Population and reproductive ecology of the direct-developing sea stars *Parvulastra parvivipara* and *Cryptasterina hystera*

Lana M. Roediger

B. Sc. Mar. Biol. (Hons)

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School of Biological Sciences

Flinders University

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Abstract

The population dynamics and reproductive ecology of direct-developing marine invertebrates are poorly understood. The absence of an ecologically decoupling dispersive larval stage between adults and offspring in these species was thought to increase population stability relative to species with complex life-histories, but recent evidence suggests that they are less stable because population fluctuations are not dampened over time by recruitment of larvae from other populations. Recent studies have also shown that some marine invertebrates adaptively alter offspring phenotype (size) in response to environmental conditions experienced by the adults. Offspring size has profound implications for all life-history stages of marine invertebrates, as well as their population dynamics. The capacity to adaptively alter offspring phenotype should be greater among direct developers than species with dispersive larvae because their offspring are more likely to experience similar conditions to adults, and there are no conflicting selective pressures acting on life-history stages that occupy different ecological niches. I examined the population dynamics and brood characteristics of two Australian intertidal asterinids that reproduce via direct development—*Parvulastra parvivipara* (3 temperate populations, South Australia) and *Cryptasterina hystera* (4 tropical populations, Queensland). High structural complexity of tide pools predicted the likely presence and high abundances of both species; however, while population sizes of *P. parvivipara* and *C. hystera* were stable over 3 and 2-years, respectively, their distributions were highly dynamic. Both species disappeared from large proportions of tide pools that offered ideal conditions and recolonised these pools with no apparent periodicity. I suggest that metapopulation dynamics operating among tide pools stabilise population abundances in circumstances where unpredictable changes in tide pool conditions can lead to 100 % mortality. Small proportions of *P. parvivipara* gave birth during autumn and winter, but most individuals gave birth in late spring to summer. Offspring size was greatest and brood sizes smallest during summer. Experiments showed that larger

offspring had greater fitness (survivorship) than smaller offspring during this period. I suggest that *P. parvivipara* adaptively alters brood characteristics during summer to maximise offspring fitness during this period. Intragonadal cannibalism among siblings may facilitate these changes in brood characteristics. I also suggest that *P. parvivipara* exhibits plasticity in the timing of births; that is, mean offspring fitness is maximised by matching the characteristics of an individual's brood and timing of births to prevailing environmental conditions. The coefficients of variation in offspring sizes of both species were high, but based on this sampling *P. parvivipara* was more variable than *C. hystera*. The higher variability exhibited by *P. parvivipara* may be a bet-hedging strategy that maximises mean offspring fitness in a temperate habitat that is more variable than the tropical habitat of *C. hystera*. The CV in offspring size of *P. parvivipara* did not differ among intertidal zones as would be expected if a greater offspring size was used as a bet-hedging strategy in the more variable upper areas of its intertidal zone. I suggest that intertidal habitats are inherently variable and the high CV in their brood characteristics may increase mean offspring fitness via bet-hedging.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Lana M. Roediger

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Chapter 1

General Introduction

1.1 Overview

The life-history strategies of marine invertebrates are very diverse, ranging from broadcast spawning of gametes and pelagic larval development, to internal fertilisation and direct development of offspring within the adult (see reviews by Strathmann 1985, McEdward & Janies 1993, Havenhand 1995, Levin & Bridges 1995, McEdward & Janies 1997). While this diversity is of applied importance in the management of exploited species (e.g. Caddy 1989, Jamieson 1993, Thorpe et al. 2000) and marine pests (e.g. Lafferty & Kuris 1996, Bax et al. 2001), it has also driven a large body of research aimed at understanding the evolutionary ecology of divergent life-history strategies (see Strathmann 1985, Havenhand 1995). Offspring size is perhaps the most important life-history characteristic of marine invertebrates because it influences ecological processes at the levels of individual species, populations and communities (e.g. George et al. 1990, Levitan 2000, Moran & Emlet 2001, Eckert 2003, Jeffery & Emlet 2003, Marshall et al. 2003, Marshall 2005), and differs by more than an order of magnitude among some closely related clades (e.g. Byrne 2006).

Understanding the evolutionary ecology of different offspring sizes requires comparative investigations into the fitness consequences of offspring size and how species allocate energy to offspring (i.e. trade-offs between offspring size and fecundity) under different ecological conditions. To date, however, most studies have focused on comparisons among species that are confounded by our inability to control for the fact that they are different species with different evolutionary histories (see Harvey & Pagel 1991, Bernardo 1996). While such studies have provided valuable insights into the evolutionary ecology of life-history strategies among species, it has recently become more widely appreciated that there is considerable phenotypic variation in offspring size within species of marine invertebrates, and indeed within broods of individual adults (reviewed in Marshall & Keough 2008a). Moreover, it is becoming increasingly apparent that intraspecific phenotypic variation in offspring size in marine invertebrates can be adaptive, and is likely to be under a degree of maternal control in response to changes in environmental conditions that they experience (Allen et al. 2008, Marshall et al. 2008b). These findings are important because they provide the opportunity to make comparisons of the ecological consequences of offspring size without the confounding inherent in among species comparisons, and also make it possible to investigate how individuals allocate energy to offspring under different environmental conditions.

Of the diverse life-history strategies exhibited by marine invertebrates, those that reproduce via direct development and viviparity are among the most rare and have consequently received relatively little attention in the literature (Levin and Bridges 1995; McEdward and Miner 2001; Pechenik 1999). These species, however, can potentially provide particularly strong insights into the ecological consequences of variation in offspring size and how species allocate energy to offspring under different ecological conditions for two reasons. First, the absence of an intervening pelagic larval stage, that is ecologically decoupled from the adult stage and acted on by different selective pressures, means that mothers should be able to predict the environmental conditions that their offspring will experience (Marshall and Keough 2008a). Consequently, inferences into relationships between environmental conditions and potentially adaptive alterations in energy allocation to offspring can likely be made through investigations of species with direct development than of species with pelagic larval stages. Second, it has recently been recognised that the degree of variation in offspring size within species, and within the broods of individual adults, is considerably higher among species with direct development than in species with other life-history strategies (reviewed in Marshall and Keough 2008a; Marshall et al. 2008b). This relatively high level of within species variation in offspring size could potentially provide powerful insights into relationships between environmental conditions and how adults allocate energy to offspring.

I examined variation in offspring size within and among broods of offspring produced by two species of viviparous, direct-developing sea stars: *Parvulastra parvivipara* (formerly *Patiriella parvivipara* see Keough & Dartnall 1978, O'Loughlin & Waters 2004) and *Cryptasterina hystera* (Dartnall et al. 2003) (Phylum: Echinodermata, Class: Asteroidea, Family: Asterinidae). These species have highly-restricted distributions, and are two of only five sea stars known to exhibit viviparity and direct development of offspring (Byrne 2006). I examined what determines the distributions and abundances of these species and how offspring size and brood characteristics vary with the environment. The following sub-sections outline background information to subsequent chapters of this thesis.

1.2 Diversity of marine invertebrate life-histories

The diversity of life-history strategies exhibited by marine invertebrates has led researchers to develop classification systems that broadly categorise strategies based on characteristics of offspring development, including nutritional modes, sites of development, types of morphogenesis, and dispersal potentials (see Thorson 1946, 1950, Mileikovsky 1971, Chia 1974, Strathmann 1985, McEdward & Janies 1993, Levin & Bridges 1995, McEdward & Janies 1997). Three broad modes of offspring development are generally recognised: (1) feeding pelagic larval development (planktotrophy) followed by metamorphosis into the adult form; (2) non-feeding pelagic larval development (often imprecisely termed “lecithotrophy” as not all non-feeding larvae feed on a yolk source) followed by metamorphosis into the adult form and; (3) direct development, in which offspring are released as juveniles of the adult form. Viviparity, in which the morphogenesis of offspring takes place within the gonad of the mother, coupled with direct development, is the most evolutionarily derived life-history strategy exhibited by marine invertebrates (reviewed in McEdward & Janies 1997). While these classifications are useful for broadly defining offspring development types, the life-history strategies of marine invertebrates collectively form a continuum of variation that makes simple classification systems inherently problematic.

The majority of marine invertebrates reproduce by releasing gametes into the water surrounding them where fertilisation occurs (interchangeably termed “broadcast-spawning” and “free-spawning” in the literature), followed by an often lengthy pelagic larval development stage, and subsequent metamorphosis into the adult form (McEdward & Janies 1993, Havenhand 1995, Levin & Bridges 1995, McEdward 1997, McEdward & Janies 1997, Pechenik 1999). Free-spawning followed by pelagic larval development is considered to be the ancestral life-history strategy among marine invertebrates, and has been lost in many species (Strathmann 1985, McEdward & Janies 1993, Hart et al. 1997, McEdward & Janies 1997, Byrne 2006). A four-stage series of transformations has been hypothesised in the evolution of direct development from pelagic larval development in marine invertebrates. The first transition is thought to be the development of non-feeding pelagic larvae from species with feeding pelagic (planktotrophic) larvae. A secondary transition from a pelagic larval stage to the development of non-feeding larvae in benthic egg masses is thought to follow, with subsequent transitions to the retention of the eggs/larvae within mothers and ultimately direct development (i.e. viviparity and direct development) (Figure 1) (Strathmann 1985, McEdward & Janies 1993, 1997). Two primary morphological changes are thought to occur in the evolutionary transition from pelagic feeding larval development to direct development of offspring: the loss of feeding structures in the transition from feeding to non-feeding larval development, and the loss of the larval body plan in the transition from non-feeding larval development to direct development (McEdward & Janies 1997) (Figure 1). These changes are considered to be irreversible due to marked transformations in larval morphology that they involve (Hart et al. 1997, McEdward & Janies 1997).

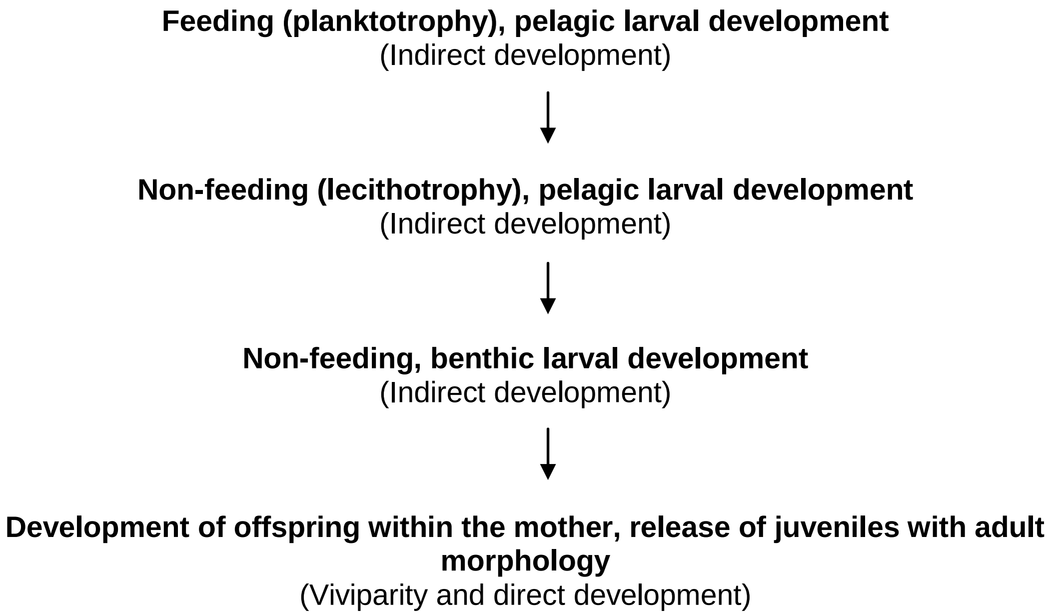

Figure 1.1. Suggested evolutionary pathway from feeding pelagic larval development to viviparity and direct development in marine invertebrates. Bold text = broad characteristics of larval development involved in life-history strategy, and non-bold text = terminology generally applied to life-history strategies with these larval development characteristics (modified from McEdward & Janies 1993, McEdward 1995).

1.3 Evolutionary ecology of marine invertebrate life-histories

Differences in life-history strategies among marine invertebrates have profound implications for their fecundity, mortality rates and dispersal potentials, as well as the genetic structuring of populations, population distributions, and rates of evolution and extinction. These implications are overviewed in the following sub-sections.

1.3.1 Fecundity and mortality

The evolution of different life-history strategies among marine invertebrates involves trade-offs between offspring size and the number of offspring produced (i.e., fecundity), and offspring size and their mortality rates. The production of larger offspring is thought to result in reduced fecundity but higher offspring fitness and hence lower mortality, while the production of smaller offspring results in higher fecundity, but at the expense of increased mortality rates (Vance 1973, Smith & Fretwell 1974, Christiansen & Fenchel 1979, Parker & Begon 1986).

Species that reproduce via direct development generally produce larger offspring than species with other life-history strategies and have relatively low fecundity. While their lower fecundity is presumably balanced by lower mortality rates of offspring, the production of large offspring via direct development requires high inputs of maternal energy that can result in mortality of adults through starvation, predation, or due to the energetic cost of giving birth (Menge 1975, Keough & Dartnall 1978, Byrne 1996, Bosch & Slattery 1999). Mortality rates of small, feeding larvae are also likely to be high because food resources in the plankton are spatially and temporally variable, the larvae may be dispersed to unfavourable habitats, and because larvae are susceptible to predation during their often lengthy planktonic developmental periods prior to settlement. It should be noted, however, that while the trade-off between offspring size and offspring mortality rates are highly likely, there remains little empirical evidence to support the notion because of the inherent difficulties of tracking the fate of small planktonic larvae (Underwood & Fairweather 1989, Pechenik 1999, Vaughn & Allen 2010).

Species with non-feeding planktonic larvae have the benefit of being maternally provisioned with the energy required for their generally short pelagic larval period, but this advantage is traded-off against lower fecundity compared to species with planktotrophic larval forms, and they remain susceptible to other sources of planktonic mortality, albeit for a shorter time (Menge 1975, Havenhand 1993, McEdward 1995, Pechenik 1999).

1.3.2 Dispersal, population distributions, and population structures

Dispersal is a major process regulating populations of marine invertebrates, and for most species it occurs during the pelagic larval phase (Pechenik 1999). Dispersal via a pelagic larval phase can be beneficial because it reduces competition for food and space (see Havenhand 1995), reduces the probability of extinction by spreading the risk of mortality among different environments (i.e. "bet-hedging") (Emlet 1989, Jeffery & Emlet 2003), and facilitates large geographic ranges with reduced inbreeding (Emlet 1995, Pechenik 1999). These advantages are traded-off against the potential for dispersal away from favourable habitats, a greater risk of mortality in the plankton, and greater susceptibility to environmental stresses (e.g. food limitation).

A major cost associated with direct development is the lack of dispersive larvae that results in more restricted distributions compared to species that possess a pelagic larval phase (Pechenik 1999). These species potentially face increased competition with conspecifics, and are vulnerable to changes in environmental conditions (Dybdahl 1994, Pechenik 1999, Johnson et al. 2001). Limited dispersal can, however, be beneficial in environments where favourable habitats are rare or patchily distributed because dispersal would present a high probability of mortality (Menge 1975, Palmer & Strathmann 1981). Recent work by Keever (2010) on the population genetics of Australian sea stars shows that species exhibiting direct development have high levels of genetic population structuring in comparison to those that have a dispersive pelagic larval stage. These generalities, however, are not always apparent: for example, the sea star *Parvulastra exigua* (formally classified in the genus *Patiriella*, see O'Loughlin & Waters 2004), which broods its offspring in benthic egg masses, is widely distributed and it is believed to have adapted a strategy of rafting to disperse to other areas (Waters & Roy 2004).

1.3.3 Population stability over ecological and evolutionary time-scales

The processes underlying the population dynamics of marine invertebrates have received considerable research attention. Many factors are known to regulate populations, including pre and post-settlement processes such as competition (e.g. Connell 1961, Menge 1972), predation (Fairweather 1988a, Stevenson 1992), recruitment (e.g. Underwood & Fairweather 1989), mortality (e.g. Connell 1985), food availability (e.g. Underwood et al. 2004) and behavioural interactions (e.g. Fairweather 1988b, Crowe & Underwood 1998). In the intertidal zone, additional environmental factors such as desiccation (e.g. Gosselin & Qian 1997, Chan et al. 2006, Miller et al. 2009), exposure to wave action (e.g. McGuinness 1987, Metaxas & Scheibling 1993, Denny 1995, Lindegarth & Gamfeldt 2005), salinity and temperature fluctuations (e.g. Chen & Chen 1993, Harley 2008) and gradients in these parameters across the intertidal zone (e.g. Underwood 1981, Somero 2002) are also known to affect distributions and abundances of populations, as well as their population dynamics.

Investigations into the effects of these variables on the distributions, abundances and dynamics of marine invertebrate populations have been largely restricted to species that have dispersive larval stages, and relatively little is known about processes influencing the population dynamics of species with direct development. This is important because the population dynamics of species with pelagic larvae largely dependent upon the experiences of their larval stages during their pelagic period, while the population dynamics of species exhibiting direct development are dependent on processes that influence the adult and offspring within the same environment. Factors regulating populations and population stability are therefore likely to be different among species of marine invertebrates exhibiting these life-history strategies (Eckert 2003).

A long-standing notion in marine ecology is that species with pelagic larval phases undergo greater fluctuations in population abundances than species with direct development due to the hazards associated with a pelagic larval phase. High planktonic mortality rates are thought to contribute to variable recruitment success, and hence variable population abundances (Thorson 1950, Underwood & Fairweather 1989, Pechenik 1999, Vaughn & Allen 2010). In contrast to this notion, recent investigations suggest that dispersive pelagic larval stages may actually have a stabilising influence on the abundance of marine invertebrate populations over ecological time scales, due to a greater potential for replenishment of populations by recruits from other populations, which may act to dampen population fluctuations (Grantham et al. 2003). In a review of relationships between the length of the planktonic period (i.e. long, short or none) and the stability of populations of marine invertebrates, Eckert (2003) found that population fluctuations were not affected by the duration of the planktonic larval period (short versus long), but that species that did not have a planktonic period exhibited greater population fluctuations than those with a dispersive larval phase.

Fossil evidence suggests that over evolutionary time scales species with direct development have smaller distributions, higher speciation rates and higher extinction rates than species with pelagic larval phases (Hansen 1978a, 1980, Emlet 1989, Jablonski & Hunt 2006, Crampton et al. 2010). For example, in an analysis of Australian Tertiary echinoids, Jeffery and Emlet (2003) found that species that did not have a pelagic larval phase had higher speciation rates, shorter longevity and more restricted distributions than those with pelagic larval stages. Although the various life-history trade-offs associated with each life-history strategy are widely accepted, there remains remarkably little empirical evidence from *in situ* studies of species with direct development. Furthermore, most investigations of trade-offs associated with different life-history strategies are confounded by comparisons that have been made among species.

1.4 Intraspecific variation in offspring size

Small differences in maternal investment, and hence offspring size, can have profound implications for fundamental life-history characteristics and it is now recognised to be a highly variable phenotypic trait among marine invertebrates (reviewed in Marshall & Keough 2008a). Offspring size can vary among populations within species (George 1994, 1996), among broods within populations (Bingham et al. 2004, Monro et al. 2010) and within the broods of individuals (Turner & Lawrence 1977, Byrne 1996, Marshall et al. 2000). Indeed, Marshall and Keough (2008a) and Monro et al. (2010) note that it is becoming increasingly evident that phenotypic plasticity in offspring size is widespread, and that it appears that mothers rarely produce offspring of a constant size throughout their lifetime. This variation is important for two reasons: (1) variation in offspring size within species is likely to be adaptive as a basis on which natural selection operates; and (2) it enables investigations into the ecology of variation in maternal investment in offspring within species, eliminating the confounding influence of differences among species. However, investigations into the effects of intraspecific variation in offspring size on population dynamics of marine invertebrates have only recently begun.

Intraspecific variation in offspring size can be attributed to numerous factors, including maternal, genetic, and environmental variables. Maternal effects are defined as those resulting from the maternal environment or maternal phenotype on offspring phenotype (Marshall et al. 2008a). Maternal size has long been known to influence offspring size: among many species, larger mothers produce larger offspring (Sakai & Harada 2001, Marshall & Keough 2008a). The reasons for this relationship are not yet clear, but have been proposed to result from larger mothers being able to provision resources to offspring more efficiently than smaller mothers (Sakai & Harada 2001, Marshall & Keough 2004a), or because larger mothers may have access to better resources (Hendry et al. 2001). It has also been proposed that larger mothers should produce bigger offspring in conditions when competition among offspring at the population level is likely to be high (Parker & Begon 1986). Reproductive output can also be affected by maternal nutritional state. For example, Steer et al. (2004) found that when the squid *Euprymna tasmanica* was fed a nutritionally rich diet, mothers produced significantly larger offspring than when they were fed a relatively poor diet. Similarly, George (1996) showed that in several species of echinoderms a reduction in food supply to adults resulted in the production of smaller offspring.

The quality of the environment is also a well known cause of intraspecific variation in offspring size in marine invertebrates. Environmental variables that can influence offspring size include temperature, salinity, and wave exposure. For example, George (1994) found that in the sea star *Leptasterias epichlora*, mothers that came from populations exposed to wave energy and an abundant food supply produced larger and higher quality eggs compared to eggs produced by mothers in less food resource rich sheltered environments. Similarly, Collin and Salazar

(2010) showed that the gastropod *Crepidula ustulatulina* produced smaller eggs and hatchlings at higher temperatures than at lower temperatures. Latitudinal differences in environmental variables can also influence offspring size in some species. For example, Lardies and Castilla (2001) showed a decrease in egg volume, but an increase in egg number (fecundity) with decreasing latitude in populations of the crab *Pinnaxodes chilensis.* Biotic interactions including competition and predation events can also cause variation in offspring size within species: Marshall and Keough (2004a) showed that following a predation event, colonies of the bryozoan *Bugula neritina* produced smaller offspring.

Intraspecific variation in offspring size has also been viewed as a response to the environment that maximises offspring fitness (McGinley et al. 1987, George 1996, Marshall & Keough 2008b). In many species, larger offspring have higher fitness than smaller offspring, but this relationship can differ depending upon the environment. For example, the production of larger offspring is more advantageous under stressful (e.g. competitive) conditions compared to benign environments in several species of marine invertebrates. Using the bryozoan *B. neritina* as an experimental model, Allen et al. (2008) showed that mothers produced larger offspring in response to an increase in intraspecific competition, and that larger offspring had higher dispersal potentials than smaller offspring that may enable them to better escape unfavourably crowded conditions. However, the production of larger offspring in stressful environments is not always advantageous. For example, Moran and Emlet (2001) found that larger hatchlings of the direct-developing gastropod *Nucella ostrina* had greater growth and survivorship than smaller hatchlings during winter, but that this relationship was absent during harsh summer conditions. Thus, the benefits of producing larger offspring were only apparent under certain environmental conditions.

There is also a growing body of research to suggest that mothers can adaptively alter the size of their offspring in response to the environmental conditions that they experience. For example, Marshall and Keough (2009) showed that, under high levels of intraspecific competition, the bryozoan *Watersipora subtorquata* released larger offspring with greater dispersal capacity compared to offspring from mothers that were not exposed to competition.

Explanations for the production of variable offspring size within broods of the same mother are more challenging. Variation in offspring size within broods has previously been viewed as a by-product of physiological constraints on reproduction that prevent mothers evenly distributing resources among their offspring, and as therefore having little or no adaptive value (Fox & Czesak 2000, Einum & Fleming 2004). Recent modelling conducted by Marshall et al. (2008b) suggested that, rather than just being a result of physiological constraints on mothers, the production offspring of variable sizes within broods may be adaptive when environmental conditions are unpredictable. In circumstances where environmental conditions are variable, and therefore difficult or impossible for a mother to predict (e.g. inconsistent changes in habitat conditions), mothers may employ a bet-hedging strategy through the production of a range of offspring sizes that increases the potential that at least some of the offspring may be of an optimal size for the conditions that they experience (Marshall et al. 2008b, Crean & Marshall 2009).

A mother’s ability to predict the environment that her offspring will experience and adaptively adjust offspring size accordingly is likely to be dependent on their life-history strategy. Offspring produced by species with pelagic larval phases do not necessarily settle in the same habitat as their mothers, and these offspring are also likely to encounter a range of environmental conditions that are unpredictable to their mothers (Marshall & Keough 2006, Marshall et al. 2008b). The generally non-dispersive offspring of species with direct development are much more likely to experience the same environmental conditions as their mothers, and the ability of these mothers to predict these conditions and adaptively alter offspring phenotype should therefore be greater than for species with complex life-histories. These species therefore provide the greatest opportunity to examine the extent to which mothers may be able to predict the environment into which their offspring are released and adjust offspring size accordingly.

Furthermore, species with direct development exhibit greater levels of intraspecific variation in offspring size than species with other life-history strategies that may make them particularly informative towards understanding relationships between intraspecific maternal provisioning to offspring and changes in environmental conditions. In a review of variation in offspring size in marine invertebrates, Marshall and Keough (2008a) found that species with direct development exhibited a mean coefficient of variation (CV) in offspring size within populations of 15 %, while species with other life-history strategies exhibited an average CV in offspring size of 9 %. Thus, marine invertebrates with direct development exhibit approximately 40 % more intraspecific variation in offspring size than species with all other life-history strategies; however, their findings were limited to offspring size variation in only nineteen species with direct development. The apparent high level of variation in offspring size exhibited by species with direct development, together with absence of ecologically decoupled life-history stages (i.e. larvae), make them likely to be highly informative towards understanding the adaptive nature of offspring size variation in marine invertebrates (Marshall & Keough 2008a) because they should have the greatest ability to predict the environment that their offspring will experience and adjust offspring size accordingly. Consequently, further studies of offspring size variation and the environment are needed on species that exhibit direct development.

1.5 Implications of intraspecific variation in offspring size

Just as interspecific variation in offspring size has profound implications for other life-history traits of marine invertebrates (see 1.2 above), intraspecific variation in offspring size also appears to have similar consequences that have only recently started to be investigated. Intraspecific variation in offspring size profoundly influences fecundity (Marshall 2005), pre and post-settlement growth and mortality rates (Marshall et al. 2003, Marshall & Keough 2004b), dispersal potentials (Marshall & Keough 2003a), fertilisation success (Marshall et al. 2000, 2002), and embryonic development time (Marshall & Bolton 2007). Differences in offspring size can also influence future reproductive output, offspring survivorship, and growth. For example, Marshall et al. (2003) showed that colonies that came from larger offspring of the bryozoan *Bulgula neritina* had higher survivorship, greater growth and reproduced sooner than those from smaller offspring. Similarly, Dias and Marshall (2010) recently found that larger offspring of the encrusting bryozoan *Celleporaria* sp. had greater growth and subsequent reproductive output than smaller offspring, but that offspring performance was dependent on the duration of the planktonic period.

The research of Marshall and others (see Marshall & Keough 2008a and references therein) has substantially changed the way that we think about variation in offspring size and its ecological significance. It is becoming increasingly accepted that intraspecific variation in offspring size can have significant effects on offspring fitness and population structures among marine invertebrates. There are currently few studies into how offspring size varies within species of marine invertebrates with direct development, nor what the consequences of this variation are for offspring fitness. In this thesis I investigate these topics in two species of sea stars (*Parvulastra parvivipara* and *Cryptasterina hystera)* that reproduce via viviparity and direct development of offspring. A potentially critical difference between *P. parvivipara* and *C. hystera*, and the species examined in recent offspring size investigations (e.g. Marshall et al. 2006; Allen et al. 2008; Marshall and Keough 2009) is that they do not have a dispersive larval stage. Both species brood offspring within their gonads and give birth to fully formed, non-dispersive juveniles. Therefore, while plasticity in offspring traits mediated by the environment are observed in some species with dispersive offspring may be related to adaptive alterations in their dispersive capacity (e.g. Marshall et al. 2006, Allen et al. 2008, Leips et al. 2009, Marshall & Keough 2009), plasticity in species with no dispersal capacity has not been extensively examined. Studies across species with diverse life-history strategies examining plasticity in offspring and brood traits and also offspring fitness in relation to a range of potential environmental influences are needed to address this problem.

1.6 Species examined in the investigations presented here

Parvulastra parvivipara and *Cryptasterina hystera* (Figure 1.2) are two of only five sea stars (Phylum Echinodermata, Class Asteroidea, Family Asterinidae) known to exhibit viviparity and direct development of offspring (Byrne 2006).

Both species give birth to non-dispersive, fully developed juveniles that emerge through the gonopores of adults, and begin independent existence immediately (Keough & Dartnall 1978, Dartnall et al. 2003) (Figure 1.3).

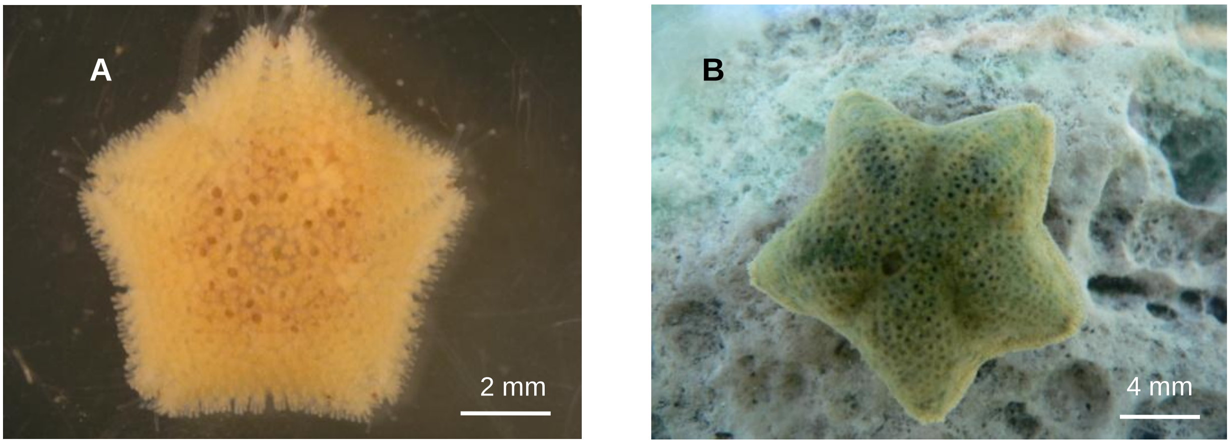

Figure 1.2. The viviparous, direct-developing sea stars (A) *Parvulastra parvivipara*, and (B) *Cryptasterina hystera*.

P. parvivipara is the world's smallest viviparous sea star with a diameter ranging from 1 to 11 mm. It is restricted to tide pools on seven small granite intertidal zones within 200 km of coastline between Point Labatt and Cape Vivonne, on the west coast of Eyre Peninsula, South Australia (Keough & Dartnall 1978, Roediger & Bolton 2008) (Figure 1.4A). Adults of *P. parvivipara* are hermaphroditic and reproduce only once before dying soon after giving birth. Reproduction is asynchronous among individuals: a peak in births occurs during spring, but some individuals within populations reproduce at other times of the year. Viable offspring can be dissected from adults at any time of the year suggesting that reproductively mature individuals are capable of giving birth at any time. Up to 36, non-dispersive juvenile offspring are born to each breeding specimen, with up to a 5-fold difference in juvenile size (i.e. diameter) within and among broods (approximate range 0.5 – 3.5 mm diameter) (Byrne & Cerra 1996) (Figure 1.3). Byrne (1996) provided evidence of intragonadal cannibalism among offspring in *P. parvivipara*.

C. hystera is found on the central coast of Queensland between Yeppoon and Emu Bay and on One Tree Island in the Great Barrier Reef (Figure 1.4B & C) (Dartnall et al. 2003, Byrne & Walker 2007). *C. hystera* are hermaphroditic and synchronously release their offspring during spring (Byrne et al. 2003). Adults give birth to up to a thousand offspring that are generally more uniform in size than those of *P. parvivipara* (~ 800 µm in diameter, although some attain diameters of up to 3 mm, (Byrne 2005). Evidence of intragonadal cannibalism has also been reported in *C. hystera* (Byrne 2005).

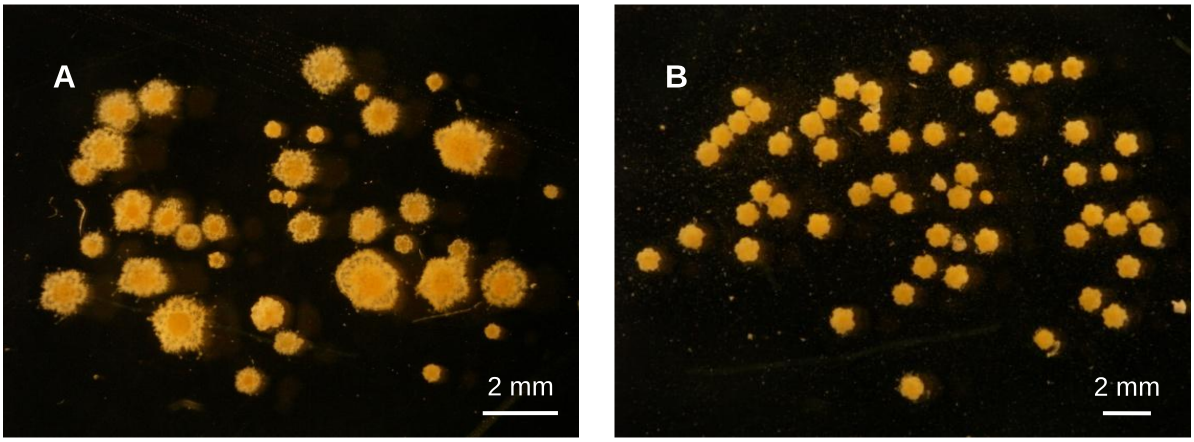

Figure 1.3. Examples of variation in the size of offspring within broods released by (A) *P. parvivipara*, and (B) *C. hystera*.

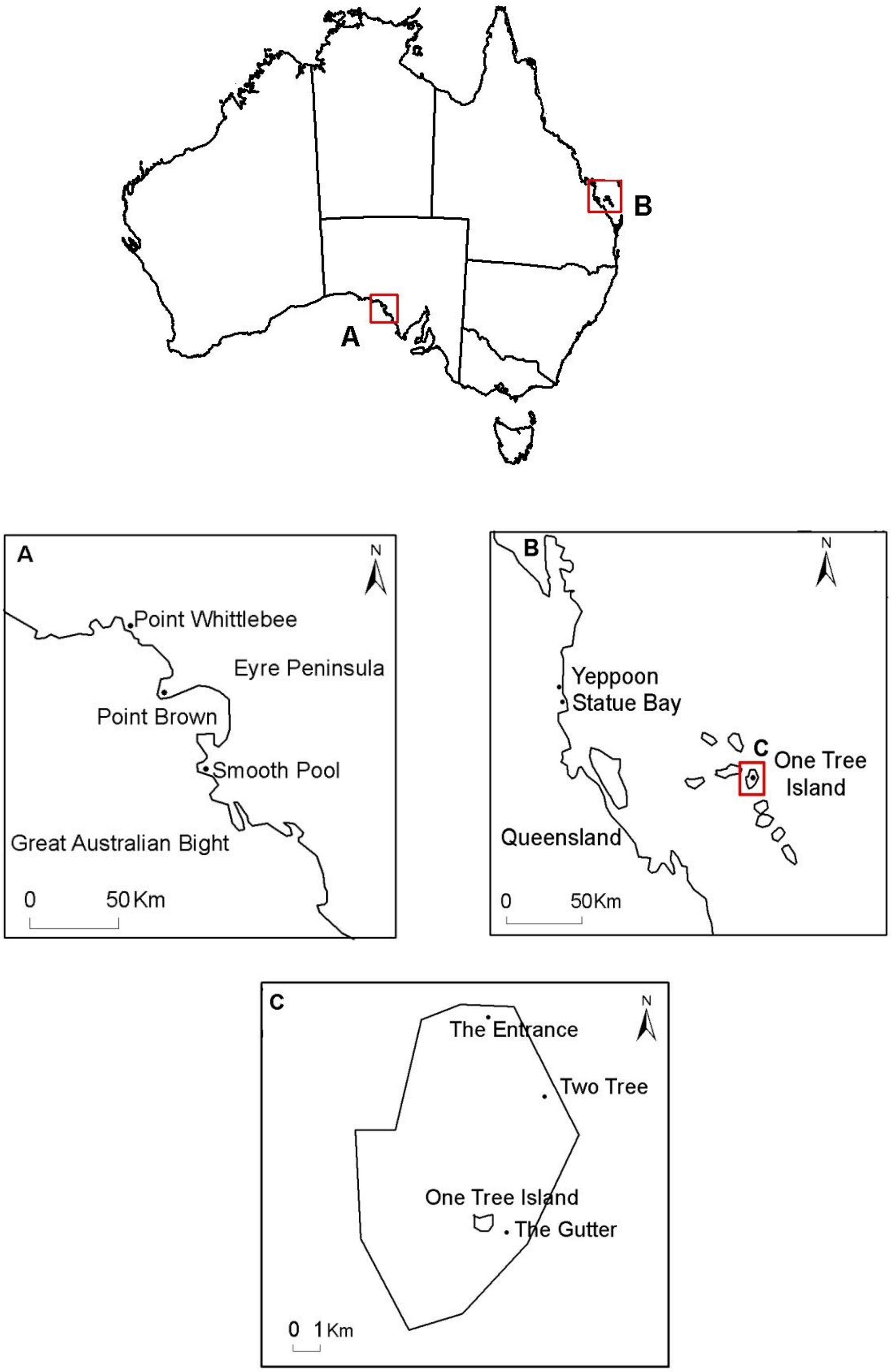

Figure 1.4. The populations of (A) *P. parvivipara* on the west coast of the Eyre Peninsula, South Australia, and of *C. hystera* in (B) Central Queensland, and (C) on One Tree Island, that were examined in this study.

P. parvivipara is patchily distributed among tide pools that differ substantially in their ecological characteristics both spatially and temporally (Roediger & Bolton 2008), while *C. hystera* is found in tide pools in coral rubble and cobble boulder habitats that are relatively more uniform (Dartnall et al. 2003, Byrne & Walker 2007) (Figure 1.5). The tide pools that these species inhabit are subject to considerable variations in physical, chemical and biological characteristics over daily and seasonal weather cycles, although this variability is more pronounced in the temperate habitat of *P. parvivipara*. Temperate intertidal habitats generally tend to undergo more pronounced seasonal, intra-seasonal and inter-annual variability in water temperatures, irradiance, and nutrient availability than tropical intertidal habitats (Lardies & Castilla 2001, Muller-Parker & Davy 2001).

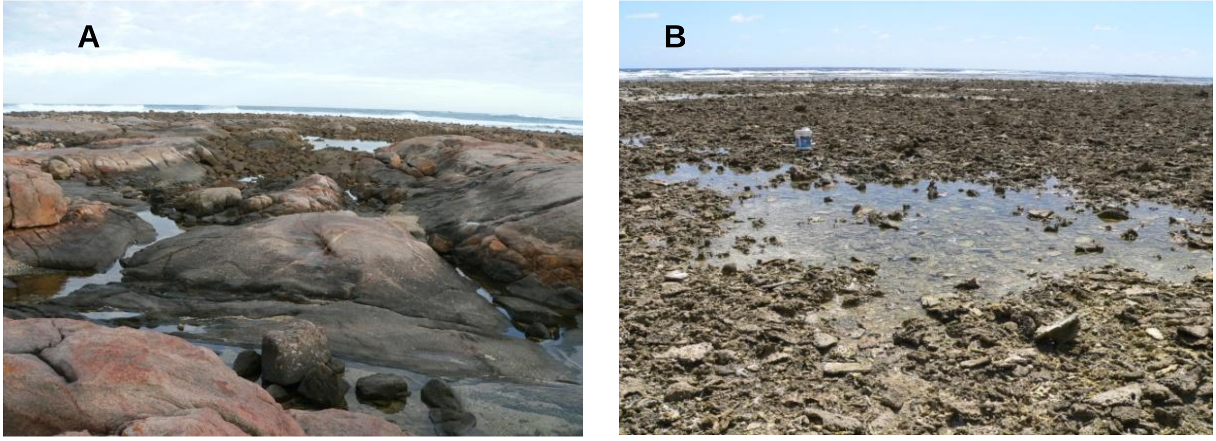

Figure 1.5. Examples of tide pool habitats of (A) *P. parvivipara* at Smooth Pool, on the west coast of the Eyre Peninsula, South Australia, and (B) *C. hystera* at Two Tree, on One Tree Island, Queensland, Australia.

1.7 Thesis aims

The main aim of this research was to increase the understanding of population dynamics and reproductive characteristics of marine invertebrates that exhibit viviparity and direct development. Given that there is no decoupling larval stage between viviparous direct-developing mothers and their offspring, these species should have the greatest ability of all marine invertebrates to predict the environment that their offspring will experience and therefore the greatest capacity to adjust offspring size to best suit prevailing conditions. Here, I address the knowledge gaps on how viviparous direct-developing species allocate energy to offspring under different environmental conditions. I aim to determine if mothers adjust the size of their offspring in response to the environment and whether these changes are adaptive. Furthermore, given the strong effects of offspring size on offspring fitness in some direct-developing species (see Moran and Emlet 2001), it is likely that the population dynamics of these species are susceptible to variation in offspring size. I also examine relationships between variation in offspring size and population dynamics of two direct-developing Asterinids within and among populations over time. The findings from these examinations will increase our understanding of how environmental conditions influence maternal provisioning to offspring and will also increase our knowledge of the population dynamics of viviparous, direct-developing species.

Specifically I examined:

- Variation in the abundances of populations of *P. parvivipara* and *C. hystera* at the scales of individual tide pools within populations, and within and among geographically separated populations over time;
- The influence of ecological variables on the distribution and abundance of *P. parvivipara* and *C. hystera* over time;
- Variation in offspring and brood sizes within and among broods of three populations of *P. parvivipara*, and in relation to an environmental gradient across the intertidal zone over seasons;

- The effect of population density on offspring size and brood size of *P. parvivipara*.
- Variation in offspring size in two viviparous, direct-developing marine invertebrates that respectively inhabit a temperate and a tropical intertidal environment;
- The effects of variation in offspring size on offspring fitness (growth and survivorship) in *P. parvivipara*

1.8 Thesis structure

This thesis consists of six chapters. Chapters 2 through 5 contain the results of field and experimental investigations and are presented in the format of four manuscripts for submission to peer-reviewed journals. The introductory material to each manuscript necessarily contains some overlap due to the related nature of each element of my research. Chapter 6 provides a synthesis of findings, future research directions and conclusions.

Marine invertebrates that reproduce via direct development have been thought to have more stable populations than species with life-histories that include a pelagic larval phase, but recent evidence suggests otherwise. There are a large number of studies that examine the population dynamics of intertidal marine invertebrates, but the majority of these focus on species that have a pelagic larval stage in their life-history. There are relatively few studies examining the population dynamics of direct-developing, viviparous species. In Chapter 2 I examine the distribution, abundance and stability of *P. parvivipara* and *C. hystera* populations in relation ten ecological variables that were identified as potential determinants of these population parameters. Three populations of *P. parvivipara* were surveyed four times a year (once per season) over three consecutive years, while four populations of *C. hystera* were examined annually (spring) over two years. Population abundances, sizes of individuals within populations and ecological variables were measured within individual tide pools to examine temporal (between surveys, seasons and years) and spatial variation in these parameters. I also examined size-frequency distributions and the coefficients of variation in their abundances within and among populations.

Direct-developing marine invertebrates exhibit variably sized offspring within broods that can vary up to 5-fold in size. Changes in the brood characteristics among intertidal environments or among seasons have not been extensively examined in these species, but may reflect adaptive changes to the environment. I examine the reproductive periodicity and brood characteristics of *P. parvivipara* in Chapter 3. I examined: (1) seasonal variation in brood size and offspring size within and among broods of three populations that differ in their spatial extent; 2) variation in offspring and brood size in relation to an environmental gradient across the extensive intertidal zone at one location; (3) relationships between the sizes of adults and those of their offspring and broods; and (4) relationships between naturally occurring differences in densities of adults of *P. parvivipara* and the size characteristics of their offspring and broods in the field and in a controlled laboratory experiment.

During the course of research presented in Chapters 2 and 3, and from previous research conducted by Byrne (1996, 2005), it was apparent that although *P. parvivipara* and *C. hystera* share the same broad life-history strategy and a close phylogenetic affinity, the characteristics and levels of size variation in their offspring and broods differs markedly. I hypothesised that a potential reason for these differences is the relative levels of environmental stability that each species is exposed to: *P. parvivipara* inhabits a variable temperate intertidal zone, while *C. hystera* inhabits a relatively stable tropical intertidal zone. In Chapter 4 I present the results of my investigations into the variation in offspring size exhibited by *P. parvivipara* and *C. hystera* during the period immediately preceding their peak reproductive seasons, and relate coefficients of variation in offspring size to the environments that they inhabit.

Differences in offspring sizes can influence fundamental life-history traits including survival, growth and reproduction. The large variation in offspring sizes exhibited within broods of *P. parvivipara* (Chapters 3 & 4) is likely to influence offspring fitness. In Chapter 5 I experimentally examined the fitness consequences (i.e. offspring growth and survivorship) of differences in offspring size exhibited by *P. parvivipara*. The survivorship and growth (both net and as proportion of size at birth) were measured using offspring of different sizes obtained from within broods. Measurements were made at 3-month intervals (i.e. seasonally) over the course of a year from the time of birth. *P. parvivipara* were maintained in an outdoor laboratory aquarium system that was designed to mimic daily and seasonal variations in environmental conditions that they experience in tide pools. In this chapter I also relate observed seasonal changes in the phenotypic characteristics of offspring within broods reported in Chapter 3 to the fitness consequences of differences in offspring size reported here.

The findings from these investigations are synthesised and discussed in Chapter 6, and future research directions are discussed.

Chapter 2

Population dynamics of the direct-developing sea stars *Parvulastra parvivipara* and *Cryptasterina hystera*

2.1 Abstract

An enduring notion in marine ecology has been that species with reproductive modes that incorporate a long pelagic larval period undergo greater fluctuations in population sizes than species with relatively short larval periods or direct development because highly variable planktonic mortality rates result in variable recruitment rates. In contrast to this idea, recent evidence suggests that species with dispersive pelagic larval phases may actually have more stable populations than species with no pelagic phase because population fluctuations are dampened by the recruitment of dispersive offspring from other populations. I examined the population dynamics of the direct-developing sea stars *Parvulastra parvivipara* and *Cryptasterina hystera* in their respective temperate and tropical habitats over 3-years. Abundances of *P. parvivipara* and *C. hystera* across populations were stable over the study period but their distributions among tide pools within populations were highly dynamic. While high structural complexity within tide pools was a consistent positive predictor of the presence and high abundances of both sea stars, specimens disappeared from many tide pools and recolonised others (up to 45 % of all tide pools inhabited). The stable abundances of both species at the population level, coupled with highly dynamic distribution patterns within populations, suggests that abundances may be maintained by small-scale metapopulation relationships, in which individuals are passively transported among tide pools enabling ephemerally unsuitable pools to be recolonised as conditions across the intertidal platforms change. *P. parvivipara* exhibited a mean CV of abundance within tide pools among years of 104 %, while *C. hystera* had a mean CV of 80 %. These levels of variation are similar to the mean values

reported for species with a dispersive pelagic larval phases and therefore don't tend to support the notions that species with direct development have either higher or lower levels of population stability than those with pelagic larval phases.

2.2 Introduction

Distributions and abundances of marine invertebrates vary in space and time in response to complex interactions among organismal traits (e.g. Connell 1961, 1972, Fairweather 1988a, Lively et al. 1993, Menge 2000a), physical forcings (e.g. McGuinness 1987, Metaxas & Scheibling 1993, Denny 1995, Lindegarth & Gamfeldt 2005), ecological processes (e.g. Gosselin & Qian 1997, Schreider et al. 2003, Underwood et al. 2004), and stochastic factors (e.g. Underwood & Fairweather 1989, Bascompte et al. 2002, Navarrete et al. 2008). Most studies into the determinants and dynamics of distributions and abundances of species in intertidal systems have focussed on temperate environments, while intertidal systems in tropical environments remain relatively understudied (Przeslawski et al. 2008). These studies have also overwhelmingly focussed on species with life-history strategies that incorporate a pelagic, dispersive larval phase (e.g. Astles 1993, Chapman 1994, Underwood & Chapman 1996, Menge 2000a). The determinants and dynamics of distributions and abundances of intertidal species that do not have dispersive pelagic larval stages (i.e. species with direct development) are also likely to be different to those of species with dispersive larval stages, but have rarely been studied in the field.

The influence of life-history strategies on population dynamics of marine invertebrates has been of particular interest because of the extraordinary diversity that they exhibit (e.g. Menge 1975, Connell & Sousa 1983, Pechenik 1999, Eckert 2003). Most marine invertebrates exhibit complex life cycles in which relatively sedentary adult life-history phases are decoupled from dispersive, pelagic larval phases that have different morphologies, and occupy different ecological niches to the adults prior to metamorphosis into the adult form (see Havenhand 1995, Levin & Bridges 1995). At the other end of a continuum of variation in life-history strategies among marine invertebrates are species that reproduce via viviparity and direct development, in which there is no decoupling dispersive phase between adults and their offspring (e.g. Hart et al. 1997, McEdward 1997, Pechenik 1999).

Over evolutionary time scales, fossil evidence suggests that populations of species with long dispersive pelagic larval phases have wider distributions, slower speciation rates and slower extinction rates than species with short pelagic larval phases or direct development (Hansen 1978b, 1980, Emlet 1989, Jeffery & Emlet 2003, Jablonski & Hunt 2006, Crampton et al. 2010). Relationships between life-history strategies and the population dynamics of marine invertebrates over relatively short ecological time scales are, however, more contentious. An enduring notion in marine ecology since the pioneering work of Thorson (1946, 1950) has been that species with life-histories that incorporate a long planktonic larval period undergo greater fluctuations in population sizes than species with relatively short larval periods or direct development because highly variable planktonic mortality rates result in variable recruitment rates (i.e. unstable) (e.g. Thorson 1946, 1950, Roughgarden et al. 1988, Underwood & Fairweather 1989). This appealing idea has been difficult to test because the mortality rates of pelagic larvae are difficult to determine and consequently there is little evidence to support it (reviewed in Olafsson et al. 1994, Vaughn & Allen 2010).

In contrast to this notion, recent evidence suggests that species with dispersive, pelagic larval phases may actually have more stable populations than species with no pelagic larval phase because population fluctuations are dampened by the recruitment of dispersive offspring from other populations. A stable population can be defined as one in which the abundance of individuals is relatively constant over time, versus an unstable population which undergoes large fluctuations in abundance of individuals over time. In a review of relationships between the length of the planktonic period (i.e. long, short or none) and population fluctuations among marine invertebrates, Eckert (2003) found that population fluctuations among species with complex life-history strategies are not affected by the duration of the planktonic larval period (short versus long), but that species that have no planktonic period exhibit greater population fluctuations than those with a dispersive pelagic larval phase. The inability of direct-developing species to disperse away from unfavourable conditions, such as environmental stress, is thought to result in high mortality rates, and hence large fluctuations in population sizes (Dybdahl 1994, Eckert 2007). Conversely, species that possess a dispersive pelagic larval phase are capable of receiving recruits from other populations that may act to dampen population fluctuations (Eckert 2003, Grantham et al. 2003, Grimm et al. 2003, Eckert 2009). A major impediment to our ability to understand relationships between life-history strategies and the dynamics of distributions and abundances of marine invertebrates is the lack of information on species with direct development, particularly those that inhabit tropical systems. Additional long-term investigations into the population dynamics of species with direct development are likely to be particularly informative towards understanding relationships between life-history strategies and population stability because of the lack of ecological decoupling between adults and their offspring.

In a previous study examining determinants of the distribution and abundance of the direct-developing sea star *Parvulastra parvivipara* at a discrete time interval, I found that their abundance was markedly variable among tide pools within geographically isolated tidal platforms that they inhabit (Roediger & Bolton 2008). Between the pilot and the quantitative survey of this study, I noted that some of the tide pools that were unoccupied by *P. parvivipara* during the pilot study subsequently contained high densities. I found that the abundance of *P. parvivipara* was higher in tide pools with greater structural complexity, and decreased with increasing exposure to wave energy. I also found that the likelihood of finding sea stars in a tide pool decreased with increasing wave-exposure and elevation within the tidal platform, while higher levels of structural complexity within tide pools increased the likelihood of their presence. However, these patterns were markedly variable as 13 % of tide pools that had seemingly ideal ecological attributes to support large numbers of *P. parvivipara* did not contain them. Furthermore, while densities of *P. parvivipara* were generally high in tide pools that were occupied (range 3 to > 1000 specimens per m^2), these tide pools contrasted strikingly with the absence of specimens in adjacent pools with similar ecological attributes.

The aims of this study were to examine the population dynamics of the viviparous, direct-developing sea stars *Parvulastra parvivipara* (Keough and

Dartnall 1978) and *Cryptasterina hystera* (Dartnall et al. 2003) (Echinodermata: Asteroidea) in relation to ecological attributes of individual tide pools over time. *P. parvivipara* and *C. hystera* are two of only five sea stars known to exhibit viviparous, direct development (Keough & Dartnall 1978, Byrne 1996). *P. parvivipara* reproduces throughout the year and gives birth to up to 36 non-dispersive juvenile offspring that can vary 5-fold in size. *C. hystera* gives birth to hundreds of non-dispersive fully formed offspring during the austral spring that are approximately 0.8 mm in diameter (Byrne et al. 2003).

Three geographically separate populations of *P. parvivipara* located at Smooth Pool, Point Brown and Point Whittlebee on the west coast of the Eyre Peninsula, South Australia were examined. While my previous study on the distribution and abundance of *P. parvivipara* was only conducted at a discrete time interval (Roediger & Bolton 2008), in this study I examined populations during each season across 3-years to identify changes in abundance and recruitment events, and to determine whether seasonal changes in the ecological attributes of tide pools influence the dynamics of their distribution and abundance. The intertidal platform at Smooth Pool is much larger than those at Point Brown and Point Whittlebee, extending up to 200 metres between the low and high intertidal zones. Smooth Pool therefore provided the additional opportunity to examine differences in the population dynamics of *P. parvivipara* across the intertidal gradient over the study period. To examine reproductive periodicity of *P. parvivipara*, the size-frequency structure and coefficient of variation of the sizes of specimens within tide pools were also examined over time.

The distribution and abundance of *C. hystera* were examined in relation to ten ecological variables during spring over 2-years at four populations: The Gutter, Two Tree and The Entrance on One Tree Island on the Great Barrier Reef, and Statue Bay on the central coast of Queensland, Australia. The ecological variables were identified in preliminary investigations as likely determinants of the distribution and abundance of *C. hystera*, and are similar to those previously identified as potential drivers of the distribution and abundance of *P. parvivipara* (see Roediger and Bolton 2008). In order to provide a basis to ongoing investigations into the population stability of *C. hystera*, I recorded size-frequency distributions and the coefficient of variation in abundance in tide pools within populations over two consecutive years.

2.3 Methods

2.3.1 Study populations

Populations of *P. parvivipara* are restricted to seven small, geographically isolated locations along a 200 km stretch of the west coast of the Eyre Peninsula, South Australia. The population dynamics of *P. parvivipara* were examined at three of these locations: Smooth Pool (32^{o} 92′ S, 134^{o} 07′ E), Point Brown (32^{o} 53′ S, 133^{o} 86′ E) and Point Whittlebee (32^{o} 20′ S, 133^{o} 73′ E) (Figure 1.4A). The intertidal platform of each population consists of granite outcrops that encompass hundreds of tide pools at low tide. Water temperatures in these tide pools can fluctuate by up to 26^{o} C in a single day (data presented here). They are also subject to strong wave energy in the low intertidal zones, and are susceptible to high evaporation rates and hence salinities and in the high intertidal zone (pers. obs.).

C. hystera is restricted to tropical intertidal coral-rubble fields on One Tree Island on the Great Barrier Reef, and to intertidal cobble boulder fields on the central coast of Queensland, Australia (Byrne et al. 2003, Byrne & Walker 2007). The distribution and abundance of four populations of *C. hystera* were examined in Central Queensland, Australia: The Gutter (23^{o} 51′ S, 152^{o} 09′ E), Two Tree (23^{o}49 S, 152^{o}09 E) and The Entrance (23^{o} 48′ S, 152^{o} 07′ E), which are located on One Tree Island, southern Great Barrier Reef, and Statue Bay (23^{o} 17′ S, 150^{o} 78′ E), which is located on the mainland south of Yeppoon (Figure 1.4). One Tree Island is surrounded by a coral cay comprised of coral rubble and at low tide hundreds of tide pools form (Davies et al. 1976, Byrne & Walker 2007). Populations of *C. hystera* at One Tree Island are located on the semi-exposed eastern side of the coral cay (Figure 1.5B). At Statue Bay, *C. hystera* inhabits shallow tide pools where it is found on the underside of boulders and smaller rocks (Dartnall et al. 2003). Statue Bay is comprised of rocks ranging in diameter from approximately 1 to 30 cm that are scattered over a muddy-sand substratum. *C. hystera* from One Tree Island have a distinctive pale green colour, while those from Statue Bay are dark brown to olive green (Figure 1.2B).

2.3.2 Distribution, abundance and size structure

Surveys of the abundance of *P. parvivipara* at each population were conducted at low tide four times per year (i.e. once per season) over 3-years between June 2007 and April 2010. Tide pools were randomly selected using numbered reference points that were superimposed on aerial photographs of the intertidal zones of each population (Smooth Pool n = 33, Point Brown n = 11, Point Whittlebee n = 11). In order to re-locate each tide pool over the study period, rocks adjacent to each pool were marked with a unique identifier using red spray paint and their latitude and longitude were recorded using a GPS (Garmin GPSMAP 96). The abundance of *P. parvivipara* was determined from counts of specimens within four 25 × 25 cm (625 cm^2) quadrats that were haphazardly thrown into each tide pool. In order to compare variation in the abundance of *P. parvivipara* among populations and among intertidal zones at Smooth Pool independently of population sizes, the coefficients of variation (CV) of abundance in tide pools (SD / mean × 100) were calculated for each year.

The large intertidal area of Smooth Pool was divided into low, mid and high intertidal zones based on iso-lines of immersion times of each tide pool throughout one tidal cycle from low to high tide in May 2007 (tidal range 2.4 m). Boundaries of the intertidal zones were defined at 2-hour intervals from low tide using photographs taken from an elevated position overlooking the intertidal platform (i.e. 0-hrs = low zone margin, 2-hrs = mid zone margin, 4-hrs = high zone margin). All tide pools across the area were assigned to an intertidal zone and numbered on an aerial photograph. Tide pools were then randomly selected within each intertidal zone (low n = 15, mid n = 10, high n = 7) using the methods outlined above.

To determine the size-frequency structures of *P. parvivipara* in tide pools among populations and among intertidal zones at Smooth Pool, measurements of the diameter of randomly selected specimens (~ 20 per pool) were made in each tide pool during each survey. Measurements were taken from the tip of one arm to the tip of the opposite arm with a vernier caliper (accuracy ± 1 mm). To compare variation in the size-frequency structure of *P. parvivipara* among populations and among intertidal zones at Smooth Pool independently of the mean specimen size, the coefficients of variation (CV) of the size structure (SD / mean ×100) were calculated for each season. The CV in the size structures of specimens within tide pools were calculated to identify pulses in recruitment that, based on previous observations, do not necessarily correspond to increases in abundance.

The distribution and abundance of *C. hystera* were determined for each study population in October of 2008 and 2009. This period immediately precedes the birth of *C. hystera* offspring, and therefore avoids the recruitment pulse and subsequent mortalities that would introduce a source of substantial variability to the abundance data. At each study population, a 100 metre-long transect was positioned parallel to the shoreline at the interface of the rubble margin and coral pavement (low tide margin) during low tide. Seven distances along this transect were randomly selected, and at each point a 50 metre transect was positioned perpendicular to it. Tide pools that were closest to four randomly selected distances along each of these transects were sampled. A tide pool was defined as an isolated body of water or a low-lying area where moisture was evident underneath the rubble after the tide had retreated. The abundance of *C. hystera* was determined from counts of specimens within four 0.5 × 0.5 m (0.25 m^2) quadrats that were haphazardly thrown into each tide pool. To determine the size structure of *C. hystera* in tide pools, the diameter of each specimen sampled was measured from the tip of one arm to the tip of the opposite arm with a vernier caliper (accuracy ± 1 mm).

2.3.3 Ecological variables

An earlier investigation identified ecological variables of tide pools that may explain the distribution and abundance of *P. parvivipara* (Roediger & Bolton 2008). Ten variables were identified as potential determinants of the distribution and abundance, and were therefore recorded for each tide pool sampled during this study. These variables were: 1) structural complexity; 2) size; 3) depth; 4) position within the rock platform; 5) whether it was tidal or wave fed; 6) exposure to wave energy; 7) the presence or absence of an anoxic layer in the sediment; 8) water temperature; 9) salinity; and 10) pH (Table 2.1, also see Roediger & Bolton 2008).

Salinity, temperature and pH were measured using a CyberScan PC300 waterproof hand-held pH/conductivity/TDS/temperature electronic meter (Eutech Instruments, Singapore). Wave energy was estimated using the weight loss of plaster balls (5 cm diameter), that were deployed in randomly selected tide pools for 24-hours. The balls were deployed in the low (n = 10), mid (n = 10) and high (n = 8) intertidal zones of Smooth Pool, and randomly across the intertidal platforms at Point Brown (n = 11) and Point Whittlebee (n = 11). The balls were retrieved and dried in an oven (30^{o} C, 24-hrs) and then weighed (accuracy ± 0.001g) before and after deployment. A steel bracket was used to secure the plaster balls in the tide pools. The temperature range and the degree of variation in temperatures in tide pools in the low, mid and high intertidal zones at Smooth Pool were determined. Temperature recordings were made at 2-hour intervals between June 2007 and April 2009 using data loggers (Thermochron ibutton, Temperature Technology, Adelaide, South Australia) that were deployed in 5 randomly selected tide pools in each intertidal zone. Each logger was encased in a plastic container that was attached to a boulder at the bottom of the tide pool with durable adhesive tape (3M duct tape). The mean temperatures and temperature ranges were determined from graphical outputs obtained from the loggers at each survey period.

Ten ecological variables that may influence the distribution and abundance of *C. hystera* were identified from a preliminary investigation conducted at One Tree

Island in 2007. Each variable was measured during low tide (Table 2.1). The variables investigated were: 1) salinity; 2) temperature; 3) pH; 4) distance from the pavement (low tide margin); 5) structural complexity; 6) area; 7) depth; 8) tide pool type; 9) wave exposure; and 10) the number of other macro-benthic organisms present. Details of each variable and the measurement scales used were defined *a priori* and are given in Table 2.1 (also see Roediger & Bolton 2008). Where existing scales of measurement were not available, measurements were made on ordinal scales that were defined *a priori* on the basis of the pilot investigation. Water temperature, salinity and pH were measured using a CyberScan PC300 waterproof hand-held pH/conductivity/TDS/temperature electronic meter (Eutech Instruments, Singapore).

2.3.4 Statistical analyses

Repeated-measures linear mixed models (LMM) were used to examine changes in the abundance and mean size-frequency of *P. parvivipara* and *C. hystera* in each population and *P. parvivipara* in each intertidal zone at Smooth Pool (SPSS v 15.0). Because populations were repeatedly surveyed, measurements of both abundance and size structure were correlated across surveys. LMMs were therefore used for these analyses because they are able to correctly model correlated errors when observations are not independent, and because the assumptions of homogeneity of variances are relaxed (Leech et al. 2008, Norušis 2008, Bolker et al. 2009, West 2009). An auto-regressive (AR1) covariance structure was used because the degree of correlation between surveys of abundance and size-frequencies (i.e. same specimens being counted and measured) was higher between surveys made at the beginning of the study compared to those made at later time intervals throughout the study, and therefore provided the best fit of the model to the data (see SPSS Inc. 2002, Leech et al. 2008, Norušis 2008, West 2009). Bonferroni pairwise post hoc comparisons were used to identify the location of differences among survey times ($\alpha = 0.05$).

The mean abundance and size of *P. parvivipara* and *C. hystera* in tide pools during all survey periods were separately compared among populations and for *P. parvivipara* among intertidal zones at Smooth Pool using a one-way ANOVA (α

= 0.05). Abundances were transformed (√ or fourth root) prior to analyses to meet the assumptions of ANOVA.

To compare variation in the abundance and size structure of *C. hystera* among populations, the coefficients of variation (CV = SD/mean × 100) of abundance and size-frequency were calculated for each population. The CV of abundance was calculated between surveys for each tide pool within a population and was compared among populations using a one-way ANOVA (α = 0.05). The CV in the size structures of specimens within populations were calculated for each population for each survey to identify pulses in recruitment that may not necessarily correspond to increases in abundance over longer time periods. A two-way ANOVA was used to compare the CV in size structure among populations and between surveys. Population was treated as a fixed factor because all known populations of *C. hystera* with specimens present were surveyed and survey was also a fixed factor. Bonferroni pair-wise post-hoc comparisons (α = 0.05) were used to identify the locations of significant differences where they were detected by ANOVA.

Two-way ANOVAs (α = 0.05) were used to compare the CV in abundance of *P. parvivipara* in each tide pool among populations or among intertidal zones at Smooth Pool among years. In these analyses, year and population were fixed factors. Similarly, two-way ANOVAs (α = 0.05) were also used to compare the CV in size structure of *P. parvivipara* among populations or among intertidal zones at Smooth Pool at each survey. In these analyses, intertidal zone was defined as a fixed factor. To understand the seasonal population dynamics of changes in sizes of *P. parvivipara*, surveys were assigned to their respective seasons (3 surveys per season over the study period) and the analysis was performed on the CV in size structure across seasons (a fixed factor). Where significant differences were detected, Bonferroni pair-wise post-hoc test comparisons (α = 0.05) were used to determine their locations.

The proportion of tide pools from which sea stars disappeared but subsequently re-colonised was determined for both species in each population or intertidal zone at Smooth Pool. For *P. parvivipara*, the percentage of time that tide pools were uninhabited over the three year period were calculated and compared among populations and among intertidal zones using a Kruskal-Wallis one-way non-parametric ANOVA.

Relationships between ecological variables and the abundance of *P. parvivipara* were compared using multiple linear regression analyses for each season in one year of the surveys (2008). To maximise the inferential power of the model, data from all populations were pooled. The temperature range in tide pools were initially included in the model as a potential predictor of abundance: however, as temperature loggers were not deployed in every tide pool examined, the temperature range could only be included as an average value applied to the intertidal zone that tide pools were located in. This reduced the power of the model because only 33 of the 55 pools were included in the analysis. Inclusion of temperature range data also resulted in multi-collinearity with two other potential predictor variables (salinity and tide pool position). Notwithstanding these problems, preliminary analyses run with temperature ranges included showed that it was not a significant predictor of abundance and it was therefore removed from further analyses. No multi-collinearity was detected among the remaining ecological variables, all of which were included in the subsequent analysis. Using the same data set, multiple logistic regression analyses were employed to determine whether any of the ecological variables predicted the presence or absence of sea stars. Because the tide pool temperature data showed co-linearity with other ecological variables, the mean temperature and temperature range in tide pools at Smooth Pool across the intertidal zone were subsequently examined independently. Two-way ANOVAs were used to determine whether mean water temperatures or temperature ranges differed among tide pools located in the low, mid and high intertidal zones over time. In the analyses, intertidal zone and survey were random factors, and mean water temperatures or temperature ranges were the dependent variables. Temperature ranges were transformed ($\sqrt{}$) to meet the assumptions of ANOVA. The degrees of wave exposure within and among the populations were compared using one-way ANOVAs ($\alpha = 0.05$) and subsequent Bonferroni post-hoc comparisons.

Relationships between ecological variables and the abundance of *C. hystera* were also examined using multiple linear regression analysis. The analysis was conducted on the 2009 data only because the variables were not measured at Statue Bay during 2008. To strengthen the regression model, data from all populations were pooled to increase the ratio of the response variable (*C. hystera* abundance in 123 tide pools) to predictor variables (ecological variables) (see Quinn & Keough 2003). *C. hystera* were absent from many tide pools at each location resulting in a large number of zero values for abundance: therefore, data were fourth-root transformed to meet the model's assumption of distributional normality (see Quinn & Keough 2002). A full model including all of the variables was initially run. Variables that were found to be non-significant predictors of abundance in this model were removed from the data set, and a reduced model was subsequently run to increase the ratio of the response variable to the predictor variables. Variables included in the reduced model were tide pool structural complexity, area, depth, distance from the pavement (low tide margin), and the number of other macro-benthic organisms present. No co-linearity was detected among the remaining predictor variables. Multiple logistic regression analysis was employed to determine whether any of the ecological variables predicted the presence or absence of *C. hystera*. A full model including all variables was initially performed on the 2009 data from all populations. Variables that were non-significant determinants of presence or absence were removed from the analysis, and a reduced model was run to increase the strength of the analysis. The variables included area, depth, distance from the pavement (low tide margin), the number of other macro-benthic organisms present and tide pool structural complexity. Assumptions of the parametric model were explored and tested using normality plots and Levene's homogeneity of variances test.

2.4 Results

2.4.1 Abundance and size structure

2.4.1.1 *P. parvivipara*

Populations of *P. parvivipara* were stable over the 3-year study period with the exception of significant spikes in their abundance at Smooth Pool and Point Whittlebee in January of 2009 (Table 2.2, Figure 2.1A). Abundances in these populations had returned to levels that were not significantly different from other surveys periods by April of the same year (Figure 2.1A). The abundance of *P. parvivipara* at Point Brown did not differ among surveys over the 3-year study period (Table 2.2, Figure 2.1A). The spike in population abundance that occurred in January 2009 at Smooth Pool and Point Brown was also apparent in the mid and high intertidal zones at Smooth Pool (Table 2.2, Figure 2.1B). Abundance within the high intertidal zone fluctuated significantly during several periods of the study. Specimens disappeared from more than half of the tide pools (57 %) in the high intertidal zone during the summer and autumn of 2010.

The mean density (± SE) of *P. parvivipara* among populations was 124 ± 21 per m^2 and no statistically significant difference in abundance were detected among populations (ANOVA: $F_{2, 52} = 1.961$, $P = 0.151$) (Figure 2.2A). The mean densitiy of *P. parvivipara* derived among intertidal zones at Smooth Pool was 163 ± 32, but the mean abundance in tide pools among intertidal zones were not statistically different (ANOVA: $F_{2, 30} = 1.667$, $P = 0.206$) (Figure 2.2B). The highest abundance of *P. parvivipara* within a tide pool was recorded at Smooth Pool during January 2009 (1852 per m^2) and among tide pools ranged from 4 to 1852 per m^2.

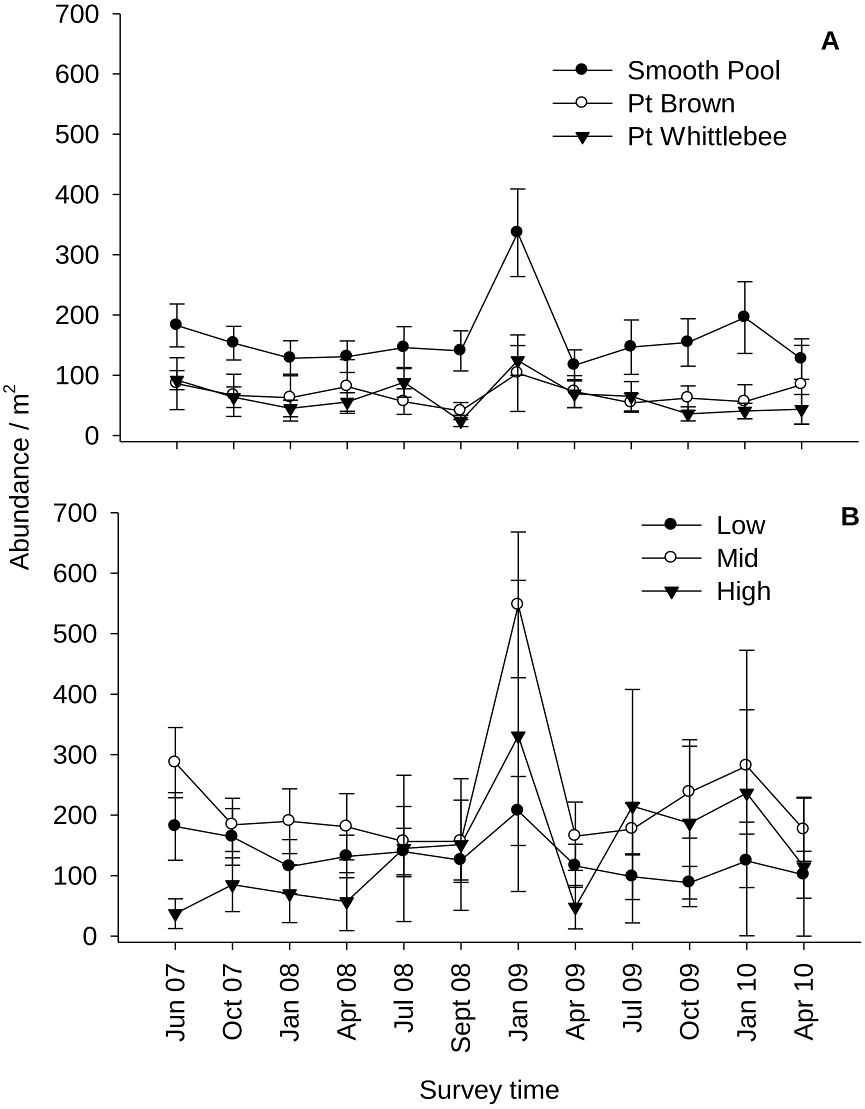

Figure 2.1. Mean (± SE, *n* = 7-15) abundances of *P. parvivipara* in tide pools at (A) three populations, and (B) in the intertidal zones at Smooth Pool. Data were collected between 2007 and 2010.

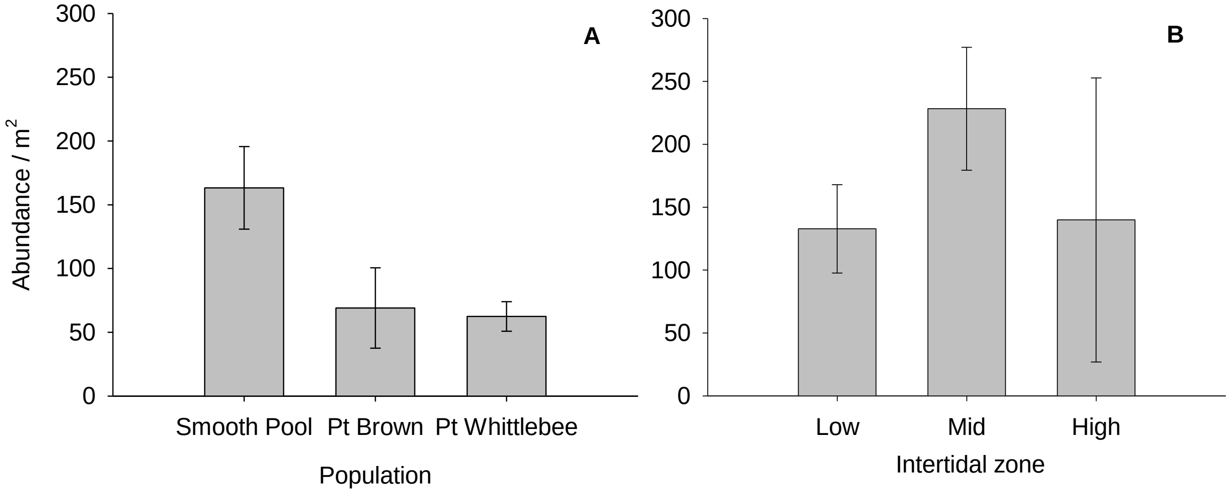

Figure 2.2. Mean (± SE, *n* = 7-15) abundances of *P. parvivipara* in tide pools of (A) three populations, and (B) in the low, mid and high intertidal zones at Smooth Pool.

The CV of *P. parvivipara* abundance within tide pools did not differ among populations (mean among populations ± SE 105 ± 9 %). The CV in abundance of sea stars was significantly higher in the high intertidal zone (mean 118 ± 11 %), while the CV in abundance did not differ between the low and mid intertidal zones (mean 68 ± 7 and 81 ± 9 %, respectively). The CV in abundance did not differ significantly among years (Table 2.3).

P. parvivipara periodically disappeared from tide pools in which they were once abundant in each of the populations. Over the study period, *P. parvivipara* disappeared on at least one occasion from 55 % of the tide pools at Point Brown, 46 % of tide pools at Smooth Pool, and 36 % of tide pools at Point Whittlebee. Within the intertidal gradient at Smooth Pool, *P. parvivipara* disappeared from more tide pools in the high intertidal zone (71 %) than in the mid and low intertidal zones (30 % and 44 %, respectively).

The frequency of disappearance events was highest between the January and April surveys. The proportion of time that tide pools were uninhabited ranged from means of 17 % at Smooth Pool to 11% and 8 % at Point Whittlebee and Point Brown, respectively: however, these differences were not statistically significant (P = 0.793). On average, tide pools within the low intertidal zone of Smooth Pool were uninhabited for 16 % of the time compared to 11 % and 29 % of the time for the mid and high intertidal zones, but these differences were not statistically significant (P = 0.193).

The mean size of *P. parvivipara* differed among surveys at all study populations (Figure 2.3A, Table 2.4), and between the low and mid intertidal zones at Smooth Pool (Figure 2.3B, Table 2.4). The largest specimens measured in the field were 11 mm in diameter and were recorded at Point Brown and Smooth Pool. At all populations, except the high intertidal zone at Smooth Pool, the mean size of specimens was larger in June 07 and April 08 (winter and autumn) compared to other surveys (Figure 2.3A & B).

The mean diameter (± SE) of *P. parvivipara* among populations was 4.21 ± 0.06 mm, but did not differ significantly among study populations (ANOVA: $F_{2,\ 52}$ = 2.661, P = 0.079, Figure 2.4A). The mean size of *P. parvivipara* among intertidal zones at Smooth Pool was 4.18 ± 0.06 mm and was not significantly differernt among intertidal zones (ANOVA: $F_{2,\ 30}$ = 0.973, P = 0.390, Figure 2.4B).

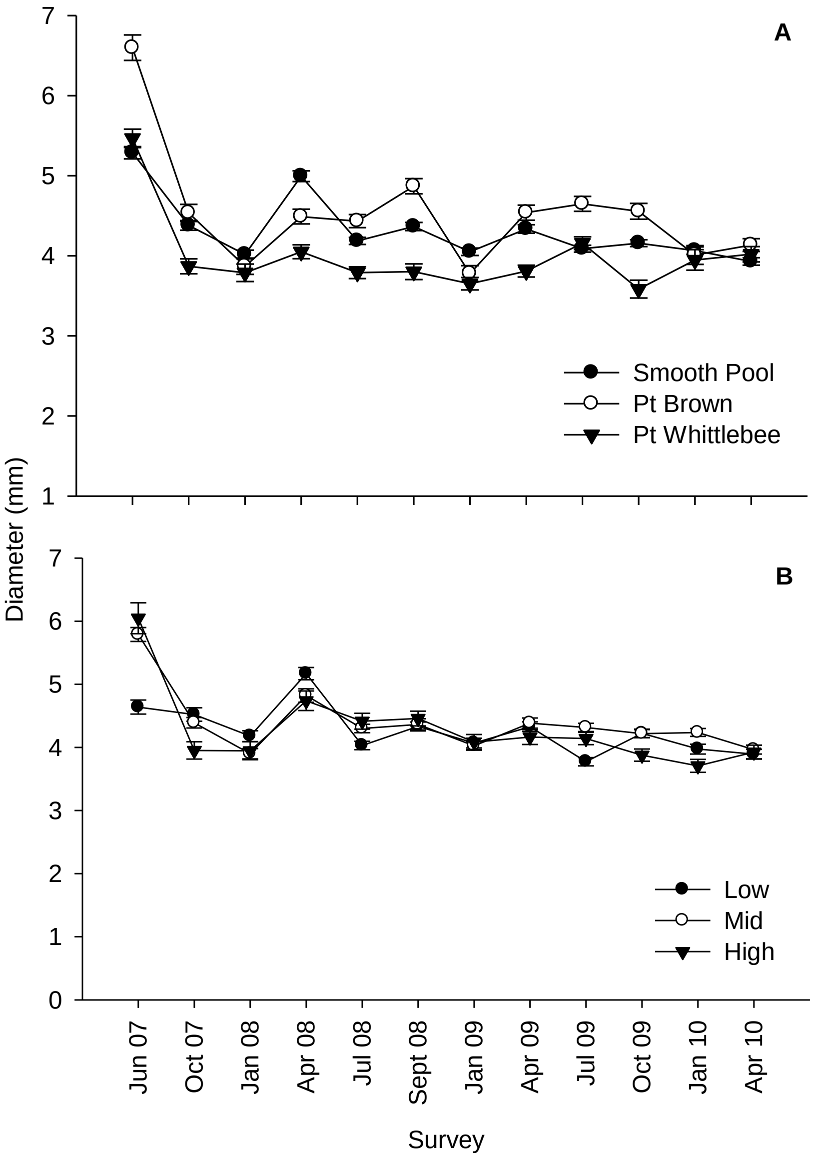

Figure 2.3. Mean (± SE, *n* = 7-15) size structure of *P. parvivipara* in tide pools at (A) three populations, and (B) among the low, mid and high intertidal zones at Smooth Pool. Data were collected between 2007 and 2010.

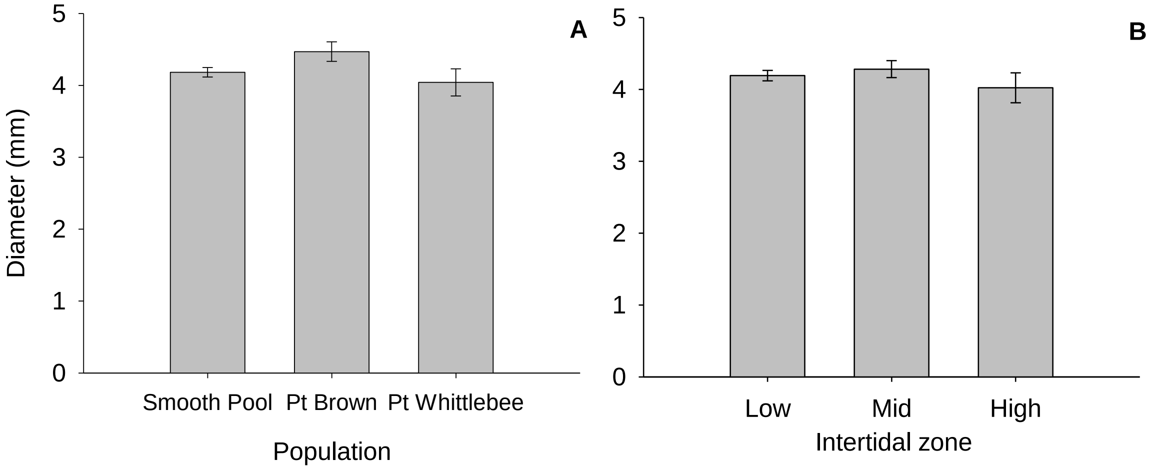

Figure 2.4. Mean (± SE, *n* = 7-15) size structure of *P. parvivipara* in tide pools at (A) three populations, and (B) among the low, mid and high intertidal zones at Smooth Pool.

The CV in the size structure of *P. parvivipara* was significantly greater at Smooth Pool (mean ± SE, 38 ± 1 %) than at Point Brown and Point Whittlebee (mean 33 ± 2 % and 34 ± 1 %, respectively) but no difference was detected between Point Brown or Point Whittlebee (Table 2.5). No differences were apparent in the CV of the size of *P. parvivipara* among intertidal zones at Smooth Pool (Table 2.5), although the CV of the sizes of specimens was significantly greater during summer (January) than during winter survey periods in all populations and intertidal zones.

2.4.1.2 *C. hystera*

The mean abundance of *C. hystera* in tide pools across both surveys was 2.6 specimens per m^2 among populations, and increased from 2.1 per m^2 in 2008 to 3.1 per m^2 in 2009. Among populations and surveys, the mean abundance of *C. hystera* ranged from 0.5 to 32 per m^2. The abundance of *C. hystera* in tide pools was significantly higher in 2009 compared to 2008 at The Gutter and Statue Bay, but no significant difference in abundance was detected between surveys at Two Tree or The Entrance (Table 2.6 Figure 2.5A). The mean abundance of C. *hystera* was significantly higher at the Gutter than at The Entrance and Statue Bay, but was not significantly different from the abundance at Two Tree ($F_{3,\ 116} = 14.216$, MS = 4.799, $P < 0.001$) (Figure 2.5A). At Two Tree, the mean abundance of *C. hystera* was not significantly different from that at Statue Bay, while abundances at the Entrance were significantly lower than those of all other populations (Figure 2.5A).

The CV of *C. hystera* abundance within tide pools did not differ significantly among populations (ANOVA: $F_{3,\ 76} = 0.0587$, MS = 156.289, $P = 0.981$). The mean CV in abundance within tide pools was 80 %, and ranged from 76 % at the Entrance to 84 % at the Gutter. Among populations, *C. hystera* was found in 50 % of the tide pools sampled in 2008, compared to 62 % of tide pools in 2009. A greater proportion of tide pools were inhabited during 2009 compared to 2008 among all populations (proportional increases: Gutter 12 %, Two Tree 29 %, Entrance 3 % and Statue Bay 4 %). The proportion of tide pools occupied by *C. hystera* ranged from 86 % at the Gutter in 2009 to 21 % at Statue Bay in 2008. Of all the tide pools sampled ($n = 120$), 6 % of those previously inhabited by *C. hystera* were no longer occupied by *C. hystera* in 2009. However, 16 % of the tide pools that previously did not contain *C. hystera* in 2008 were colonised by at least one specimen in 2009.

On average, *C. hystera* were significantly larger in 2009 compared to 2008 at Two Tree, but no significant difference was apparent between surveys at The Gutter, The Entrance, or Statue Bay (Table 2.7, Figure 2.5B). Across populations and surveys, the average (± SE) diameter of *C. hystera* was 15.9 ± 0.1 mm and individual sizes ranged from 24 mm to 5 mm. The mean sizes of specimens did not differ significantly among populations ($F_{3,\ 49} = 0.110$, MS = 0.266, $P = 0.954$) (Figure 2.5B), and no significant differences in the CV in the size-structures of *C. hystera* were detected between surveys or among populations (Table 2.8).

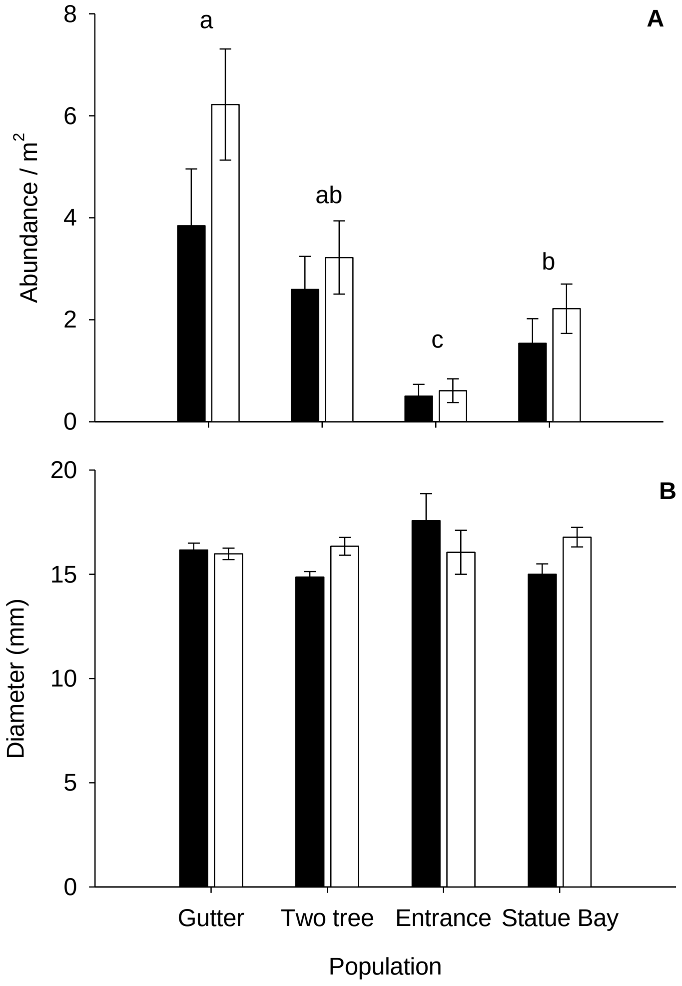

Figure 2.5. Mean (± SE) (A) abundance of *C. hystera* in tide pools (*n* = 28-32), and (B) size (*n* = 14-188) in four populations during 2008 (black bars) and 2009 (white bars). Letters denote significant differences between populations averaged across surveys that were identified by ANOVA (no letters above bars means a NS result).

2.4.2 Ecological variables

2.4.2.1 *P. parvivipara*

Multiple linear regression analyses showed that the abundance of *P. parvivipara* at Smooth Pool during summer was higher with increasing structural complexity of tide pools, while abundance decreased with increasing levels of wave exposure or the presence of an anoxic layer in the sediment ($F_{10, 44} = 5.259$, MS = 3.716, $P < 0.001$, $R^2 = 0.441$) (Table 2.9). These patterns were also apparent in the autumn survey, where abundance was greater with increasing structural complexity and depth of tide pools. However, abundance was lower in tide pools with higher temperatures in the autumn survey ($F_{10, 44} = 5.016$, MS = 3.401, $P < 0.001$, $R^2 = 0.426$) (Table 2.9). Data from the winter survey indicated that abundance was significantly greater in tide pools with higher structural complexity, but no other variables significantly increased abundance at this time ($F_{10, 44} = 4.806$, MS = 2.602, $P < 0.001$, $R^2 = 0.413$) (Table 2.9). During the spring survey the abundance of *P. parvivipara* in tide pools was also positively related to increasing structural complexity, but negatively related to increasing wave exposure ($F_{10, 44} = 4.958$, MS = 3.048, $P < 0.001$, $R^2 = 0.423$) (Table 2.9).

Logistic regression analyses showed that the likelihood of *P. parvivipara* being present in tide pools was greatest in those with high structural complexity across all survey seasons (Table 2.10). However, the likelihood of *P. parvivipara* being present in tide pools decreased with the presence of an anoxic layer in the sediment in the summer, and decreased with increasing water temperature in the autumn. The winter survey data showed that the presence of *P. parvivipara* was less likely in tide pools that were elevated or which had an anoxic layer in the sediment (Table 2.10). The Hosmer-Lemeshow goodness of fit test showed a significant result ($P = 0.048$) for the autumn model, indicating that these results need to be treated with some caution. For all other seasons the fit of the models to the data were robust ($P > 0.89$ in all cases).

The amount of material eroded from the plaster balls did not differ among populations of *P. parvivipara*, suggesting that the degree of wave energy that they were exposed to was also the same (ANOVA: $F_{2,\,49} = 2.688$, $P = 0.078$) (Figure 2.6A). Within the intertidal platform at Smooth Pool, the amount of material lost from the plaster balls from associated wave exposure was significantly greater in tide pools located in the low intertidal zone compared to tide pools in the mid and high intertidal zones (ANOVA: $F_{2,\,27} = 17.657$, $P < 0.001$) (Figure 2.6B).

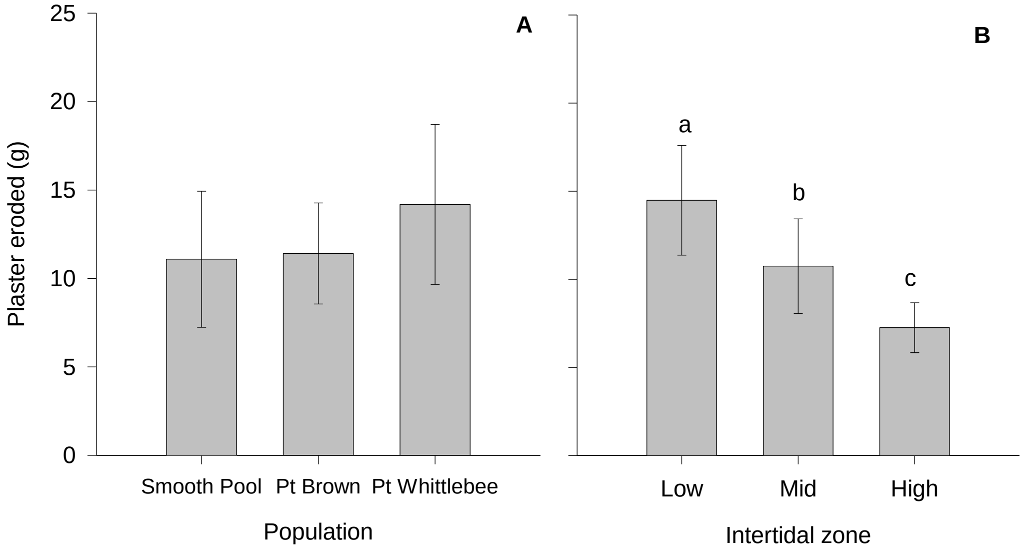

Figure 2.6. Mean (± SE, $n = 8$-11) amount of plaster (g) eroded from plaster balls (as a measurement of wave exposure) in (A) three populations of *P. parvivipara*, and (B) among the low, mid and high intertidal zones at Smooth Pool.

Temperatures across all tide pools ranged from 2^{o} C to 37.5^{o} C over the study period and the mean temperatures were significantly higher during summer than winter (Table 2.11). However, no significant differences were apparent in mean temperatures of tide pools among intertidal zones within seasons at Smooth Pool (Figure 2.7A). The greatest temperature range over the year recorded within an individual tide pool was 26.5^{o} C. Tide pool temperatures varied significantly more during summer than winter, and variations were significantly greater in tide pools located in the high intertidal zone than those in the low and mid intertidal zones (Table 2.11, Figure 2.7B).

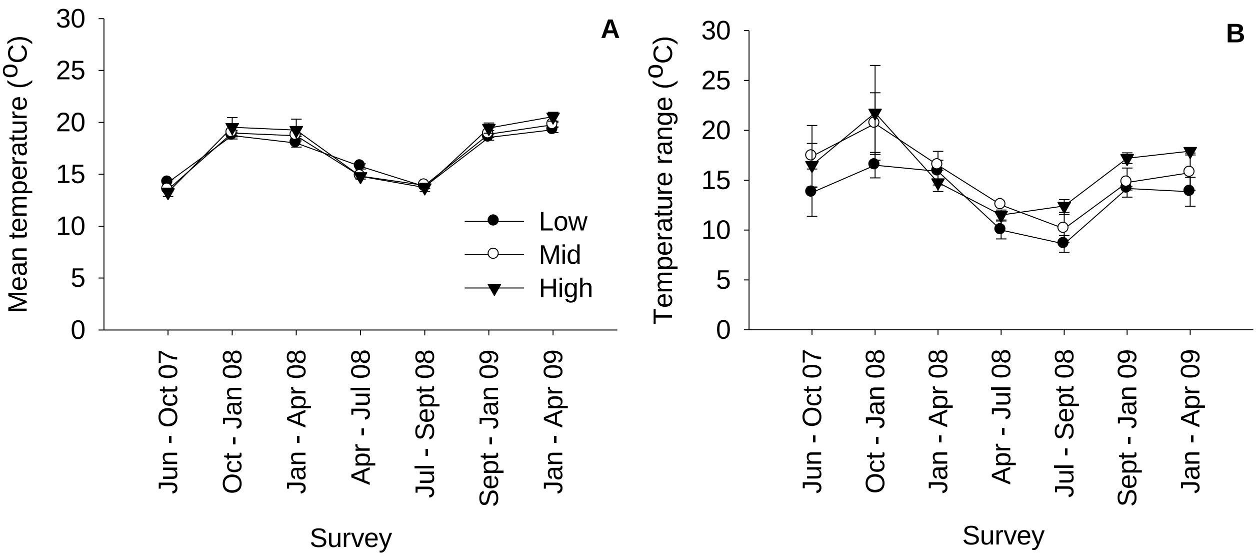

Figure 2.7. The (A) mean (± SE, *n* = 2-5) temperatures, and (B) temperature ranges (difference between minimum and maximum recorded during the year) of tide pools in the low, mid and high intertidal zones at Smooth Pool between June 2007 and 2009.

2.4.2.2 *C. hystera*

Multiple linear regression analysis showed that the mean abundance of *C. hystera* increased with increasing depth and complexity of tide pools, but decreased with increasing size (area) of tide pools ($F_{5,\ 117}$ = 8.332, MS = 3.439, $P < 0.001$, R^2 = 0.231) (Table 1.12).

Multiple logistic regression analysis showed that the likelihood of finding *C. hystera* in a tide pool increased with increasing depth and structural complexity, and also increased in tide pools that contained a greater number of other macro-benthic organisms (Table 2.13). The odds ratio from the logistic regressions suggests that the complexity of tide pools is the most important ecological variable determining the presence or absence of *C. hystera* (Table 2.13).

2.5 Discussion

The degree of variability in the abundances of populations of *P. parvivipara* differed across spatial scales. Abundances of *P. parvivipara* were stable over the 3-year study period at the scale of each population, but were highly variable among tide pools within populations. Statistically significant spikes in population abundances were observed in populations at Smooth Pool and Point Whittlebee during January 2009. These spikes coincided with an increase in the coefficient of variation (CV) in the size of *P. parvivipara*, suggesting that they were the result of recent recruitment events. However, abundances of *P. parvivipara* in these populations had returned to levels that were not significantly different from those of other survey periods by the autumn survey (April) of the same year. This corresponded with a decrease in the CV in the sizes of specimens in these populations to values that were not significantly different from those observed in other survey periods suggesting that abundance increases due to summer recruitment pulses were balanced by mortalities of either new recruits, or mortalities of older, larger specimens and concomitant growth of new recruits. The CV of abundances were similar across years indicating that population abundances were stable over time.

The apparent stability of abundances of *P. parvivipara* at the population scale contrasts sharply with variation in abundances at the scale of individual tide pools within populations. *P. parvivipara* temporarily disappeared from large proportions of tide pools within each of the populations. Across the three populations, specimens disappeared from 45 % of the tide pools surveyed. At Point Brown, specimens disappeared from more than half of the tide pools (55%) at least once over the 3-year study period, while 46 and 36 % of ostensibly suitable tide pools were unoccupied on at least one occasion at Smooth Pool and Point Whittlebee, respectively. Disappearance events in tide pools were higher in the summer and autumn months (January and April). Tide pools in which *P. parvivipara* disappeared were eventually recolonised at all populations, and the average proportion of time that they remained unoccupied over the 3-year study period was 14 %. Spatial differences in the proportions of tide pools from which *P. parvivipara* disappeared were also apparent across intertidal zones at Smooth

Pool. In the high intertidal zone, 71 % of tide pools experienced at least one disappearance and re-colonisation event, compared to 44 % and 30 % of the tide pools in the low and mid intertidal zones, respectively. The CV in abundance in the high intertidal zone was also significantly greater than in mid and low intertidal zones. Tide pools located within the high intertidal zone also remained uninhabited for the longest period of time (29 % on average over the study period) and therefore provide highly ephemeral habitat for *P. parvivipara*.

The distribution and abundance of *Cryptasterina hystera* was variable within and among populations. While the distribution and abundance of *C. hystera* were only examined at one time interval in each survey year, the dynamics of their populations seem to be similar to those that I found for *P. parvivipara*: abundances of *C. hystera* appear to be stable at the population level across surveys, but their distributions among tide pools within populations are highly dynamic. *P. parvivipara* disappeared from up to half of the tide pools examined between survey periods both within and between years, whereas *C. hystera* disappeared from only 22 % of tide pools examined. These observations need to be treated with caution due to the limited time periods over which surveys of each species were conducted. For *P. parvivipara* 12 surveys were conducted over 3-years where as for *C. hystera* only 2 surveys were conducted over 2-years. Consequently, there is a potential confounding of temporal scales between the two species as differences in the distribution and abundance of *P. parvivipara* may also be attributable to differences between surveys within years. Nonetheless these results seem valid given differences in the topographical structure and structural diversity of the environments inhabited by each species. The intertidal habitat of *C. hystera* is flatter and much more homogenous than that of *P. parvivipara* (see Figure 1.5) and may therefore present fewer topographic obstacles to the recolonisation of tide pools or movement of *C. hystera* among tide pools within the intertidal platform.

Similar patterns of variability within populations have been shown in other species with direct development. For example, Johnson et al. (2001) found that direct-developing gastropod species showed more spatial variability within than between shores because it took a long time for them to recolonise habitat after within-shore disturbances. Similarly, Eckert (2007) found that the direct-developing holothurian *Pachythyone rubra* showed high levels of spatial variability within populations in response to spatial differences in environmental conditions, predation, sedimentation and food supply. For *P. parvivipara*, irregular intensities of post-recruitment mortality rates between tide pools could also explain variability in *P. parvivipara* abundance within populations.

High structural complexity in tide pools was a consistent predictor of the likely presence and high abundance of *P. parvivipara* across seasons among populations. These results are consistent with those reported in a previous study conducted at a discrete time interval (Roediger & Bolton 2008). However, the relative importance of other ecological variables appears to change across seasons and is likely to explain the disappearance events of *P. parvivipara* from many tide pools that appear to provide suitable habitat for them throughout most of the year. Shallow tide pools contained low abundances or no *P. parvivipara* during the autumn surveys. *P. parvivipara* inhabiting shallow tide pools are therefore susceptible to mortality during the preceding summer months.

Seasonal changes in biotic and abiotic variables are well known determinants of seasonal mortalities of intertidal species (Astles 1993, Metaxas & Scheibling 1996, Chan et al. 2006, Harley 2008) and high thermal stress and/or salinities are likely to explain the periodic disappearance of *P. parvivipara* in many tide pools. Tide pools located on the margins of intertidal platforms are often associated with more variable biotic and abiotic conditions that can affect distributions and abundances of species (Sousa 1979, Metaxas & Scheibling 1993, Somero 2002, Harley 2008, Miller et al. 2009). This appears to be particularly true for *P. parvivipara* as disappearance events from tide pools were most frequent and persistent in the high intertidal zone, and in tide pools that were in elevated positions. Temporal variability in the habitability of many tide pools, combined with the absence of a discrete dispersive life-history stage, are likely to explain the highly dynamic distribution of *P. parvivipara* within each intertidal platform.

Increasing depth and structural complexity of tide pools were significant positive predictors of higher abundances of *C. hystera*, while decreasing area of tide pools had a significant negative effect on their abundance. Similarly, *C. hystera* were most likely to be present in tide pools that were deeper and more structurally complex, but also in tide pools that had a greater number of other organisms present. Thus, a combination of 3 of the 10 ecological variables examined had a significant positive effect on the distribution and abundance of *C. hystera* in their tropical intertidal habitat. Deeper tide pools may be favoured by *C. hystera* because they are less susceptible to completely evaporating compared to shallower pools, and are therefore more likely to provide a more consistently suitable habitat for *C. hystera* during low tide. In contrast, increasing area of tide pools negatively influenced the abundance of *C. hystera*. Although the larger pools may be less susceptible to drying out, their proximity in the intertidal zone was often closer to the low tide margin (i.e. the pavement interface), which receives more wave action during higher tide levels (Walker et al. 2008). Consequently, some of the larger tide pools inhabited by *C. hystera* may be exposed to greater levels of exposure to wave energy that may be responsible for lower abundances within these pools. While exposure to wave energy was not a significant predictor of *C. hystera* abundance in the analysis, tide pools sizes may be confounded with levels of wave energy. Direct measurements of wave energy within individual tide pools using chalk balls or clod cards would clarify this. Similarly, differences in wave exposure on the coral cay surrounding One Tree Island may contribute to the restricted distribution of *C. hystera* on only the eastern side of the coral cay. The distribution and abundance of *P. parvivipara* is negatively influenced by increased exposure to wave energy within the intertidal platforms it inhabits and it may therefore explain the lower abundances of *C. hystera* found in some tide pools.

High structural complexity of the coral rubble within the tide pools was a consistent predictor of high abundances of *C. hystera* among all populations. *C. hystera* was found on the underside of coral rubble or rocks stacked in multiple layers, which they appeared to utilise for shelter from daylight and exposure during low tide. The complex layers of rubble and rocks are likely to provide greater area for grazing, greater food resources, protection from predation, and protection from dislodgement from water movement during high tide, which may also explain their high abundances within these pools. These results are similar to those for *P. parvivipara*, although some predictors of abundance (wave exposure, presence of an anoxic layer and temperature) varied with season. Seasonal variability in the distribution and abundance of *C. hystera* could easily be investigated and would provide valuable information on the temporal variability, recruitment, and mortality events in tropical direct-developing species that inhabit intertidal environments.

C. hystera and *P. parvivipara* were found in tide pools located within the high intertidal zones, where the diversity and abundance of other organisms was low, but they were also found in tide pools in the mid and lower intertidal areas that were inhabited by a diverse array of macro-benthic species that included bryozoans, gastropods, hermit crabs, and ascidians. A characteristic of viviparous marine invertebrates is their distribution in the high zones of the intertidal areas and their ability to tolerate the harsh environmental conditions in these regions (e.g., salinity and temperature fluctuations) (Byrne et al. 2003). The presence of other organisms in tide pools was, however, a significant predictor of the presence of *C. hystera* suggesting that, while they can tolerate the harsh conditions in the high intertidal zone, their survivorship is higher and their presence is more likely in regions that also provide suitable habitat for a wide range of other organisms. Among tide pools the density of *C. hystera* ranged from 0.5 to 32 specimens per m^2 and 4 to 1852 for *P. parvivipara*, with individuals often found aggregated in within tide pools. The reasons for this aggregation are not clear, but may be related to behavioural patterns associated with reproduction or ecological factors that are were not apparent during the surveys. For example, the benefit of species aggregating in the intertidal zone to reduce desiccation stress has been documented in a variety of species (Garrity 1984, Chapman 1995, Chapman & Underwood 1996).

The mean CV in abundance of *P. parvivipara* in tide pools across the three populations was 104 % over the study period, compared to 80% for *C. hystera*. These values are below the mean CV of 134 % reported by Eckert (2003) for 39 other marine invertebrates with non-planktonic development and also fall outside of the 95 % confidence interval range of expected CV’s in abundance based on her data for species with non-planktonic development. Eckert (2003) found that species with a short dispersive pelagic larval phase had a mean CV in abundance of 88 %, while those with a long dispersive larval phase had a mean CV in abundance of 109 %. Thus *P. parvivipara* exhibited a mean CV in abundance in tide pools among populations that was 16 % higher but 5 % lower than species examined by Eckert (2003) that had short and long pelagic larval phases, respectively. While *C. hystera* exhibited a mean CV in abundance that was lower than the mean CV's derived by Eckert (2003) for species with any pelagic larval phase. Although species that contain dispersive pelagic larval phases may be faced with considerable risks of planktonic mortality (Underwood & Fairweather 1989, Pechenik 1999, Vaughn & Allen 2010), a dispersive larval stage generally appears to result in lower fluctuations in population abundance compared to species with direct development based on Eckert (2003). For *P. parvivipara* and *C. hystera*, however, this generality does not apply. The comparatively low CV in abundance within tide pools may be the result of regular successful recruitment of offspring via direct development into tide pools inhabited by *P. parvivipara* and *C. hystera.*

The mean CV of abundance of *C. hystera* was lower than the mean value derived for *P. parvivipara* (mean 80 versus 104 %). These differences may be partially explained by differences in the sampling protocol and the biological differences in reproductive periodicity of the species. The populations of *P. parvivipara* were examined four times in each of 3-years and the surveys therefore identified variability in population sizes that are attributable to a main recruitment pulse in spring. Moreover, additional variability in the *P. parvivipara* data is probably attributable to the fact that some individuals of this species reproduce at other times of the year (Chapter 4). Sampling of *C. hystera* populations was conducted over 2-years only and in the austral spring before the main period of births (Byrne et al. 2003) and therefore missed some of the variability in abundance that must be apparent in these populations. Ongoing investigations of *C. hystera* populations would provide valuable data on the population dynamics of a species with its relatively uncommon mode of reproduction in a tropical environment. Despite the differences in sampling protocols and differences in the reproductive periodicity of these species, similar patterns of abundance and distribution were found in both species.

The frequent disappearance and re-colonisation events of both species in tide pools within tidal platforms may indicate the existence of small-scale metapopulation dynamics operating at the scale of the tidal platform. The concept of marine metapopulation dynamics has been previously established in intertidal tide pools systems, where despite tidal flushing and water movement, separate dynamics are maintained within tide pools (see Johnson et al. 2001, McAllen & Taylor 2001, Grimm et al. 2003). Recent findings by Keever (2010) showed that populations of *P. parvivipara* and *C. hystera* exhibit high levels of genetic differentiation among populations that are geographically close (within 10 km), suggesting that genetic exchange via dispersal among populations is limited or no non-existent. However, dispersal of individuals among tide pools within populations must be common because frequent disappearances of specimens within tide pools are balanced by re-colonisation of tide pools, such that abundances at the population scale are stable. According to Grimm et al. (2003), a marine metapopulation exists when: 1) patches contain discrete local populations; 2) dynamics of populations are not synchronous; 3) occupants of patch face risk of local extinction; and 4) patches are linked by dispersal with the possibility of new populations becoming established where none currently exist. These species appear to meet these criteria because they inhabit discrete pools and the population dynamics among those pools differs in space and time, with extinction and re-colonisation being common place.

Three potential means by which *P. parvivipara* and *C. hystera* may disperse between tide pools include rafting on objects such as shell grit or sea grass, via dispersal from dislodgement and transport by wave action, or active movement via walking within or among tide pools. Dispersal via wave action has been observed in some intertidal marine invertebrates as a means of colonizing areas of the intertidal zone (De Magalhaes 1998, Thiel & Gutow 2005, Miller et al. 2007). For example, De Magalhaes (1998) found that the small littorinid *Nodilittorina lineolata* was passively transported via wave action to upper levels of the shore. Mechanisms of rafting are reported to assist dispersal in the sea star *Parvulastra exigua* (Waters & Roy 2003) and in brooding marine invertebrates that have limited dispersal abilities due to the absence of a dispersive larval stage (Highsmith 1985). Rafting on shell grit and wave action are likely means of dispersal that would explain the distribution patterns of *P. parvivipara* and *C. hystera*, but they have not been observed in the field. For *P. parvivipara*, the more persistent absence of specimens in tide pools in the high intertidal zone, which were observed to sustain large populations at some times over the study period, may be the result of infrequent transport of individuals to these areas that would be reliant upon particularly high tides and/or storm events.

The movement of sea stars via actively walking within and among tide pools is another potential mechanism of dispersal. During high tides sea stars may be able to move between tide pools that become interconnected or during low tide may migrate through wet areas in crevices or under boulders. In order to elucidate the existence of a metapopulation existing within each of the habitats of each species, direct evidence of dispersal is required in which individuals are tracked from one tide pool to another. Tagging large numbers of individuals, and tracking their position across the intertidal platform over time, or removal experiments that monitor recolonisation may provide means of determining the frequency and mechanisms of dispersal.

Storm events and tropical cyclones also have the potential to alter intertidal populations (Underwood 1999), and *P. parvivipara* and *C. hystera* may be vulnerable to these events. For example, in 2006 category 5 Tropical Cyclone Larry crossed the coast of northern Queensland resulting in the disappearance of an entire population of the intertidal sea star *Aquilonastra petagona* (M. Byrne pers. comm.). This finding supports previous suggestions by Pechenik (1999) that direct development of offspring is probably beneficial to species that inhabit patchily distributed habitat because it ensures local recruitment, but that this advantage is likely to be traded-off against a higher vulnerability to localised or widespread extinction resulting from environmental disturbances. The relatively high vulnerability to extinction of species with restricted distributions and direct development compared to species with wide distributions and dispersive life-history stages has been demonstrated in the fossil record (Hansen 1980, Jeffery & Emlet 2003, Crampton et al. 2010). Limited dispersal is thought to be beneficial for some species in which favourable habitats are rare or patchily distributed (Menge 1975, Palmer & Strathmann 1981). The highly distinct and patchily distributed habitats inhabited by *P. parvivipara* and *C. hystera* along small sections coastline in South Australia and Queensland respectively, may have been a strong selective pressure in the evolution of viviparity and direct development in these species or visa-versa. While direct development may reduce the likelihood that recruits are not lost to unfavourable habitats in which they cannot survive, this benefit likely comes at the cost of a high probability of extinction resulting from their reduced ability to escape rapid changes in existing habitats and the dynamic nature of their populations.

2.6 Tables

Table 2.1. Potential ecological predictors of the distribution and abundance of *P. parvivipara* and *C. hystera* recorded in each tide pool. Scales on which variables were measured are given. Scales of measurement for variables that do not have existing scales were defined *a priori* based on preliminary surveys.

Predictor variable	Measurement Scale	Definition
Salinity	‰	Measured in the centre of the pool
Temperature	^{o}C	Measured in the centre of the pool
pH	pH	pH units
Distance from Pavement	m	Taken from the discrete band where the rubble edge meets the pavement coral in the low intertidal zone (low tide margin).
Structural complexity of tide pool (scale: 1 – 6)	1	Smooth granite bottom; or few coral rubble/ rocks.
	2	Smooth granite bottom with few large boulders; or medium density coral rubble/ rocks on sand.
	3	Smooth granite bottom with few boulders of both small and large sizes; or dense layer of large and small sized rubble/ rocks on sand.
	4	Smooth granite bottom with boulders (both small and large) and coarse shell grit; or multiple and denser layers of large, mid and small coral rubble/ rocks, sand, shell grit, whole shells and/or a denser mix of smaller rubble /rocks.
	5	Smooth granite bottom with boulders, shell grit and sand.
	6	Denser mixture of rocks with layering, sand, shell grit, whole shells and / or denser mix of smaller rocks.
Tide pool size (scale: 1 – 5)	1	Small: < 5 m^2
	2	Small-Medium: 5-10 m^2
	3	Medium: 10-50 m^2
	4	Medium-Large: 50-100 m^2
	5	Large: > 100 m^2
Depth of tide pool	m	Derived from measurement of depth in deepest part of each tide pool.
Tide pool position (scale: 1 -2)	1	Elevated above the intertidal zone with water exchange facilitated by wave action. (i.e. tide pools that are on granite rocks elevated above the intertidal zone).
	2	Non-elevated – lies at the intertidal level with water exchange via tidal movement.
Source of water to tide pool type (scale: 1 – 3)	1	Discrete tide pool with water exchange via wave action
	2	Discrete water exchange via tidal movement.
	3	Non-discrete – tide pool is permanently connected to adjacent coastal water.
Anoxic layer in the	1	Anoxic layer present.

sediments at the base of tide pool (scale: 1 – 2)	2	Anoxic layer absent.
Exposure to wave energy (scale: 1 – 4)	1	Low – protected from wave energy.
	2	Medium - moderately exposed to wave energy, but energy largely dissipated by surrounding rocks.
	3	High - exposed but energy partly dissipated by surrounding rocks.
	4	Very high - constantly exposed to wave energy.
Number of other macro-invertebrate organisms present	Count of all specimens in quadrats	Combined number of macro-invertebrate specimens present in quadrats.

Table 2.2. Comparisons of the abundances of three populations of *P. parvivipara* (Smooth Pool, Point Brown and Point Whittlebee) and of intertidal zones (low, mid and high) at Smooth Pool across surveys using repeated-measures linear mixed models (auto-regressive [AR1] covariance structure). The abundance of *P. parvivipara* among intertidal zones at Smooth Pool, and the interaction between surveys and intertidal zones are similarly analysed.

Population/ Intertidal zone	**Source**	**df**	**MS**	***F***	***P***
Smooth Pool	Intercept	1	43.023	86.207	< 0.001
	Survey	11	279.226	8.738	**< 0.001**
	Intertidal zone	2	43.023	2.644	0.083
	Survey × intertidal zone	22	279.226	2.374	**0.001**
Pt Brown	Intercept	1	12.606	26.802	< 0.001
	Survey	11	87.578	0.560	0.856
Pt Whittlebee	Intercept	1	22.232	54.634	< 0.001
	Survey	11	73.538	3.799	**< 0.001**
Low	Intercept	1	21.270	50.758	<0.001
	Survey	11	136.219	1.301	0.230
Mid	Intercept	1	14.351	70.353	< 0.001
	Survey	11	78.358	6.251	**< 0.001**
High	Intercept	1	8.636	16.558	0.003
	Survey	11	57.399	4.114	**< 0.001**

Table 2.3. Two-way ANOVAs comparing the CV in abundance of three populations of *P. parvivipara* in tide pools over 3-years (Smooth pool, Point Brown and Point Whittlebee), and among years across intertidal zones at Smooth Pool.

Source	**df**	**MS**	***F***	***P***
Population				
Population	2	636.093	0.258	0.773
Year	2	1691.246	1.417	0.505
Population × year	4	1193.353	0.485	0.747
Residual	156	2461.143		
Intertidal zone				
Intertidal zone	2	17874.067	7.622	< **0.001**
Year	2	4652.342	2.251	0.144
Intertidal zone × year	4	2067.293	0.882	0.478
Residual	90	2345.145		

Table 2.4. Comparisons of the size structure of three populations *P. parvivipara* (Smooth Pool, Point Brown and Point Whittlebee) and of intertidal zones (low, mid and high) at Smooth Pool across surveys using repeated-measures linear mixed models (auto-regressive [AR1] covariance structure). The abundance of *P. parvivipara* among intertidal zones at Smooth Pool, and the interaction between surveys and intertidal zones are similarly analysed.

Population/ Intertidal zone	**Source**	**df**	**MS**	***F***	***P***
Smooth Pool	Intercept	1	87.076	9845.850	< 0.001
	Survey	11	184.622	6.453	**< 0.001**
	Intertidal zone	2	84.200	0.170	0.844
	Survey × intertidal zone	22	179.957	1.502	0.078
Pt Brown	Intercept	1	31.504	2088.917	< 0.001
	Survey	11	66.343	8.204	**< 0.001**
Pt Whittlebee	Intercept	1	13.681	2796.615	< 0.001
	Survey	11	39.333	5.596	**< 0.001**
Low	Intercept	1	40.827	6858.591	< 0.001
	Survey	11	86.499	4.955	**< 0.001**
Mid	Intercept	1	26.771	3833.410	< 0.001
	Survey	11	57.188	5.708	**< 0.001**
High	Intercept	1	13.770	1500.797	< 0.001
	Survey	11	30.603	1.309	0.267

Table 2.5. Two-way ANOVAs comparing seasonal changes in the CV in size-structures of three populations of *P. parvivipara*, and seasonal changes in the CV in size-structures across intertidal zones at Smooth Pool. Data from each survey were assigned their respective season for the analyses.

Source	**df**	**MS**	***F***	***P***
Population				
Season	3	95.890	9.861	< **0.001**
Population	2	82.719	8.506	**0.002**
Season × population	6	11.634	1.196	0.342
Residual	24	9.724		
Intertidal zone				
Season	3	66.722	3.122	**0.045**
Intertidal zone	2	63.556	2.973	0.070
Season × intertidal zone	6	11.551	0.540	0.772
Residual	24	21.374		

Table 2.6. Comparisons of the abundances of four populations of *C. hystera* over 2-surveys (across 2-years) using repeated-measures linear mixed models (auto-regressive [AR1] covariance structure).

Population	**Source**	**df**	***F***	***P***
Gutter	Intercept	31	25.903	< 0.001
	Survey	1	7.558	**0.010**
Two Tree	Intercept	31	21.779	< 0.001
	Survey	1	1.198	**0.282**
Entrance	Intercept	27	6.405	0.018
	Survey	1	0.465	**0.501**
Statue Bay	Intercept	27	16.425	<0.001
	Survey	1	5.791	**0.023**

Table 2.7. Comparisons of size structures of *C. hystera* of four populations of *C. hystera* over 2-surveys (over 2-years) using repeated-measures linear mixed models (auto-regressive [AR1] covariance structure).

Population	**Source**	**df**	***F***	***P***
Gutter	Intercept	31	1923	< 0.001
	Survey	1	3.55	0.075
Two Tree	Intercept	31	1688.609	< 0.001
	Survey	1	6.640	**0.019**
Entrance	Intercept	27	39.694	0.003
	Survey	1	7.305	0.054
Statue Bay	Intercept	27	3472.262	< 0.001
	Survey	1	2.054	0.190

Table 2.8. Two-way ANOVA comparing the CV in the size structure of *C. hystera* between surveys and among four populations (the Gutter, Two Tree, the Entrance and Statue Bay). NS = a non-statistically significant relationship.

Source	**df**	**MS**	***F***	***P***
Survey	1	17.510	5.751	0.096
Population	3	21.958	7.212	0.069
Survey × population	3	3.045	0.200	NS
Residual	7	13.217		

Table 2.9. Ecological variables identified by multiple linear regression analyses as seasonal predictors of the abundance of *P. parvivipara*. Abundance data were from surveys conducted in each season during 2008 and the data were pooled across populations in order to increase the power of the analyses. Variables that increased abundance are denoted (+), and those that decreased abundance are denoted (-).

Predictor variable	***t***	***P***
Summer		
Complexity (+)	4.114	**< 0.001**
Wave exposure (-)	- 2.114	**0.040**
Anoxic layer (-)	3.758	**< 0.001**
Autumn		
Depth (+)	2.338	**0.024**
Complexity (+)	5.172	**< 0.001**
Temperature (-)	-2.982	**0.005**
Winter		
Complexity (+)	5.717	**< 0.001**
Spring		
Complexity (+)	4.278	**< 0.001**
Wave exposure (-)	-2.348	**0.023**

Table 2.10. Ecological variables identified by multiple logistic regression analyses as seasonal predictors of the presence or absence of *P. parvivipara*. Presence/absence data were from surveys conducted in each season during 2008 and data were pooled across populations in order to increase the power of the analyses. Variables that predicted the presence of *P. parvivipara* are denoted (+), and those that predicted their absence are denoted (-).

Independent variable	**Coefficient**	**SE**	**Wald Statistic**	***P***	**Odds Ratio**	**5% Conf. Lower**	**95% Conf. Upper**
Summer							
Complexity (+)	1.572	0.607	6.704	**0.010**	4.818	1.465	15.842
Anoxic (-)	3.222	1.646	3.833	**0.050**	25.069	0.996	630.677
Autumn							
Complexity (+)	2.749	1.076	6.529	**0.011**	15.632	1.897	128.782
Temperature (-)	-2.530	1.271	3.964	**0.046**	0.0797	0.00661	0.961
Winter							
Complexity (+)	2.668	1.265	4.448	**0.035**	14.416	1.208	172.114
Anoxic layer (-)	3.327	1.551	4.604	**0.032**	27.849	1.334	581.580
Type (-)	-3.661	1.865	3.856	**0.050**	0.0257	0.000665	0.993
Spring							
Complexity (+)	10.201	5.027	4.119	**0.042**	26940.526	1.418	511806591.6

Table 2.11. Two-way ANOVAs comparing the mean temperatures and temperature ranges of tide pools at Smooth Pool in the low, mid and high intertidal zones across seasons. Temperature data were collected during each season (summer, autumn, winter and spring) over two years (2007-2009).

Source	**df**	**MS**	***F***	***P***
Mean Temperature				
Survey	6	78.625	150.653	< **0.001**
Intertidal zone	2	0.654	1.252	0.294
Survey × intertidal zone	12	0.831	1.593	0.123
Residual	52	0.522		
Temperature Range				
Survey	6	1.650	10.096	< **0.001**
Intertidal zone	2	0.860	5.263	**0.008**
Survey × intertidal zone	12	0.0749	0.458	0.930
Residual	52	0.163		

Table 2.12. Ecological variables that were identified as significant predictors of *C. hystera* abundance by multiple linear regression analyses (indicated in bold). Abundance data in tide pools were pooled across populations in order to increase the power of the analyses (*n* = 123). Variables that increased abundance are denoted (+), and those that decreased abundance are denoted (-).

Predictor variable	***T***	***P***
Distance from pavement	-1.574	0.118
Size (-)	-2.455	**0.016**
Depth (+)	3.825	**< 0.001**
Complexity (+)	3.222	**0.002**
Number of macro-invertebrate organisms present	1.933	0.056

Table 2.13. Ecological variables that were identified as significant predictors of the presence or absence of *C. hystera* by multiple logistic regression analyses (indicated in bold). Presence/absence data were pooled across populations in order to increase the power of the analyses (*n* = 123). Variables that predicted the presence of *C. hystera* are denoted (+), and those that predicted their absence are denoted (-).

Predictor	β	**s.e.** β	**Wald's** x^2	P	**Odds ratio**	**5 % Conf. Lower**	**95 % Conf. Upper**
Constant	-2.277	1.247	3.334	0.068	0.103	0.00819	1.182
Distance	-0.0175	0.0144	1.476	0.224	0.983	0.955	1.011
Size	-0.657	0.358	3.356	0.067	0.519	0.257	1.047
Depth (+)	30.335	11.663	6.765	**0.009**	1.495	1764	1.266
Complexity (+)	0.821	0.322	6.516	**0.011**	2.274	1.210	4.272
Other organisms (+)	0.0285	0.0131	4.767	**0.029**	1.029	1.003	1.056

Chapter 3

Variation in offspring size and brood size in the direct-developing sea star *Parvulastra parvivipara* across the intertidal environment

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3.1 Abstract

Offspring size of marine invertebrates differs substantially among and within species, and within individual broods. This variation in offspring size can have profound implications for all life-history phases, as well as evolutionary, population and community level processes. Recent evidence suggests that some marine invertebrates adaptively alter offspring phenotype (size) in response to the environmental conditions experienced by adults. Species that reproduce via direct development should have the greatest ability to predict the conditions that their offspring will experience because there is no decoupling between adult and offspring life-history stages, but relationships between environmental conditions and offspring phenotypes have not been extensively examined in these species. I examined variation in offspring size of the direct-developing sea star *Parvulastra parvivipara* among seasons, populations and across an extensive intertidal platform in which environmental variability is highest in the high intertidal margin of its distribution. I also examined the reproductive output of specimens among different population densities. The CV in offspring size within and among broods of *P. parvivipara* did not differ among intertidal zones (mean 36 %), suggesting that environmental conditions may not differ sufficiently across these zones to affect offspring size variability. *P. parvivipara* produced larger brood sizes and more variably sized offspring at higher densities in the field, but smaller broods and smaller, less variable, offspring sizes in the laboratory. The high CV in offspring size exhibited by *P. parvivipara* may be an adaptive bet-hedging response to conditions that are inherently variable across the intertidal environment. Individuals of *P. parvivipara* released offspring at different times

throughout the year and died soon after giving birth. However, the majority of births occurred over the spring and summer months and brood characteristics differed during these periods. Broods produced by *P. parvivipara* during spring were larger, and contained smaller and more variably sized offspring than broods produced by *P. parvivipara* during the summer. I propose that *P. parvivipara* may employ a complex interplay of bet-hedging strategies in which adults not only adaptively alter offspring phenotypes within broods in response to seasonal factors, but can also alter the timing of births in response to environmental conditions (i.e. plasticity in reproductive timing) and the characteristics of their brood at a particular time in order to maximise mean offspring fitness.

3.2 Introduction

Offspring size is perhaps the most important life-history characteristic of marine invertebrates because it influences fecundity (e.g. Marshall et al. 2003, Marshall 2005), pre- and post-settlement growth and mortality rates (e.g. Moran & Emlet 2001, Marshall et al. 2003, Marshall & Keough 2004b) and dispersal potentials (e.g. Havenhand 1993, Levitan 2000, Marshall & Keough 2003a), as well as population dynamics (e.g. Connell & Sousa 1983, Eckert 2003) and evolutionary processes (e.g. Emlet 1989, Jeffery & Emlet 2003). Among marine invertebrates, offspring size differs by more than an order of magnitude, even among some closely related clades in which species exhibit divergent reproductive strategies (e.g. Byrne 2006). Understanding the evolutionary ecology of this diversity requires comparative investigations of the fitness consequences of offspring size and how species allocate energy to offspring under different ecological conditions. To date, however, most studies have focused on comparisons among species that are confounded by our inability to control for the fact that they are different species with different evolutionary histories (see Harvey & Pagel 1991, Bernardo 1996, Marshall & Keough 2008a). While such studies have provided valuable insights into the evolutionary ecology of life-history strategies among marine invertebrates, it has recently become more widely appreciated that the considerable variation in offspring size that is apparent within species provides the opportunity to make comparisons without the confounding influence of species (Marshall & Keough 2008a).

Offspring size has been shown to vary considerably among populations of marine invertebrates (George 1994, 1996), among individuals within populations (Bingham et al. 2004), and within the broods of individual adults (Turner & Lawrence 1977, Marshall et al. 2000). The causes of this variation include genetic differences between individuals and non-genetic maternal effects (reviewed in Marshall et al. 2008a). Maternal effects are defined as those resulting from the maternal environment or phenotype on offspring phenotype (Marshall et al. 2008a). Sources of maternal effects on phenotypic variation in offspring size include maternal size (Sakai & Harada 2001), latitude (Lardies & Castilla 2001, Monaco et al. 2010), environmental quality (Gimenez & Anger 2001, Collin & Salazar 2010), and stress (George 1996, Steer et al. 2004).

There is also increasingly strong evidence to suggest that mothers can adaptively alter the size of offspring in response to the environmental conditions that they experience. Marshall and Keough (2009) showed that under high levels of intraspecific competition the bryozoan *Watersipora subtorquata* released larger offspring with greater dispersal capacity compared to offspring from mothers that were not exposed to competition. These researchers also demonstrated seasonal variation in the offspring size of *W. subtorquata*, in which mothers produce larger offspring during summer when a steeper relationship between offspring size and performance is evident (Marshall & Keough 2008b). Because the larval stage of *W. subtorquata* is short, the environment that they are released into is likely to be similar to that experienced by mothers. In these circumstances, mothers may be adaptively adjusting the phenotype of their offspring according to the environmental conditions that they experience.

While these studies suggest directional adaptive shifts in offspring size in response to specific ecological conditions, they still do not explain variation in offspring size that is often apparent within broods. However, recent modelling of the fitness consequences of offspring size under different environmental conditions by Marshall et al. (2008b) suggests that species may produce more variably sized offspring within broods in response to unstable environmental conditions in which mothers cannot predict the conditions that their offspring will experience. Their modelling shows that the production of variably sized offspring in unpredictable environments increases mean offspring fitness, and that within brood variation in offspring size could therefore also represent an adaptive response to prevailing environmental conditions.

Marshall and Keough (2008a) note that the most powerful inferences into relationships between environmental conditions experienced by mothers and the manner in which energy is consequently allocated to offspring within broods are likely to be provided by species in which there is no ecological decoupling between adult and offspring life-history phases. The diverse life-history strategies of marine invertebrates collectively form a continuum of variation in the degree to which parental energy is invested in individual offspring. The majority of marine invertebrates exhibit complex life-cycles in which sedentary adults reproduce via a small pelagic larval phase that is ecologically decoupled from the adult and undergoes metamorphosis following settlement (reviewed in Levin & Bridges 1995, Pechenik 1999). At the other end of the continuum of variation in life-history strategies among species are those with direct development, in which there is no decoupling phase between adults and their relatively large offspring (reviewed in Levin & Bridges 1995, McEdward & Janies 1997). Few studies of potentially adaptive variation in offspring size within broods of species with direct development have previously been made, but these species should have the greatest ability to predict the environment that their offspring will experience and hence to adjust offspring phenotype adaptively. Marshall and Keough (2008a) suggested that species in which there is a decoupling of the adult and offspring phases (albeit brief in some cases), and metamorphic rearrangement of the pelagic larval forms into the sedentary adults, may have a relatively limited capacity to adaptively adjust offspring size because of conflicting selection pressures between life-history stages. These authors also showed that the degree of variation in offspring size within broods of species with direct development are considerably higher than those of species with intervening larval stages, and suggest that direct developers should therefore have the greatest capacity to adaptively alter offspring size within broods in response to prevailing environmental conditions.

Consequently, species with direct development provide model species that can be experimentally exploited towards a better understanding of phenotypic variation in offspring characteristics within and among broods.

The viviparous, direct-developing sea star *Parvulastra parvivipara* inhabits seven small, geographically separated locations along a 200 km stretch of the west coast of the Eyre Peninsula, South Australia, where it is found on the underside of rocks within tide pools (Keough & Dartnall 1978, Roediger & Bolton 2008). The density of *P. parvivipara* within ostensibly similar tide pools varies dramatically within and among populations from only a few individuals to more than 1000 per m^2 (Roediger & Bolton 2008). *P. parvivipara* is one of only five sea stars known to exhibit viviparity and direct development and the hermaphroditic adults give birth to up to 36 non-dispersive juveniles (Byrne 1996, Roediger, unpublished data). Offspring also exhibit up to 5-fold differences in size within and among broods (Byrne 1996), thereby providing a strong basis for potentially adaptive shifts in offspring phenotype to ecological conditions.

The aims of this study were to determine: (1) seasonal variation in offspring and brood size within and among broods among three populations of *P. parvivipara* that differ in their spatial extent; (2) variation in offspring and brood size in relation to an environmental gradient across the extensive intertidal zone in one population; (3) relationships between the sizes of adults and those of their offspring and broods; and (4) relationships between population density and the sizes of offspring and broods. I examined variation in offspring size and brood size in three populations of *P. parvivipara* (Smooth Pool, Point Brown and Point Whittlebee) over a 2-year period. Each location is characterised by granite outcrops extending across the intertidal zone that encompass large numbers of tide pools, and form a complex, dynamic mosaic of environmental variation in which some pools are only ephemerally suitable for *P. parvivipara* (see Chapter 2). The three populations allowed a comparison of reproductive traits across localities that differ in their intertidal size and ecological variables. The intertidal zone at Smooth Pool is much more extensive than at the other locations: in a previous investigation examining the distribution and abundance of *P. parvivipara* I found that tide pools in the high intertidal zone appear to provide more variable environmental conditions than pools in the mid and low intertidal zones (see Chapter 2). Smooth Pool therefore provided the opportunity to examine relationships between the levels of environmental variation that *P. parvivipara* experiences across the intertidal platform and the phenotypic characteristics of their offspring and broods. I also examined the relationship between population density and the sizes of offspring and broods in *P. parvivipara* using a combination of field measurements that exploited naturally occurring differences in densities at Smooth Pool, and laboratory experiments in which densities were manipulated. The laboratory experiment allowed for a controlled investigation of the effect of density on brood characteristics that were independent of adult size and additional ecological factors present in natural tide pools (e.g. predation, wave exposure etc.).

3.3 Methods

3.3.1 Study populations

Three populations of *Parvulastra parvivipara* were examined along the west coast of the Eyre Peninsula, South Australia: Smooth Pool (32° 92′ S, 134° 07′ E), Point Brown (32° 53′ S, 133° 86′ E) and Point Whittlebee (32° 20′ S, 133° 73′ E) (Figure 1.4A).

3.3.2 Specimen collections

To investigate variation in the sizes of offspring and broods of *P. parvivipara*, adult specimens were collected from tide pools at each of the study populations four times a year (once per season) across 2-years from June 2007 to April 2009. Tide pools were randomly selected using numbered reference points that were superimposed on aerial photographs of the intertidal zone of each population (Smooth Pool n = 15, Point Brown n = 5, Point Whittlebee n = 5). In order to re-locate each tide pool over the study period, rocks adjacent to each pool were marked with red paint and their latitude and longitude were recorded using a GPS (Garmin GPSMAP 96). In July 2008, densities of *P. parvivipara* were determined within tide pools at Smooth Pool from counts made within four 25 × 25 cm (625 cm^2) quadrats that were haphazardly positioned in the 15 randomly selected tide pools.

The extensive intertidal platform at Smooth Pool was further used to investigate the influence of environmental variability across the intertidal zone on the sizes of offspring and broods. At Smooth Pool the large intertidal area was divided into low, mid and high intertidal zones based on iso-lines of immersion time throughout one tidal cycle (tidal range = 2.4 m). Intertidal zone boundaries were defined at 2-hour time intervals from low to high tide using photographs taken from an elevated position overlooking the intertidal platform (i.e. 0-hrs from predicted low tide = low zone margin, 2-hrs = mid zone margin, 4-hrs = high zone margin). All tide pools across the area were assigned a reference number within an intertidal zone on an aerial photograph, and five tide pools were then randomly selected within each intertidal zone using the methods outlined above. Ten specimens with a diameter greater than 4 mm were collected from each tide pool and preserved in 90 % ethanol. Specimens above this size were consistently reproductively mature (see Byrne 1996).

3.3.3 Measurements of offspring size and brood size

To examine relationships between the size of adults and the sizes of their offspring and broods, the radius of each specimen was measured along the axis from the tip of the longest arm to the middle of the stomach on the actinal surface using the ocular micrometer in a dissecting microscope (accuracy ± 0.01 mm). Adult specimens were subsequently dissected by cutting around the perimeter of the body with a scalpel to separate the upper and lower surfaces of their body cavities. The gonads were then removed from the body cavity and the offspring that they contained were dissected from them under the microscope. Offspring that were dissected from adults from each population throughout the year were able to feed, survive and grow in the laboratory. Juvenile specimens within the size range of dissected offspring were also released naturally by adults in the laboratory and also found in tide pools throughout the year (see Chapter 2): therefore the sizes of the juveniles obtained by dissection were considered to be within the size range that they would be naturally born at. Offspring dissected from each adult were counted (brood size) and their diameters were measured from the tip of one arm to the tip of the opposite arm using an ocular micrometer (accuracy ± 0.01 mm).

3.3.4 Density experiment

Adult *P. parvivipara* (*n* = 90, radius 3 – 4 mm) were collected from the underside of granite rocks in one tide pool at Smooth Pool in April 2009 and placed into separate vials containing sea-water for transport to the Lincoln Marine Science Centre, Port Lincoln, South Australia. Thirty small pink granite rocks (~ 5 × 5 cm) that are utilised by *P. parvivipara* for shelter and grazing substrata were also collected from the tide pool and transported to the laboratory in an insulated container. Each specimen was subsequently transferred into a separate 250 mL beaker containing 200 mL of sea-water. These containers were partially immersed in a flow-through aquarium system to maintain their temperature at that of ambient sea-water (~ 16^{o} C at the time of collection). Gentle aeration was supplied to each container through PVC tubing (inner diameter 0.8 mm; IsoflexKartell™) connected to the laboratory's air compressor. Specimens were maintained in the aquarium for 24-hours prior to transfer into the experimental treatments.

To examine the effect of density on offspring and brood sizes of *P. parvivipara*, three experimental densities were examined (120, 240, and 720 adults per m^2) that are within the natural range of densities recorded in tide pools at Smooth Pool (Chapter 2). These densities were equivalent to 1, 2, and 6 specimens per experimental container. Experimental containers were constructed from sections of PVC pipe (height = 8 cm, radius = 51.5 mm and volume = 667 mL). To allow water to flow through the experimental container while retaining the sea stars, one end of each section of pipe that formed their base was covered with nylon mesh (Nitex®, 200 µm pore size). PVC legs were attached to the base of each container (1 cm high) to allow water to flow underneath them. Two circular vents (40 mm diameter) were drilled on opposite sides of each container and were also covered with nylon mesh (200 µm) to facilitate additional water movement through the containers.

Because the planar area of tide pools was used to estimate densities of *P. parvivipara* in the field, I used the area of the base of the experimental containers (83.3 cm^2) to calculate experimental densities of *P. parvivipara*. The containers were immersed in a flow-through sea-water aquarium to a depth of 7 cm giving each container a water volume of 584 mL. Sea stars were randomly allocated to 30 containers at densities equivalent to 120, 240, and 720 individuals per m^2 (i.e. 1, 2, or 6 individuals per container, and *n* = 10 containers per density treatment). *P. parvivipara* feed on biofilms that develop on granite rocks (Keough & Dartnall 1978, Byrne 1996): therefore, to provide a food source for the sea stars I added a granite rock collected from Smooth Pool to each container (1 per container). The rocks also provided shelter from UV light during the day, thereby providing similar conditions to those that *P. parvivipara* experiences in natural tide pools. To further mimic natural tide pool conditions, the containers were randomly positioned in a flow-through sea-water aquarium (2 × 1 m, water depth = 8 cm, volume = 160 L) that was located outdoors in full sun.

All containers were randomly allocated to positions within the aquarium that received sunlight throughout the day so that no containers offered preferential conditions to the sea stars. The interspersion of treatments resulting from their random allocation to positions was visually examined and deemed acceptable because treatments were not clumped together (see Hurlbert 1984). Each container was separated from adjacent containers by 10 cm, and a lockable cage was placed over the aquaria to ensure that they were not disturbed. A continuous supply of filtered sea-water from the laboratories flow-through aquarium system maintained the exchange of sea-water. This allowed for the slow exchange of water but at a rate that still allowed the sea-water in the experimental tank to experience daily heating and cooling that was dependent on the daily and seasonal weather conditions.

Offspring released by adults were removed from each container using fine forceps or a pipette every four weeks between May 2009 and January 2010. The offspring were enumerated and their diameters determined using the methods given above. The containers and aquaria were cleaned every 4-weeks. In instances when adults died within treatments, the dead specimens were removed from the containers.

3.3.5 Statistical analyses

Two-way ANOVAs ($\alpha = 0.05$) were used to determine whether offspring size, brood size and adult size differed among populations and seasons. In these analyses population and season were fixed factors. Similarly, two-way ANOVAs ($\alpha = 0.05$) were used to examine whether offspring size, brood size and adult size differed among intertidal zones and seasons at Smooth Pool, where intertidal zone and season were fixed factors. The data from each year of the study were pooled to increase sample sizes within seasons as seasonal differences in parental investment in offspring were of primary interest. To compare variation in the size of offspring of *P. parvivipara* among populations and among intertidal zones at Smooth Pool independently of the mean specimen size, the coefficients of variation (CV) of offspring size (SD / mean × 100) were calculated for broods obtained from individual adults and among broods from all adults. Two-way ANOVAs were used to compare the CV in offspring size from individual adults among seasons and populations and among seasons and intertidal zones. Population, intertidal zones and season were analysed as a fixed factors. Bonferroni pair-wise post-hoc comparisons ($\alpha = 0.05$) were conducted where significant differences were detected by ANOVA.

Relationships between the sizes of adults and the sizes of their offspring and broods were examined using linear regression analyses. The relationship between offspring size and brood size were similarly investigated. Linear regression analyses were also used to examine relationships between adult size and the CV in offspring size within broods and among adults. In order to increase the power of these comparisons, data from all populations were pooled. To ensure that the data met the assumptions of parametric analysis, distributional normality and homogeneity of variances were examined prior to analyses using quartile plots and Levene’s tests. In cases where these assumptions were violated, outliers were removed (i.e. brood sizes ≥ 25), or square-root transformations of the data were conducted to alleviate the violation.

Relationships between the density of adults in tide pools (i.e. the unit of replication) at Smooth Pool and the sizes of adults, offspring and broods in July 2008 were examined using linear regression analyses (SigmaPlot v 11.0). The coefficients of variation (CV) in offspring size within and among broods were calculated in order to normalise the effect of adult size on offspring size (see Chapter 4). Linear regression analyses were used to examine relationships between the density of sea stars and the coefficient of variation in offspring size within and among broods.

One-way ANOVAs ($\alpha = 0.05$) were used to determine whether the density of individuals in the experimental containers affected offspring size, mean brood size, and the CV in offspring size among treatments. To ensure that the data met the assumptions of parametric analysis, their distributions and homogeneity of variances were examined prior to analyses using quartile plots and Levene's tests. In cases where these assumptions were violated, the data were square-root or log transformed to meet these assumptions.

3.4 Results

3.4.1 Adult size

The average size of *P. parvivipara* collected from tide pools was greatest during the spring surveys (i.e. during September and October) across all populations (Figure 3.1A). There was a small but significant increase in the average size of specimens between the winter and spring surveys (Figure 3.1A). This was followed by another small but significant decrease in the average size of specimens between the spring and summer (January) surveys that ranged across 7 % (0.22 mm) at Smooth Pool to 6 % (0.19 mm) and 9 % (0.24 mm) at Point Brown and Point Whittlebee, respectively (Figure 3.1A). Differences in the sizes of *P. parvivipara* collected from tide pools were found among the three populations, and among intertidal zones within the population at Smooth Pool. On average across seasons (i.e. pooled data), specimens of *P. parvivipara* collected at

Smooth Pool were significantly larger than those at Point Brown and Point Whittlebee (~10 % and 18 %, respectively) (Table 3.1, Figure 3.2A). The size range of *P. parvivipara* collected at Smooth Pool was also greater than that of those at Point Brown and Point Whittlebee (Table 3.2).

Within the population at Smooth Pool, the average size of specimens collected from tide pools across seasons in the mid intertidal zone was significantly larger than those in the low and high intertidal zones (by ~10 % and 5 %, respectively) (Table 3.2D). However, the size range and CV in the sizes of specimens in the low intertidal zone was approximately 17 % greater than those of specimens in the mid and high intertidal zones (Table 3.2). Two-way ANOVA and subsequent post-hoc tests showed that while the average sizes of *P. parvivipara* did not differ between the low and high intertidal zones during autumn, winter and spring, those in the high intertidal zone were significantly larger than those in the low intertidal zone during the summer (Table 3.3, Figure 3.3A).

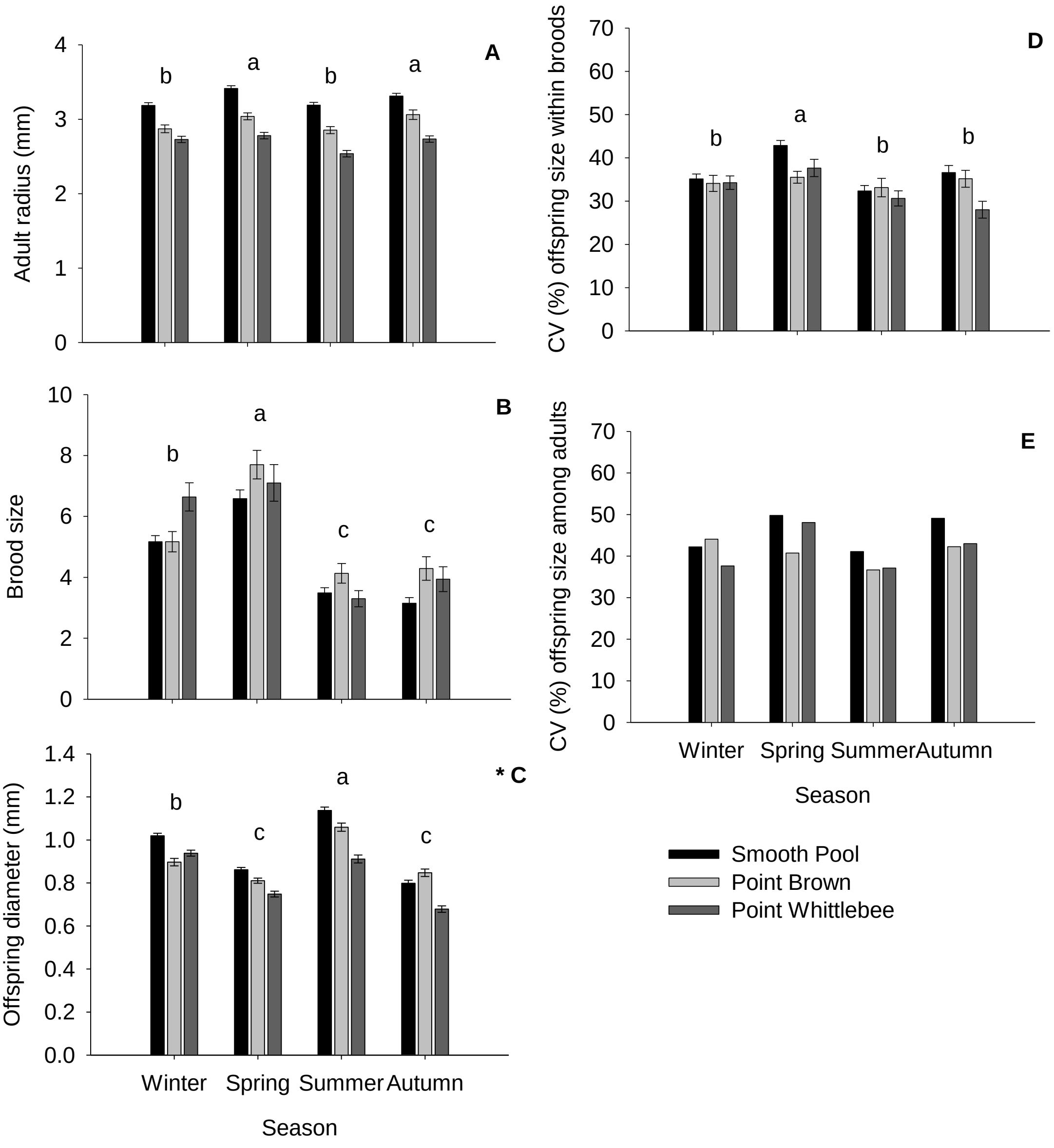

Figure 3.1. Seasonal differences in adult sizes (mean ± SE, *n* = 281- 460) and the sizes of offspring and broods in three populations of *P. parvivipara*: (A) adult size; (B) brood size; (C) offspring size; (D) CV in offspring size within broods; and (E) CV offspring size among adults. Letters denote significant differences among seasons averaged across populations (i.e. main effect only) that were identified by two-way ANOVAs. *Denotes a significant interaction detected between season and population.

3.4.2 Brood size

Offspring that were dissected from adults from each population throughout the year were able to feed, survive and grow in the laboratory for periods exceeding a year. Thus, *P. parvivipara* produced viable offspring throughout the year. Brood sizes among populations were largest during spring (mean ± SE 6.9 ± 0.2), and smallest during summer (3.6 ± 0.1) and autumn (3.6 ± 0.2) (Figure 3.1B). Brood sizes decreased by 52% between the spring surveys and those conducted in summer or autumn. These results indicate that while a peak in births of *P. parvivipara* occurs during spring, reproduction in each population is spread throughout the year (Figure 3.1B). The mean brood size of *P. parvivipara* across populations was 4.93 ± 0.09 and ranged from 0 to 36 (Table 3.2). Brood sizes were not significantly different among populations despite the larger average size of specimens sub-sampled from Smooth Pool (Table 3.1, Figure 3.2B). Linear regression analyses showed a significant positive relationship between adult size and brood size (Table 3.4, Figure 3.4A).

On average, brood sizes within Smooth Pool were significantly larger in the low intertidal zone compared to the high intertidal zone, but brood sizes in the low and high intertidal zones did not differ significantly from those in the mid intertidal zone (Table 3.3, Figure 3.2E). Brood sizes were significantly larger in spring compared to other seasons across all intertidal zones (Figure 3.3B). However, a significant interaction was detected between intertidal zone and season, which showed that brood sizes were significantly larger in the low intertidal zone than those in the high intertidal zone during all seasons apart from spring (Table 3.3, Figure 3.3B).

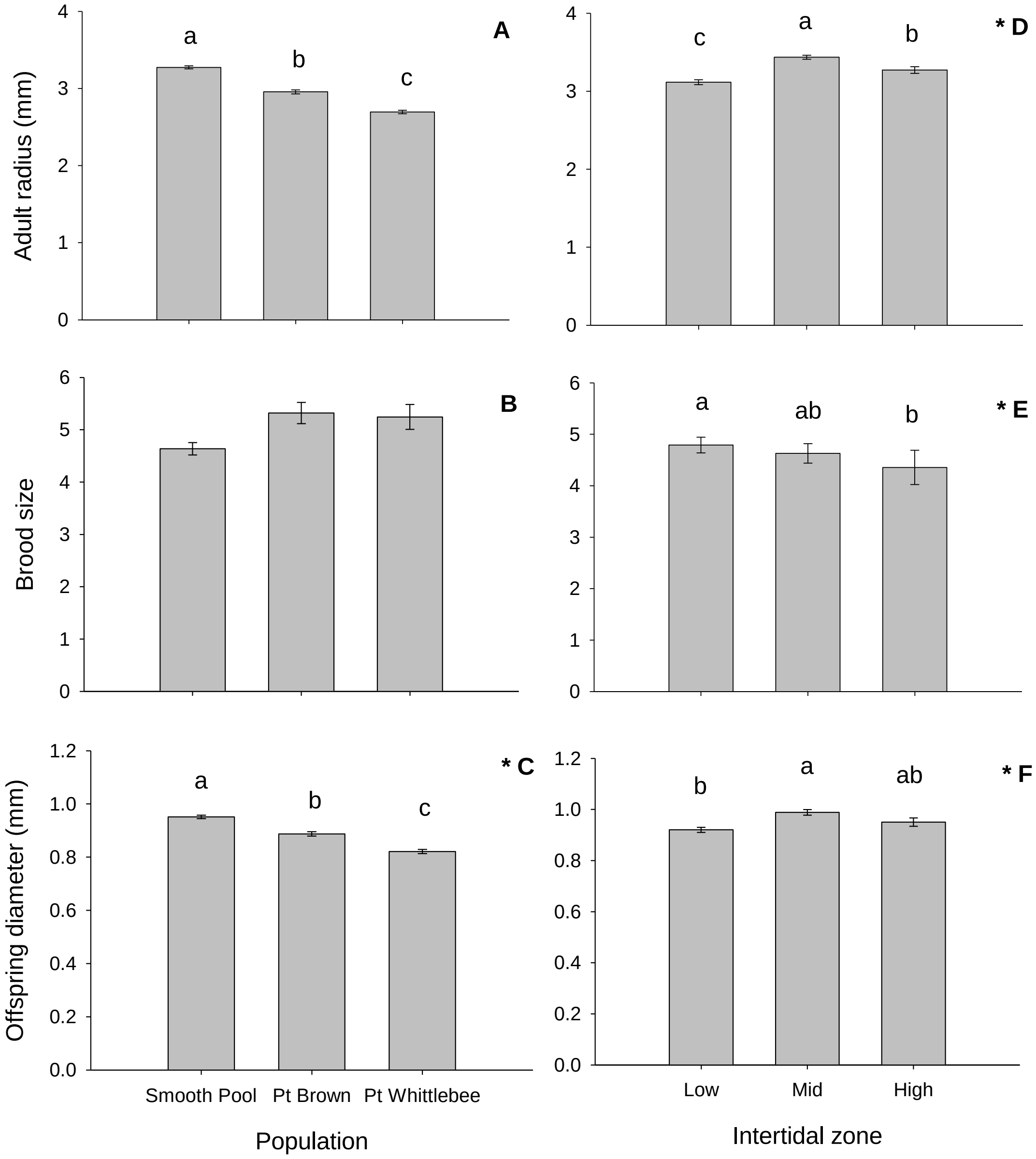

Figure 3.2. Mean (± SE, *n* = 127 - 992) (A) sizes of adults, (B) broods, and (C) offspring of three populations of *P. parvivipara*, and (D, E & F) among intertidal zones at Smooth Pool. Data were pooled across seasons. Letters denote significant differences among populations averaged across seasons (i.e. main effect) that were identified by two-way ANOVAs (so all bars having the letter 'a' means a NS result). *Denotes significant interactions between seasons and populations, and between seasons and intertidal zones that were identified by ANOVA (no letters above bars means a NS result).

3.4.3 Offspring size

The average size of *P. parvivipara* offspring was 0.90 ± 0.01 mm, and individuals ranged from 0.2 mm to 3.55 mm in diameter (range = 3.35 mm) among populations (Table 3.2). Mean offspring size was significantly larger in summer (1.07 mm ± 0.01) than in other seasons, and smallest during autumn (0.78 ± 0.01) (Table 3.1, Figure 3.1C). Pooled data across seasons showed that offspring from Smooth Pool were significantly larger on average than those from Point Brown and Point Whittlebee, while those from Point Brown were significantly larger than those from Point Whittlebee (Figure 3.2C). A significant interaction was detected between population and season, which showed that offspring from Smooth Pool were larger on average than those from Point Brown during winter, spring and summer, but no difference in offspring size was apparent between these populations in autumn (Figure 3.1C). Linear regression analyses showed a significant but weak positive relationship between adult size and mean offspring size (Table 3.4, Figure 3.4B), and a weak, but significant negative relationship between brood size and mean offspring size (Table 3.4, Figure 3.4C).

Offspring from the mid intertidal zone of Smooth Pool were significantly larger on average than those from the low intertidal zone, but offspring size in the low and mid intertidal zones were not significantly different to those in the high intertidal zone (Figure 3.2F, Table 3.3). Mean offspring size was significantly larger during the summer than during the other seasons (Figure 3.2F, Table 3.3). Mean offspring size also differed seasonally among intertidal zones: during winter and summer offspring were larger in the mid than in the low intertidal zone but no significant differences were apparent among intertidal zones during spring (Table 3.3, Figure 3.3C).

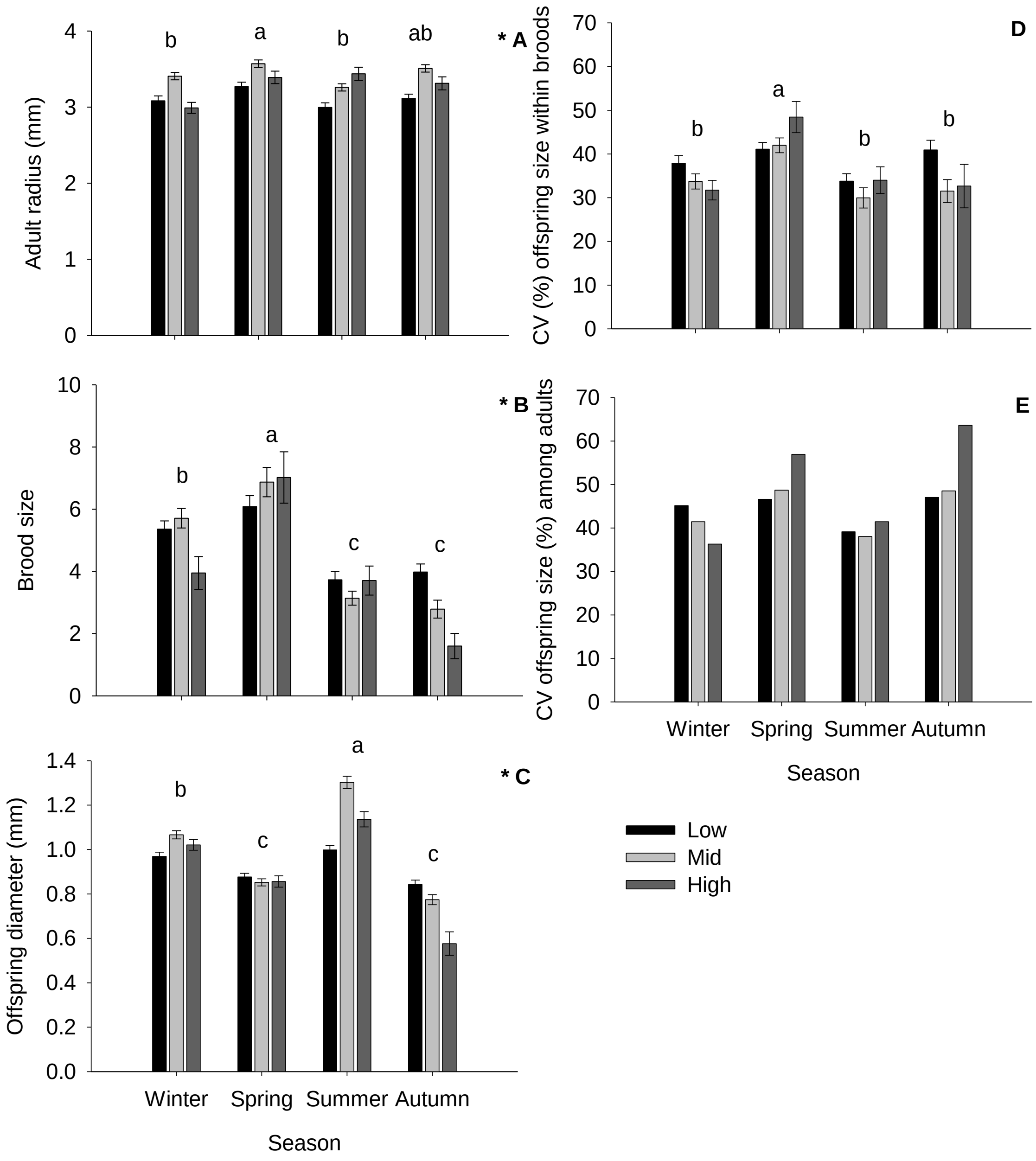

Figure 3.3. Seasonal changes in mean (± SE, *n* = 150 - 260) adult sizes of *P. parvivipara* and the sizes of their offspring and broods among intertidal zones at Smooth Pool: (A) adult size; (B) brood size; (C) offspring size; (D) CV in offspring size within broods; and (E) CV offspring size among adults. Letters denote significant differences among seasons averaged among populations (i.e. main effect only) that were identified by two-way ANOVAs. *Denotes significant interactions between seasons and intertidal zones.

3.4.4 Coefficient of variation in offspring size

The coefficient of variation (CV) in offspring size within the broods of individual adults was significantly greater during spring compared to the other seasons (Figures 3.1D, 3.3D). Between the winter and spring surveys, the CV in offspring sizes increased by 8 % at Smooth Pool, 2 % Point Brown and 3 % at Point Whittlebee (Figure 3.1A). The average CV in offspring size within broods across all seasons was 36 % (± 0.5), and ranged from 0 % to 104 % among individuals (Table 3.2). Differences in the CV in offspring size were also apparent among populations of *P. parvivipara* and among intertidal zones within the population at Smooth Pool (Table 3.1, 3.3). The CV in offspring size was significantly higher at Smooth Pool than Point Whittlebee, but no difference was apparent between Point Brown and the other populations (Figure 3.5A). Within the population of *P. parvivipara* at Smooth Pool, the CV in offspring size across all seasons was significantly lower in the mid intertidal zone than in the low intertidal zone, but the CV in offspring size in the high intertidal zone was not significantly different to those in the mid and low intertidal zones (Figure 3.5B). No interactions were detected between populations and seasons or between seasons and tide pool location within the intertidal zone (Tables 3.1, 3.3). Linear regression analysis showed significant but weak, positive relationship between brood size and the CV in offspring size within broods (Table 3.4, Figures 3.4D).

The CV in offspring size among broods from all adults was consistently greater than the CV in offspring size within broods from individual adults across all populations and intertidal zones, (Figures 3.5A, 3.5B). The CV in offspring size among adults ranged from 50 % at Smooth Pool in spring to 36 % at Point Brown in summer (Figure 3.1E). The CV in offspring size among broods in the high intertidal zone at Smooth Pool ranged from 63 % in autumn to 36 % in winter (Figure 3.3E).

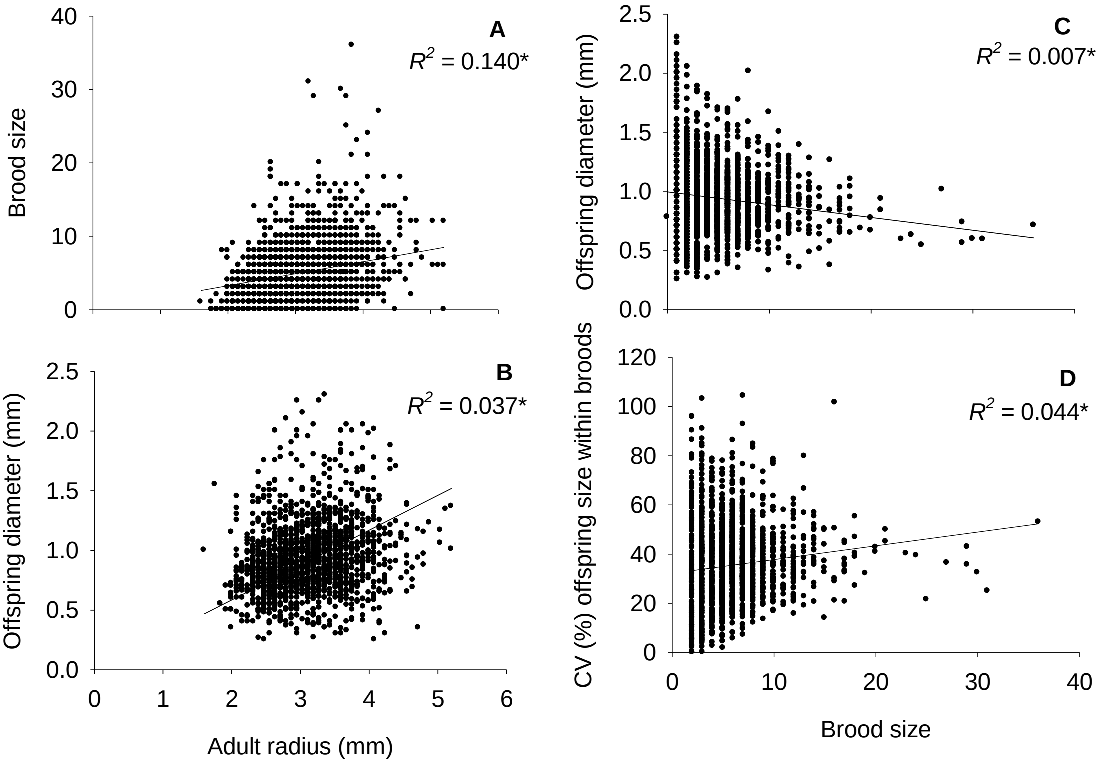

Figure 3.4. Linear regression analyses (n = 1417-1785) of adult sizes and (A) brood sizes and (B) mean offspring sizes. Relationships between brood sizes and (C) mean offspring size, and (D) the CV in offspring sizes within broods were similarly analysed. NB: while regression analyses showed significant relationships ($*P < 0.05$) in all of these comparisons, the low coefficients of determination indicate that the models explain little (i.e. < 1 to 14 %) of the observed variation. Lines on plots represent the regression equations.

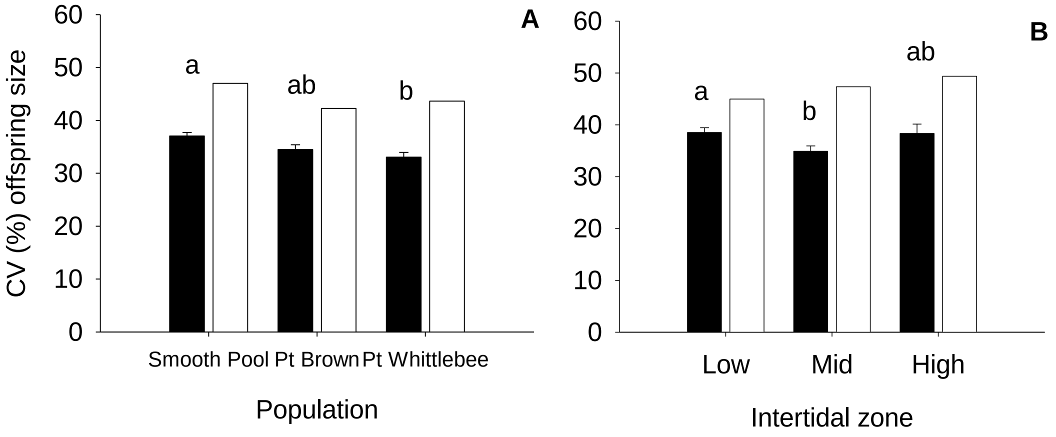

Figure 3.5. Mean (± SE, *n* = 127 - 793) coefficients of variation (CV) in offspring size within broods (black bars; letters show main effects of population [A] or intertidal zone [B] on CV offspring size within broods) and among adults (white bars) among (A) three populations of *P. parvivipara*, and (B) among intertidal zones at Smooth Pool.

3.4.5 Adult density and sizes of offspring and broods

The density of *P. parvivipara* in tide pools at Smooth Pool ranged from 4 to 572 specimens per m^2 during July 2008. The average size of adult *P. parvivipara* was significantly greater in tide pools where they were present at higher densities than lower densities (Table 3.5, Figure 3.6A). Linear regression analyses also showed that there was a significant positive relationship between the density of *P. parvivipara* and their brood sizes (Table 3.5, Figure 3.6C), and between density and the coefficient of variation (CV) in offspring size within broods (Table 3.5, Figure 3.6D). However, the density of *P. parvivipara* in tide pools did not significantly affect the mean size of offspring produced (Figure 3.6B), or the CV in offspring size among adults.

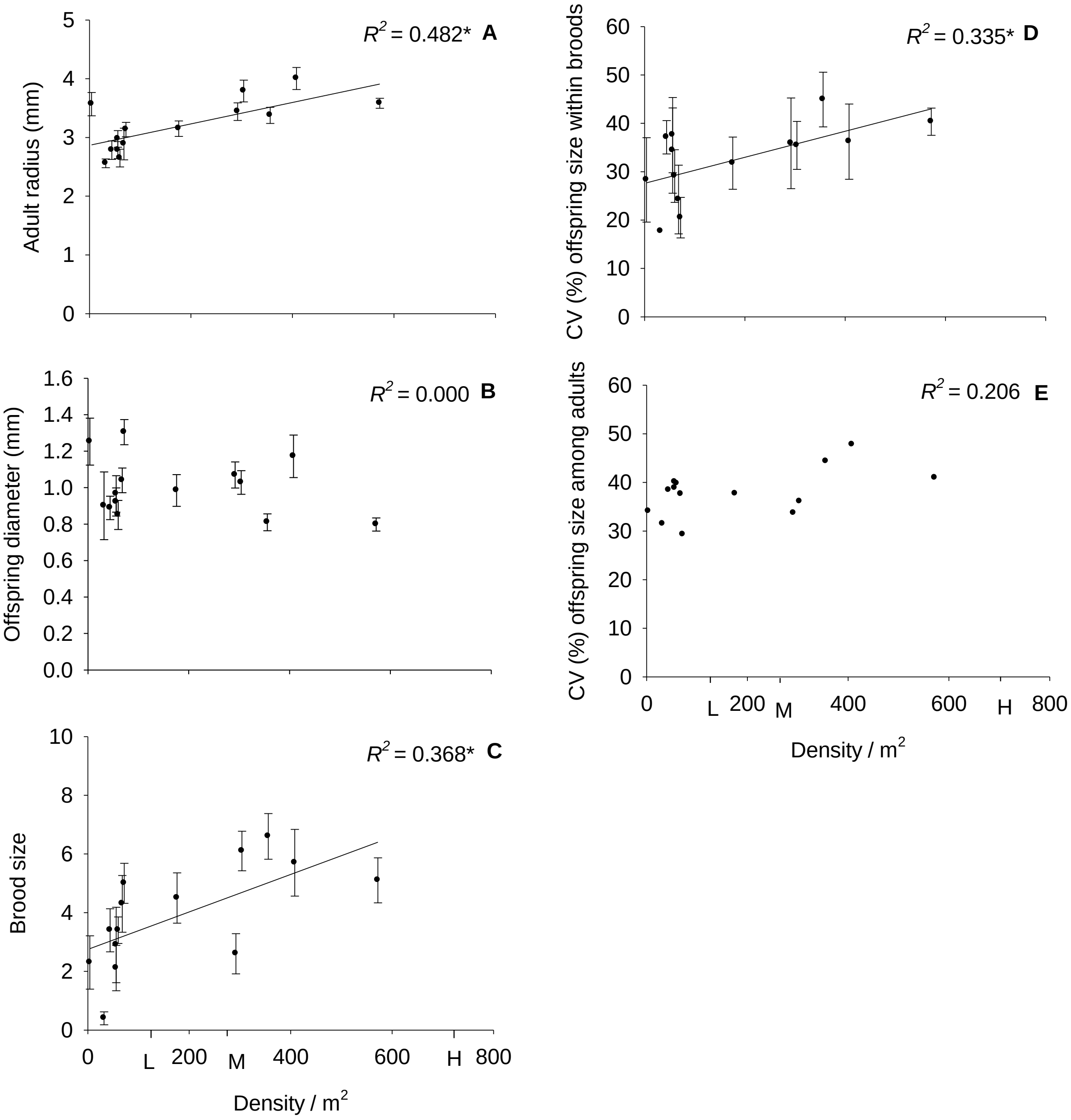

Figure 3.6. Linear regression comparisons of the relationships between the density of *P. parvivipara* in tide pools (n = 14) at Smooth Pool and the mean ± SE (A) adult size (n = 10), (B) offspring size, (C) brood size, (D) CV in offspring size within broods, and (E) CV in offspring size among adults. Letters on x-axis represent the experimental treatment densities examined: low = 120 /m^2, medium = 240 /m^2, and high = 720 /m^2. * indicates P < 0.05. Lines on plots represent significant regression equations.

3.4.6 Density experiment

The diameter of offspring released by adults ranged from 0.6 to 3.0 mm. The average CV in offspring size was 22.73 ± 0.81 % and there were no differences apparent among the three treatment densities (P = 0.206) (Figure 3.7C). There was a significant inverse relationship between the density of *P. parvivipara* and the size of offspring that they produced (ANOVA: $F_{2, 27}$ = 17.323, MS = 0.263, $P < 0.001$). The average diameter of offspring in the low-density treatment was 7 % and 19 % bigger than those in the medium and high-density treatments, respectively (Figure 3.7A). Tukey's post-hoc comparisons showed that offspring produced in the high-density treatment were significantly smaller than those in the low and medium-densities, while offspring released from adults in the low-density were significantly larger than those in the medium-density treatment (Figure 3.7A).

ANOVA and subsequent Tukey's tests showed that the average sizes of broods released by *P. parvivipara* in the low and medium-density treatments were also significantly larger than those in the high-density treatment, but that no difference was apparent between the low and medium-density treatments (ANOVA: $F_{2, 27}$ = 11.285, MS = 0.527, $P < 0.001$) (Figure 3.7B).

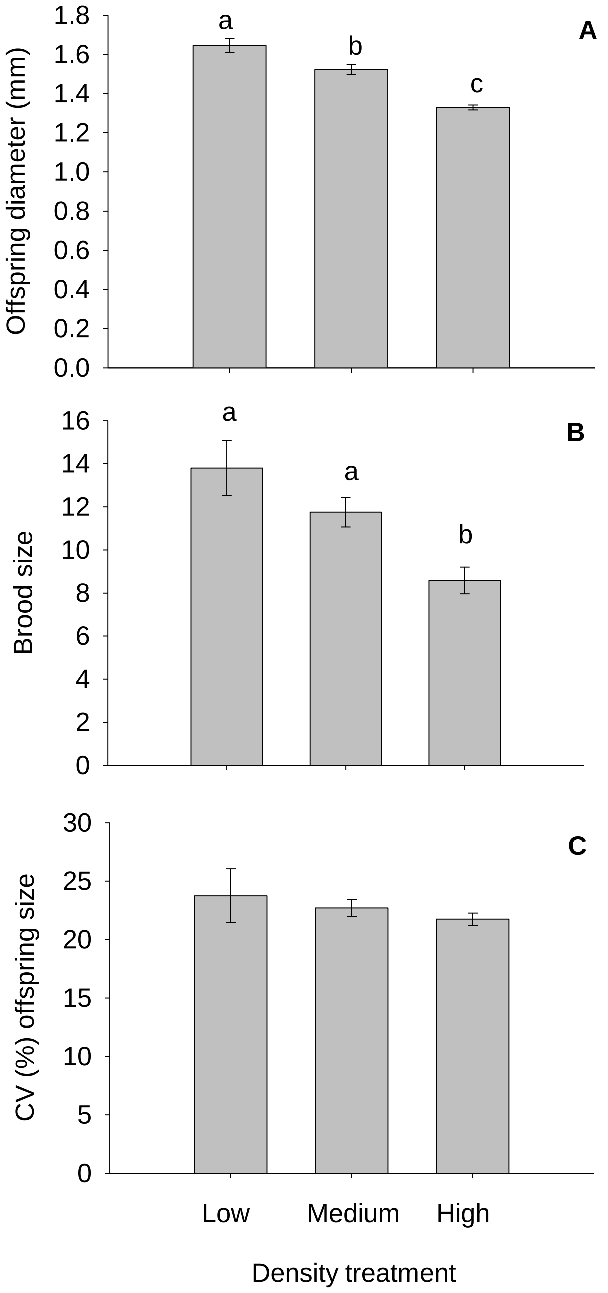

Figure 3.7. Mean (± SE) (A) offspring sizes, (B) brood sizes, and (C) CV's in offspring sizes among treatments of *P. parvivipara* (*n* = 10) that were maintained at three densities in the laboratory. Treatment densities were low = 120 /m^2, medium = 240 /m^2, and high = 720 /m^2. Letters above bars denote significant differences among treatments that were identified by ANOVA (no letters above bars means a NS result).

3.5 Discussion

There is increasingly strong evidence to suggest that some marine invertebrates adaptively alter offspring phenotype (especially size) in response to the environmental conditions experienced by adults (Allen et al. 2008, Marshall & Keough 2008a, Marshall & Keough 2009, Monro et al. 2010), and that under conditions where environmental conditions fluctuate unpredictably, the production of variably-sized offspring within a brood increases mean offspring fitness (Marshall et al. 2008b, Crean & Marshall 2009). My results suggest that variation in offspring size of *P. parvivipara* does not increase with the level of environmental variability across an intertidal platform, and therefore tends to not support the notion that this represents an adaptive bet-hedging strategy to maximise offspring fitness in more unpredictable environmental conditions within populations. However, across populations and seasons, differences are apparent in both brood sizes and offspring sizes that may indicate a bet-hedging strategy based on offspring size variation. Offspring size within broods was more variable during spring, which also coincides with when temperature conditions within tide pools become more variable (see Chapter 2, Figure 2.7). Perhaps more importantly, the data suggest that *P. parvivipara* may employ a much more complex interplay of bet-hedging strategies in which adults not only alter offspring phenotypes within broods in response to seasonal environmental factors, but that each adult may alter the timing of births in response to environmental conditions to adaptively match the phenotypic characteristics of their brood.

The density of *P. parvivipara* in tide pools at Smooth Pool, South Australia ranged from 4 to 572 sea stars per m^2 during July 2008, and there was a positive relationship between density and both the size of broods and the CV in offspring size within broods. However, no relationship was apparent between density and the mean size of offspring produced by *P. parvivipara* in the field. In contrast, *P. parvivipara* held at higher densities in the laboratory produced smaller offspring and smaller broods than at low densities, but there was no relationship between density and the CV in offspring size within treatments.

Unlike many marine invertebrates, *P. parvivipara* adults are capable of giving birth to viable broods of offspring at any time of the year. Adults across a wide range of sizes within each of the populations examined released offspring during all seasons (see Chapter 2). However, a distinct increase in the number of births and presence of offspring within populations was observed between the spring and summer collections in each population in each year of this study (Chapter 2). Therefore, the data suggest that while the release of offspring during spring is likely to be optimal, adults may be induced to give birth at other times of the year for reasons that could be genetically predetermined and/or the result of some environmental cue. My observations of large numbers of *P. parvivipara* that were held in aquarium systems for periods exceeding a year indicate that changes in environmental conditions can act as a cue for some adults to give birth. For example, sudden changes in temperature and salinity that are not lethal to *P. parvivipara* were observed to induce the release of offspring by some adults of widely-varying sizes (Roediger, unpublished data), suggesting that while the timing of births are linked to environmental cues, the response is not uniform across adults, nor linked to differences in their sizes. Testing whether the timing of offspring release affects offspring fitness in the field is the next step to determining the adaptive nature of the differences in the reproductive characteristics displayed by *P. parvivipara* among seasons and populations.

The implications of these patterns of births may be further complicated if the effects of environmental cues on the timing of births are related to the phenotypic characteristics of broods held by adults at that particular time. In other words, the mean fitness benefit of giving birth in response to an environmental cue may differ among adults according to the phenotypic characteristics of their brood at that point in time. Data presented here show that across all populations and across intertidal zones at Smooth Pool, the coefficient of variation (CV) in offspring size among adults is consistently greater than the variation within broods from individual adults. Therefore, there are clear differences in the characteristics of the broods of adults throughout the year and across populations, providing a basis for potential variation in the timing of births according to brood characteristics and environmental conditions. Further insights into these potential relationships are beyond the scope of the data presented here: experiments are required in which likely environmental cues for inducing birth are manipulated and compared to the phenotypes of broods released by some adults and the broods of other adults that did not give birth. The range of potential environmental cues for inducing births in adults are large, but based on previous investigations into determinants of the distribution and abundance of *P. parvivipara* (Roediger & Bolton 2008, Chapter 2), changes in temperature and salinity are logical starting points for future manipulative investigations.

The extensive intertidal platform at Smooth Pool provided the opportunity to examine how characteristics of broods of *P. parvivipara* varied among the high, mid and low intertidal zones, across seasons and among tide pools that varied in the density of *P. parvivipara*. The level of environmental variability differs among the intertidal zones, and is reflected in variation in the physical characteristics of tide pools and dynamics of the distribution and abundance of *P. parvivipara* previously reported (Chapter 2). In the high intertidal zone, the physical characteristics of tide pools and the distribution and abundances of *P. parvivipara* are highly variable. Indeed, *P. parvivipara* disappears from many of the tide pools in the high intertidal zone for extended periods and, while they subsequently recolonise them, there is no clear periodicity to these events or consistency in these patterns among tide pools (Chapter 2). Despite this, the results show that across all seasons the CV in offspring size does not differ between the high or low and mid-intertidal zones, as would be expected if *P. parvivipara* produced more variably sized offspring within broods in more unpredictable environments. This was unexpected because abundances are highest in the mid-intertidal zone, and this intertidal zone appears to provide the most consistently suitable habitat for them (Chapter 2).

In additional research (Chapter 5), I demonstrated that small *P. parvivipara* offspring exhibit similar survivorship to large offspring in benign and relatively stable environments, but that larger offspring have greater survivorship under more variable environmental conditions. Other researchers have predicted that the production of smaller offspring, rather than fewer large offspring in favourable conditions should maximise fitness (Smith & Fretwell 1974, Parker & Begon 1986), and the relationship between offspring size, survivorship and environmental conditions found in *P. parvivipara* support this prediction. Tide pools containing low densities of *P. parvivipara* appear to represent relatively unfavourable habitats compared to tide pools containing high densities (Roediger and Bolton, 2008; Chapter 2). Therefore, a reasonable prediction based on the relationships between offspring size and survivorship in *P. parvivipara* is that adults should produce larger offspring in the less favourable and less densely populated tide pools.

However, the results obtained from the field showed that offspring produced by adults at lower densities were the same size as those produced by adults from tide pools containing high densities. Brood sizes were higher in tide pools where high densities of the sea stars were present, suggesting that resource limitation in less favourable and less populated tide pools may be a determinant of the patterns observed here. Alternatively, there may have been demographic age differences among tide pools which may have affected adult sizes and consequent offspring size variability or brood size relationships, or larger sized adults present in the higher density tide pools may have led to potential confounding and greater reproductive output for size related or genetic reasons. The higher CV in offspring sizes in broods from adults in high-density pools is more likely to be a by-product of their larger brood sizes than any adaptive bet-hedging through the production of variable offspring sizes in response to variable environmental conditions because all tide pools are presumably subject to the same levels of environmental variability (Chapter 2). Field based experiments in which the habitat quality of tide pools and the density and size of *P. parvivipara* are manipulated are needed to determine whether the results obtained here are a consequence of differences in resource availability among tide pools. Such experiments should also use newly born offspring as experimental models rather than adults as this would eliminate any potential confounding influence of an adult’s previous environmental experience on its offspring characteristics, which may have been apparent in my experiments.

The density of *P. parvivipara* in tide pools in the field differed in its effect on brood characteristics to those observed among densities in controlled laboratory conditions. The laboratory experiments were highly controlled and the results are therefore more likely to reflect density-mediated effects on offspring and brood sizes than the field experiments. The production of smaller offspring and broods at higher densities in the laboratory could be the result of either density-mediated food limitation through grazing on the limited substrata available (rock), or of a density-mediated shift in how mothers allocate energy to offspring. However, as increasing density resulted in a reduction in offspring and brood size, density-mediated resource limitation is the most likely reason for these results because brood size should have remained the same or increased if resources were not limited. There is, however, a potential confounding effect between the lowest density and the other densities as no other specimens were present in the low-density containers. Despite any potential confounding that may have arisen due to the absence of other specimens, the density comparison is still valid between the medium and high densities and does not change the results.

There are at least several possible explanations for the patterns in offspring size and variability observed among populations and among intertidal zones. First, the definition of environmental variability based on the physical characteristics of tide pools across the intertidal zone, and the patterns of distribution and abundance of *P. parvivipara* reported previously, may not be important determinants of potential adaptive bet-hedging in offspring size in this species (e.g. temperature range). A potential factor may include the complexity of the habitat itself, which may in turn drive adaptive variation in offspring size in response to the potentially greater number of variably sized physical spaces in shell grit and other substratum that they inhabit.

Second, any potentially important differences in offspring size that may indicate bet-hedging are at least partially confounded by differences in the mean size and CV of size of adults in different areas of the intertidal zone. Data reported here suggest that, on average, adults within the mid-intertidal zone are larger than those in the low intertidal zone, but that the CV in the size of adults in the low intertidal zone is greater than in the mid intertidal zone. Unfortunately, the data set collected in this study was too small to enable comparisons in which adult size is adequately controlled. Further, potentially high mortalities of specimens within tide pools (prior to collection) may also mitigate any effects of offspring size.

Third, the level of variation in offspring size produced by *P. parvivipara* is extremely high (mean CV in offspring size = 36 %) in comparison to many other marine invertebrates, including those that reproduce via direct development (see Marshall & Keough 2008a). Marshall and Keough (2008a) showed that the average CV in offspring size among 102 species of marine invertebrates with different life-history strategies was approximately 9 %, while those with direct development exhibited a mean CV of 14 %. Consequently, the large variability in offspring size exhibited somewhat evenly by *P. parvivipara* across intertidal zones and populations may act as a bet-hedging strategy to maximise fitness in inherently unpredictable temperate tide pool environments.

Lastly, there may not be any bet-hedging in offspring size or timing of release going on at all among populations or seasons as speculated above. Increased environmental variation in the immediate maternal habitat within populations (i.e. among intertidal heights) does not lead to more variably size offspring at the Smooth Pool population. Therefore, experiments in which the size of adults is controlled and potential drivers of variation in offspring size are then compared would be needed to establish any potential causal or adaptive bet-hedging relationships among different environmental conditions.

Differences in brood size, offspring size and the CV in offspring size within broods were apparent among seasons in the three populations examined. During the spring when there was a clear spike in the frequency of births (Chapter 2), *P. parvivipara* contained larger broods consisting of smaller offspring with a higher CV in offspring size than during the summer months. During summer, offspring within broods were on average 23 % larger than during the spring, but the CV in offspring size was 20 % lower. Although no causality can be established between seasons and these differences in offspring size and the CV of broods, other studies have demonstrated that they may indeed be adaptive shifts that increase mean offspring fitness. Marshall and Keough (2008b) observed that the bryozoan *Watersipora subtorquata* produced larger offspring in summer compared to other seasons, which corresponded to the period when the strength of the relationship between offspring size and performance was greatest. In a separate investigation, I examined the effect of offspring size on offspring fitness in *P. parvivipara* in laboratory experiments that exploited the variation in offspring size with broods of this species to control for potential fitness differences among individuals (see Chapter 5). In these experiments, larger offspring showed higher survivorship than smaller offspring, but only during summer months. These results support the notion that increases in the size of offspring in broods of *P. parvivipara* from spring to summer may represent an adaptive response to seasonal shifts in environmental conditions.

The change in the CV in offspring size within the broods of adults from spring to summer may similarly represent an adaptive seasonal response in *P. parvivipara*. Environmental conditions are highly variable in both spring and summer in southern Australia, but extreme temperatures and diurnal temperature fluctuations are much more common during summer. Indeed, temperatures in tide pools that *P. parvivipara* inhabits often exceed 30° C and can range from 15 to 36^{o} C during the summer months (Chapter 2). Therefore, while the relatively high CV in offspring size within broods may increase mean offspring fitness during spring by bet-hedging against unpredictable environmental conditions, this advantage may be lost during the summer when experimental evidence suggests that bigger offspring have higher survivorship than smaller offspring. In a number of other marine invertebrate species, increasing the mean offspring size in harsh environmental conditions can increase offspring fitness (Marshall et al. 2006, Allen et al. 2008, but see Moran & Emlet 2001). Whether the differences observed in the mean offspring size and variability in offspring sizes of *P. parvivipara* between seasons is an adaptive response to environmental conditions remains unknown.

Alternatively, the change in offspring size characteristics and brood sizes of *P. parvivipara* between spring and summer may be due to either genetically predetermined differences in the timing and allocation of reproductive energy to offspring, or the result of adaptive plasticity facilitated by intra-gonadal cannibalism of siblings within broods. Intragonadal cannibalism is a potential means by which the allocation of energy to adults or offspring (size) and brood sizes of *P. parvivipara* could be adaptively altered in response to changing environments and competitive conditions. Intragonadal cannibalism among siblings has been reported to occur in *P. parvivipara* (Byrne & Cerra 1996), *Cryptasterina hystera* (Byrne 2005) and other echinoderms (Byrne & Cerra 1996, Frick 1998), but the extent and consequences of this relatively uncommon behaviour have never been examined. Intragonadal cannibalism may act to (a) directly increase the size of offspring within the gonad prior to birth and (b) indirectly reduce sibling numbers and subsequent competition for resources. I did not see any direct evidence of cannibalism in my investigation here. When offspring were dissected from live adults, all offspring removed from the gonads were viable (i.e. all showed active movement of tube-feet, regardless of their size). While some offspring dissected from adults were extremely large (3.55mm) (potentially attaining their large size from intragonadal cannibalism), they were not common among broods. Byrne (2005) suggested that the release of some offspring of the sea star *C. hystera* during spring might free up space in the gonad allowing the remaining juveniles to attain a larger size via intragonadal cannibalism. However for *P. parvivipara*, smaller brood sizes did not result in significantly greater variation in offspring size within broods. Because *P. parvivipara* die soon after giving birth, the increase in offspring size observed between spring and summer is unlikely to be the result of the release of some offspring. Rather, intra-gonadal cannibalism could provide a mechanism to shift the allocation of energy among siblings within a brood to substantially increase offspring size during the summer when a larger size may influence offspring fitness. Testing this idea is likely to be experimentally challenging because it would require direct evidence of intra-gonadal cannibalism shifting the allocation of energy within the brood to influence offspring size in response to an environmental factor such as temperature. Non-invasive imaging techniques (e.g. ultrasound, magnetic resonance imaging, or x-rays) could provide methods for quantifying intragonadal cannibalism in *P. parvivipara* and for understanding any role that it may have in mediating changes in offspring and brood characteristic in relation to environmental conditions.

While my research does not appear to indicate that there is any relationship between population density and adaptive plasticity in offspring and brood traits in *P. parvivipara*, recent research by others suggests that in more competitive environments some marine invertebrates produce larger offspring that exhibit higher performance than smaller offspring (e.g. Marshall et al. 2006, Allen et al. 2008, Leips et al. 2009, Marshall & Keough 2009). For example, Allen et al. (2008) found that at intermediate population densities, the bryozoan *Bugula neritina* produced larger larvae than those at low densities that were capable of dispersing further, which presumably enables them to escape competitive conditions. However, research by Menge (1974) on the predatory sea star *Leptasterias hexactis* suggests that there is no relationship between the energy invested in offspring and level of competition that adults experience in this species.

A potentially critical difference between *P. parvivipara* and *L. hexactis*, and the species examined by Marshall et al. (2006), Allen et al. (2008), and Marshall and Keough (2009) is that they do not have a dispersive larval stage. *P. parvivipara* broods offspring within its gonads and gives birth to fully formed, non-dispersive juveniles, while *L. hexactis* broods offspring underneath its body and also releases non-dispersive juveniles (Chia 1966, Menge 1975). Therefore, while plasticity in offspring traits mediated by increased competition that are observed in some species with dispersive offspring may be related to adaptive alterations in their dispersive capacity, similar plasticity in species with no dispersal capacity would be irrelevant. Studies examining plasticity in offspring and brood traits in relation to a range of potential influences across species with diverse life-history strategies are needed to address this problem.

On average, *P. parvivipara* were larger and produced larger offspring at Smooth Pool than at Point Brown and Point Whittlebee. These differences were not surprising given that among-population differences in the sizes of adults and offspring have been reported in other species (e.g. Menge 1974, George 1994, Bingham et al. 2004, Gosselin & Rehak 2007)**.** *P. parvivipara* from Smooth Pool were on average 14 % larger than those at Point Brown and Point Whittlebee. The intertidal platform at Smooth Pool is much larger (3926 m^2) than those at Point Brown and Point Whittlebee (1523 and 642 m^2, respectively), and also provides a much more structurally-complex environment than the other locations. It therefore seems reasonable to suggest that the larger average size of *P. parvivipara* at Smooth Pool reflects higher habitat quality over at least parts of the extensive intertidal platform than those at Point Brown and Point Whittlebee. Indeed, the average size of *P. parvivipara* collected from the mid intertidal zone of Smooth Pool was greater than those collected in the high and low intertidal zones, and the mid intertidal zone exhibits higher structural complexity that appears to provide better habitat for *P. parvivipara* (Chapter 2). Alternatively, other models could also explain the differences observed in *P. parvivipara* among populations independent of habitat quality. For example, the age of sea stars examined or the strength of a particular cohort among populations may influence the size of specimens. Differences in dispersal capabilities may also explain size differences observed among intertidal zones (e.g. smaller offspring may disperse more easily into the high or low zones). These models require further testing.

Nevertheless, genetic differences among populations cannot be precluded because recent molecular analyses suggest that there is very little, if any, genetic exchange among populations of *P. parvivipara* (Keever 2010). Differences in offspring size among populations are at least partially attributable to differences in the sizes of adults because, across populations of *P. parvivipara*, larger adults produced larger offspring. This relationship is now reported to be widespread among marine invertebrates (Marshall et al. 2003, Marshall & Keough 2003b, Marshall & Keough 2008a). The reasons for this relationship are not yet clear but have been proposed to result from increased efficiency of provisioning to offspring by larger mothers than smaller mothers (Sakai & Harada 2001, Marshall & Keough 2004a), or because larger mothers may have access to better resources resulting in the production of larger offspring (Hendry et al. 2001). It has also been proposed that larger mothers should produce bigger offspring in conditions where competition between sibling offspring is probable (Parker & Begon 1986). Data pooled across populations also showed that there was a significant positive relationship between the size of adults and brood size. However, differences in the average size of adults among populations did not appear to be large enough to influence average brood sizes because no statistical differences were apparent among them, but does not eliminate adult size as a potential confounding factor.

Recent genetic evidence suggests that there is little or no genetic exchange among populations of *P. parvivipara* (Keever 2010). Given that this species is also hermaphroditic, and potentially self-fertilising, the genetics of populations may be essentially invariant and each of the isolated populations may therefore be made up of genetically identical individuals. This possibility is a potentially important determinant of the reproductive patterns observed in the field because a reproductive bet-hedging strategy may operate at the scale of the population. The clear spike in births in the spring and early summer suggests that this is the time at which offspring fitness may be greater at this time. However, the presence of individuals in the population giving birth at other times of the year may function as a bet-hedging approach that spreads the risk of recruitment failure over the year at the scale of the population (i.e. offspring are released at different times throughout the year to avoid offspring mass mortalities during events such as heat waves or storm events), or as a means of reducing competition among offspring during the peak period of births.

Although no relationship was found between the variability of offspring size within broods of *P. parvivipara* and apparent levels of environmental variation across an intertidal gradient, differences in brood sizes and offspring sizes across seasons suggests a more complex interplay of bet-hedging strategies in this species. *P. parvivipara* may not only alter offspring phenotypes within broods in response to seasonal environmental factors, but each adult may alter the timing of birth in response to environmental conditions to potentially adaptively match the phenotypic characteristics of their brood. Births may therefore be triggered by a combination of environmental cues and phenotypic characteristics of the brood that may increase the mean fitness of offspring. Investigating this idea would require experiments in which environmental cues that induce birth are manipulated and then offspring compared to the phenotypes of broods released by some adults within a group and the broods of other adults that did not give birth under the same conditions. In addition, experiments demonstrating that broods with different phenotypes confer fitness benefits under different environmental conditions would be required. While such investigations would be experimentally challenging, I believe that they have the potential to provide substantial insights into adaptive plasticity in offspring phenotypes among marine invertebrates.

3.6 Tables

Table 3.1. Two-way ANOVAs comparing adult size, brood size, offspring size and the CV's of offspring size among seasons of three populations of *P. parvivipara* (Smooth Pool, Point Brown and Point Whittlebee).

Test	**Source**	**df**	**MS**	***F***	***P***
Adult size	Season	3	0.322	13.987	**<0.001**
	Population	2	4.275	185.554	**<0.001**
	Season × population	6	0.0310	1.345	0.234
	Residual	1781	0.0230		
Brood size	Season	3	62.551	69.241	**<0.001**
	Population	2	2.652	2.935	0.053
	Season × population	6	1.576	1.745	0.107
	Residual	1781	0.903		
Mean offspring size	Season	3	1.327	68.730	**<0.001**
	Population	2	0.620	32.131	**<0.001**
	Season × population	6	0.0840	4.352	**<0.001**
	Residual	1574	0.0193		
CV offspring size within broods	Season	3	23.665	9.968	**<0.001**
	Population	2	11.878	5.003	**0.007**
	Season × population	6	4.601	1.938	0.072
	Residual	1416	2.374		

Table 3.2. Size ranges of *P. parvivipara* adults, offspring and broods of three populations (Smooth Pool, Point Brown and Point Whittlebee). Size parameters are also given for *P. parvivipara* in the low, mid and high intertidal zones of Smooth Pool.

Population or intertidal zone	**Adult radius range (mm)**	**CV (%) Adult radius**	**Offspring diameter range (mm)**	**Maximum brood size**	**Mean CV (%) offspring size within broods**	**CV (%) offspring size among adults**
Smooth Pool	1.6 – 5.2	16.62	0.20 – 3.55	36	37.04 ± 0.65	47.01
Point Brown	1.9 – 5.0	17.04	0.25 – 2.35	29	34.49 ± 0.91	42.26
Point Whittlebee	1.8 – 4.2	16.24	0.25 – 2.35	31	33.03 ± 0.93	43.65
Low	1.6 – 5.2	18.87	0.20 – 3.55	21	38.52 ± 0.89	44.97
Mid	2.2 – 5.2	13.69	0.20 – 3.30	36	34.89 ± 1.04	47.31
High	1.9 – 4.9	14.94	0.25 – 2.70	25	38.33 ± 1.81	49.35

Table 3.3. Two-way ANOVA comparisons of seasonal changes in adult sizes, brood sizes, offspring sizes and the CV of offspring sizes of *P. parvivipara* among the low, mid and high intertidal zones at Smooth Pool.

Test	**Source**	**df**	**MS**	***F***	***P***
Adult size	Season	3	2.706	8.898	< **0.001**
	Intertidal zone	2	10.312	33.912	< **0.001**
	Season × intertidal zone	6	1.231	4.047	< **0.001**
	Residual	981	0.304		
Brood size	Season	3	39.459	53.315	< **0.001**
	Intertidal zone	2	11.210	15.147	< **0.001**
	Season × intertidal zone	6	5.171	6.986	< **0.001**
	Residual	891	0.740		
Mean offspring size	Season	3	1.558	74.682	< **0.001**
	Intertidal zone	2	0.0850	4.072	**0.017**
	Season × intertidal zone	6	0.169	8.098	< **0.001**
	Residual	879	0.0209		
CV offspring size within broods	Season	3	35.324	14.271	< **0.001**
	Intertidal zone	2	15.429	6.233	**0.002**
	Season × intertidal zone	6	4.592	1.855	0.086
	Residual	781	2.475		

Table 3.4. Linear regression analyses of relationships between sizes of adult *P. parvivipara* and the sizes of their offspring and brood sizes. Relationships between brood sizes and the sizes of offspring and the coefficient of variations in offspring size within broods are similarly compared. Note: data across the three populations and four seasons were pooled to increase the power of these analyses.

Regression	**Sign**	**df**	**MS**	***F***	***P***
Brood size vs adult size	+	1785	246.913	290.743	**< 0.001**
Mean offspring size vs adult size	+	1583	1.430	61.620	**< 0.001**
Mean offspring size vs brood size	-	1574	0.303	12.680	**< 0.001**
CV offspring size within broods vs brood size	+	1417	152.578	64.181	**< 0.001**

Table 3.5. Linear regression comparisons between the density of *P. parvivipara* in tide pools at Smooth Pool and the sizes of adults and their brood characteristics. Comparisons were made between the density of *P. parvivipara* and the mean sizes of adults, offspring, and broods, and the coefficients of variation (CV) in offspring size within and among broods. NS = a non-statistically significant relationship.

Reproductive trait	**Sign**	**df**	**MS**	***F***	***P***
Adult size	+	13	1.345	13.073	**0.004**
Offspring size	NS	13	0.017	0.648	0.436
Brood size	+	13	16.557	8.571	**0.013**
CV within broods	+	13	293.958	7.550	**0.018**
CV among adults	NS	13	82.626	4.374	0.058

Chapter 4

Variation in offspring size in two species of sea stars exhibiting viviparity and direct development

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4.1 Abstract

Levels of maternal energy investment in offspring of marine invertebrates, and hence offspring size, differ markedly among and within species, and within broods of individuals. These differences in offspring size are widely held to have implications for individuals, populations and community level processes. Recent studies suggest that mothers may be capable of adaptively altering offspring phenotypes to maximise offspring fitness in response to the environment, and that when environmental conditions fluctuate unpredictably the production of variable offspring sizes within broods may represent an adaptive bet-hedging strategy that increases mean offspring fitness. The capacity to adaptively alter offspring phenotype should be greater among direct developers than species with dispersive larvae because their offspring are more likely to experience similar conditions to adults, and there are no conflicting selective pressures acting on life-history stages that occupy different ecological niches. I examined the coefficient of variation (CV) in offspring size within broods between two species of closely related direct-developing sea stars, *Parvulastra parvivipara* and *Cryptasterina hystera*, which inhabit a temperate and tropical intertidal zone in South Australia and Queensland, respectively. These species provided the opportunity to simultaneously examine the characteristics of their offspring in relation to differing levels of environmental variation and habitat characteristics. The CV in offspring size within broods of *P. parvivipara* was higher than that of *C. hystera* (mean = 40 % and 14 %, respectively), supporting the idea that *P. parvivipara* may produce more variably sized offspring than *C. hystera* to maximise offspring mean offspring fitness in response to an environment that is less predictable. I

suggest that the habitats of both species are inherently variable and the high variability in their brood characteristics may increase mean offspring fitness via bet-hedging. Manipulative experiments in which brood characteristics are examined in relation to different environmental conditions are needed to establish causal relationships between them.

4.2 Introduction

While the large degree of variation in offspring sizes among species of marine invertebrates has long been recognised (e.g., Thorson 1950, Vance 1973, Emlet et al. 1987), the degree to which offspring size varies among adults and broods within species, has only recently been recognised. Offspring size in marine invertebrates can be influenced by environmental and maternal factors, and is now known to be a highly plastic trait that can differ considerably among populations, among individuals within populations, and within broods of offspring from individuals (reviewed in Marshall & Keough 2008a). This variation in offspring size is important because small differences can strongly influence fundamental life-history parameters, including dispersal potentials (Marshall & Keough 2003a, Marshall & Bolton 2007), growth (Marshall & Keough 2004b), survivorship (Moran & Emlet 2001, Marshall et al. 2003), population stability (Eckert 2003), and evolutionary or extinction rates (Emlet 1989, Jeffery & Emlet 2003).

Recent investigations indicate that offspring size can be under a degree of maternal control such that adults can adaptively adjust the sizes of their offspring according to the environmental conditions that they are likely to experience. For example, Allen et al. (2008) showed that the bryozoan *Bugula neritina* produced larger offspring in response to an increase in intraspecific competition, and that larger larvae had higher dispersal potentials, presumably in order to escape unfavourable conditions. Similarly, Marshall and Keough (2009) showed that under high levels of intraspecific competition, the bryozoan *Watersipora subtorquata* released larger offspring with greater dispersal capacity compared to offspring from adults that were not exposed to competition.

Variation in offspring size within broods has been more difficult to explain and was previously viewed as a by-product of physiological constraints on reproduction that prevented adults evenly distributing resources among their offspring, and thus having little, or no, adaptive value (Fox & Czesak 2000, Einum & Fleming 2004). However, recent examinations of offspring size variation within broods suggest that when environmental conditions fluctuate unpredictably, the production of variable offspring sizes within broods may represent an adaptive bet-hedging strategy that increases mean offspring fitness (Marshall et al. 2008b, Crean & Marshall 2009, Olofsson et al. 2009). Conversely, adults should produce broods of more uniform offspring size when the conditions that they are experiencing are stable (Marshall et al. 2008b).

The ability of individuals to adaptively adjust their allocation of resources to offspring depending on the environment is likely to be limited by their capacity to predict the environment that their offspring will experience. Species that reproduce via a dispersive pelagic larval stage may have limited ability to predict the environment that their offspring will experience because they are often dispersed far from the adult (Pechenik 1999). In contrast, species that reproduce via direct development, in which offspring are released into the environment occupied by the adult at an advanced juvenile stage, should have the greatest ability to predict the environment that their offspring will experience and adaptively vary offspring phenotype to suit the environment accordingly (Marshall et al. 2008b, Crean & Marshall 2009). The majority of studies that have examined determinants of offspring size within species have focused on species with pelagic larval stages and benthic adult stages, yet species with viviparous, direct development exhibit the highest variation observed in offspring size among marine invertebrates (reviewed in Marshall & Keough 2008a). Viviparous, direct-developing marine invertebrates are likely to provide powerful insights into the adaptive significance of variation in offspring size for later life-history stages because they are not subject to the de-coupling influence of the adult and offspring environment.

I compared variation in offspring size in two viviparous, direct-developing sea stars, *Parvulastra parvivipara* and *Cryptasterina hystera*, that respectively inhabit a temperate and a tropical intertidal environment. Differences in offspring size variation between two closely related viviparous species that inhabit a temperate and tropical environment has not been examined among marine invertebrates (see Marshall & Keough 2008a). Temperate intertidal environments undergo pronounced seasonal cycles, within season variability and inter-annual variability in water temperatures, irradiance, and nutrient availability, compared to tropical intertidal environments that are relatively stable (Lardies & Castilla 2001, Muller-Parker & Davy 2001). Consequently, *P. parvivipara* inhabits an environment that is more unpredictable than that of *C. hystera* and I therefore predicted that offspring size would be more variable within and among broods of *P. parvivipara* than *C. hystera*. Populations of *P. parvivipara* are found distributed along 200 km of coastline between Cape Vivonne and Point Labatt on the west coast of the Eyre Peninsula, South Australia (Roediger & Bolton 2008). Within its limited range, *P. parvivipara* occurs in tide pools across the intertidal zones of seven small, geographically isolated granite platforms. Populations of *C. hystera* are found on coastal and an offshore island in central Queensland. Its current known distribution includes Yeppoon, Statue Bay and One Tree Island (Byrne 2005, Byrne & Walker 2007). *C. hystera* is found in tide pools on the underside of boulders and coral rubble within intertidal fields. Both *P. parvivipara* and *C. hystera* are hermaphroditic. To examine variation in offspring size between *P. parvivipara* and *C. hystera*, I calculated the coefficient of variation in offspring size within broods from individual adults and among broods from adults of both species. Three populations of each species were examined between 2 surveys conducted over 2-years to examine spatial and temporal variability in offspring size. The relationships between reproductive output and adult size were also examined in each species. As a measure of environmental variability in the temperate and tropical intertidal habitats, I also examined daily variations in tide pool temperatures because differences in temperature variations between temperate and tropical habitats may have different selective pressures on variation in offspring sizes.

4.3 Methods

4.3.1 Study populations

Three populations of *P. parvivipara* were examined in South Australia: Smooth Pool (32^{o} 92′ S, 134^{o} 07′ E), Point Brown (32^{o} 53′ S, 133^{o} 86′ E) and Point Whittlebee (32^{o} 20′ S, 133^{o} 73′ E) (Figure 1.4A). Three populations of *C. hystera* were examined in Queensland: The Gutter (23^{o} 51′ S, 152^{o} 09′ E) and Two Tree (23^{o} 49′ S, 152^{o} 09′ E), which are located on the coral rubble cay at One Tree Island in the Great Barrier Reef, and Statue Bay (23^{o} 17′ S, 150^{o} 78′ E), Central Queensland (Figure 1.4B & C).

4.3.2 Specimen collections

Populations of *P. parvivipara* were examined in the austral spring during October 2007 and September 2008 because this corresponds to the period immediately preceding the release of most offspring (Chapter 4). Similarly, populations of *C. hystera* were examined in the austral spring during October in 2008 and 2009 because this also corresponds to the period preceding the release of offspring in this species (Byrne 2005). Specimens of *P. parvivipara* were collected from tide pools at Smooth Pool, Point Brown and Point Whittlebee each year. Tide pools were randomly selected using numbered reference points that were superimposed on aerial photographs of the intertidal zones of each population (Smooth Pool n = 15, Point Brown n = 5, Point Whittlebee n = 5). Ten specimens were randomly collected from each tide pool and preserved in 90% ethanol for subsequent dissection. Specimens of *C. hystera* were collected from tide pools in the intertidal fields at The Gutter, Two Tree and Statue Bay. Four tide pools were randomly selected at each population from random numbers along a 100 metre long transect that was positioned parallel to the shoreline. The tide pool closest to the random number on the transect line was sampled. Five specimens from each tide pool were randomly collected and placed into vials containing seawater and subsequently preserved in 90% ethanol for dissection.

4.3.3 Specimen dissections

To examine variation in offspring size in each species, offspring were dissected from adult specimens and measured. Offspring dissected from each species were viable, capable of independent existence, and within the size range of offspring naturally released by adults. Juvenile specimens within the size range of dissected offspring were released naturally by adults in the laboratory and also found in the field: therefore, the sizes of the dissected offspring were considered be representative of those that both species release during natural births. The radius of each adult specimen was measured prior to dissection along the axis running from the tip of the longest arm to the middle of the stomach on the actinal surface. Adult specimens of *P. parvivipara* were measured using the ocular micrometer in a dissecting microscope (accuracy ± 0.01 mm), while specimens of *C. hystera* were digitally imaged through a microscope and subsequently measured using image analysis software (ImageJ 1.43s 2007; accuracy ± 0.01 mm). Adults were dissected by cutting around the perimeter of the body cavity with a scalpel, which enabled the actinal and dorsal surfaces to be separated exposing the gonads. The offspring contained within the gonads were removed using forceps and needles under a dissecting microscope. The diameters of offspring of *P. parvivipara* were measured from the tip of one arm to the tip of the opposite arm using an ocular micrometer (accuracy ± 0.01 mm) in a dissecting microscope, and the number of offspring within the gonads of each adult was recorded. The relatively small offspring of *C. hystera* were digitally imaged under a dissecting microscope and their diameters subsequently measured using the image analysis software (accuracy ± 0.01 mm). The number of offspring within the gonads of each adult (brood size) was also recorded.

4.3.4 Temperature variation

As an indication of environmental variability in the temperate and tropical intertidal habitats of *P. parvivipara* and *C. hystera*, I recorded water temperatures within tide pools. Temperatures at Smooth Pool were recorded between June 2007 and April 2009, while those at One Tree Island were recorded between October 2008 and April 2009 and during October 2009. Temperature data loggers

(Thermochron ibutton, Temperature Technology, Adelaide, South Australia) were randomly deployed in the intertidal habitats of each species. One logger was placed into randomly selected tide pools inhabited by *P. parvivipara* at Smooth Pool (n = 15) and into tide pools inhabited by *C. hystera* at The Gutter (n = 7). Each logger was encased in a plastic container that was attached to a boulder or piece of coral rubble at the bottom of the tide pool with durable adhesive tape (3M duct tape). The loggers were programmed to record water temperatures at 2-hour intervals. The temperature range (minimum and maximum) of each tide pool were determined within each habitat.

4.3.5 Statistical analyses

To compare variation in offspring size of each species independently of absolute differences in offspring size, I calculated the coefficients of variation (CV) in offspring size in each population of each species in both years of the study. The CV in offspring size was also calculated within and among broods of individuals of each species. A non-parametric Kruskal-Wallis ANOVA ($\alpha = 0.05$) was used to examine whether the CV in offspring size within adults differed between *P. parvivipara* and *C. hystera.* The assumptions of parametric ANOVA were not met using raw or transformed data, and the non-parametric analysis was therefore used. To increase the power of the analyses, data from all populations and both years were pooled for each species. A one-way ANOVA was used to compare the temperature range recorded in tide pools between the temperate and tropical habitat.

A two-way ANOVA ($\alpha = 0.05$) was used to determine whether the CV in offspring size of each species differed among populations and between years. Population was considered a fixed factor in analyses, while year was considered a random factor in the analyses for both species. Assumptions of homogeneity of variances and distributional normality were checked using Levene’s tests and quartile plots, respectively. Bonferroni pair-wise post-hoc comparisons (at $\alpha = 0.05$) were conducted where significant differences were detected by ANOVA. Linear regression analyses were used to examine the relationships between adult size and offspring size, brood size and the CV in offspring size within broods. To increase the power of these regression analyses, data for each species was pooled across populations and years. In instances where the data did not meet the assumptions of parametric tests, transformations were applied (square-root, fourth-root or log transformed), or outliers were removed to correct these problems.

4.4 Results

4.4.1 Variation in offspring size between species

A significant difference was found between the CV in offspring size of *P. parvivipara* and *C. hystera* (Kruskal-Wallis one-way ANOVA: $H = 180.16$, $P < 0.001$). The mean CV in offspring size within broods of *P. parvivipara* was 40 % compared to 14 % for *C. hystera* (Figure 4.1). The CV in offspring size within broods ranged from 0 to 103 % for *P. parvivipara,* and from 2 to 40 % for *C. hystera*. The CV in offspring size among adults was also higher for *P. parvivipara* (48 %) compared to *C. hystera* (16 %). The CV in offspring size was greater among adults than within broods released by individual adults in both species (Figure 4.1).

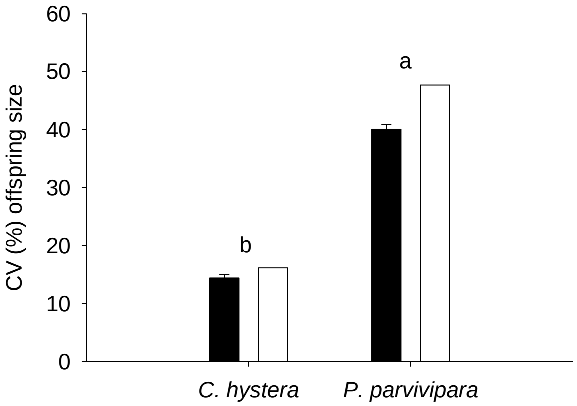

Figure 4.1. Mean (± SE, *n* = 28 - 235) coefficients of variation (CV) in offspring size within broods (black bars) and among adults (white bars) of *P. parvivipara* and *C. hystera*. Letters denote significant differences in the CV of variation in offspring size within broods between species.

The radii of *P. parvivipara* adults ranged from 1.9 mm to 5.2 mm, while those of *C. hystera* adults ranged from 9.1 mm to 17.9 mm (Figure 4.2A). The mean brood size of *P. parvivipara* was 7 offspring compared to 378 for *C. hystera* (Figure 4.2B). Brood sizes of *P. parvivipara* ranged from 0 to 36, while those of *C. hystera* ranged from 0 to 2311 (Figure 4.2B). *P. parvivipara* produced offspring that were on average 17 % larger in diameter than those of *C. hystera* (Figure 4.2C). The mean (± SE) diameter of offspring produced by *P. parvivipara* was 0.82 ± 0.01 mm and 0.68 ± 0.01 mm in *C. hystera* (Figure 4.2C). The diameter of *P. parvivipara* offspring ranged from 0.2 to 3.45 mm (3.25 mm range), while those of *C. hystera* ranged from 0.27 mm to 3.17 mm (2.89 mm range).

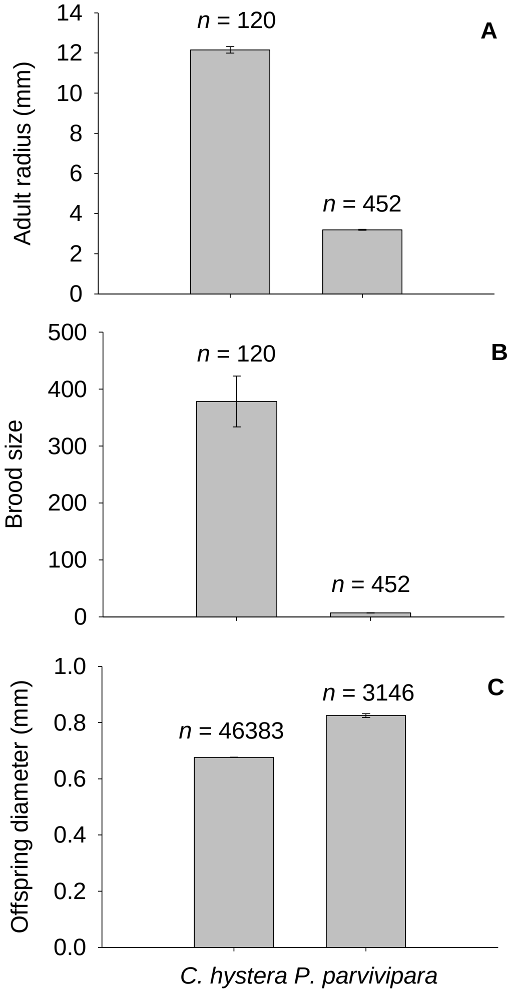

Figure 4.2. Mean sizes (± SE, *n* = 120 - 46383) of (A) adults, (B) broods, and (C) offspring of *P. parvivipara* and *C. hystera*. Data are pooled across years and populations.

4.4.2 Variation in offspring size within species

Two-way ANOVA showed that there were significant differences in the CV in offspring size within broods of *P. parvivipara* among years and populations (Table 4.1). The CV in offspring size was consistently greater in 2008 than in 2007 in all three populations, and was greater at Smooth Pool than at Point Whittlebee and Point Brown (Figure 4.3A). No interaction was detected between populations and years (Table 4.1). Two-way ANOVA showed that the CV in offspring size within broods of *C. hystera* was significantly greater at Statue Bay than in the populations on One Tree Island (Table 4.1, Fig 4.3B). The CV in offspring size of *C. hystera* did not differ significantly between years (Table 4.1).

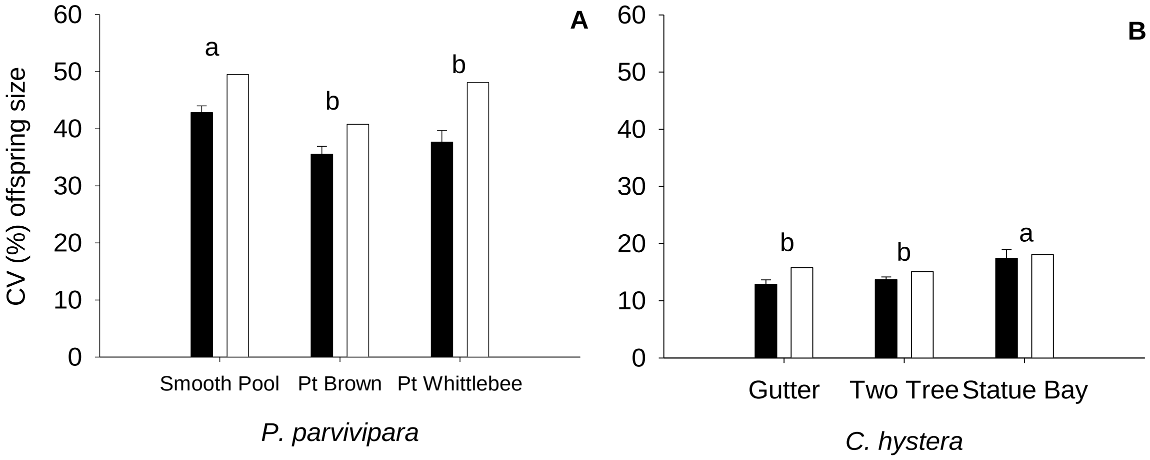

Figure 4.3. Mean (± SE, *n* = 28 - 235) coefficients of variation (CV) in offspring size within broods (black bars) and among adults (white bars) of three populations of (A) *P. parvivipara*, and (B) *C. hystera*. Letters denote significant differences in the CV of variation in offspring size within broods among populations (pooled across years) for each species.

Linear regression analyses failed to detect a significant relationship between brood sizes of *P. parvivipara* and the CV in offspring size within broods (Table 4.2), but a weak, significant and negative relationship was apparent between brood size and the CV in offspring size within broods in *C. hystera* (Table 4.2, Figure 4.4). Larger adults of both species produced larger broods than smaller adults (Table 4.2). On average, larger adults of *P. parvivipara* also produced larger offspring; however, no relationship was found between adult size and offspring size in *C. hystera* (Table 4.2, Figure 4.4). Linear regression analyses failed to detect an effect of brood size on mean offspring size of *P. parvivipara*, but detected a significant negative relationship in *C. hystera* (Figure 4.4). Across all of these comparisons, low coefficients of determination indicated poor fits of the linear regression models to the data, and therefore only a small amount of the variation is explained by the regression models (Table 4.2, Figure 4.4).

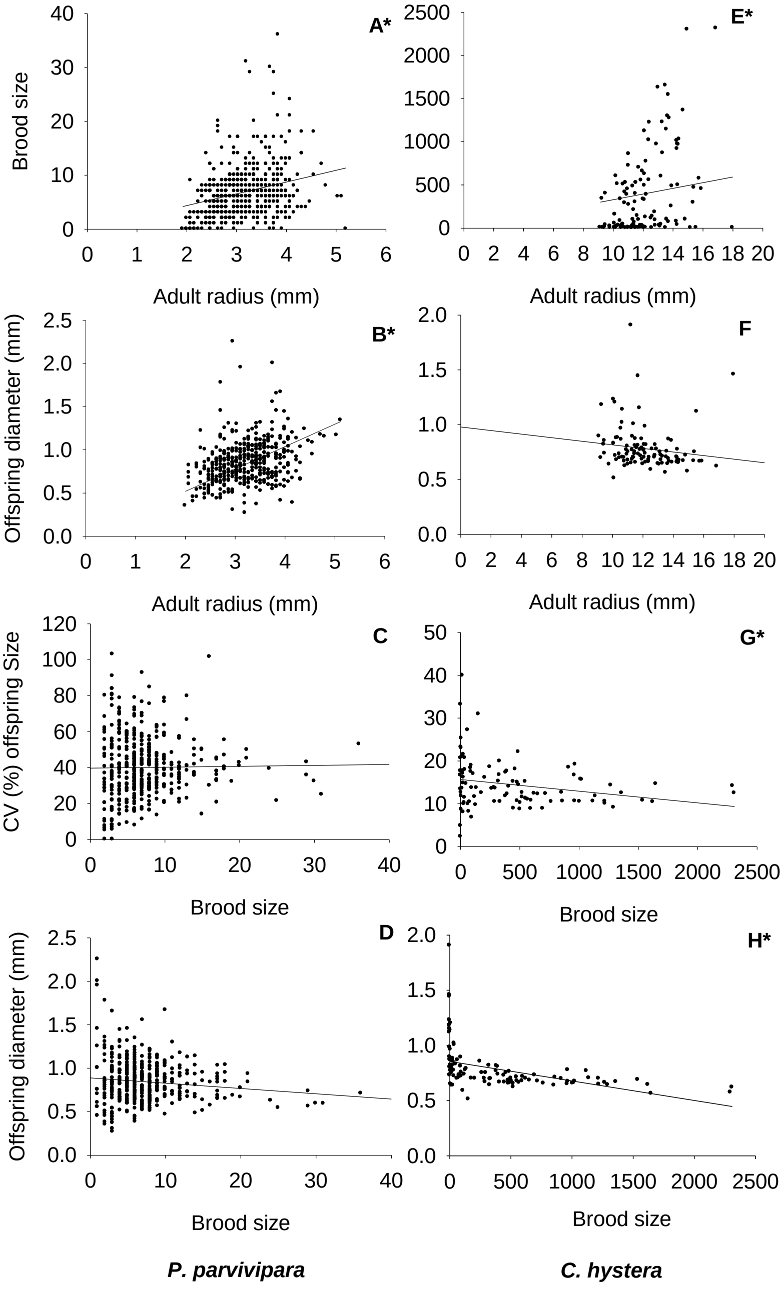

Figure 4.4. Linear regression analyses (n = 97 - 450) of adult size against (A, E) brood sizes, and (B, F) mean offspring sizes. Relationships between brood sizes and (C, G) the CV in offspring size within broods, and (D, H) mean offspring sizes are similarly analysed for *P. parvivipara* and *C. hystera*, respectively. NB: while regression analyses showed significant relationships ($*P < 0.05$) in some of these comparisons, the low coefficients of determination (Table 4.2) indicate that the models explain little of the observed variation (i.e. < 1 to 22 %). Lines on plots represent the regression equations of untransformed data.

4.4.3 Temperature variation

The temperature of tide pools in the temperate habitat of *P. parvivipara* ranged from 2 to 37.5° C, while those in the tropical habitat of *C. hystera* ranged from 15.5 to 41.5° C. Thus, while maximum temperatures in tide pools in the tropical habitat of *C. hystera* were greater than those in the temperate habitats of *P. parvivipara*, the overall temperature range experienced among tide pools was 11.5 ° C greater in the temperate habitat. However, no significant difference was detected between the temperature ranges recorded within tide pools between the temperate (mean ± SE = 24.4 ± 5.0) and tropical habitat (19.9 ± 5.0) (ANOVA: $F_{1, 19} = 3.435$, MS = 84.868, $P = 0.079$) (Figure 4.5).

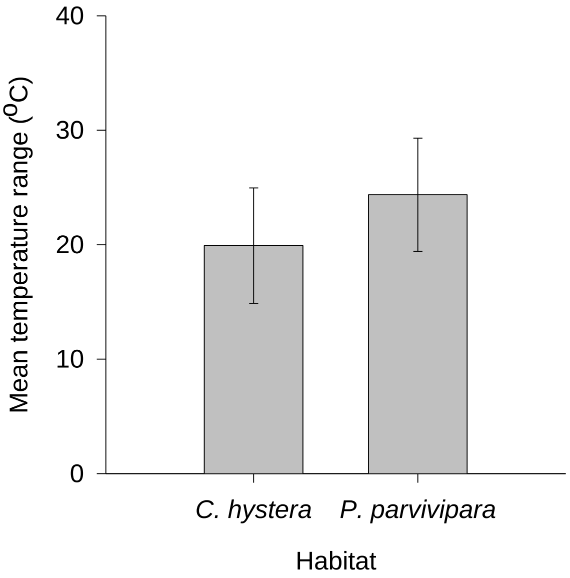

Figure 4.5. The mean temperature range recorded among tide pools in the temperate (*P. parvivipara*) and tropical (*C. hystera*) habitat.

4.5 Discussion

Recent theoretical modelling suggests that the production of variably sized offspring within broods of marine invertebrates increases mean offspring fitness when environmental conditions fluctuate unpredictably, and is therefore an adaptive response to such conditions (Marshall et al. 2008b). I found that the coefficient of variation (CV) in offspring size within broods of the temperate viviparous sea star *P. parvivipara* was over twice that exhibited by the tropical viviparous sea star *C. hystera* (mean = 40 and 14 %, respectively). The average size of offspring produced by *P. parvivipara* was also 17 % bigger than *C. hystera*: however, *C. hystera* produced significantly larger broods than *P. parvivipara*.

The intertidal area that the two species inhabit differed considerably in their temperature regimes, with tide pools inhabited by *P. parvivipara* having an overall temperature range among tide pools that was 11.5 ^{o} C greater than the tropical habitat. While no significant difference in temperature ranges was recorded among tide pools between the habitats, on average the temperatures of tide pools were also 4.5^{o}C more variable in the temperate habitat. Although temperature was the only parameter recorded in this study as a measure of environmental variability, the temperate environment also appeared to differ substantially more than the tropical environment in salinity regimes, and in the frequency and unpredictability of periods of high wave energy (pers. obs.). Because the tide pools that *P. parvivipara* inhabit are subject to greater temperature changes than those inhabited by *C. hystera*, the higher CV in offspring size exhibited by *P. parvivipara* may represent an adaptive bet-hedging strategy that increases mean offspring fitness in its relatively unpredictable environment. Additional data on physical and non-physical factors would provide greater insight into the environmental differences between the intertidal conditions experienced by each species.

While no causal relationships between levels of environmental variability and the respective levels of variation in offspring size of each species can be determined from this study, the results provide the first comparison of offspring size variation between two closely related viviparous, direct-developing species that inhabit a temperate or tropical environment. However, caution also needs to be taken in the interpretation of these findings because this comparison was undertaken on only two species that have separate evolutionary pathways to viviparity (Byrne 2006) and is therefore un-replicated at the species level. Because these two species have independent pathways to viviparity strengthens the argument about the effect of viviparity on offspring size variation in the respective habitats per se.

The production of variably sized offspring in response to unpredictable environments has also been observed in several other species. For example, Koops et al. (2003) found that adults of the trout *Salvelinus fontinalis*, which were exposed to variable, and hence less-predictable environmental conditions, produced more variably sized offspring than those that were exposed to a relatively stable environment. Similarly, Crean and Marshall (2009) using data of Gagliano and McCormick (2007) showed that adults of the coral reef fish *Pomacentrus amboinensis* that were exposed to more variable environmental conditions (food and competition), produced more variably sized offspring than to those on less variable reefs.

Differences in the heterogeneity of the two environments inhabited by *P. parvivipara* and *C. hystera* may be responsible for the contrasting variability in the size of their offspring. The tide pools inhabited by *P. parvivipara* vary substantially in their position across the intertidal zone (i.e. elevation, location relative to low tide margin), water temperature, flushing times between tidal cycles, exposure to wave action, and in the density of *P. parvivipara* present. For example, tide pools inhabited by *P. parvivipara* in the high intertidal zone experience higher temperature ranges and lower levels of wave exposure than those in the mid and low intertidal zones at Smooth Pool (Chapter 2). These environmental variables are likely to be more stable and consistent among the tide pools that *C. hystera* inhabits. Consequently, it may not only be the unpredictability of the environment that *P. parvivipara* inhabits driving their relatively high level of variation in offspring size, but the greater heterogeneity of the tide pools that they inhabit. Carefully designed experiments are needed in order to establish whether the differences in offspring size variation in *P.*

parvivipara and *C. hystera* reported here are causally related to differences in the predictability of their respective habitats.

A potential alternative, but not mutually exclusive explanation for differences in the variability of offspring size between the two species is the occurrence of intragonadal sibling cannibalism in both species. *P. parvivipara* and *C. hystera* brood offspring in their gonads to an advanced juvenile stage and the offspring have been reported to cannibalise each other within the gonad, presumably to enhance offspring size (Byrne 1996, 2005). However, there is little direct evidence as to the extent to which cannibalism occurs in either species, although it is thought to be more common in *P. parvivipara* (Byrne 2005). The implications of cannibalism for population dynamics, reproductive outputs, and offspring fitness have not been examined extensively in marine invertebrates (but see Spight 1976, Cubillos et al. 2007). Cubillos et al. (2007) found that cannibalism in the marine gastropod *Crepipatella fecunda* decreased larval development times and increased the size of larvae within capsules. In land snails, cannibalism can act as a population regulator (Baur 1988), and can result in faster growth and increased survival of offspring (Baur 1990). The extent and adaptive nature of this behaviour in either of the sea stars examined here is yet to be determined, but is a potentially important life-history trait that warrants further investigation.

Both species exhibited differences in the CV in offspring size among populations and *P. parvivipara* had greater CV in offspring size during 2008. Differences in the CV in offspring size among populations in each species may be explained by additional physical and non-physical factors acting at the scale of the population such as the level of competition, predation, intragonadal cannibalism or food availability. For *P. parvivipara* differences in offspring size variability between years may be attributable to climatic variations influencing environmental conditions among seasons. Salinities, temperatures and upwelling events can vary between seasons from year to year and may flow on to affect the degree of variability in offspring size produced. These ideas would require longer-term studies and independent testing for each species.

In both species of sea stars, larger adults produced more offspring in broods. However, while offspring size was positively related to adult size in *P. parvivipara,* it was negatively related to adult size in *C. hystera.* A positive relationship between adult (maternal size in non-hermaphroditic species) size and offspring size is widespread in many species of marine invertebrates, although some species show no relationship at all (see Marshall et al. 2008a, Marshall & Keough 2008a). Adult size may correlate with adult age in *P. parvivipara*: larger adults may also be older adults that may have brooded their offspring over longer periods resulting in the production of larger offspring. However, in both instances the linear regression models showed that adult size explained very little of the apparent variation in offspring size of *P. parvivipara* and *C. hystera,* and the nature of these relationships therefore remains unclear.

P. parvivipara produces much larger offspring than *C. hystera*. A potential reason for this difference may be that larger offspring convey more fitness benefits for *P. parvivipara* than for *C. hystera.* In a separate investigation, I found that larger offspring of *P. parvivipara* had higher survivorship than smaller offspring during the summer months, indicating that size does indeed matter for *P. parvivipara* under some conditions (Chapter 6). Whether similar relationships exist in *C. hystera* are unknown. The production of larger offspring may also be advantageous for *P. parvivipara* because high levels of competition among offspring seems likely due to the high densities in which they are found in tide pools at certain times of the year (Chapter 2). Parker and Begon (1986) predicted that, in conditions where sibling competition is probable (i.e. releasing offspring into the same habitat), the production of larger offspring will be favoured. This notion is supported by a study by Marshall et al. (2006) who found that in the colonial ascidian *Botrylloides violaceus,* the advantages of producing larger sized offspring were increased under high levels of intraspecific competition because they were capable of dispersing further and escaping these unfavourable conditions. The densities at which *P. parvivipara* are found in tide pools is highly variable, but range up to several hundred specimens per square metre (Roediger & Bolton 2008). In contrast, the density of *C. hystera* in the field is relatively low (maximum = 32 per m^2) (Chapter 3). While direct-developing species do not need to produce larger offspring at higher densities for dispersal advantages in the plankton; larger offspring may have other advantages against smaller offspring in competitive environments. How the density of conspecifics influences the offspring size and fitness in marine invertebrates with direct development could be further investigated through additional experimental manipulations of density (see Chapter 3).

C. hystera produced smaller offspring that were less variable in size than those of *P. parvivipara*: however, the mean brood size of *C. hystera* was 54 times higher than that of *P. parvivipara* (mean brood size = 378 for *C. hystera*, versus 7 for *P. parvivipara*). The different evolutionary pathways to viviparity that these species have followed may be responsible for the differences in offspring size, brood size and offspring size variability reported here. *P. parvivipara* is thought to have evolved viviparity from a species with benthic non-feeding development, whereas *C. hystera* is believed to have evolved viviparity much more recently from a species with non-feeding planktonic development (Byrne 1996, 2005, 2006). A major limitation of my study is that offspring size variability was examined in only one viviparous species within each habitat type. Additional viviparous species to consider for future examinations across multiple habitats include the sea stars *Parvulastra vivipara* in Tasmania, Australia and *Cryptasterina pacifica* in Japan (Byrne 2006). However, the rarity of species with this life-history strategy inevitably limits the number of comparisons that can be made.

While *C. hystera* exhibited a comparatively lower CV in offspring size within broods compared to *P. parvivipara* (26 % less), there was evidence of potentially very high levels of plasticity in offspring size (i.e. the largest offspring dissected from *C. hystera* was 3.17 mm in diameter). While variable offspring sizes were found in some individuals of *C. hystera*, it was not very common and this may be reflective of their relatively stable environment. Both *P. parvivipara* and *C. hystera* exhibited greater variation in offspring sizes among broods than within broods from individual adults (on average 8 % and 2 %, respectively). Similar observations were made by Marshall et al. (2008b), who showed that direct-developing marine invertebrates consistently produced greater variation in offspring size among broods than within broods.

Although my results tend to support current theoretical predictions that adults may adaptively vary the size of their offspring in more unpredictable habitats, they are confounded by the interspecies comparison, ecological differences between habitats, and different evolutionary pathways to viviparity that *P. parvivipara* and *C. hystera* have undergone. Experiments to establish whether CV in offspring size changes in response to experimental manipulations of environmental stability, and whether potential differences in offspring size result in increased offspring fitness, would provide strong evidence for a causal relationship between environmental variation and adaptive alterations of offspring size in these species.

4.6 Tables

Table 4.1. Two-way ANOVAs comparing of the CV in offspring size within broods of *P. parvivipara* (temperate) and *C. hystera* (tropical) among years and populations. Populations of *P. parvivipara* examined were located at Smooth Pool, Point Brown and Point Whittlebee, and those of *C. hystera* were located at The Gutter, Two Tree and Statue Bay.

Species	**Source**	**df**	**MS**	***F***	***P***
P. parvivipara	Year	1	16.182	8.011	**0.005**
	Population	2	11.969	75.277	**0.003**
	Year × population	2	0.159	0.079	0.924
	Residual	411	2.020		
C. hystera	Year	1	0.209	0.464	0.497
	Population	2	2.156	1.955	**0.010**
	Year × population	2	1.103	2.453	0.091
	Residual	95	0.450		

Table 4.2. Linear regression analyses of relationships between sizes of adult *P. parvivipara* and *C. hystera* against and the sizes of their offspring, the coefficient of variation (CV) of offspring sizes within broods, and brood sizes. Relationships between brood sizes, and the sizes of offspring and the CV in offspring size within broods, are similarly compared for each species.

Regression	**Sign**	**df**	**MS**	***F***	***P***	***R*²**
P. parvivipara						
Brood size vs adult size	+	450	43.064	51.81	< **0.001**	0.101
Mean offspring size vs adult size	+	429	4.030	53.92	< **0.001**	0.110
CV offspring size within broods vs brood size	+	408	7.087	3.36	0.068	0.006
Mean offspring size vs brood size	-	429	0.0581	3.340	0.066	0.006
C. hystera						
Brood size vs adult size	-	118	53.862	16.27	< **0.001**	0.144
Mean offspring size vs adult size	-	105	0.0289	2.95	0.089	0.018
CV offspring size within broods vs brood size	-	97	2.784	5.79	**0.018**	0.047
Mean offspring size vs brood size	-	103	0.235	30.23	< **0.001**	0.219

Chapter 5

Consequences of variation in offspring size within broods on offspring fitness during the first year of development in the direct-developing sea star *Parvulastra parvivipara*

5.1 Abstract

Offspring size differs markedly among and within species of marine invertebrates and has profound implications for all life-history stages, as well as their population dynamics. Those species that reproduce via direct development exhibit comparatively high levels of variation in offspring size within broods than species with pelagic larval phases, yet its implications have rarely been examined in this suite of species. I examined the consequences of differing offspring sizes within broods of the direct-developing sea star *Parvulastra parvivipara* on offspring growth and survival. Survival, net growth and growth as a proportion of initial size were examined among broods in small (< 2 mm in diameter) and large (≥ 2 mm) offspring throughout the first year of their development. Only 23 % of all offspring examined survived to one year of age. Large offspring had significantly higher survival than small offspring during summer conditions and this persisted throughout the first year of development. Small offspring exhibited greater net and proportional growth than large offspring throughout development. Brood identity did not significantly influence offspring survival; although the survival of offspring within broods ranged from 0 to 50 %. My finding that larger offspring have a fitness advantage over smaller offspring during summer (higher survivorship) suggests that adults may adaptively alter the sizes of their offspring in response to the environment to maximise mean offspring fitness among seasons.

5.2 Introduction

The evolutionary ecology of substantial variation in offspring size among marine invertebrates has been studied extensively (reviewed in Vance 1973, Smith & Fretwell 1974, Marshall & Keough 2008a) because of its profound consequences for offspring survivorship (e.g. Moran & Emlet 2001), growth (e.g. Marshall & Keough 2004b, 2005), reproduction (e.g. Marshall et al. 2003, Marshall 2005), fertilization success (e.g. Levitan 1996, Podolsky & Strathmann 1996, Styan 1998), development times and dispersal potentials (e.g. Emlet et al. 1987, Havenhand 1993), competition (e.g. Marshall et al. 2006) and predation (e.g. Rivest 1983). Studies of relationships between offspring size and offspring fitness have focused primarily on comparisons among species because of the obvious differences among them. While such studies have provided substantial insights into these relationships, they are confounded by our inability to control for the fact that they are different species with different evolutionary histories (see Harvey & Pagel 1991, Bernardo 1996, Marshall & Keough 2008a). However, recent research has shown that offspring size also varies substantially among and within broods of offspring within species, and that this variation can be exploited experimentally to investigate relationships between offspring size and fitness without the inherent confounding of interspecies comparisons (Marshall & Keough 2008a).

Intraspecific variation in offspring size has been shown to occur among populations (George 1994, 1996), within populations (Bingham et al. 2004), and between and within broods of individuals within populations (Byrne 1996, Marshall & Keough 2003b, Gosselin & Rehak 2007, Van Der Sman et al. 2009). Sources of this variation include differences in adult (maternal) size (Sakai & Harada 2001), environmental quality (Gimenez & Anger 2001, Collin & Salazar 2010), nutrition (George 1996, Steer et al. 2004) and latitude (Lardies & Castilla 2001, Monaco et al. 2010).

While the size of offspring can change in response to adult condition and to the environment, it has recently been shown that changes in offspring size may be adaptive responses by mothers to prevailing environmental conditions that increase offspring fitness. In other words, mothers have some ability to predict the environment that their offspring will experience and to adaptively alter offspring phenotype accordingly. For example, Allen et al. (2008) and Marshall and Keough (2009) showed that under competitive conditions the bryozoans *Bugula neritina* and *Watersipora subtorquata* produced larger offspring that were capable of dispersing further than smaller offspring, presumably enabling them to escape competitive conditions and increasing the likelihood that the offspring will obtain resources that will increase their fitness.

An explanation for variation in offspring sizes within broods from individuals has been more problematic and has, until recently been viewed as a by-product of physiological constraints associated with reproduction (Fox & Czesak 2000, Einum & Fleming 2004). A plausible alternative, but not mutually exclusive explanation proposed by Marshall et al. (2008) and Crean & Marshall (2009) is that the production of variably sized offspring within broods acts an adaptive bet-hedging strategy that increases mean offspring fitness in circumstances where environmental conditions fluctuate unpredictably. While theoretical modelling provides support for this idea (Marshall et al. 2008b), empirical studies examining the fitness consequences of variation in offspring size within broods of individuals are limited.

To date, most studies examining the fitness consequences of offspring size variation among and within species have focused on species that have dispersive pelagic larval stages in their life-cycles (reviewed in Marshall & Keough 2008a), and studies of species with direct development are relatively rare (but see Rivest 1983, Moran & Emlet 2001, Van Der Sman et al. 2009). Species that reproduce via direct development have the potential to provide valuable insights into the adaptive nature and consequences of offspring size variation because they exhibit greater levels of variation in offspring size within broods than species with dispersive pelagic larval stages (see Marshall et al. 2008b, Marshall & Keough 2008a). Indeed, some direct-developing species can exhibit up to 5-fold differences in offspring size among individuals and within broods (e.g. Byrne 1996). Thus, the fitness consequences of large differences in offspring size can be examined in direct-developing marine invertebrates without the confounding influences of inter-species or inter-individual comparisons.

The direct-developing sea star *Parvulastra parvivipara* exhibits substantial variation in offspring size within and among broods: the size of offspring adults give birth to can range from 0.6 to 3.5 mm in diameter. *P. parvivipara* is one of only five sea stars known to exhibit viviparity and direct development: hermaphroditic adults give birth to broods of up to 36 non-dispersive offspring in a single brood, and die soon after giving birth (Chapter 4). *P. parvivipara* is found at seven small, geographically separated locations along a 200 km stretch of the west coast of the Eyre Peninsula, South Australia, where it is found on the underside of rocks within tide pools (Keough & Dartnall 1978, Roediger & Bolton 2008). The tide pools that *P. parvivipara* inhabits undergo large daily and seasonal fluctuations in environmental conditions (e.g. temperature and salinity), and some pools are only ephemerally suitable for *P. parvivipara* (Chapter 2). I examined relationships between the size of offspring and their growth and survivorship during their first year of development. I maintained specimens in an experimental tide pool that underwent daily and seasonal fluctuations in temperature regimes to also examine if offspring fitness (growth and survivorship) relationships changed with seasonal changes in the environment.

5.3 Methods

5.3.1 Collection of adults and offspring

P. parvivipara from which offspring were subsequently obtained were collected from Smooth Pool (32^o 92 S, 134^o 07 E) on the west coast of the Eyre Peninsula, South Australia in July 2009. This period precedes the period of peak births in *P. parvivipara* populations that occur in the austral spring, although some individuals in all populations are known to give birth at other times of the year (Chapter 4). Twenty reproductively mature specimens (with a radius between 3 and 4 mm) were collected from the underside of granite rocks in one tide pool and placed into separate vials containing sea-water. Forty small granite rocks (pink granite, ~ 5 × 5 cm) were also collected from one tide pool and transported with *P. parvivipara* specimens in an insulated container to the Lincoln Marine Science Centre, Port Lincoln, South Australia.

Specimens were transferred into separate 250 mL beakers containing 200 mL of sea-water at the laboratory. These beakers were then partially immersed in a flow-through aquarium system to maintain their temperatures at that of ambient sea-water (~ 16^{o} C). Gentle aeration was supplied to each container through PVC tubing (inner diameter 0.8 mm; IsoflexKartell™) connected to the laboratory's air compressor, and the sea-water in each container was exchanged with filtered sea-water obtained from the flow through aquarium system every 3 – 4 days. Specimens of *P. parvivipara* held under these conditions gave birth to cohorts of offspring naturally. This enabled offspring from individual adults to be collected and for the naturally occurring variation in offspring size within broods to be experimentally exploited to eliminate experimental confounding that may be attributable to differences among adults. Four offspring were collected from each adult (n = 10) that gave birth within 3-weeks of collection, and were maintained in separate beakers prior to experimentation so that the adult (maternal) identity of offspring was known. The radius of each adult and their offspring (n = 4 per adult) was measured along the axis from the tip of the longest arm to the middle of the stomach on the actinal surface using the ocular micrometer in a dissecting microscope (accuracy ± 0.01 mm). The diameters of offspring used in the subsequent experiment ranged from 0.95 to 2.5 mm within broods.

5.3.2 Offspring growth and survivorship

To examine the consequences of offspring size on offspring fitness in *P. parvivipara*, the survivorship, growth and proportional growth relative to initial size (i.e. normalised for initial size) were measured throughout their first year of development. Each offspring was placed into an experimental container constructed of PVC pipe (height = 8 cm, radius = 51.5 mm and volume = 667 mL). To facilitate water exchange through the container, Nylon mesh (Sefar Nitex®, 200 μm pore size) was glued to one end forming a base. PVC legs (1 cm high) were then glued to the base of the containers to allow water to flow underneath them. To facilitate further water exchange through the container, two circular vents (40 mm diameter) were drilled on opposite sides and were similarly covered with nylon mesh. Because *P. parvivipara* appear to feed on biofilms that develop on granite rocks within tide pools that they inhabit (Keough and Dartnall 1978), a granite rock obtained from the tide pools that adults were collected from was placed in each container. These rocks also offered shelter, thereby providing similar conditions to natural tide pools for *P. parvivipara*.

Each experimental container that was randomly positioned within a flow-through sea-water aquarium (2 × 1 m, water depth = 7 cm, volume = 160 L). The aquarium was located outdoors in full sun to mimic natural tide pool conditions, and to allow a biofilm to be maintained on the granite rocks as a source of food for the offspring. To ensure that no positions offered preferential conditions to the sea stars, all randomly allocated positions within the aquarium received sun throughout the day. The interspersion of randomly positioned experimental containers was examined visually and deemed acceptable because there was no positional clumping of containers holding particular size classes or broods of *P. parvivipara* (see Hurlbert 1984). Adjacent experimental containers were separated by 10 cm to ensure sufficient water exchange. Sea-water within the aquarium was continuously exchanged with filtered sea-water plumbed via the laboratory's flow-through sea-water system. This allowed for the slow exchange of water but at a rate that still allowed the sea-water in the experimental tank to experience daily heating and cooling that was dependent on the daily and seasonal weather conditions. A lockable cage was placed over the aquarium to ensure that the sea stars and containers could not be disturbed.

Offspring were retrieved from each container using forceps or a pipette, and measured using methods given above at 3-month intervals over a year from August 2009 to August 2010. Mortalities were also recorded at each sampling interval from the presence of remnants of their bodies, or from microscopic observations of their tube feet, which are highly active in live specimens. The net growth (diameter, mm) and the percentage growth of initial offspring size (100 × size at time *x* /original size, %) were also determined at each sampling interval (calculated from time zero) for each offspring. Offspring were subsequently returned to their respective containers and positions within the aquarium. A temperature data logger (Thermochron ibutton, Temperature Technology, Adelaide, South Australia) was placed into the experimental tank to monitor the water temperature fluctuations over the duration of the experiment. The temperature range (minimum and maximum) of the experimental tank was determined for the periods July to September, October to December, December to March and March to June.

5.3.3 Statistical analyses

To examine the effects of initial offspring size on survivorship, I used two-way ANOVAs ($\alpha = 0.05$) in which offspring size was a categorical, fixed factor and the brood/adult that offspring came from was a categorical random factor. Each offspring was assigned to a size category based on their diameter: (1) < 2 mm (n = 26), or (2) ≥ 2 mm (n = 14). Offspring sizes were divided at 2 mm in diameter because this represents the approximate median size of offspring produced by *P. parvivipara* (range 0.2 – 3.55 mm in diameter; see Chapter 4). Analyses were conducted on data collected at 3-month intervals (i.e. seasonally). Because brood was deemed as non-significant in the analyses, and because mortalities of offspring occurred throughout the experiment and affected sample sizes, the net growth (mm) and proportional growth (%) of offspring against offspring size (category 1 or 2) were analysed using one-way ANOVAs ($\alpha = 0.05$). To ensure that the data met the assumptions of parametric analysis, distributional normality and homogeneity of variances were examined prior to analyses using quartile plots and Levene's tests. On occasions where the data did not meet these assumptions, square-root transformations were applied to rectify these problems.

Linear regression analyses were used to examine relationships between the size of adults (mothers, n = 10) and the mean growth and survivorship of their offspring within broods after 12-months of development. The coefficient of variation in offspring size within broods was calculated for each adult as a measure of variation that was independent of any differences in size among them. Linear regressions were also used to examine whether there was a relationship between the CV in offspring size within broods and survivorship of offspring within broods to determine if broods that had greater CV in offspring size had higher survivorship.

5.4 Results

5.4.1 Survivorship

During the first 3-months of development, 81 % of offspring < 2 mm in diameter survived, while 100 % of offspring ≥ to 2 mm survived. Neither offspring size, nor the brood that offspring came from, significantly affected offspring survivorship during the first 3-months of the experiment (Table 5.1). However, after 6-months, and following the onset of the austral summer, the proportion of larger offspring (≥ 2) that survived was significantly greater than that of smaller offspring (Table 5.1). Only 31 % of offspring within the smaller size class survived to 6-months, compared to 61 % of the offspring in the larger size class. Larger offspring had a significantly greater survivorship than smaller offspring throughout the remainder of the experimental period at 9 and 12-month periods (27 % and 10 % greater survival, respectively) (Table 5.1, Figure 5.1). Following 12-months of development, 29 %, of offspring ≥ 2 mm in diameter survived compared to 19 % of offspring < 2 mm (Figure 5.1). Brood identity did not influence the survivorship of offspring (Table 5.1).

After 3-months of development, 35 offspring survived across the 10 broods; however, this declined to only 17 offspring from 9 broods after 6-months. After 9-months, offspring from 9 of the 10 broods remained alive (n = only 13 offspring), and at the conclusion of the experiment only 9 offspring remained alive from 6 broods. Linear regression analyses failed to detect an effect of adult size on the mean survivorship of their offspring after 12-months ($P = 0.135$). Over the course of the experiment, all offspring ($n = 4$) died in 4 of the broods examined, and only one offspring survived in each of another 4 broods over the same period. Only two offspring survived in each of the 2 remaining broods after 12-months. The CV in offspring size within broods ranged from 10 to 33 %: however, linear regression failed to detect an effect of the CV in offspring size within broods from each adult did on the proportions of offspring surviving with broods after 12-months (*P* = 0.598).

In the first 3-months of the experimental period, which corresponded with the austral spring, 80 % of all offspring survived: however, after the austral summer (6-months into the experimental period) only 42 % of the offspring across all size classes survived. The survivorship of offspring across all size classes was 23% after 12-months.

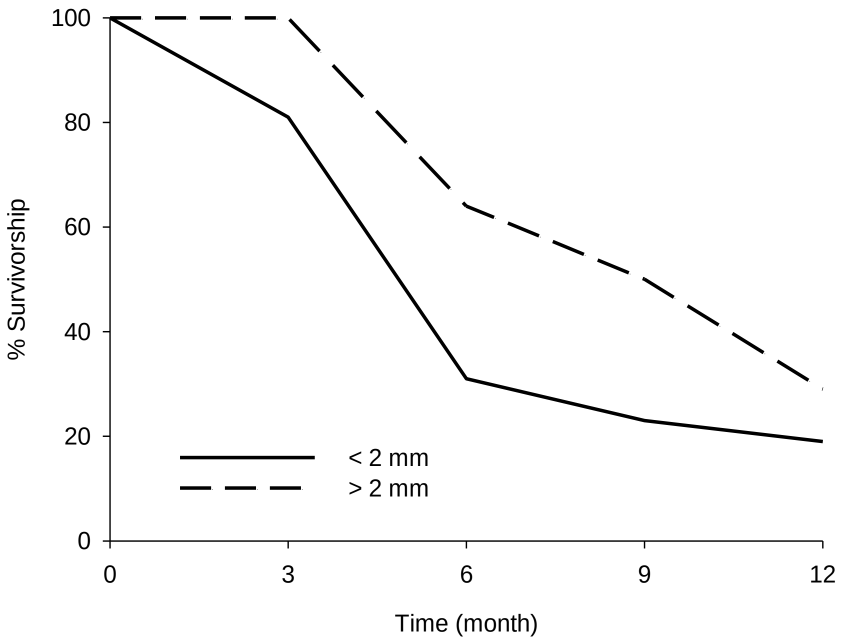

Figure 5.1. Cumulative percentages of survivorship of *P. parvivipara* offspring < 2 mm (solid line) and ≥ 2 mm in diameter (dashed line) over time during the first year of development.

5.4.2 Growth

After the first 3-months of development, which corresponded with the austral spring, there was no statistical difference in the net growth of small (< 2 mm diameter) or large offspring (≥ 2 mm) (mean ± SE increase in diameter 0.67 ±

0.09 and 0.81 ± 0.12 mm, respectively) (Table 5.2). Similarly, there was no statistical difference in the proportional growth of offspring from each size class after 3-months of development (mean 46 ± 5 and 37 ± 6 %, respectively) (Table 5.3). However, after 6-months of development over the spring and summer months, offspring within the small size class exhibited significantly greater proportional growth compared to offspring in the large size class (mean 128 ± 12 and 52 ± 11 %, respectively) and net growth (Table 5.2 & 5.3). After 9-months of development these differences remained significant. At the conclusion of the experiment (12-months), offspring in the small size class had greater net and proportional growth than those in the large size class, but only the proportional growth was significantly greater (71 % greater) (Table 5.2 & 5.3). Due to low survivorship of offspring, the likely power of the tests conducted following 12-months of the experiment were low (i.e. $n = 9$ after 12-months), thereby limiting the inferences that can be drawn from these results.

Linear regression failed to detect an effect of adult size on the mean net growth ($P = 0.329$) or mean proportional growth ($P = 0.576$) of offspring after 12-months of development. Similarly, linear regression analyses did not detect a significant relationship between the initial offspring size and the final size of offspring attained after one year ($P = 0.631$): sizes of offspring from the small and large size classes were statistically the same after 12-months of development. The statistical power of this comparison was substantially reduced due to the high mortality of offspring after 12-months ($n = 9$) and should therefore be treated with caution.

5.4.3 Temperature variation

Temperature fluctuations recorded in the experimental tank were greater between the months of October 2009 and March 2010 (Figure 5.2). The temperature range recorded between July and September was and June. The temperature data logger failed between December and March (summer) and therefore the data for this period is missing.

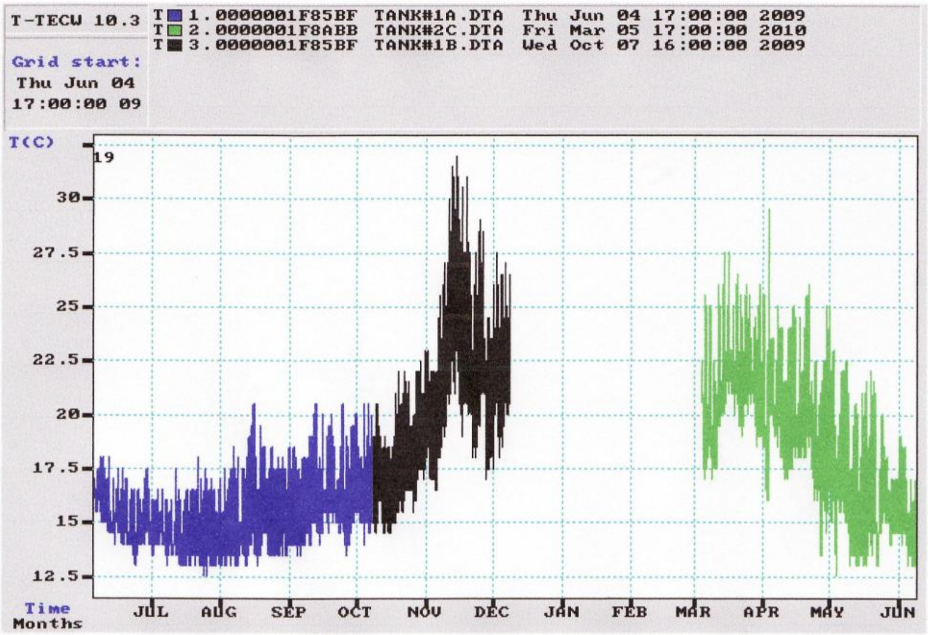

Figure 5.2. Raw data on daily temperature ranges experienced within the experimental tank (T-TECW Graph Viewer, Temperature Technology, Adelaide, South Australia). Note: daily temperature ranges are greater during late spring and early summer. Due to logger failure data is absent between December and March.

5.5 Discussion

Offspring size significantly affected the survival and growth of *P. parvivipara* during the first year of development, but varied in strength and direction over time. In the first 3-months of development, which corresponded with the austral spring, offspring size did not significantly affect offspring fitness (survival, net growth increment, or percentage growth of initial size). However, after 6-months of development, and following the summer months, larger offspring had higher survivorship than smaller offspring, and this persisted throughout the rest of the experimental period. Smaller offspring also exhibited higher growth as a proportion of their initial sizes at this time. The survival of offspring across all size classes was 80 % during the first 3-months: however, higher mortalities of offspring occurred during summer across all size classes (38 % died between December and January), but the mortality was highest among the smaller size class. Sixty percent of offspring that were larger than or equal to 2 mm in diameter survived the summer, while only 9 % of offspring smaller than 2 mm survived this period. After one year of development, the initial offspring size did not significantly affect the final size attained by offspring. Therefore, a major fitness advantage associated with producing larger offspring by *P. parvivipara* during the first year of development appears to be increased survivorship due to the disproportionately high mortality rate of small offspring during the summer months.

The higher levels of offspring mortality during the summer months are likely attributable to the relatively high levels and fluctuations in temperature, or salinity and irradiance over this period. *P. parvivipara* exhibit a peak in births during the spring and early summer months, but some individuals release their broods of offspring at other times of the year, and the characteristics of broods differ seasonally (Chapter 4). I previously found that fewer but larger offspring are produced by *P. parvivipara* during summer compared to offspring that are released at other times of the year (Chapter 4). Moreover, the coefficient of variation in offspring size within broods of *P. parvivipara* is also lower during the summer months (Chapter 4). These findings support the idea that the release of larger offspring in summer months may be an adaptive response by *P. parvivipara* to maximise offspring survival because the mortality rate of larger offspring was lower during this period. These findings also support the notion that the offspring size-performance relationships are steeper in harsher environments (Einum & Fleming 1999, Marshall & Keough 2008b) because survival rates of *P. parvivipara* offspring were lower across all size classes during summer, but highest among the largest individuals. However, further studies are needed to examine any disadvantages of producing larger offspring at other times of the year to determine if the response of changes in the mean offspring size displayed by *P. parvivipara* across seasons is indeed adaptive.

Recent modelling conducted by Marshall et al. (2008b) suggests that variation in offspring size within broods of marine invertebrates can be an adaptive strategy that increases mean offspring fitness in variable (unpredictable) environments via bet-hedging. The mean coefficient of variation in offspring size within broods of *P. parvivipara* used for this experiment (i.e. CV calculated on $n = 4$ offspring per brood) was 25 %, and the diameter of offspring within broods differed by approximately 3-fold (range = 0.9 – 2.5 mm in diameter). While differences in the coefficients of variation in offspring size within broods did not affect the percentage of offspring surviving from each brood under laboratory conditions utilised here, differences in mean offspring fitness may be apparent among broods in the field. Predictable versus unpredictable variation in environmental conditions may also influence the level of variation exhibited in offspring sizes and offspring survivorship. Seasonal fluctuations in environmental variables maybe somewhat predictable to *P. parvivipara*, however, events such as heatwaves and associated temperature rises are likely to be less predictable and may result high mortalities of offspring that are independent of their size and brood. While conditions within the experimental tanks were attempted to be maintained as close to field like conditions as possible, the conditions may not have been as unpredictable or harsh to show any affect of CV in offspring size within broods on offspring survival.

Tide pools that *P. parvivipara* inhabit undergo pronounced daily and seasonal fluctuations in environmental conditions (Chapter 2) that become more pronounced during the summer (Chapter 2). Indeed, daily fluctuations in water temperatures within tide pools often exceed 30^{o} C during the summer (Chapter 2). However, the amplitude and duration of daily and seasonal fluctuations in environmental conditions are highly variable among years (pers. obs.), and *P. parvivipara* may consequently have little ability to predict the severity of fluctuations with the exception that they are generally more pronounced during the summer. These daily, seasonal and yearly fluctuations in the level of environmental variability may be the driving force underlying the exceptionally large variation in offspring size within all broods of offspring released by *P. parvivipara*: as suggested by Marshall et al. (2008b) and Crean and Marshall (2009), this within brood variation in offspring size may represent an adaptive bet-hedging strategy that acts to maximise mean offspring fitness in an environment that fluctuates unpredictably. *P. parvivipara* populations present an exceptional opportunity to empirically test this idea in the field through experimental exploitation of naturally occurring variation in offspring size within and among broods, and variation in environmental conditions that are apparent among tide pools, seasons and years.

I found that the strength of the effect of offspring size on offspring growth depended on the timing of development: the higher net growth in the small size class of offspring did not persist throughout development. One possible explanation for this finding is that smaller offspring invest their energy into growth during harsh conditions (summer months) of development in order to escape the size range that is vulnerable to mortality during summer conditions. In contrast to my findings, higher growth rates have been observed in larger offspring in a variety of other marine invertebrates, including some direct developers (e.g. Rivest 1983, Moran & Emlet 2001, Marshall et al. 2003, Marshall & Keough 2005). However, observations of offspring growth have generally only taken place during the first few weeks or months of development, but the direction or strength of the relationship can change over longer times (Hendry et al. 2001, Dias & Marshall 2010). For example, Marshall and Keough (2004b) showed that initial larval size positively affected survival and growth in the bryozoan *Watersipora subtorquata*, but after 3-weeks there was no relationship between offspring size and growth. The absence of significant effects of the size of *P. parvivipara* offspring on survivorship during early development, and the subsequent changes in the pattern of growth observed throughout the study, highlight the importance of extended observations of the effects of offspring size on offspring fitness. Juvenile sea stars will also invest energy to forage for food, for movement, to digest food and also to develop internal organs such as reproductive organs at different stages throughout their development. Therefore energy invested in growth may also depend on the energy invested elsewhere in combinations with the environmental conditions experienced by offspring. It would be valuable to investigate how differences in the allocation of energy invested into growth by smaller offspring influence subsequent reproductive output and the characteristics of their broods.

5.6 Tables

Table 5.1. Two-way ANOVA comparisons of the survival of *P. parvivipara* offspring from 2 size classes (< 2 mm or ≥ 2 mm in diameter) and 10 broods at 3, 6, 9 and 12-month intervals throughout their first year of development.

Stage of development (transform)	**Source**	**df**	**MS**	***F***	***P***
3 months (√)	Size	1	0.571	3.708	0.223
	Brood	9	0.298	0.819	0.605
	Size × brood	7	0.154	0.424	0.877
	Error	22	0.364		
6 months	Size	1	22.527	19.058	**0.025**
	Brood	9	4.938	1.271	0.306
	Size × brood	7	1.182	0.304	0.944
	Error	22	3.886		
9 months	Size	1	56.679	11.784	**0.028**
	Brood	9	10.349	1.012	0.460
	Size × brood	7	4.810	0.470	0.846
	Error	22	10.227		
12 months	Size	1	81.027	7.614	**0.042**
	Brood	9	18.003	1.035	0.444
	Size × brood	7	10.642	0.612	0.740
	Error	22	17.368		

Table 5.2. One-way ANOVAs comparing the size of *P. parvivipara* offspring at birth (< 2 mm or ≥ 2 mm in diameter) and their net growth (mm) at 3, 6, 9 and 12-month intervals over the first year of development.

Stage of development	**Source**	**df**	**MS**	***F***	***P***
3 months	Size	1	0.348	1.818	0.186
	Error	36	0.192		
6 months	Size	1	2.976	12.921	**0.003**
	Error	15	0.230		
9 months	Size	1	3.909	10.337	**0.008**
	Error	11	0.378		
12 months	Size	1	1.512	4.588	0.069
	Error	7	0.330		

Table 5.3. One-way ANOVAs comparing the size of *P. parvivipara* offspring at birth (< 2 mm or ≥ 2 mm in diameter) and their growth as a proportion of initial size at 3-month intervals over the first year of development (calculated as size at time *x* /initial size × 100, where *x* is the 3, 6, 9 and 12-month sampling intervals).

Stage of development	**Source**	**df**	**MS**	***F***	***P***
3 months	Size	1	130.738	0.187	0.668
	Error	36	700.577		
6 months	Size	1	20926.243	33.986	< **0.001**
	Error	15	615.725		
9 months	Size	1	25495.167	34.139	< **0.001**
	Error	11	746.803		
12 months	Size	1	11360.556	18.362	**0.004**
	Error	7	618.714		

Chapter 6

General discussion

The population dynamics of marine invertebrates are highly complex and largely dependent on the life-history strategies that they employ (Pechenik 1999). While the population dynamics of intertidal marine invertebrates that have dispersive, pelagic larval stages have received substantial attention in the literature (e.g. Connell 1961, Underwood 1981, Menge & Sutherland 1987, Chapman 1994, Chapman & Underwood 1996, Menge 2000a), studies on the dynamics of species that reproduce via direct-development are lacking and are generally poorly understood. Coupled with this is the recent recognition that intraspecific variation in offspring size can significantly affect offspring fitness and the population dynamics of marine invertebrates, and that the level of this variation is particularly high among species with direct development (reviewed in Marshall & Keough 2008a). Previous studies into the consequences of intraspecific variation in offspring size in marine invertebrates have predominantly focused on species with dispersive larval phases. Our knowledge of offspring size-fitness relationships in direct-developing species is limited, as are its effects on the population dynamics of these species.

The main aims of my research were to (1) examine determinants of the distribution, abundance and population dynamics of *Parvulastra parvivipara* and *Cryptasterina hystera*, and (2) to examine relationships between environmental conditions and variation in the brood characteristics of these species. My research therefore aimed to contribute towards an improved understanding of population dynamics and the reproductive ecology in species with direct development, and to provide insight into the potentially adaptive nature of offspring size variation within direct-developing marine invertebrates. More broadly, my research aimed to contribute to our understanding of the fields of marine invertebrate reproduction and ecology.

The key findings of my research are:

- Populations of the direct-developing sea star *P. parvivipara* (Chapter 2) are highly variable within tide pools within populations, but abundances are stable at the population level and among years;
- The ecological variables that determine *P. parvivipara* distributions and abundances change seasonally, and a combination of tide pool structural complexity, wave exposure, depth, temperature and the presence of an anoxic layer within sediments in tide pools determine their abundance and presence (Chapter 2);
- The distribution and abundance of *C. hystera* (Chapter 2) can be predicted from a combination of tide pool structural complexity, size, depth, and the number of other organisms present;
- Population abundances of *C. hystera* are also stable across surveys but their distributions are dynamic, although to a lesser degree than those of *P. parvivipara* (Chapter 2);
- The brood characteristics of *P. parvivipara* (Chapter 3) change among seasons but the intertidal gradient inhabited by *P. parvivipara* does not influence brood characteristics including offspring size;
- Offspring sizes produced by *P. parvivipara* are larger in summer and exhibit the highest coefficient of variation (CV) in spring (Chapter 3);
- At higher population densities, *P. parvivipara* produced smaller offspring and brood sizes in controlled laboratory conditions, but larger and more variably sized offspring in the field (Chapter 3).
- The CV in offspring size (Chapter 4) was lower in the tropical sea star *C. hystera* compared to the temperate species *P. parvivipara*; and
- Offspring size affects offspring fitness in *P. parvivipara* (Chapter 5): larger offspring have higher survivorship following summer conditions, but smaller offspring exhibit greater rates of growth throughout the first year of development.

My research enhances the understanding of the population dynamics and reproductive ecology of direct-developing viviparous marine invertebrates in at least five ways. First, the absence of a pelagic larval stage does not result in large fluctuations in abundance at the population level for either of the direct-developing, viviparous sea stars examined here. Therefore, despite recent evidence suggesting that direct-developing species may undergo greater fluctuations in abundance than species with a dispersive larval stage (Eckert 2003) this generality does not apply to *P. parvivipara* and *C. hystera,* and in the short-term the population abundances of these species appear to be relatively stable.

Second, due to the absence of an ecologically decoupled life-history stage (i.e. larvae), these direct-developing species should have the greatest ability of all marine invertebrates to predict the environment that their offspring will experience and adjust offspring size to suit the conditions accordingly. The coefficient of variation (CV) in offspring size within and among broods of *P. parvivipara* did not differ among intertidal zones (mean 36 %), suggesting that environmental conditions may not differ sufficiently across these zones to affect offspring size variability. However, the density of *P. parvivipara* in tide pools affected brood characteristics in the field and lab and it is likely that other ecological variables may also do the same, but this requires further controlled testing. Despite the maternal and offspring habitat being the same, thereby maximising a mothers ability to predict her offspring environment, the high CV in offspring size exhibited by *P. parvivipara* within broods may be an adaptive response to maximise reproductive success in conditions that are inherently variable and unpredictable (i.e. no one offspring size is optimal in environmental conditions that are constantly changing).

Third, the levels of variation in offspring size differed significantly between *P. parvivipara* and *C. hystera*: in the temperate environment more variable offspring sizes were produced. The differences in variability in offspring size exhibited between these two species may have some adaptive value in response to the different environmental conditions experienced between temperate and tropical environments: the temperature range was greater in the temperate habitat. This requires further testing, but the evidence suggests that there are different selective pressures acting on these species reproductive ecology and offspring size variability. I also suggest that intertidal habitats are inherently variable and the high CV in their brood characteristics may increase mean offspring fitness via bet-hedging.

Fourth, while plasticity in offspring traits mediated by the environment are observed in some species with dispersive offspring (larvae) (e.g. Marshall et al. 2006, Allen et al. 2008, Leips et al. 2009, Marshall & Keough 2009), little is known about plasticity in species with no dispersal capacity (i.e. direct developers). The adaptive nature of the differences in mean offspring size exhibited by *P. parvivipara* across seasons and populations is potentially related to differences in offspring fitness: larger offspring had greater survivorship following harsh summer environmental conditions, which is also when significantly larger offspring were produced at all populations in the field. Thus, the maternal habitat and the environment experienced by direct-developing species such as *P. parvivipara* appear to have some influence on long-term maternal provisioning to offspring.

Lastly, the reproductive traits exhibited by *P. parvivipara* and *C. hystera* have most likely evolved to maximise survival and maintain their population persistence. My findings suggest that the complex reproductive traits that these species exhibit is ensuring successful recruitment and that in the short-term their stability in abundance at the population level does not appear to be compromised by variation in offspring sizes.

More generally, surveys conducted on populations of *P. parvivipara* and *C. hystera* show that they undergo dramatic variations in abundance within populations at the small localised scale of individual tide pools (Chapter 2). The tide pools that they inhabit appear to act as separate pockets of populations that have isolated dynamics. Patterns of disappearances of sea stars from numerous ostensibly suitable tide pools, and subsequent recolonisation events occurred among tide pools within populations. In contrast to the dynamic nature of their distributions among tide pools, abundances of *P. parvivipara* and *C. hystera* were relatively stable over time at the population level (Chapter 2) and the existence of small-scale metapopulation relationships may be responsible for this stability: while individual tide pools undergo large fluctuations in abundance, population level abundances appear to be stabilised by the passive transport of individuals between tide pools. Passive transport of sea stars may occur sporadically through the water column during extremely high tides and/ or during period of intense wave energy (i.e. storms events). Furthermore, active movement of specimens may also occur between some tide pools during high tide or specimens may move through damp crevices or under boulders where moisture remains.

The concept of marine metapopulation dynamics has been previously established in intertidal tide pools systems, where despite tidal flushing and water movement, separate dynamics are maintained within tide pools (see Johnson et al. 2001, McAllen & Taylor 2001, Grimm et al. 2003). Future examinations of passive dispersal or active movement of *P. parvivipara* and *C. hystera* between tide pools will elucidate whether this phenomenon is indeed occurring within populations of these species and other direct-developing marine invertebrates that inhabit tide pool environments. While some tagging methods I attempted failed (nail-polish and super-gluing tags—data not presented here), alternative techniques such as discoloration of calcified body structures or tissues with chemical dyes (i.e. tetracycline) could be attempted in order to track individuals within the complex tide pool system that they inhabit. Alternatively, molecular genetic studies using high-resolution markers (e.g. microsatellites) or removal and recolonisation experiments could potentially be used to determine levels of connectivity within populations.

In a review of relationships between the length of the planktonic period and population fluctuations among marine invertebrates, Eckert (2003) found that population fluctuations among species that have no planktonic period exhibited a CV in abundance that ranged from 5 to 359 %, but had a mean of 134 ± 8 %. Eckert (2003) also found that those species that exhibited a short versus a long pelagic larval stage had an average CV in abundance of 88 % and 109 %, respectively. The CVs in abundance of *P. parvivipara* and *C. hystera* (104 and 80 %, respectively) fit within the range of values found by Eckert (2003) for direct-developing species, and on average do not undergo greater fluctuations in abundance than the mean values of species with dispersive pelagic larval phases reported by Eckert (2003). They also fall outside of the outside of the 95 % confidence interval range of expected CV's in abundance based on her data for species with non-planktonic development. Furthermore, in comparison with the mean values in Eckert's (2003) data, the fluctuations in abundances of *P. parvivipara* and *C. hystera* also show that direct-developing species do not necessarily undergo less variation than those with a pelagic larval stage, as originally described by Thorson (1950). However, my results only captured a small window of the population dynamics of *P. parvivipara* and *C. hystera*, and longer-term studies examining their distributions and abundances of additional populations are needed to adequately test this conclusion. Similar examinations of closely related species that exhibit direct development (e.g. *Parvulastra vivipara, Cryptasterina pacifica*) and other life-history strategies (e.g. *Patiriella regularis, Parvulastra exigua, Cryptasterina pentagona, Meridiastra calcar*) would also be valuable.

To enhance our understanding of the reproductive ecology of direct-developing marine invertebrates, I examined the reproduction and brood characteristics of *P. parvivipara* among populations and seasons, and across an intertidal platform with varying levels of environmental stability (Chapter 3). My results from Chapter 3 suggest that variation in offspring size of *P. parvivipara* does not increase with the level of environmental variability across an intertidal platform, and therefore tends to not support the notion that this represents an adaptive bet-hedging strategy to unpredictable environmental conditions within a species (Marshall et al. 2008b). Nor did variation in population densities consistently alter the reproductive output of *P. parvivipara* among tide pools in the field or in the laboratory. It is important to note, however, that conditions across the intertidal zone may not have differed enough in their variability to detect any change. Furthermore, it is also possible that the level of variability in offspring size is so great in response to environmental conditions that are inherently capricious that further variation in offspring size among intertidal zones may be adaptively inconsequential. The lack of field estimates of juvenile mortality is also a limiting factor on the inferences that can be made from this work. Field-based measurements of the mortality rates of the non-dispersive offspring of *P.*

parvivipara may be feasible and would contribute substantially to our understanding of the ecology of species with direct development.

While my research does not appear to indicate that there is any relationship between density and adaptive plasticity in offspring and brood traits in *P. parvivipara*, recent research by others suggests otherwise (e.g. Marshall et al. 2006, Allen et al. 2008, Leips et al. 2009, Marshall & Keough 2009, but see Menge 1974). However, a potentially critical difference between *P. parvivipara* and the species examined by Marshall et al. (2006), Allen et al. (2008), and Marshall and Keough (2009) is that they do not have a dispersive larval stage. Therefore, while plasticity in offspring traits mediated by increased competition that are observed in some species with dispersive offspring may be related to adaptive alterations in their dispersive capacity, similar plasticity in species with no dispersal capacity would be irrelevant. Furthermore, the reproductive output of marine invertebrates can be can be influenced by a range of other biotic and abiotic variables and it is likely that additional variables including temperature (Lardies & Castilla 2001, Collin & Salazar 2010), salinity (McAllen & Brennan 2009), predation (Marshall & Keough 2004a) and other sources of competition (Allen et al. 2008, Marshall & Keough 2009) may have influenced the brood characteristics of *P. parvivipara* among densities. Studies examining plasticity in offspring and brood traits in relation to a range of potential environmental and ecological factors across species with diverse life-history strategies are needed to address this problem.

However, across populations and seasons there were differences apparent in the sizes of offspring released by *P. parvivipara* that may indicate a bet-hedging strategy based on changes in offspring size over time. During summer, offspring within broods were on average 23 % larger than at other times of the year, and also had higher survivorship (fitness) than smaller offspring during summer. Perhaps more importantly, my findings suggest that *P. parvivipara* may employ a much more complex interplay of bet-hedging strategies in which adults not only adaptively alter offspring phenotypes within broods in response to seasonal environmental factors, but that each adult alters the timing of birth in response to environmental conditions and the phenotypic characteristics of their brood.

To further explore the relationship between offspring size variability and the environment, I compared the coefficient of variation in offspring size between the temperate or tropical direct-developing sea stars *P. parvivipara* and *C. hystera* (Chapter 4). I used temperature range and mean temperature variation as a proxy to compare the variability of the temperate and tropical environment. It is likely that a range of other biotic and abiotic variables vary between the two habitats and contribute to differences in environmental stability between tropical and temperate intertidal environments. Measurements of these variables would provide valuable information towards increasing our understanding of the notion of relative environmental stability in these environments (e.g. food supply, salinity, pH). This study showed that variation in offspring size is significantly lower in *C. hystera*, which inhabits a tropical environment, compared to *P. parvivipara*, which inhabits a temperate environment. These findings are, however, sensitive to the confounding influence of the interspecific comparison and absence of replication at the species level. Controlled experiments in which environmental stability is manipulated are needed to determine whether there is a causal relationship between environmental stability and variation in offspring size that we could interpret as an adaptive shift in offspring phenotypes.

The level of variation in the size of offspring exhibited by *P. parvivipara* is extremely high in comparison to many other marine invertebrates, including those that reproduce via direct development (see Marshall and Keough 2008). The diameter of offspring observed among broods ranged from 0.2 to 3.5 mm. Previous studies have shown that intraspecific variation in offspring size can significantly affect offspring fitness of marine invertebrates (e.g. Moran & Emlet 2001, Marshall et al. 2003, Marshall & Keough 2004a, Marshall 2005, Allen et al. 2008, Marshall and Keough 2008a). In Chapter 5, I found that the size of offspring produced by *P. parvivipara* significantly affected offspring growth and survivorship: larger offspring had greater survivorship, but smaller offspring had higher percentage growth of initial size after the first year of development. However, the effect of offspring size on survivorship was not evident until offspring experienced summer conditions. The fitness benefits associated with producing offspring that are larger corresponded to seasonal differences observed in the brood characteristics of *P. parvivipara*: offspring are larger, and broods are smaller with less variably sized offspring during the summer months (Chapter 3), which is when large offspring have higher survivorship than small offspring. Seasonal variation in offspring size and brood characteristics of *P. parvivipara* may therefore act to maximise offspring fitness of *P. parvivipara*. Moran and Emlet (2001) have also demonstrated that the fitness advantages associated with offspring size can change among seasons and environmental conditions in a direct-developing species, but they conversely showed that advantages associated with differences in offspring size were lost in harsh conditions. They did not, however, note changes in brood characteristics that align with offspring fitness advantages.

An investigation of the potential consequences of smaller offspring allocating greater energy into growth than larger offspring during the first year of development on future life-history traits (e.g. reproductive output) was beyond the scope of this study, but may provide substantial insights into the ecology of direct-development. Field based experiments to monitor such traits would provide insight into how populations may be affected by differences in offspring size and the cost or benefits of the variation in brood characteristics exhibited by *P. parvivipara*. The adaptive nature of the variation in offspring size exhibited by direct-developing species remains poorly understood (Marshall and Keough 2008a) and warrants further research into the long-term implications of variation in offspring size throughout development and subsequent reproduction.

In conclusion, while the population dynamics of intertidal marine invertebrates are well documented and the effects of offspring size variation in marine invertebrates are beginning to be understood, large gaps remain in our understanding of the ecology of direct-developing species. In addition to potential research directions described above, future avenues for research on direct-developing species include their roles in food chains, relative predation risk, likelihood of competition, and their longevity. Species come and go, and the fossil record shows that with direct-developing, viviparous species the "going" is likely to be more rapid than for species with dispersive life-history stages due to their highly restricted distributions and limited ability to inhabit new environments (Emlet 1989, Jeffery & Emlet 2003). Consequently, direct-developing species are susceptible to accelerated extinction rates and this may be more rapid than usual given that the sea level rise that is anticipated to occur as a result of global climate change (Harley et al. 2006, Helmuth et al. 2006, Mislan et al. 2009). This susceptibility is, however, tempered by the possibility that sea level rise may open up new habitat for them to expand into.

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