a larval bucephalid trematode. However, amongst patches (64 m^{-2} areas) there were significant skews in sex ratios and also higher prevalences of castrating parasites than would be expected if scallops had been randomly assigned to patches.

Using (exact) information about the spatial distribution of scallops within Largs Bay, a simulation model was run to predict the fertilisation success of *C.bifrons* within a real population. Predictions of average fertilisation rates in the higher density (0.13 scallops m^{-2}) pre die-off population were low (~6 %), but were significantly greater than average rates predicted for the lower density (0.06 scallops m^{-2}) post-die-off population (~2%). I noted that average rates of fertilisation across populations essentially reflected fertilisation in the few high density patches in both the seagrass and silt habitats, because these patches contained most of the sampled scallop population. There was a high amount of variability in success amongst individuals in both of these populations, though there was more variability in the lower density post die-off population. In each population only a small number of individual males and females had very high rates of fertilisation success (>50%), with most other scallops having much lower success rates than this. Given that scallops can move and may participate in numerous spawning events during their lifetime, a simulation was run in which individuals' success each year came from distributions of success within populations similar to those predicted in the first part of the chapter. This illustrated that spawning numerous times (with intervening spatial redistribution) greatly reduced variation amongst individuals in lifetime reproductive success and perhaps is a mechanism by which "chaotic genetic patchiness" could be generated.

In addition to simply describing the dynamics of external fertilisation in different habitats/patches and predicting how success varies between individuals and populations, in the light of some of the patterns uncovered I speculated about some of the selective forces associated with this form of reproduction (recognising that creating such post-hoc "explanations" may be a largely unproductive exercise, Peters 1991). For example, I asked whether fertilisation "works" more

effectively in some habitats/locations than others because of the physical nature of these places - which appears to not be the case if densities are uniform across habitats. I also suggested that perhaps scallops actively avoid being very close to each other (which would be in accord with dispersion patterns described in chapter 7), because there is little advantage in being very close, due the possible negative effects of polyspermy.

Future research directions

In a strict sense, this thesis has simply generated a detailed hypothesis about what the fertilisation success of *C.bifrons* might be in a range of conditions. Various parts of the hypothesis were tested during its development, but the major predictions of this hypothesis remain untested. To test these, however, will not be easy as direct observations of spawning events will be needed. Indeed, generally there is a critical need for more observations of natural spawning events, especially those that incorporate careful, planned sampling designs. Even better would be these sorts of (replicated) measurements of spawning success following manipulations of population density and/or the age- or size-structure of populations. Whilst desirable, direct measures will be difficult and infrequently obtained simply because spawning events are so rarely witnessed (see chapter 1). Certainly, if such events are fortuitously encountered, then workers must be ready to make the most of opportunities and be ready at all times with plans for what sort of information should be collected (i.e. what specific hypotheses will be tested) and the appropriate sampling gear (e.g. Styan 1997) ready to do this (Levitan 1995). In part, work such as that in chapter 4 may help to narrow down the searching in a temporal sense, but much more work is still needed before we will be able to predict spawning of *C.bifrons* precisely enough that workers can realistically plan when they should be underwater.

One possible direction for future research is to assess whether indirect, genetic methods can be used to identify in field populations the sorts of Allee Effects that have been predicted here. Importantly, reductions in rates of fertilisation success in sperm limited conditions were not spread evenly across a population. Associated with reductions in average rates of fertilisation success were increases in the variance of success amongst individuals. At least potentially, a tell-tale

change in reproductive skewing might be detectable using genetic markers, which then might tell us that an associated reductions in average success rates has occurred. For example, techniques used to estimate effective genetic population size (e.g. Waples 1989, 1991) might be used to detect differences in the distribution of fertilisation success within experimentally manipulated populations (as a change in Ne). There are, however, numerous problems with available techniques in this area (Nunney 1995). Doubtless, additional work is required (and beyond the scope of this thesis) before existing techniques can be used reliably, but given the rapid rate of developments in the area of molecular ecology, both the necessary sensitive genetic markers, and the appropriate statistical tools, should become available sooner rather than later.

A major, unresolved issue that has been raised here is the potential effects that population structure may have on the process of external fertilisation. Somewhat to my surprise, in chapter 4 I found little evidence that the size of males had any effect on the rate at which they released sperm. This led me to propose an alternative model of spawning within populations in which male size directly affects spawning synchrony. In this model, because population structure affects the effective density of the spawning populations, this then has direct affects on both the overall zygote production and the degree of reproductive skewing within a population.

Obviously, discriminating between the proposed spawning frequency model and a model in which animals spawn synchronously may be quite important for our understanding of the dynamics of external fertilisation. If the spawning frequency model were true, then managers of free-spawning populations would probably want to be aware of potential multiplicative effects that may result from various fishing strategies. For example, removing large individual males in the belief that these are not much more important reproductively than small individuals might be a mistake because this harvesting strategy might not only reduce the number of animals present to release sperm, but it may further reduce the effective density of male spawners because spawning synchrony levels may be lower (which in turn reduces average fertilisation success rates and increases reproductive skewing).

At this stage the spawning frequency model is simply a viable alternative model to a model in which animals spawn synchronously; there is little direct supportive evidence other than the non-allometric relationship between size and spawning rates. Whilst direct observations will be the best way to discriminate between these models, getting these observations in the field (and in concert with experimental manipulations of age- or size-structure), will be very difficult. Numerous indirect tests may, however, be possible. For example, under the fecundity/synchrony model large individuals should be able to be induced into spawning more often than small individuals and if individuals release a fixed amount of gametes each time, then the number of releases should be allometrically related to individual size in the same way that gonad investment is related to size; neither of these will be the case under the synchronously spawning model. Measurement of spawning rates and testing if they are allometrically related to size in the same way as reproductive effort (gonad mass) is another test that can be conducted (as was done in this thesis). I explained in chapter 4 that it may be possible to discriminate between models of spawning behaviour using indirect measures of spawning activity (such as gonad indices) because the different models predict different temporal patterns in the within sample variances of populations of large and small individuals. Finally, because different degrees of reproductive skewing are produced under the alternative spawning behaviour models, then if this can be measured in either the subsequent larval or recruit pool (perhaps with yet to be developed genetic methods - see above), then this could be compared across a range of populations with contrasting population structures.

In chapters 6 and 7, I suggested that large reproductive skews amongst individuals predicted in a single spawning event may be "averaged out" when individuals have the opportunity to spawn numerous times during their lifetime (as long as they disperse between spawning events). This again, suggests a mechanism by which the age structure of a population might directly influence its dynamics - at least for low density (sperm limited) populations, those populations whose individuals live longer (and so spawn more often in their lifetime) will have larger effective genetic population sizes than populations whose individuals live less long. Whether this mechanism occurs or is important remains to be tested. Again, direct observations of spawning (following individuals over multiple spawning events) are needed, but perhaps indirect (genetic)

comparisons which contrast the effective genetic population sizes of populations with differing age or size-structure and /or redispersing capabilities (mobility) of individuals may also be instructive.

Implications for managing free-spawning populations

In terms of how scallop (or other) free-spawning populations might be managed in the light of the recognition of the potential for sperm limitation, some important issues have been raised. For example, to maintain some minimum amount of *C.bifrons* egg (larval) production in the face of fishing pressure, at least some areas of high densities will need to be maintained. This was probably intuitively obvious without the work in this thesis, but perhaps the unexpected point was just how high the density will need to be in order to achieve high rates of fertilisation. Though the details are likely to vary from species to species and predicted values here may be somewhat imprecise (remembering that the models included many assumptions, for example, those about spawning synchrony), three attributes of a population should generally increase average fertilisation success and minimise the lifetime variance in individual reproductive success (and so preserve maximum genetic diversity) within it. First, higher density populations result in lower variation amongst individuals in fertilisation success rates in spawning events. Second, a population of larger/more fecund individuals may decrease the skew in reproductive success within individual spawning events (i.e. larger individuals may spawn more synchronously than less fecund individuals - see chapter 4). Third, as illustrated in chapter 7, variance in lifetime reproductive success of older (larger) individuals should be lower than for a population of younger (smaller) individuals.

In situations where usually anthropogenic disturbances such as fishing and toxic algal blooms have already reduced stocks of economically or culturally important free-spawning animals like scallops, restocking schemes are increasingly being implemented in an attempt to restore or enhance populations (e.g. Kassner and Malouf 1982, Tettelbach and Wenczel 1993, Peterson et al. 1996). If these restocked populations are to be self-sustaining, it will be necessary to ensure replacement spawners are introduced at sufficiently high density such that fertilisation does not fail (of course, consideration will also need to be given to factors such as the oceanographic

transport of larvae e.g. Shanks 1995). This will require an understanding of how external fertilisation works in the species in question (similar to those models developed here) and, because external fertilisation is a spatially sensitive process, might also necessitate an understanding of the processes that determine the dispersion of adult spawners (see chapter 7). If the models developed here and in chapters 5 and 6 are indicative of what could be expected in other scallop species elsewhere (and, of course, they might not be - see chapter 1), then generally any restocking that is undertaken will need to be done at reasonably high densities (probably >1-2 scallops m^{-2}) and preferably with large, fecund individuals.

As suggested in chapter 6, because scallop populations are often extensive, they will usually be large enough such that the effective genetic population size will be reasonably large (i.e. > 500), even with the extreme skews in reproductive success that may sometimes occur as a result of sperm limitation. Nonetheless, maximising effective genetic population size might still be a goal for the scientific management of free-spawning populations, in which case, managers might aim to avoid the large skews in lifetime reproductive success which the models here suggests can be generated. Given the many alternative ways in which fished populations can be managed (for example, with TAC, size limits, reserved areas etc), future research might assess what form of management (harvesting) not only preserves the greatest larval production (cf. Botsford et al 1993, Quinn et al 1993), but also best preserves the largest effective genetic size of a harvested population. The effect illustrated in figure 7.7 suggests that longevity and repeated reproduction are important not only in demography (Chesson 1984), but also in maintaining high effective genetic population size and hence genetic variation; harvsting systems typically reduce longevity! Sophisticated restocking programmes might also consider the genetic consequences of any such restocking, especially given that the genetic diversity of (perhaps hatchery produced) restocked individuals is often low to start with. Again, high densities of large restocked individuals, that are distributed spatially as evenly possible, may be needed in order to minimise variation in fertilisation success amongst individuals and so maximise the effective genetic size of populations.

Of course, even if possible, maintaining populations such that they achieve high and invariant fertilisation success may not always be easy or practical. The indentification of areas likely to to contribute disproportionately to reproduction (i.e. clearly identifying what the real reproducing stock is) might enable these to be set aside to allow reproduction to take place unaffected, but a practical difficulty with such a strategy might be that high density patches of individuals are likely to be dynamic in both space and time, and so would be very difficult to identify and preserve. This is likely to be especially true for animals such as scallops which are mobile and which also have notoriously variable larval settlement and recruitment rates on a range of spatial scales (Brand et al. 1991, Oresanz et al. 1991). High density patches are probably just the scallops that fishers would most likely want to target, simply because they would get the best returns for their effort in fishing those areas. Similarly, though large, fecund individuals (both female and male) might be disproportionately important to reproduction in a range of ways, these might also be the most commercially valuable. In terms of restocking, it is plausible that individuals at high densities might suffer increased mortality or parasitic castration rates, or perhaps spreading individuals across wide areas might be a necessary strategy to hedge against catastrophic events in isolated locations.

A final comment

This thesis has focussed on the fertilisation ecology of the scallop *C.bifrons* in certain areas where it is common in South Australia. As outlined in Levitan (1995) and reiterated in chapter 1, the details of the process of external fertilisation are probably quite species-specific and patterns uncovered for one species in certain situations need not necessarily be repeated in other species or even the same species elsewhere. Obviously, for other scallop species, specific studies of those species' fertilisation ecology are needed (this is also true for other groups) -extrapolating widely from the work here may be inappropriate, but, given the dearth of other information, is probably inevitable. Given the worldwide commercial importance of scallops, other studies should hopefully appear in the near future which will allow for comparisons with the work I have produced here. Long term, my hope is that this thesis will prove to have been an useful step in developing our overall understanding of the dynamics of the process of external fertilisation and the

importance of this life-stage in the population dynamics of *C.bifrons* and free-spawning species in general.

Literature Cited

Ackerman, J. D. and Okubo, A. (1993) Reduced mixing in a marine macrophyte canopy. Functional Ecology 7, 305-309.

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

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

Arsenault, D. J. and Himmelman, J. H. (1996a) Ontogenetic habitat shifts of the Iceland scallop, *Chlamys islandica* (Muller, 1776), in the northern Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences 53, 884-895.

Arsenault, D. J. and Himmelman, J. H. (1996b) Size-related changes in vulnerability to predators and spatial refuge use by juvenile Iceland scallops *Chlamys islandica*. Marine Ecology Progress Series 140, 115-122.

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

Babcock, R. C. and Mundy, C. N. (1992) Reproductive biology, spawning and field fertilization rates of *Acanthaster planci*. Australian Journal of Marine and Freshwater Research 43, 525-534.

Babcock, R.; Mundy, C.; Keesing, J. and Oliver, J. (1992) Predictable and unpredictable spawning events: *in situ* behavioural data from free-spawning coral reef invertebrates. Invertebrate Reproduction and Development 22, 213-228.

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

Baker, P. F. and Presley, R. (1966) A direct method of measuring the rate of entry of sperm into sea urchin eggs. Proceedings of the Physiological Society 166, 47-49.

Barbeau, M. A.; Scheibling, R. E.; Hatcher, B. G.; Taylor, L. H. and Henniger, A. W. (1994) Survival analysis of tethered juvenile sea scallops *Placopecten magellanicus* in field experiments : effects of predators, scallop size and density, site and season. Marine Ecology Progress Series 115, 243-256.

Barber, B. J. and Blake, N. J. (1991) Reproductive physiology. In: Scallops: biology, ecology and aquaculture. (Ed: Shumway, S. E.) Elsevier, Amsterdam, New York, 377-428.

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.

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

Beaumont, A. R. and Budd, M. D. (1983) Effects of self-fertilisation and other factors on the early development of the scallop *Pecten maximus*. Marine Biology 76, 285-289.

Belding, D. L. (1910) A report upon the scallop fishery of Massachussetts; including the habitats, life history of *Pecten irradians*, its rate of growth and other facts of economic value. The Commonwealth of Massachussetts.

Beninger, P. G.; Donval, A. and Le Pennec, M. (1995) The osphradium in *Placopecten magellanicus* and *Pecten maximus*: histology, ultrastructure, and implications for spawning synchronisation. Marine Biology 123, 121-129.

Benzie, J. A. H. and Dixon, P (1994) The effects of sperm concentration, sperm:egg ratio, and gamete age on fertilization success in Crown-of-Thorns Starfish *Acanthaster planci* in the laboratory. Biological Bulletin 186, 139-152.

Benzie, J. A. H.; Black, K. P.; Moran, P. J. and Dixon, P. (1994) Small-scale dispersion of eggs and sperm of the Crown-of-thorns starfish *Acanthaster planci* in a shallow coral reef habitat. Biological Bulletin 186, 153-167.

Bolton, T. F. and Havenhand, J. N. (1996) Chemical mediation of sperm activity and longevity in the solitary ascidians *Ciona intestinalis* and *Ascidiella aspersa*. Biological Bulletin 190, 329-335.

Bomadelli, J. C. and Himmelman, J. H. (1995) Examination of assumptions critical to body component indices: application to the giant scallop *Placopecten magellanicus*. Canadian Journal of Fisheries and Aquatic Sciences 52, 2457-2469.

Bonardelli, J. C.; Himmelman, J. H. and Drinkwater, K. (1996) Relation of spawning of the giant scallop, *Placopecten magellanicus*, to temperature fluctuations during downwelling events. Marine Biology 124, 637-649.

Botsford, L. W.; Quinn, J. F.; Wing, S. R. and Brittnacher, J. G. (1993): Rotating spatial harvest of a benthic invertebrate, the red sea urchin, *Strongyloncentrotus franciscanus*. Management of exploited fish. Proceedings of the international symposium on management strategies for exploited fish population, Alaska Sea Grant.

Braley, R. M. (1984) Reproduction in the giant clams *Tridacna gigas* and *T.derasa in situ* on the north-central Great Barrier Reef, Australia, and Papua New Guinea. Coral Reefs 3, 221-227.

Brand, A. R. (1991) Scallop ecology: distributions and behaviour. In: Scallops: biology, ecology and aquaculture. (Ed: Shumway, S.E.) Elsevier, Amsterdam, New York, 517-584.

Brawley, S. H. (1987) A sodium dependent, fast block to polyspermy occurs in eggs of fucoid algae. Developmental Biology 124, 390-397.

Brawley, S. H. (1992) Fertilization in natural populations of the dioecious brown alga *Fucus ceranoides* and the importance of the polyspermy block. Marine Biology 113, 145-157.

Brazeau, D. A. and Lasker, H. R. (1990) Sexual reproduction and external brooding by the Caribbean gorgonian *Briareum asbestinum*. Marine Biology 104, 465-474.

Brazeau, D. A. and Lasker, H. R. (1992) Reproductive success in the Caribbean octocoral *Briareum asbestinum*. Marine Biology 114, 157-163.

Bricelj, V. M.; Epp, J. and Malouf, R. E. (1987) Intraspecific variation in reproductive and somatic growth cycles of bay scallops *Argopecten irradians*. Marine Ecology Progress Series 36, 123-137.

Butler, A. J.; Vicente, N. and de Gaulejac B. (1993) Ecology of the pteroid bivalve *Pinna bicolor* Gmelin and *Pinna nobilis* L. Marine Life 3: 37-35.

Byrd, E. W. Jr., and F. D. Collins. 1975. Absence of fast block to polyspermy in eggs of the sea urchin *Strongylocentrotus purpuratus*. Nature 257, 675-677.

Caddy, J. F. (1970) A method of surveying scallop populations form a submersible. Journal of the Fisheries Research Board of Canada 27, 535-549.

Caddy, J. F. (1989) A perspective on the population dynamics and assessment of scallop fisheries, with special reference to the sea scallop *Placopecten magellanicus* Gmelin. In: Marine invertebrate fisheries: their assessment and management. (Ed. Caddy, J. F.) John Wiley and Sons, 559-574.

Carsen, A. E.; Hatcher, B. G.; Scheibling, R. E.; Hennigar, A. W. and Taylor, L. H. (1995) Effects of site and season on movement frequencies and displacement patterns of juvenile sea scallops *Placopecten magellanicus* under natural hydrodynamic conditions in Nova Scotia, Canada. Marine Ecology Progress Series 128, 225-238.

Carsen, A. E.; Hatcher, B. G. and Scheibling, R. E. (1996) Effect of flow velocity and body size on swimming trajectories of sea scallops, *Placopecten magellanicus* (Gmelin): a comparison of laboratory and field measurements. Journal of Experimental Marine Biology and Ecology 203, 223-243.

Chernoff, H. (1987) Factors affecting mortality of the scallop *Chlamys asperrima* (Lamarck) and its epizooic sponges in South Australian waters. Journal of Experimental Marine Biology and Ecology 109, 155-171.

Chesson, P. L. (1984) The storage effect in stochastic populations. Lecture Notes in Biomathematics 54, 76-89.

Chia, Fu-S. and Bickell, L.R. (1983) Echinodermata. In: Reproductive biology of invertebrates, Volume II: Spermatogenesis and sperm function. (Eds: Adiyodi, K. G. and Adiyodi, R. G.) John Wiley and Sons, New York, 545-620.

Christen, R.; Schackmann, R. W. and Shapiro, B. M. (1985) Ionic regulation of sea urchin sperm motility, metabolism and fertilizing capacity. Journal of Physiology 370, 347-365.

Clavier J. (1992) Fecundity and optimal sperm density for fertilization in the ormer (*Haliotis tuberculata* L.). In: Abalone of the world: biology, fisheries and culture. (Eds: Shepherd, S. A.; Tegner, M. J. and Guzman Del Proo, S. A.) Fishing News Books, Oxford, 86-92.

Clotteau, G. and Dube, F. (1993) Optimizing of fertilization parameters for rearing surf clams (*Spisula solidissima*). Aquaculture 114, 339-353.

Coll, J. C.; Bowden, B. F.; Meehan, G. V.; Konig, G. M.; Carol, A. R.; Tapiolas, D. M.; Alino, M.; Heaton, A.; Nys, R. D.; Leone, P. A.; Maida, M.; Aceret, T. L.; Willis, R. H.; Babcock, R. C.; Willis, B. L.; Florian, Z.; Clayton, M. N. and Miller, R. L. (1994) Chemical aspects of mass spawning in corals. I. Sperm-attractant molecules in the eggs of the scleractinian coral *Montipora digitata*. Marine Biology 118, 177-182.

Coma, R.; Llobet, I.; Gilet, J-M. and Zabala, M. (1996) Quantification of sexual reproduction in the marine benthic hydroid *Campanularia everta*. Marine Biology 125, 365-373.

Coma, R. and Lasker, H. R. (1997a) Effects of spatial distribution and reproductive biology on *in situ* fertilization rates of a broadcast-spawning invertebrate. Biological Bulletin 193, 20-29.

Coma, R. and Lasker, H. R. (1997b) Small-scale fertilization success in a broadcast spawning octocoral. Journal of Experimental Marine Biology and Ecology 214, 107-120.

Cragg, S. M. and Crisp, D. J. (1991) The biology of scallop larvae. In: Scallops: biology, ecology and aquaculture. (Ed: Shumway, S.E.) Elsevier, Amsterdam, New York, 75-132.

Croll, R. P.; Too, C. K. L.; Pani, A. and Nason, J. (1995) Distribution of serotonin in the sea scallop *Placopecten magellanicus*. Invertebrate Reproduction and Development 28, 125-135.

Csanady, G. S. (1973) Turbulent diffusion in the environment. D. Reidel, Boston.

Denny, M. W. (1988). Biology and mechanics of the wave-swept environment. Princeton University Press, Princeton, N J.

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

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

Desrosiers, R. R. and Dube, F. (1993) Flowing seawater as an inducer of spawning in the sea scallop *Placopecten magellanicus* (Gmelin, 1791). Journal of Shellfish Research 12, 263-265.

Desrosiers, R. R.; J. Desilets, and Dube, F. (1996) Early developmental events following fertilization in the giant scallop *Placopecten magellanicus*. Canadian Journal of Fisheries and Aquatic Sciences 53, 1382-1392.

Desrosiers, R. R.; Desilets, J. and Dube, F. (1996) Early developmental events following fertilization in the giant scallop *Placopecten magellanicus*. Canadian Journal of Fisheries and Aquatic Sciences 53, 1382-1392.

Dix, T. G. (1976) Larval development of the queen scallop *Equichlamys bifrons*. Australian Journal of Marine and Freshwater Research 27, 399-403.

Downing, J. A.; Rochon, Y. and Perusse, M. (1993) Spatial aggregation, body size, and reproductive success in the freshwater mussel *Elliptio complanata*. Journal of the North American Benthological Society 12, 148-156.

Dredge, M. C. L. (1981) Reproductive biology of the saucer scallop *Amusium japonicum balloti* (Bernardi) in central Queensland waters. Australian Journal of Marine and Freshwater Research 32, 775-787

Dredge, M. C. L. (1988) Recruitment overfishing in a tropical scallop fishery? Journal of Shellfish Research 7, 233-239.

Dufresne-Dube, L.; Dube, F.; Guerrier, P. and Couillard, P. (1983) Absence of a complete block to polyspermy after fertilization of *Mytilus galloprovincialis* (Mollusca, Pelecypoda) oocytes. Developmental Biology 97, 27-33.

Eckman, J. E. (1996) Closing the larval loop: linking larval ecology to the population dynamics of marine benthic invertebrates. Journal of Experimental Marine Biology and Ecology 200, 207-237.

Fong, P. P.; Kyozuka, K.; Duncan, J.; Rynkowski, S.; Mekasha, D. and Ram, J. L. (1995) The effect of salinity and temperature on spawning and fertilization in the zebra mussel *Dreissna polymorpha* (Pallas) from North America. Biological Bulletin 189, 320-329.

Fonseca, M. S.; Fisher, J. S.; Zieman, J. C. and Thayer, G. W. (1982) Influence of seagrass, *Zostera marina* L., on current flow. Estuarine, Coastal and Shelf Science 15, 351-364.

Freeman, G., and Miller, R. L. (1982) Hydrozoan eggs can only be fertilized at the site of polar body formation. Developmental Biology 94, 142-152.

Fuentes, H. R. (1994) Population biology of the commercial scallop (*Pecten fumatus*) in Jervis Bay, NSW. Memoirs of the Queensland Museum 36, 247-259.

Gambi, M. C.; Nowell, A. R. M. and Jumars, P. A. (1990) Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. Marine Ecology Progress Series 61, 159-169.

Garcia-Esquival, Z. and Bricelj, V. M. (1993) Ontogenetic changes in microhabitat distribution of juvenile bay scallops, *Argopeten irradians irradians* (L.), in eelgrass beds, and their potential significance to early recruitment. Biological Bulletin 185, 42-55.

Gibbons, M. C. and Castagna, M. (1984) Serotonin as an inducer of spawning in six bivalve species. Aquaculture 40, 189-191.

Ginzburg, A. S. (1972) Fertilization in fishes and the problem of polyspermy. U.S. Department of Commerce. Springfield, Virginia.

Goshima, S. and Fujiwara, H. (1994) Distribution and abundance of cultured scallop *Patinopecten yessoensis* in extensive sea beds as assessed by underwater camera. Marine Ecology Progress Series 110, 151-158.

Greenwood, P. J. and Bennett, T. (1981) Some effects of temperature-salinity combinations on the early development of the sea urchin *Parechinus angulosus* (Leske) fertilization. Journal of Experimental Marine Biology and Ecology 51, 119-131.

Grosberg, R. K. (1987) Limited dispersal and proximity-dependent mating success in the colonial ascidian *Botryllus schlosseri*. Evolution. 41, 372-384.

Grosberg, R. K. and Levitan, D. R. (1992) For adults only? Supply-side ecology and the history of larval biology. Trends in Ecology and Evolution 7, 130-133.

Gruffydd, L. D. and Beaumont, A. R. (1970) Determination of the optimum concentration of eggs and spermatozoa for the production of normal larvae in *Pecten maximus* (Mollusca, Lamellibranchia). Helgolander wiss. Meeresunters 20, 486-497.

Hamel, J. -F. and Mercier, A. (1996) Evidence of chemical communication during the gametogenesis of holothuroids. Ecology 77, 1600-1616.

Hamilton, P. V. and Koch, K. M. (1996) Orientation toward natural and artificial grassbeds by swimming scallops, *Argopecten irradians* (Lamarck, 1819). Journal of Experimental Marine Biology and Ecology 199, 79-88.

Hartnoll, R. G. (1967) An investigation of the movement of the scallop *Pecten maximus*, Helgolander wiss. Meeresunters 15, 523-533.

Hatcher, B. G.; Scheibling, R. E.; Barbeau, M. A.; Hennigar, A. W.; Taylor, L. H. and Windust, A. J. (1996) Dispersion and mortality of a population of sea scallop (*Placopecten magellanicus*) seeded in a tidal channel. Canadian Journal of Fisheries and Aquatic Sciences 53, 38-54.

Havenhand, J. N. (1991) Fertilisation and the potential for dispersal of gametes and larvae in the solitary ascidian *Ascidia mentula* Muller. Ophelia 33, 1-15.

Hedgecock, D (1994a) Does variance in reproductive success limit effective population sizes of marine organisms? In: Genetics and evolution of marine organisms (Ed. Beaumont, A.). Chapman and Hall, London. 122-134.

Hedgecock, D. (1994b) Temporal and spatial genetic structure of marine animal populations in the California current. California Cooperative Oceanic Fisheries Investigations Report 35, 73-81.

Hedgecock, D. V.; Chow, V. and Waples, R. S. (1992) Effective population numbers of shellfish broodstock estimated from temporal variance in allelic frequencies. Aquaculture 108, 215-232.

Hill, W. G. (1972) Effective size of populations with overlapping generations. Theoretical Population Biology 3, 278-289.

Hoppe, W. F. and Reichert, M. J. M. (1987) Predictable annual mass release of gametes by the coral reef sponge *Neofibularia nolitangere* (Porifera: Dermospongiae). Marine Biology 94, 277-285.

Hurlburt, S. H. (1990) Spatial distribution of the montane unicorn. Oikos 58, 257-271.

Husband B. C. and Barrett, S. C. (1995) Estimating effective population size: a reply to Nunney. Evolution 49, 39-394.

Jaffe, L. A. (1976) Fast block to polyspermy in sea urchins is electrically mediated. Nature 261, 68-71.

Jaffe, L. A. and Gould, M. (1985) Polyspermy-preventing mechanisms. In: Biology of fertilization. Vol 3. (Eds: Metz, C. B. and A. Monroy, A.) Academic press, New York. 223-250.

Johnson M. S. and Black R. (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. Evolution 38, 1371-1383.

Johnson M. S.; Holborn, K. and Black, R. (1993) Fine-scale patchiness and genetic heterogeneity of recruits of the corallivorous gastropod *Drupella cornus*. Marine Biology 117, 91-96.

Johnson, K. G. (1992) Synchronous planulation of *Manicina areolata* (Scleractinia) with lunar periodicity. Marine Ecology Progress Series 87, 265-273.

Joll, L. M. and Caputi, N. (1995) Geographic variation in the reproductive cycle of the saucer scallop, *Amusium balloti* (Bernardi, 1861) (Mollusca: Pectinidae), along the Western Australian coast. Marine and Freshwater Research 46, 779-792.

Kaczmarska, I. and Dowe, L. L. (1997) Reproductive biology of the red alga *Polysiphonia lanosa* (Ceramiales) in the Bay of Fundy, Canada. Marine Biology 128, 695-703.

Kailola, P. J.; Williams, M. J.; Stewart, P. C.; Reichelt, R. E.; McNee, A. and Grieve, C. (1993) Australian fisheries resources. Bureau of Resource Sciences and the Fisheries Research Corporation, Canberra.

Kassner, J. and Malouf, R. E. (1982) An evaluation of 'spawner transplants' as a management tool in Long Island's hard clam fishery. Journal of Shellfisheries Research 2, 165-12.

Keesing, J. and Babcock, R. (1996) Fertilisation rates of abalone in the field. Report to Department of Industry Sciences and Technology (DIST). Bilateral Science and Technology Collaboration Program – Project 93/7794. South Australian Research and Development Institute, West Beach, Australia.

Kinzie, R. A. III (1993) Spawning in the reef corals *Pocillopora verrucosa* and *P. eydouxi* at Sesoko Island, Okinawa. Galaxea 11, 93-105.

Koehl, M. A. R. and Powell, T. M. (1994): Turbulent transport of larvae near wave-swept shores: Does water motion overwhelm larval sinking ? In: Reproduction and development of marine invertebrates. (Eds: Wilson, W. H.jr; Strickler, S. A. and Shinn, G. L.) John Hopkins University Press, Baltimore, 261-274.

Krebs, C. J. (1979) Ecological methodology. Harper and Row, New York.

Lambert, C. C. and Lambert, G. (1981) Formation of the block to polyspermy in ascidian eggs: time course, ion requirements, and role of the accessory cells. Journal of Experimental Zoology 217, 291-295.

Lasiak, T. (1990) Asynchronous reproductive activity in the broadcast spawner *Cellana capensis* (Gmelin, 1791) (Gastropoda: Patellidae). Journal of Molluscan Studies 56, 69-81.

Lasker, H. R.; Brazeau, D. A.; Calderon, J.; Coffroth, M A.; Coma, R. and Kim, K. (1996) *In situ* rates of fertilization amongst spawning gorgonian corals. Biological Bulletin 190, 45-55.

Levitan, D. R. (1988): Asynchronous spawning and aggregative behaviour in the sea urchin *Diadema antillarum* Philipi. In: Echinoderm Biology : 6th International Echinoderm Conference. (Ed: Burke, R.) A H Balkema Press, pp. 1-6.

Levitan, D. R. (1991) Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biological Bulletin 181, 261-268.

Levitan, D. R. (1993) The importance of sperm limitation to the evolution of egg size in marine invertebrates. The American Naturalist 141, 517-536.

Levitan, D. R. (1995). The ecology of fertilization in free-spawning invertebrates. In: Ecology of marine invertebrate larvae. (Ed: McEdward, L. R.) CRC Press, Boca Raton.123-156.

Levitan, D R. (1996a) Effects of gamete traits on fertilization in the sea and the evolution of sexual dimorphism. Nature 382, 153-155.

Levitan, D. R. (1996b) Predicting optimal and unique egg sizes in free-spawning marine invertebrates. The American Naturalist 148, 174-188.

Levitan, D. R.; Sewell, M. A. and Chia, Fu-S. (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 Chia, Fu-S. (1992) How distribution and abundance influence fertilization success in the sea urchin *Strongyloncentrotus franciscanus*. Ecology 73, 248-254.

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

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

Ludbrook, N. H. and Gowlett-Holmes, K. L. (1989) Chitons, gastropods and bivalves. In: Marine invertebrates of southern Australia, Part II (Eds: Shepherd, S. A. and Thomas, I. M.) South Australian Government Press, Adelaide. 504-724.

MacDonald, B. A. and Thompson, R. J. (1988) Intraspecific variation in growth and reproduction in latitudinally differentiated populations of the giant scallop *Placopecten magellanicus* (Gmelin). Biological Bulletin 175, 361-371.

MacDonald, B.A. and Bajdik, C.D. (1992) Orientation and distribution of individual *Placopecten magellanicus* (Gmelin) in two natural populations with differing production. Canadian Journal of Fisheries and Aquatic Sciences 49, 2086-2092.

Malakoff D. (1997) Extinction on the high seas. Science 277, 486-488.

Marconato, A. and Shapiro, D. Y. (1996) Sperm allocation, sperm production and fertilization rates in the bucktooth parrotfish. Animal Behaviour 52, 971-980.

Marconato, A.; Shapiro, D. Y.; Petersen, C. W.; Warner, R. R. and Yoshikawa, T. (1997) Methodological analysis of fertilization rate in the bluehead wrasse *Thalassoma bifascatum*: pairs versus group spawns. Marine ecology Progress Series 161, 61-70.

Marconato, A.; Tessari, V. and Marin, G. (1995) The mating system of *Xyrichthys novacula*: sperm economy and fertilization success. Journal of Fish Biology 47, 292-301.

Martinez, G.; Saleh, F.; Mettifogo, L.; Campos, E. and Inestrosa, N. (1996) Monomanias and the release of gametes by the scallop *Argopecten purpuratus*. The Journal of Experimental Zoology 274, 365-372.

McGarvey, R.; Serchuk, F. M. and McLaren, I. A. (1993) Spatial and parent-age analysis of stock-recruitment in the Georges bank sea scallop (*Placopecten magellanicus*) population. Canadian Journal of Fisheries and Aquatic Sciences 50, 564-574.

Mead, K. S. and Denny, M. W. (1995) The effects of hydrodynamic shear stress on fertilization and early development of the purple sea urchin *Strongylocentrotus purpuratus*. Biological Bulletin 188, 46-56.

Miller, R. L. (1966) Chemotaxis during fertilization in the hydroid *Campanularia*. Journal of Experimental Zoology 162, 22-44.

Miller, R. L. (1975) Chemotaxis of *Ciona intestinalis*. Nature 354, 244-245.

Miller, R. L. (1977) Chemotactic behaviour of the sperm of chitons (Mollusca: Polyplacophora). Journal of Experimental Zoology 202, 203-212.

Miller, R. L. (1985) Demonstration of sperm chemotaxis in Echinodermata: Asteroidea, Holothuroidea, Ophiuroidea. Journal of Experimental Zoology 234, 383-414.

Minchin, D. (1989) Up-slope movements in the scallop *Pecten maximus*. Journal of Molluscan Studies 55, 423-425.

Minchin, D. (1992) Multiple species, mass spawning events in an Irish sea lough: the effect of temperatures on spawning and recruitment of invertebrates. Invertebrate Reproduction and Development 22, 229-238.

Mita, M.; Hino, A. and Yasumasu, I. (1984) Effect of temperature on interaction between eggs and spermatozoa of sea urchin. Biological Bulletin 166, 68-77.

Moore, P. A. and Atema, J. (1991) Spatial Information in the three-dimensional fine structure of an aquatic odor plume. Biological Bulletin 181, 408-418.

Morgan, G. (1995) The timing of larval release. In: Ecology of marine invertebrate larvae (Ed: McEdward, L.R.).CRC Press, Boca Raton. 157-191.

Morisita, M. (1971) Composition of the I-index. Research in Population Ecology 13, 1-27.

Morris, A. 1994. The effect of population parameters on the fertilization success of the asteroid *Acanthaster planci* (L.), a modeling approach. B.Sc.(Hons) Thesis, Dept Zoology, University of Queensland, Brisbane.

Nowell, A. R. M. and Jumars, P. A. (1984) Flow environments of aquatic benthos. Annual Review of Ecology and Systematics 15, 303-328.

Nunney, L. (1995) Measuring the ratio of effective population size to adult numbers using genetic and ecological data. Evolution 59, 389-392.

Nunney, L .(1996) The influence of variation in female fecundity on effective population size. Biological Journal of the Linnean Society 59, 411-425.

Nunney, L. and Elam, D. R. (1994) Estimating the effective population size of conserved populations. Conservation Biology 8, 175-184.

O'Connor, W. A. and Heasman, M. P. (1995) Spawning induction and fertilisation in the doughboy scallop *Chlamys* (*Mimachlamys*) *asperrima*. Aquaculture 136, 117-129.

O'Connor, W. A. and Heasman, M. P. (1996) Temporal patterns of reproductive condition in the doughboy scallop. *Chlamys* (*Mimachlamys*) *asperrima* Lamarck, in Jervis Bay, Australia. Journal of Shellfish Research 15, 237-244.

Olive, P. J. W. (1992) The adaptive significance of seasonal reproduction in marine invertebrates: the importance of distinguishing between models. Invertebrate Reproduction and Development 22, 165-174.

Oliver, J. and Babcock, R. (1992) Aspects of the fertilization ecology of broadcast spawning corals: sperm dilution effects and *in situ* measurements of fertilization. Biological Bulletin 183, 409-417.

Oresanz, J. M.; Parma, A. M. and Iribarne, O. (1991) Population dynamics and management of natural stocks. In: Scallops: biology, ecology and aquaculture. (Ed: Shumway, S. E.) Elsevier, Amsterdam, New York, 625-713.

Parsons, G. J.; Robinson, S. M. C.; Chandler, R. A.; Davidson, L. A.; Lanteigne, M. and Dadswell, M. J. (1992) Intra-annual and long-term patterns in the reproductive cycle of giant scallops *Placopecten magellanicus* (Bivalvia:Pectinidae) from Passamaquoddy Bay, New Brunswick, Canada. Marine Ecology Progress Series 80, 203-214.

Pearse, J. S.; McClary, D. J.; Sewell, M. A.; Austin, W. C.; Perez-Ruzafa, A. and Byrne, M. (1988) Simultaneous spawning of six species of echinoderms in Barkley Sound, British Columbia. Invertebrate Reproduction and Development 14, 279-288.

Pearson, G. A. and Brawley, S. H. (1996) Reproductive ecology of *Fucus distichus* (Phaeophyceae): an intertidal alga with successful fertilization. Marine Ecology Progress Series 143, 211-233.

Pennington, J. T. (1985) The ecology of fertilization of echinoid eggs: The consequences of sperm dilution, adult aggregation. and synchronous spawning. Biological Bulletin 169, 417-430.

Peters, R. H. (1991) A critique for ecology. Cambridge University Press, Cambridge.

Petersen, C. W. (1991a) Variation in fertilization rate in the tropical reef fish, *Halichoeres bivattatus*: correlates and implications. Biological Bulletin 181, 232-237.

Petersen, C. W. (1991b) Sex allocation in hermaphroditic sea basses. The American Naturalist 138, 650-667.

Petersen, C. W.; Warner, R. R.; Cohen, S.; Hess, H. C. and Sewell, A. T. (1992) Variable pelagic fertilization success: implications for mate choice and spatial patterns of mating. Ecology 73, 391-401.

Peterson, C. H. (1983) A concept of quantitative reproductive senility: application to the hard clam *Mercenaria mercenaria* (L.)? Oecologia 58, 164-168.

Peterson, C. H. and Summerson, H. C. (1992) Basin-scale coherence of population dynamics of an exploited marine invertebrate, the bay scallop: implications of recruitment limitation. Marine Ecology Progress Series 90, 257-272.

Peterson, C. H.; Ambrose, W. G. J. and Hunt, J. H. (1982) A field test of the swimming response of the bay scallop (*Argopecten irradians*) to changing biological factors. Bulletin of Marine Science 32, 939-944.

Peterson, C. H.; Summerson, H. C. and Leuttich, R. A. jr (1996) Response of bay scallops to spawner transplants: a test of recruitment limitation. Marine Ecology Progress Series 132, 93-107.

Pielou, E. C. (1977) Mathematical ecology. Wiley, New York.

Pitcher, C. R. and Butler, A. J. (1987) Predation by asteroids, escape response, and morphometrics of scallops with epizoic sponges. Journal of Experimental Marine Biology and Ecology 112, 223-249.

Podolsky, R. D., and Strathmann, R. R. (1996) Evolution of egg size in free spawners: consequences of the fertilization-fecundity tradeoff. American Naturalist 148, 160-173.

Pohle, D. G.; Bricelj, V. M. and Garcia-Esquivel, Z. (1991) The eelgrass canopy: an above-bottom refuge from benthic predators for juvenile bay scallops *Argopecten irradians*. Marine Ecology Progress Series 74, 47-59.

Pulliam, H. R. (1988) Sources, sinks, and population regulation. American Naturalist 132, 652-661.

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 *Strongyloncentrotus franciscanus*. American Zoologist 33, 537-550.

Riveros, A.; Zuniga, M.; Larrain, A. and Becarra, J. (1996) Relationships between fertilization of the Southeastern Pacific sea urchin *Arbacia spatuligera* and environmental variables in polluted coastal waters. Marine Ecology Progress Series 134, 159-169.

Rose, R. A. and Dix, T. G. (1984) Larval and juvenile development of the doughboy scallop *Chlamys* (*Chlamys*) *asperrimus* (Lamarck) (Mollusca: Pectinidae). Australian Journal of Marine and Freshwater Research 35, 315-323.

Rothschild, L. and Swann, M. M. (1951) The fertilization reaction in the sea-urchin. The probability of a successful sperm-egg collision. Journal of Experimental Biology 28, 403-416.

Rumrill, S. S. (1990) Natural mortality of marine invertebrate larvae. Ophelia 32, 163-198.

Rupp, J. H. (1973) Effects of temperature on fertilisation and early cleavage of some tropical echinoderms, with emphasis on *Echinometra mathaei*. Marine Biology 23, 183-189.

Sanders, M. J. (1966) Parasitic castration of the scallop *Pecten alba* (Tate) by a bucephalid trematode. Nature 212, 307-308.

Sanders, M. J. and Lester, R. J. G. (1981) Further observations on a bucephalid trematode in scallops (*Pecten alba*) in Port Phillip Bay, Victoria. Australian Journal of Marine and Freshwater Research 32, 475-478.

Sause, B. L.; Gwyther, D.; Hanna, P. J. and O'Connor, N. A. (1987) Evidence for winter-spring spawning of the scallop *Pecten alba* (Tate) in Port Phillip Bay, Victoria. Australian Journal of Marine and Freshwater Research 38, 329-337.

Schneider, D. C.; Gagnon, J. M. and Gilkinson, K. D. (1987) Patchiness of epibenthos megafauna on the outer Grand Banks of Newfoundland. Marine Ecology Progress Series 39, 1-13.

Schuel, H. (1984) The prevention of polyspermic fertilization in sea urchins. Biological Bulletin 167, 271-309.

Serrao, E. A.; Pearson, G.; Kautsky, L. and Brawley, S. H. (1996) Successful external fertilization in turbulent environments. Proceedings of the National Academy of Sciences 93, 5286-5290.

Sewell, M. A. and Levitan, D. R. (1992) Fertilization success during a natural spawning of the dendrochirote sea cucumber *Cucumaria miniata*. Bulletin of Marine Science 51, 161-166.

Shanks, A. L. (1995) Mechanisms of cross-shelf dispersal of marine invertebrates. In: Ecology of marine invertebrate larvae (Ed: McEdward, L. R.) CRC Press, Boca Raton. 123-156

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 Sciences 50, 2001-2009.

Sinclair, M. (1988) Marine populations: an essay on population regulation and speciation. Washington Sea Grant Program and University of Washington University Press.

Sokal, R. R. and Rohlf, F. J. (1995) Biometry -the principles and practice of statistics in biological research. W.H. Freeman Company, New York.

Spinelli, G. and Albanese, I. (1990) Echinodermata: molecular and cellular biology of the sea urchin embryo. In: Reproductive biology of invertebrates. (Eds: K. G. Adiyodi, and R. G. Adiyodi eds), John Wiley & Sons Ltd. 283-390.

Sprung, M., and Bayne, M. (1984). Some practical aspects of fertilizing the eggs of the mussel *Mytilus edulis* L. Journal du Conseil International pour L'Exploration de la Mer 41: 125-128.

Starr, M.; Himmelman, J. H. and Therriault, J. -C. (1990) Direct coupling of marine invertebrate spawning with phytoplankton blooms. Science 247, 1071-1074.

Stokesbury, K. D. E. and Himmelman, J. H. (1993) Spatial distribution of the giant scallop *Placopecten magellanicus* in unharvested beds in the Baie des Chaleurs, Quebec. Marine Ecology Progress Series 96, 159-168.

Stokesbury, K. D. E. and Himmelman, J. H. (1995) Biological and physical variables associated with aggregations of the giant scallop *Placopecten magellanicus*. Canadian Journal of Fisheries and Aquatic Sciences 52, 743-753.

Stokesbury, K. D. E. and Himmelman, J. H. (1996) Experimental examination of movement of the giant scallop, *Placopecten magellanicus*. Marine Biology 124, 651-660.

Styan, C. A. (1997) Inexpensive and portable sampler for collecting eggs of free-spawning marine invertebrates underwater. Marine Ecology Progress Series 150, 293-396.

Systat (1992) Systat for windows. Systat Corporation.

Temkin, M. H. (1996) Comparative fertilization biology of gymnolaemate bryozoans. Marine Biology 127, 329-339.

Tettelbach, S. T. and Wenczel, P. (1993) Reseeding efforts and the status of bay scallop *Argopecten irradians* (Lamarck, 1819) populations in New York following the occurrence of 'brown tide' algal blooms. Journal of Shellfish Research 12, 423-431.

Thomas, F. I. M. (1994a) Physical properties of gametes in three species of sea urchins. Journal of Experimental Biology 194, 263-284.

Thomas, F. I. M. (1994b) Transport and mixing of gametes in three free-spawning polycheate annelids, *Phragmatopoma californica* (Fewkes), *Sabellaria cementarium* (Moore) and *Schizobranchia insignis* (Bush). Journal of Experimental Marine Biology and Ecology 179, 11-27.

Thouzeau, G.; Robert, G. and Smith, S. J. (1991) Spatial variability in distribution and growth of juvenile and adult sea scallops *Placopecten magellanicus* (Gmelin) on eastern Georges Bank (Northwest Atlantic). Marine Ecology Progress Series 74, 205-218.

Underwood, A. J. and Fairweather, P. G. (1989) Supply-side ecology and benthic marine assemblages. Trends in Ecology and Evolution 4,16-20.

Vacquier, V. D. and Payne, J. E. (1973) Methods for quantitating sea urchin sperm-egg binding. Experimental Cell Research 82, 227-235.

Vahl, O. (1981) Age-specific residual reproductive value and reproductive effort in the Iceland Scallop, Chalmys islandica (O.F. Muller). Oecologia 51, 53-56.

Vogel, H.; Czihak, G.; Chang, P. and Wolf, W. (1982) Fertilization kinetics of sea urchin eggs. Mathematical Biosciences 58, 189-216.

Warner, R. R.; Shapiro, D. Y.; Marconato, A. and Petersen, C. W. (1995) Sexual conflict: males with highest mating success confer the lowest fertilization benefit to females. Proceedings of the Royal Society of London, B Series 262:135-149.

Watts, R. J.; Johnson, M. S. and Black, R. (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. Marine Biology 105, 145-151.

Weissburg, M. J. and Zimmer-Faust, R. K. (1993) Life and death in moving fluids: hydrodynamic effects on chemosensory-mediated predation. Ecology 74, 1428-1443.

West, G. (1990) Methods of assessing ovarian development in fishes: a review. Australian Journal of Marine and Freshwater Research 41, 199-222.

Williams, M. E.; Bentley, M. G. and Hardedge, J. D. (1997) Assessment of field fertilization success in the infaunal polycheate *Arenicola marina* (L.). Invertebrate Reproduction and Development 31, 189-197.

Winet, H. (1973) Wall drag on free-moving cilliated micro-organisms. Journal of Experimental Biology 59: 753-766.

Winter, M. A. and Hamilton, P. V. (1985) Factors influencing swimming in bay scallops, *Argopecten irradians* (Lamarck, 1819). Journal of Experimental Marine Biology and Ecology 88, 227-242.

Wolf, B. M. and White, R. W. G. (1995) Age and growth of the Queen scallop, *Equichlamys bifrons*, in the D'Entrecastreaux Channel and Huon River Estuary, Tasmania. Marine and Freshwater Research 46, 1127-1135.

Wolf, B. M. and White, W. G. (1997) Movements and habitat use of the queen scallop, *Equichlamys bifrons*, in the D'Entrecasteaux Channel and Huon River estuary, Tasmania. Journal of Shellfish Research 16, 533-539.

Worcester, S. E. (1995) Effects of eelgrass beds on advection and turbulent mixing in low current and low shoot density environments. Marine Ecology Progress Series 126, 223-232.

Wright, S. (1938) Size of population and breeding structure in relation to evolution. Science 87: 430-431.

Young, C.M.; Tyler, P.A.; Cameron, J. L. and Rumrill, S. G. (1992) Seasonal breeding aggregations in low-density populations of the bathyal echinoid *Stylocidaris lineata*. Marine Biology 113, 603-612.

Yund, P. O. (1990) An *in situ* measurement of sperm dispersal in a colonial marine hydroid. The Journal of Experimental Zoology 253, 102-106.

Zacharin, W. Reproduction and recruitment in the doughboy scallop (*Chlamys asperrimus*) in the D'Entrecasteaux Channel, Tasmania. Memoirs of the Queensland Museum 36, 299-306.

Zar, J. H. (1984) Biostatical analysis. Prentice-Hall, New Jersey.

Zeeck, E.; Harder, T.; Beckman, M. and Muller, C. T. (1996) Marine gamete-release pheromones. Nature 382, 214.

Zimmer-Faust, R. K.; Finelli, C. M.; Pentcheff, N. D. and Wethey, D. S. (1995) Odor plumes and animal navigation in turbulent water flow: a field study. Biological Bulletin 188: 111-116.

Ziomek, C. A. and Epel, D. (1975) Polyspermy block of *Spisula* eggs is prevented by cytochalasin B. Science 189, 139-141.

Appendix

Vol. 150: 293–296, 1997 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published April 30

NOTE

Inexpensive and portable sampler for collecting eggs of free-spawning marine invertebrates underwater

Craig A. Styan*

Department of Zoology, University of Adelaide, South Australia 5005, Australia

ABSTRACT: A simple device is described that can be used to collect the eggs of free-spawning marine invertebrates and that is cheap, portable and fool-proof. The air-displacement sampler was used to collect eggs from female sea urchins, *Heliocidaris erythrogramma*, downstream from experimentally induced spawning males. Average fertilisation rates of eggs decreased downstream and were 87, 48, 40, 17 and 7% at 10, 100, 200, 400 and 1000 cm respectively.

KEY WORDS: Fertilisation ecology · Sampling · Sea urchin · *Heliocidaris* · Marine invertebrate

There is increasing evidence that eggs released by free-spawning marine invertebrates and fish are not always successfully fertilised (reviewed in Levitan 1995, Levitan & Peterson 1995). Predicting when and where incomplete fertilisation is likely to occur is important for the conservation of these species and understanding their evolutionary ecology (Levitan 1995, Levitan & Peterson 1995). Measurement of fertilisation success in field experimental studies (Pennington 1985, Levitan 1991, Babcock et al. 1994) or direct observations of natural spawning events (Peterson 1991, Babcock & Mundy 1992, Babcock et al. 1992, Peterson et al. 1992, Sewell & Levitan 1992, Shapiro et al. 1994, Marconato et al. 1995) require that isolated samples of free-spawned eggs be collected *in situ* and safely transported back to the laboratory. To facilitate the collection of such data, I describe here a new diver-operated device (the 'air-displacement sampler') that can be used to collect eggs underwater. As a demonstration of its use in the field, I report the results of a simple experiment in which the sampler was used to collect the eggs of female sea urchins (*Heliocidaris erythrogramma*) that were induced to spawn at varying distances downstream from a spawning male. As a broad test of the sampler, it was expected that the results of this experiment would show a pattern of decreasing fertilisation success with increasing distance downstream, similar to that found in previous experimental (Pennington 1985, Levitan 1991, Babcock et al. 1994) and theoretical work (Denny 1988). These data are also likely to be important in their own right, for they are the first indicating the scale (of distance between spawners and hence population density) over which sperm limitation effects may occur in *H. erythrogramma*. For commercially harvested populations of urchins such as *H. erythrogramma*, incorporating 'Allee Effects' (reduced *per capita* larval production through sperm limitation effects) into population models may be important for effective management (Quinn et al. 1993).

The air-displacement suction sampler. The air-displacement sampler, shown in Fig. 1, is based upon a 2 l, air filled, clear solid plastic container with a sealable, screw-top lid. The sampler works underwater by drawing water (and eggs) through a lower inlet tap into an attached (empty) plastic bag as air is released from the container through an upper outlet tap (bore of both taps = 19 mm). The sampler needs to be held upright (as in Fig. 1B) with both the inlet and outlet taps open for this to occur. Once the plastic bag is full or sufficient numbers of eggs have been collected, the inlet tap is closed, sealing the sample for return to the laboratory, and the bag/lid simply unscrewed.

Taking further samples is a simple process if working in shallow water—the container is simply opened above water (refilling the main container with air) and a new bag/lid attached. Underwater, the main container can be refilled by closing the outlet tap and purging a diver's octopus regulator into it, then screwing a new bag/lid onto the air filled container. Note that the container does not have to be completely filled with air for

*E-mail: cstyan@zoology.adelaide.edu.au

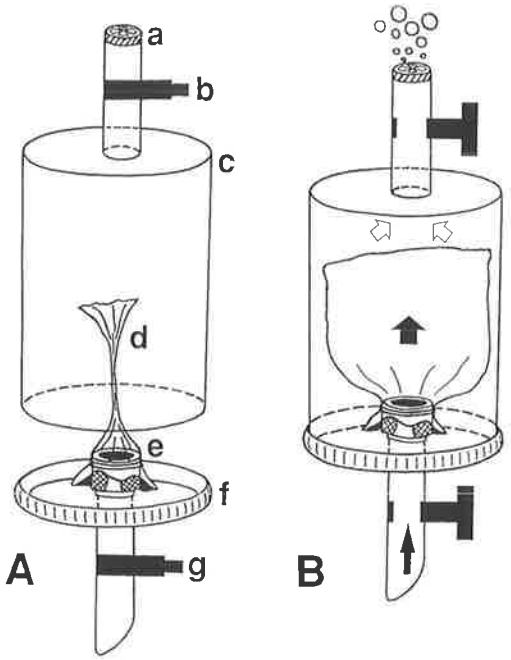


Fig. 1. The air-displacement sampler. (A) Outlet and inlet taps closed. (a) 1-way snorkel valve, (b) outlet tap, (c) 2 l plastic container, (d) plastic bag attached to (e) snap-on garden hose connector, (f) screw on, sealable lid, (g) inlet tap. (B) Outlet and inlet taps open. Air (hollow arrows) escapes from within container through upper one-way valve, drawing eggs and water (solid arrows) through inlet tap into attached internal plastic bag

the sampler to work. If a clear plastic container is used and filled underwater, adjusting air volume within the container to a calibration mark before attaching the bag/lid (by filling then venting excess air through the outlet tap) allows consistent sampling of a fixed, known volume of water (volume of sample = volume of air released). A one-way snorkel valve on the outlet tap allows this tap to be opened without escape of air once the bag/lid is attached, priming the sampler for action as soon as the inlet tap is also opened. To avoid the bulk of multiple lid/inlet taps, plastic 'snap-on' garden hose connections (Nylex™) can be used to attach replacement plastic bags to a single lid/inlet tap, with plugs and one-way valves within the hose fittings used to seal removed, filled bags.

The main features of this sampler are its simplicity and portability. The air-displacement sampler is extremely inexpensive and easy to make. Parts cost less than A$60 but most could be found (at no cost) lying around an aquarium or workshop. The other benefit of its extreme simplicity is that it cannot suffer circuit or battery failure, nor can it fail through flooding. The air-displacement sampler is easily carried and operated by a single worker on SCUBA and the whole sampler, diver's slate and 12 replacement bags can be carried in a medium sized catchbag that weighs very little above (<1 kg) or below the water. This is in contrast to some other samplers (e.g. the 'COTSucker'; Mundy et al. 1994).

Fertilisation success of *Heliocidaris erythrogramma*. Ripe urchins were collected from a shallow subtidal rocky reef at Wool Bay, Yorke Peninsula, South Australia, in late November 1995 and were taken to a nearby site at Edithburgh Jetty where they were induced to spawn by injecting 3 to 5 ml of a 0.5 M KCl solution into the body cavity. To simulate rocky reef habitat where *Heliocidaris erythrogramma* are commonly found, rows of 18 × 18 × 18 cm concrete blocks were arranged in line with the prevailing current and a single spawning male was placed on a central block. After 10 minutes of male spawning, a spawning female was placed on the furthermost downstream block and, as they were released, her eggs were collected using the air-displacement sampler. The female was then moved to the next block upstream where a new sample of eggs was collected as released. The female was moved upstream until egg samples had been collected at 1000, 400, 200, 100 and 10 cm downstream of the (still spawning) male. An egg sample was also collected 800 cm upstream of the male, which was assumed to be free of sperm from the experimental male, to act as a control for background fertilisations. All sample bags were filled at the maximum rate (valves fully open). Three hours later, at least 200 eggs per sample were examined under a dissecting microscope and scored for the presence or absence of fertilisation membranes or cleavage lines that indicated successful fertilisation. This was repeated 5 times over 3 consecutive days, using a new pair of ripe urchins each time. Water was 4 m deep and at 18°C and current speeds were between 5 and 12 cm s^{-1}.

With valves opened fully, the sampler collected 1.3 l samples of eggs and water at a rate of 3 l min^{-1}. *Heliocidaris erythrogramma* eggs are large (approx. 400 µm), buoyant, often stick together in strings and are a highly visible orange colour and so multiple strings could be collected some distance downstream (up to 1.5 m) from the female using several sampling 'sucks' by opening and closing the inlet valve. Eggs remained in good condition in the plastic bags and many hundreds were easily filtered out to be examined later.

Fertilisation success (displayed in Fig. 2) decreased with increasing distance from the male, presumably because of decreasing sperm concentration downstream. The average rates of fertilisation were 87, 48, 40, 17 and 7% at 10, 100, 200, 400 and 1000 cm downstream respectively. All control samples indicated no background fertilisation during trials. These values are similar, but perhaps slightly greater than, rates found in equivalent field experiments for the urchins *Strongylocentrotus*

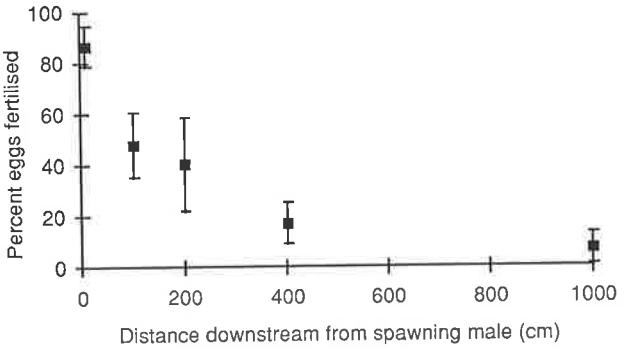


Fig. 2. Mean (± SE) fertilisation success of female *Heliocidaris erythrogramma* with increasing distance downstream from a spawning male (n = 5)

droebachiensis (Pennington 1985) and *Diadema antillarum* (Levitan 1991). Higher fertilisation rates were found over these distances for the crown of thorns starfish *Acanthaster planci* (Babcock et al. 1994) but much lower rates found for the scallop *Chlamys bifrons* (Styan unpubl.). That the pattern of the *Heliocidaris erythrogramma* data fits well within previous estimates tentatively suggests that the air-displacement sampler returns realistic fertilisation estimates, or at least estimates with similar biases to other sampling devices. Assuming no bias, these data also suggest that large Allee Effects are unlikely in populations of *H. erythrogramma* where there is a reasonable likelihood of male spawners within 400 cm upstream of females. Although it provides an indication of the scale of inter-spawner distance over which sperm limitation may occur, care must be taken in extrapolating data from simple linear arrays to population models of fertilisation success. To be reasonable, such models require further data about sperm dispersal upstream and across current, population dispersion patterns and level of spawning synchrony between individuals (Styan unpubl.).

Any device used to collect free-spawned eggs potentially may bias estimates of fertilisation success (Levitan 1995) and at this point it is not possible to determine to what degree the air-displacement sampler has affected the *Heliocidaris erythrogramma* data. Upwards bias in fertilisation rate may have been caused by containing eggs at high concentrations of sperm for longer than would be experienced otherwise (Morris 1994). However, models of fertilisation kinetics (Vogel et al. 1982, Styan unpubl.) and laboratory experiments (Levitan et al. 1991) indicate that most sperm and egg collisions occur within the first few seconds of exposure of eggs to sperm, suggesting any bias will be slight. Killing contained sperm or removing samples as quickly as possible to the surface where eggs can be washed with/into sperm-free water are probably the best ways to avoid this problem (S. T. Mead & R. C. Babcock unpubl.). Collecting eggs as long as possible after spawning should also help to reduce sampling biases associated with preventing eggs from mixing freely in natural conditions, but needs to be weighed against increased difficulty in capturing dilute eggs. Further work is required to determine whether apparent differences in the scale over which fertilisation works that occur between species reflect true differences in the dynamics of external fertilisation or instead are related to techniques used or environmental conditions experienced during experiments by different workers.

As expected, the sampler worked without failure in all trials and the reasonably large volume of water sampled (1.3 l) allowed capture of an acceptable number of eggs at some distance downstream from the female. The air-displacement sampler can collect much more than the largest presently available plastic syringe (60 ml), but other pump/filter samplers (Mundy et al. 1994, Taylor et al. 1995) or Nitex mesh nets (Peterson 1991, Peterson et al. 1992) may be better for sampling barely visible eggs at very low concentrations. Larger samples might be taken with a scaled up version of the air-displacement sampler, but changes in buoyancy associated with the displacement of >2 l of air may lead to dangerous problems for incorrectly weighted divers. Modifications such as an air collection/recycling container attached to the outlet tap and appropriate weighting with lead may circumvent this, but would negate the primary advantages (size, portability and simplicity) of the air-displacement sampler.

The small construction costs, easy storage and foolproof reliability means that the air-displacement sampler could be easily carried on nearly all field ventures where free-spawning events may be encountered. Given the highly sporadic and unpredictable nature of most sightings of free-spawning events (Pennington 1985, Pearse et al. 1988, Minchin 1992, Babcock & Mundy 1992, Babcock et al. 1992, Sewell & Levitan 1992), I would encourage marine ecologists to do this and be ready at all times to add to the regrettably small number of fertilisation rate measurements made during natural spawning events.

Acknowledgements. Joanna Strzelecki and Ian Magraith provided invaluable help in the field and Alan Butler, Joanna Strzelecki, Andy Davis and Michael M. Beck provided comments on this note. I was supported by an Australian Postgraduate Research Award, a Queens Trust Award and a University of Adelaide Alumni Award.

LITERATURE CITED

Babcock RC, Mundy CN (1992) Reproductive biology, spawning and field fertilization rates of *Acanthaster planci*. Aust J Mar Freshwat Res 43:525–534

Babcock R, Mundy C, Keesing J, Oliver J (1992) Predictable and unpredictable spawning events: *in situ* behavioural data from free-spawning coral reef invertebrates. Inv Reprod Develop 22:213–228

Babcock RC, Mundy CN, Whitehead D (1994) Sperm diffusion models and *in situ* confirmation of long-distance fertilization in the free-spawning asteroid *Acanthaster planci*. Biol Bull 186:17–28

Denny MW (1988) Biology and mechanics of the wave-swept environment. Princeton University Press, Princeton

Levitan DR (1991) Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. Biol Bull 181:261–268

Levitan DR (1995) The ecology of fertilization in free-spawning invertebrates. In: McEdward LR (ed) Ecology of marine invertebrate larvae. CRC Press, Boca Raton, p 123–156

Levitan DR, Peterson CW (1995) Sperm limitation in the sea. TREE 10:228–231

Levitan DR, Sewell MA, Chia FS (1991) Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: interaction of gamete dilution, age, and contact time. Biol Bull 181:371–378

Marconato A, Tessari V, Marin G (1995) The mating system of *Xyrichthys novacula*: sperm economy and fertilization success. J Fish Biol 47: 292–301

Minchin D (1992) Multiple species, mass spawning events in an Irish sea lough: the effect of temperatures on spawning and recruitment of invertebrates. Inv Reprod Develop 22: 229–238

Morris A (1994) The effect of population parameters on the fertilization success of the asteroid *Acanthaster planci* (L.), a modelling approach. BSc (Hons) thesis, Dept Zoology, University of Queensland, Brisbane

Mundy C, Babcock R, Ashworth I, Small J (1994) A portable, discrete-sampling submersible plankton pump and its use in sampling starfish eggs. Biol Bull 186:168–171

Pearse JS, McClary DJ, Sewell MA, Austin WC, Perez-Ruzafa A, Byrne M (1988) Simultaneous spawning of six species of echinoderms in Barkely Sound, British Columbia. Inv Reprod Develop 14:279–288

Pennington JT (1985) The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation. and synchronous spawning. Biol Bull 169:417–430

Peterson CW (1991) Variation in fertilization rate in the tropical reef fish, *Halichoeres bivattatus*: correlates and implications. Biol Bull 181:232–237

Peterson CW, Warner RR, Cohen S, Hess HC, Sewell AT (1992) Variable pelagic fertilization success: implications for mate choice and spatial patterns of mating. Ecology 73:391–401

Quinn JF, Wing SR, Botsford LW (1993) Harvest refugia in marine invertebrate fisheries: models and applications to the red sea urchin *Strongylocentrotus franciscanus*. Am Zool 33:537–550

Sewell MA, Levitan DR (1992) Fertilization success during a natural spawning of the dendrochirote sea cucumber *Cucumaria miniata*. Bull Mar Sci 51:161–166

Shapiro DY, Marconato A, Yoshikawa T (1994) Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. Ecology 75:1334–1344

Taylor RB, Blackburn RI, Evans JH (1995) A portable battery-powered suction device for the quantitative sampling of small benthic invertebrates. J Exp Mar Biol Ecol 194:1–7

Vogel H, Czihak G, Chang P, Wieland W (1982) Fertilization kinetics of sea urchin eggs. Math Biosci 58:189–216

This note was presented by A. J. Underwood (Senior Editorial Advisor), Sydney, Australia

Manuscript first received: September 24, 1996
Revised version accepted: March 3, 1997

Amendments made following submission

Chapter 2

page 29: add " E_o - concentration of eggs (eggs μl^{-1})"

Chapter 3

page 49, line 6: delete the word "egg"

page 49, line 11: delete phrase ", according to which model produced the best fit by eye". Replace with ", depending on which model best fitted the data"

page 49, line9: change "3.5 hrs" to "3 hrs"

page 52, line: change "an" to "a"

page 73, figure caption: change "contact time (t)" to "contact time (t seconds)"

Chapter 4

Page 88, line 22: change "figure 4.8.C" to "figure 4.7.C"

Chapter 5

page 125, line 8: The sentence "After 10 minutes of dye flow, samples of ...." should read "After 10 minutes of dye flow, but before dye release had been turned off, samples of....."

page 127, line 7: change "form" to "from"

page 128, line 3: delete "used, " replace with "were"

page 129, line 23" replace "for" with "from"

page 141, line 18: replace "its sampling in two dimensions and" with " sampling plumes in two dimensions with"

page 145, caption: Data were log(x+1) transformed prior to analysis

page 156, caption: males were spawning at 5.4 x 10^6 sperm sec^{-1}

page 157, caption: males spawning at 1.5 x 10^6 sperm sec^{-1}, 5.4 x 10^6 sperm sec^{-1} and 3.7 x 10^7 sperm sec^{-1} are shown as hollow squares (dashed line), solid diamonds (solid line) and hollow triangles (dotted line) respectively

page 158, caption: males were spawning at 5.4 x 10^6 sperm sec^{-1}

Chapter 6

page 161, line 13: N_e should be defined as " the effective genetic population size"

Chapter 7

page 182, line 15: add "(NN)" immediately after the first "Nearest neighbour"

page192, line 11, replace "he" with "the"

page 203, line 16: "Watt et al." should be "Watts et al."