

Early life history of the introduced seastar *Asterias amurensis* in the
Derwent estuary, Tasmania: The potential for ecology-based
management

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Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

School of Zoology, University of Tasmania (April 2002)

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Abstract

The early life-history of the introduced Northern Pacific seastar (*Asterias amurensis*) in the Derwent Estuary, Tasmania, is investigated with an aim to identify opportunities for improved management. A discrete non-equilibrium model of gamete dispersal and fertilization success is developed. The model is parameterized with species-specific gamete traits measured in the estuary population, and is validated using empirically determined estimates of fertilization success, which required containing gametes in collection flasks. *In situ* measurements of the proportion of eggs fertilized ranged from 0.80 ± 0.08 (SE) adjacent to the spawning male, to 0.16 ± 0.04 (SE) for separation distances of 16 m. The model is then adapted to simulate dispersal of gametes without the experimental artifact of containing gametes in flasks. There is good concordance between empirical estimates and model predictions when the model simulates the experimental procedure. However, when simulations allow for dispersal and fertilization without confinement of gametes in the collection device, predicted fertilization success is substantially lower at 0.05 adjacent to the spawning male and 0.03 at 16 m.

The model is extended to predict the reproductive potential of discrete populations of the seastar in the Derwent Estuary. The effects of population density, group size, spawning synchrony, sex ratio, and water depth on the total number of eggs fertilized and the proportion of eggs fertilized is predicted. Within the range of parameters tested, group size, density and water depth had the most significant effects on fertilization success. The model predicted a 300% increase in fertilization success when density is increased from 0.025 to 0.2 individuals m^{-2} . Spatial variability in the reproductive potential of populations in the estuary is also assessed based on gonad indices of seastars determined at 9 sites in the estuary. Gonad indices of starfish at yacht clubs were 3 times higher than 'control' and wharf sites. Given results of the gamete dispersal model, and spatial variability in density and gonad indices, it is likely that discrete populations in the Derwent Estuary contribute differentially to larval production, and particular populations might potentially be targeted for management.

Dispersal of larvae of the introduced seastar *Asterias amurensis* in the Derwent Estuary, and advection of larvae out of the estuary, is predicted using an

inverse transport model incorporating the behavioural responses of larvae at different stages of development to salinity and light. In laboratory conditions larvae demonstrate reverse diel migration, and do not swim into water < 26 ppt salinity. This behaviour influenced the predicted mortality and retention of larvae in the Derwent Estuary. However, regardless of the swimming behaviour, the transport model suggests that the majority (> 99%) of late-stage larvae are advected out of the estuary.

Substrata that induce settlement and metamorphosis of laboratory reared larvae were determined by introducing competent brachiolaria into wells containing substrata commonly available in SE Tasmania. Larvae settled at high rates when exposed to non-geniculate coralline algae (0.98 ± 0.02 SE after 2 days) and at moderate rates on mud and bare rock (0.37 ± 0.06 and 0.44 ± 0.06 respectively after 7 days). On day 7, larval settlement on sand and in the control (filtered seawater) were low (0.01 ± 0.01 and 0.05 ± 0.02 respectively). Based on the distribution of these substrata in the Derwent Estuary, these results suggest that larvae potentially settle at low rates in the lower estuary where the benthos is largely sand, and at moderate rates in the mid-estuary where fine sediments dominate. Settlement at high rates might potentially occur on fringing reefs in the mid and lower estuary, but this habitat comprises a small portion of the estuary.

Declaration

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

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Alice Morris

18 April 2002

ACKNOWLEDGMENTS

First I would like to thank my two supervisors: Ron Thresher and Craig Johnson. Ron provided good advice during the project and I thank him for his candour. I am also grateful to Ron for facilitating a stimulating work environment and logistic support at CRIMP. Craig provided valuable advice during the project and I particularly thankful to him for his assistance during initial stages of the project and during the write-up.

Many people from CSIRO provided ideas, assistance, and support and I thank them for their contribution. Nic Bax, Alan Butler, and Jemery Day provided valuable suggestions on drafts of the various chapters and I thank them for their input. I am grateful to Russ Bradford, Barry Bruce, Mark Green, and Caroline Sutton for providing technical advice during the project and a fun work environment. Val Latham and Trevor McDougall assisted with the design of salinity experiments. I am also thankful to the unidentified fairy that left relevant papers on my desk intermittently throughout the project.

I am grateful to John Keesing for sharing his practical knowledge of how to rear larvae on a large scale. Alan Beech and Bill Wilkinson also provided technical advice and assistance with larval rearing and algal culture. Russ Babcock provided valuable input at the initial stages of the project. The inspiration for a lot of this work came from Russ and Craig, and I am particularly indebted to Russ for teaching me how to cook a nasty pizza. I would also like to thank Jeff Ross and Piers Dunstan for assistance in the field and the rest of Craig's lab for their support.

Chad Hewitt and Britta Schaffelke provided sound advice. Chad also gave me a flexible work environment in the final stages of the thesis, which helped considerably. I thank Alan Jordan who generously gave permission for me to include his habitat mapping data (figure 6.4). I would also like to thank I. P. Freely for offering the loan of a wetsuit, thanks but no thanks, and Dirk Welsford for helping me find Amanda Huggenkiss.

Finally, I thank my friends and family for their support.

This thesis is dedicated to Aunty Chris who'd wake me up in the early hours with a phone call whenever my uni results came out.

Chapter 1: Introduction

The threat of marine introductions

Over the last two decades, marine introductions have been recognised as a major threat to marine environments, marine-based industries, and human health (Carlton 1996, 1999; Carlton and Geller 1993; Hallegraeff 1998; Harvell *et al.* 1999; Lafferty and Kuris 1994, 1996; McLoughlin and Thresher 1994). Many marine invasive species are introduced as larvae through ballast water exchange (Carlton 1996; Carlton and Geller 1993; Ruiz *et al.* 2000) and arrive in the donor port without predators, pathogens or parasites (Lafferty and Kuris 1994). This lack of natural enemies can result in outbreaks of marine invaders which amplifies the impact of these species (Lafferty and Kuris 1994). *Asterias amurensis*, native to the north-west Pacific (Davenport and McLoughlin 1993; Fisher 1930), is an invasive species that has arrived in Australian waters with no parasites (Goggin 1998) and has attained high densities through population outbreak.

Asterias amurensis in Australian waters

Asterias amurensis was accidentally introduced into the Derwent Estuary, Tasmania in the early 1980's (Davenport and McLoughlin 1993). Since the introduction, the seastar has become prolific in the estuary, with adult densities reaching up to 9.44 individuals m⁻² (Buttermore *et al.* 1994), and larval densities among the highest of any marine invertebrate (Bruce *et al.* 1995). The density of *A. amurensis* in the middle reaches of the estuary is higher than densities considered as outbreaks in Japan (Buttermore *et al.* 1994; Grannum *et al.* 1996; Morrice 1995; Nojima *et al.* 1986). Given the unprecedented abundance in the Derwent Estuary and the major impact of the seastar on marine-based industries within the native range (Hatanaka and Kosaka 1959; Kim 1969; Nojima *et al.* 1986) *Asterias amurensis* was identified as a target pest species by the Australian Ballast Water Management Advisory Committee (ABWMAC). Over the last decade the seastar was also introduced into Port Phillip Bay, Victoria, and the population size has escalated from 340 000 in 1998 to 75 million in 2000 (Parry and Cohen 2001). The seastar directly impacts marine based industries in Tasmania by settling in scallop spat collectors and oyster trays (Martin and Proctor 2000), and has potential to impact other marine based industries and the marine environment in southern

Australia (Buttermore *et al.* 1994; McLoughlin and Thresher 1994; Turner 1992). The significant economic and environmental impacts, and a public perception that the seastar is undesirable, warrants investigation into potential management strategies to reduce *A. amurensis* numbers in the Derwent Estuary, Tasmania.

Ecology-based management of introduced marine pests

Eckman (1996) raised the challenge to marine ecologists to 'close the larval loop' and develop a population model incorporating the various factors affecting abundance and distribution of free-spawning marine invertebrates from gamete release to settlement. Free-spawning marine invertebrates commonly produce large numbers of gametes and there is massive mortality between spawning and settlement. Determining critical points in the life history of free-spawning introduced marine pests where high variability in survivorship occurs may be useful for management.

The potential for population models to improve management of commercially harvested free spawning marine invertebrates has been recognised (Levitan and Sewell 1998; Babcock and Keesing 1999) although the approach is still in its infancy. The framework required to manage commercial species to avoid fisheries collapse is equally applicable to managing populations of marine invaders (Kuris *et al.* 1996). In the context of fisheries management, populations that represent areas of high larval production (sources) which are situated so that predominant currents transport larvae to areas of low larval production (sinks), are ideally conserved. In invasion biology, areas that represent sources of large numbers of larvae may potentially be targeted for improved management. The two disciplines have opposing aims (conservation in fisheries management, and elimination in invasion biology), but they stem from a similar conceptual basis.

In this thesis the early life-history of *Asterias amurensis* is investigated with an aim to acquire knowledge that may be useful to improve management of the introduced pest in the Derwent Estuary, Tasmania. Predicting the relative importance of various factors acting from gamete release to larval settlement that affect the abundance and distribution of free-spawning marine invertebrates requires a multi-disciplinary approach (Eckman 1996). Factors acting at spatial scales

spanning several orders of magnitude can affect the distribution and abundance of larvae at the end of the pelagic phase (see Eckman 1996; Levitan 1995 for reviews).

Factors affecting the distribution and abundance of competent brachiolaria

Spatial variability in growth and condition of adults can affect somatic and gonadal development in free spawning marine invertebrates, which can in turn affect reproductive output (Babcock *et al.* 1994; Guilou and Lumingas 1999; Levitan 1989; Meidel and Scheibling 1999; Qian and Chia 1991). Empirical studies and theoretical models also suggest that population characteristics at the time of spawning such as density, group size, and depth can influence reproductive output (Babcock *et al.* 2000; Claereboudt 1999; Levitan 1991; Levitan *et al.* 1992; Levitan and Young 1995; Pennington 1985). The interaction of larval behaviour and hydrodynamics can also affect larval dispersal, governing the spatial distribution of larvae (Dippner 1987; Smith and Stoner 1993; Tremblay *et al.* 1994; Hinckley *et al.* 1996; Verdierbonnet *et al.* 1997; Jenkins *et al.* 1999). Larval feeding, growth, and mortality affects abundance throughout the pelagic phase (Boidron-Mètarion 1995; Hart and Strathmann 1995; Morgan 1995; Rumrill 1990). Finally, many marine invertebrate larvae display species-specific responses to chemical cues emanating from various substrata and many die or develop abnormally if an appropriate substratum is not encountered to induce settlement and metamorphosis (Johnson *et al.* 1997; Knight-Jones 1953; Pawlik 1992; Williams 1964; Wilson 1968).

Fertilization ecology as a management tool

Historically, it has generally been accepted that fertilization success in free-spawning marine invertebrates is low and limited by the availability of sperm (Allee 1931; Mortensen 1938). These predictions have been supported by *in situ* experiments that predict decreasing fertilization success with increasing male/female separation distances (Babcock and Mundy 1992; Babcock *et al.* 1994; Brazeau and Lasker 1992; Levitan 1991; Levitan and Young 1995; Levitan *et al.* 1992; Pennington 1985; Yund 1990). More recently, models of gamete dispersal and fertilization success predict that sperm limitation can be overcome by increased group size, density, degree of aggregation, and spawning in shallow water (Babcock *et al.* 1994; Levitan and Young 1995; Claereboudt 1999). These models adopt a two-step process in which a plume diffusion model (Csanady 1973) is used to

predict equilibrium gamete concentrations at various distances from a spawning individual, and then fertilization success is estimated using a random walk model (Vogel *et al.* 1982). The emerging picture from the results of these models and *in situ* experiments is that fertilization success in free-spawning marine invertebrates is highly variable and dependent on population characteristics. Identifying populations of *Asterias amurensis* that have potential to produce large numbers of zygotes may be useful for improved management.

Larval dispersal as a vector and priority

Predicting rates of spread of introduced marine species has been a major focus of invasion biology over the last decade (Grosholz 1996; Hastings 1996). The impact of an introduced species on the marine environment and marine-based industries is dependent in part on its rate of spread. Information on larval dispersal can be used to predict the rate and direction of spread of an introduced species so that areas where range extension are likely can be identified.

Vertical migration is common in marine invertebrate larvae (see Forward 1976, 1988; Mileikovsky 1973; Thorson 1964; Young 1990, 1995; Young and Chia 1987, for reviews) and can be an important factor in governing horizontal dispersal (Smith and Stoner 1993) and, for estuarine species, may be important in retaining larvae in estuaries (Cronin and Forward 1982; Sulkin and Van Heukelem 1982; Young and Chia 1987). Recent advances in larval dispersal models have fused hydrodynamics with vertical migration patterns of larvae to predict horizontal dispersal (Dippner 1987; Hinckley *et al.* 1996; Jenkins *et al.* 1999; Rothlisberg *et al.* 1995, 1996; Smith and Stoner 1993; Tremblay *et al.* 1994; Verdierbonnet *et al.* 1997).

Thesis outline

Some of the factors that potentially influence the distribution and abundance of larvae of *Asterias amurensis* have been measured in previous studies, for example population parameters prior to the onset of spawning (Grannum *et al.* 1996; Morrice 1995), larval distribution (Bruce *et al.* 1995), and the length of the pelagic phase (Bruce *et al.* 1995; Kas'yanov 1988; Komatsu 1975). Other potentially important factors governing settlement rates, such as spatial variability in fecundity, the effect

of population parameters on fertilization success, larval dispersal and larval settlement cues, will be addressed in this thesis.

In Chapter 2 species-specific gamete traits are determined for *Asterias amurensis* in the Derwent Estuary to parameterise the Vogel-Czihak-Chang-Wolf fertilization model. Laboratory studies were undertaken to determine egg and sperm release rates, egg diameter, sperm velocity, sperm longevity, gamete fall velocities, and the effect of sperm and egg concentration on fertilization success. Variability in the reproductive potential of discrete populations within the estuary is considered by comparing gonad indices of seastars at three habitats with differing anthropogenic influence *viz* wharves, yacht clubs, and 'control' sites without anthropogenic structure.

In Chapter 3 the fertilization model is extended to simulate gamete dispersal. Fertilization success of eggs released at increasing distance downstream of a spawning male were measured by collecting eggs in a plankton sampler. The gamete dispersal/fertilization model was validated by simulating the experimental procedure and comparing the empirical and predicted patterns of fertilization success. After validating the model by simulating the effect of the plankton sampler, dispersal and fertilization without impediment in the collecting device is simulated, with all other parameters held constant.

Given acceptable validation of the gamete dispersal/fertilization model when experimental artifacts are simulated, in Chapter 4 the model is extended to simulate the effect of various population parameters on fertilization success. Of the parameters tested, density, group size and depth had the most substantial effects on fertilization success.

In Chapter 5 a model of larval dispersal in the Derwent Estuary is developed incorporating larval vertical migration behaviour. The response of laboratory reared larvae at 4 stages of development (early, mid, and late bipinnaria, and early brachiolaria) to salinity and light were measured. The number of larvae retained in the estuary is determined for various release points, river flow regimes, and larval vertical migrating behaviours.

In Chapter 6, substrata that induce settlement and metamorphosis in competent brachiolaria are identified by introducing laboratory reared competent brachiolaria into wells containing substrata common in the Derwent Estuary and scoring larval settlement and metamorphosis over 7 days. Larvae settle at high rates on non-geniculate coralline algae, and a follow-up experiment indicated a role of bacteria on the surface of the algae in inducing settlement.

In the general discussion (Chapter 7) the potential for practical management outcomes of the work is discussed, and priorities for future research are suggested.

Chapter 2: Fertilization kinetics of the introduced seastar, *Asterias amurensis*, in the Derwent estuary, Tasmania

Abstract

Predicting fertilization success in free-spawning marine invertebrates requires knowledge of how sperm and eggs behave at several spatial scales. In this chapter species-specific gamete traits are determined for the introduced sea star *Asterias amurensis* in the Derwent estuary, Tasmania. Laboratory studies were undertaken to determine egg and sperm release rates, egg diameter, sperm velocity, sperm longevity, gamete fall velocities, and the effect of sperm and egg concentration on fertilization success. Two constants necessary to parameterize the Vogel-Czihak-Chang-Wolf fertilization model were determined from experiments that measure decreasing fertilization success with increasing sperm dilution. The fertilization equation was tested by comparing the results of egg dilution experiments to model predictions and the fit of empirical and theoretical predictions was reasonable. Of the parameters investigated, sperm concentration had the most substantial effect on fertilization success.

Variability in the reproductive potential of discrete seastar populations within the Derwent Estuary was also determined by comparing gonad indices of seastars at three habitat types with differing types of anthropogenic influence: wharves, yacht clubs and 'control' sites without anthropogenic structure. There were significant differences in gonad indices between habitats. The relationship between gonad index and total seastar weight, and the mass of dry gamete release during spawning suggest that populations at yacht clubs release between 1.5 to 2.6 times the amount of dry gamete than populations at control sites.

Key words: Fertilization success; free-spawning marine invertebrates; sperm limitation; gamete release rates, Asterias amurensis, marine pests.

Introduction

Historically, it was generally accepted that fertilization success in free-spawning marine invertebrates is low, and limited by the availability of sperm (Allee 1931; Mortensen 1938). However, Thorson (1950) suggested that behaviour at the time of spawning, such as aggregation and synchrony in spawning, may overcome the problem of sperm limitation. More recent empirical studies of fertilization success indicate that sperm is not always limiting in free-spawning invertebrates, and that high levels of fertilization success result from the massive numbers of gametes produced (Babcock *et al.* 1994; Levitan *et al.* 1991; Pennington 1985; Yund 1990, 2000). The emerging picture is that fertilization success in free-spawning marine invertebrates is highly variable and depends on factors acting at several temporal and spatial scales. Because gametes dilute rapidly when released into the water column (Denny 1988; Denny and Shibata 1989), the proportion of eggs fertilized in free spawners increase with high densities, increased aggregation of spawning individuals, spawning in shallow water, and increased synchronicity in spawning (Babcock *et al.* 1994; Babcock *et al.* 2000; Babcock and Keesing 1999; Babcock and Mundy 1992; Benzie *et al.* 1994; Benzie and Dixon 1994; Coma and Lasker 1997; Levitan 1991, 1996; Levitan *et al.* 1992; Oliver and Babcock 1992; Pennington 1985; Yund 1990). In addition to these population traits, theoretical models predict that species-specific parameters, including sperm velocity and egg diameter can also affect zygote production in free spawning invertebrates (Levitan 1996, 1998; Podolsky and Strathman 1996). Thus, predicting reproductive success in free spawning marine invertebrates may require knowledge of factors acting across spatial scales of 10^{-4} to 10^3 m.

In addition to the inherent variability of fertilization success, there are unavoidable artifacts in methods to determine fertilization success in the field, adding another layer of complexity to assessing fertilization dynamics in nature. Most commonly, fertilization in the field is measured by containing gametes in apparatus such as nitex bags or plankton samplers (Babcock *et al.* 1994; Babcock and Mundy 1992; Coma and Lasker 1997; Levitan 1991; Levitan *et al.* 1992; Oliver and Babcock 1992; Pennington 1985; Yund 1990). These methods may overestimate fertilization rates because gametes are contained in the sampling apparatus

and do not disperse (and thus dilute) naturally, or under-estimate fertilization if gamete contacts are hindered by nets separating sperm and eggs (see Levitan 1995 for discussion). Because of this sampling artifact, field experiments alone are not sufficient to estimate fertilization in free spawning marine invertebrates.

For this reason, field and laboratory studies have been combined to develop models that predict fertilization success in free spawning marine invertebrates. Specifically Vogel *et al.* (1982) developed a model that predicts fertilization success based on sperm and egg concentrations (S_0 and E_0), sperm and egg contact time (τ), and two constants (β and β_0). The Vogel-Czihak-Chang-Wolf (VCCW) model predicts fertilization success for various sperm and egg concentrations by determining the probability that sperm and eggs collide using a random walk model (Vogel *et al.* 1982; pp. 203). The model assumes that sperm attach to the first egg they contact even if fertilization does not occur. The fertilization ratio is calculated as:

$$F = 1 - \exp(-\beta/\beta_0 \times S_0/E_0 (1 - e^{-\beta_0 E_0 \tau})) \quad (1)$$

The gamete contact time (τ) is either the time that sperm and eggs interact or the sperm half-life, whichever is smaller. The bimolecular reaction constant, β , is the product of the sperm swimming velocity and the egg fertilization area, where the egg fertilization area is defined as the area of the egg that a sperm must bind in order to fertilize (Vogel *et al.*, 1982). β_0 is the rate constant of sperm-egg collision and is estimated as the product of the total cross-sectional area of the egg and sperm swimming velocity. The two constants, β and β_0 , scale the model for unsuccessful collisions due to non-viable sperm, or sperm contacts with the impenetrable area of the egg. The usefulness of the VCCW model has been determined using four species of sea urchin, for which it accurately predicts empirical measurements of fertilization success (Levitan 1991, Vogel *et al.* 1982).

A combination of laboratory observation and experiments and field trials are used to fully parameterise the VCCW model for the northern Pacific seastar, *Asterias amurensis*, after its accidental introduction to Tasmania. The model is developed to assess spatial variation in reproductive success over the range of habitats the seastar has invaded in the Derwent estuary, south-east Tasmania. I

hypothesise that there are habitats where the seastar has disproportionately high reproductive output due to improved food-related growth and condition. These habitats could be focal areas for management action directed at reducing reproductive output of this pest.

Materials and Methods

Induced spawning

Seastars were collected from Hobart wharves in the Derwent estuary, Tasmania, from June-August in 1996 - 1999 (figure 2.1). Seastars were held in aquaria at 12 °C for < 2 days before being used in experiments; the storage time had no effect on fertilization success. Seastars were sexed macroscopically by extracting a sample of the gonad with a 14-gauge syringe. Spawning was induced in 55 and 35 minutes in females and males after injection with 5-7ml of 10^{-3} M 1-methyladenine. Unless stated otherwise, the gamete mixtures were incubated for 1 hour at 10 °C and fixed in 5% formalin. Fertilization was confirmed by development of a fertilization membrane and fertilization success estimated by examining 100 eggs selected randomly from each sample.

Sperm density

To determine mean (and standard error) sperm density (sperm ml^{-1}), 11 spawning males were blotted dry and their undiluted gametes ('dry' sperm) collected by pipette as they were exuded from the gonopore. The dry sperm was diluted 1000 fold and sperm density determined by counting the total number of sperm in each of 10 haemocytometer chambers for each male.

Egg density

'Dry' female gametes were collected by inverting spawning females ($n = 6$) over beakers. The number of eggs in a 1 ml aliquot of 'dry' gamete was determined by counts under a dissecting microscope to estimate average egg density (eggs ml^{-1}).

Sperm and egg release

Ray length and total wet weights of ripe seastars were measured before seastars were induced to spawn by injection of 5-7ml of 10^{-3} M 1-methyladenine. Adults were blotted dry and inverted over beakers to collect dry gametes and the duration of spawning was measured. Once spawning had ceased, the dry gamete mass was weighed. Sperm and egg release rates were estimated as the product of the gamete release rate (gs^{-1}) and gamete concentration (ml^{-1}). Spawning duration of

adult seastars induced in aquaria were similar to that of seastars inverted over beakers (A Morris *pers obs*).

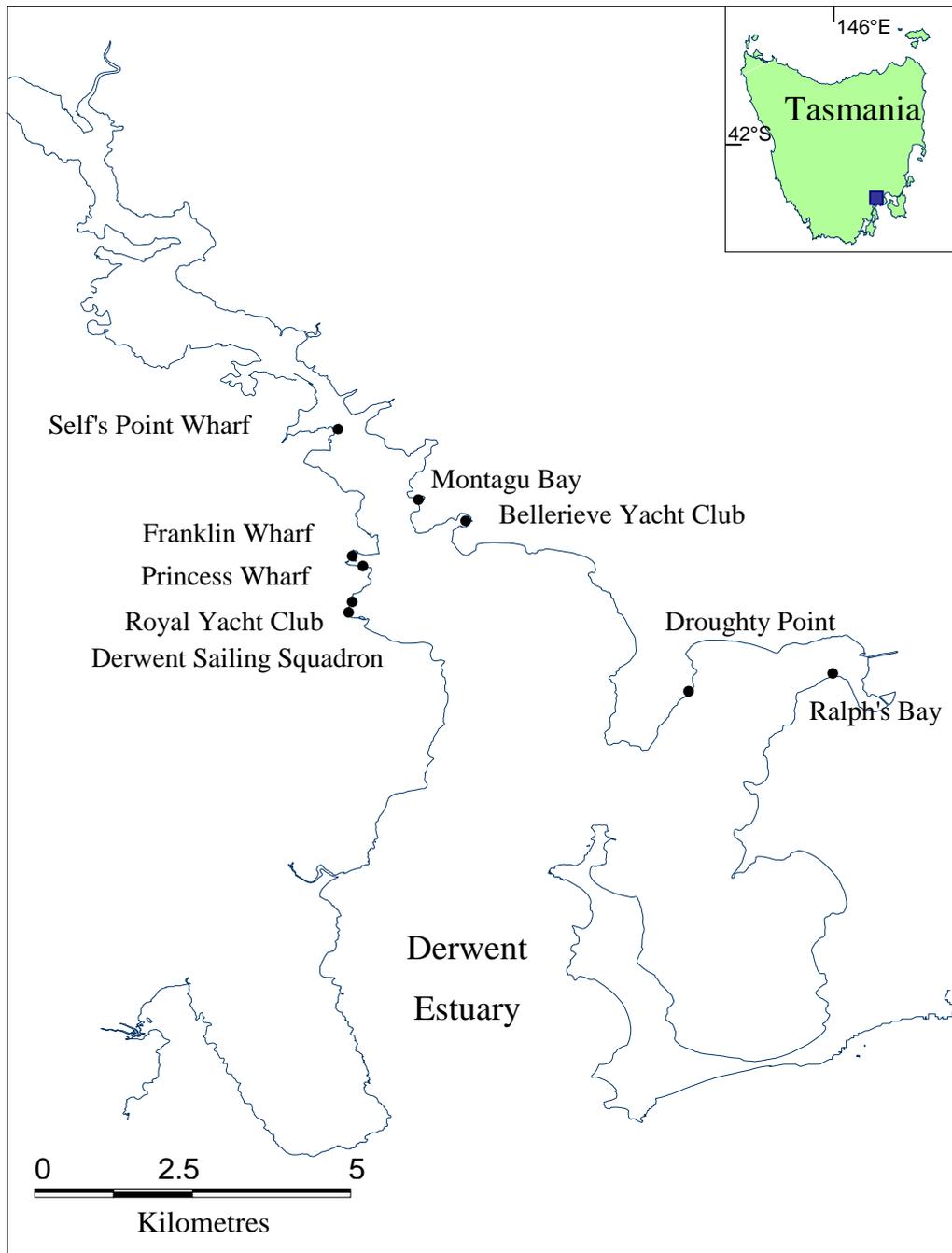


Figure 2.1. The Derwent estuary, Tasmania, showing sites where starfish were collected to determine gonad indices. There were 3 site types, *viz* wharves (Princess, Self's Point, and Franklin), yacht clubs (Derwent Sailing Squadron, Royal, and Bellerieve), and 'controls' without anthropogenic structures (Montagu Bay, Ralph's Bay, and Droughty Point).

Ova diameter

Late stage eggs were obtained by inducing 10 females to spawn and collecting the dry gametes. The diameter of 20 eggs selected haphazardly from each seastar were measured under a compound microscope fitted with a calibrated eye piece micrometer.

Sperm half-life

A 10 000 - fold sperm dilution was aged for 0, 5, 10, 15, 20, 30, 40, 60, 80, and 100 mins. Once sperm were aged for the appropriate time, 0.2 ml of dry female gamete was pipetted into vials containing 9.8 ml of the sperm solution. Sperm and egg solutions were incubated at 10 °C. Sperm half-life was defined as the time for the proportion of eggs fertilized to drop to 50% of fertilization observed with fresh sperm and eggs. Because it was not possible to have a continuous supply of fresh eggs, fertilization success was also determined for fresh sperm, and egg mixtures aged for 140 minutes to ensure that the observed decrease in the proportion of eggs fertilized with time was due to sperm aging not loss in egg viability. These methods were repeated for gamete solutions incubated at 17 °C to determine if there was an effect of temperature on sperm aging. There were four replicates of each treatment.

Sperm velocity

A 1000 - fold dilution of dry sperm was made and sperm were videoed under a compound microscope. Sperm were replaced every 30 s during the experiment to ensure that measurements of sperm velocity were not affected by sperm aging. Temperature during the procedure was maintained at 10 °C, consistent with the ambient temperature in the Derwent Estuary during the seastar spawning season. Distance was calibrated against a graticule and time was kept by the video internal clock. The video was digitized, and 30 sperm trajectories were measured using the Scion Image Beta 4.0.2 software (<http://www.scioncorp.com>).

Effect of sperm dilution on fertilization success and estimation of rate constants

Dry gametes were collected by blotting dry seastars and inverting spawning individuals over beakers. Twelve serial sperm dilutions (10^{-1} , 10^{-2} , 10^{-3} etc) were made immediately with sterile filtered (0.2 μm) seawater to a volume of 9 ml.

Female gametes were diluted in sterile filtered seawater by 10^{-1} and 1 ml of this solution was added to each sperm dilution. The final egg concentration in vials was $1060 \text{ eggs ml}^{-1}$. Vials were agitated gently for 1 hr.

The sperm-egg collision constant (β_0) was given as the product of the cross-sectional area of an egg ($\sigma \text{ mm}^2$), and sperm swimming velocity ($v \text{ mm s}^{-1}$). The bimolecular reaction constant, β , was then determined using data of the effect of sperm dilution on fertilization success. The estimate of β that resulted in the best fit of experimental data and VCCW model predictions was determined by minimising the difference between fertilization equation predictions and observations of the proportion of eggs fertilized with increasing sperm dilution (Microsoft Excel SR-2 97).

Effect of sperm aging and dilution on fertilization success

The interaction between sperm dilution and sperm viability was determined in a 2-way design with treatments of sperm dilution (10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7}) in factorial combination with aging treatments (15, 30, 60, and 120 mins). Methodologies were as above with 4 replicates of each treatment.

Effect of egg concentration on fertilization success

Fertilization success of gamete mixtures at 4 egg dilutions (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4}) crossed with 3 sperm dilutions (2×10^{-5} , 2×10^{-6} , 2×10^{-7}) were determined. Gamete solutions were incubated at 10°C for 1 hr and then fixed in 5% formalin. There were 4 replicates of each treatment.

The results of egg dilution experiments were compared to the predictions of the VCCW fertilization equation predictions given a fertilization time of 3600 s. All other parameters were estimated empirically using the procedures described above. Model predictions were compared with the results of egg dilution experiments.

Effect of egg diameter on fertilization success

A sensitivity analysis was performed on the VCCW model to predict the effect of sperm chemotaxis on fertilization success. Sperm chemotaxis can be

simulated by applying the VCCW model using larger egg diameter since chemotaxis effectively increases egg size. The effect of increasing egg diameter on fertilization success was determined for three sperm concentrations (2.67×10^{-3} , 2.67×10^{-2} , and 2.67×10^{-1} sperm ml^{-1}) and three egg concentrations (2.12, 0.212, and 0.0212 egg ml^{-1}). The fertilization time of 3600s was held constant for each simulation. Egg diameter was increased from 0.14 to 0.40 mm in the simulations, thus varying the value of β_0 , while the ratio of β/β_0 was held constant at 7.9×10^{-3} (i.e. proportion of sperm collisions to successful fertilizations was held constant). The minimum egg diameter of the range of egg diameters investigated approximates the average egg diameter measured in seastars from the Derwent Estuary (0.149 mm) and the maximum exceeds the combined distances of average egg diameter and the distance over which chemotaxis may operate (Miller 1985).

Temporal and spatial variability in gonad indices

Seastars ($n = 30$) were collected on 5 occasions from 9 sites in the Derwent Estuary (figure 2.1) to determine spatio-temporal variability in gonad indices over the spawning period. Sampling was spatially hierarchical with 3 sub-sites within each of 3 site types, *viz* wharves, yacht clubs and controls. Control sites did not support wharf, yacht clubs or other anthropogenic structures. Sites were sampled between the 15th and the 23rd of every month from May to September 1998 except for two wharf sites that were not sampled in August. Hermaphrodites, found only in wharf and yacht club sites, were rare (6 in total) and were excluded from the analysis.

Adults were housed in aquaria for < 24 hr before dissection. Juvenile seastars (ray length < 60 mm) were not included in the analysis. Seastars were blotted dry, and wet weight and ray length recorded. The first arm clockwise from the madreporite was used to measure ray length. If this arm had been lost the next intact arm was measured. The gonad index was determined as the proportion of total seastar wet weight devoted to gonad. Results were analyzed using a nested ANOVA with main effects of time, site type, and sub-site nested within site type.

Dependence of amount of gametes released on gonad index and seastar size

The relationship between gonad index and the amount of dry gamete released was determined for 30 seastars. Ripe adults were blotted dry, weighed, and induced to spawn by an injection of 5 - 7ml of 10^{-3} M 1-methyladenine. The mass of dry gamete released was measured and the remaining gonad was removed and weighed. Gonad indices (GI) were defined as the sum of dry gamete released and remaining gonad weight expressed as a proportion of total seastar wet weight. Dependence of total gamete release (g) on gonad index and total seastar wet weight was determined.

Results

Fertilization parameters

Adult male seastars, weighing on average 233 g, spawned 27.5 g of dry sperm at a rate of 1.30×10^8 sperm s^{-1} (table 2.1). Females, weighing an average of 260 g, spawned 26.24 g at a rate of approximately 600 eggs s^{-1} and spawned for approximately 15 min longer than males (table 2.1). Average egg diameter was 149 μm (SE = 1.13). The minimum and maximum diameters measured were 105 and 190 μm respectively. There were significant differences in egg size between individual females (1-way ANOVA, $F_{(9,190)} = 11.12$, $p < 0.001$). Average sperm swimming velocity at ambient temperature (10 °C) was 0.11 mm s^{-1} (SE = 0.0052).

Parameter	Mean	95% CI
Sperm density (sperm ml^{-1})	2.67×10^{10}	$\pm 6.41 \times 10^9$
Sperm release (g)	27.50	± 11.66
Spawning duration (s)	5630	± 725
Egg density (eggs ml^{-1})	106000	± 27000
Egg release (g)	26.24	± 6.64
Spawning duration (s)	4653	± 670
Sperm swimming speed (mm s^{-1})	0.11	± 0.0052
Egg Diameter (mm)	0.149	± 0.00221
β ($\text{mm}^3 s^{-1}$)	1.5E-5	
β_0 ($\text{mm}^3 s^{-1}$)	1.9E-3	

Table 2.1. Spawning parameters (mean and 95% confidence intervals) in *Asterias amurensis* determined in laboratory experiments.

Sperm half-life

Increases in water temperature resulted in decreased sperm longevity (figure 2.2). The half-life of sperm incubated at 17 °C was approximately 25 min, while at 10 °C the half life was more than 100 min (130 min by extrapolation; figure 2.2). The decline in sperm viability, for sperm aged from 0 to 100 minutes, was linear at 10 °C but declined as a power function at 17 °C. Fertilization success in treatments with fresh sperm and eggs incubated at 10 °C for 140 minutes averaged 75 % (SE =

4.14) suggesting that the decreases in fertilization with time are due to sperm aging, not reduced egg viability with time.

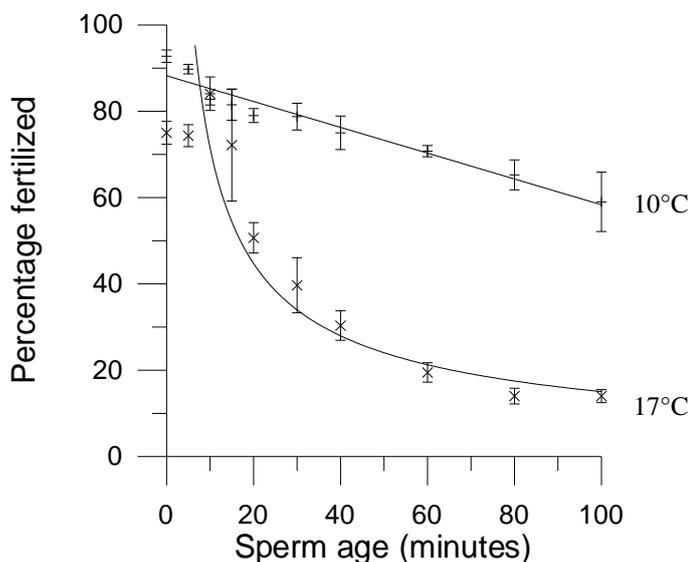


Figure 2.2. The effect of sperm age on fertilization success of gametes incubated at 10 and 17 °C. The percentage of eggs fertilized are means (\pm SE) of 2 independent male/female crosses. Sperm incubated at 10 °C follow a linear decline in viability give by: $Y = -0.299 \times X + 88.211$ ($R^2 = 0.94$). The power relationship of decreasing sperm viability with time in the 17 °C treatment is described by: $\log(Y) = -0.677 \times \log(X) + 5.827$ ($R^2 = 0.90$). Sperm and egg concentration in each replicate were constant at 2.67×10^6 sperm ml^{-1} and 2120 eggs ml^{-1} , respectively.

Effect of sperm dilution on fertilization success and estimation of rate constants

High levels of fertilization success was observed in treatments with sperm dilution in the range of 10^{-2} to 10^{-4} (figure 2.3). In treatments with sperm outside this range ($> 10^{-2}$ and $< 10^{-4}$) fertilization success was low. The rate constant of sperm-egg collision β_0 , determined from the product of sperm swimming speed and egg cross sectional area was $1.9 \times 10^{-3} \text{ mm}^3 \text{ s}^{-1}$. The bimolecular reaction constant (β) determined by nonlinear optimization (Solver-Microsoft Excel 97) was $1.5 \times 10^{-5} \text{ mm}^3 \text{ s}^{-1}$.

Effect of sperm aging and dilution on fertilization success

Sperm dilution did not have a substantial effect on sperm half-life within the ranges of sperm concentrations tested (figure 2.4). Sperm half-lives, measured as the

time for fertilization success to drop to 50% of the original percentage, for sperm diluted by 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} were 97.5, 95, 95, and 100 min respectively.

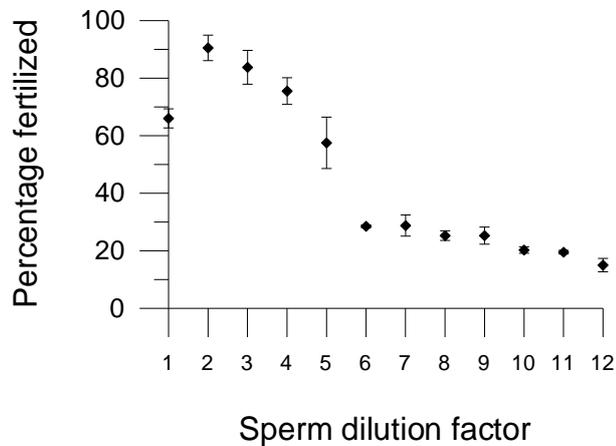


Figure 2.3. The effect of sperm dilution on fertilization success in *Asterias amurensis*.

Fertilization success for 12 serial sperm dilutions (10^{-1} , 10^{-2} , 10^{-3} , etc.) is shown where the concentration of undiluted ('dry') sperm is 2.67×10^{10} sperm ml^{-1} and egg concentration was 1060 eggs ml^{-1} . Mean percent fertilization (\pm SE; $n=4$) is optimal at dilutions of 10^{-2} – 10^{-4} , and decreases at higher and lower concentrations.

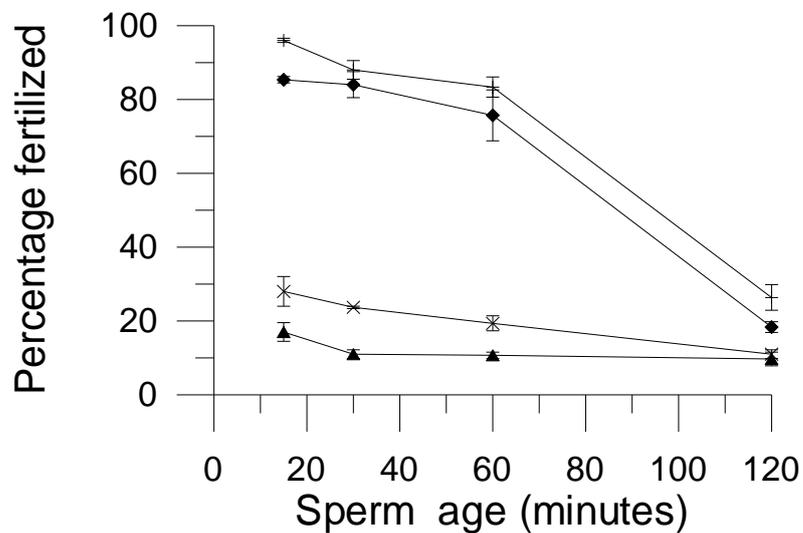


Figure 2.4. The influence of sperm dilution on the decline in sperm viability with age. Mean percent fertilization (\pm SE) of gamete mixtures with sperm at four dilutions: 10^{-4} (dash), 10^{-5} (diamond), 10^{-6} (cross) and 10^{-7} (triangle) are shown (where the concentration of 'dry' sperm is 2.67×10^{10} sperm ml^{-1}) for sperm aged for 15, 30, 60, and 120 minutes. Egg concentration was 1060 eggs ml^{-1} in each treatment. Sperm half-life for sperm diluted by 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} are approximately 97, 95, 95, and 100 minutes respectively.

Effect of egg concentration on fertilization success

The effect of egg concentration on fertilization success depended on sperm dilution (figure 2.5). Egg concentration had a large effect on fertilization success in treatments with high sperm concentrations. However at low sperm concentrations, egg concentration had little effect on fertilization success since fertilization was already limited by the availability of sperm. Predicted percent fertilized based on the VCCW model showed similar patterns to the experimental data (figure 2.5).

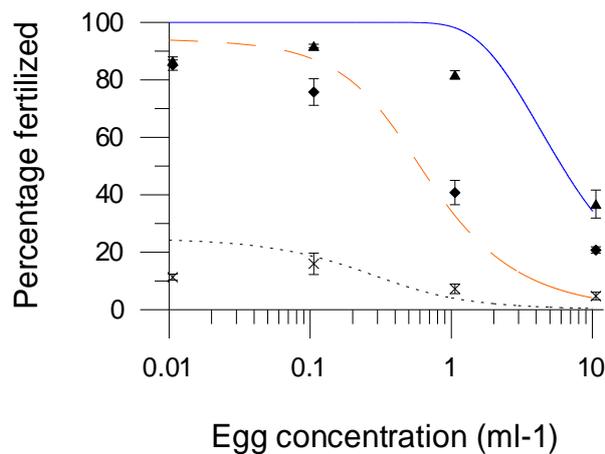


Figure 2.5. The effect of egg dilution on fertilization success at three sperm dilutions: 2×10^{-5} (triangle), 2×10^{-6} (diamond), and 2×10^{-7} (cross) where the concentrations of 'dry' sperm and eggs are 2.67×10^{10} sperm ml^{-1} and 106000 eggs ml^{-1} respectively. Data are means (\pm SE; $n=4$). Fertilization success showed a sigmoidal decline with increasing egg concentrations at all sperm dilutions. VCCW model predictions are also shown for sperm dilutions of 2×10^{-5} (solid line), 2×10^{-6} (dashed line), and 2×10^{-7} (dotted line).

Effect of egg diameter on fertilization success

The effect of increasing egg diameter on fertilization success was dependent on sperm and egg concentrations (figure 2.6). When sperm and egg concentrations were high larger egg size did not improve fertilization success, even for a 3 – fold increase in egg diameter. When sperm and egg concentrations were low, the VCCW model predicts that egg diameter has a significant effect on fertilization success (figure 2.6).

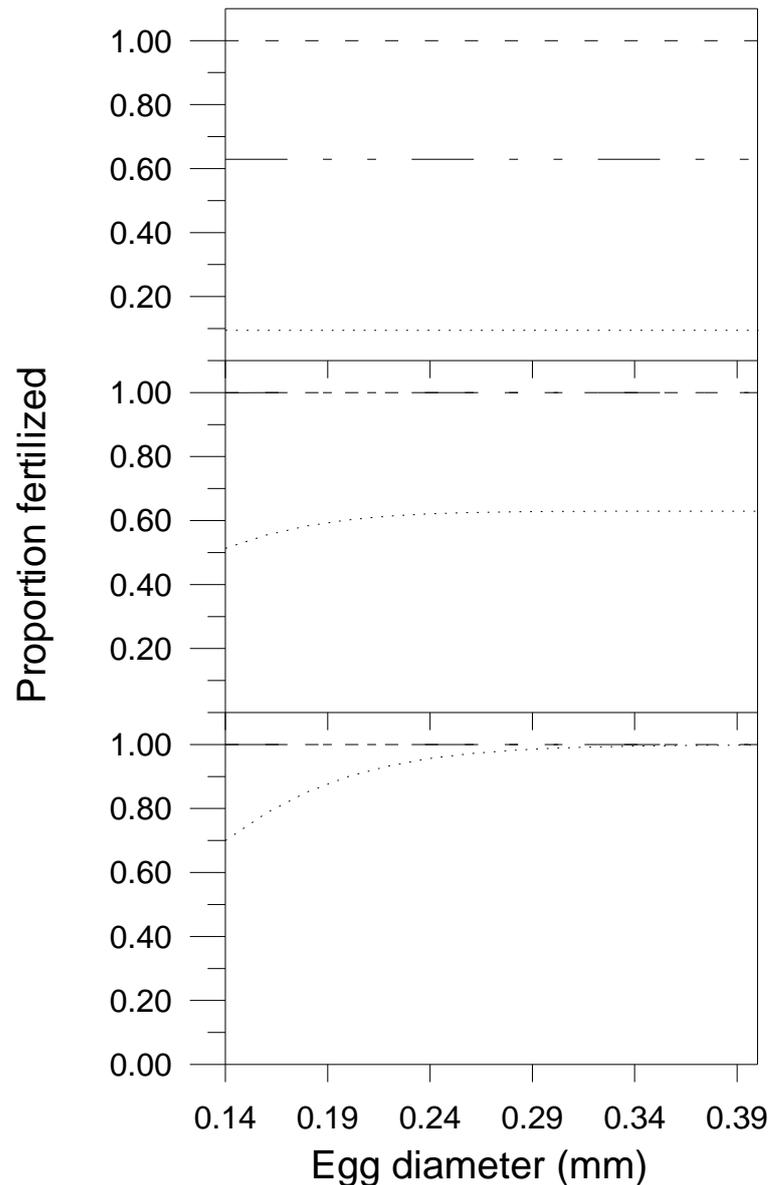


Figure 2.6. VCCW model predictions of the effect of egg diameter on fertilization success at three sperm concentrations: 2.67×10^3 (dashed), 2.67×10^2 (dash and dots), and 2.67×10^1 (dots) sperm ml^{-1} . VCCW model predictions are shown for 3 egg densities: 2.21 (top), 0.221 (middle), and 0.0221 (bottom) eggs ml^{-1} . Fertilization success of eggs with increasing diameter at egg concentrations of 0.221 (middle) and 0.0221 (bottom) and sperm concentrations of 2.67×10^3 and 2.67×10^2 sperm ml^{-1} follow the same trajectory.

Temporal and spatial variability in gonad indices

Gonad indices reached maximum values in mid-July (winter) and decreased in August and September (figure 2.7). There were significant differences in mean gonad indices between sites; seastars at yacht clubs have significantly higher indices (0.16) than those at wharf (0.11) and control (0.096) sites ($F_{(2,12)} = 7.00$, $p < 0.05$).

There was also a significant interaction effect of time and sex with male gonad indices decreasing earlier than females ($F_{(3, 844)} = 4.87, p < 0.05$).

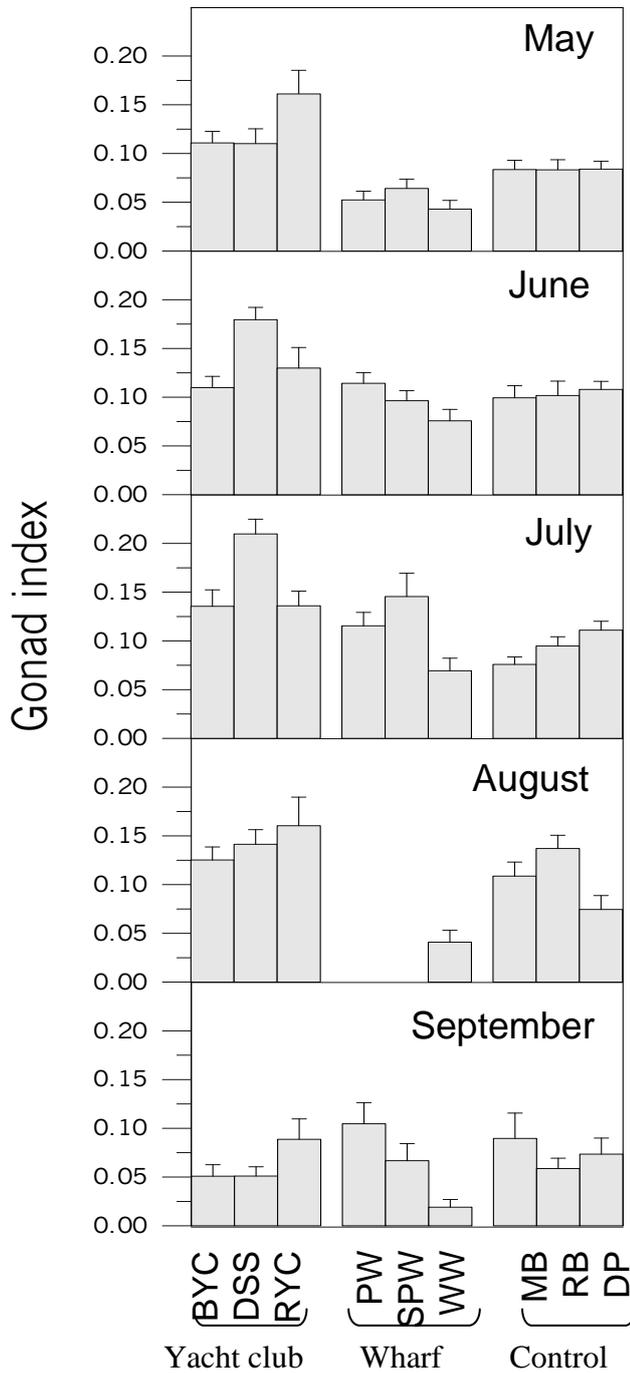


Figure 2.7. Temporal and spatial variability in gonad index of seastars at yacht clubs: Bellerieve Yacht Club (BYC), Derwent Sailing Squadron (DSS) and Royal Yacht Club (RYC); wharves: Princes Wharf (PW), Self's Point Wharf (SPW) and Waterman's Wharf (WW); and 'control' sites: Montagu Bay (MB), Ralph's Bay (RB) and Droughty Point (DP). Data are means (\pm SE) for populations sampled from May (top) to September (bottom) 1998.

Dependence of amount of gametes released on gonad index and seastar size

There was a linear increase in dry gamete release with gonad index in both male and female seastars (figure 2.8a). There was also a positive relationship between the weight of dry gamete released and seastar weight (figure 2.8b).

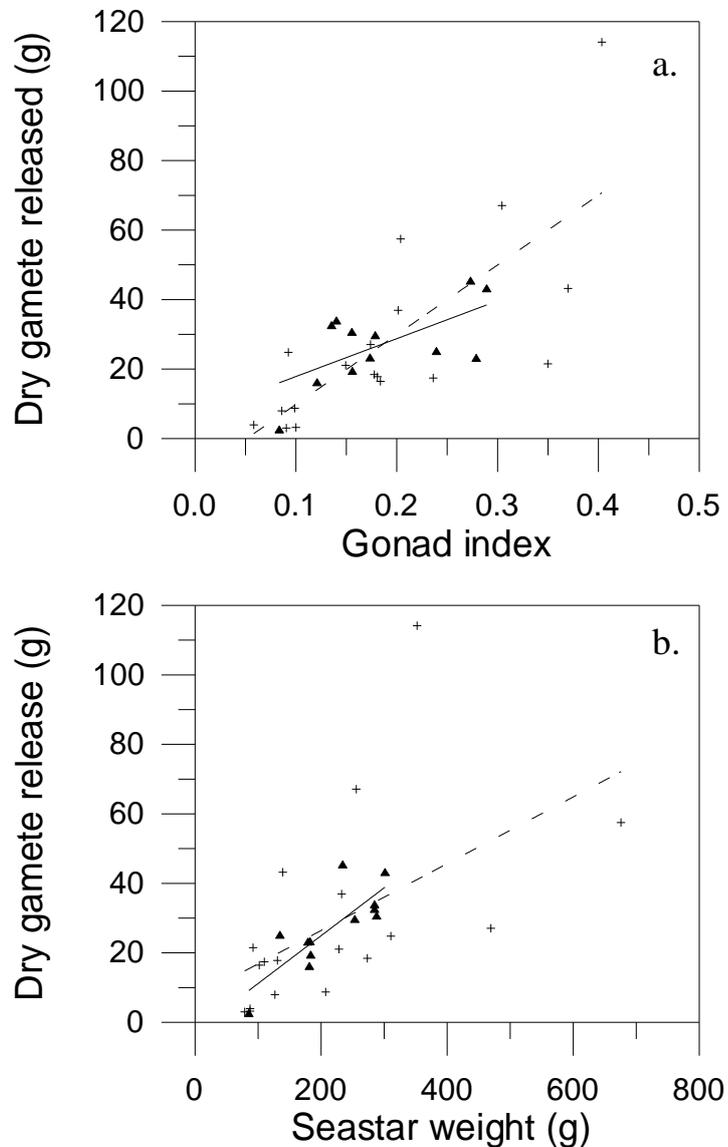


Figure 2.8. Total release of dry gametes is positively related to gonad index (a) and seastar weight (b), in both male (crosses) and female (triangles) seastars. The linear increase in dry gamete release with gonad index is given by: $Y = 200.9 \times X - 10.27$ ($R^2 = 0.56$) (dashed line) for males, and $Y = 108.9 \times X - 6.95$ ($R^2 = 0.40$) (solid line) for females. There was also a linear increase in dry gamete released with seastar weight, given by: $Y = 0.96 \times X + 7.22$ ($R^2 = 0.29$) for males (dashed line), and $Y = 0.14 \times X - 2.59$ ($R^2 = 0.66$) for females (solid line).

Discussion

Variability in fertilization success

Factors acting at spatial scales spanning several orders of magnitude have the potential to affect fertilization success in free-spawning marine invertebrates. Previous workers have found that variability in fertilization parameters at the level of the gamete, individual, and population significantly affect fertilization success in free spawning invertebrates, but with significant variation in the nature of effects both between species and within species (see Levitan 1995 for review). Strong positive relationships have been found between food availability and somatic and gonadal development in many free-spawning marine invertebrates (Guilou and Lumingas 1999; Levitan 1989; Meidel and Scheibling 1999; Qian and Chia 1991). Echinoderms are known for a high degree of plasticity in development and variation in fertilization potential within species has been observed in several asteroid populations (Babcock *et al* 1994; Oliver and Babcock 1992; Wahle and Peckhman 1999). This plasticity can result in substantial variation in the reproductive potential of discrete populations when there is spatial variability in the quantity and/or quality of food resources. Development of a model of fertilization success for *Asterias amurensis* in the Derwent Estuary requires knowledge of parameters that are species-specific, such as gamete release rates and egg diameter, and site specific—such as gonad indices.

There was significant spatial variation in the reproductive potential of seastar populations in the estuary. Peak gonad indices of populations at yacht club sites were more than 50% higher than those at control sites (figure 2.7). The linear increase in the mass of dry gamete released with gonad index suggests that the yacht club populations spawn between 1.5 and 2.6 times the amount of dry gamete per individual than populations at control sites (19 and 26 g for males and females at yacht clubs, 7.2 and 18 g for males and females at control locations). The average weight of individuals at yacht clubs and wharves were also higher than at control sites (A. Morris unpublished data). Given these results and higher densities of animals around anthropogenic structures (*pers obs*) it is likely that some areas in the estuary contribute more to larval production and therefore could be targeted for

management. Whether the observed differences in gonad indices between sites has a significant effect on larval production, depends, in part, on fertilization success.

The percentage of eggs fertilized in *Asterias amurensis* was sensitive to sperm concentration with maximum success attained at sperm dilutions between 10^{-2} and 10^{-4} (figure 2.3). Because gametes disperse rapidly when released into the water column, sperm limitation can inhibit fertilization success in free spawning marine invertebrates (see Levitan 1995 for discussion). In this study sperm concentration had a large effect on fertilization. Egg concentration and sperm age also had significant effects on fertilization success in laboratory studies (figures 2.2 and 2.5).

Sperm half-life

The effect of sperm age on fertilization depended strongly on temperature. Sperm half-life was estimated at ca. 128 minutes at 10 °C (ambient at the time of spawning), while the half-life of sperm in treatments incubated at 17 °C was less than 20 minutes. However, given that sperm dilute rapidly when released in the water column (Denny 1988; Denny and Shibata 1989), and fertilization success is extremely sensitive to sperm concentration (this study, Benzie and Dixon 1994; Levitan *et al.* 1991; Pennington 1985), it is unlikely that sperm aging will significantly alter fertilization success in the Derwent Estuary because dilution is likely to reduce fertilization to low levels before a significant effect of aging is evident. Sperm aging may have a much greater effect in inhibiting fertilization success if *A. amurensis* establishes in warmer water further north on the east Australian coast.

Although the respiratory dilution effect (showing decreased longevity of sperm with dilution) is well established (see Chia and Bickell 1983 for review), I found no relationship between sperm dilution and sperm half-life, with half lives ranging from ca. 95 to 100 minutes over 4 orders of magnitude of dilution (10^{-4} – 10^{-7}). The difference may reflect the range of sperm dilutions investigated. Here, the initial sperm dilution was 4 orders of magnitude, while in other studies the effect of sperm dilution on viability was determined for gamete dilutions from 0 to 4 (Chia and Bickell 1983; Styan 1998) and 2-7 (Levitan *et al.* 1991) orders of magnitude.

Polyspermy

Fertilization success was not only sensitive to low sperm concentration, but high sperm concentration also resulted in low levels of fertilization, probably due to polyspermy (Levitan *et al.* 1991; Styan 1998). Styan (1998) adjusted the VCCW model to account for reduced fertilization due to polyspermy, which significantly improved the fit to empirical results of laboratory experiments. The results of sperm dilution experiments in *Asterias amurensis* yielded low levels of fertilization at sperm dilutions of 10^{-1} , however, at sperm concentrations of 10^{-2} and below, fertilization success was high. Since gametes disperse rapidly in the field, and average sperm release rates in *Asterias amurensis* are relatively low ($5 \times 10^{-3} \text{ ml}^{-1}$), it is highly unlikely that polyspermy is an important factor in the fertilization dynamics of *Asterias amurensis* in nature. In these circumstances there is no case to account for polyspermy in modelling fertilization success.

Chemotaxis and fertilization

Chemotaxis can attract sperm up to 200 μm from a point source containing egg extracts (Miller 1985), effectively increasing the diameter of the egg. However, the VCCW fertilization equation is a random walk model that does not account for sperm chemotaxis. If chemotaxis does influence fertilization in laboratory fertilization trials, the fertilization equation would under-estimate fertilization success because sperm swimming would be directed towards the egg, not random. Assuming chemotaxis arises, its effect can be simulated by increasing egg diameter in the VCCW model. It is clear that increasing egg diameter in the VCCW model affects fertilization success when sperm and egg concentrations are low (figure 2.6). In the most extreme case, the model predicts a 28% increase in the proportion of eggs fertilized (from 0.72 to 0.92) when egg diameter is increased from 0.14 to 0.20 mm, simulating chemotaxis operating within only 30 μm of the egg. Despite the sensitivity of the VCCW to egg diameter when sperm and egg concentrations are low, the VCCW fertilization model adequately predicted the results of egg dilution experiments for the sperm and egg dilutions investigated (figure 2.5). Given this performance of the model over a wide-range of sperm and egg concentrations, it is unnecessary to simulate sperm chemotaxis in the fertilization equation.

Gonad indices

Seastar populations at different sites in the estuary exhibited dissimilar gonad indices (figure 2.7). In July, gonad indices at yacht club sites averaged 0.16 compared to 0.096 at control sites. The higher relative gonad mass at yacht clubs, and the linear increase in dry gamete release with relative gonad weight (figure 2.8), points to a 1.5 to 2.6 -fold difference in amount of gametes released per individual at yacht clubs and control sites. Coupled with higher densities of seastars at sites with anthropogenic structure, these results suggest that anthropogenic activity may affect the reproductive potential of discrete seastar populations in the Derwent Estuary. To predict how the observed differences in reproductive potential translate into spatial variability in zygote production requires further knowledge of how population parameters (eg. density, sex ratio and spawning synchronicity) and environmental factors (eg. current velocity and depth) affect fertilization success (Babcock *et al.* 1994; Babcock *et al.* 2000; Babcock and Keesing 1999; Babcock and Mundy 1992; Claereboudt 1999; Coma and Lasker 1997; Levitan 1991, 1996; Levitan *et al.* 1992; Oliver and Babcock 1992; Pennington 1985; Yund 1990).

Chapter 3: A discrete model of gamete dispersal and fertilization success
in the introduced seastar *Asterias amurensis*, in the Derwent Estuary,
Tasmania

Abstract

A discrete model of gamete dispersal and fertilization success in the introduced seastar, *Asterias amurensis*, is developed to predict fertilization success for various male-female separation distances. In validating the model, the effect of collecting gametes into flasks during the experimental procedure is simulated. The model is then adapted to simulate dispersal of gametes without the experimental artifact of collection of gametes. When the model simulates the experimental procedure, the results approximate empirical estimates of fertilization success. *In situ* measurements of the proportion of eggs fertilized ranged from 0.80 ± 0.05 (SE) for eggs released adjacent to the spawning male, to 0.16 ± 0.04 (SE) for separation distances of 16 m. However, when the experimental artifact is removed, and natural gamete dispersal is simulated, predicted estimates of fertilization success are substantially lower. Model predictions of the proportion of eggs fertilized for gametes that disperse without impediment are 0.05 adjacent to the spawning male, and 0.03 at separation distances of 16 m. A sensitivity analysis was performed on the effect of sperm release rate on the predictions of the cellular gamete dispersal and fertilization model. The model is extremely sensitive to sperm release rates within range of values measured in *Asterias amurensis* in the Derwent Estuary.

Introduction

Measuring fertilization success in free spawning marine invertebrates has proven a major challenge because of logistic constraints in sampling gametes as they disperse. High levels of fertilization success have been reported for the asteroid *Acanthaster planci*, even when spawning males and females were separated by large distances (23% fertilization at 64 m separation; Babcock *et al.* 1994), while fertilization success measured in other free-spawning invertebrates are substantially lower, dropping to less than 5% for separation distances greater than 5 m (Levitan 1991; Pennington 1985; Yund 1990). Large differences in fertilization success among free spawning marine invertebrates is often difficult to interpret because of differences in experimental techniques. In their experiments with *Acanthaster planci*, Babcock *et al.* (1994) retained gametes in 1 litre flasks for 1-2 hours before the gametes were fixed. Pennington (1985) also retained gametes in experiments with the sea urchin *Strongylocentrotus droebachiensis*, however the gametes were fixed after 10 minutes. Gametes of the hydroid *Hydractinia echinata* were allowed to disperse naturally in Yund's (1990) experiments, while Levitan (1991) contained eggs of the sea urchin *Diadema antillarum* in nitex bags.

Artifacts of experimental protocol may either promote or inhibit fertilization to different degrees (e.g. see Levitan 1995 for discussion), confounding the results of *in situ* experiments, and therefore comparisons among species where dissimilar methodologies are employed should be interpreted cautiously. In experimental work in which gametes are contained in vessels, the gametes are held at higher concentrations than if they had dispersed naturally. In some special circumstances experimental artifacts may be small, for example when sperm half-life is short (Petersen *et al.* 1992) or where gametes do not disperse long distances (Brazeau and Lasker 1992; Yund 1990). In free-spawning asteroids, where sperm half-life can be as high as 180 minutes (figure 2.2), and where approximately neutrally buoyant gametes may disperse far, artifacts associated with retention of gametes can be high. Estimates of fertilization success in free spawning invertebrates with relatively long-lived, neutrally buoyant gametes must be adjusted to account for artifacts in empirical estimates. One method to estimate fertilization success of naturally dispersing gametes is to validate models against empirical data by simulating

experimental artifacts and then, if a good validation is realized, the artifact can be removed from the model while other parameters remain unchanged, and unimpeded dispersal can be simulated (Morris and Johnson submitted MS).

To date, modelling fertilization success in free spawning marine invertebrates has used a two-step process. First, gamete concentrations are estimated using a plume diffusion model (Csanady 1973; Denny 1988; Denny and Shibata 1989) and then, only after gametes attain equilibrium concentrations, the proportion of eggs fertilized is estimated using a random walk model based on the Vogel-Czihak-Chang-Wolf (VCCW) fertilization equation (Vogel *et al.* 1982). The VCCW equation requires an estimate of the time that gametes interact (the fertilization time), however determining a realistic value for the fertilization time is problematic when gametes disperse unimpeded. Moreover, the assumption that gamete concentrations and fertilization success attain equilibrium soon after spawning commences in the field has not been scrutinised.

Recent developments of the plume diffusion approach by Levitan and Young (1995) and Claereboudt (1999) are spatially-explicit and allow simulations of more than one spawning pair so that, for example, the effect of population density and sex ratio can be investigated. The results of these studies (Claereboudt 1999, Levitan and Young 1995) suggest that high population density and spatial aggregation both significantly increase fertilization success. While these models are improvements of earlier efforts, they are still limited in that they decouple the processes of gamete dispersal and fertilization, and they require equilibrium concentrations of gametes.

Here I combine empirical studies with laboratory derived measurements of spawning parameters in *Asterias amurensis* (Chapter 2) to parameterise and test a cellular gamete dispersal and fertilization model (Morris and Johnson submitted MS) for the introduced seastar, *Asterias amurensis*, in the Derwent Estuary, Tasmania. Fertilization success measured empirically is compared with model predictions when the artifacts of experimental methods are simulated. I also estimate fertilization success of gametes simulated to disperse without the impediment of a collecting device. Predictions of fertilization with and without constraint of gametes in a

collecting device are compared to illustrate the effect of experimental artifacts on measurements of fertilization rates.

Materials and Methods

In situ estimates of fertilization success

Experiments were performed at Sandy Bay Point (42°54.5'S 147°21.4'E) in the Derwent Estuary, Tasmania in July 1996 at a depth of 5 m. Adult seastars were collected and moved to the experimental site where they were held in cages until required. Seastars were sexed macroscopically by extracting a gonad sample with a 14-gauge syringe, and a male and female seastars were induced to spawn by injection of 5-7 ml 10^{-3} M 1-methyladenine. Once seastars were spawning, the direction of current was determined by the release of fluorescein dye and the male was placed at the point of dye release. A transect line was placed at the spawning male and run downstream parallel to the main axis of flow. Current velocity was estimated by releasing pulses of dye released approximately 1 m above the substrata, and measuring the time for the dye cloud to advect 3 – 5 m down the transect line.

Egg samples were taken at 0, 1, 2, 4, 8, 16, and 32 m directly downstream from the spawning male. Sampling always commenced at the downstream end of the transect line and progressed upstream so that the sperm plume was not disrupted during sampling. The female was placed at the required distance downstream of the spawning male and a plankton sampler containing 12 individual 1 litre flasks (described in Mundy *et al.* 1994) was placed 0.15 m above the spawning female to collect gametes. Each point was sampled using a different flask in the plankton sampler. A sample was also taken 16 m upstream of the spawning male ($x = -16$ m) to control for possible sperm contamination from other seastars in the vicinity. In addition to the samples taken directly downstream from the main axis of flow, samples were also taken at 1, 2, 4, 8, and 12 m offset from the main axis of flow ($y = 1$, $y = 2$, etc.). Samples were collected and fixed with 10% formalin approximately 2 hours after collection. The proportion of eggs fertilized, as evidenced by the development of a fertilization membrane, was determined from a sample of 100 eggs.

Flushing experimental flasks with sperm free water

To determine whether containing gametes in flasks promoted fertilization success, flushing experiments were performed in which the 1 litre sampling

cartridges were flushed with sperm-free water *ca.* 30 s after gametes were collected. The male was placed at the point of dye release and the female placed 4 m directly downstream ($x = 4$ m, $y = 0$ m). On obtaining the sample, the flask was flushed with approximately 3 l of sperm-free water piped in from 10 m perpendicular to the main axis of flow ($x = 4$ m, $y = 10$ m). It was assumed that sperm concentration at this distance offset to from the main axis of flow was low. A control whereby the cartridge was not flushed with sperm free water was performed at the same position. There were 6 replicates of each treatment. Control and flushed samples were fixed with formalin 1-2 hrs after collection and processed in the laboratory. Fertilization, as evidenced by the development of a fertilization membrane, of 100 haphazardly selected eggs was determined for each treatment.

Gamete vertical velocities

Seastars were collected from the Derwent estuary, and housed in aquaria overnight. A female seastar was induced to spawn by an injection of 5-7 ml 10^{-3} M 1-methyladenine and eggs were injected gently into a 0.15 m x 0.15 m x 1 m column filled to 0.95 m with filtered seawater through a valve at a height of 0.85 m. After eggs had descended 0.1 m, vertical velocities were measured through the next 0.1 m. There were 20 replicates.

A male seastar was induced to spawn by injection of 5-7 ml 10^{-3} M 1-methyladenine and inverted over a beaker to collect undiluted gametes ('dry' sperm). Vertical velocities were determined for dry sperm and two dilutions of dry sperm (10^{-1} and 10^{-2}). Sperm was injected into the column (as above) and the time for each sperm cloud to descend 0.1 m was measured. If the sperm cloud was dispersed, the time for the centre of the cloud to sink 0.1 m was measured.

Model structure

The model is a 3-dimensional cellular model, with changes to the system occurring in discrete time steps. Each cell in the array represents a volume of 1 m^3 water and fertilization rates and dispersal are modelled at 1 s intervals. It is assumed that gametes do not disperse beyond 1 m in each 1 s interval. Each cell in the model (excepting boundaries) is treated identically, and in each time step a

fixed proportion of gametes in each cell disperse to cells to the north, south, east and west in the horizontal (x, y) plane, and up and down in the vertical (z) plane.

At each time step, a fixed proportion of particles in each cell of the array move to adjacent cells to the east and west (x - axis), north and south (y - axis) and to cells above and below (z - axis), simulating processes of diffusion and advection. The proportion of gametes moving to neighbouring cells are given as:

$$p_e = V_x \frac{\Delta t}{2\Delta x} + D_x \frac{\Delta t}{\Delta x^2}$$

$$p_w = -V_x \frac{\Delta t}{2\Delta x} + D_x \frac{\Delta t}{\Delta x^2}$$

$$p_n = D_y \Delta t / \Delta y^2$$

$$p_s = D_y \Delta t / \Delta y^2$$

$$p_a = D_z \Delta t / \Delta z^2$$

$$p_b = D_z \Delta t / \Delta z^2$$

The diffusivities are given after Csanady (1973):

$$D_x = \sigma^2 V_x / 2x$$

$$D_y = \sigma^2 V_x / 2y$$

$$D_z = \sigma^2 V_x / 2z$$

where σ^2 is the spatial standard deviation of diffusion, and x, y and z are the distances from the source on the x, y and z axes respectively.

Since x, y and z are equivalent and arbitrarily given as unity, the diffusivities D_x, D_y and D_z are equal and reduce to $=\sigma^2 V_x / 2$. The estimate of spatial standard deviation for diffusion as derived in Denny and Shibata (1989) is given as:

$$\sigma_y = \alpha_y \left(\frac{u^*}{V_x} \right) x^\beta$$

where u^* is the shear velocity, α_y the plume constant, and β the dispersal constant. These equations provide the concentration of gametes in each cell at each time

step, and fertilization rates for particular sperm and egg concentrations are estimated by the VCCW fertilization equation (equation 1).

Model validation

Fertilization success predicted by the spatial (cellular) simulation model was compared to empirical estimates of fertilization success. Maximum sperm release rates measured in *Asterias amurensis* in the Derwent Estuary are 2.3×10^8 sperm s^{-1} (table 2.1). Maximum sperm release rates were used in calibration simulations because seastars were collected at a site in the estuary where maximum gonad indices were measured, and there is a positive relationship between gonad index and the mass of dry gamete released (figure 2.8). The dispersal of sperm was simulated for 2000 iteration seconds and egg release and dispersal was modelled to simulate the experimental protocol of containing gametes in flasks at constant concentration for 7200 simulation seconds. The fertilization time of 7200 s accounts for the 2 hours that sperm and eggs were contained in the sampling device before fertilization success was determined. Egg density in the experimental flasks was estimated at 9×10^6 m^{-3} based on release of 600 eggs s^{-1} for 15 s directly into a 1 litre flask. Egg concentration in the flask does not have a significant effect on fertilization success within several orders of magnitude of the estimate (i.e. from 9×10^3 to 9×10^6 m^{-3}). The rate collision constants for *Asterias amurensis*, β and β_0 , are 1.5×10^{-14} and 1.9×10^{-12} $m^3 s^{-1}$ respectively (table 2.1).

The average current measured during the experimental work was 0.03 ms^{-1} (SE = 0.007). The shear velocity was estimated at 0.003 ms^{-1} (i.e. 10% of the mean current velocity: see Denny 1988; Denny and Shibata 1989), and the plume constant was estimated at 2.5. The plume constant is within the range of values suggested by Denny and Shibata (1989), and was the value used in simulations to predict fertilization rates in *Acanthaster planci* (Morris and Johnson submitted MS).

Sperm release rate sensitivity analysis

The effect of sperm release rate on fertilization success was simulated by parameterizing the cellular model with mean, maximum, and minimum (95% CI) sperm release rates measured in the Derwent Estuary seastar population (Chapter 2). The maximum and minimum sperm release rates were 2.3×10^8 and 5.7×10^7 sperm

s⁻¹. All other parameter values were held constant from the model calibration. The proportion of eggs fertilized of eggs released from 0 –32 m downstream of the spawning male was plotted.

Model predictions for gametes simulated to disperse naturally

Predicted fertilization success of continuously dispersing gametes (allowing simultaneous fertilization) were determined and compared to predictions simulating experimental collection of gametes into flasks. Other than effects of containing gametes, all other parameters were identical in the two runs. The duration of gamete release in males and females was 5630 and 4650 iteration seconds, respectively, being the average spawning duration measured in *Asterias amurensis* (Chapter 2). The time for predicted fertilization rates to attain equilibrium in simulations of continuous dispersal of gametes was determined by plotting fertilization success of eggs released 0 m, 4 m, and 16 m directly downstream of the spawning male against time. Gamete dispersal and fertilization was simulated for 10000 seconds to record fertilization that occurred after seastars ceased releasing gametes.

Results

Empirical estimates of fertilization success

Empirically measured fertilization success of eggs released directly downstream of the spawning male decreased rapidly with increasing male-female separation distances (figure 3.1). The proportion fertilized of eggs released adjacent to the spawning male was 0.80 (SE = 0.077), while at 16 m downstream from the male fertilization success dropped to 0.16 (SE = 0.037). In contrast, fertilization of eggs released at 1 m and 2 m offset from the main axis of flow ($y = 1$ and $y = 2$) increased with increasing distance along the x-axis from 0 – 4 m ($x = 0$ to $x = 4$) (figure 3.2). The proportion of eggs fertilized at the control site ($x = -16$) was 0.03 (SE = 0.04). The average current prevailing during field experiments was 0.031 ms^{-1} (SE = 0.007).

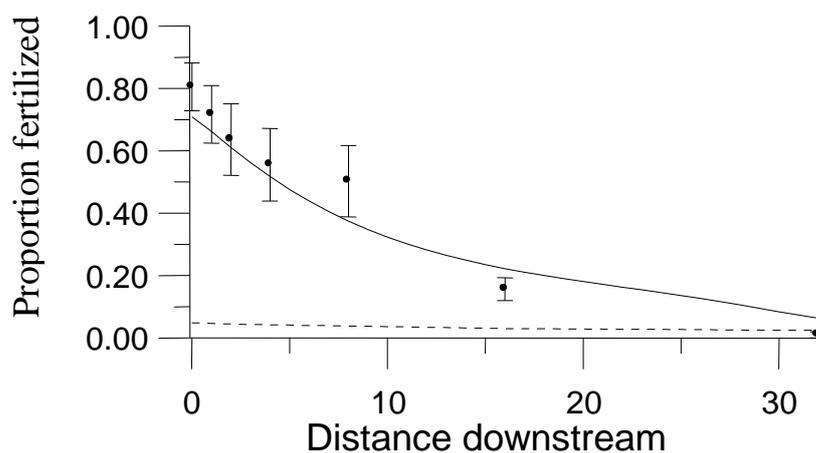


Figure 3.1. Empirically measured fertilization success of eggs released directly downstream from the spawning male (solid circles) and model predictions for gametes treated under experimental regime (solid line). Predicted fertilization success of gametes that are prevented from further dispersal by collecting in the sampling apparatus (dotted line) are much lower than for gametes contained in the sampling flasks.

Flushing experimental flasks with sperm-free water

Fertilization success 4 m directly downstream from males in samples flushed with sperm-free water (0.52, SE = 0.09) was significantly lower than in controls (0.79, SE = 0.05) (figure 3.3).

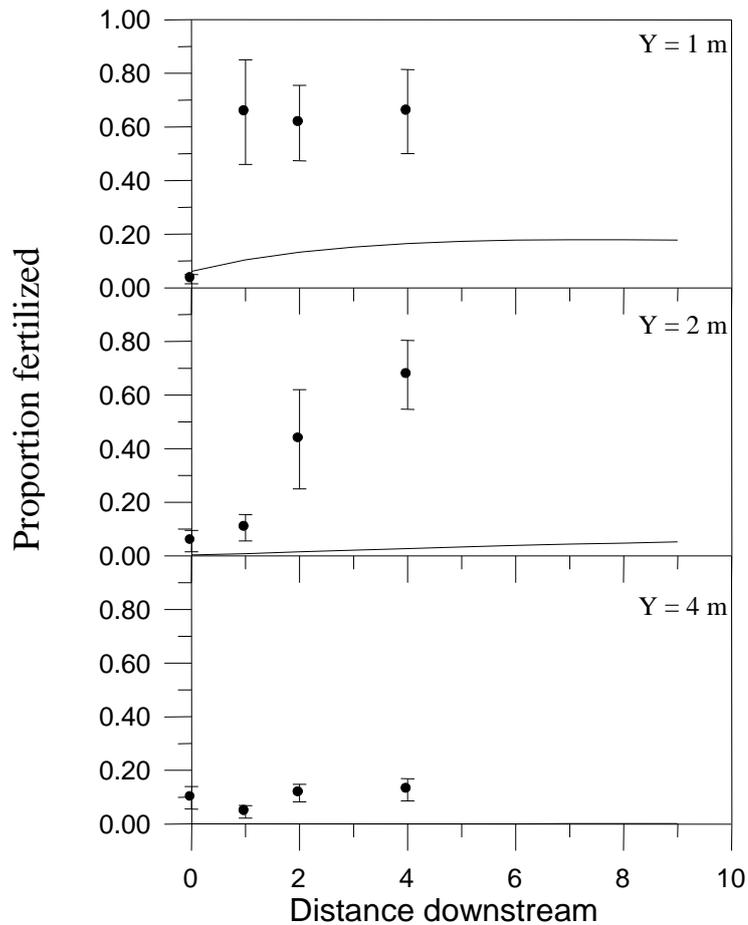


Figure 3.2. Fertilization success of *Asterias amurensis* for downstream females offset from the axis of current flow. Empirically determined fertilization (solid circles) and model predictions when collection of gametes into flasks is simulated (solid line) are shown for egg released 1 m (top), 2 m (centre), and 4 m (bottom) offset from the main axis of flow.

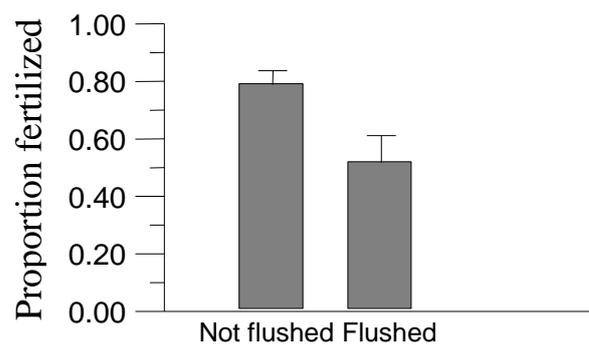


Figure 3.3. Comparison of fertilization success of eggs released 4 m directly downstream from the spawning male with and without flushing flasks with sperm free water (mean \pm SE). When sampling flasks were flushed with sperm-free water, the proportion of eggs fertilized was significantly lower than in treatments where sperm and eggs were contained in the flasks ($t_{10} = 1.89$, $P < 0.05$).

Gamete vertical velocities

Male and female gametes are approximately neutrally buoyant, with eggs and sperm sinking at mean vertical velocities of 8.15×10^{-4} and $4.13 \times 10^{-3} \text{ ms}^{-1}$ for individual eggs and dry sperm respectively (table 3.1). Diluting sperm decreased the vertical velocity of the sperm cloud. For the purposes of modelling, because gametes were approximately neutrally buoyant, it was assumed that there is no significant effect of vertical velocities on gamete dispersal and fertilization.

Gamete fall velocity	Mean (m s^{-1})	SE
Egg fall rate	8.15×10^{-4}	1.17×10^{-4}
Dry sperm	4.13×10^{-3}	5.46×10^{-4}
Diluted sperm (10^{-1})	1.34×10^{-3}	6.53×10^{-5}
Diluted sperm (10^{-2})	1.10×10^{-3}	6.53×10^{-5}

Table 3.1. Mean (and SE) vertical velocity of individual eggs, and sperm clouds at three concentrations. Diluted sperm sink more slowly than undiluted sperm.

Model validation

Predicted fertilization when containment of gametes in flasks is simulated is in good agreement with the empirical estimates of fertilization success of eggs released from 0 – 32 m directly downstream from the spawning male (figure 3.1). For eggs released at 1, 2, and 4 m offset from the main axis of flow, model predictions are lower than empirically determined fertilization (figure 3.2).

Sperm release rate sensitivity analysis

Fertilization success was sensitive to increases in sperm release rates within the 95% confidence interval of rates measured in *Asterias amurensis* in the Derwent estuary. At maximum release rates ($2.3 \times 10^8 \text{ sperm s}^{-1}$) the proportion of eggs released 1 m directly downstream from the spawning male fertilized is 0.71 while the proportion at the same position in a simulation using minimum release rates ($5.7 \times 10^7 \text{ sperm s}^{-1}$) is 0.33 (figure 3.4).

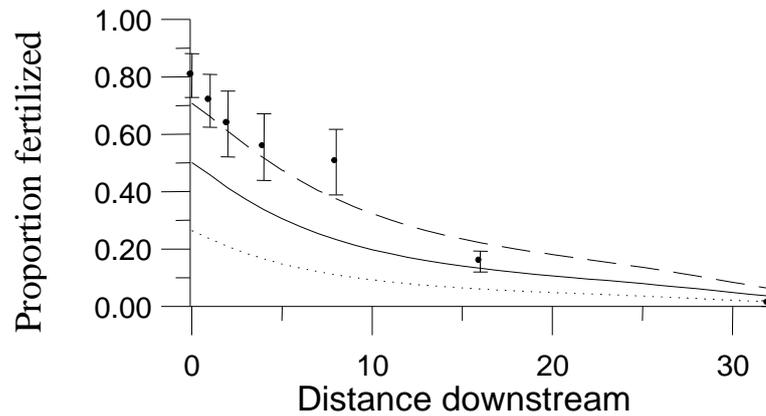


Figure 3.4. Sensitivity of predicted fertilization success to sperm release rates. Data show predicted fertilization success of eggs released directly downstream from the spawning male in simulations using sperm release rates of 1.3×10^8 (solid line), 2.3×10^8 (dashed) and 5.7×10^7 (dotted) sperm s^{-1} . These values represent the average sperm release rate measured in the Derwent Estuary, and the maximum and minimum extremes of the 95% confidence interval of measurements, respectively. Empirically determined fertilization in *Asterias amurensis* eggs released directly downstream from the spawning male is shown for comparison (solid circles).

Model predictions for gametes simulated to disperse naturally

When gametes are simulated to disperse without confinement in the plankton sampler but all other parameters are unaltered, the model predicts that fertilization is an order of magnitude lower (figure 3.1). For example, predicted fertilization success for eggs released adjacent to the spawning male when the experimental collecting device is simulated is 0.7, however, when gametes are allowed to disperse under identical conditions but without retention in flasks the proportion of eggs fertilized drops to 0.05 (figure 3.1).

Attaining equilibrium

The proportion and total number of eggs fertilized reached equilibrium approximately 6000 iteration seconds after the onset of spawning, i.e. approximately 2000 iteration seconds after gamete release ceased (figure 3.5). The increase in slope of the fertilization curves at 4650 s is due to fertilization of eggs after gamete release ceased.

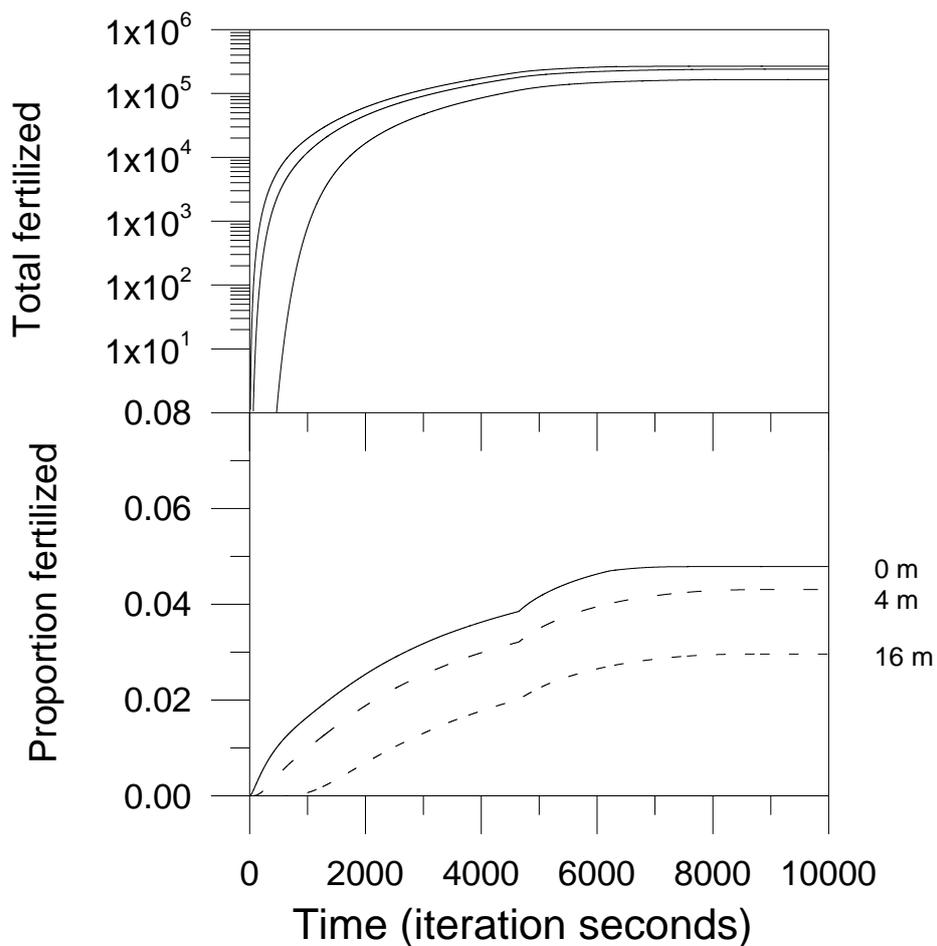


Figure 3.5. Model predictions of total number of eggs fertilized (top) and proportion fertilized (bottom) of eggs released 0 m (top line), 4 m (middle line), and 16 m (bottom line) directly downstream from a spawning male for continuous gamete dispersal (i.e. with experimental artifact removed). Fertilization success is shown from commencement of gamete release. The duration of gamete release in males and females were 5630 and 4650 simulation seconds, respectively, determined from empirical measurements of spawning duration. The increase in fertilization rates from 4650 to 10000 s represent fertilization after cessation of egg release.

Discussion

Empirical estimates of fertilization

Fertilization measured empirically in *Asterias amurensis* was lower than measured in the larger *Acanthaster planci* using identical experimental methodology. The proportion of eggs fertilized in *A. amurensis* was 0.80 (SE = 0.077) adjacent to the spawning male and dropped to 0.50 (SE = 0.11) 8m downstream (figure 3.1), while estimates for *A. planci* using identical methodology was 0.90 (SE = 0.021) at the source and as high as 0.72 (SE = 0.095) 8 m directly downstream from the spawning male (Babcock *et al.* 1994). Fertilization success of *Coscinasterias muricata*, also determined using the same experimental technique, are of the same order as in *Asterias amurensis* (Babcock *et al.* 2000). High fertilization success in *Acanthaster planci* relative to the two temperate species probably reflect that sperm release rates in the tropical asteroid (6.14×10^8 sperm s^{-1}) are 2.6 times higher than those in *C. muricata* and *A. amurensis*, (2.4×10^8 and 2.3×10^8 sperm s^{-1} respectively) (Babcock *et al.* 1994 and 2000).

Fertilization success in other free spawning marine invertebrates including urchins (Pennington 1985; Levitan 1991), soft corals (Brazeau and Lasker 1992), and hydroids (Yund 1990) is universally lower than that measured in the present study and for other free spawning seastars (Babcock *et al.* 1994 and 2000). These differences probably reflect differences in experimental protocols rather than a real trend of higher fertilization success in asteroids compared to other free spawning invertebrates. In experiments investing fertilization success in other free spawning marine invertebrates, gametes were either fixed after being contained in flasks for a short time (Pennington 1985), or contained in semi-permeable bags that allowed for sperm transfer (Levitan 1991).

The experimental technique adopted in this study over-estimates fertilization success because gametes are contained in flasks for approximately 2 hours before being fixed. When the sampling device was flushed with sperm-free water *in situ* the proportion of eggs fertilized decreased by almost 30% (from 0.79 to 0.52 for control and flushed respectively, figure 3.3), indicating that containing gametes in experimental flasks promotes fertilization success. However, flushing in this way

does not enable predicting fertilization rates of freely-dispersing gametes because gametes are still contained at high concentrations in flasks before sperm are washed out, there is some time lag before sperm are flushed, and it is likely that sperm remain in the sampling device. An alternative approach to estimating fertilization rates in free spawning invertebrates without artifacts of experimental procedure is to validate simulation models that include the effects of experimental protocols, and then remove the effect of the experiment to simulate gamete dispersal unimpeded by artifacts.

Model calibration

The discrete model reasonably predicted observed fertilization when the practice of retaining gametes in flask was simulated (figure 3.4). Model predictions were close to empirical estimates for egg released directly downstream from the spawning male when maximum sperm release was simulated. Maximum gamete release rates were used here because gonad indices of adults used to determine sperm release rates were about one-third of those used in the experiment, and there is a positive relationship between gonad index and the amount of dry gamete released in *Asterias amurensis* (figure 2.8). Model predictions of fertilization was sensitive to sperm release rates at the extremes of the 95% confidence interval of average rates measured in the Derwent Estuary seastar population. For example, the model predicts the proportion of eggs fertilized was 0.7 and 0.3 for eggs released adjacent to the spawning male when maximum and minimum sperm release rates were simulated, respectively (figure 3.4). The result of predicted fertilization success being slightly lower than those measured may well reflect that gonad indices of animals used in the experiment were so much greater than those used to measure sperm release rates.

When the artifact of retaining gametes in collection flasks was removed, predicted fertilization fell by more than an order of magnitude. This effect was greatest at higher gamete concentrations. For eggs released adjacent to the spawning male, predicted proportion fertilized was 0.7 when the effect of the collection flasks was simulated, but fell to 0.05 in the same position when gametes were simulated to disperse unimpeded (figure 3.1). These results suggest the importance of accounting for experimental artifacts when predicting fertilization success.

The two-step plume diffusion approach, used to predict fertilization success in free-spawning marine invertebrates to date, is limited because of the assumption that gamete concentrations and fertilization rates attain equilibrium instantaneously. Fertilization rates predicted in simulations of naturally dispersing gametes did not reach equilibrium for more than 6000 iteration seconds after the onset of spawning (figure 3.5). The plume diffusion model is therefore likely to over-estimate fertilization success as a result of over estimating sperm densities. The discrete model simulates continuous gamete dispersal and fertilization and therefore does not rely on the assumption of equilibrium gamete concentrations. Another advantage of the discrete model over the existing two-step plume diffusion approach is that the model can be validated by simulating experimental protocols and then fertilization of gametes that disperse without impediment can be estimated. Using this approach, fertilization success in free-spawning marine invertebrates with long-lived neutrally buoyant gametes may be estimated more reliably without the error associated with experimental artifacts.

Chapter 4: Variability in reproductive success of discrete populations of the introduced seastar *Asterias amurensis* in the Derwent Estuary, Tasmania: Model predictions

Abstract

Because gametes are released directly into the water column in free-spawning marine invertebrates, fertilization success can be limited by rapid dilution of sperm and eggs. The behaviour of individuals prior to the onset of spawning can increase the probability of successful fertilization. In this chapter a model of gamete dispersal and fertilization success is used to predict the effect of various population parameters on fertilization success in the introduced seastar *Asterias amurensis* in the Derwent Estuary, Tasmania. The effect of density, sex ratio, group size, degree of spawning synchrony, and water depth on the proportion and total number of eggs fertilized is predicted. The model predicts non-linear responses of fertilization success to changes in density, sex ratio, group size and water depth. The model predicts a 3-fold increase in the proportion of eggs fertilized among 40 individuals when density is increased from 0.025 to 0.2 individuals m^{-2} . This range of densities is well within densities commonly observed in the Derwent Estuary. For a population density of 0.02 individuals m^{-2} the model predicts more than a 5 – fold decrease in the proportion of eggs fertilized for an increase in depth from 1 to 2 m, (from 0.81 in 1 m compared to 0.14 in 2 m), and a substantial drop in the total number of eggs fertilized (2.04×10^8 in 1 m, 3.23×10^6 in 2 m). Overall the model results suggest that some discrete populations in the Derwent Estuary contribute more to reproductive output, and therefore may be targeted for active management.

Introduction

Variability in fertilization success

Estimating fertilization success of discrete populations of an introduced marine pest may provide opportunities for management. If there is spatial heterogeneity in reproductive potential among discrete populations of the pest, and these populations can be identified, targeting populations that contribute disproportionately to larval production may be worthwhile in management. Several free-spawning marine invertebrates, including populations of *Asterias amurensis* in the Derwent estuary, have a high degree of spatial variability in reproductive potential depending on various environmental factors that affect growth and condition (chapter 2; and Babcock *et al.* 1994; Guilou and Lumingas 1999; Levitan 1989; Meidel and Scheibling 1999; Oliver and Babcock 1992; Qian and Chia 1991; Wahle and Peckman 1999). In addition to this, various population parameters, including group size, degree of aggregation, water depth, and density, can affect fertilization success in free-spawning marine invertebrates (Babcock *et al.* 2000; Levitan 1991; Levitan *et al.* 1992; Pennington 1985). Given the numerous factors affecting reproductive success in free-spawning marine invertebrates, it is likely that discrete populations experience substantial variation in reproductive potential.

Behaviour and fertilization success

Low sperm concentration is generally regarded as the most significant limiting factor in fertilization success in free-spawning invertebrates (Levitan 1993, 1995; Levitan and Petersen 1995; Levitan and Young 1995; Yund 2000). Because gametes disperse rapidly when released into the water column (Denny 1988; Denny and Shibata 1989), sperm concentration can decrease such that fertilization rates of eggs are low, even at short distances from a spawning male (Chapter 3; Levitan 1991; Levitan *et al.* 1992; Pennington 1985; Yund 1990). Despite this, free-spawning marine invertebrates are successful in nature, so an evolutionary argument would suggest that some mechanism must overcome low fertilization success in free-spawning marine invertebrates. The limited observations of spawning in nature suggest that free-spawning marine invertebrates invoke behaviours that increase fertilization success such as aggregation, spawning in shallow water, and spawning synchronously (Babcock and Mundy 1992; Babcock *et al.* 1992; Coma and Lasker

1997; Gladstone 1992; Sewell and Levitan 1992; Yund 2000; Young 2001). For example, Babcock and Mundy (1992) observed high fertilization success during a mass spawning in the coral reef seastar, *Acanthaster planci*, in which over 88 individuals spawned within 2 hours. Spawning seastars moved to shallow water (1 - 4 m) and individuals adopted an arched position and/or climbed onto coral outcrops to elevate the release point above the seafloor. Theoretically, greater synchrony in spawning and spawning in shallow water should maximise fertilization success.

Population characteristics and fertilization success

The experiments of Pennington (1985) with the sea urchin *Strongylocentrotus droebachiensis* were the first to show that the number of spawning males significantly affected fertilization rates of eggs. Similar experiments with *Diadema antillarum* (Levitan 1991) and *Strongylocentrotus franciscanus* (Levitan *et al.* 1992) also demonstrated increased fertilization with larger numbers of males and increased aggregation. While these experiments indicate the effect of some population characteristics on fertilization success, there are severe logistical difficulties in conducting experiments to measure the effect of population parameters in large populations and in testing the effect of a wide variety of parameters on fertilization success (Levitan 1995). Because of these restraints empirical estimates of the effect of population characteristics on fertilization success are problematic.

To address this issue, models of fertilization success in free-spawning marine invertebrates have been developed that allow for the effect of population parameters on fertilization success to be simulated (Claereboudt 1999; Levitan and Young 1995). In their study, Levitan and Young (1995) found that population size and density both affect fertilization success over a large range of group sizes, and there was an interaction between the density and absolute number of animals. The effect of density on fertilization success was less for populations with large group sizes (Levitan and Young 1995). Using a similar modelling approach, Claereboudt (1999) also predicted significant effects of population density, aggregation, and gamete release on fertilization success in free-spawning marine invertebrates. These models have the same limitations as those discussed in earlier chapters, i.e. decoupled fertilization and dispersal, and reliance on equilibrium gamete concentrations.

Predicting the impact of potential management strategies on larval production of an introduced marine pest requires knowledge of how various population parameters, such as density and sex ratio, affect fertilization success. In this chapter a model of gamete dispersal and fertilization success, parameterized for *Asterias amurensis* in the Derwent Estuary (chapters 2 and 3), is used to simulate spawning scenarios describing particular behaviours and population features. In this way properties of potential target populations can be identified and options for management can be considered.

Materials and Methods

Model landscape

The effect of various population parameters on fertilization success was predicted using a spatially explicit model that simulates gamete dispersal and fertilization success in discrete time intervals (described in Chapter 3). In simulations designed to predict the effect of population parameters on fertilization success, gamete release was simulated in an area representing 40 m x 40 m. Seastar distribution in this area was random. Gamete dispersal and fertilization was simulated in an area 200 m x 60 m to estimate fertilization of gametes released in the 40 m x 40 m area but then dispersed by advection and/or diffusion. Under the advection regime examined the 200 m x 60 m area is sufficiently large to account for the great majority of fertilization of gametes released in the 40 m x 40 m array.

Parameterization

Unless stated otherwise the basic parameter set is as outlined following. Spawning duration was 4650 s and 5630 s for male and female seastars respectively, estimated from empirical observation (table 2.1). Simulations were 10000 seconds to allow fertilization after cessation of egg release. A sperm release rate of 2.3×10^7 sperm s^{-1} and an egg release rate of 600 eggs s^{-1} was used as determined in Chapter 2. The current velocity (U) of 0.03 m s^{-1} was the mean measured during *in situ* experiments (Chapter 3). The shear velocity (u^*) was set at the suggested value of 10% of the mean water velocity, i.e. 0.003 m s^{-1} (Denny 1988; Denny and Shibata 1989). The plume constant was held constant at 2.5 this value is within the range suggested by Denny and Shibata (1989).

Effect of population density

The effect of density on fertilization success was simulated for three population sizes (40, 90 and 130 individuals). The area in which seastars were randomly distributed was increased from 5 m x 5 m to 40 m x 40 m to simulate densities of 0.9 to $0.025 \text{ seastar m}^{-2}$, while holding group size constant. Spawning was synchronous and there was a 1:1 sex ratio. The fertilization response was estimated as the mean of 5 replicate runs. The total number of eggs fertilized and proportion of eggs fertilized were determined for each density.

Effect of sex ratio

Sex ratio was varied from 10:1 to 1:10 male : female. The effect of sex ratio was simulated for population densities of 1, 0.49 and 0.218 seastars m^{-2} . Group size was held constant at 196 individuals, and seastars were randomly distributed through 13 m x 13 m, 20 m x 20 m, and 30 m x 30 m to obtain a range of densities. The proportion fertilized and total number of eggs fertilized was estimated as the mean of 5 replicate runs

Effect of synchrony in spawning

Fertilization success was compared among populations differing in spawning synchrony from exactly synchronous spawning to random spawning over 600, 1200, 2400, 3600, 4600, and 5400 s. Gamete dispersal and fertilization was simulated for 11000, 12000, 13000, 14000, 15000 and 16000 s for these spawning scenarios respectively, to capture fertilization occurring after cessation of gamete release. Seastar populations were simulated in a 30 m x 30 m array at densities of 1.0, 0.2 and 0.02 individuals m^{-2} . The proportion fertilized and total number of eggs fertilized was estimated as the mean of 5 replicate runs.

Effect of group size

Fertilization success was determined for group sizes between 9 and 1600 individuals while holding density constant by increasing the seastar spawning area. Limits to the maximum spawning area restricted the maximum group size that could be simulated while holding density constant. The total number of eggs fertilized and the proportion fertilized was estimated for population densities of 1.0, 0.2, and 0.02 individuals m^{-2} . The proportion and total number of eggs fertilized was estimated as the mean of 5 replicate runs.

Effect of water depth

Fertilization success was predicted for depths of the spawning population ranging from 1 - 10 m. Gamete dispersal and fertilization success of population densities of 0.02, 0.2 and 1.0 seastars m^{-2} were simulated for each depth. The proportion and total number of eggs fertilized was estimated as the mean of 5 replicate runs.

Results

General

The gamete dispersal and fertilization model predicts that fertilization success in *Asterias amurensis* is highly variable and dependent on several population parameters. The simulation model predicts that density, sex ratio, group size, spawning depth, and synchrony in spawning all affect fertilization success in *Asterias amurensis* in a non-linear manner. Within the ranges of parameters tested, the proportion of eggs fertilized was most sensitive to group size, density, and water depth.

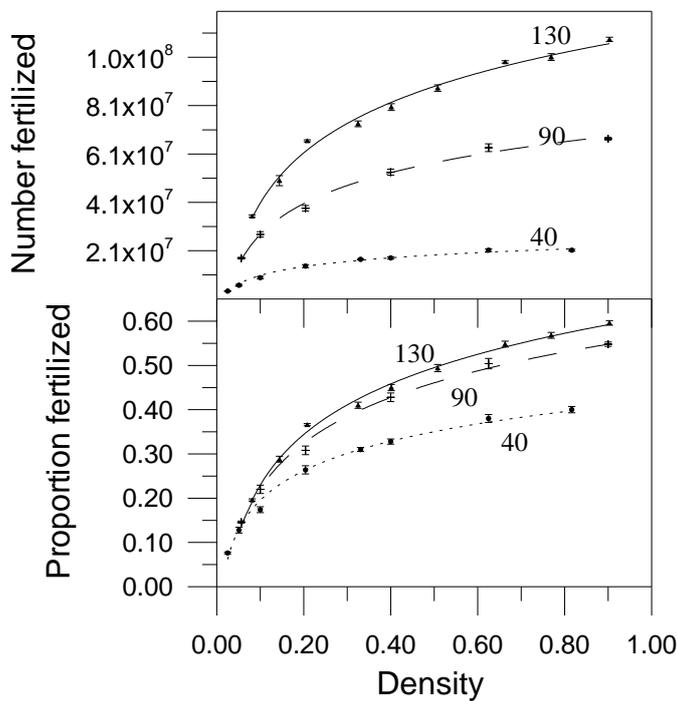


Figure 4.1. The effect of density of *Asterias amurensis* on fertilization success when the total number of animals is held constant. The total number of eggs fertilized (top) and proportion fertilized (bottom) are shown for seastars of fixed group sizes of 130 (solid), 90 (dashed), and 40 (dotted) individuals. The number of eggs fertilized for increasing population density follow logistic curves given by $Y = 3.00 \times 10^7 \times \log(X) + 1.10 \times 10^8$ ($R^2 = 0.99$), $Y = 1.85 \times 10^7 \times \log(X) + 7.02 \times 10^7$ ($R^2 = 0.99$), and $Y = 5.25 \times 10^6 \times \log(X) + 2.28 \times 10^7$ ($R^2 = 0.99$) for group sizes of 130, 90 and 40 individuals respectively, where $X = \text{density (individuals m}^{-2}\text{)}$ and $Y = \text{total number of eggs fertilized}$. The response of the proportion fertilized for increasing density also follow logistic curves given by $Y = 0.165 \times \log(X) + 0.609$ ($R^2 = 0.99$), $Y = 0.149 \times \log(X) + 0.564$ ($R^2 = 0.99$), and $Y = 0.096 \times \log(X) + 0.417$ ($R^2 = 0.99$) for group sizes of 130, 90, and 40, respectively, where $X = \text{density (individuals m}^{-2}\text{)}$ and $Y = \text{proportion fertilized}$.

Effect of population density

The model predicts that the proportion and total number of eggs fertilized increase with increasing seastar density (figure 4.1). For all group sizes simulated, the rate of increase of fertilization success was greatest at the lowest densities. For populations of 40 individuals, the model predicts more than a 3 – fold increase in the proportion of eggs fertilized (from 0.08 to 0.26) for an order of magnitude increase in density (from 0.025 to 0.2 individuals m^{-2}) (figure 4.1).

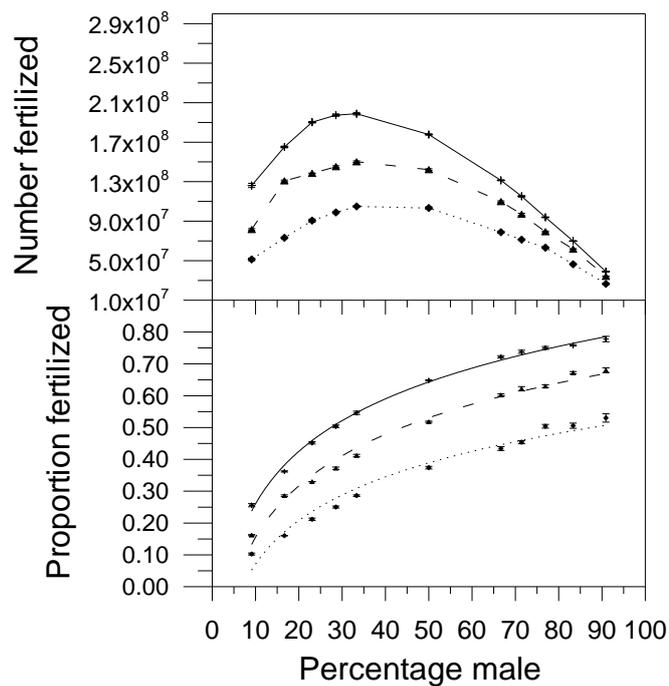


Figure 4.2. The effect of sex ratio of *Asterias amurens* populations on predicted fertilization success when sex ratio is varied from 10:1 to 1:10 male : female, and group size held constant. The total number of eggs fertilized (top) and proportion fertilized (bottom) are shown for populations of 196 individuals at densities of 1.0 (solid line), 0.49 (dashed), and 0.218 (dotted) seastars m^{-2} . The proportion of eggs fertilized for populations with increasing male bias follow logistic curves given by $Y = 0.238 \times \log(X) - 0.288$ ($R^2 = 0.99$), $Y = 0.233 \times \log(X) - 0.382$ ($R^2 = 0.99$), and $Y = 0.197 \times \log(X) - 0.384$ ($R^2 = 0.97$) for population densities of 1, 0.49 and 0.218 seastars m^{-2} , respectively, where X = the proportion of the population male, and Y = total number of eggs fertilized. While the proportion of eggs fertilized increases with increasing male bias (bottom), the absolute number of eggs available decreases, resulting in fewer total zygotes produced (top).

Effect of sex ratio

Simulations indicate that fertilization success is sensitive to sex ratio within the ranges tested. Increasing male bias in the spawning population increased the proportion of eggs fertilized for all densities (figure 4.2). The sex ratio that maximized the number of eggs fertilized was dependent on the density of the spawning population (figure 4.2). The model predicts maximum zygote production for densities of 1.0, 0.2, and 0.02 individuals m⁻² in populations consisting of ca. 30, 35, and 40 % males respectively. Shifting the male bias above 50 percent resulted in a rapid decline in the number of eggs fertilized for all densities simulated.

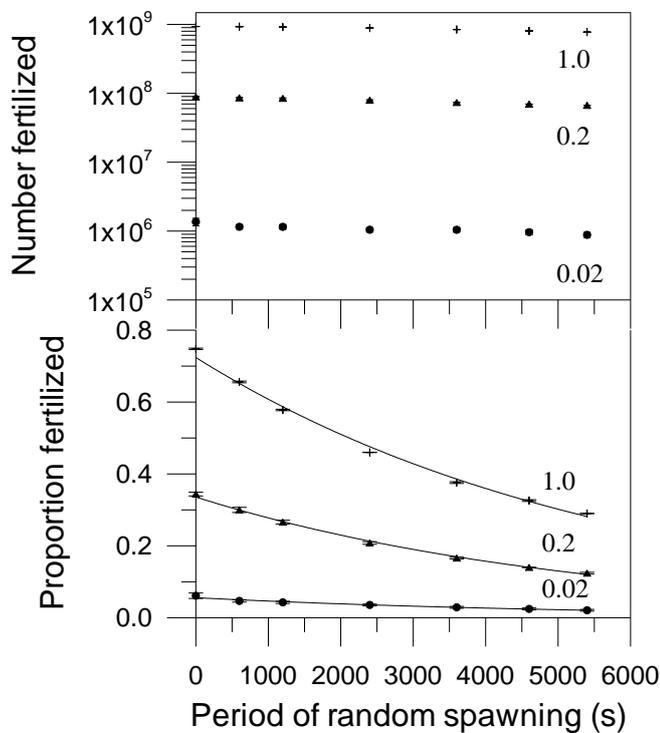


Figure 4.3. The effect of spawning synchrony of *Asterias amurensis* populations on fertilization success when the period over which individuals commence spawning is varied from 0 (exactly synchronous) to 5400 s. The effect of increasing the period over which spawning commences on the total number of eggs fertilized (top) and proportion fertilized (bottom) is shown for densities of 1.0 (cross), 0.2 (triangle), and 0.02 (circle) seastars m⁻².

There was an exponential decline in the proportion fertilized with decreasing synchronicity for population densities of 1.0, 0.2 and 0.02 individuals m⁻² given by: $\log(Y) = -1.748 \times 10^{-4} \times X - 0.323$ ($R^2 = 0.99$), $\log(Y) = -1.90 \times 10^{-4} \times X - 1.09$ ($R^2 = 0.99$) and $\log(Y) = -1.825 \times 10^{-4} \times X - 2.887$ ($R^2 = 0.98$) where X = period of random spawning and Y = proportion fertilized.

Effect of spawning synchrony

There was an exponential decline in the proportion of eggs fertilized with decreasing spawning synchrony for the three population densities simulated (figure 4.3). The total number of eggs fertilized was of the same order of magnitude irrespective of the degree of synchrony (figure 4.3).

Effect of group size

There was a power relationship between the total number of eggs fertilized and group size (figure 4.4). The proportion of eggs fertilized increased with increasing group size with the rate of increase greatest for population densities of 1.0 individuals m^{-2} (figure 4.4). The increase in the proportion of eggs fertilized with group size plateaus at approximately 400 and 125 individuals for densities of 1.0 and 0.2 individuals m^{-2} .

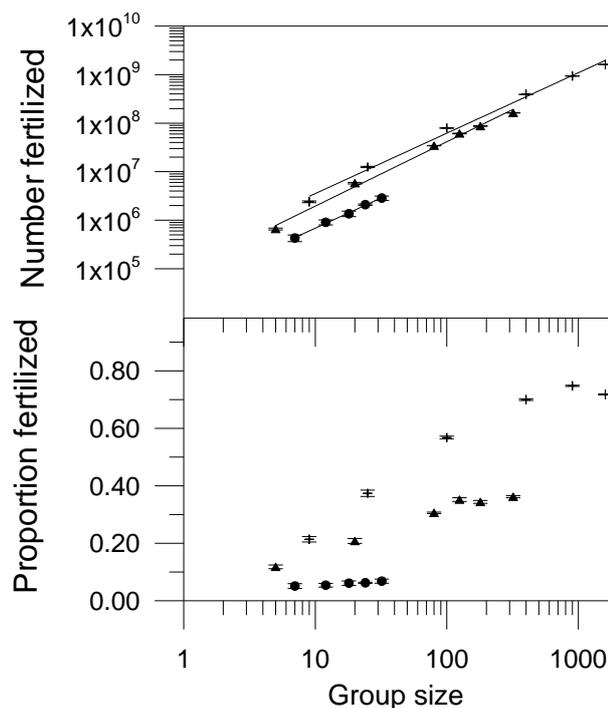


Figure 4.4. The effect of group size of *Asterias amurensis* on fertilization success where density is held constant. For populations densities of 1.0 (cross), 0.2 (triangle), and 0.02 (circle) seastars m^{-2} , both the total number of eggs fertilized (top) and proportion fertilized (bottom) increased with group size. The total number of eggs fertilized increased with group size as a power relationship given by $\log(Y) = 1.25 \times \log(X) + 12.21$ ($R^2 = 0.99$), $\log(Y) = 1.33 \times \log(X) + 11.43$ ($R^2 = 0.99$), and $\log(Y) = 1.24 \times \log(X) + 10.57$ ($R^2 = 0.99$) for densities of 1.0, 0.2, and 0.02 individuals m^{-2} , where X = group size, and Y = total number of eggs fertilized.

Effect of water depth

The simulation model predicts that the total number and proportion of eggs fertilized are reduced with increasing depth of the spawning population (figure 4.5). The effect was most pronounced for changes in depth between 1 and 5 m, and was greatest between 1 and 2 m. For population densities of 0.02 individuals m⁻², an increase in depth from 1 to 2 m resulted in more than a 5 – fold decrease in fertilization success (0.81 for 1 m compared to 0.14 for 2 m), and a substantial drop in the total number of eggs fertilized (2.04 x 10⁸ for 1 m, compared to 3.23 x 10⁶ for 2 m) (figure 4.5).

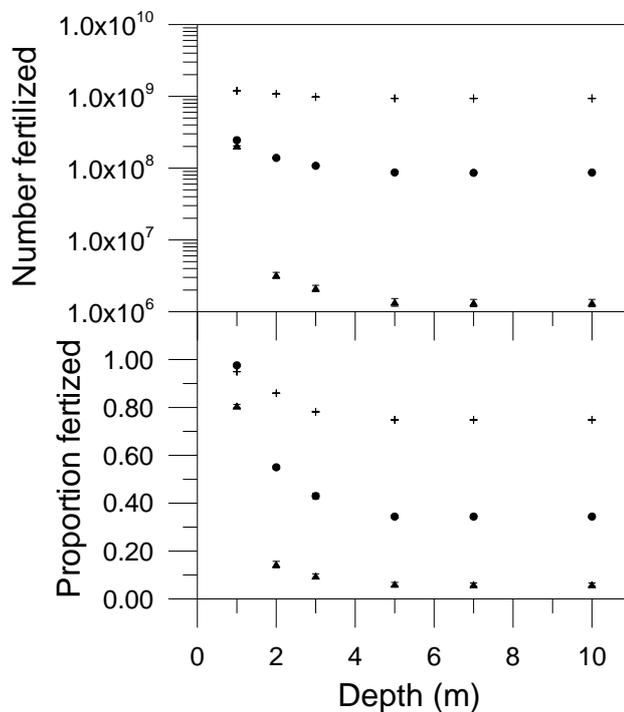


Figure 4.5. The effect of depth of spawning populations on fertilization success. Increasing depth reduced both the total number of eggs fertilized (top) and proportion fertilized (bottom) at densities of 1.0 (cross), 0.2 (triangle), and 0.02 (circle) seastars m⁻². The effect is most pronounced at shallow depths.

Discussion

The Allee effect

Sperm limitation in free-spawning marine invertebrates is regarded as the most important limiting factor in fertilization success (Denny and Shibata 1989; Levitan 1993; Levitan and Petersen 1995; Levitan and Young 1995; Yund 2000). Sperm limitation can be overcome if invertebrates spawn in large populations with high density (the Allee effect, Allee 1931). Theoretical and empirical studies suggest the benefit of large crowded populations to fertilization success ceases at a critical point when adult populations become resource limited (Levitan 1991; Levitan and Young 1995; Meidel and Scheibling 1999). Given that the *Asterias amurensis* is a generalist predator and there is a large amount of potential food introduced into the Derwent Estuary via anthropogenic action (*pers obs*; Grannum *et al.* 1996; Morrice 1995) it is unlikely that the seastar is resource limited unless density is extremely high.

Factors affecting fertilization success

The model of gamete dispersal and fertilization success predicts that fertilization success in *Asterias amurensis* is extremely sensitive to changes in population parameters within the ranges tested. Of the parameters simulated, fertilization success, measured as either the total number of eggs fertilized or proportion fertilized, was most sensitive to density, group size and water depth (figures 4.1, 4.4, 4.5). These results are in agreement with the limited number of *in situ* experiments that illustrate a positive effect of density (Levitan 1991), group size (Levitan *et al.* 1992, Pennington 1985), and shallow water depths (Babcock *et al.* 2000) on fertilization success. The predictions of existing models of fertilization success in free spawning marine invertebrates also suggest that increased density and group size improve fertilization success (Claereboudt 1999; Levitan and Young 1995). However, Levitan and Young (1995) also found an interaction effect between density and group size with density being less significant in large populations.

Effect of population density

The proportion of eggs fertilized was sensitive to changes in density from 0.02 to 0.2 individuals m⁻² (figure 4.1). Seastar densities in the Derwent Estuary at

the time of spawning range from 0 – 7 individuals m^{-2} (Grannum *et al.* 1996; Morrice 1995). The density of *A. amurensis* is highest in the mid-estuary with more than 1 seastar m^{-2} near the port of Hobart, and less than 0.1 individual m^{-2} in the lower estuary (Grannum *et al.* 1996; Morrice 1995). The simulation model predicts that fertilization success varies substantially over this range of densities tested (figure 4.1). For example, the model predicts a 250% increase in the proportion of eggs fertilized (from 0.22 to 0.55) when population density is increased from 0.1 to 0.9 individuals m^{-2} and group size is held constant at 90 individuals. The model also predicts a 2.4 – fold increase in the total number of eggs fertilized over this range of densities (from 2.77×10^7 to 6.73×10^7 eggs fertilized for densities of 0.1 to 0.9 individuals m^{-2} , respectively), suggesting that the population in the mid-estuary produces substantially more larvae than the population in the lower estuary.

Effect of sex ratio

The simulation model predicts maximum zygote production in populations with female bias (figure 4.2). There was an interaction between the sex ratio and density, with the optimal sex ratio (i.e. the population structure that resulted in the greatest number of eggs fertilized) being more female biased in populations with higher densities. These results suggest that management options that eliminate only males (such as parasitic castrators) would need to force the sex ratio below 1 : 5 (males: females) before sperm limitation significantly affects fertilization success.

Effect of spawning synchrony

For densities of seastars commonly observed in the Derwent estuary, varying the period of random spawning from complete synchrony to random over 1.5 hours did not substantially affect the total number of eggs fertilized (figure 4.3). Naturally increasing the period of random spawning beyond 1.5 hours will result in decreased fertilization success. These results suggest that, while the period of spawning is important to fertilization success, high degrees of synchronicity are not required for high levels of fertilization success.

Effect of water depth

The simulation model predicts a sharp increase in fertilization success with increasing depth when all other parameters are held constant. In a similar study

Babcock *et al.* (2000) found a significant effect of depth on fertilization success providing that eggs are released directly downstream of the spawning male, however they predicted no effect where eggs are released offset from the main axis of flow. In the present study randomly distributed individuals were simulated, and increases in depth from 1 – 2 m had a substantial affect on fertilization success. For an increase in depth from 1 to 2 m, the model predicts more than a 5 – fold decrease in the proportion of eggs fertilized (from 0.806 in 1 m compared to 0.144 in 2 m), and a 100-fold drop in the total number of eggs fertilized (2.04×10^8 in 1 m, compared to 3.23×10^6 in 2 m) (figure 4.5).

There have been several observations of free spawning marine invertebrates, including *Asterias amurensis*, moving into shallower water prior to spawning (*pers obs*, Babcock and Mundy 1992, Breen and Adkins 1980, Gladstone 1992, Levitan 1998, Morrice 1995). Individuals moved to shallow water (1-4 m) in a natural spawning of the coral reef seastar *Acanthaster planci* (Babcock and Mundy 1992). Given results to indicate that fertilization success increases with decreasing depth, moving into shallow water may be an adaptation to maximize fertilization success in free-spawning marine invertebrates.

Setting management priorities

The model predictions suggest populations of *Asterias amurensis* attain maximum fertilization success in shallow water in the mid-estuary, where density is highest, such as near wharves and yacht clubs, and in shallow water (Ling 2000). The seastar density in the mid estuary is > 1.0 individuals m^{-2} (Grannum *et al.* 1996; Morrice 1995) while the model predicts that decreases in density, for all group sizes simulated, are only effective in significantly reducing fertilization success of populations densities of 0.2 individuals m^{-2} and below (figure 4.1). For a management strategy to be effective in reducing fertilization success requires reducing population density below this critical limit therefore it would be necessary to remove massive numbers of individuals, and/or remove any stimulus that may cause adults to aggregate in these areas of high anthropogenic impact. Even if effective in reducing fertilization success substantially, whether reduced larval production would result in reductions in numbers of larvae at the end of the pelagic stage, and finally recruitment requires further investigation. However, reducing the

number of larvae produced in the Derwent Estuary would reduce the risk that larvae are transported to other Australian ports via ballast water, and minimise the risk of larvae settling in marine farms in south-east Tasmania.

Model limitations

The model of gamete dispersal and fertilization developed to predict fertilization success in populations of *Asterias amurensis* was validated by independent empirical measures of fertilization success for various male/female separation distances (chapter 3). The degree to which these results can be extrapolated to simulate larger populations has not been tested empirically due to the logistical difficulties involved in inducing large populations to spawn simultaneously and measuring fertilization success of gametes as they disperse. Extrapolating model results to predict fertilization success in larger populations should be treated with caution because fertilization is extremely sensitive to population size (figure 4.4; Levitan *et al.* 1992; Levitan 1995). Furthermore the model assumes that current speed and sheer velocity remains constant throughout the estuary, and for example, does not account for low flow regimes commonly observed in protected areas such as yacht clubs. The predictions of the proportion and total number of eggs fertilized for each spawning scenario are designed as a tool to illustrate the effect of population parameters on fertilization success so that populations that should be targeted for management can be identified.

Chapter 5: Dispersal of an introduced seastar is influenced by behavioural responses of larvae to salinity and light

Abstract

Assessing the rate and direction of dispersal of an introduced species is an important component in defining its threat to the marine environment and marine-based industries. Dispersal of larvae of the introduced seastar *Asterias amurensis* in the Derwent Estuary, Tasmania, and advection of larvae out of the estuary, was predicted using an inverse transport model incorporating the behavioural responses to salinity and light at different stages of larval development. The estuary was modelled as a set of contiguous cells arranged in 3 layers vertically and 30 columns horizontally. The flux of salinity and larvae at the interface between adjacent cells was determined by advective and diffusive components at each time-step in the model. The vertical distribution of larvae was governed by empirical estimates of larval vertical swimming speed.

Vertical migration patterns of four larval stages (early, mid and late bipinnaria, and early brachiolaria) in response to various light and salinity regimes were determined in laboratory trials and these data incorporated into the transport model. Patterns of vertical migration depended on larval age, the salinity profile, and light. Bipinnaria display reverse diel migration, swimming towards the surface in response to light, and sinking in darkness at an average velocity of $3 \times 10^{-5} \text{ ms}^{-1}$. The maximum swimming velocity measured was $3 \times 10^{-4} \text{ ms}^{-1}$. Ascending larvae will not pass into water of salinity < 26 ppt irrespective of the light regime. Early brachiolaria larvae were negatively buoyant.

Model runs examined the effect of (1) larval swimming velocity and response to salinity, (2) river flow, and (3) the location of spawning populations in the estuary, on both dispersal and the number of larvae retained in the estuary. Results indicate that the majority ($> 99\%$) of larvae are advected out of the estuary. Under average river flow conditions, the model predicts that at the end of the pelagic phase, larvae with average swimming velocities are diluted by 7 orders of magnitude from initial concentrations in the estuary.

Behavioural responses of larvae to salinity and the behaviour of vertical migration affect the proportion of larvae retained in and advected from the estuary. Larvae swimming at the maximum velocities measured empirically were more likely to be retained in the estuary than slower larvae. Under conditions of average river flow, larvae with average migration velocities were less likely to be advected out of the estuary than passive larvae, however there was also high mortality of these larvae due to advection upstream in the lower portion of the salt wedge, below the halocline. Larvae moved upstream by this mechanism were eventually advected into low salinity water in which they did not survive. If larvae were simulated as passive particles, the number retained in the estuary was extremely sensitive to river flow, with estimates of dilution of these larvae varying by 10 orders of magnitude depending on the flow regime. Advection patterns of larvae simulated to vertically migrate were less sensitive to changes in river flow than advection of passive larvae.

Key words: larval dispersal; larval behaviour; hydrodynamic model; Asterias amurensis; vertical migration; salinity; introduced species

Introduction

Predicting rates of spread in introduced species has been a major focus of invasion biology over the last decade (Grosholz 1996; Hastings 1996). The impact of an introduced species on the marine environment and marine industries is dependent, in part, on its rate of spread. Assessing the rate and direction of dispersal is an important component in defining the threat of an introduced species to the marine environment and marine resources. Knowledge of the contribution that established populations make to recruitment is also a key issue in the development of management plans for introduced marine pests. Information on larval dispersal is therefore needed to develop an appropriate response to marine incursions.

In several examples of marine introductions, there is a lag between local establishment of an introduced species and its subsequent spread (Crooks and Soule 1999; Grosholz 1996). These lags may be due to environmental barriers, vagaries of dynamics of small populations or impediments to dispersal such as retention of larvae in estuaries. In the case of the introduced pest *Asterias amurensis* there is an apparent disparity between high adult densities in the Derwent Estuary and low recruitment elsewhere in south-eastern Tasmania. Compared with high densities of seastars in the estuary (peak densities exceed 1 m^{-2}), densities of adults outside the estuary are very low (Grannum *et al.* 1996; Morrice 1995). This is peculiar given that in its native environment the seastar is common in subtidal coastal waters from 0 - 200 m in a large expanse of the north-west Pacific from the Bering Straits to Korea and around the Japanese coastline (McLoughlin and Bax 1993). In developing management plans, it is critical to know whether this situation arises because larvae are largely retained in the estuary, or whether they are advected out of the system but other processes limit recruitment outside the estuary. Previous models based on wind-driven circulation of surface waters, and which did not consider the behaviour of larvae, indicated that larvae are largely retained in the estuary (Bruce *et al.* 1995; Lyne 1993). The present work, which factors in behaviour of larvae in the water column, offers a contrary view.

The effect of larval vertical migration on dispersal

Vertical migration is common in marine larvae (see Forward 1976, 1988; Mileikovsky 1973; Thorson 1964; Young 1990, 1995; Young and Chia 1987, for reviews) and can be an important factor in governing horizontal dispersal and, for estuarine species, may be important in retaining larvae in the estuarine environment (Cronin and Forward 1982; Sulkin and Van Heukelem 1982; Young and Chia 1987). Recent advances in larval dispersal models have fused hydrodynamics with the vertical migration of larvae to predict horizontal dispersal (Dippner 1987; Hinckley *et al.* 1996; Jenkins *et al.* 1999; Rothlisberg *et al.* 1995, 1996; Smith and Stoner 1993; Tremblay *et al.* 1994; Verdierbonnet *et al.* 1997). These models use observed larval behaviour to predict the vertical distribution of larvae in the water column, and then model dispersal. This approach is necessary in systems where water currents move in different directions depending on depth, for example when propagules are released in a stratified estuary such as the Derwent Estuary.

Materials and Methods: experimental

Larval culture

Ripe adult seastars (7 females and 4 males) freshly collected from the Derwent Estuary were induced to spawn by injection of 5-7 ml of 10^{-3} M 1-methyladenine. A mixture of sperm, diluted to avoid polyspermy, and eggs in filtered seawater was aerated for 1-2 hrs, realising fertilization success > 95%. Fertilized embryos were transferred to 5 325 l conical tanks filled with 1 μ m filtered seawater (average salinity 33.8 ppt) and aerated with small conical air-stones (10 mm diameter). The airflow was kept low to ensure larvae were not damaged by turbulence. Water temperature was within 2-3 degrees of ambient, increasing from 8-13 °C over the 3 month rearing. From day 4 larvae were fed daily on 3 microalgae species: *Rhodomonas sp.*, *Isoscrysis sp.* and *Dunaliella teriolecta*, at 5000-10000 ml⁻¹. The tanks were cleaned on days 2, 3, and 4, then weekly for the remainder of the rearing. Larvae were not exposed to the air during water exchanges.

Vertical swimming responses of larvae to various salinity and light regimes were determined for early, mid and late bipinnaria and early brachiolaria. Twenty larvae were used in each replicate trial for experiments on early and mid bipinnaria. Because availability of robust larvae decreased throughout the rearing, fewer larvae (10 and 6 respectively) were used in trials with late bipinnaria and early brachiolaria. There were six replicates of each stage/treatment combination.

Larval response to salinity

Experiments to determine behavioural responses of larvae to salinity were conducted in rectangular columns 0.15 m x 0.15 m x 1.0 m filled to a depth of 0.95 m. Columns were marked at 100 mm intervals starting at a height of 50mm, dividing the columns into 10 depth stratum (figure. 5.1). Valves in the middle of each depth strata allowed access to water samples to measure salinity. Larvae were injected via a valve 100 mm from the bottom of the column (depth stratum 2) at the beginning of experiments.

The control treatment was a fully mixed system of 1 μ m filtered seawater (33.8 ppt). In the salt wedge treatment there was a sharp halocline at a depth of 650

mm (depth stratum 6) with salinity below the halocline at 33.8 ppt and above at 4 ppt. The halocline was formed by filling the experimental column with seawater (33.8 ppt) to 650 mm, and then slowly pouring fresh water (4 ppt) through a floating sponge to fill the chamber to 950 mm. The resultant halocline was stable for several hours. The two salinities used in halocline experiments approximate the extremes of salinities commonly observed in the Derwent estuary (Bruce *et al.* 1997).

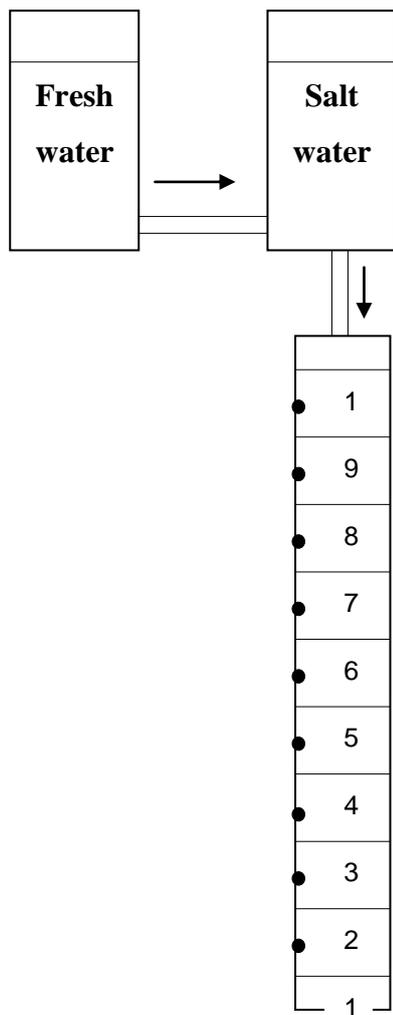


Figure 5.1. Schematic of the method used to establish a salinity gradient in experimental columns (after Coombs 1981). The experimental chamber was initially filled to a depth of 50 mm with filtered seawater. A valve between the fresh and saltwater reservoir was opened to gradually reduce salinity in the salt water reservoir. Water entering the experimental chamber was passed through a floating sponge to ensure that the salinity gradient was not disrupted. Water in the saltwater reservoir was stirred continuously. The salinity of each depth stratum (1-10) was measured from water samples taken by syringes attached to valves (●) in the middle of each stratum.

The third treatment was a salinity gradient with a gradual increase in salinity with depth from 4 ppt at 0.90 m to 33.8 ppt at the base of the column. The salinity gradient was formed following the procedure of Coombs (1981). The column was initially filled with seawater (33.8 ppt) to a depth of 50 mm. The column was then filled with water from two containers, one filled with fresh water (4 ppt), and the other with salt water (33.8 ppt). The fresh water container was connected to the salt water container, and the salt water was piped directly into the experimental chamber (figure. 5.1). To establish the gradient the valve between the fresh and salt water containers was opened, and then the water from the salt water reservoir to the experimental column was opened. Water entering the column was passed through a floating sponge to minimise mixing in the chamber. Water in the saltwater reservoir was mixed continuously so that water entering the experimental column gradually decreased in salinity. The resulting salinity gradient was stable for several hours.

Larvae were sorted under dissecting microscope to ensure they were active, morphologically normal, and at the appropriate stage. Larvae required for each replicate in < 3ml of seawater (33.8 ppt) were injected at the base of the column with a syringe attached to a valve 100 mm above the base (depth stratum 2). The process was gentle and larvae were not damaged.

After 30 min, the number of larvae in each depth stratum was counted. Experiments using salinity treatments were conducted between 06:30 and 19:30 hours, corresponding to the hours of daylight in the rearing tanks. Experimental columns were illuminated by fluorescent lights from above. The fluorescent lights were > 3 m from the top of the experimental column to minimise potential artifacts associated with high light intensities. At the completion of each trial, water samples were taken from valves in the middle of each depth stratum and salinity measured by refractometer.

Larval response to light

Experiments to determine the effect of light on the vertical migration of late bipinnaria examined larval vertical swimming responses in three light regimes: normal photoperiod, reverse daylight and continuous darkness. Normal photoperiod followed the day/night cycle in the larval rearing facility, with fluorescent lights on

at 06:30 h and off at 19:30 h. In the reverse daylight trial, lights were turned on at 19:30 h and off at 06:30 h. Trials in continuous darkness ran for 24 h. All trials commenced at 14:30 h when larvae were introduced to the column 10 mm from the base (depth stratum 2). Larval height was measured by counting the number of larvae in each depth stratum at 15:00, 19:00, 20:00, 23:00, 06:00, 07:00, 10:00 and 14:30 hours. Six fluorescent lights were suspended 3 m above the experimental chambers. During dark periods, a flash light was used to briefly scan the water column to locate larvae.

Larval swimming speeds were estimated by determining the mean rate of change of larval vertical position in the experimental chamber. Larval movements in the first 30 min after introduction into the experimental column were not included in estimating larval swimming speed.

Materials and Methods: modelling

Model description

Larval dispersal was modelled using a transport model composed of a one-dimensional sequence of 30 'columns' arranged along the estuary (figure 5.2). Each column is divided vertically into three layers. Exchanges at the horizontal and vertical interfaces between adjacent cells is prescribed by a pair of opposing exchange fluxes (of units: volume per unit time), which simulate the processes of advection, diffusion, vertical swimming and settling (figure 5.3). While figure 5.3 shows the exchange fluxes associated with an interior cell, the cases for bottom (layer 0) and surface (layer 2) cells are similar but rather simpler. For a cell in layer 0, there is no advective or diffusive exchange through the lower interface, although sinking of larvae is allowed through this interface and out of the model. For a cell in layer 2, there is no exchange through the upper interface (the water surface).

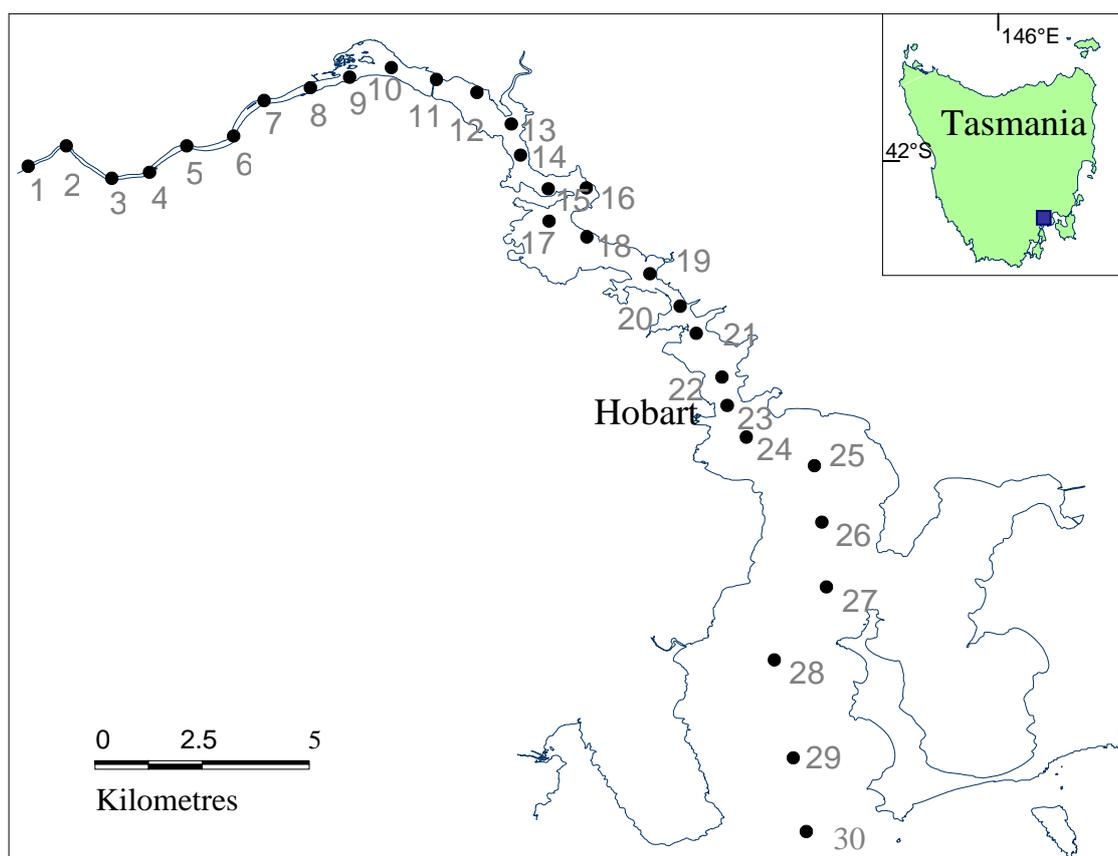


Figure 5.2. The Derwent Estuary, Tasmania, showing the distribution of column centres (1-30) in the inverse transport model. The estuary was represented by 30 columns horizontally and 3 layers vertically. Tracer concentrations were averaged within cells.

	Larval age (days)		
	0-90		91-120
	Day	Night	Day/night
Salinity < 18 ppt	Mortality	Mortality	Mortality
Salinity 18<26 ppt	↓	↓	↓
Salinity ≥26 ppt	↑	↓	↓

Table 5.1. Summary of parameters used in the inverse transport model to simulate larval vertical swimming behaviour (arrows indicate direction of larval movement) in response to salinity, and mortality due to exposure to low salinity water. Hatched coeloblastula (day 1) to early brachiolaria (day 90) displayed reverse diel migration although larvae would not swim into waters of salinity < 26ppt. Early brachiolaria (day 91) have a sinking velocity for the rest of the pelagic phase. Larvae were not tolerant to salinities below 18 ppt (Sutton and Bruce 1996).

The advective and diffusive components of the exchange fluxes are temporally variable depending on river flow. The fate of larvae and salt within each cell is simulated by discrete time-steps, and includes both the physical transport processes and, for the larvae, vertical swimming and settling, and larval survival.

The time-varying advective and diffusive exchange fluxes used in the transport model were derived from an extended version of the steady-state inverse model of Pritchard (1969). Extensions include: an increase of the number of layers from two to three; the inclusion of horizontal turbulent diffusive processes; and the use of singular value decomposition to solve the resultant under-determined set of conservation equations (Parslow *et al.* 1998). Steady transport fields were derived from salinity distributions for four ‘standard’ river flow conditions: 64.7 m³s⁻¹, 94.9 m³s⁻¹, 164.5 m³s⁻¹ and 217.6 m³s⁻¹. Transport fields for other river flows were derived by weighted linear interpolation, based on the transport fields for the neighbouring pair of standard river flows. The salinity observations used for the inversions were based on those collected by the CSIRO Coastal Zone Program during the years 1992 to 1994 (e.g. Walker and Hunter 1995).

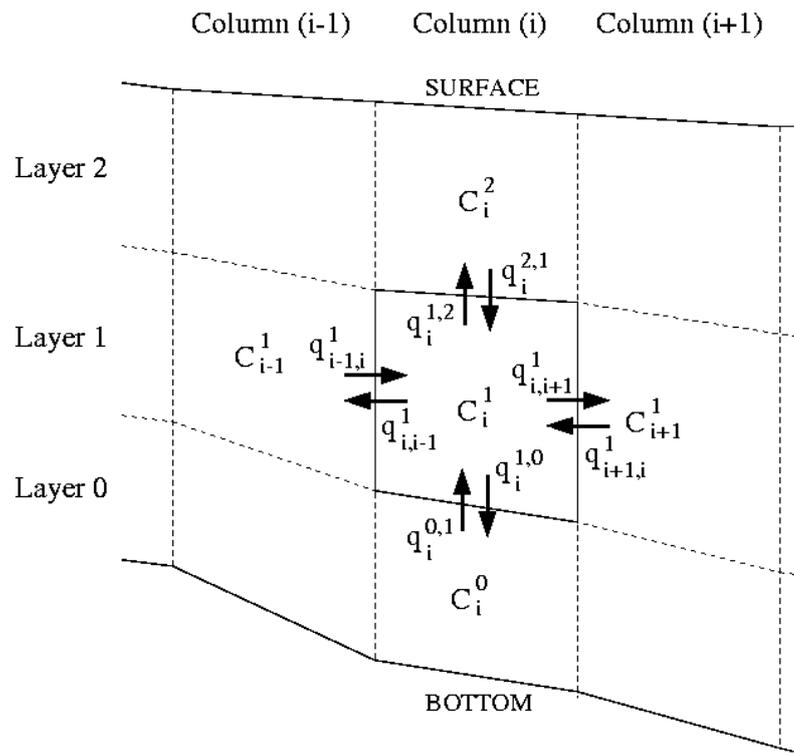


Figure 5.3. Schematic of one cell of the transport model. Subscripts denote the column index and superscripts the layer index. The flux of salt or larvae to neighbouring cells is determined at each cell interface by the difference of two components. Each component is derived from the product of an exchange flux (e.g. $q_{i-1,i}^1$, which passes from the cell in column $i-1$ and layer 1 to the cell in column i and layer 1) and the concentration of salt or larvae in the cell at the tail of the arrow (in this example, the concentration of salt or larvae in the cell in column $i-1$ and layer 1 is denoted as C_{i-1}^1). The total flux of salt or larvae between these two cells is therefore given by $(C_{i-1}^1) q_{i-1,i}^1 - (C_i^1) q_{i,i-1}^1$. The conservation equations are fully described by Parslow *et al.* (1998).

Parameterization

General

The transport model was used to simulate the concentrations of larvae and salt (which affects the larval behaviour). Each model run simulated 140 days of flux, the first 20 days being an initialisation phase to establish a stable salinity profile, after which larvae were released and the 4 month (120 days) larval pelagic phase was simulated. Unless stated otherwise, larval release was simulated in cells 21-29 in layer 0 (on the sea floor) for 1 day (86400 simulation seconds) to a final concentration of 1 larvae m^{-3} . The dilution of larvae from initial concentrations was predicted at the end of the pelagic phase. Pilot runs showed that the model was not sensitive to actual versus average day lengths and therefore average day lengths

were used in all simulations both for simplicity and in order to minimise computing time.

Scenario 1: Effect of larval vertical migration

In simulations designed to predict the effect of larval diel vertical migration on dispersal, river flow was constant at $145 \text{ m}^3 \text{ s}^{-1}$ (this was the average river flow in the estuary between 1st August - 30th November in 1992 and 1993; CSIRO, unpublished data), and day length was held constant at 12 hrs (the average during the larval pelagic phase).

The dispersal of larvae with three different swimming behaviours was simulated. Larvae were designated either as passive and neutrally buoyant, or to change depth at mean ($3 \times 10^{-5} \text{ ms}^{-1}$) or maximum ($3 \times 10^{-4} \text{ ms}^{-1}$) velocities in the manner observed in laboratory trials.

Scenario 2: Effect of river flow

The effect of river flow on larval dispersal was determined for the three swimming behaviours of larvae (i.e. passive neutrally buoyant larvae, and mean and maximum vertical swimming velocities). Five scenarios of river flow were investigated. Three were constant flow regimes representing average, maximum, and minimum daily flows measured during the pelagic phase of the seastar in 1992 and 1993. The two regimes of variable river flow were the empirical time-series of river flow measured daily from 1/8/92 - 1/12/92 and 1/8/93 - 1/12/93 (CSIRO unpublished data). The mean and standard deviation of the two time-series were $158.97 \text{ m}^3 \text{ s}^{-1}$ (SD = 40.75) and $124.14 \text{ m}^3 \text{ s}^{-1}$ (SD = 27.71) for 1992 and 1993 respectively. All other parameter values were the same as those used in scenario 1.

Scenario 3: Effect of the site of larval release

The effect of the site of larval release in the estuary on dispersal was simulated for larvae with the three swimming behaviours (i.e. passive, and mean and maximum swimming velocities). Larval release was simulated as either 100% of larvae from Hobart (cell 23) or from Halfmoon Bay towards the mouth of the estuary (cell 29), and compared with simulations of uniform larval release across cells 21-29. These two sites comprise the spatial extremes of significant larval

release given the present distribution of seastars in the estuary. Other parameter values were the same as in scenario 1.

Scenario 4: Effect of salinity dependent vertical migration

The effect of larval avoidance of low salinity on dispersal was predicted by comparing dispersal of larvae that followed the observed response to both light and salinity, with dispersal of larvae simulated to migrate solely in response to light. The density and distribution of larvae simulated with each migration pattern was plotted at the end of the pelagic phase. To ascertain whether larval dilution was due to advection out of the estuary or mortality due to low salinity, these two scenarios were repeated under identical conditions but without osmotically induced mortality. In these simulations larvae swam at average velocity, and other parameters were the same as in scenario 1.

Results: experimental

Larval response to salinity

Early, mid, and late bipinnaria responded similarly to salinity treatments (figure 5.4). In the control treatment where salinity was constant with depth, larvae distributed relatively uniformly throughout the water column (figure 5.4). In wedge treatments most bipinnaria were distributed just below the halocline (figure 5.4). Larvae were never observed above the halocline in any treatment. When introduced into a column with a salinity gradient, bipinnaria larvae swam upwards until salinity was reduced to 26 ppt, and did not venture into low salinity water (figure 5.4). Brachiolaria larvae were largely distributed near the bottom of the column (depth stratum 1) irrespective of the salinity treatment (figure 5.4).

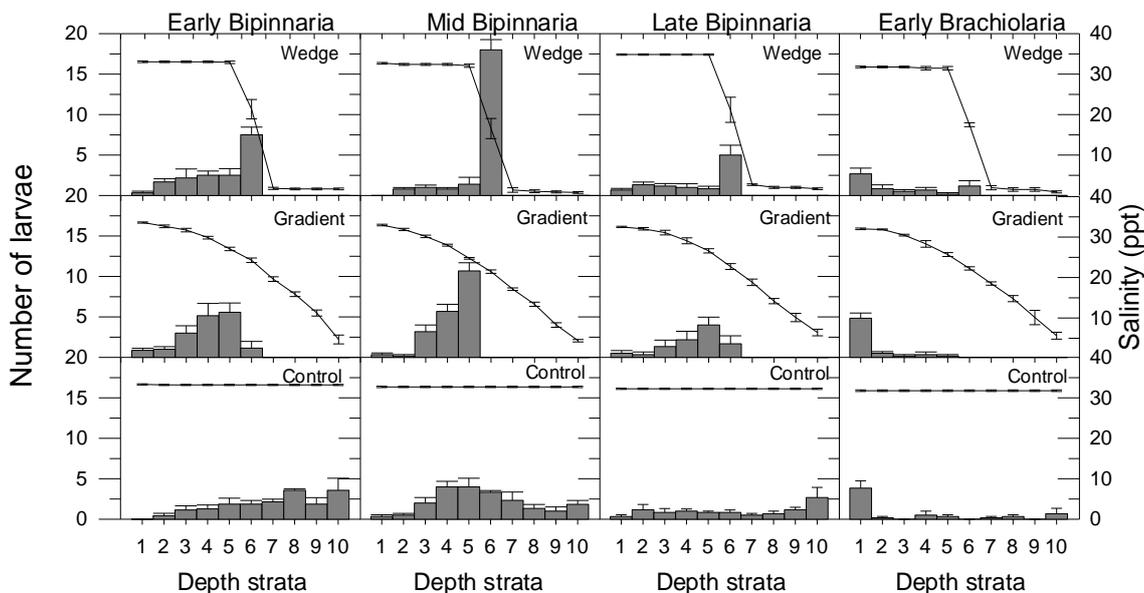


Figure 5.4. Vertical swimming response of *Asterias amurensis* larvae to salinity. The number of larvae (bars) and salinity (line plot) in each of 10 depth strata is shown. Chamber 10 represents the surface water layer. The swimming response of early, mid and late bipinnaria, and early brachiolaria larvae were measured separately (there were 20, 20, 10, and 6 larvae of each age class respectively in each trial). Data are means (\pm SE) of six replicate trials for each stage/treatment combination.

Larval response to light

Late bipinnaria showed a pattern of reverse diel migration, swimming upwards during the day and sinking at night (figure 5.5). Average and maximum

swimming velocities measured during the day were $3 \times 10^{-5} \text{ms}^{-1}$ and $3 \times 10^{-4} \text{ms}^{-1}$ respectively. Downward movement at night was initially faster than the speed of upward swimming during the day, and then after 8 hours much slower, averaging over the course of the night to approximate the rate of daylight swimming. For the purposes of modelling it was assumed that the day and night swimming speed are equivalent. This migration pattern had a strong endogenous component, as similar migration patterns were observed irrespective of the light regime (figure 5.6).

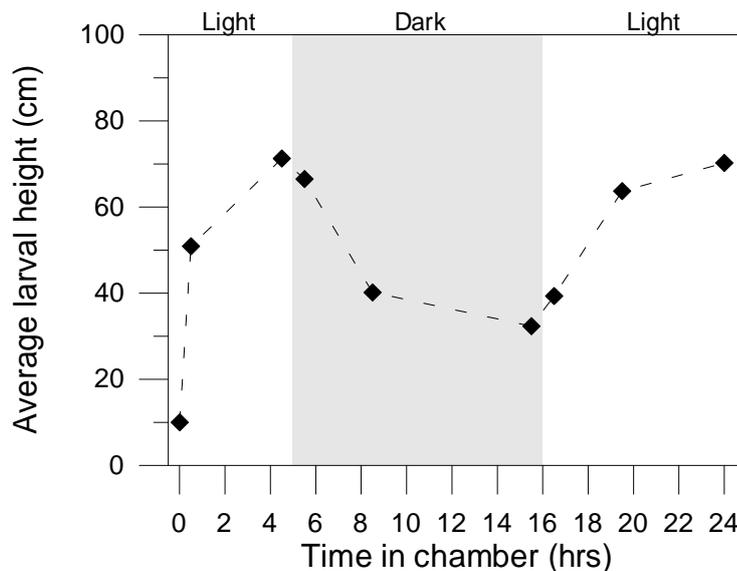


Figure 5.5. Changes in depth distribution of late bipinnaria larvae of *Asterias amurensis* over 24 hours. Larvae were injected into chambers at a height of 10 cm and the average height of the 20 larvae was plotted against time in chamber. The average swimming speed of larvae was $3 \times 10^{-5} \text{ms}^{-1}$. Larval movements in the first 30 min of each trial was not considered in the analysis. Data show mean average depths from 3 replicate trials under normal photoperiod.

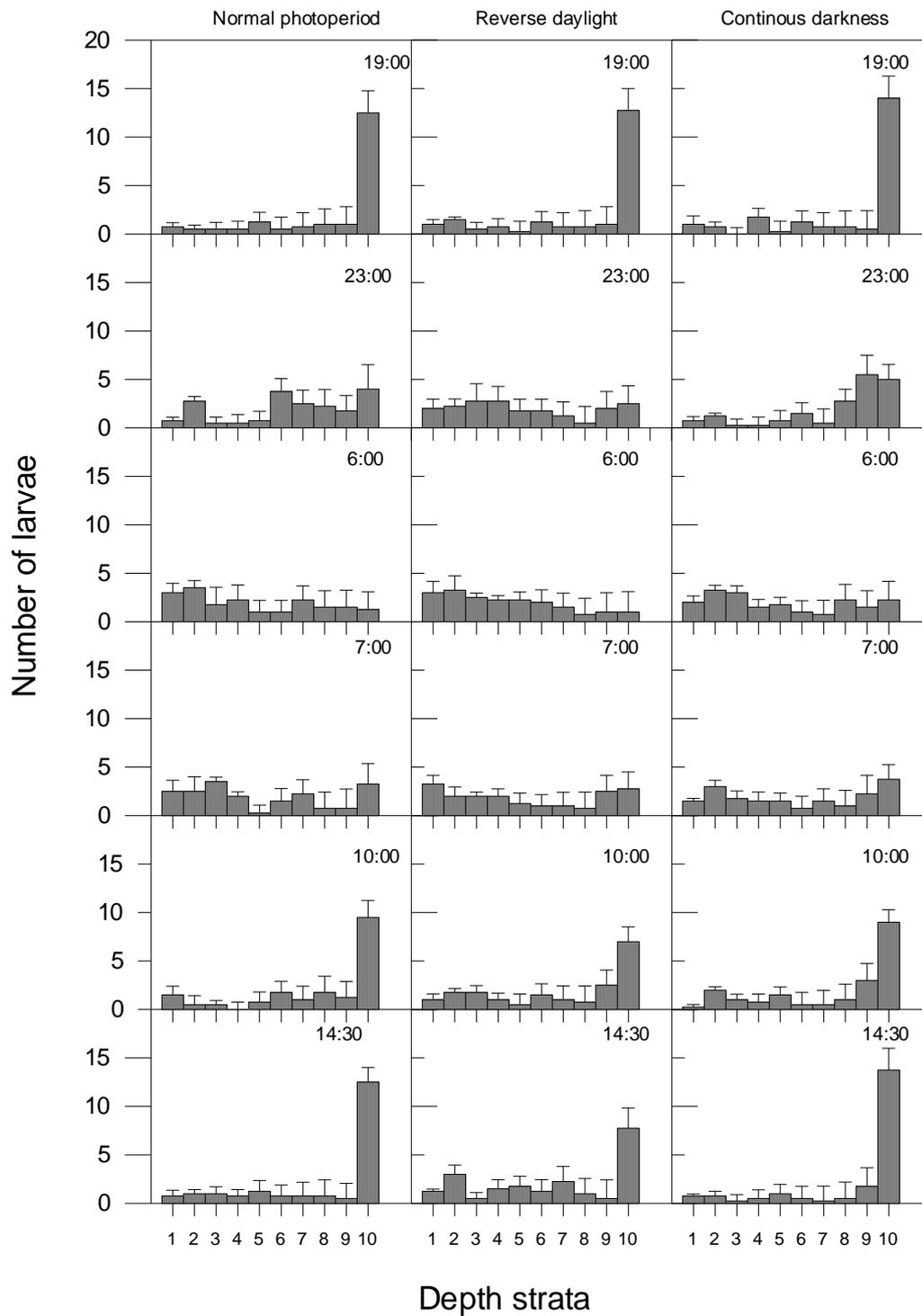


Figure 5.6. Depth distribution of late bipinnaria in response to three light regimes: normal photoperiod, reverse daylight, and continuous darkness. The height of larvae in the water column was measured by counting the number of larvae in 10 chambers with chamber 1 being the bottom water layer and chamber 10 being the surface layer. Data are means (\pm SE) of three replicate trials. All trials commenced at 14:30 hours.

Results: modelling

General

Results of the behavioural experiments were used to set model inputs (table 5.1). Specifically bipinnaria display reverse diel migration, swimming upwards in daylight and sinking at night. For the purposes of modelling the vertical swimming behaviour of larvae, it was assumed that larvae swim vertically upwards during the day and downwards at night at average velocities of $3 \times 10^{-5} \text{ ms}^{-1}$. The vertical swimming behaviour of larvae is salinity and age dependent. Bipinnaria swim vertically towards light unless salinity drops below 26ppt when vertical swimming ceases. Early brachiolaria are negatively buoyant irrespective of salinity and light. Larval *Asterias amurensis* undergo cell lysing when exposed to salinities below 17.5 ppt for 5-10 mins (Sutton and Bruce 1996), so larval mortality was simulated if larvae were advected into waters of salinity $< 18\text{ppt}$.

Treatment	Passive	Dilution of larvae	
		Mean Velocity ($3\text{E-}5 \text{ ms}^{-1}$)	Max Velocity ($3\text{E-}4 \text{ ms}^{-1}$)
Minimum river flow ($85 \text{ m}^3\text{s}^{-1}$)	8.38E-06	4.55E-05	4.55E-03
Average river flow ($145 \text{ m}^3\text{s}^{-1}$)	4.08E-05	7.62E-07	5.46E-04
Maximum river flow ($235 \text{ m}^3\text{s}^{-1}$)	5.29E-15	1.71E-06	1.94E-03
River flow time series 1 (1992)	2.95E-07	1.29E-06	8.90E-04
River flow time series 2 (1993)	1.57E-05	4.93E-06	1.50E-03
Upstream release of larvae (cell 23)	2.89E-05	6.11E-07	6.85E-04
Downstream release of larvae (cell 29)	4.87E-05	8.65E-07	3.09E-04

Table 5.2. Summary of larval dilutions (proportion of larvae retained in the estuary at the end of the pelagic phase) under different conditions of water flow, point of release of larvae, and swimming behaviour of larvae. Results are shown for dilution of larvae under minimum, average, and maximum river flow regimes, as well as two time-series of river flow measured daily in 1992 and 1993 during the pelagic phase. The dilution of larvae released in cell 23 (upstream), and 29 (downstream) is also shown; in other scenarios in the table larvae were released uniformly across cells 21-29.

Scenario 1: Effect of larval vertical migration

Simulation results suggest that the vast majority of larvae are advected out of the estuary (figure 5.7). The model predicts that larval swimming behaviour significantly affects the number of larvae retained in the estuary throughout the pelagic phase (figure 5.8). Under average river flow, the total number of larvae retained in the estuary spanned 4 orders of magnitude depending on larval swimming behaviour (table 5.2). Larvae with maximum swimming velocities were retained in the estuary at highest rates with dilution of 4 orders of magnitude from original concentrations at the end of the pelagic phase. Under average river flow, more passive larvae are retained than larvae migrating vertically at mean swimming velocity. Larvae swimming at mean velocity suffer slightly higher mortality due to low salinity than passive larvae because migrating larvae are more likely to be distributed further upstream than passive larvae (figures 5.7 and 5.8).

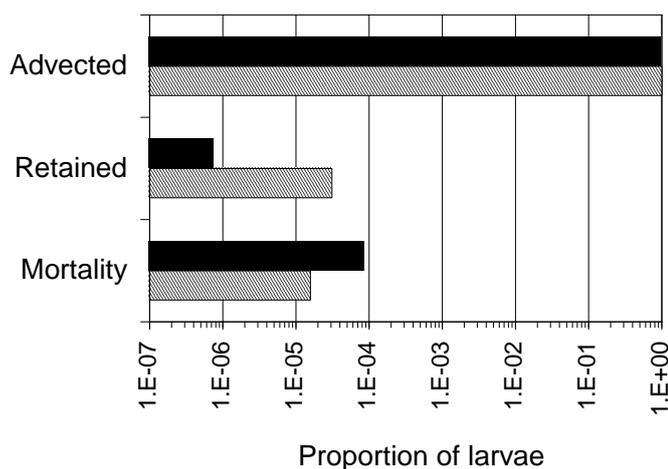


Figure 5.7. The fate of larvae released in the Derwent Estuary in simulations with constant average river flow after 120 days of dispersal. The proportion of passive larvae (striped) and larvae migrating vertically at mean swimming velocity (solid) that are advected out of the estuary (top), retained in the estuary (middle), and die from exposure to low salinity water (bottom) is shown.

Larval swimming behaviour has a small effect on the distribution of larvae in the estuary at the end of the pelagic phase. Passive larvae were slightly more abundant in cells towards the mouth of the estuary (cells 25-30), while larvae with average and maximum swimming speeds were distributed more evenly throughout the estuary (figure 5.8). The model predicts low numbers of larvae upstream from

cell 10 under average river flow (figure 5.8). The majority of larvae that were upstream from cell 10 were in layer 0 (bottom), which is more saline than the surface and middle water layers.

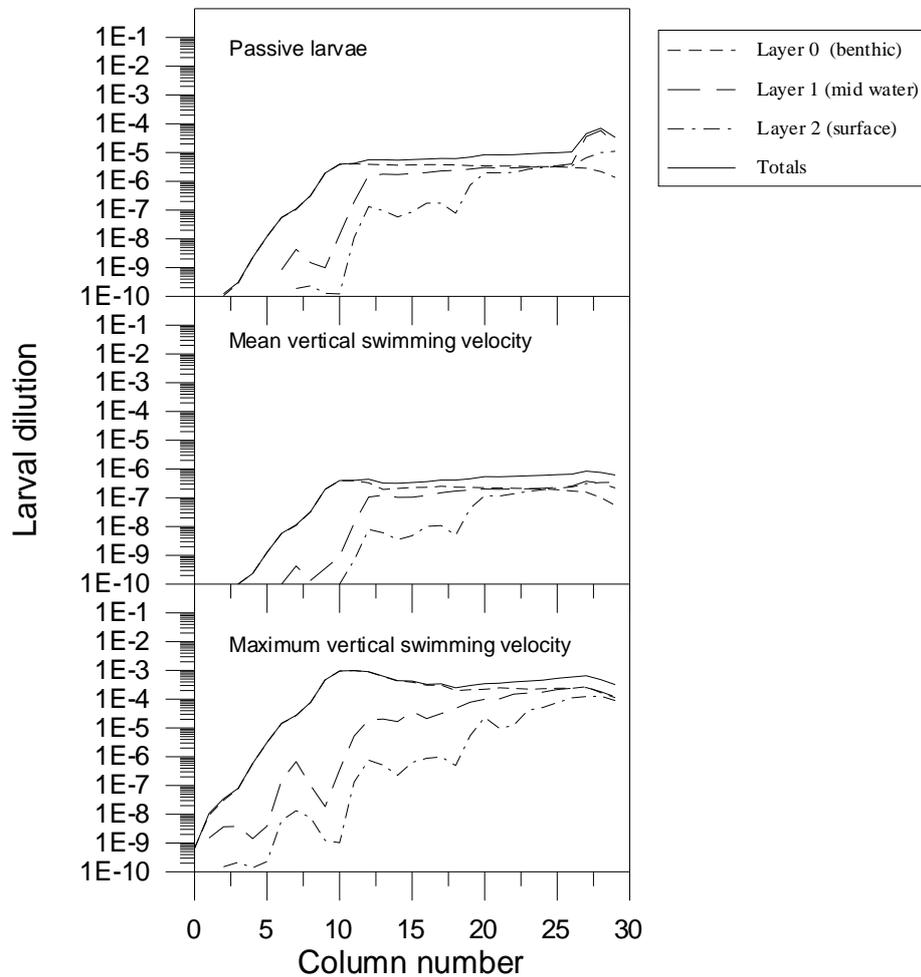


Figure 5.8. The effect of swimming behaviour of larvae on the horizontal distribution of larvae in the Derwent Estuary at the end of the pelagic phase. Three larval swimming behaviours were investigated: passive and neutrally buoyant (top), mean swimming velocity (middle), and maximum velocity (bottom). Total larval numbers (solid line), and the vertical distribution of larvae in 3 layers are plotted. Column 30 is at the mouth of the estuary.

Scenario 2: Effect of river flow

River flow has a large effect on the number and distribution of larvae retained in the estuary for the three swimming behaviours investigated (Figure 5.9). The number of passive larvae retained was particularly sensitive to river flow, fluctuating by approximately 10 orders of magnitude between the five flow regimes

investigated (table 5.2). Under conditions of high flow, passive larvae were diluted by 15 orders of magnitude from initial concentrations. Larvae with mean and maximum swimming velocities were more resilient to fluctuations in river flow, with retention rates varying by less than 2 orders of magnitude across the 5 scenarios of river flow.

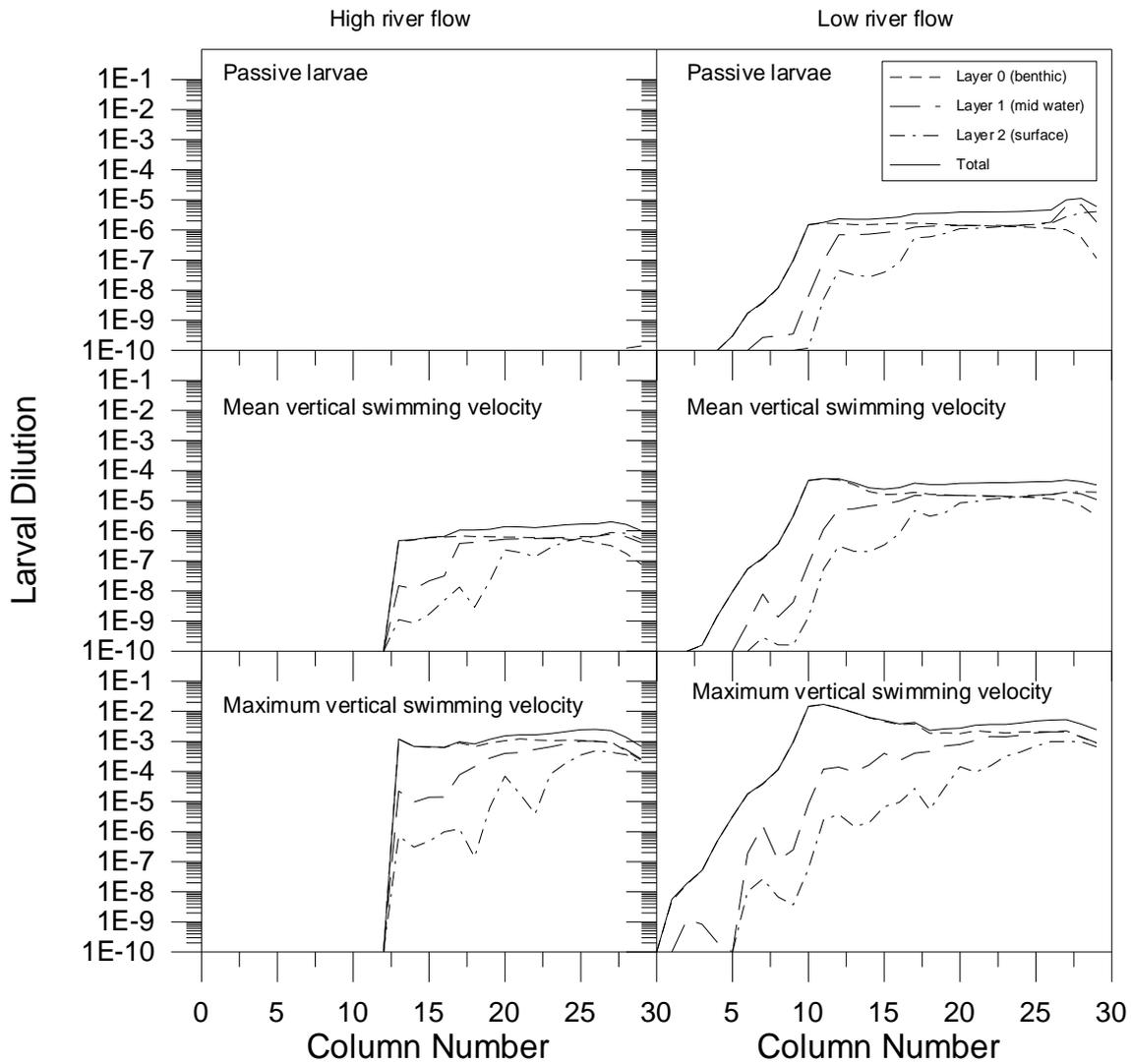


Figure 5.9. The effect of high ($235 \text{ m}^3\text{s}^{-1}$) (LHS) and low ($85 \text{ m}^3\text{s}^{-1}$) (RHS) river flow on larval dispersal. Simulation results for passive larvae and larvae with mean and maximum vertical swimming velocities are shown. Total larval numbers (solid line), and the vertical distribution of larvae in 3 layers are plotted. Column 30 is at the mouth of the estuary.

The distribution of larvae at the end of the pelagic phase is also affected by river flow (figures 5.8 and 5.9). Under high flow regimes the distribution of larvae

shifts downstream (figure 5.9). Conversely, under low river flow regimes, the distribution of larvae shifts upstream (figure 5.9).

River flow at the time when larvae are present was generally higher in 1992 than 1993. Despite this, predicted retention rates of larvae with average and maximum swimming velocities are relatively constant between years (table 5.2), while inter-annual differences in river flow had a larger effect on retention of passive larvae. Retention of passive larvae was predicted to be 2 orders of magnitude greater under the conditions of lower river flow in 1993 than in 1992 (table 5.2). In contrast, there was less than 1 order of magnitude difference in larval retention between the two simulations for larvae that vertically migrate (table 5.2).

Scenario 3: Effect of the distribution of larval release

The number of larvae retained in the estuary was relatively insensitive to the point of larval release compared with variability associated with changes in river flow (table 5.2). For the three swimming behaviours investigated, differences in the number of larvae retained in the estuary were less than one order of magnitude for larvae released adjacent to Hobart (cell 23) or at the mouth of the estuary (cell 29). Larvae migrating vertically at average swimming velocity were slightly more likely to be retained in the estuary if released towards the mouth than further upstream (table 5.2).

Scenario 4: Effect of salinity dependent vertical migration

Larvae that avoid low salinity layers are more likely to be retained in the estuary, as a result of advection upstream in the salt wedge, than larvae that migrate vertically solely in response to light (figure 5.10). However, since these larvae subsequently suffer high levels of mortality due to transport to areas of low salinity, there is little net effect of the larvae responding to salinity on retention (cf. figures 5.10a and 5.10c).

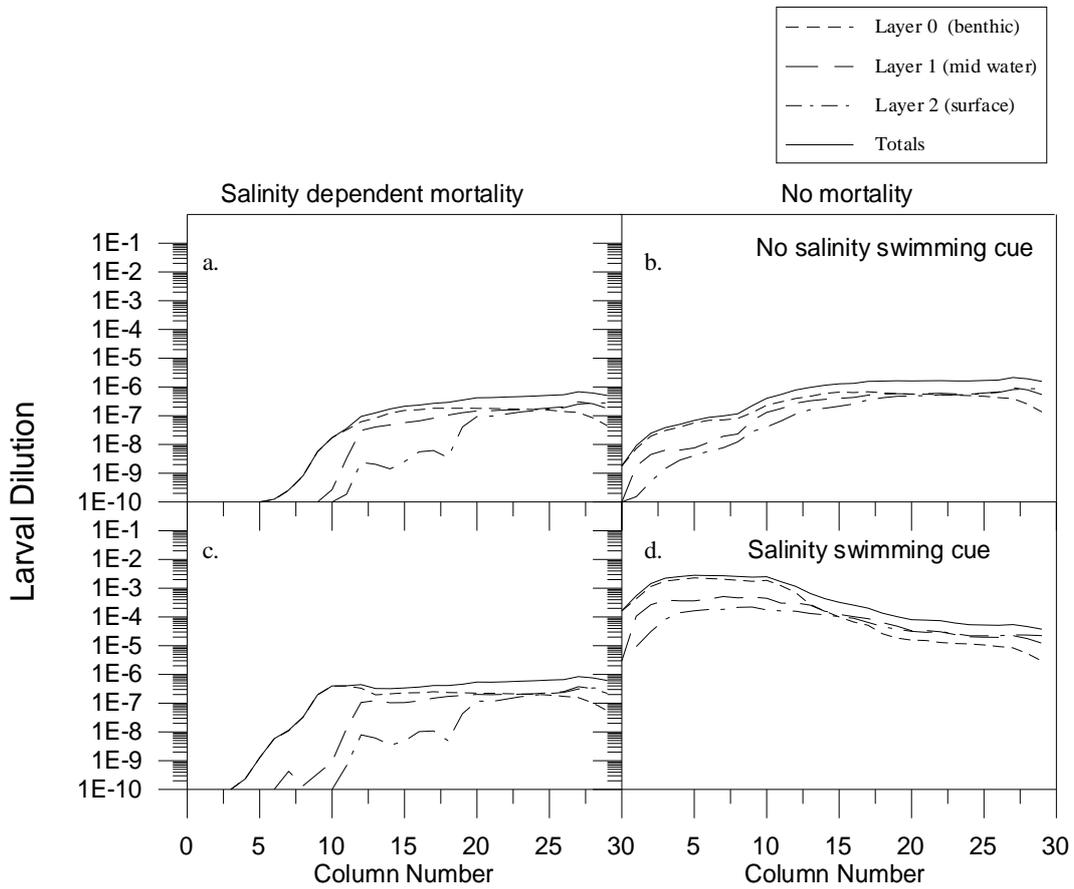


Figure 5.10. Effect on larval dilution of larval behavioural response to salinity and salinity-dependent mortality. Larval retention was predicted for vertically migrating larvae swimming at mean velocity that respond to light only (a and b) and for larvae that respond to both light and salinity (c and d). Total larval numbers (solid line), and the vertical distribution of larvae in 3 layers are plotted. The mouth of the estuary is at column 30.

Discussion

General

Hydrodynamic models used to simulate dispersal of marine invertebrate larvae, including larvae of *Asterias amurensis* often assume that larvae behave as passive particles (Black and Moran 1991, Bruce *et al.* 1995, Hill 1990, 1991, Lyne 1993, Richards *et al.* 1995). Furthermore, previous models of dispersal of *A. amurensis* larvae in the Derwent Estuary considered only advection by wind driven currents in the top few centimetres of water (Bruce *et al.* 1995, Lyne 1993). Given that many marine invertebrate larvae vertically migrate in response to light and salinity (see Forward 1976, 1988, Mileikovsky 1973, Thorson 1964, Young and Chia 1987, and Young 1995 for reviews), and other simulation models have emphasised the importance of larval vertical migration in affecting horizontal dispersal (Dippner 1987; Hinckley *et al.* 1996; Jenkins *et al.* 1999; Rothlisberg *et al.* 1995, 1996; Smith and Stoner 1993; Tremblay *et al.* 1994; Verdierbonnet *et al.* 1997), the assumptions that larvae are passive and transported largely by wind driven surface currents warrants scrutiny.

The inverse transport model developed here predicts that the majority of larvae (> 99%) are advected out of the Derwent Estuary during the 120 day pelagic phase irrespective of larval swimming behaviour, river flow, release point, or day length. These results are in concordance with an observed decline in larval density in the estuary throughout the pelagic phase (Bruce 1998). The inverse transport model results are diametrically opposed to those of previous simulations of dispersal of *Asterias amurensis* larvae in the Derwent Estuary, which predicted that most larvae are retained in the estuary (Bruce *et al.* 1995; Lyne 1993). The divergent results reflect different assumptions about larval behaviour and external forcings of circulation. The early work assumed that larvae do not vertically migrate but accumulate in the top 50 mm of the water column where they are dispersed by wind driven currents (Bruce *et al.* 1995; Lyne 1993). Assuming observations of larvae in laboratory trials occur in the estuary, and larvae in the water column are less affected by surface circulation than the dynamics of river flow and the salt wedge, a majority of larvae are advected. Given the very clear response of larvae to salinity (figure 4; Sutton and Bruce 1996), and similar responses in larvae of other estuarine species

(Forward 1976, 1988; Mileikovsky 1973; Thorson 1964; Young and Chia 1987), advection of large numbers of larvae from the estuary is likely.

If large numbers of larvae are transported in this way, there is significant potential for the Derwent Estuary population to act as a source of larvae for other regions in south-east Tasmania. The estuary population clearly poses a risk to both native communities and marine farms in surrounding coastal waters. My results suggest that the estuary population could be the source of small juvenile seastars detected in scallop and oyster spat collectors on marine farms outside the Derwent Estuary in south-east Tasmania (Buttermore *et al.* 1994; Martin and Proctor 2000; McLoughlin and Thresher 1994; Turner 1992). It follows that management strategies to minimise reproductive output of seastars in the Derwent Estuary are likely to have important flow on benefits to surrounding coastal waters by reducing the risk of infection.

Despite predictions that most larvae are advected out of the estuary, because of the capacity for high larval production, the absolute number of larvae retained in the estuary is still likely to be substantial and is clearly sufficient to maintain high population densities in the Derwent. Bruce *et al.* (1995) measured larval densities above 1100 m^{-3} in the port of Hobart. These are among the highest densities of invertebrate larvae ever recorded. Hence large numbers of larvae could still be retained in the estuary despite model predictions that the majority are advected out.

Swimming behaviour affects larval retention

The simulations indicate that larval vertical swimming behaviour has a large effect on larval dispersal. This highlights the importance of incorporating larvae behaviour into models of larval dispersal. Simulating conditions of high river flow, dilution of passive larvae in the estuary could be as much as 9 orders of magnitude greater than that of larvae vertically migrating at average velocity (table 5.2). In general, there were notable differences in retention rates depending on whether larvae moved at average or maximum velocities or were passive. Larvae with maximum swimming velocities were retained in the river at highest rates under all of the conditions investigated, and advection rates of swimming larvae were less sensitive to river flow than were passive larvae.

The distribution of competent brachiolaria in the estuary

The model predicts that swimming behaviour also affects the distribution of larvae in the estuary at the end of the pelagic phase. At the end of the pelagic phase, passive larvae were distributed mostly in the lower Derwent (cells 26 to 30), while larvae simulated with mean and maximum velocities were distributed more evenly among cells 10 - 30 (figure 5.8). Larvae were rare upstream (cells 1 – 10) irrespective of swimming behaviour because of high mortality rates in low salinity water. Empirical data on the horizontal distribution of larvae in the estuary (Bruce *et al.* 1995) are broadly consistent with our predictions for swimming larvae, but do not reflect results of simulations with passive larvae. In agreement with simulation results for larvae that vertically migrate, field surveys also show that larvae are slightly more abundant with distance upstream up to the boundary of the salt wedge (Bruce *et al.* 1995; Bruce 1998).

Development of the model

The inverse transport model developed in this paper averages larval concentration across the width of the estuary. Hence the coriolis effect is averaged across the estuary. It is further assumed that the effects of wind and tide on advection are negligible in comparison to the effect of salt flux and river flow. It is reasonable to suggest that small-scale dispersal patterns, such as the coriolis effect and water movement in bays and anthropogenic structure, could affect the number of larvae retained in the estuary. These factors could be included in development of the model.

The only source of larval mortality simulated was through salinity stress therefore predictions of the number of larvae retained in the estuary are liberal. Including estimates of other sources of larval mortality in the field would improve the predictions of larval density in the estuary at the end of the pelagic phase, however obtaining these estimates is difficult (Levin 1990).

The larval advection model predictions could be tested by measuring larval density in the estuary throughout the larval phase. These experiments would also be useful to validate the assumptions that larvae behave the same in the field as in

experimental chambers. The vertical distribution of larvae over 24 hours could be measured to ground truth the assumption that larvae behaviour in the field is the same as that observed in the laboratory.

Summary of conclusions

1. The model predicts that the majority of larvae are advected out of the estuary for the three swimming behaviours investigated. The Derwent Estuary population of *Asterias amurensis* has the potential to act as a source of larvae to adjacent south-east Tasmanian waters.
2. Larvae migrating vertically at the maximum velocity observed in laboratory trials are more likely to be retained in the estuary than passive neutrally buoyant larvae and larvae vertically migrating at average swimming velocities. Retention of larvae swimming at maximum velocity also showed least sensitivity to effects of river flow, and the distribution of larval release.
3. The rate at which larvae are flushed from the estuary is extremely variable depending on river flow, larval response to salinity, and their swimming speeds. Vertically migrating larvae that respond to salinity are more likely to be retained than neutrally buoyant passive particles.
4. There is substantial inter-annual variation in predicted retention of larvae based on measured river flow for 1992 and 1993. It is therefore likely that there are 'good' and 'bad' years for *A. amurensis* recruitment in the estuary.

Chapter 6: Settlement and metamorphosis of the introduced asteroid
Asterias amurensis

Abstract

Determining settlement preferences of introduced marine pests is a useful component of a strategic management plan. If settlement and metamorphosis is induced particular substrata, and these substrata have a limited distribution then there is potential in management responses to target key habitats that promote larval recruitment. In this chapter laboratory and field studies are combined to determine settlement ecology of the introduced seastar *Asterias amurensis* in the Derwent Estuary, Tasmania. Substrata that induce settlement and metamorphosis were determined in laboratory reared larvae by introducing competent brachiolaria to wells containing 1 of 6 substrata common in the estuary. Larvae settled at high rates when exposed to non-geniculate coralline algae (NCA) (0.98 ± 0.02 SE after 2 d exposure) and at moderate rates in mud and rock treatments (0.37 ± 0.06 and 0.44 ± 0.06 after 7 d of exposure for mud and rock, respectively). Larval settlement in treatments consisting of sand and the control (filtered seawater) were uniformly low for the duration of the experiment (0.01 ± 0.01 and 0.05 ± 0.02 for sand and control, respectively). Larvae exposed to NCA treated with antibiotic had lower rates of settlement than larvae exposed to NCA that was reinfected with bacteria (0.06 and 0.67 on day 6 respectively). Settlement intensity of larvae at 7 sites in the Derwent Estuary, Tasmania, was determined using standardised collectors consisting of 'bioballs'. Settlement of larvae was higher on the western side of the estuary relative to the eastern side. Settlement of larvae on the sampling apparatus was highly variable both within sites and between sites at scales of $10^1 - 10^3$ m².

Introduction

The relationship between larval distribution and abundance at the end of the pelagic phase and subsequent recruitment is one of the most poorly quantified aspects of marine invertebrate ecology. The few studies that have investigated processes important at the transitional stage between the pelagic phase and recruitment suggest that mortality during this period is high (eg. Babcock and Mundy 1996; Keesing *et al.* 1996; Keogh and Downes 1982). Determining the contribution of settlement and immediate pre- and post-settlement processes to recruitment rates of free spawners is problematic because it is difficult to isolate these processes at this stage of the life history. An important process linking the larval phase and recruitment to the benthos is induction of metamorphosis. Larvae of benthic marine invertebrates require a chemical cue usually associated with the substratum to induce metamorphosis (see Chia *et al.* 1984; Pawlik 1992; Johnson *et al.* 1997 for review). Larvae have a limited longevity and limited period of competency during which metamorphosis is possible, and they may die or develop abnormally if an appropriate substratum or other cue is not encountered to induce settlement and metamorphosis (Knight-Jones 1953, Williams 1964, Wilson 1968). Further, settlement and metamorphosis on appropriate substrata may improve survivorship of juveniles if it ensures that newly settled juveniles are near appropriate food, in appropriate habitats, and/or avoid predators (Babcock and Mundy 1996; Denley and Underwood 1979; Gosselin and Qian 1997; Hunt and Scheibling 1997; Johnson *et al.* 1991a). Clearly, the distribution of, and nature of larval responses to, settlement cues potentially affect the number and distribution of larvae that recruit as metamorphosed individuals.

Determining morphogenic cues for introduced marine pests is of interest because it may indicate areas that promote recruitment. If morphogenic cues are specific, and localised this information may be useful in developing management plans. Knowledge of settlement cues may be used in conjunction with information on the distribution and abundance of competent brachiolaria of *Asterias amurensis* in the Derwent Estuary to identify areas at high risk of establishment of new populations.

Cues inducing settlement and metamorphosis of larvae from a wide range of marine invertebrate species may be highly specific. In some cases invertebrates are induced by a single species of fleshy or non-geniculate coralline algae (NCA) (Barker 1977; Brancato and Woollacott 1982; Crisp and Meadows 1962; Gee 1965; Johnson et al. 1991a and b; Johnson and Sutton 1994; Knight-Jones 1953; Morse and Morse 1984; Morse *et al.* 1998; Ryland 1959; Thompson 1958, 1962; Williams 1964, Wilson 1968). In many cases bacteria provide the morphogenic cue (Johnson *et al.* 1997), and bacteria associated with the surface of coralline algae are known to induce metamorphosis in the crown-of-thorns seastar *Acanthaster planci* (Johnson and Sutton 1994) and some corals (Negri *et al.* in press). While several other species of seastar are also induced by NCA (Barker 1977; Yamaguchi 1973) the role of bacteria in this process has been indicated only for *A. planci* (Johnson *et al.* 1991).

Competent larvae have potential to delay metamorphosis if an appropriate cue is not encountered (see Pechenik 1990, for review). Provided larvae are resuspended into the water column and advected away from substrata that do not induce metamorphosis, delaying metamorphosis can increase the probability that larvae will encounter a substratum that induces settlement and metamorphosis (Thorson 1950, Scheltema 1961, Meadows and Campbell 1972, Crisp 1974). However, the period over which larvae can delay metamorphosis is limited, and if larvae do not come into contact with appropriate cues, larvae may become hyper-competent in which case specificity for particular substrata declines (Thorson 1950, Jarrett 1997, Knight-Jones 1953, McCormick 1999; Pechenik *et al.* 1996; Wilson 1953).

The principal aim of this study was to identify substrata that induce settlement and metamorphosis in the introduced seastar *Asterias amurensis* from a variety of substrata common in the Derwent Estuary, Tasmania. Field measurements of settlement of larvae onto standardized collectors were also made to determine the availability of competent larvae in the estuary. These results are then discussed in the context of the distribution of sediment types in the Derwent Estuary to predict areas of maximum larval settlement.

Materials and Methods

Larval culture

Males (4) and females (7) seastars collected from the Derwent Estuary, were placed in separate buckets and induced to spawn by injection of 5-7 ml of 10^{-3} M 1-methyladenine. Once spawning commenced, males were removed from buckets, blotted dry, and undiluted gametes ('dry' sperm) collected by pipette before diluting in filtered seawater by 10^3 to avoid polyspermy. Eggs were collected by inverting spawning females over beakers. Sperm and egg mixtures were placed in 6 aerated beakers (2 l) with filtered seawater ($1 \mu\text{m}$) for 1 – 2 hours. Fertilization success in beakers, as evidenced by the development of a fertilization membrane, was high (95-100%) and cell development appeared normal. Larvae were reared in 350 l tanks (average 35 ppt salinity and $13 \text{ }^\circ\text{C}$) at initial stocking densities of $10 \text{ larvae ml}^{-1}$. Larvae were fed daily a mixture of *Dunaliella tertiolecta*, *Isochrysis sp.*, and *Rhodomonas sp.* at $10^4 \text{ cells ml}^{-1}$ during the 3 month larval phase. Larvae were examined microscopically and only competent brachiolaria (with developed primordia, and oral and adhesive discs) were selected for experiments.

Settlement and metamorphosis of larvae on different substrata

Five substrata common in the Derwent Estuary (*viz.* sand, mud, rock, non-geniculate coralline algae (NCA), mussel shell, and a control consisting of sterile seawater) were used in experiments to measure rates of metamorphosis of competent larvae. Thin shards of NCA were prepared by shaving the algae from rocks with a scalpel, being careful to avoid the sulfide-reducing layer. Substrata were collected 3 days before experimental trials and were stored separately in aerated tanks with sterile seawater. Pieces of each substratum of approximately equal surface area (*ca.* $2\text{-}3 \text{ cm}^2$) was placed in separate plastic wells (16.5 ml) filled with sterile seawater. Ten competent brachiolaria, with starfish primordia and oral and adhesive discs, were placed in each experimental well. There were 10 replicate wells of each treatment. The number of larvae metamorphosed on each day of the 7-day trial was counted.

Assessment of the role of bacteria on the surface of NCA in inducing metamorphosis

The role of bacteria associated with the surface of non-geniculate coralline algae (NCA) in inducing metamorphosis of larvae was determined by comparing rates of metamorphosis of larvae exposed to NCA with normal bacterial cover, with that exposed to NCA with many bacteria removed by antibiotic treatment (after Johnson and Sutton 1994). The experiment included 3 replicates of each of 4 treatments: NCA treated with antibiotics and incubated in sterile seawater for 24 hrs (T1); NCA treated with antibiotics and then reinfected with bacteria for 24 hrs (T2); NCA freshly treated with antibiotics (T3); and untreated NCA (T4). Settlement of 6 competent brachiolaria was scored over 6 days in each treatment. Treatments involving the reduction of NCA surface bacteria were prepared by shaving thin shards of NCA (*ca.* 2-3 cm²) from rocks and placing them in beakers with sterile seawater and an antibiotic mixture consisting of tetracycline (30 mg l⁻¹), streptomycin sulfate (30 mg l⁻¹) and chloramphenicol (50 mg l⁻¹). This antibiotic mixture successfully reduced the diversity of bacteria on tropical NCA (Johnson and Sutton 1994). Shards were swirled periodically for 24 hrs in the antibiotic mixture, washed through 6 changes of sterile seawater, and separated into 2 treatments. To re infect shards with NCA bacteria (T2), antibiotic treated shards were placed in beakers and sterile seawater with shards of untreated NCA, separated by 100 µm mesh, for 24 hrs. This technique has previously been successful in re infecting antibiotic treated NCA with bacterial colonies, yielding a diversity of epiphytic bacteria comparable to untreated NCA (Johnson and Sutton 1994).

Field measurements of availability of larvae

Settlement collectors consisting of 100 'bioballs' (37 x 29 mm) enclosed tightly in a mesh bag were deployed at 7 sites in the Derwent Estuary (figure 6.1) over 1st – 4th October 1997 and collected from 4th – 6th December 1997. It was assumed that larvae released during the peak of spawning (August) would be competent to settle in early December given the 3-4 month larval duration. Bioballs are plastic spheres with a high surface to volume ratio that are used in aquaculture for bio-filtration, and have been useful as settlement collectors of *Acanthaster planci* (Keesing 1993). The bags of bioballs were attached to rope 1 m above the seafloor, at depths of 3 - 5 m. Three replicate collectors were deployed at each site, which covered habitats ranging from sand, mud, rock, and seagrass. Collectors were

retrieved into large plastic bags and frozen until sorting. Each bioball was washed individually until only sessile animals remained, and the sample was passed through a 100 μm sieve. The sieve contents were viewed under a dissecting microscope to count the number of newly settled recruits.

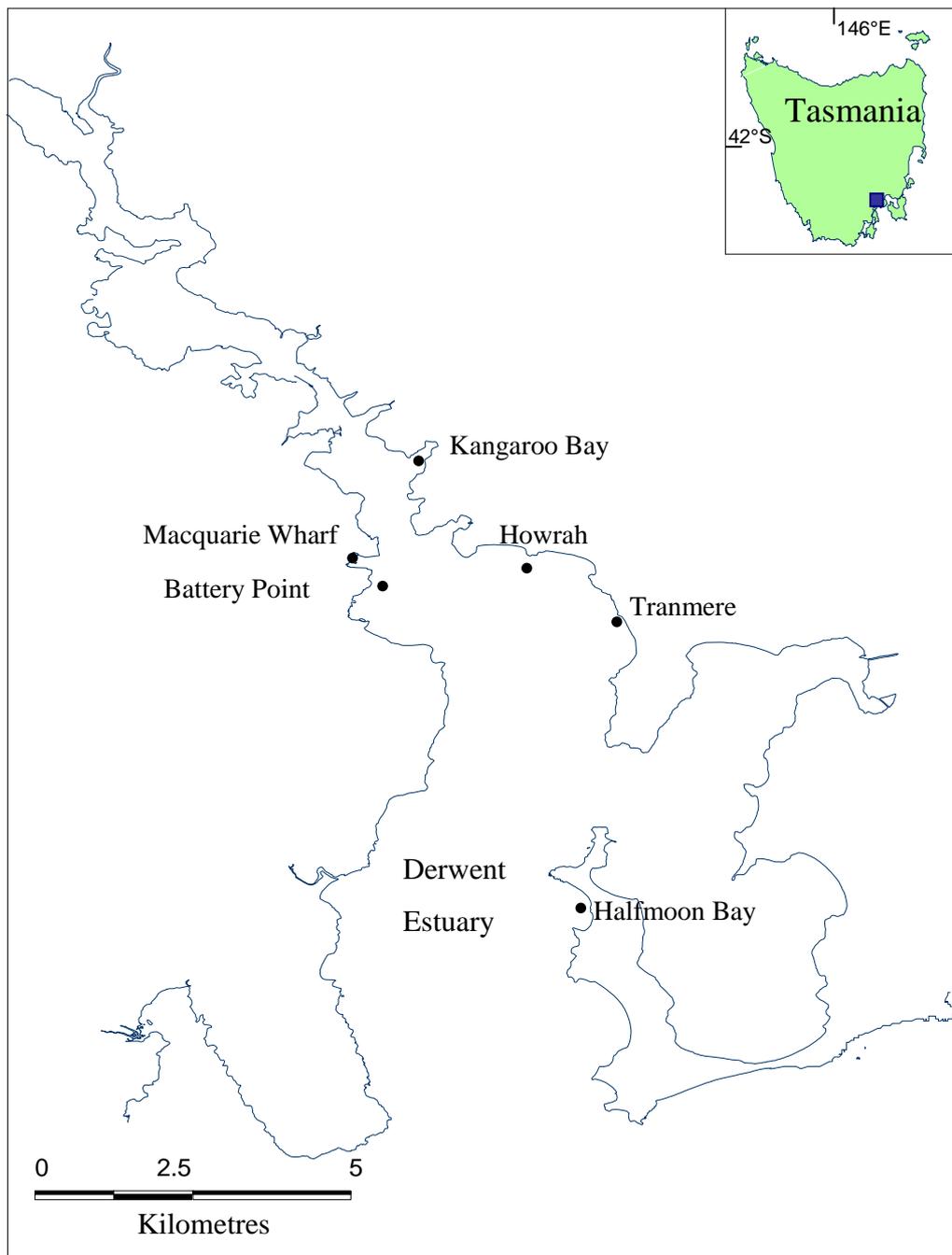


Figure 6.1. The Derwent estuary, Tasmania showing sites where settlement sampling devices were deployed.

Results

Settlement and metamorphosis of larvae on different substrata

Differences in settlement rates of larval *Asterias amurensis* on the different substrata after 7 days were highly significant (figure 6.2). Non-geniculate coralline algae (NCA) induces settlement in *Asterias amurensis* at high rates (0.98 ± 0.02 SE by day 2) and at moderate rates in mud and rock treatments (0.37 ± 0.06 and 0.44 ± 0.06 SE by day 7 for mud and rock respectively). Settlement on mud and rock was much lower than on NCA, and it was not until day 4 that > 20% of larvae settled on mud and rock. Larval settlement on sand and in the control (sterile seawater) were low (0.01 ± 0.01 and 0.05 ± 0.02 SE for sand and control respectively) (figure 6.2).

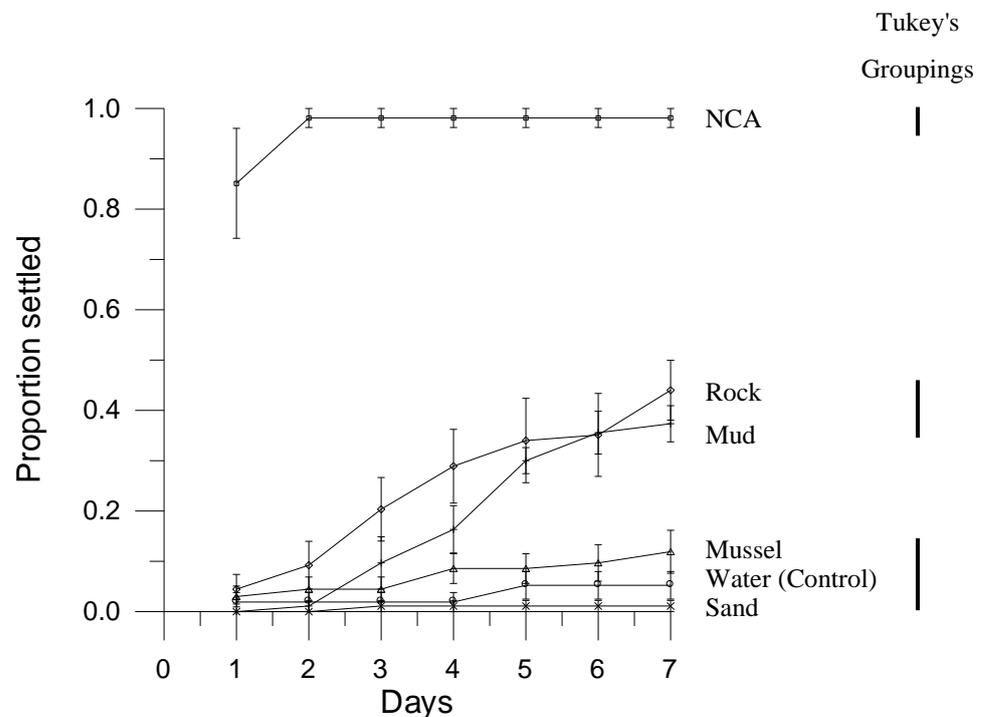


Figure 6.2. Settlement of laboratory reared *Asterias amurensis* larvae exposed to 6 substrata common in the Derwent estuary. The number of larvae metamorphosing each day was scored and plotted as the total number settled divided by the total number in each well. Settlement of competent brachiolaria on different substrata after 7 d was significantly different (1-way ANOVA, $F_{(5,8)} = 100.35$, $P < 0.001$). Tukey's HSD grouping results for settlement on day 7 at $p < 0.05$ were $NCA > Rock = Mud > Mussel = Water = Sand$.

Assessment of the role of bacteria on the surface of NCA in inducing metamorphosis

High rates of settlement and metamorphosis of larvae exposed to untreated NCA (T4) (67% by day 3) showed that larvae used in the experiments were competent (figure 6.3). There were significant differences between rates of settlement in larval *Asterias amurensis* exposed to the 4 NCA treatments (1-way ANOVA, $F_{(3,8)} = 5.37$ $P = 0.026$). Larvae exposed to NCA that had been treated with antibiotic (T1) had lower rates of settlement than larvae exposed to NCA that had been reinfected with bacteria (T2) (0.06 and 0.67 on day 6 respectively) (Figure 6.3.).

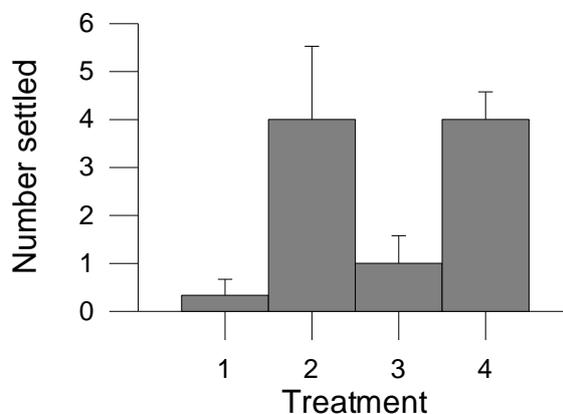


Figure 6.3. The effect of bacteria on the settlement and metamorphosis of competent brachiolaria. Larvae were introduced into wells with NCA exposed to 4 antibacterial treatments: 1. NCA exposed to antibiotics and incubated in sterile seawater for 24hrs; 2. NCA exposed to antibiotics and reinfected with NCA bacteria (i.e. incubated in sterile seawater with untreated NCA); 3. NCA freshly exposed to antibiotics; 4. Untreated NCA. Larval settlement on day 6 in treatments with relatively high levels of bacteria (2 and 4) were significantly higher than treatments exposed to antibiotic (1 and 3) (planned a priori orthogonal contrast, $P = 0.004$, transformation = $Y^{0.6}$)

Field measurements of settlement of larvae on standardised collectors

Settlement of seastars on standardised collectors was higher on the western side of the estuary than the eastern side, however, settlement was highly variable both within and between sites (table 6.1). There were large variances in the number of settlers at each site, suggesting that the availability of larvae in the water column may be patchy at scales of $10^1 - 10^3$ m^2 . Recruitment to the standardised collectors

was highest at sites characterised by mud (table 6.1), which comprise the great majority of benthic habitat in the estuary (figure 6.4).

Site	Substratum at site	Average abundance (\pm SE) per collector
Kangaroo Bay	Rock	3.3 (\pm 0.3)
Howrah	Sand	1.0 (\pm 0.6)
Howrah	Rock	0.5 (\pm 0.4)
Tranmere	Mud	6.7 (\pm 0.9)
Tranmere	Rock	0.7 (\pm 0.7)
Battery Point	Mud	17.0 (\pm 3.5)
Macquarie Wharf	Mud	18.7 (\pm 9.2)
Halfmoon Bay	Sand	0.5 (\pm 0.4)
Halfmoon Bay	Seagrass	0.5 (\pm 0.4)

Table 6.1. Recruitment of asteroid larvae (average \pm SE) in the Derwent Estuary. Recruitment was highest on the western side of the estuary and at sites dominated by mud.

Discussion

Larval *Asterias amurensis* were induced to settle at high rates (0.98 ± 0.02 SE by day 2) by non-geniculate coralline algae (NCA). This is consistent with other echinoderm species which are also induced to settle by NCA (Barker 1977; Johnson *et al.* 1991a and b; Johnson and Sutton 1994; Yamaguchi 1973). While rapid induction of metamorphosis occurred only on NCA, larvae settled at lower rates on mud and rock substrata, and sand and mussel shells were poorly inductive (figure 6.2). Accordingly, as competent larvae aged during the experiment they became less discriminating and settled on a wider variety of substrata (figure 6.2). This response towards hyper-competency after prolonged exposure to non-preferred substrate has been observed in several marine invertebrate larvae (see Peckenik 1990, for review).

Settlement and metamorphosis of the crown-of-thorns seastar, *Acanthaster planci*, requires a morphogenic signal from bacteria associated with the surface of the coralline, while to manufacture the signal the bacteria requires a chemical substrate from the NCA (Johnson and Sutton 1994). Experiments designed to determine the role of bacteria on the surface of NCA in *Asterias amurensis* suggest that bacterial colonies on the surface of the coralline are also important in inducing settlement and metamorphosis. Settlement and metamorphosis of larvae in treatments with bacteria were higher than treatments where bacteria had been removed (figure 6.3).

There were large variances in the number of larvae recruiting to collectors at each site, suggesting that the availability of competent larvae in the water column may be patchy at scales of $10^1 - 10^3 \text{ m}^2$ (table 6.1). Larval distribution determined from vertical plankton tows do not reflect a similar pattern with concentrations across the river approximately equal for mid-estuary sites (Bruce *et al.* 1995). Recruitment of larvae on standardised collectors was highest at sites dominated by mud (table 6.1).

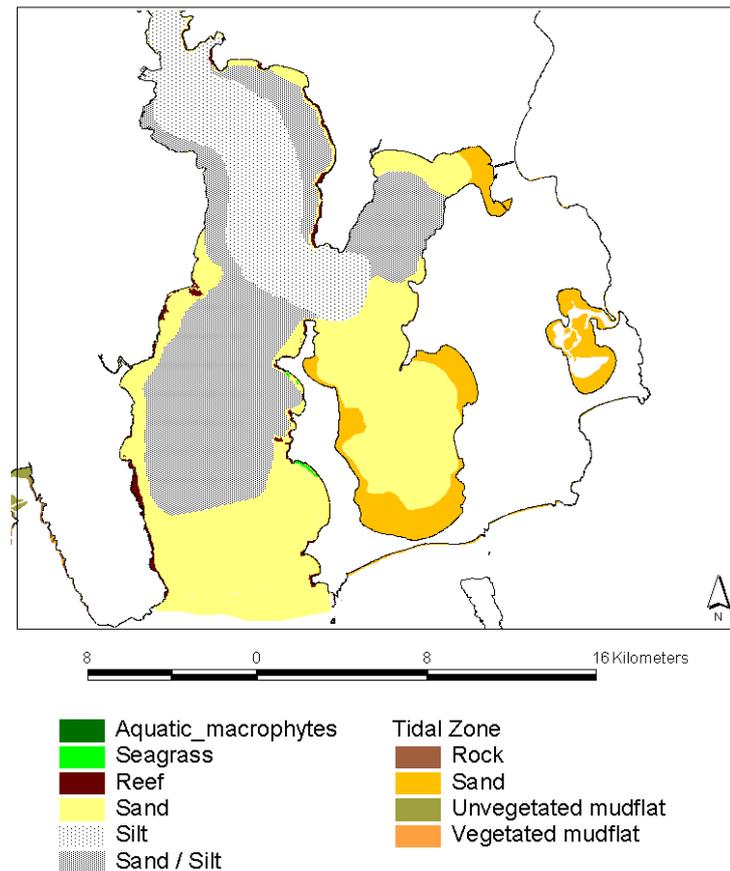


Figure 6.4. Distribution of habitat types in the lower reaches of the Derwent Estuary at 1:100,000. From Jordan *et al.* (2001).

Mud (= silt) is the dominant substratum in the middle reaches of the Derwent Estuary (figure 6.4). If larval settlement behaviour in the field is similar to behaviour observed in laboratory trials, I would predict moderate settlement and metamorphosis in the mid estuary. Similarly, in the lower reaches of the estuary, where sand is the dominant substrate (figure 6.4), I would predict low rates of settlement and metamorphosis. Based on the results of laboratory trials I would predict high rates of settlement on fringing reefs that support NCA in the middle to lower reaches (figure 6.4), however these areas constitute only a small fraction of the available substrata. Given that laboratory experiments predict that larvae settle at moderate rates on mud, and mud is the third most common substrata in the estuary, comprising 53.82 km² (Jordan *et al.* 2001), targeting newly settled juveniles is not a feasible management option unless survival of recruits is spatially stratified.

Chapter 7: General discussion

Setting management goals

In this thesis the early life history of *Asterias amurensis* is investigated with an aim to identify opportunities for improved management of the introduced pest in the Derwent Estuary, Tasmania. The feasibility of management alternatives should be evaluated in the context of the desired management outcome. While eradicating *A. amurensis* entirely from the estuary is the most desirable outcome, this eventuality is highly unlikely given available technology. However, by targeting the seastar strategically, it may be possible to reduce the number of seastars in the estuary to a level that reduces the impact on the natural environment and marine farms, and the risk of larval transport via ballast water exchange. The risk assessment and cost benefit analysis required to justify a focussed management strategy is outside the scope of this project.

Implications of fertilization ecology for management.

Factors acting at spatial scales spanning several orders of magnitude have potential to affect fertilization success of *Asterias amurensis* in the Derwent Estuary. The reproductive potential of discrete populations of echinoderms can vary with environmental factors that affect somatic and gonadal growth (Babcock *et al.* 1994; Oliver and Babcock 1992; Guilou and Lumingas 1999; Levitan 1989; Meidel and Scheibling 1999; Qian and Chia 1991; Wahle and Peckman 1999). Seastar gonad index varied significantly between sites with different levels of anthropogenic impact (figure 2.7). The positive relationship between mass of dry gamete released and gonad index (figure 2.8) suggests that gamete release of individuals at yacht clubs is between 1.5 and 2.6 that at control sites. Given that fertilization in free-spawning marine invertebrates is extremely sensitive to sperm concentration (figure 3.4, Benzie and Dixon 1994; Levitan *et al.* 1991; Pennington 1985), and individuals at yacht clubs spawn more dry gamete per individual, it is reasonable to assume that the higher gonad indices at yacht clubs translate into higher zygote production.

In addition to variable reproductive potential acting at the level of the individual, density, group size, and depth of spawning populations affect fertilization success in free-spawning marine invertebrates (Chapter 4, Babcock *et*

al. 2000; Claereboudt 1999; Levitan 1991; Levitan and Young 1995; Pennington 1985). The density of seastars in the Derwent Estuary generally decreases with distance from the port of Hobart (Grannum *et al.* 1996; Morrice 1995), and the distribution of high density populations (> 0.4 individuals m^{-2}) is aggregated (Grannum *et al.* 1996). *Asterias amurensis* also moves into shallow water prior to the spawning season (*pers obs*, Morrice 1995). The model of gamete dispersal and fertilization success predicts non-linear increases in fertilization success with increased density, increased group size, and decreased water depth (figures 4.1, 4.4, and 4.5) suggesting differential larval production between sites in the estuary.

The combination of variability in dry gamete release, and population characteristics that affect fertilization success such as density, group size, and depth, is likely to result in large variation in larval production between sites. Based on the model results, maximum larval production would be expected in areas associated with high anthropogenic impact in the mid-estuary where high gonad indices and density co-occur. It may be useful to focus management effort in these areas. Whether variability in the number of larvae produced by discrete populations of the seastar affects the distribution and abundance of competent brachiolaria is dependent on larval dispersal, growth and mortality.

Implications of larval dispersal for management

The model of larval dispersal predicts that the vast majority ($> 99\%$) of larvae are advected out of the estuary over the 120-day larval phase (table 5.1). If the model predictions are accurate, the Derwent Estuary could be the source of larvae that settle on scallop lines and in mussel trays in marine farms in south-eastern Tasmania (Martin and Proctor 2000). Any management effort effective in reducing the number of larvae produced in the estuary would reduce the risk of the Derwent Estuary population to marine-based industries in southern Tasmania.

The model of larval dispersal predicts that the number of larvae retained in the estuary is not sensitive to the point of larval release (table 5.1). If the model predictions are realistic, larvae produced from populations that spawn in the mid and lower estuary have a similar probability of being retained. There are no management implications of this finding, so the suggestion that targeting populations in the mid-

estuary (where larval production is high) may reduce advection of larvae from the estuary remains.

Implications of settlement ecology for management

Laboratory reared competent brachiolaria settled at high rates on non-geniculate coralline algae, and at moderate rates on mud and rock (figure 6.2). These results suggest that there may be spatial variability in settlement of larvae in the Derwent Estuary. Assuming metamorphosis of larvae in the field is induced by similar cues to those in laboratory experiments, these results suggest moderate rates of settlement on fine sediments in the mid-estuary, and low rates of settlement on sandy benthos in lower reaches of the estuary. High rates of settlement may occur on fringing reef in the mid and lower estuary but this habitat comprises a small portion of the estuary (figure 6.4). Recruitment of *Asterias amurensis* to artificial collectors was highest at sites where mud was the dominant substrata, in the middle reaches of the estuary (table 6.1). Since mud is widespread in the estuary (covering 53.82 km² Jordan *et al.* 2001), management intervention focusing on particular substrata does not appear feasible unless survival of newly settled recruits is spatially stratified.

Future research

The results of this work suggest several key areas of research that may be useful for improved management of *Asterias amurensis* in the Derwent Estuary. In order to develop a population model incorporating the various factors affecting abundance and distribution of *Asterias amurensis* from gamete release to settlement, or to 'close the larval loop' (Eckman 1996) requires further research. The key points of interest are outlined below.

Observations of natural spawning

Observations of spawning in nature suggest that free-spawning marine invertebrates invoke behaviours that increase fertilization success such as aggregation, spawning in shallow water, and spawning synchronously (Babcock and Mundy 1992; Babcock *et al.* 1992; Coma and Lasker 1997; Gladstone 1992; Sewell and Levitan 1992; Yund 2000). Information on the density, degree of aggregation, degree of synchrony, group size, sex ratio and water depth of spawning in *Asterias amurensis* would be useful to improve predictions of the fertilization model.

Also of interest is a measurement of the spatial variability in the amount of dry gamete released by discrete populations in the estuary. In Chapter 2, I proposed that populations at yacht clubs have potential to spawn more dry gamete per individual based on the higher gonad indices at yacht clubs and the relationship between gonad index and the amount of dry gamete released. Testing this experimentally, for populations in the mid and lower estuary, would be of interest to quantify the difference in reproductive potential between populations.

Validating the larval dispersal model

Vertical migration patterns observed in laboratory experiments were used to parameterise the larval dispersal model. While efforts were made to decrease experimental artifacts such as using large experimental columns (relative to the size of larvae), larval behaviour *in situ* may not reflect observations in the laboratory. The vertical distribution of larvae throughout 24 hrs could be measured to determine whether the depth distribution of larvae is explained by model predictions. To further validate the larval dispersal model, the distribution of larvae outside of the estuary could be measured to test the prediction that the vast majority of larvae are advected from the Derwent Estuary. Furthermore, the only source of larval mortality simulated in the model of larval dispersal was through salinity stress. Measuring mortality during the planktonic phase would be useful to further parameterise the larval dispersal model, however, obtaining these estimates is difficult (Levin 1990).

Conclusion

Eckman (1996) described 'closing the larval loop' in free-spawning marine invertebrates a major challenge to marine ecologists because processes that are important to distribution and abundance of animals with both a benthic and pelagic phase act over a wide range of temporal and spatial scales. In particular, our understanding of the various processes acting from spawning to settlement and metamorphosis is patchy. In this thesis I have filled some of the gaps required to develop a model of population dynamics for *Asterias amurensis* in the Derwent Estuary, with an aim to improve management of this introduced pest. I have outlined some ideas for improved management of this introduced species and suggested priorities for further research. Given the variability in a number of parameters

affecting population dynamics in *Asterias amurensis* in the Derwent Estuary, these results should be regarded as species and site specific.

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