Population biology of the sand dollar, *Peronella lesueuri*, in Cockburn Sound, southwest Australia

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I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

Peronella lesueuri, commonly known as the pink sand dollar, is an irregular echinoid that can be found in the sandy sediment substrates of Cockburn Sound, southwest Australia. The numbers in which they are found indicate that they are likely to be an ecologically significant species. This is the first study of the biology and ecology of this species.

A survey of current spatial distribution and abundances of *Peronella lesueuri* populations at a range of sites within Cockburn Sound shows the presence of the sand dollars on the shallower, sandy sills that surround the Sound. *P. lesueuri* was notably absent from the deeper central basin of Cockburn Sound, where silt is a major component of the sediment. The population densities of *P. lesueuri* ranged from 0.3 to 2.5 individuals m^{-2} at sites no deeper than ten metres. Mean test lengths were larger at sites less than five metres in depth. Sediment grain size preference and food availability were likely factors that determined the spatial distribution of the sand dollars in Cockburn Sound.

Detailed monthly sampling for population demographics was conducted at Jervoise Bay over a 23-month period in which significant temporal variations in the densities of the *P. lesueuri* were recorded. Two spikes in population densities in December

2010 and January 2011 were indicative of clear annual recruitment events. The spike in densities caused by recruitment, however, were quick to return to what appeared to be the stable population density of 0.13 – 0.3 individuals m^{-2}.

This study showed that the *P. lesueuri* reproductive cycle is annual, with peak spawning in summer. Gametogenesis was recorded in spring, spawning in summer, spent gonads in autumn and gonads recovered during winter. The gametogenic cycle of *P. lesueuri* underwent five distinct morphological stages; Recovery, Growth, Mature, Partially Spawned and Spent, similar to those described in other echinoderm gametogenic cycles.

In the recovery stage, nutritive phagocytes filled the lumen of both the testes and ovaries. Primary gametocytes (Stage 1 gametes) were present large numbers. Growing gonads contained increasing numbers of secondary gametocytes. Mature gametes are present in some gonads while nutritive phagocytes decreased in volume. In fully mature male gonads, lumens were densely packed with spermatozoa. Two variants of the mature stage were observed in the ovaries; one was packed full of ova with few oocytes, the other contained oocytes in all stages of development. Some female sand dollars produced only one cohort of ova which are spawned out once all the ova are released, while others can produce several cohorts of ova throughout the spawning period. Partially spawned gonads contained large numbers of mature gametes although spaces left by spawned gametes were observed. Lumens of spent gonads were mostly empty, although unspawned gametes in various stages of degeneration were occasionally present. Unspawned gametes eventually broke down and were incorporated into the

nutritive phagocytes. The gametogenic stages were highly synchronous between sexes. Juveniles attained sexual maturity at between one and a half and two years of age.

Size frequency distributions as well as density variation over a 23-month period indicated that *Peronella lesueuri* recruits annually. Juveniles appeared in the adult population around May/June, five to six months after the start of the spawning period. Annual recruitment strength was variable and juvenile mortality was high. Up to 88% juvenile mortality was measured in the first year alone.

Pooled length-at-age data derived from the 2009 and 2010 cohorts of recruits was combined with length-at-age data for large “adult” sand dollars obtained from growth zone counts to construct a scatter plot to fit a growth curve. Growth in *P. lesueuri* is sigmoidal and is best described by the Richards’ Growth Curve. Juvenile *P. lesueuri* grow quickly, with a maximum growth rate of 60 mm yr^{-1} at two years of age. Intermediate-sized sand dollars were scarcely encountered. The rapid growth rate of sand dollars of intermediate size predicted by the Richards’ growth curve coupled with high juvenile mortality provides an explanation for the scarcity of sand dollars between 50 and 100 mm. The growth rate slowed to close to zero at approximately two and a half years of age, and this may be associated with the attainment of sexual maturity. Maximum size in the population studied was 182 mm. The maximum life expectancy is approximately five years.

This study indicated that P. lesueuri has diurnal and seasonal patterns of activity throughout the year, with greater movement rates in summer (mean of 5.3 cm hr^{-1}, day; 3.9 cm hr^{-1}, night) than in the winter (mean of 2.7 cm hr^{-1}, day; 2.0 cm hr^{-1}, night). Seasonal changes in temperature and physiological requirements by the sand dollar are the most likely reason for the seasonal differences; however reasons for diurnal movement variation were unclear. Direction of movement was found to be random at both times of the year. Based on the movement rates, and at a density estimate of 0.5 sand dollars per m^{-2}, it is estimated that the sand dollars can rework the entire area of the sediments in the habitats they occupy in approximately 20 days.

This study provided the first histological description of the gametogenic cycle in *Peronella lesueuri* and established that the reproductive cycle is annual. The growth rate and life expectancy of *P. lesueuri* was also determined. The study of diurnal and seasonal patterns of activity in *P. lesueuri* indicated that it was ecologically significant as a sediment bioturbator. While further research is required to fully assess the biological and ecological significance of *P. lesueuri* in Cockburn Sound, the densities in which *P. lesueuri* is found on the shallow sediment bottoms imply that they have a potentially important role as an agent in mediating biogeochemical processes in the surface sediments.

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Chapter 1 Introduction and Review of the Literature

1.1 Introduction

Sand dollars are commonly observed, abundant macro-organisms in sandy marine sedimentary substrates across a range of depths in intertidal and subtidal zones. *Peronella lesueuri* (L. Agassiz, 1841), commonly known as the pink sand dollar is a large abundant species in south-western Australia. Prior to this study, little was known of its biology and ecology.

1.1.1 Taxonomy and Distribution of Peronella lesueuri

Classification of *Peronella lesueuri*:

PHYLUM: Echinodermata
    CLASS: Echinoidea
        ORDER: Clypeasteroida
            FAMILY: Laganidae
                GENUS: *Peronella*
                    SPECIES: *lesueuri* (L. Agassiz, 1841)

In his 1938 monograph, Echinoderms from Australia, Clark (1938) noted the distribution of *Peronella lesueuri* around Australia from southern Queensland, northward and westward towards Darwin and Broome, and then along the west coast down to Fremantle and Albany. *P. lesueuri* was also encountered in more recent marine surveys in the Dampier Archipelago (Marsh and Morrison 2004) and

Kimberley regions (Keesing et al. 2011). *P. lesueuri* has a wide Indo-Pacific distribution (Figure 1.1) and has been recorded in Hong Kong (Agassiz and Clark 1914; Clark 1921), Southern Japan (Agassiz and Clark 1914; Clark 1921), Singapore (Agassiz and Clark 1914) and the Philippines (Agassiz and Clark 1914). *Peronella lesueuri* can be found in coarse to fine sandy sedimentary substrates. In Cockburn Sound, it is found on the shallow banks and sills that surround the deep basin, in densities of up to six individuals m^{-2} (Marsh and Devaney 1978; Forehead and Thompson 2010). *P. lesueuri* has not been reported in the fine muddy substrate of the deep basin (Marsh and Devaney 1978; Cary et al. 1995; Forehead and Thompson 2010).

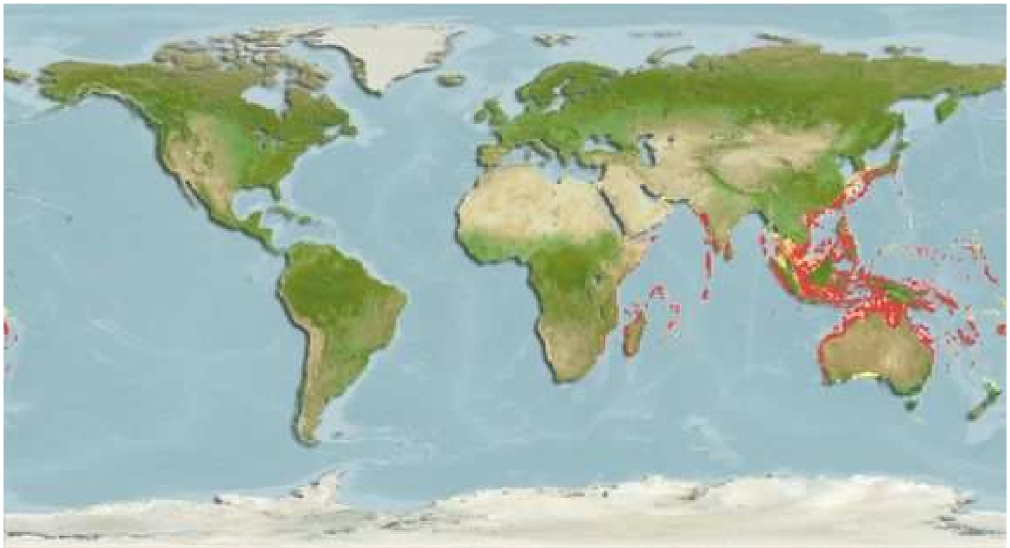

Figure 1.1 Predicted worldwide distribution of *Peronella lesueuri* (Aquamaps 2011). The distribution is shown by the red dots.

The class Echinoidea includes sea urchins, heart urchins and sand dollars. Sand dollars, such as *Peronella lesueuri*, have a flattened, rigid test formed by 10 rows of interlocking plates, covered by a velvet-like layer of spines and podia, which are used for locomotion and feeding (Mooi and Telford 1982; Ellers and Telford 1984; Telford et al. 1985). The test consists of five alternate ambulacral and interambulacral areas, each made up of two rows of plates. With a distinct pink colour, described as old rose by Clark (1938), *Peronella lesueuri* can grow up to lengths of 18 centimetres, although in Cockburn Sound, lengths of 13-15 centimetres are more common. Sand dollars are bilaterally symmetrical and have an anterior-posterior axis which passes through the peristome and periproct on the oral surface and through the madreporite, which is centrally located on the aboral surface of the test. Four gonopores are located in the genital plate adjacent to the madreporite. Oval in shape, *P. lesueuri* is slightly elongated along its anterior-posterior axis.

Compared to the extensive literature available on regular echinoid population ecology, there is far less published scientific literature about irregular echinoids and the only published literature to date on the reproductive biology of *Peronella lesueuri* reported that individuals from a lagoon near Misaki in Japan had ripe gonads in late June and July (Mortensen 1921). Mortensen (1921) also noted that *P. lesueuri* had yolk-laden ova with diameters of 0.3-0.4 mm, which suggested lecithotropic development.

1.2 Review of the Literature

Extensive ecological and biological research has been conducted on echinoderms; however the majority of the research has not focused on irregular echinoids, which includes heart urchins and sand dollars. This review of the literature will therefore include articles on all Echinoidea; however, emphasis will be placed on irregular echinoids and sand dollars in particular.

1.2.1 Sand dollar distribution

Echinoids have a worldwide distribution which encompasses a bathymetric depth range from the intertidal to abyssal zones. They occupy a range of habitats, including coral and limestone reefs, benthic sediment and intertidal beaches and rock pools. Sand dollars are found in benthic communities and appear to be common in soft sediment habitats in shallow tropical to subtropical regions. They have also been encountered in temperate zones and at depth. Species such as *Echinarachnius parma* and *Dendraster excentricus* occur in the temperate zones of the North American continent (Birkeland and Chia 1971; Stanley and James 1971; Cabanac and Himmelman 1996). At Sable Island, Southeast Canada, *E. parma* was encountered at a maximum depth of 256 metres (Stanley and James 1971), while *D. excentricus* has been encountered at depths of 90 metres (Clark 1948; as cited in Birkeland and Chia 1971).

1.2.1.1 Habitats

Many sand dollar species are known to occur both in the intertidal shallows as well as subtidal zones. Oftentimes distribution across the beach is size related. In Mexico, juveniles of the sand dollar *Encope grandis* were found throughout the intertidal zone, whereas larger individuals were more abundant in the lower intertidal zone (Ebert and Dexter 1975). At Pallarenda Beach in Queensland, Australia, *Arachnoides placenta* had a similar distribution, with larger individuals found towards the lower section of the beach (Haycock 2004). Lane and Lawrence (1980) observed that adults of *Mellita quinquiesperforata* move into deeper water, while juveniles settle in shallow, inshore areas. The presence of juvenile *Dendraster excentricus* higher up on the shore than adults was also observed along the Pacific coast of California and Baja California, Mexico (Merrill and Hobson 1970). In these cases, it is possible that juvenile sand dollars are less capable of manipulating the finer sediment and the coarser sand higher up the beach provides a more stable environment for them (Haycock 2004). Conversely, adults of the subtidal sand dollar *Echinarachnius parma* in the Gulf of St Lawrence have a propensity to move upstream into shallower waters, with the juveniles occurring at greater depth (Cabanac and Himmelman 1996). The authors suggested that larger individuals were better adapted for the shallows and less likely to be transported away by water turbulence than juveniles.

There are few observations on the habitat preferences of *Peronella lesueuri* in the literature. However, Mortensen (1921) observed *Peronella lesueuri* on the sandy sediment in a shallow lagoon in Misaki, Japan and within Cockburn Sound, *P. lesueuri* has been observed at a depth of 4-8 metres (Forehead and Thompson 2010), suggesting that this species occurs subtidally. It is unclear if *P. lesueuri* can survive in the intertidal zone.

1.2.1.2 Sediment preferences

Many irregular echinoid species display preferences for a particular substrate. Sediment grain size has a significant effect on the burrowing, locomotive and feeding behaviours of irregular echinoids (Ghiold 1979; Lane and Lawrence 1982; Telford et al. 1987; Schinner 1993; Cabanac and Himmelman 1996). *Schizaster* *canaliferus*, a heart urchin common in the North Adriatic Sea, only displayed burrowing activity in fine sediments (2-20 µm) with burrowing activity not observed in sandy sediments larger than 63 µm (Schinner 1993). Although the sand dollar *Echinocyamus pusillus* can be found in coarse substrate (>1000 µm) where it nestles between pebbles (Telford et al. 1987), the preferred mean/largest grain size of most species of sand dollars is usually between 100-200 µm (Ghiold 1979; Telford et al. 1987; Telford 1990; Pomory et al. 1995). In sediment size preference experiments, sand dollar *Mellita quinquiesperforata* was unable to burrow in sediment that was too large (>1000 µm) or too small (<63 µm) (Ghiold 1979). However, Pomory et al. (1995) found that *Mellita tenuis* had the capability to burrow and move in grain sizes larger than their preferred size. Sand dollars manipulate sediment while moving as well as feeding; this led Pomory et al. (1995) to suggest that the dimensions of the feeding podia of each species of sand dollar may play a larger role in determining the preferred sediment grain size than the ability to burrow or move.

Unlike heart urchins, which commonly burrow in fine, silty sediment (Schinner 1993; Hollertz and Duchene 2001; Lohrer et al. 2005), silt is detrimental to some species of sand dollars. In Beaufort, North Carolina, Weihe and Gray (1968) observed a drop in abundance of juvenile *Mellita quinquiesperforata* from the sand flats at Bird Shoal when dredging suspended and deposited mud and silt over the sand flats. When placed in mechanically sorted fine sediments (63 µm) in the laboratory, *M. quinquiesperforata* began burrowing until the fine sediment coated their tests and caused burrowing to cease; the fine layer of sediment on the test obstructs spine movement, eventually causing death (Ghiold 1979).

In Cockburn Sound, *Peronella lesueuri* has been observed at depths of 4-8 metres in medium and fine sands (Forehead and Thompson 2010), but was notably absent from the deeper main basin, where silt dominates the sediment (Marsh and Devaney 1978; Cary et al. 1995).

1.2.1.3 Diet and feeding mechanisms

Sand dollars are size selective deposit feeders (Ellers and Telford 1984; Telford et al. 1985; Telford and Mooi 1986; Telford 1990; Hilber and Lawrence 2009). The diet of *P. lesueuri* is not known but sand dollars are known to feed on diatoms from the microphytobenthos, foraminifera and other microeukaryotes, as well as lipid and bacterial components coating sedimentary particles (Lane and Lawrence 1982; Mooi and Telford 1982; Findlay and White 1983; Ellers and Telford 1984; Telford et al. 1987).

In the literature, two mechanisms were suggested for feeding in these sand dollars. Both methods assumed that smaller particles were selected from the surrounding sediment.

The sieve hypothesis suggests that particle size selection occurs when aboral spines behave like a sieve (Chia 1969; Ghiold 1979). Ciliary currents were used to sweep suspended matter (<100 µm), which contains food particles like diatoms, onto the test surface and then into food tracts, where they are caught in mucus. The mucus strings are then carried by the ambulacral and buccal tube feet into the mouth. This hypothesis assumes that the role played by podia in food selection and transport is secondary. Authors who subscribe to this hypothesis believe that *Dendraster excentricus* (Chia 1969), *Echinarachnius parma* (Mooi and Telford 1982), *Lovenia elongata* (Lawrence and Ferber 1971), *Mellita quinquesperforata* (Lane and Lawrence 1982) and *Mellita sexiesperforata* (Goodbody 1960) feed in this manner.

The second hypothesis suggests that feeding podia on the oral surface of the sand dollar select particles out of the substrate (Ellers and Telford 1984; Telford et al. 1985). The particles are then passed along the podia to the food grooves where they are collected in mucus cord. Another set of specialised podia then transport the mucus cord to the mouth where the modified Aristotle's Lantern crushes the sand grains and diatoms (Ellers and Telford 1984; Telford et al. 1985). *Mellita quinquiesperforata* was observed to feed in this manner by Telford et al. (1985) in contradiction to Lane and Lawrence (1982).

While most sand dollars are found buried or prone on the surface of the sediment, some sand dollar species adopt a rare and novel feeding method: suspension feeding. Suspension feeding has been reported in three scutellid sand dollars – *Dendraster excentricus* (Chia 1969; Morin et al. 1985; Francisco and Herzka 2010), *Dendraster vicainoensis* and *Encope michelini* (Lawrence et al. 2004), and two laganid sand dollars – *Heliophora orbiculus* and *Rotula augusti* (Dartevelle 1935 as cited in Lawrence et al. 2004) but has, however, only been studied in *D. excentricus* and *Encope michelini*.

When suspension feeding, these sand dollars bury their anterior end in the sand and assume a vertical (inclined) posture. This vertical position places the sand dollars in an ideal position to intercept suspended food particles that are swept over the sediment beds. In areas of moderate water movement, *Dendraster excentricus* adults are usually positioned vertically (Merrill and Hobson 1970). At low tidal levels, however, *D. excentricus* changed its position from vertical to being prone on the sediment and its feeding method from suspension to deposit feeding (Francisco and Herzka 2010). In areas of high water movement, *D. excentricus* is usually buried in the sediment (Merrill and Hobson 1970).

There is a significant gap in the literature pertaining to the diet and feeding mechanisms of *Peronella lesueuri.* While the feeding mechanism of *P. lesueuri* is unknown, the few documented observations of *P. lesueuri* in the literature have noted them on or buried in the sediment (Mortensen 1921; Forehead and Thompson 2010). There is no evidence that *P. lesueuri* adopts an inclined position for suspension feeding.

1.2.1.4 Aggregation and patchiness

Echinoderms commonly form dense aggregations (Konnecker and Keegan 1973; Garnick 1978; Young et al. 1992; Lauzon-Guay and Scheibling 2007a; Lauzon-Guay and Scheibling 2007b; Alvarado 2008; Westerberg et al. 2008). Sea urchins have the ability to migrate towards a food source (Ceccherelli et al. 2009) and form feeding aggregations despite the randomness of their movements (Hereu 2005; Lauzon-Guay et al. 2006; Dumont et al. 2007). *Strongylocentrotus droebachiensis* has a tendency to move further in areas where food is scarce, concentrating in areas of greater food availability (Lauzon-Guay and Scheibling 2007) and aggregating into a feeding front (Abraham 2007). Feeding fronts can be destructive (Lauzon-Guay and

Scheibling 2007) with the urchins grazing all the foliose algae from an area, leaving a “barrens habitat” (Langdon et al. 2011).

Along the Pacific coast of North America, the sand dollar *Dendraster excentricus* has been known to occur in large, densely aggregated beds of up 1,200 individuals m^{-2} (Merrill and Hobson 1970; Birkeland and Chia 1971; Highsmith 1982; Morin et al. 1985). This close spacing allows the sand dollars to be in the inclined position to feed, with the highest densities occurring in the deepest regions of the beach where surge is lower (Morin et al. 1985). Substrate and water movement appeared to limit the distribution of sand dollars shoreward (Morin et al. 1985).

Mellita quinquiesperforata, a sand dollar common to the tropical and subtropical Atlantic coast of North and South America, has a propensity to aggregate. Instead of forming dense beds like *D. excentricus*, they tend to form clearly defined clusters (Weihe and Gray 1968; Bell and Frey 1969; Lane and Lawrence 1980). While large-scale aggregations of benthic organisms can be attributed to physical environmental factors, patchiness could be attributed to the distribution of sediment grain size composition and food availability at a smaller scale (Swigart and Lawrence 2008). *M. quinquiesperforata* have been observed to aggregate around depressions in the sand or behind sand bars that act as silt traps (Weihe and Gray 1968; Lane and Lawrence 1980). Off the Central Florida Gulf Coast, *Mellita tenuis* and *Encope michelini* were also found in dynamic clusters that changed within two to five hours after initial observation (Swigart and Lawrence 2008). Swigart and Lawrence (2008) attributed the aggregations to local, short-term changes in food concentration and that the sand dollars disperse when the food concentration is decreased .

Other reasons for echinoid aggregation include breeding and defence from predators. As fertilization is external, conspecific aggregations, especially in small populations, may increase breeding success (Levitan and Young 1995). The sea urchin *Echinus esculentus* has been shown to be attracted to conspecific gametes (Campbell et al. 2001). Aggregations of sea urchin *Stylocidaris lineata* were only observed when individuals had ripe gonads, suggesting that aggregation may facilitate spawning synchrony and cause gametes to be retained at high concentrations near the adults long enough for fertilization to occur (Young et al. 1992).

Aggregations reduce individual predation pressure (Bernstein et al. 1981). The presence of crabs increased the aggregation tendency of large *Strongylocentrotus purpuratus* individuals as aggregations served as a better defence than hiding and allowed urchins to continue feeding during the day (Bernstein et al. 1981).

It is unknown if *Peronella lesueuri* forms aggregations. While several authors have observed *P. lesueuri* in considerable numbers in their studies, there has been no mention of aggregations of this species (Mortensen 1921; Marsh and Devaney 1978; Forehead and Thompson 2010).

1.2.2 Reproductive Biology

Echinoid reproduction has been extensively researched both in the field and laboratory, and studies have been primarily focused on the effect of environmental factors on reproductive cycles. Echinoids are dioecious and undergo gametogenesis. Most species release their gametes into the water column for fertilization, although some species have been known to brood (Pearse and McClintock 1990; Schatt and Feral 1996). Cyclic reproductive patterns with annual, seasonal, monthly and/or lunar periodicities are common. Some species, however, have been found to exhibit continuous reproduction, like *Echinometra mathaei* in Western Australia (Pearse and Phillips 1968). It is possible that the standard sampling techniques (e.g. monthly) used were not able to detect whether the population had gonads in a constantly mature state or if the reproductive cycle exhibited a shorter cyclic pattern (e.g. monthly). *Centrostephanus coronatus* at Santa Catalina Island in California was found to have monthly reproductive rhythms closely corresponding to lunar cycles (Kennedy and Pearse 1975). Lima et al. (2009) suggested that a population of *Echinometra lucunter* from Muro Alto beach in Brazil showed continuous reproduction with seasonal peaks.

Echinoid reproduction occurs in a cycle through the stages of growth, development, maturation of the gonads and spawning of gametes, after which gonad tissues recover and accumulate a nutrient store to fuel the following cycle (Chatlynne 1969; Gonor 1973; Byrne 1990).

Displaying a wide range of reproductive strategies and periodicities, echinoids time their gamete release to coincide with optimal environmental conditions, which likely results in higher rates of fertilization and larval survival (Pearse and Cameron 1991; Walker etal. 2007; Mercier and Hamel 2009). Separate populations of the same species have been known to have different cycles, with timing based on local conditions. Reproductive cycles are often seasonal indicating that a wide range of external cues and factors, such as photoperiod (Pearse et al. 1986; Bay-Schmith 1989; Byrne et al. 1998; Alsaffar and Lone 2000), sea temperature (Spirlet et al. 1998; Brewin et al. 2000), nutrient availability (Starr et al. 1993; Muthiga and Jaccarini 2005; Kino and Agatsuma 2007) and lunar periodicity (Kennedy and Pearse 1975; Iliffe and Pearse 1982) may play a part in their regulation. However, in the field it is difficult to isolate a single initiating factor, as most environmental conditions change seasonally. Different species of echinoids also seem to respond differently to cues and factors at various points in the reproductive cycle. The perfect recurrence of the reproductive cycle of *Echinocardium cordatum* in the North Sea over three consecutive years strongly suggests that a decrease followed by a gradual increase in seawater temperature initiated gametogenesis (Nunes and Jangoux 2004); while in the St Lawrence Estuary in Canada, *Strongylocentrotus droebachiensis* seems to spawn in response to phytoplanktonic food availability (Starr et al. 1993).

There is a significant gap in the literature as little is known about the reproductive biology of *Peronella lesueuri*.

1.2.2.1 Temperature effects

Research suggests that a lack in temperature variation can inhibit or initiate gametogenesis. Nunes and Jangoux (2004) showed that gametogenesis in *Echinocardium cordatum* commenced after minimum temperatures in the study area had been reached, suggesting that the seasonal increase of sea temperature were the factor that initiated gametogenesis. However, experiments under laboratory conditions indicated that gametogenesis in *Hemicentrotus pulcherrimus* could only be initiated by a drop in temperature from 25°C to 15°C (Ito et al. 1989; Sakairi et al. 1989). When held at a constant temperature of 15°C, Sakairi (1989) observed that gametogenesis in *H. pulcherrimus* was inhibited. In the waters off southern Japan, Agatsuma (2007) also observed gametogenesis of *H. pulcherrimus* being initiated by decreasing sea temperatures.

Temperature has also been shown to be an inhibitor of gametogenesis. Lares and McClintock (1991) found that gametogenesis in *Eucidaris tribuloides* was suppressed when held at 30°C for two months, with no mature male specimens observed. In southern California, when sea temperatures exceeded 17°C in the summer, no mature individuals of *Strongylocentrotus purpuratus* were found (Cochran and Engelmann 1975). In the laboratory, Cochran and Engelmann (1975) also found that *S. purpuratus* gametogenesis was inhibited at 17°C.

1.2.2.2 Photoperiod effects

While Agatsuma (2007) found that photoperiod did not play a role in gametogenesis of *Hemicentrotus pulcherrimus* in Japan, gametogenesis in other echinoids has been frequently correlated with photoperiod. There is still a lack of understanding as to how photoperiod acts as a cue for gametogenesis (Walker et al. 2007). It is likely that the length of photoperiod, like temperature, can play a role in facilitating or impeding the gametogenic cycle.

Holland (1967) suggested that the change in the length of the photoperiod has an effect on the gametogenesis of the sea urchin *Stylocidaris affinis* from the Gulf of Naples. Holland (1967) also suggested that photoperiod could play an indirect role on gametogenesis by affecting quality and quantity of food supply. When held at photoperiods of 12 hours or less in laboratory conditions, gametogenesis occurred in *Strongylocentrotus purpuratus*, but when the photoperiod was increased to 16 hours, gametogenesis was inhibited (Pearse et al. 1986; Bay-Schmith 1989). This suggests that as daylight hours get shorter in autumn, there is a critical daylength that will trigger the initiation of gametogenesis in *S. purpuratus*.

In the northern Gulf of Mexico, gametogenesis and spawning in the sand dollar *Clypeaster ravenelii* was found to be highly synchronised with increasing photoperiod, suggesting that it is a reproductive cue (Vernon et al. 1993). Laboratory experiments also suggested that lengthening photoperiod is a significant cue for *Psammechinus miliaris* to complete gametogenesis (Kelly 2001).

1.2.2.3 Effects of other abiotic factors

Food availability is an annually variable factor, dependant on locale and weather, and as such, is unlikely to be directly involved in the initiation and/or inhibition of the reproductive cycle (Eckelbarger and Watling 1995). Spirlet *et al.* (1998) observed no change in the gametogenic period in *Paracentrotus lividus* despite starvation, although there was a marked reduction in gamete numbers. Quality and quantity of food resources have been shown to have an effect on gonad size, with individuals exposed to a more abundant food resource producing larger gonads (Gonor 1973; Meidel and Scheibling 1998; Kelly 2000). Nutrient allocation trade-off between gonad and somatic growth in times of poor food availability has also been observed (Ebert 1968; Gonor 1972; Ebert 1982).

Studies showed that spawning in *Strongylocentrotus droebachiensis* is timed to coincide with seasonal phytoplankton blooms (Himmelman 1975; Starr et al. 1990; Starr et al. 1993). On the Kenyan coast, Muthiga and Jaccarini (2005) observed that spawning in *Echinometra mathaei* peaks in tandem with phytoplankton abundance. This suggests that spawning is timed to ensure optimal food availability for planktonic larvae, thereby increasing survival rates.

The principles of how lunar cycles affect reproductive rhythms of echinoids remain unclear. There is, however, some evidence to suggest that some echinoid species spawn in synchrony with specific lunar phases (Kennedy and Pearse 1975; Iliffe and Pearse 1982; Coppard and Campbell 2005; Muthiga 2005), although any lunar spawning has to be a consequence of a synchronised gametogenic cycle. Naylor (1999) suggested that lunar cycles of behaviour are a response to the tidal cycles that are related to the lunar phase.

1.2.2.4 Gonad analysis

Studies on echinoid reproduction typically use the Gonad Index (GI) to identify the reproductive stages. The GI is the gonad weight as a fraction of body weight. This method, however, assumes that the proportion of gonad to body size is constant for all sizes and can be inaccurate when animals of various sizes are used (Gonor 1972; Ebert et al. 2011); it should therefore only be used on animals of similar size. Several variations on the GI have been used. The most commonly used index is the ratio of wet gonad weight to wet total body weight (Dotan 1990; Drummond 1995; Meidel and Scheibling 1998; Kino and Agatsuma 2007; James and Heath 2008; Fabbrocini and D'Adamo 2010; Schuhbauer et al. 2010). Another common index used is the ratio of dry gonad weight to dry total body weight (Byrne 1990; Nunes and Jangoux 2004; Tavares and Borzone 2006; Lima et al. 2009).

There are two other reproduction staging methods, both of which are quantitative and can only be used on female gonads. The size frequency method, in which oocytes are measured individually, can be time-consuming and tedious. In the stage frequency method oocytes are classified by stage and counted, which, while lacking in the accuracy of the size frequency method, is quicker, more efficient and gives acceptable results (Gonor 1973). Reproductive stages for male gonads can only be differentiated qualitatively from histological slides.

1.2.3 Growth

1.2.3.1 Life expectancy

Echinoids possess a wide range of longevities with one estimate for the red sea urchin, *Strongylocentrotus franciscanus,* suggesting that they can live for more than 100 years (Ebert and Southon 2003). Longevity estimates for sand dollars, however, suggest that in general, life expectancies are shorter (Ebert and Dexter 1975). Maximum age for *Echinarachnius parma* was estimated at 15-18 years (Steimle 1990; Cabanac and Himmelman 1996), while *Encope stokesii* had a life expectancy of less than one year (Dexter 1977). The life expectancy of *Peronella lesueuri* is unknown and will be one of the aspects covered in this study.

1.2.3.2 Factors affecting growth in echinoids

Rates of growth in echinoids are dependent on a variety of factors including inclement weather, which can result in test damage; food availability and consumption; and physical environmental conditions (Ebert 1968; Niesen 1977).

Echinoids generally exhibit a sigmoidal growth rate curve, with a rapid initial growth rate before a reduction in growth rate. In some species, a decline in growth is observed after the maximum size of the individual is reached. Using shifts in modes of size distributions, Ebert (1975) found that two sand dollar species, *Encope grandis* and *Mellita grantii* in the Gulf of California, Mexico, took six and five years respectively to attain 95% of their maximum size. Both species are intertidal and their populations were found to be limited by environmental factors, such as sand movement, storms, and high temperatures at low tides. Similarly, growth bands present in the interambulacral plates of *Astriclypeus manni* Verrill and *Clypeaster japonicus* suggested that initial growth was exponential, with up to 93% of skeletal growth occurring within the first four years and growth discontinuing after year five or six (Kang et al. 2007). Crapp and Willis (1975) also observed growth slowing in sea urchin *Paracentrotus lividus* after four years and Lane and Lawrence (1980) found a declining rate of growth in the sand dollar *Mellita quinquiesperforata* as it increased in age. In British Colombia, Zhang et al. (2008) observed that the growth rate of the red sea urchin, *Strongylocentrotus franciscanus*, remained constant or even increased in the first few years, before declining with test diameter or age.

Negative growth rates in echinoids have been observed in many instances. Fluctuations in environmental conditions have been suggested as a factor for negative growth in *Strongylocentrotus purpuratus* of maximum size, as calcite in the test is reabsorbed (Ebert 1967). Seasonal negative growth was also observed in larger *Mellita quinquiesperforata* in the fall following a high summer growth rate (Lane and Lawrence 1980). This was attributed to a response to an energy deficit in storage tissues after high energy requirements for reproductive activities over summer. This highlights the ability of echinoids to control nutrient allocation to either gonadal or somatic growth depending on the requirement at the time (Ebert 1967; Gonor 1972; Ebert 1982).

Environmental conditions affect growth rates as well as the maximum attainable size in echinoids. *Dendraster excentricus* in two different habitats in Alki Point, Seattle exhibited different rates of growth, with a population living in a cobble and hard clay substrate showing a greater juvenile growth rate and a slower adult growth rate than a population living in deep sands (Birkeland and Chia 1971). Adults in the cobble substrate population were also smaller and occurred in much higher densities than the deep sand population (Birkeland and Chia 1971). A combination of shelter and abundant food resource make it an optimal environment for juvenile growth, but recruitment and retention success leads to overcrowding in the area, which then limits maximum adult size. In another study, *Dendraster excentricus* at an exposed site were smaller and had ripe gonads for a shorter period compared to a sheltered site (Niesen 1977).

1.2.3.3 Echinoid growth zones

Growth bands on the Aristotle's Lantern (jaw) and the ambulacral and interambulacral test plates of the echinoid skeleton are commonly used as a means of ascertaining age and charting growth (Birkeland and Chia 1971; Crapp and Willis 1975; Pearse and Pearse 1975; Ebert 1988; Gage 1991; Gage 1992a; Gage 1992b; Tan and Lawrence 2001; Agatsuma and Nakata 2004; Kang et al. 2007; Schuhbauer et al. 2010). Birkeland and Chia (1971) used cedar oil to highlight growth rings to estimate age distribution within *Dendraster excentricus* populations in Seattle, USA, whereas Pearse and Pearse (1975) enhanced growth rings by immersing charred test plates in xylene to reveal alternating translucent and opaque bands that reflect seasonal changes in growth rates. The translucent bands are formed during the slower skeletal growth over autumn and winter while the opaque bands are wider and are formed during the spring and summer when plate growth is fastest.

Annual growth bands or rings have been verified in several species. Annual periodicity of the growth rings in *Loxechinus albus* were verified separately by Gebauer and Moreno (1995) and Schuhbauer et al. (2010) through marginal increment analysis. Tetracycline tagging of *Echinus esculentus* showed that a single growth band forms annually (Gage 1992b).

Tan and Lawrence (2001) found that the number of growth lines in *Mellita tenuis* were independent of size, suggesting that seasonality of growth and growth rate were not dependent on each other. While there appeared to be an annual periodic component to the growth bands in *Strongylocentrotus purpuratus* and *Echinometra mathaei*, Ebert (1988) observed growth lines added by the sea urchins for other reasons that were indistinguishable from annual growth lines. Ebert (1988) also observed that rapidly growing individuals may show one growth band or more per year. However for slower growing or non-growing individuals annual lines may not be distinct and so age may be overestimated for the smaller, rapidly-growing individuals and underestimated for the larger, slower-growing individuals.

1.2.3.4 Recruitment

Echinoderms commonly exhibit seasonal recruitment patterns (Ebert and Dexter 1975; Cameron and Rumrill 1982; Hunte and Younglao 1988; Kenner 1992; Ebert et al. 1994) although some species, such as *Encope stokesii*, have year-round recruitment with recruitment peaks during favourable seasons (Dexter 1977). Seasonal recruitment as a result of seasonal reproductive cycles allows larvae and newly settled juveniles the highest chances of survival when environmental conditions are favourable (Birkeland and Chia 1971; Cameron and Rumrill 1982).

Annual recruitment strength can be affected by a myriad of environmental factors. Cameron and Rumrill (1982) found that the varying recruitment strength reflected by annual size distribution frequencies could be correlated to small-scale variations in current conditions in Monterey Bay, California. This prevented larvae from reaching sand dollar beds for settlement.

Juveniles of many echinoid species seem to show a higher propensity to settle near adults (Birkeland and Chia 1971; Cameron and Schroeter 1980; Highsmith 1982; Hunte and Younglao 1988). Birkeland and Chia (1971) suggested that recruitment of juveniles in one population of *Dendraster excentricus* at Alki Point, USA, was stronger due to the cobble substrate providing the juveniles better protection than the shifting sandy sediments which support another nearby population. Adults of *D. excentricus and Echinarachnius parma* have also been observed to release a chemical cue into the sediment, which induces larval settlement and metamorphosis (Highsmith 1982; Pearce and Scheibling 1990). Cameron and Schroeter (1980), however, observed that juveniles of *Strongylocentrotus* *purpuratus* and *Strongylocentrotus franciscanus* were found in higher densities near adults. The presence of adult urchins or substrates associated with adult urchins did not enhance settlement. Instead, both juvenile mortality and migration provided more influence on the distribution of juvenile urchins.

1.2.4 Movement and Behaviour

Echinoids exhibit a wide range of movement patterns in response to a range of environmental biotic and abiotic factors. Movement patterns are species or population-dependent and may vary in response to seasonal changes, predation pressure, food availability and reproduction.

1.2.4.1 Seasonal and Diel Activity Rhythms

Echinoids commonly exhibit seasonal and diel activity rhythms that are associated with a variety of life functions, such as feeding, reproduction and predator avoidance.

Seasonal activity patterns are commonly observed amongst sub-tropical and temperate echinoids and are usually correlated to seasonal cycles in photoperiod, temperature and food availability. Seasonal changes in water temperature play a small part in increasing the metabolic rate of marine invertebrates (Brockington and Clarke 2001). The main cause of metabolic rate increase was caused by the increasing physiological activity associated with feeding, growth and reproduction. Seasonal cycles of feeding intensity exhibited by sea cucumbers in Canada were related to the availability (Hamel and Mercier 1998) and quality (Singh et al. 1999) of food rather than temperature and photoperiod.

There is a high occurrence of nocturnal activity observed amongst a variety of sea urchin species. Research suggests that nocturnal activity acts as a predator avoidance strategy (Freeman 2003; Hereu 2005; Miyamoto and Kohshima 2006; Young and Bellwood 2011), especially in reef and seagrass habitats, where fish, the major predators of echinoderms, are visual predators and are mostly diurnally active.

1.2.4.2 Directionality

The position of the anus directly behind the central mouth on the oral surface of sand dollar *Mellita quinquiesperforata* led Weihe and Gray (1968) to conclude that polarization is sufficient for the direction of movement to only be forward and the anterior end of to always take the lead. The sand dollar *Dendraster excentricus* has a predominantly forward movement, but also has the ability to move in reverse (Chia 1969).

Movement responses of the sand dollar *Echinarachnius parma* to slope and current were tested in the laboratory (Cabanac and Himmelman 1998). Individuals of all sizes displayed a preference for upslope movement, but adults and juveniles were found to exhibit different responses to current. When placed in a flume, adult sand dollars had a preference for upstream movement while similar proportions of juveniles chose to move upstream and downstream. This supports the hypothesis that adult *E. parma* migrate to shallower waters to take advantage of the food resources as water turbulence is less likely to dislodge and transport them (Cabanac and Himmelman 1996). The sand dollar *Encope grandis* has the ability to sense its position on the beach, orient itself and move towards the water (Ebert and Dexter

1975). Weihe and Gray (1968), however, noted that the directional choice of sand dollar *M. quinquiesperforata,* after being placed in a particular orientation, was random and was not affected by the prevailing winds. Similarly, *D. excentricus* was observed lying randomly, not uniformly oriented to the direction of the current (Chia 1969).

1.2.4.3 Burrowing and Bioturbation

Echinoids have a global distribution in marine habitats and are found at all depths, latitudes and ecosystems. Most echinoids inhabit benthic ecosystems and play key ecological roles as bioturbators of sediment (Uthicke 1999; Michio et al. 2003; Vopel et al. 2007) and as grazers of seagrass or macroalgae (Alcoverro and Mariani 2002).

When bioturbating echinoids dominate a soft-bottomed habitat they have the ability to turn over enough sediment to affect infaunal abundances (Dahlgren et al. 1999), lower levels of organic matter and inhibit eutrophication (Michio et al. 2003). Bioturbating echinoids rework the sediment through their burrowing and to a lesser extent, feeding activity. When heart urchins burrow, they constantly displace sediment, increasing the area available for oxygen exchange between sediment and seawater (Vopel et al. 2007). An increase in temperature had a significant effect on the bioturbation activity of the spatangoid *Brissopsis lyrifera*, suggesting that it is more active when water temperatures are higher (Hollertz and Duchene 2001). The amount of sediment reworked by *B. lyrifera* due to burrowing was also found to be 60-150 times higher than the volume ingested (Hollertz and Duchene 2001). In Norway, the bioturbation activity of *B. lyrifera* was vital in increasing oxygenation of the sediment, increasing the precipitation of phosphate and decreasing the rates of denitrification (Widdicombe and Austen 1998). The volume of sediment reworked at a site in New Zealand, where heart urchins of the genus *Echinocardium* were abundant, suggests that the surface sediment could be reworked every three days (Lohrer et al. 2005). In Antarctic benthic sediments, the bioturbation activity of the heart urchin *Abatus ingens*, which burrows and feeds on surface sediments, significantly contributes to the reworking of sediments (Thompson and Riddle 2005).

In Puget Sound, Washington, Backman (1984) discovered that sand dollars played a part in limiting the distribution of seagrass *Zostera marina* by burrowing under and disrupting rhizome matrixes and uprooting plants. Competing for the same substrate, clear patches of sediment were quickly colonised by sand dollars, excluding the seagrass. Studies on different species of seagrass and sand dollars in Japan and Mexico, however, have not been able to show conclusively that bioturbation by sand dollars affects distribution of various seagrass species in those regions (Valentine et al. 1994; Matsuda et al. 2008). At the southern end of Cockburn Sound, Western Australia, *P. lesueuri* was found on sand flats in and around existing and transplanted seagrass meadows.

Under laboratory conditions the average burrowing time for *Mellita quinquiesperforata* was 7.2 minutes, while in the field the average time taken to burrow was 4.05 minutes, although some sand dollars were completely covered within two minutes (Weihe and Gray 1968). A quick burrowing time allows for the sand dollars to return to the safety of being buried within the sediment, where they are not exposed to the strong currents and turbulence in the water column (Weihe and Gray 1968; Merrill and Hobson 1970). Burrowing behaviour in sand dollars that reside in the intertidal zone also allows them to shelter from the extreme temperature and water level fluctuations as well as from predators (Weihe and Gray 1968). In sheltered areas where water movement was less, populations of adult *Dendraster excentricus* were not inclined to burrow and were usually found aggregated in inclined positions, feeding by filtering suspended particles from the water column (Merrill and Hobson 1970).

Prior to this study, there were no published studies on movement patterns of *Peronella lesueuri*. As part of this study, a paper describing the seasonal and diel movement variations of *P. lesueuri* was published recently (Yeo et al. 2013).

1.3 Study Site - Cockburn Sound

Cockburn Sound is a semi-enclosed embayment, located south of Perth, Western Australia, (32°09'S; 115°45'E), in an area with industrial, defense as well as recreational uses. With an estimated area of 124 square kilometres, Cockburn Sound is approximately 16 kilometres in length and 9 kilometres in width (Steedman and Craig 1983) . Semi-enclosed by shallow Parmelia and Success Banks to the north and Southern Flats to the south, the central basin is 17-22 metres deep and consists of mud and silt, and is edged with shallower (2-10 m) sandy sills and beaches (Figure 1.2; Marsh and Devaney 1978). It is in the shallow sandy benthic substrates of Cockburn Sound that the sand dollar, *Peronella lesueuri,* forms a dominant part of the fauna.

Cockburn Sound is bound by the mainland to the east and Garden Island to the west. Garden Island, where the Royal Australian Navy has a base, is linked to the mainland by a solid causeway with two openings to the sea (Steedman and Craig 1983). One opening is 300 metres wide with a depth of 2.8 metres, while the other is 600 metres wide and 4.5 metres deep. Cockburn Sound has a mean maximum daily tidal range of 0.55 m and wind-driven currents with a mean of 0.05 m s^{-1}. The bathymetry of the Sound is such that it acts mainly as a closed system, with the water circulation in the Sound predominantly wind-driven.

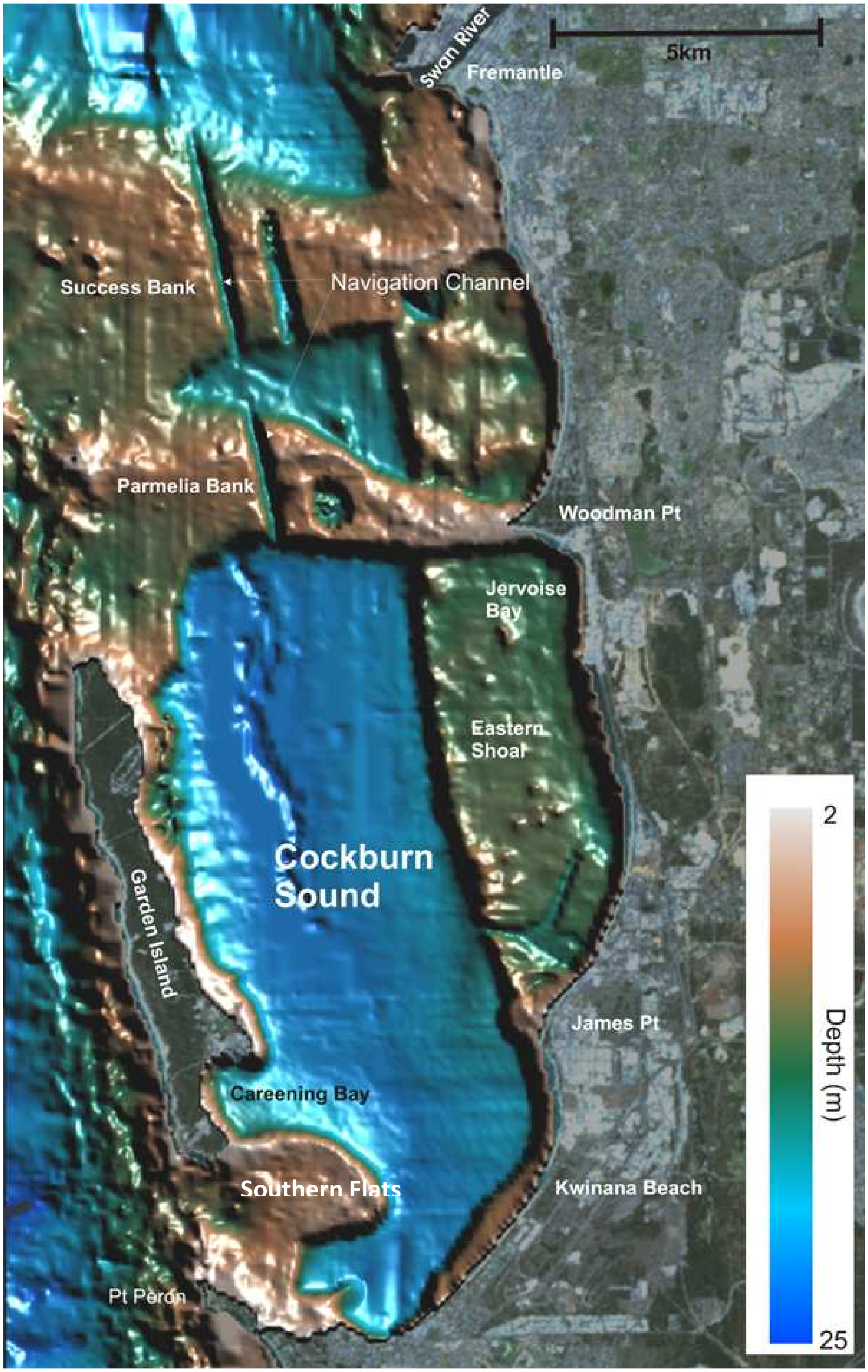

Figure 1.2 Bathymetric map of Cockburn Sound. Jervoise Bay is to the northeast of the Sound (Ozcoasts 2009).

Sea surface temperatures in the area range from a low of 16 °C in winter to a high of 23 °C in summer (Pearce et al. 1999). In February and March 2011, Western Australia experienced a significant marine heat wave with seawater temperatures of 2-4°C above the long-term mean (Pearce et al. 2011). In Cockburn Sound, the heatwave persisted for eight weeks and seawater temperatures at depths between 10 and 20 metres were significantly higher than those recorded in the nine years previous (Rose et al. 2012).

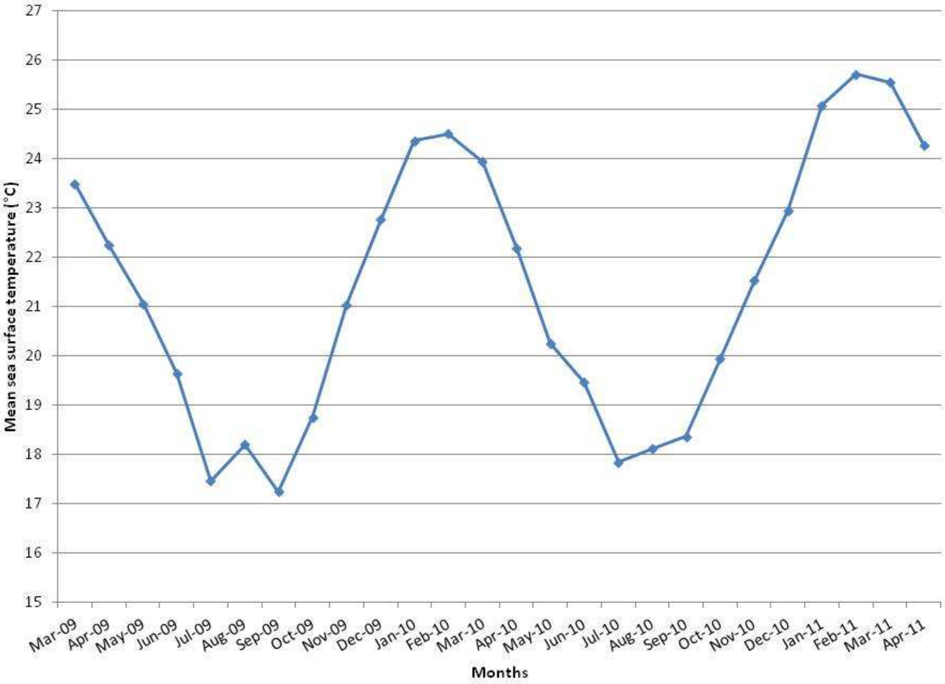

Figure 1.3 Mean sea surface temperatures in Cockburn Sound from March 2009 until April 2011 (IMOS 2011).

Cockburn Sound has a long history of pollution. Heavy industries on the eastern shore are heavily regulated and monitored today, but in the 1970s industrial and community waste released into Cockburn Sound caused problems associated with heavy metal, hydrocarbon and pathogen contamination (DEP 1996). More recently, reduced contaminant inputs, improved waste treatment practices and relocation of some industries have allowed the benthic invertebrate fauna to recover (DEP 1996).

Seagrass cover on the shallow, subtidal sandbanks of Cockburn Sound used to be extensive, but during the period of major industrial development on the shore in the 1950s, extensive loss of seagrass occurred due to the discharge of effluents rich in plant nutrients (Cambridge and McComb 1984) as well as shellsand mining (DEP 1996). Between 1954 and 1978, it was estimated that the area of seagrass meadows decreased from 4,200 to 900 ha (Cambridge and McComb 1984). The nutrient-enriched waters enhanced the growth of seagrass epiphytes and filamentous algae which were the most likely cause of the decline of seagrass meadows (Cambridge et al. 1986). In recent years, large-scale seagrass rehabilitation has been underway in Cockburn Sound to aid in the recovery of seagrass meadows.

Today, Cockburn Sound also serves a major recreational function, with activities like fishing, snorkelling and diving that take place in and around the Sound. Cockburn Sound is also home to a blue mussel aquaculture venture.

1.4 Study Aims

It is evident from the review of the literature that little is known about *Peronella lesueuri.* A pilot study in 2009 showed the feasibility of *Peronella lesueuri* for the study of its population biology and ecology and its potential role in ecosystem function (e.g. bioturbation effects). Individuals are large and relatively easy to spot by divers. Sand dollar density also seemed sufficiently abundant at the main Jervoise Bay sampling site to sustain monthly benthic sled sampling over the two-year proposed study. Although there was scope to study a wide range of aspects of *P. lesueuri*, the lack of knowledge of its basic biology determined the foci for this study.

Thus the aims of this study were to: 1) ascertain the distribution and density; 2) describe in detail and establish the seasonality of the reproductive cycle; 3) determine the growth and recruitment rates as well as 4) seasonal and diel variations in movement rates of *Peronella lesueuri* in Cockburn Sound.

Previous surveys had indicated that the sand dollars were found in the sediment of the shallower banks and sills that encircled the Sound and were absent from the deeper central basin (Marsh and Devaney 1978; Cary et al. 1995; Forehead and Thompson 2010). In the comprehensive survey by Marsh and Devaney (1978), exact densities were not recorded and at sites where *P. lesueuri* were present, the "density" was only described as "single sighting" or "abundant". Chapter 2 provides an updated distribution and density figures of *Peronella lesueuri* within Cockburn Sound and discusses the factors that are likely to affect its spatial distribution and density.

The gametogenic cycle of *Peronella lesueuri* is described histologically in Chapter 3. The histological changes in gonads of *P. lesueuri* were tracked over a 25-month period to determine the periodicity of the reproductive cycles. Within the constraints of a field study, this chapter will discuss the impact of environmental factors on gametogenesis and spawning in *P. lesueuri*.

In Chapter 4, juvenile recruitment and growth were determined through size frequency distribution, and growth rings on the test plates of large individuals were used to determine the life expectancy and growth rate of *P. lesueuri*.

Chapter 5 is focused on the seasonal and diel movement rates and patterns of *P. lesueuri* in Cockburn Sound and discusses their ecological importance in the bioturbation of the surface sediment in the habitats they occupy.

Chapter 6 highlights the major findings of this study and discusses their implications.

Chapter 2 Distribution of *Peronella lesueuri* in Cockburn Sound

2.1 Introduction

Echinoids, which include regular sea urchins as well as irregular urchins and sand dollars, have a worldwide distribution and can be found within a large bathymetric range, from the intertidal to the abyssal zone. Sand dollars, however, are more common in benthic communities of shallower, tropical to subtropical regions. *Peronella lesueuri*, commonly known as the pink sand dollar, is one such species. Records of *P. lesueuri* exist from West India, Bay of Bengal, northern Australia, the Philippines, Hong Kong, Singapore and southern Japan (Agassiz and Clark 1914; Clark 1921; Clark and Rowe 1971), indicating that they have a tropical to subtropical Indo-Pacific distribution. In 1938, Clark noted in his monograph, *Echinoderms of Australia*, that *P. lesueuri* had a distribution from southern Queensland, north and westward towards Darwin and Broome and then along the west coast, down to Fremantle and Albany. *P. lesueuri* was also encountered in more recent marine recent surveys in the Dampier Archipelago (Marsh and Morrison 2004) and Kimberley (Keesing et al. 2011). *P. lesueuri* is common in and around Cockburn Sound, approximately 25 kilometres south of Fremantle (Marsh and Devaney 1978; Forehead and Thompson 2010).

Sand dollars, as the name suggests, are found in benthic communities, commonly occurring buried in soft sediment habitats (Mortensen 1921; Ebert and Dexter 1975; Steimle 1990; Cabanac and Himmelman 1996; Haycock 2004). Some species, including *Arachnoides placenta* from Queensland (Haycock 2004) and *Encope grandis* and *Mellita grantii* in the Gulf of California (Ebert and Dexter 1975), can be found in rock pools and wet sands in the intertidal region, while other species, for example *Dendraster excentricus*, can be found from the intertidal to depth of 90 metres (Merrill and Hobson 1970; Clark 1948 as cited in Birkeland and Chia 1971).

Most echinoids appear to have preferences for particular sediment grain sizes as this has a significant effect on their burrowing and feeding behaviour. Bell and Frey (1969) suggested that the distribution of *Mellita quinquiesperforata* in Beaufort, North Carolina, is closely correlated with sediment grain size and the sand dollars' ability to burrow through it. In laboratory experiments, *M. quinquiesperforata* was observed to burrow most efficiently in medium sand, burrowing slowly and less efficiently in mud and unwilling or unable to burrow in coarse sand (Bell and Frey 1969). In a similar experiment, the clypeasteroid sand dollar *Echinocyamus pusillus* from the west coast of Scotland also did not burrow in very fine or very coarse sand, probably due to a relationship between sediment particle size and podia size of the sand dollar (Ghiold 1982). In Queensland, adult *Arachnoides placenta* were most abundant in substrates with fine to medium particle size, although their occurrence in coarse sand areas on the beach indicates a tolerance to a wide range of particle sizes (Aung 1975 as cited in Haycock 2004).

Some echinoderms are known to form patchy distributions and dense aggregations (Birkeland and Chia 1971; Konnecker and Keegan 1973; Ebert and Dexter 1975; Swigart and Lawrence 2008; Vargas and Solano 2011). Spatial distributions of sand dollar populations can be variable, with several species know to form dense aggregations. The sand dollar *Dendraster excentricus* has been observed at densities up to 629 individuals m^{-2} at Alki Point, Seattle (Chia 1969) and 1,200 individuals m^{-2} at Zuma Beach in California (Morin et al. 1985). *Encope grandis* and *Mellita grantii* at Playa Hermosa in the northern Gulf of California, Mexico were observed at densities of 380 individuals m^{-2} and 56 individuals m^{-2} respectively, although a year later densities of 0.1 individuals m^{-2} and 0.8 individuals m^{-2} respectively were recorded.

A survey of the benthic fauna of Cockburn Sound between 1956 and 1960, as well as later collections in the ’60s and ’70s indicated that *Peronella lesueuri* was absent from most locations in the deep main basin (~25 metres deep) (Marsh and Devaney 1978). Later observations of *P. lesueuri* in Cockburn Sound indicated that they occurred at densities of up to 6 individuals m^{-2} at four and eight metre depths at Parmelia Bank (Forehead and Thompson 2010).

Although there have been instances of *Peronella lesueuri* being described as common and/or abundant in the literature (Mortensen 1921; Marsh and Devaney 1978), there have been no records of dense aggregations of this sand dollar. In Cockburn Sound, *P. lesueuri* is relatively common and where it occurs it is a dominant organism within the benthic community; however its distribution is only known from surveys carried out more than 30 years ago (Marsh and Devaney 1978). Temporal variation in sand dollar densities can be large (Ebert and Dexter 1975). This study was carried out to determine the current spatial distribution and abundances of *P. lesueuri* populations at various sites within Cockburn Sound. Temporal variation was also monitored at one site over a period of 23 months to determine if there were any annual variations in density of *P. lesueuri* populations in Cockburn Sound.

2.2 Materials and Methods

2.2.1 Distribution and density around Cockburn Sound

Benthic sites around Cockburn Sound were sampled on the 11th and 12th January 2012. Sixteen study sites within Cockburn Sound were selected based on a range of depth and benthic make-up. All the study sites selected had sediment cover. A number of sites had patchy seagrass cover as well. Densities of sand dollars were recorded at shallow sites by researchers on SCUBA while at deeper sites, an underwater drop camera (Splashcam) was used to ascertain the absence or presence of sand dollars. The deeper sites surveyed were within the deep basin of Cockburn Sound (maximum depth 20 m) and offshore from the shallower sites to determine if sand dollar distribution in the same areas of Cockburn Sound was different with respect to depth.

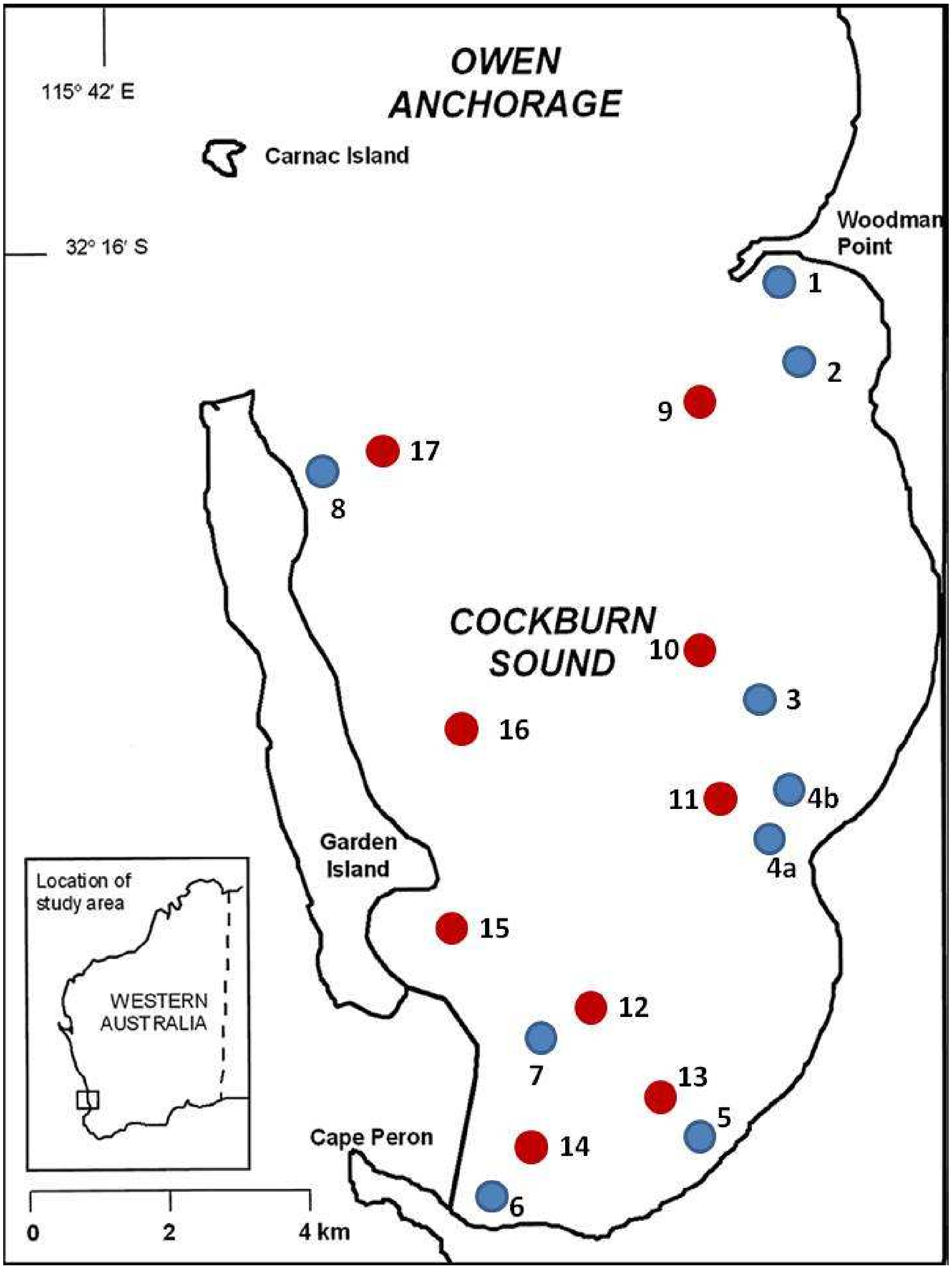

Figure 2.1 Study sites 1 – 16 in Cockburn Sound, Western Australia. Dots indicate approximate locations of sample sites. Blue dots represent the shallower sites surveyed by divers, while the red dots represent deep sites surveyed with the Splashcam. Names and coordinates for each site are detailed in Table 2.1.

Table 2.1 Site names and coordinates for sand dollar distribution study in Cockburn Sound, Western Australia.

Site Number	Site Name and Coordinates	Depth (m)
1	Woodman Point 32° 08’ 15.2” S, 115° 44’ 41.8” E	9.0
2	Jervoise Bay 32° 08’ 58.9” S, 115° 45’ 04.8” E	10.0
3	Seagrass beds near D9 wreck 32° 11’ 39.2” S, 115° 44’ 44.3” E	4.6
4a	James Point A 32° 13’ 13.5” S, 115° 44’ 56.7” E	5.5
4b	James Point B 32° 12’ 50.0” S, 115° 45’ 06.8” E	5.5
5	North Rockingham Beach 32° 15’ 42.4” S, 115° 44’ 20.2” E	8.5
6	Mangles Bay 32° 16’ 13.8” S, 115° 42’ 45.2” E	3.0
7	Southern Flats	2.9
8	Luscombe Bay 32° 10’ 01.95” S, 115° 40’ 41.11” E	3.0 - 5.0
9	North Cockburn Sound (CS) Basin 32° 09’ 660” S, 115° 43’ 925” E	17.9
10	CS Basin near D9 wreck 32° 11’ 110” S, 115° 44. 041” E	17.7
11	CS Basin off James Point 32° 12’ 686” S, 115° 44’ 228” E	17.8
12	CS Basin off Southern Flats 32° 14’ 887” S, 115° 43’ 710” E	20.0
13	CS Basin near North Rockingham Beach 32° 15. 913” S, 115° 44’ 035” E	20.0
14	CS Basin off Mangles Bay 32° 15’ 827” S, 115° 42. 732” E	17.0
15	CS Basin off Careening Bay 32° 13’ 806” S, 115° 42’ 512” E	18.7
16	CS Basin east Garden Island 32° 12’ 130” S, 115° 41’ 562” E	18.7
17	CS Basin off Luscombe Bay 32° 10’ 109” S, 115° 41’ 004” E	14.0 - 18.0

Sampling was carried out at the eight shallow study sites on 11th January 2012. These sites were located along the eastern bank, the southern flats and the western bank of Cockburn Sound (eastern shore of Garden Island). The range of sites provided a snapshot of the various types of benthic habitats that make up the shallower banks Cockburn Sound. The depths of the shallow study sites ranged from 2.5 – 10 metres. Each site was sampled in a haphazard method. Data were recorded from ten haphazard placements of a 1 m^2 quadrat around the study site. Each quadrat was placed five metres away from the last and the direction in which this was done was selected haphazardly by the lead diver. Sand dollars that were located within the quadrat were counted. The quadrat counts were then pooled for each transect to determine the mean density of sand dollars present. Size distribution at each site was sampled by measuring all the sand dollars encountered during the dive, including those located outside the sampling quadrats.

The deep study sites selected were located close to the shallow study sites within the deep central basin of Cockburn Sound and ranged in depth from 14 – 20 metres. An underwater drop camera (Splashcam) with a GARMIN GPS and black box camera GPS overlay system was used to record video footage of the bottom sediment. Live feeds from the camera were viewed on a display screen on the boat. The camera footage was also recorded for later review. Test tows with the Splashcam at Jervoise Bay showed that large sand dollars that were present in the sediment were visible in the video footage. At each deep site, the Splashcam was lowered over the side of the boat and cable was released until the camera was clearly filming the bottom sediment. The camera was then towed slowly for six minutes and the footage recorded. All video footage was then reviewed a second time on a computer to determine if sand dollars were visible in the sediment.

2.2.2 Sediment data

Sample sites were chosen close to areas where sediment grain size was readily available from previous studies conducted in Cockburn Sound (Lord & Associates 2001, Oceanica Consulting Pty Ltd 2007, Lourey 2011, Strategen 2012, Oceanica Consulting Pty Ltd unpublished). The Wentworth scale was used to classify the modal sediment grain size of the study site (Table 2.2).

Table 2.2 Classification of sediment grain size range to the Wentworth scale and sediment type.

Sediment grain size (µm)	Wentworth scale (Φ)	Sediment type
1000 – 2000	0	Very Coarse sand
500 – 1000	1	Coarse sand
250 – 500	2	Medium sand
125 – 250	3	Fine sand
62.5 – 125	4	Very fine sand
4 – 62.5	5 - 8	Silt
< 4	>8	Clay

2.2.3 Density in Jervoise Bay

As part of the wider study of *Peronella lesueuri* population biology, Jervoise Bay (Site 2) was dredged monthly from March 2009 to April 2011. The epibenthic sled used had mouth dimensions of 85 cm by 45 cm, a cutting depth of 5 cm and was enclosed by a 1 cm mesh. It is assumed that any sand dollar juveniles smaller than 1 cm was excluded. However, many individuals smaller than 1 cm were found in the dredge contents.

All sand dollars collected in the dredge samples were measured and counted. Many were broken during the collection process. Length measurements were not achievable when sand dollars were too badly broken. As each sand dollar has only one modified Aristotle's Lantern, the numbers of badly broken individuals were estimated by the number of Aristotle's Lanterns present amongst the broken pieces.

2.2.4 Statistical Analysis

All statistical analyses were carried out using JMP 9 software (SAS Institute). Multivariate correlations were used to determine the effects of depth and sediment type on sand dollar density. A one-way ANOVA was used to compare mean lengths of sand dollars from different sites and Student's t-tests were used to determine if mean lengths of sand dollars between all the sites were equal.

2.3 Results

2.3.1 Overview of sand dollar distribution and density in Cockburn Sound

Peronella lesueuri was observed at seven of the 17 sample sites. Woodman Point, Jervoise Bay, Seagrass beds near D9 wreck, James Point B, Mangles Bay and Southern Flats all had sand dollars in varying densities between 0.3 to 2.5 individuals m^{-2}. The sand dollars were observed at the Luscombe Bay site. However, densities there were so low that they were not picked up in the sampling techniques used. All seven sites where sand dollars were observed were ≤ 10 metres in depth. No sand dollars were seen on video footage taken at any of the deep basin study sites. Table 2.3 summarises the depth, sediment type and presence of sand dollars at all the sites.

2.3.2 Sediment type and size at the study sites

The modal sediment type found in the areas around the sample sites are summarised in Table 2.3. Depth and sediment size had an inverse correlation (r^2 = 0.75) in Cockburn Sound, with coarser sediments in the shallows and finer sediments in the basin. With the exception of the North Rockingham Beach site, silt appeared to be the major component of the sediment at all of the deep Cockburn Sound (CS) Basin sites (depth > 14 metres)(Oceanica Consulting Pty Ltd 2007). At the shallow sites (depth ≤ 10 metres), modal sediment type ranged from coarse to very fine sand.

Table 2.3 Depth, sediment type and density of sand dollars at sites where sand dollars were present.

Site Name and Number	Depth (m)	Sand Dollar Density (indiv. m^{-2})	Modal sediment type	Wentworth scale (Φ)	Source
Woodman Point 1	4.0 -9.0	1.4	Medium sand	2	1
Jervoise Bay 2	10.0	0.3	Medium sand	2	1
Seagrass beds near D9 wreck 3	4.6	0.3	Fine sand	3	5
James Point A 4a	5.5	0	Very fine sand	4	2
James Point B 4b	5.5	0.3	Very fine sand	4	2
North Rockingham Beach 5	8.5	0	Silt	5 - 8	4
Mangles Bay 6	3.0	2.5	Course sand	1	3
Southern Flats 7	2.9	1.3	Medium sand	2	1
Luscombe Bay 8	3.0 - 5.0	0			
North Cockburn Sound(CS) Basin 9	17.9	0	Silt	5 - 8	4
CS Basin near D9 wreck 10	17.7	0	Silt	5 - 8	4
CS Basin off James Point 11	17.8	0	Silt	5 - 8	4
CS Basin off Southern Flats 12	20.0	0	Silt	5 - 8	4
CS Basin North Rockingham Beach 13	20.0	0	Silt	5 - 8	4
CS Basin off Mangles Bay 14	17.0	0	Silt	5 - 8	4
CS Basin off Careening Bay 15	18.7	0	Silt	5 - 8	4
CS Basin east Garden Island 16	18.7	0	Silt	5 - 8	4
CS Basin off Luscombe Bay 17	14.0 - 18.0	0	Silt	5 - 8	4

Source: 1. (Lourey et al. 2011) 2. (D.A. Lord & Associates Pty Ltd 2001) 3.(Strategen 2012) 4.(Oceanica Consulting Pty Ltd 2007) 5. (Oceanica Consulting Pty Ltd, unpublished data)

2.3.3 Sand dollar distribution and density relative to depth and sediment type

Peronella lesueuri density was 0.3 individuals m^{-2} at Jervoise Bay, Seagrass beds near D9 wreck and James Point B (Table 2.3). Mangles Bay had the highest sand dollar density of 2.5 individuals m^{-2}, and Woodman Point and Southern Flats had densities of 1.3 and 1.4 individuals m^{-2} respectively. T tests showed that the sand dollar density at Mangles Bay was significantly higher than at Woodman Point ($t = 2.54$, $p = 0.03$) and Southern Flats ($t = 2.39$, $p = 0.03$).

Both depth and sediment grain size appeared to affect the distribution and density of the sand dollars. A multivariate correlation indicated that the sand dollar density was inversely correlated with depth ($r^2 = 0.56$) and sediment grain size ($r^2 = 0.86$), with no sand dollars found in the basin (depth > 14 metres). Sand dollars were not found at North Rockingham Beach or Luscombe Bay, which, at 8.5 metres deep, was well within the depth range in which sand dollars were found in this study.

Modal sediment type at the sites where *Peronella lesueuri* was present ranged from fine to coarse sands (62.5 – 1,000 µm) (Table 2.3). At the sites where silt was the modal sediment type, no sand dollars were observed.

Mean test length varied between sites (Figure 2.1). Jervoise Bay and Mangles Bay had the lowest mean lengths of 139.57 ± 4.88 mm (X ± SD) and 139.61 ± 7.87 mm respectively. The highest mean length of 150.59 ± 16.22 mm was observed at Seagrass near D9 wreck site.

A one-way ANOVA revealed significant differences in the mean test length of adult sand dollars between sites ($F_{(6, 226)} = 7.52$, $p < 0.001$). Post hoc comparisons using the Tukey's HSD test indicated that mean test lengths of sand dollars Seagrass near D9 wreck (150.59 ± 16.22 mm) and Southern Flats (148.95 ± 8.57 mm) were significantly larger than those at Jervoise Bay, James Point B, Mangles Bay and Luscombe Bay. The mean length of sand dollars at Woodman Point (148.52 ± 12.06 mm) was significantly larger than those at Jervoise Bay.

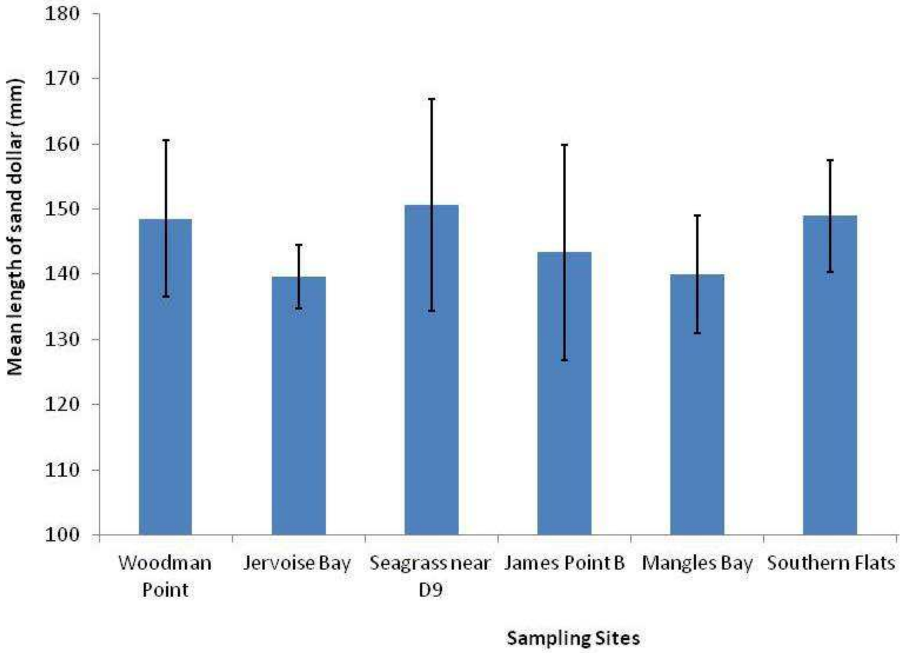

Figure 2.1 Mean test length of sand dollars with standard deviation bars at the Cockburn Sound sample sites.

2.3.4 Density in Jervoise Bay

Over the study period from May 2009 to April 2011, the density of *P. lesueuri* in Jervoise Bay varied from a high of 0.81 individuals m^{-2} in December 2009 to a low of 0.08 individuals m^{-2} in April 2011 (Figure 2.2). The average density for the entire period was 0.31 individuals m^{-2}, similar to densities recorded at Jervoise Bay (site 2), the seagrass beds near the D9 (site 3) and just north of the Kwinana Refinery (site 4b) in January 2012.

Two large spikes in density were observed in December 2009 and January 2011 (Figure 2.2). In 2009, sand dollar density started to increase in July, eventually peaking at 0.81 individuals m^{-2} in December. This was followed by a rapid decline in numbers to 0.17 individuals m^{-2} by February 2009. The second small spike in density was observed the following year in January 2011. Sand dollar density increased from 0.15 individuals m^{-2} in December 2010 to 0.4 individuals m^{-2} in January 2011. As with the previous spike, density declined sharply to 0.16 individuals m^{-2} in February 2011. Outside of the two density spikes, the density of sand dollars in Jervoise Bay hovered between 0.13 and 0.31 individuals m^{-2}.

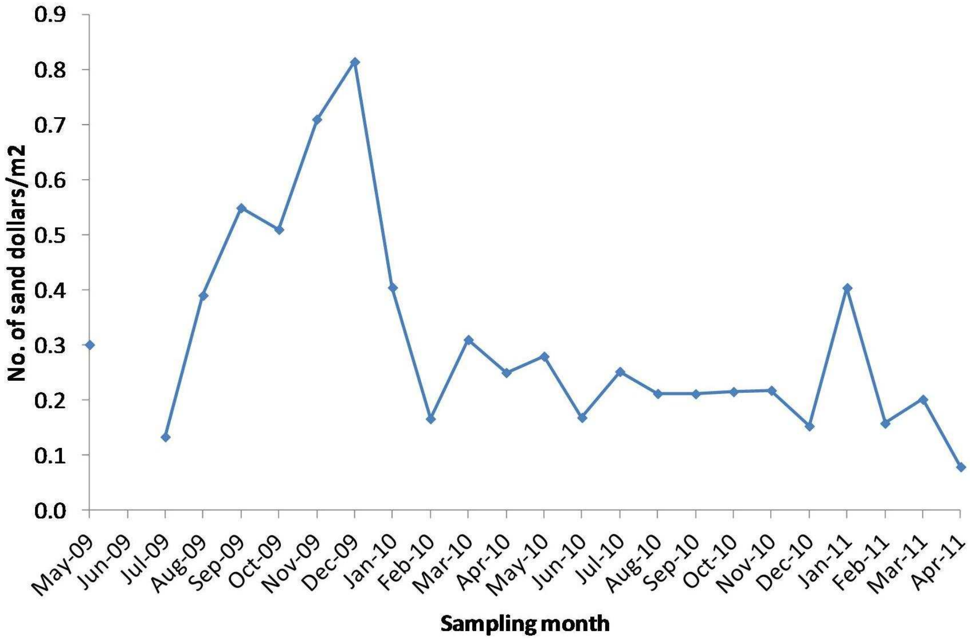

Figure 2.2 Density of *Peronella lesueuri* in Jervoise Bay from March 2009 to April 2011.

The spike in density in December 2009 at 0.81 individuals m^{-2}, was twice the density spike in January 2011. The increase in density in 2009 began in July and lasted through until December, while the density increase in 2010 did not begin until December 2010 (although small number of juveniles <8 mm were observed in July) and ended in January 2011.

2.4 Discussion

2.4.1 Distribution and densities of *Peronella lesueuri* around Cockburn Sound

The variable distribution of benthic invertebrates in marine soft sediment environments has been long known and studied at several scales (Thrush 1991; Morrisey et al. 1992; Iken et al. 2010). At a larger scale, variation in distribution of benthic organisms is attributed to physical environmental factors, like water depth and movement (Morrisey et al. 1992) and sediment type (Thrush 1991; Pomory et al. 1995). At a finer scale, distribution of benthic invertebrates can be influenced by a range of biotic and abiotic factors, such as food availability (Swigart and Lawrence 2008) and localised stochastic events (Hughes 1994; Uthicke et al. 2009). Echinoderms, in particular, are well known for their propensity to form patchy distributions or high density aggregations (Konnecker and Keegan 1973; Garnick 1978).

In Cockburn Sound, *Peronella lesueuri* is commonly encountered amongst the benthic fauna. Of the sites sampled within the Sound, however, *Peronella lesueuri* was not observed on the video footages of the deep study sites at depths of 14 metres or more. This is consistent with the results of two previous benthic fauna surveys conducted in the Sound. A benthic fauna survey carried out in 1978 for the Western Australia Department of Conservation and Environment, noted that occurrences of *P. lesueuri* within the central basin were rare (Marsh and Devaney 1978), while a more recent survey in 1995 did not encounter *P. lesueuri* at all within the deep central basin of the Sound (Cary et al. 1995). A study of marine sediments at Parmelia Bank in Cockburn Sound also stated that *P. lesueuri* were observed in the 4-8 metre depth zone but not at the very shallow 1.5 metre zone or at 14 metres (Forehead and Thompson 2010). Along with the results of other studies, the present study suggested *P. lesueuri* occurs subtidally within Cockburn Sound and that depth is a factor in determining its spatial distribution within Cockburn Sound, with no sand dollars observed at the deeper study sites.

Sediments within Cockburn Sound, however, differ with depth (Ozcoasts 2009; Forehead and Thompson 2010). Forehead and Thompson (2010) found that on Parmelia Bank in Cockburn Sound, median sediment grain size decreased with depth, with medium sands (grain size ~300 µm) in the shallows, intermediate sand (grain size ~180 µm) in the 4-8 metre zones and fine, silty sands (grain size ~150 µm) at a depth of 14 metres. All the sand dollars encountered during the study were observed between 2.9 and 9 metres in depth, where sediment grain sizes were intermediate. This suggests that distribution may be due to the sediment grain size preference of *P. lesueuri* rather than actual depth. Sand dollars move and feed concurrently by burrowing just under the surface sediments. It has been suggested that sand dollar selectivity for sediment grain size is attributed to the size of the food-collecting podia of each individual species (Telford et al. 1987) as well as the ability of the locomotory and shoe spines to manipulate the sediment (Ghiold 1979; Mooi and Telford 1982).

Fine, silty sediment has been shown to hinder movement in *Mellita quinquiesperforata* (Weihe and Gray 1968). Silt that was resuspended by dredging caused a population decline by preventing juvenile settlement (Weihe and Gray

1968), as well as by smothering adult sand dollars (Ghiold 1979). Merrill and Hobson (1970) observed that seaward margins of *Dendraster excentricus* distribution along the Pacific coast of the United States were associated with a gradual decrease in sediment grain size. Locomotory spines on the oral surface and shoe spines on the aboral surface of the sand dollar test are responsible for movement and burrowing (Mooi and Telford 1982). Locomotory spines propel the sand dollar forward into the sand while the shoe spines sort and move the larger grains along the aboral surface (Ghiold 1979; Mooi and Telford 1982). Ghiold's study on the spine morphology of sand dollar *M. quinquiesperforata* found that sediments that are too fine to be manipulated by the surface spines and podia settled and accumulated at the base of the spines on the aboral surface, eventually preventing spine and food movement, which resulted in death of the sand dollar (Ghiold 1979). The same study also found that sand dollars were unable to burrow in sediment that was too coarse as the sediment grains were too large to be handled by the shoe spines on the aboral surface. The inability of the clypeasteroid *Echinocyamus pusillus* to burrow in very fine or very coarse sediment is likely to be due to the relationship between particle weight and podia size (Ghiold 1982).

Sand dollars feed by using podia with adhesive tips on the oral surface to probe the sediment for food particles (Ghiold 1979; Mooi and Telford 1982). The food particles are then moved along to food grooves where they are swept towards the mouth by currents and podia (Ghiold 1979). The size of the food-collecting podia of the sand dollar species determines the sediment grain size selected while feeding and therefore the habitat preference of the sand dollar (Telford et al. 1987; Pomory et al. 1995). In the field, the sand dollar *Mellita tenuis* was found to have the ability to distinguish between grain size ranges in a varying mix of proportions in the sediment (Pomory et al. 1995). As such, the habitat preference and thus distribution of *P. lesueuri* within Cockburn Sound is most likely to be due to the grain size composition in the sediment that are most suitable for both feeding and burrowing.

There was significant spatial variation in the mean density of *Peronella lesueuri* populations in Cockburn Sound. Although densities of up to 6 individuals m^{-2} have been reported from Parmelia Bank (Forehead and Thompson 2010), the highest observed density during the course of this study was 2.5 individuals m^{-2} at Mangles Bay. The density of sand dollars at the Mangles Bay site was more than 8 times that of several other sites sampled. Of the three sites with the highest densities recorded during this study, Mangles Bay and Southern Flats were the shallowest at around 3 metres in depth. The Woodman Point site had a depth variable from 4 - 9 metres over a short distance. All three sites had relatively low turbidity, due to the quicker settlement of the larger sediment grain size after suspension by water movement. Decreased turbidity and higher light penetration causes greater productivity of marine sediments (Miles and Sundbäck 2000; Boer et al. 2009) and this may explain why there were higher densities of sand dollars at shallower sites.

While the exact diet of *Peronella lesueuri* is not known, other species of sand dollars have been found to graze on diatoms, foraminiferans, microeukaryotes as well as bacterial components coating sediment particles (Mooi and Telford 1982; Findlay and White 1983; Ellers and Telford 1984; Telford et al. 1987). *Peronella lesueuri* is a large sand dollar (~15 cm) and would likely have greater nutritional requirements than many of the smaller sand dollar species. Cabanac and Himmelman (1996) suggested that in the Gulf of St Lawrence in Canada, larger individuals of the sand dollar *Echinarachnius parma* move to shallower waters to take advantage of a more abundant food resource. While there is no evidence of migration in *P. lesueuri* densities of sand dollars at different sites may be an indication of the level of productivity of their food components within the sediment. Sediment analysis from Parmelia Bank in Cockburn Sound showed that sediments at depths ≤1.5 metres were less productive due to disruption of the sediment due to wave activity (Forehead and Thompson 2010). Sediments from 4, 8 and 14 metres had higher levels of primary productivity, but the uptake of silicon was the highest at 4 metres, suggesting that diatom growth was the strongest at this depth (Forehead and Thompson 2010). Diatoms are one of the known food components of sand dollars and it is therefore consistent with the highest densities of sand dollars recorded at depths where it was shallow enough for greater light attenuation for increased diatom growth, but deep enough to escape the detrimental effects of sediment destabilisation caused by wave action. Together with a coarser sediment size, it would appear that the shallower regions of Cockburn Sound (around 3 – 4 metres) may be able to support higher densities of sand dollars.

Generally, sand dollars exhibit a sigmoidal growth, with juveniles growing quickly before growth slows or ceases at a specific age (Hines and Kenny 1967; Birkeland and Chia 1971; Cabanac and Himmelman 1996; Kang et al. 2007). Environmental conditions have been known to affect maximum attainable size of *Dendraster* *excentricus* at Alki Point, USA, with the sand dollar population in the harsher environment exhibiting a smaller mean size. Given that the mean test lengths of *Peronella lesueuri* were significantly larger at three of the seven sites they were encountered at in Cockburn Sound (Figure 2.1), implied that the conditions at these sites allowed the sand dollars to attain a larger size. The seagrass beds near D9 wreck and Southern Flat sites were shallow (less than five metres in depth). Although the depth at the Woodman Point site varied from four to nine metres, most of the sampling transect was at a depth of less than five metres. A study of sediments by Forehead and Thompson (2010) indicated that on Parmelia Bank in Cockburn Sound, the sediments are autotrophic at depths of 1.5 metres and heterotrophic at 14 metres. At four metres, there was a greater amount of biomass and primary productivity. . The sediments at the shallower study sites were therefore likely to be more productive and in conjunction with fine and medium sands as the modal sediment types (Table 2.3) provided the most suitable conditions for *P. lesueuri* to attain a larger size. Although the Mangles Bay site, which had a depth of three metres and a coarse sediment grain size, had the highest sand dollar density (2.5 individuals m^{-2}), mean test length was not significantly larger. The reason for this was unclear although it was possible that the higher sand dollar density increased the competition for food, or that the grain size composition of the sediment was not ideal for feeding.

Sediment movement and hydrodynamics can play a major role in affecting sand dollar population densities (Ebert and Dexter 1975). Storms may destabilise sediment and disrupt sand dollar beds, affecting sand dollar densities (Merrill and

Hobson 1970). At Alki Point, Seattle, the lower population densities at north Alki were attributed to the impact of shifting sands on survival of juveniles (Birkeland and Chia 1971). The effect of sediment movement in Cockburn Sound on the *P. lesueuri* populations remains unknown. Enclosing the west side of Cockburn Sound, Garden Island provides shelter from strong coastal currents and waves, resulting in environmental conditions within Cockburn Sound that are relatively stable. Cockburn Sound experiences microtides of around half a metre (Pearce et al. 2011) and low tidal currents, with the water currents within the Sound being largely wind-driven (Steedman and Craig 1983). This low movement rate of water makes for minimal movement in the subtidal substrates, ideal for maintaining stable sand dollar densities. In addition, *P. lesueuri* at adult size is a larger (test length ~ 15 cm) and heavier (~130 g) sand dollar than many of the other species in the literature and when burrowed into the sediment, would be less susceptible to hydrodynamic disturbance.

During the course of this study, aggregations of *Peronella lesueuri* were not observed. Other species of sand dollars, however, are known to form aggregations. Aggregated populations of *Dendraster excentricus, Mellita quinquiesperforata, Arachnoides placenta, Encope grandis* and *Mellita grantii,* amongst others, have all been noted in the literature (Weihe and Gray 1968; Merrill and Hobson 1970; Birkeland and Chia 1971; Ebert and Dexter 1975; Haycock 2004). Aggregations may serve as an advantage for reproduction or protection from unstable sediment conditions (Birkeland and Chia 1971), or may reflect small-scale differences in food concentration within the sediment (Swigart and Lawrence 2008). The reason why *P.*

lesueuri do not form aggregations is not known; however, the sparse distribution within local populations suggests that food resources may be a limiting factor and each individual may require a larger grazing area to fulfil nutritional requirements. It also suggests that the sediments of Cockburn Sound may not be productive enough to support densities as high as those observed in other sand dollar species.

2.4.2 Temporal distribution of *Peronella lesueuri* in Jervoise Bay

Distribution of *Peronella lesueuri* at Jervoise Bay showed a significant temporal variation over the 23-month sampling period. There were two spikes in the population densities in December 2010 and January 2011, consistent with the appearance of juvenile sand dollars (>8 mm) within the dredge samples (see section 3.3.1). The spikes in density were indicative of clear annual recruitment events, with the increase in densities the result of a pulse of recruits which had settled during the recruitment period from July to January (see section 3.3.1). Following the spike in densities both in 2010 and 2011, there were rapid declines to the relatively low and stable densities of between 0.13 – 0.31 individuals m^{-2} which persisted over the rest of the sampling period. The rapid decline (over one or two months) of densities after the two spikes could be interpreted as the high mortality of newly recruited juveniles. In Monterey Bay, USA, mortality rates for early juveniles of *Dendraster excentricus* were as high as 88% while *Mellita quinquiesperforata* populations in Tampa Bay, Florida, recorded an even higher rate of 95% juvenile mortality in their first year of life (Lane and Lawrence 1980; Cameron and Rumrill 1982).

Recruitment strengths of echinoderms are highly variable and are important in determining the population density of an area. Recruitment is dependent on a range of factors, including small-scale current variations (Cameron and Rumrill 1982), substrate selection (Birkeland and Chia 1971; Cameron and Schroeter 1980), and presence of adult individuals (Hunte and Younglao 1988; Pearce and Scheibling 1990). An increase in water temperature has been shown to cause larval death, delayed metamorphosis of competent larvae and smaller juveniles in the planktotrophic larvae of the sand dollar *Arachnoides placenta* and the sea urchin *Strongylocentrotus purpuratus* (Chen and Chen 1992; Díaz-Pérez and Carpizo-Ituarte 2011). Although the literature suggests that *Peronella lesueuri* has lecithotrophic larvae (Mortensen 1921; Pearse and Cameron 1991), Hoegh-Guldberg and Pearse (1995) demonstrated that the relationship between temperature and the developmental rates of planktotrophic and lecithotrophic larvae are potentially similar. According to Reitzel et al. (2004), the duration of lecithotrophic larval life is most influenced by seawater temperature alone. Over the recruitment period of 2010-2011, the numbers of juveniles encountered were much lower than the same period in 2009-2010, which was reflected by a density spike in January 2011 that was half that for December 2009 (Fig. 22). Recruitment in 2009 also appeared to occur over a longer period, with densities of sand dollars increasing from the month of July. The increase in sand dollar density in 2010 was not noted until December and the spike in density of sand dollars occurred one month later than the previous year. From June 2010 to April 2011, the south-western coast of Western Australia experienced a “marine heat wave” and sea surface temperatures in Cockburn Sound were higher than at the same time the previous year, often by more than 2 degrees (Figure 4.6)(Pearce et al. 2011; Rose et al. 2012). This increase in water temperature in the water column may have had a detrimental effect on larval and juvenile survival as well as delaying juvenile metamorphosis, affecting the annual recruitment and thus affecting the temporal changes in density.

Both methods of sampling at Jervoise Bay produced similar results, with the quadrat method detecting in January 2012, a density (0.3 individual m^{-2}) approximate to the baseline density (0.13 - 0.31 individuals m^{-2}) recorded by the dredging method from May 2009 to April 2011. This suggested that the *P. lesueuri* population at Jervoise Bay was relatively stable and unlike some other species, was not prone to patchiness, where sand dollars move in patches and a particular area may or may not have sand dollars at any one time (Ebert and Dexter 1975; Haycock 2004). Whether this was different of populations at the other study sites is unknown, although it is unlikely.

2.5 Conclusion

Peronella lesueuri populations within Cockburn Sound show a range of spatial and temporal variation. Sand dollars were not encountered within the deep central basin of the Sound, likely due to preference for coarser sediment than the silt and mud found in the basin. The distribution of *P. lesueuri* appeared to be due to their preference for the fine to coarse sands found in the shallower areas on the edges of the Sound; densities were higher in areas around 3 metres in depth, possibly due to a higher level of available food resources.

Temporal variation in density was only studied at Jervoise Bay. Increases in density were observed over the summer, which can be attributed to recruitment events. These increases, however, were only temporary and were quick to return to what appeared to be a stable, baseline density. Although this temporal density variation was only studied at one site, mean lengths of sand dollars were similar at all the sites where populations were found, implying that growth conditions and possibly recruitment conditions were similar.

Further research is therefore required in order to better understand how sediment grain size and movement as well as sediment primary productivity affected the distribution, density and recruitment variation of *P. lesueuri*.

Chapter 3 Reproductive Biology of *Peronella lesueuri*

3.1 Introduction

Echinoid reproduction has been extensively studied and the reproduction cycles of many species have been documented through histological examination of gonad tissue which generally follows a pattern of morphologically similar stages. These comprise of growth, development and maturation of the gametocytes and spawning of gametes, after which gonad tissues recover and accumulate a nutrient store which fuels the following cycle (Chatlynne 1969; Gonor 1973; Byrne 1990). The focus of previous studies on echinoid reproduction has largely been on the regular urchins (Pearse and Phillips 1968; Gonor 1972; Gonor 1973; Gonor 1973; Byrne 1990; Dotan 1990; Drummond 1995; Byrne et al. 1998; Agatsuma and Nakata 2004; Lima et al. 2009).

Documentation of reproduction in irregular urchins, including sand dollars, is rare and the reproductive cycles are only known for a handful of sand dollar species, namely, *Sinaechinocyamus mai* (Chen and Chen 1993); *Echinarachnius parma* (Cocanour and Allen 1967); *Mellita quinquiesperforata* (Lane and Lawrence 1979; Tavares and Borzone 2006); *Clypeaster ravenelii* (Vernon et al. 1993); *Dendraster excentricus* (Niesen 1977) and *Arachnoides placenta* (Haycock 2004); and all had annual cycles. The only published literature to date on the reproductive biology of *Peronella lesueuri* reported that individuals from a lagoon near Misaki in Japan had ripe gonads in late June and July (summer) (Mortensen 1921). Mortensen (1921) also noted that *P. lesueuri* had yolk-laden ova with diameters of 0.3 – 0.4 mm, which suggested lecithotrophic development.

Echinoids commonly exhibit reproductive cycles with annual, seasonal, monthly and/or lunar periodicities although some species, like *Echinometra mathaei* in Western Australia, have been found to exhibit continuous reproduction (Pearse and Phillips 1968). Seasonal or annual reproductive cycles imply that regulation of reproduction is through one or a combination of exogenous cues and factors that change with the seasons, like sea temperatures (Spirlet et al. 1998; Brewin et al. 2000), photoperiod (Bay-Schmith 1989; Byrne et al. 1998; Alsaffar and Lone 2000), nutrient availability (Starr et al. 1993; Muthiga and Jaccarini 2005; Kino and Agatsuma 2007) and lunar periodicity (Kennedy and Pearse 1975; Iliffe and Pearse 1982). There has, however, been no identification of a single factor that can be consistently correlated with the reproductive cycles of all echinoids and it is likely that the regulating exogenous factor or combination of factors is species-specific (Mercier and Hamel 2009b).

Although there is still a lack of understanding as to how photoperiod acts as a cue for gametogenesis (Walker et al. 2007), temperature and photoperiod still are regarded as the most likely regulators of gametogenesis and spawning in echinoids (Mercier and Hamel 2009b). Temperature is thought to initiate and/or inhibit gonad growth (Iliffe and Pearse 1982; Sakairi et al. 1989) and regulate spawning (Ito et al. 1989; Spirlet et al. 1998; Brewin et al. 2000; Nunes and Jangoux 2004) in a variety of species. Photoperiod has also been identified as a possible factor affecting reproduction in echinoids, regulating gametogenesis (Holland 1967; Bay-Schmith 1989; Vernon et al. 1993) and spawning (Byrne et al. 1998; Spirlet et al. 1998)

In order to accurately determine the impacts of individual exogenous factors on reproductive cycles, each factor has to be isolated and analysed independently (Yamahira 2004). Such studies are complex and are impossible to conduct in the field. Within constraints, this field study documents the gametogenic cycle of *Peronella lesueuri* in Cockburn Sound, Western Australia, through histological analysis of gonads, determines the periodicity of the reproductive cycle and speculates on the impact of environmental factors on gametogenesis and spawning.

3.2 Materials and Methods

3.2.1 Collection

Near monthly samples of adult *Peronella lesueuri* were collected for gonad analysis from Jervoise Bay in Cockburn Sound from March 2009 to April 2011. The sand dollars were collected using an epibenthic sled that was towed behind a vessel. The sled had a 5 cm cutting depth and 1 cm mesh (Figure 3.1). The mouth dimensions of the sled were 85 cm by 45 cm. The sand dollars were manually separated from the other material collected by the sled.

Figure 3.1 The epibenthic sled and its contents after a 200 metre tow.

Peronella lesueuri displays no obvious external sexual dimorphism. Based on an assumption of a sex ratio of 1:1, the first 30 individuals encountered with a test length of ≥120 mm were collected to ensure that the sample contained at least 10 males and 10 females. There is no literature on the size at maturity of *P. lesueuri.* The approximate size of sand dollars in a preliminary sample was 150mm, therefore, sand dollars of test length ≥120 mm were assumed to have attained sexual maturity. The collected sand dollars were then placed on ice in an insulated cool box to prevent them from spawning during transport to the laboratory, where they were fixed in 10% formaldehyde in seawater for dissection.

A separate sample of 31 juvenile sand dollars varying in test length from 50 – 123 mm was also collected for a study to determine size at sexual maturity. Juvenile sand dollars were rare therefore the sample was limited in number.

3.2.2 Histology

Unlike regular sea urchins, the gonads of *Peronella lesueuri* do not occur as discrete sacs. The gonads are instead distributed throughout the main body cavity and surround the central gut. Four gonoducts transport gametes from the gonads to the gonopores on the aboral surface when spawning. It was not possible to differentiate the four discrete gonads due to the mesh-like distribution of the acini however. A preliminary histological analysis of different areas of the gonad in several individuals determined the homogeneity of the gametogenic development throughout the gonad material.

Before dissection, all sand dollars were measured for length, weighed and upon dissection, sexed. Correlation coefficients and r^2 values for test length-weight of individuals >120 mm were calculated using JMP (SAS Institute) and Microsoft Excel™. A Z-test was performed on the 466 sexed individuals to determine the sex ratio within the population. Thirty-one small individuals (50 – 122 mm), assumed to be juveniles and early adults, were also measured and weighed before dissection to determine if gonads were discernible.

All the collected sand dollars were dissected using a rotary tool with a cutting bit. A pentagonal hole was cut on the aboral surface of the test to expose the gonads (Figure 3.2). Sand dollars were then visually sexed by distinguishing their gonad material under a stereomicroscope. In mature individuals, the gonads of *Peronella lesueuri* were obvious on dissection and dominated the body cavity. Male gonads were an off-white colour and female gonads were pink to dark brown, occasionally black. The sexes were easily distinguishable from each other by eye and ova were visible under the dissecting microscope. During the spent and recovery stages, gonads of both sexes were a tan colour and sexes were difficult to distinguish. In order to determine sex, unspent ova were located using a stereomicroscope. A subsample of gonad material was extracted from ten males and ten females for histological analysis.

A portion of the gonad tissue was removed with dissecting scissors and forceps and placed in 50% ethanol. The gonad tissue samples were then dehydrated through a series of graded ethanol solutions and 100% chloroform and embedded in Paraplast embedding wax using a Leica TP 1020 automatic tissue processor. The gonad tissues were then sectioned into 10 µm thick sections on a Leica RM 2235 microtome and mounted onto glass slides. It was not possible to section any thinner than 10 µm due to the fragile condition of the gonads. Three slides were made from each gonad sample, with the sections more than 100 µm apart. As some of the ova in the females were more than 200 µm in diameter, taking the sections at more than 100 µm apart reduced the chance of encountering the same oocyte twice. Taking three sections from a sample also prevented data from being biased if the distribution of gametocytes within the gonad was not uniform (Gonor 1973). The slides were then stained with Harris' haematoxylin and 1% eosin in 95% ethanol.

The aim was to obtain 10 individuals of each sex for study. However, during the course of the study, several gonad samples were incorrectly identified and sexed and the mistakes were only discovered upon examination of the histological slides under the compound microscope. These misidentifications were taken into account when calculating proportions of gametogenic stages.

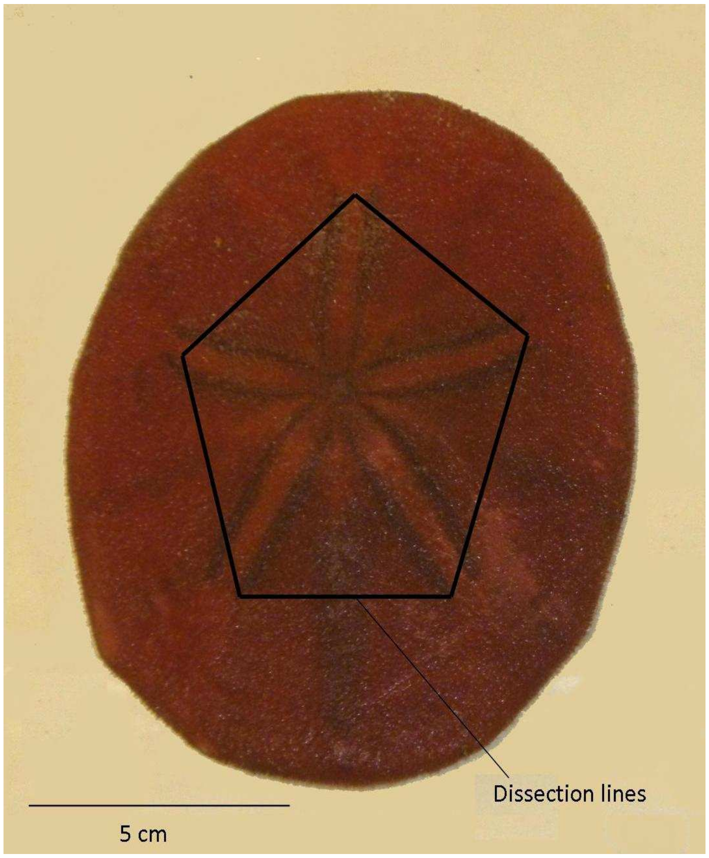

Figure 3.2 Dissection lines for *Peronella lesueuri*

3.2.3 Histological analysis

The slides were viewed using an Olympus BX51 compound microscope (Olympus Optical Co. Ltd., Tokyo, Japan) and digital images were taken of each slide. The images were taken with an Olympus DP70 camera and software (Olympus Optical Co. Ltd., Tokyo, Japan).

One image at 40x magnification was taken of each slide. Images were taken of areas where the gonad material was in good condition and best represented the gametogenic stage of the entire sample on the slide. The images were then classified into gametogenic stages. Images at higher magnification were also taken when points of interest were encountered within the gonad material.

Male and female gonads were each classed into five gametogenic stages, based on similar studies on other echinoderms (Chatlynne 1969; Byrne 1990; Falkner and Byrne 2003; MacCord and Ventura 2004). Proportions of the different gametogenic stages in each gonad sample were calculated.

Two methods were employed to study oocyte growth and differentiation through the gametogenic cycle. The change in proportion of the three main oocyte stages was used to follow the changes in the morphological stages of the gametogenic cycle. This method ensured that oocytes at different developmental stages were not grouped together when their sizes overlapped (Gonor 1973).

Oocyte size frequencies were obtained by measuring the first 50 oocytes encountered in each sample. To ensure that oocytes diameters were as accurate as possible, all Stage 2 and 3 oocytes measured had been sectioned through the nucleus and Stage 1 oocytes were only measured if sectioned through the nucleolus.

As *Peronella lesueuri* oocytes are relatively large, in order to have largest possible sample to measure from, oocytes were measured from images taken at the lowest magnification (40x) on the compound microscope camera. However the low magnification of the images used for histological analysis meant that stage 1 oocytes that were too small to have a visible nucleus or nucleolus were not measured. As a result, the mean diameter of the measured stage 1 oocytes was biased towards a slightly larger size. The oocyte diameter was calculated as the average of the longest and shortest axes of the oocyte measured through the nucleus. T-tests were used to determine if there were significant differences in oocyte diameters.

Accurate quantitative measurements were difficult for male gonads; therefore samples were only classified into five stages based on their distinct morphological features described in section 3.3.3.2.

3.2.4 Environmental data

The sea surface temperatures (SSTs) for the Cockburn Sound region were obtained from the Integrated Marine Observing System (IMOS).

The number of bright sunshine hours (BSH) was used as a measure of photoperiod. Mean number of bright sunshine hours recorded for the Perth region was obtained from the Bureau of Meteorology. The hours of “bright sunshine” is measured from midnight to midnight each day. The readings are taken from a Campbell-Stokes recorder, which uses a glass lens to focus the sun on a specially designed card. The length of the burn scorched by the intensity of the sun’s image is proportional to the number of bright sunshine hours for the day.

3.2.5 Statistical Analysis

JMP 9 software (SAS Institute) was used to perform all the statistical analyses used in this chapter. Chi-squared tests were used to compare proportions of male and females as well as proportions of oocyte stages between months.

No statistical analysis was used to test the effect of environmental variables on oocyte proportions. The stage of oocyte data was categorical and had no variance; as the assumption normally distributed continuous variables was not met, the use of parametric statistical tests was inappropriate. The correlation of environmental variables to oocyte proportions was instead described as trends visible from corresponding graphs.

3.3 Results

3.3.1 Basic Biology

To obtain gonad tissue samples for histological analysis, 466 sand dollars were dissected, of which 225 were male and 241 female. A chi-squared test showed that *Peronella lesueuri* had a sex ratio of 1:1 (χ^2=0.55, p=0.46, n=466).

Peronella lesueuri also showed no visible sexual dimorphism. There was no significant difference (t=1.24, p=0.21, n=510) between the mean length of adult males (143.49 ± 9.15mm) and adult females (142.46 ± 9.50mm). Mean weights of adult males (132.76 ± 24.01g) and adult females (133.59 ± 24.38g) also showed no significant difference (t=0.38, p=0.70, n=510).

Only 8% of juvenile sand dollars dissected to determine sexual maturity (50 – 79 mm size range) had gonad developed enough to be sexed (Table 3.1). Gonads were developed and could be sexed in 60% of individuals between 80 and 109 mm. All sand dollars larger than 115 mm had developed sexable gonads and were deemed sexually mature. Based on the data on length-at-age (Chapter 4), this indicated that *Peronella lesueuri* attains sexual maturity between 1.5 and two years of age, and all individuals above two years of age were sexually mature.

Table 3.1 Size ranges of juvenile *Peronella lesueuri* containing sexable gonads.

Size range (mm)	N	Individuals with sexable gonads (%)
50 - 79	12	8
80 - 109	10	60
110 - 123	9	89

3.3.2 Oocyte Histology

Over the course of the study, changes in the sizes and frequencies of morphologically classified oocytes were observed. Distinctive appearance features and different affinity for stains were used to classify the oocytes encountered in the histological analysis based on work performed by Gonor (1973) on *Strongylocentrotus purpuratus.* The changes in appearance and staining characteristics seem to correspond to the developmental changes within the oocyte during gametogenesis.

3.3.2.1 Oocyte stages

Stage 1 oocytes (85.63 ± 27.81 µm)

The smallest oocytes (Figure 3.3) that could be clearly identified were present along the acinal walls, usually located within a layer of nutritive phagocytes. The cytoplasm of these oocytes was strongly basophilic, staining a dense dark blue or dark purple with hematoxylin and eosin. In most cases, a pink-stained nucleus was visible, often with a distinct black nucleolus. These oocytes were rounded in shape, although they may elongate or be compressed as they grow or if they are tightly packed together. These oocytes were present in ovaries at all stages of reproduction.

Stage 2 oocytes (159.65 ± 29.65 µm)

As a stage 1 oocyte developed, the nucleus became larger and the amount of cytoplasm greatly increased. The cytoplasm of stage 2 oocytes (Figure 3.3) was basophilic and stained a bright purple with hematoxylin and eosin. Oocytes at this stage were variable in shape and possessed a relatively large size range. As stage 2 oocytes developed they remained along the acinal walls of the ovary.

Stage 3 oocytes/Ova (210 ± 33.74 µm)

Stage 3 oocytes (Figure 3.3) had a regular, round shape and consisted of a large nucleus and big volume of cytoplasm that was weakly basophilic and stained pink with hematoxylin and eosin. Although most of the stage 3 oocytes were found in the lumen of the ovary, some were attached to the acinal walls. Ova were classed together with the stage 3 oocytes because the last phases of meiosis occurred rapidly and ova were often difficult to differentiate from stage 3 oocytes. In the following text, the term "stage 3 oocyte" will also be used to include mature ova unless specified.

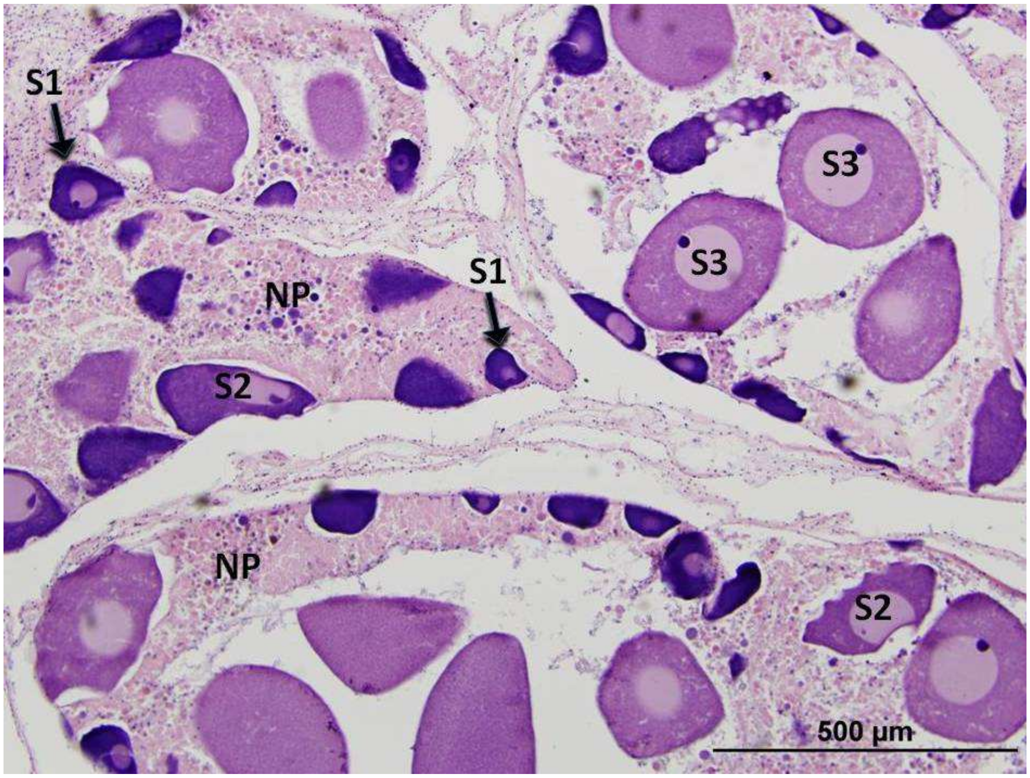

Figure 3.3 The three oocyte stages in a growing ovary. S1 = Stage 1 oocyte, S2 = Stage 2 oocyte, S3 = Stage 3 oocyte or ova. NP denotes the nutritive phagocytes, a nutrient store, that accumulates in gonads during the recovery and growth phases.

Relict oocytes

Unspawned/relict ova were broken down and reabsorbed. The cytoplasmic material appeared to break up into little globules, which were then integrated into the nutrient store of nutritive phagocytes (Figure 3.4) for the next cycle of gametogenesis. Relict ova present in the spent stages of gametogenesis were not measured or included in the counts.

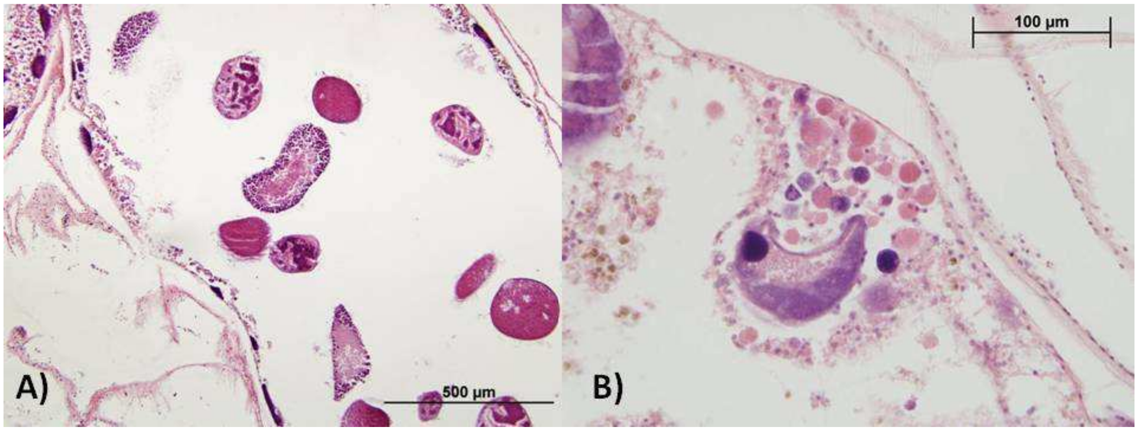

Figure 3.4 A) Relict oocytes in various stages of lysis while still retaining their shape. B) Eventually their cytoplasmic components will be assimilated into the nutrient store.

3.3.3 Gametogenic stages in *Peronella lesueuri* gonads

Histological analysis of *Peronella lesueuri* gonads revealed that the gametogenic cycle in both sexes could be classified into five morphologically distinct stages: Recovery, Growth, Mature, Partially spawned and Spent.

3.3.3.1 Gametogenic stages in the ovaries

Stage I: Recovery (Figure 3.5a and 3.5b)

On dissection, it was difficult to distinguish recovering ovaries from recovering testes. However, once subjected to histological processing and mounted on slides, ovaries could be differentiated by the presence of stage 1 oocytes. Ovaries in the recovery stage were dominated by stage 1 oocytes (S1) although some still had unspawned ova and/or stage 2 oocytes in various stages of degeneration. A thick layer of nutritive phagocytes (NP) lined the acinal wall, often filling the lumen. Nutritive phagocytes were eosinophilic and stained pink. Dark, lipofuschin-like particles (LF), probably a product of gamete breakdown, were present in some recovering ovaries (Figure 3.5b).

Stage II: Growth (Figure 3.5c)

Growing ovaries were abundant in stage 1 and stage 2 oocytes as oogenesis took place. Stage 1 and 2 oocytes remained surrounded by nutritive phagocytes and were attached to the acinal wall. Stage 3 oocytes started to appear and accumulated as ovaries moved through the growth stage. The amount of nutritive phagocytes decreased as the nutrients fuelled oogenesis.

Stage III: Mature (Figure 3.5d)

Stage 3 oocytes and ova were abundant in the lumen of mature ovaries. Some mature ovaries contained mainly mature oocytes with few stage 1 oocytes along the acinal walls and few or no nutritive phagocytes. Other ovaries contained oocytes in all stages of development along with mature oocytes in the lumen (Figure 3.5e) and some nutritive phagocytes. The two variants of the mature stage suggested that some sand dollars produced only one cohort of ova and are spawned out once all the ova are released. Other sand dollars can produce several cohorts of ova, replacing ova as they are spawned. These sand dollars probably spawned for longer through the spawning period.

Stage IV: Partially spawned (Figure 3.5f)

During the period of spawning, gonads that seemed partially spawned were observed. Large numbers of mature oocytes or ova were still present in the lumen of the acini, although spaces left by spawned ova were observed.

Stage V: Spent (Figure 3.5g)

Thin layer of nutritive phagocytes and stage 1 oocytes lined the acinal walls. Acinal lumens were mostly empty although unspawned ova in various stages of degeneration were occasionally present. Dark lipofuschin-like particles were sometimes present in the acini from the breakdown of unspawned oocytes. Unspawned oocytes eventually underwent lysis and broke down into small globules (Figure 3.5h), which were incorporated into the nutritive phagocyte layer (Figure 3.4B).

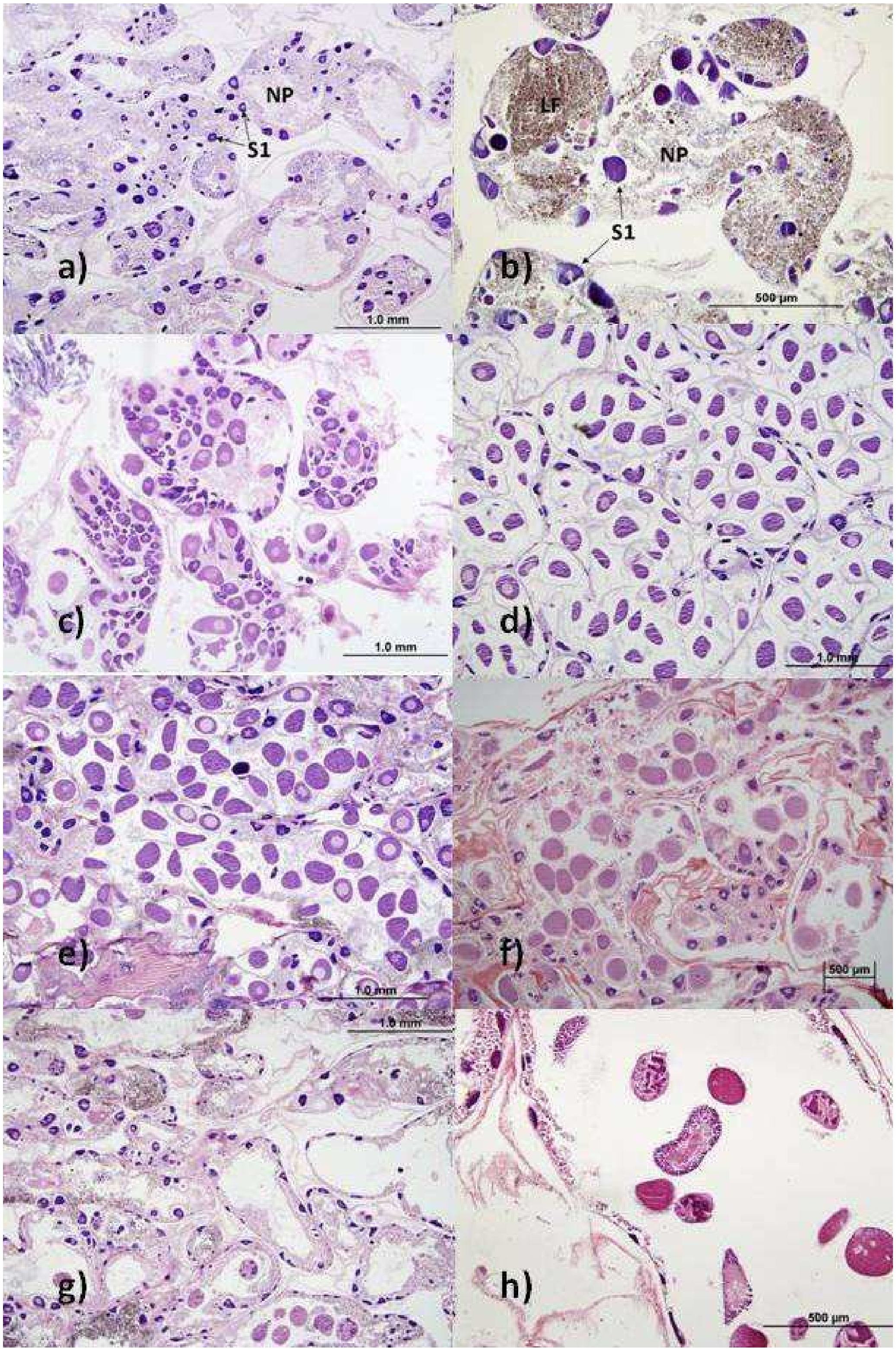
NP
S1
a)
1.0 mm
LF
NP
S1
S1
b)
500 µm
c)
1.0 mm
d)
1.0 mm
e)
1.0 mm
f)
500 µm
1.0 mm
g)
h)
500 µm

Figure 3.5 Stages of oogenesis in *Peronella lesueuri*. a) Stage I: Acinal walls with a thick layer of nutritive phagocytes and stage 1 oocytes; b) Stage I: Ovary in recovery stage showing lipofuschin-like particles (LF) in the acini; c) Stage II: Oocytes at all stages of development are present; d) Stage III: Mature ovary with no nutritive phagocytes and few stage I oocytes along acinal wall; e) Stage III: Mature ovary with oocytes in various stages of development; f) Stage IV: Spaces left by spawned ova are visible in the partially spawned ovary; g) Stage V: Spent ovary with empty lumens and thin layer of nutritive phagocytes. Unspawned ova beginning to lyse; h) Several ova undergoing lysis while still retaining their shape.

3.3.3.2 Gametogenic stages in the testes

Stage I: Recovery (Figure 3.6a)

Testes in the recovery stage were difficult to distinguish from ovaries in a similar stage at dissection. Acini of recovering testes were packed with nutritive phagocytes, digesting relict spermatozoa that were still present. A thin basophilic layer of spermatogonia could be seen lining the acinal walls.

Stage II: Growth (Figure 3.6b)

The basophilic layer of spermatogonia along the acinal walls thickened. Acini were still filled with nutritive phagocytes. As spermatogenesis progressed, spermatozoa started to accumulate in the testis lumen, displacing the nutritive phagocytes from the centre.

Stage III: Mature (Figure 3.6c)

Spermatozoa were densely packed in the lumen of mature testes. Some testes no longer contained nutritive phagocytes, but in testes where they were still present, nutritive phagocytes were displaced to the periphery.

Stage IV: Partially spawned (Figure 3.6d)

Due to gamete release, the concentration of spermatozoa decreased and acinal spaces loosely-filled with spermatozoa. Large spaces sometimes developed within dense masses of spermatozoa as spawning took place. Testes in the partially spawned stage sometimes looked similar to growing testes but could be distinguished by gaps between spermatozoa masses and the acinal walls.

Stage V: Spent (Figure 3.6e)

Spent testes had empty acini, although some still contained masses of relict spermatozoa which would eventually be broken down. An eosinophilic layer of nutritive phagocytes started to accumulate along the periphery of the acini.

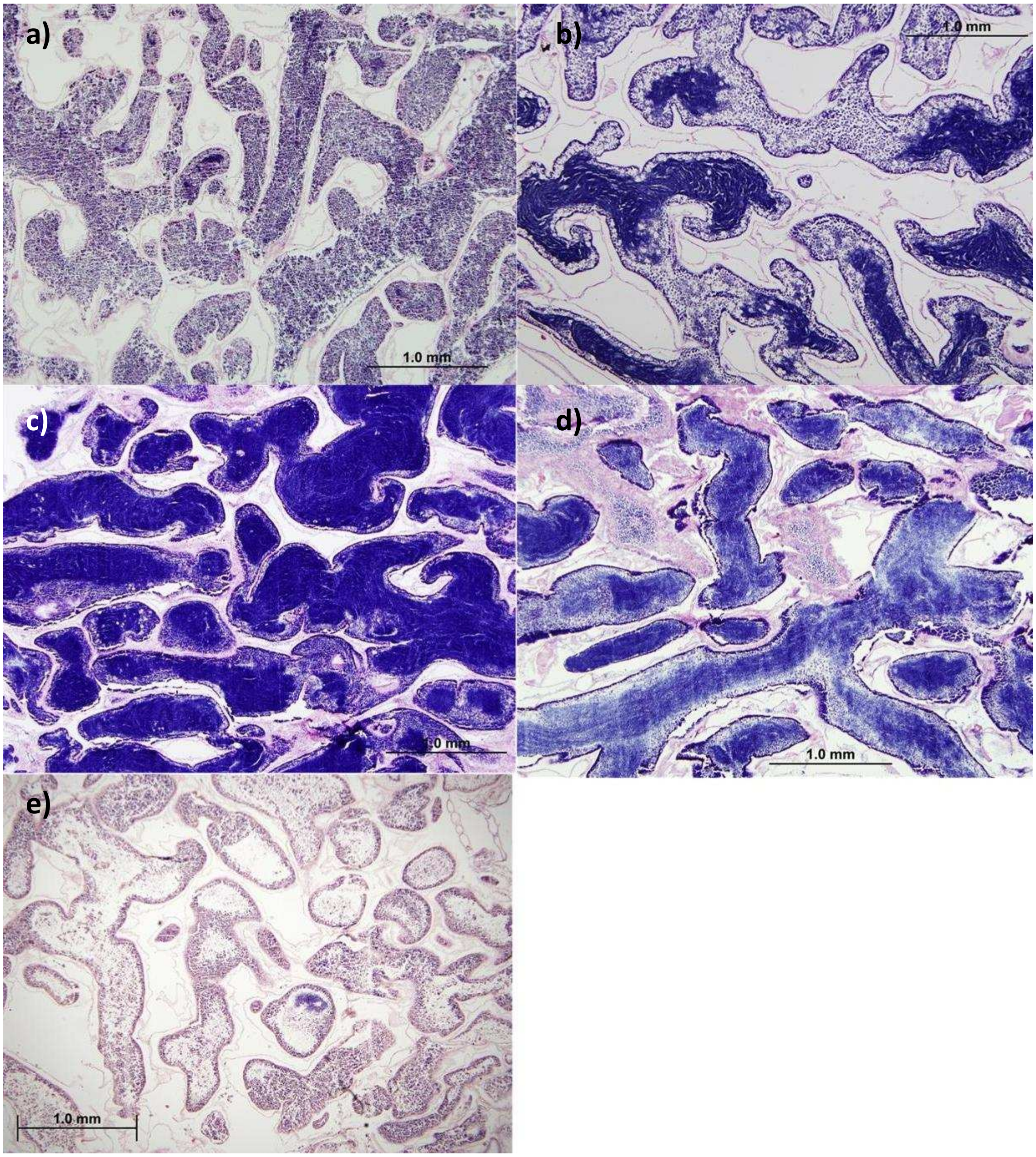

Figure 3.6 Stages of spermatogenesis in *P. lesueuri*. a) Stage I: Nutritive phagocytes fill the acini of the recovering testes; b)Stage II: Spermatozoa accumulate in the lumen of a growing testes; c) Stage III: Mature testes densely packed with spermatozoa; d) Stage IV: Partially spawned testes with gaps between loosely-packed spermatozoa and acinal wall; e) Stage V: Empty acini of spent testes.

3.3.4 The Reproductive Cycle

3.3.4.1 Annual change in gametogenic stages

The proportions of the gametogenic stages present in the monthly samples were used to ascertain the periodicity of *Peronella lesueuri* reproduction (Figure 3.7). Two distinct annual reproductive cycles were observed over the study; May 2009 – April 2010 (2009 – 2010 reproductive cycle) and May 2010 – April 2011 (2010 – 2011 reproductive cycle). Reproductive cycles in males and females appeared synchronous with corresponding gametogenic stages throughout.

Gametogenesis occurred from spring to early summer. During the 2009 – 2010 reproductive cycle, the growth stage was first observed in female gonads in August. The proportion of females with growing gonads in August and September, however, was less than 30%. No females with growing gonads were sampled in October, however, from November to January, between 90 – 100% of females had actively growing gonads. Growth in the male gonads commenced in September and between October to December, 60 – 100% of males had growing gonads. In the 2010 – 2011 reproductive cycle, gametogenesis appeared to commence in October 2010, when growing gonads were detected in 40% of females and 80% of males. No individuals with growing gonads were observed in November, but in December and January, gonads in the growth stage were observed in at least 60% of all individuals.

Mature gonads first occurred in both sexes in the summer: January 2010 and December 2010. Fully mature gonads that did not show signs of spawning were uncommon however (≤30%).

The first occurrence of gonads in the partially spawned stage indicated that spawning had commenced. Partially spawned gonads were observed in both sexes over a period of approximately 4 – 5 months; however, the spawning was assumed to be over when more than 50% of individuals contained spent gonads. Spawning took place from January to March during both reproductive cycles. During the 2009 – 2010 cycle, males appeared to commenced spawning earlier, with 80% of males sampled in January containing partially spawned gonads while spawning had yet to occur in females. With the exception of the gonad sampled on the 13 March 2009, the proportion of partially spawned gonads in males was always equal to or higher than in females.

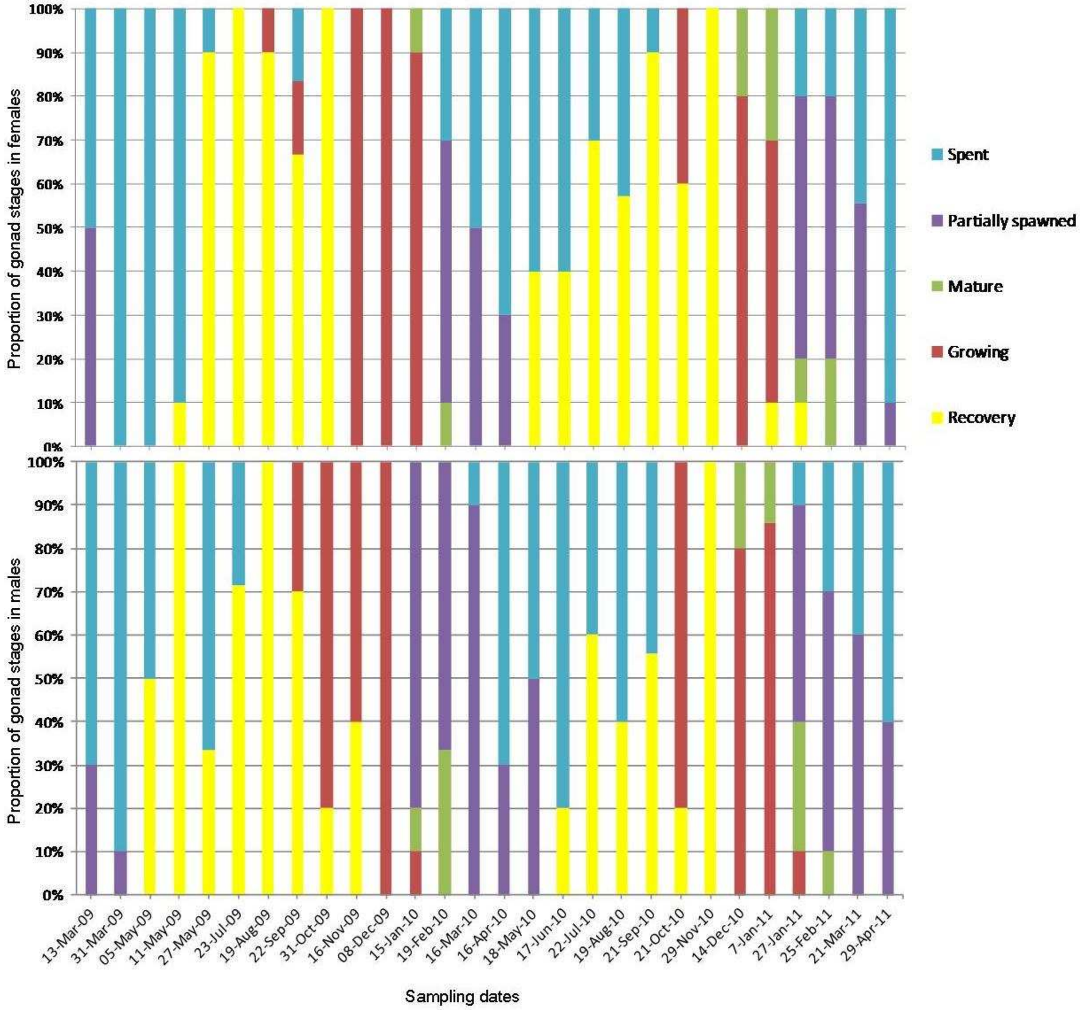

Figure 3.7 Monthly changes in the proportions of gametogenic stages in male and female *Peronella lesueuri* over the 26-month sampling period.

More than 50% of individuals of one or both sexes contained spent gonads between March – July 2009, April – September 2010 and in April 2011. The recovery stage was seen in gonads of both sexes between late autumn and late spring and was the dominant stage (≥50% of all individuals) from May – October 2009 and July to November 2010.

3.3.4.2 Annual change in oocyte proportions

Over the 26 month sampling period, temporal variation was observed in the percentages of the different oocyte stages. Stage 1 oocytes were present in varying proportions in every sample and accounted for between 38 – 100% of all oocytes. Stage 1 oocytes accounted for over 90% of all oocytes from July to September 2009 and July to August 2010 (Figure 3.8). This was when most sand dollar gonads were undergoing the recovery stage of the reproductive cycle (Figure 3.7) During this period, relict stage 2 and stage 3/ova oocytes were broken down and incorporated into the nutrient store (Figure 3.4b), while stage 1 oocytes remained undifferentiated.

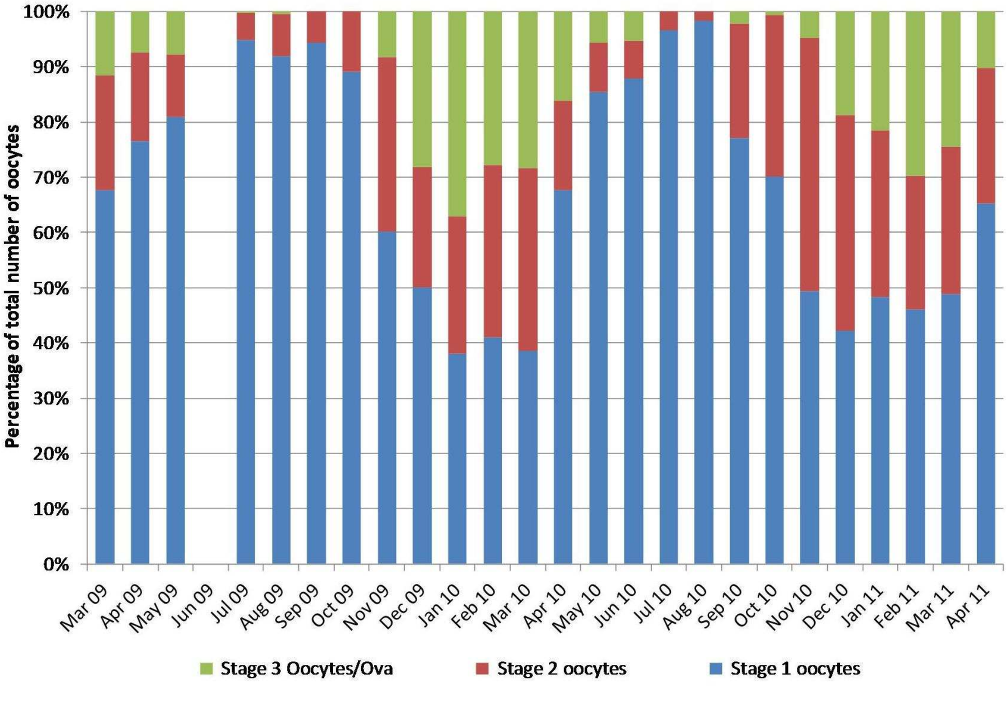

Figure 3.8 Monthly changes in the proportions of the 3 different oocyte stages encountered over the 26 month sampling period.

The proportions of stage 1 oocytes decreased by more than 20% in November 2009 and September 2010. From December to March in both 2010 and 2011, stage 1 oocytes accounted for less than 50% of all oocytes each month (Figure 3.8). This period was when most females were mature and generating ova for spawning (Figure 3.7), hence there were higher proportions of the more mature stage 2 and stage 3/ova oocytes phases.

Stage 2 oocytes were also present in every sample and were at their lowest proportions from July to August 2009 and May to August 2010, when they accounted for less than 10% of all oocytes (Figure 3.8). The sand dollars had spent gonads during this period and because no new ova were required relict stage 2 oocytes were either held in stasis or were broken down and the contents absorbed into the nutrient store. In November 2009 and September 2010, the proportion of stage 2 oocytes incresed abruptly, alongside the decrease in stage 1 oocytes. This indicated that gametogensis had commenced. In November 2009, stage 2 oocytes accounted for 31.6% of all oocytes, up from 10.9% in October. In September 2010, stage 2 oocytes made up 20.8%, up from 1.7% in August 2010. These sudden increases in the proportions of stage 2 oocytes indicated a rapid onset of oocyte growth in the sand dollars. The proportions of stage 2 oocytes then remained relatively high (between 20 – 45%) throughout the spawning periods (November 2009 – March 2010 and September 2010 – April 2011) suggesting that mature ova were constantly being generated throughout the spawning period.

Stage 3 oocytes were not present in every sample; they were absent from July – October 2009 and July – August 2010. This was when gonads were mostly spent or recovering (Figure 3.6). In each reproductive cycle, stage 3 oocytes were first observed in the same month as the abrupt increase in the proportions of stage 2 oocytes; November 2009 and September 2010 (Figure 3.7). This suggested that once gametogenesis commenced, differentiation between stage 1 and stage 3 oocytes was rapid. Stage 3 oocytes were observed in from March to May 2009, November 2009 to June 2010 and September 2010 to April 2011. Stage 3 oocyte made up more than 15% of all oocytes between December 2009 – April 2010 and December 2010 – March 2011. The stage 3 oocytes proportions peaked in January 2010 (37% of total oocytes) and February 2011 (29.7%). After the peak in spawning, proportion of stage 3 oocytes steadily dropped as differentiation of oocytes to ova slowed and mature ova were spawned out. The gonads eventually reached the spent stage where few or no stage 3 oocytes remained between July and October in both years.

3.3.4.3 Annual change in oocyte diameter

The change in mean monthly oocyte diameters followed two annual cycles over the sampling period. Mean oocyte diameters in each cycle were lowest in winter and highest in summer. The timing corresponded with the reproductive cycles observed through the analysis of gonad stage as well as the changes in oocyte proportions, which showed that gonads were spent and recovering during the winter, actively growing in spring and spawning through the summer (Figure 3.7 and 3.8).

From March 2009, mean oocyte diameters decreased gradually (Figure 3.9) to a low of 84.34 ± 33.82 µm in September. Mean oocyte diameters gradually increased from September, peaking in January 2010 (X ± SD = 160.03 ± 58.51 µm, n=500). A second gradual decline in mean oocyte diameter began in March 2010 and reached a low (X + SD = 70.15 ± 19.91 µm, n=300) in August. Oocyte diameters increased again in September 2010 as the second cycle of gametogenesis commenced. Mean oocyte diameters of the 2010-2011 reproductive cycle peaked in January 2011 (X + SD = 144.03 ± 53.22 µm, n=500) followed by a slight monthly decrease until the end of the sampling period in April.

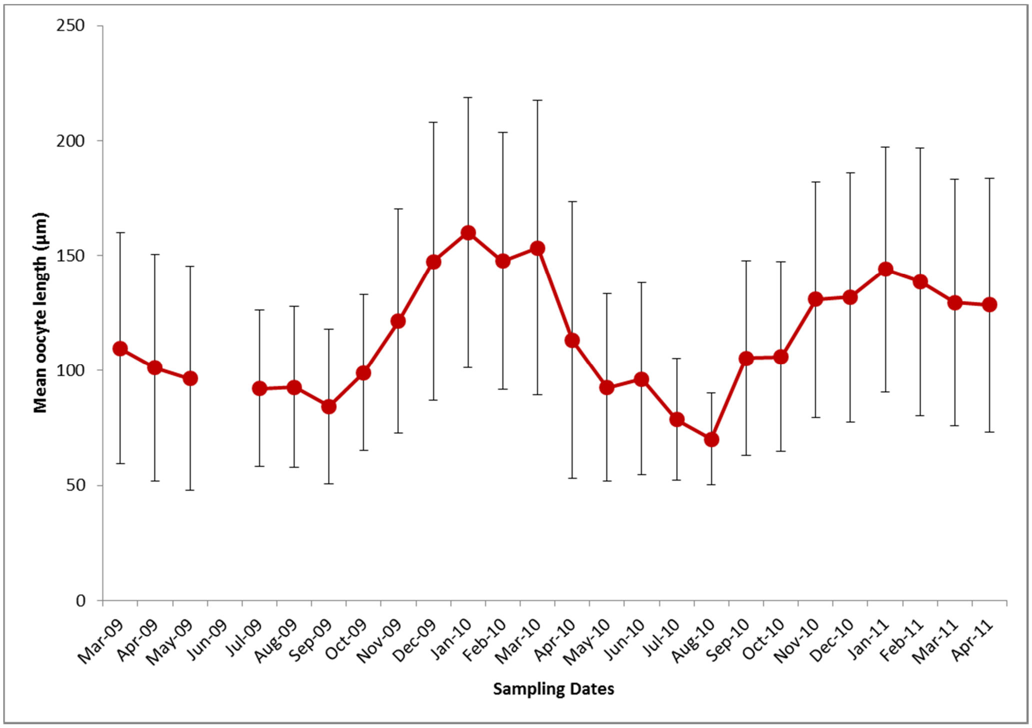

Figure 3.9 Monthly changes in the mean oocyte diameter + SD over the 26 month sampling period.

The increasing mean oocyte diameters from winter to summer (August to January) indicated the onset of gametogenesis as gonads in the growth and mature stages (Figure 3.6); stage 2 and stage 3 oocytes (Figure 3.7) also increased in proportion over the same time period. Decreasing mean oocyte diameter in from summer to autumn (February – April) reflected the decreasing proportions of stage 2 and 3 oocytes (Figure 3.7); as gametogenesis ceased, ova gradually spawned out and ovaries became spent (Figure 3.5). Further decreases in the mean oocyte diameter throughout winter reflected the high proportions of spent and recovering gonads observed during this period (Figure 3.6) and increasing proportions of stage 1 oocytes (Figure 3.7) as relict stage 2 and 3 oocytes were reabsorbed into the nutrient store.

The overall mean diameters for stage 1, 2 and 3 oocytes were 85.63 ± 27.81 µm, 159.65 ± 29.65 µm and 210.61 ± 33.74 µm respectively. However, the mean diameters for all three oocyte stages fluctuated monthly (Figure 3.10). Mean diameters of stage 1 oocytes increased approximately 30% from September 2009 to March 2010 and approximately 40% from September 2010 to April 2011, peaking in January 2010 (101.87 µm) and January 2011 (98.36 µm). Both were periods of active reproduction, when gonads were undergoing gametogenesis and spawning (Figure 3.6). Monthly mean diameters for stage 2 oocytes remained relatively constant from March 2009 to June 2010. However, decreases in stage 1 and stage 2 oocyte diameters were observed from June 2010 to August 2010.

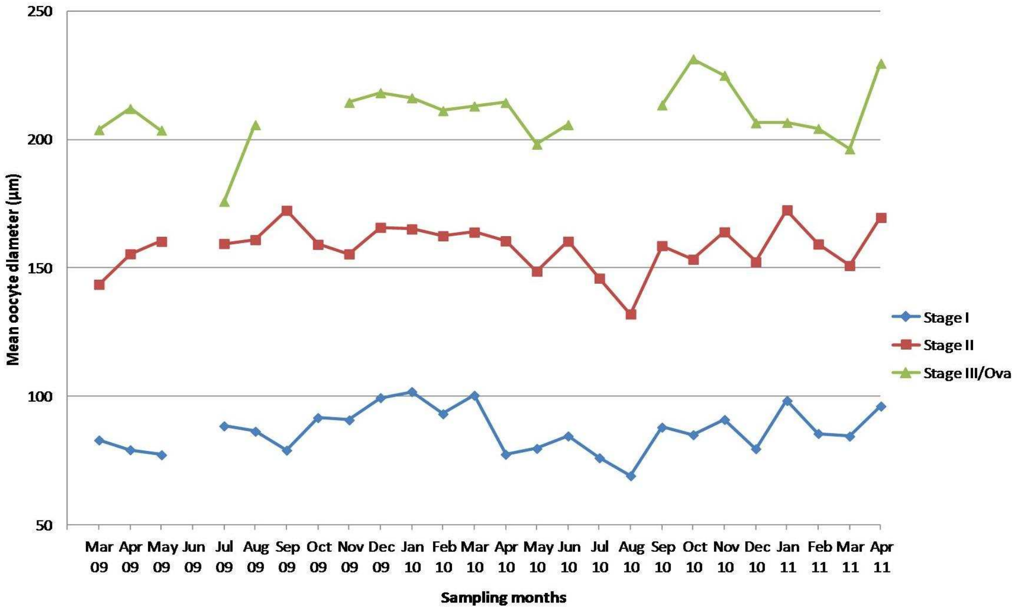

Figure 3.10 Monthly changes in mean oocyte diameters of stage 1, stage 2 and stage 3 oocytes from March 2009 to April 2011.

Stage 3 oocytes were scarce during the recovery stages of the gametogenic cycle and no stage 3 oocytes were measured in September and October 2009 and July and August 2010 amongst the randomly selected oocytes. Mean stage 3 oocyte diameters were the highest in October 2010 (231.45 µm) and April 2011 (229.71 µm) (Figure 3.10).

3.3.4.4 Comparisons between years

Two reproductive cycles were observed over the study period; May 2009 – April 2010 and May 2010 – April 2011. The seasonal periodicities of the two cycles were the same, gametogenesis taking place in spring, spawning occurring in summer and gonad recovery throughout winter (Figure 3.7). Overall, the two reproductive cycles were not significantly different from each other (F=0.12, p=0.14). Significant differences were, however, observed in the monthly proportions of oocytes and oocyte diameters between the two cycles.

In the 2010 -2011 reproductive cycle, the annual decrease in stage 1 oocytes proportions commenced in September 2010, one month earlier than the previous year (October 2009) (Figure 3.11). Proportions of stage 1 oocytes were significantly lower in September (χ^2=46.70, p<0.001), October (χ^2=54.08, p<0.001) and November (χ^2=11.22, p<0.001) 2010 than in 2009. They were also significantly higher in August 2010 (χ^2=17.57, p<0.001) January (χ^2=11.04, p<0.001) and March (χ^2=10.21, p=0.001) 2011.

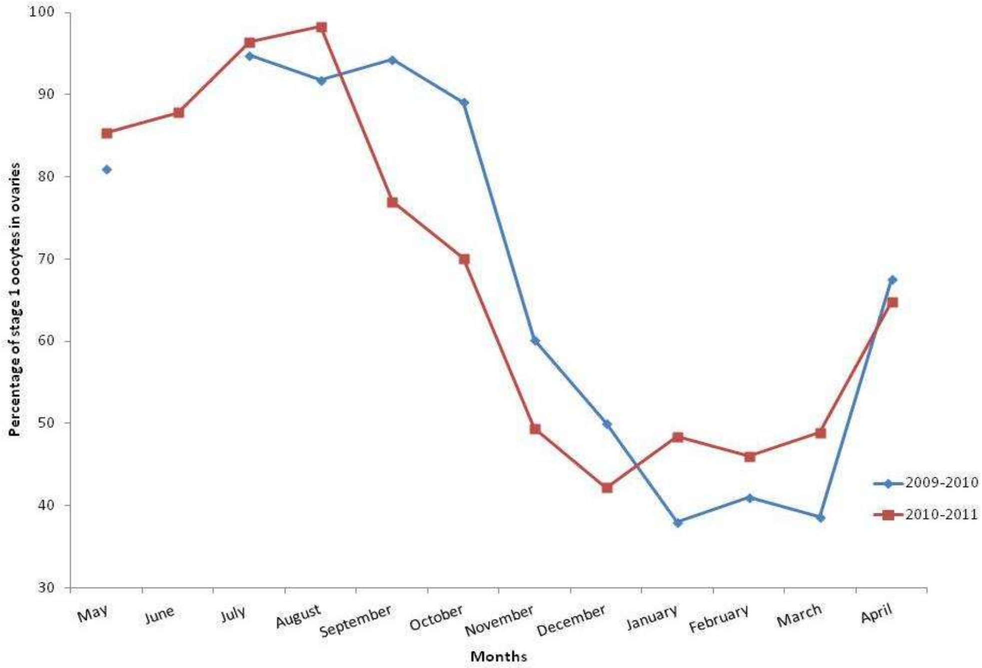

Figure 3.11 Comparison of the percentages of stage 1 oocytes present in sampled ovaries between the 2009 – 2010 and 2010 – 2011 reproductive cycles.

In 2010 – 2011, proportions of stage 2 oocytes were significantly lower in August (χ^2=16.06, p<0.001), and significantly higher in September (χ^2=37.88, p<0.001), October (χ^2=51.23, p<0.001), November (χ^2=20.33, p<0.001), December (χ^2=32.74, p<0.001) and April (χ^2=10.41, p=0.001) (Figure 3.11).

Compared with the 2009 – 2010 reproductive cycle, the 2010 – 2011 cycle presented significantly lower stage 3 oocyte proportions in December (χ^2=11.25, p<0.001), January (χ^2=28.88, p<0.001) and April (χ^2=6.97, p=0.008) (Figure 3.12).

In summary, in the 2010 – 2011 reproductive cycle, there were less stage 1 oocytes and more stage 2 oocytes present throughout gametogenesis. There were also less stage 3 oocytes produced throughout the spawning period.

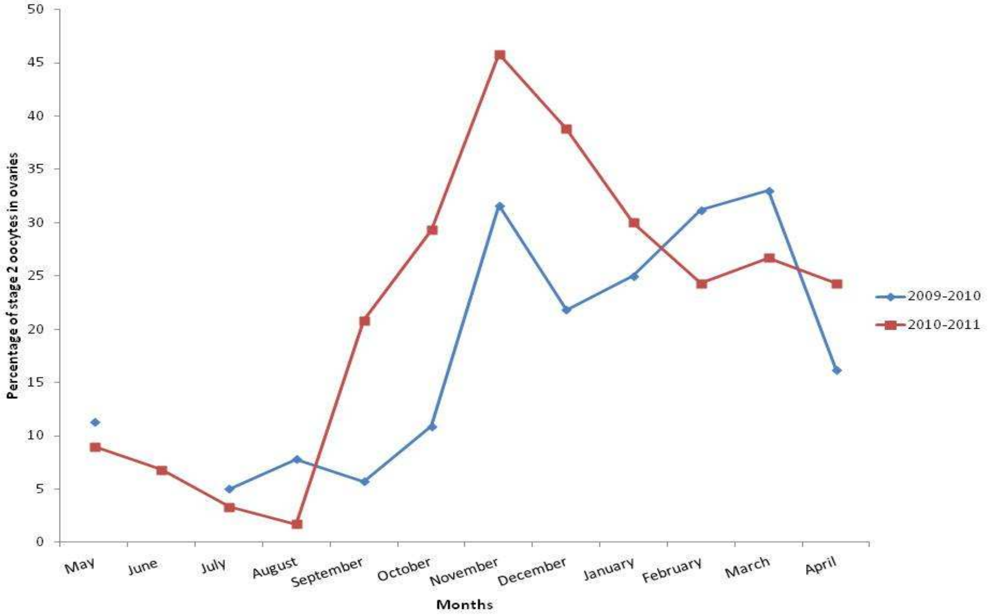

Figure 3.12 Comparison of the percentages of stage 2 oocytes present in sampled ovaries between the 2009 – 2010 and 2010 – 2011 reproductive cycles.

Significant differences in mean oocyte diameters were also observed between most corresponding months of the 2009 – 2010 and 2010 – 2011 reproductive cycles.

Tukey-Kramer HSD tests (p<0.01) showed that mean diameters were significantly lower in July, August, December, January and March and significantly higher in September, October November and April of the 2010 – 2011 reproductive cycle (Figure 3.12).

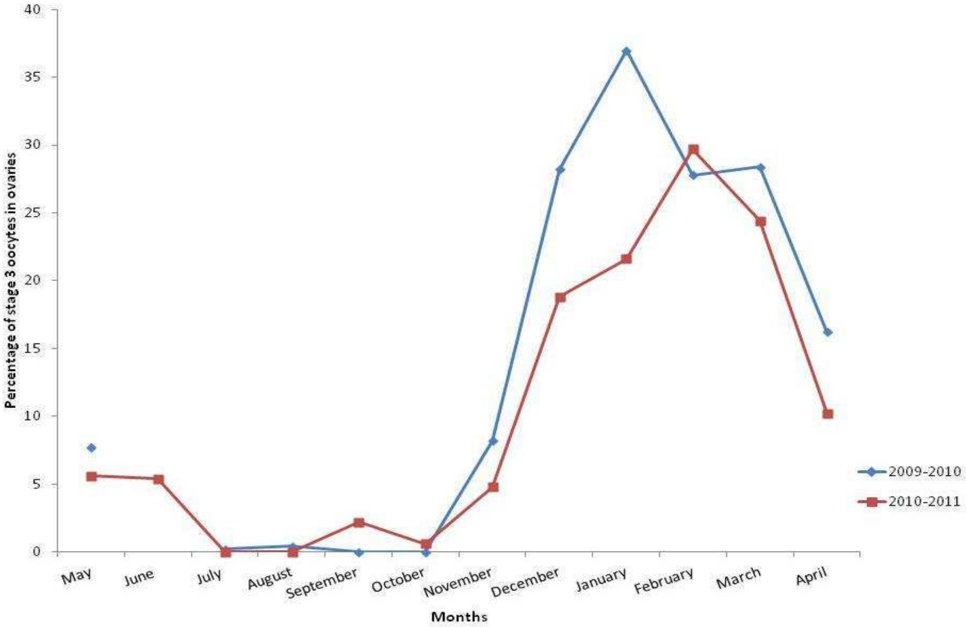

Figure 3.13 Comparison of the percentages of stage 3 oocytes present in sampled ovaries between the 2009 – 2010 and 2010 – 2011 reproductive cycles.

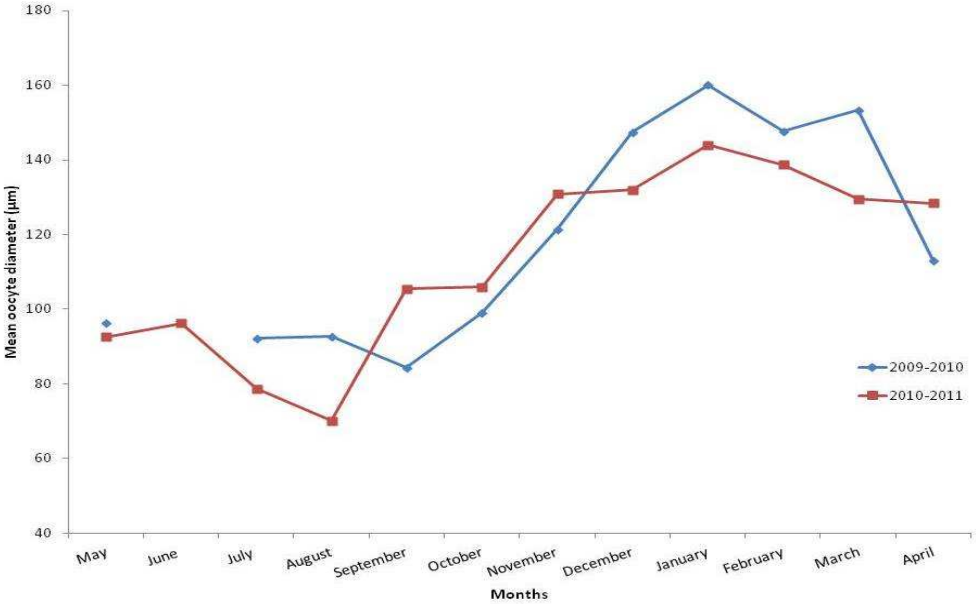

Figure 3.14 Comparison of monthly changes in mean oocyte diameters between the 2009 – 2010 and 2010 – 2011 reproductive cycles.

These significant differences in mean diameters between the reproductive cycles of both years were affected by corresponding differences in oocyte proportions observed between years. The lower mean diameters in July and August 2010 corresponded with lower proportions of the larger stage 2 oocytes and higher proportions of smaller stage 1 oocytes (Figures 3.11 and 3.12). Higher mean diameters observed in September, October and November corresponded with significantly larger proportions of stage 2 oocytes (Figure 3.12). Mean diameters were lower from December to March in the 2010 – 2011 cycle due to a combination of lower proportions of stage 2 and stage 3 oocytes (Figure 3.12 and Figure 3.13).

At the end of the spawning period in April, oocyte diameters in 2011 were significantly larger than in 2010 (Tukey-Kramer, $p<0.01$). This was due to the larger proportion of stage 2 oocytes still present in the ovaries (Figure 3.12) despite 90% of them being classed as spent (Figure 3.7).

Tukey-Kramer HSD tests (p<0.01) showed stage 1 oocyte mean diameters were significantly lower in the 2010 – 2011 reproductive cycle in the months of July, August, December and March (Figure 3.15). Stage 1 oocyte means were also significantly higher in September and April in 2010 – 2011.

Stage 2 and Stage 3 oocyte diameters showed no significant differences between the two reproductive cycles.

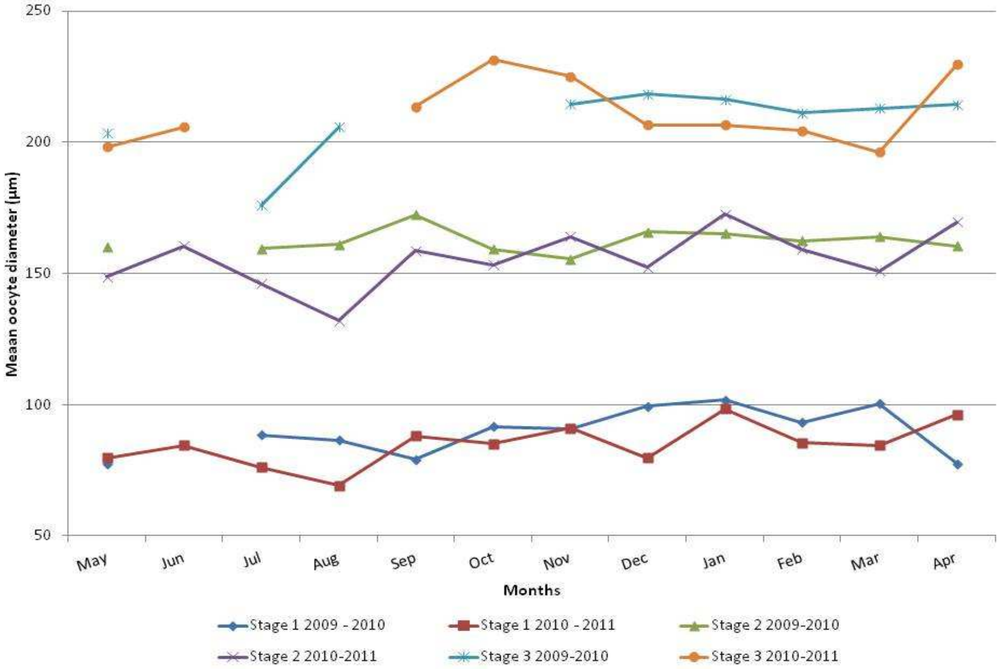

Figure 3.15 Comparison of monthly changes in mean oocyte diameters of stage 1, stage 2 and stage 3 oocytes between the 2009 – 2010 and 2010 – 2011 reproductive cycles.

3.3.4.5 Relationship to environmental variables

Sea surface temperature (SST) and number of bright sunshine hours (BSH) exhibit an annual, seasonal cycle in Cockburn Sound. SSTs in the area ranged from a low of 16°C in the winter to a high of 23°C in the summer (Pearce et al. 1999). Mean SSTs recorded in Cockburn Sound during the study period ranged from approximately 17 – 26°C (Figure 3.16). There appeared to be no significant difference between the two seasonal cycles of mean SSTs over the 2009 – 2011 study period (t=0.56, p=0.58). However, over the summer of 2010-2011, a "marine heat wave" was observed off the south-western coast of Western Australia, in which temperatures in Cockburn Sound were approximately 2°C higher than average (Pearce et al. 2011).

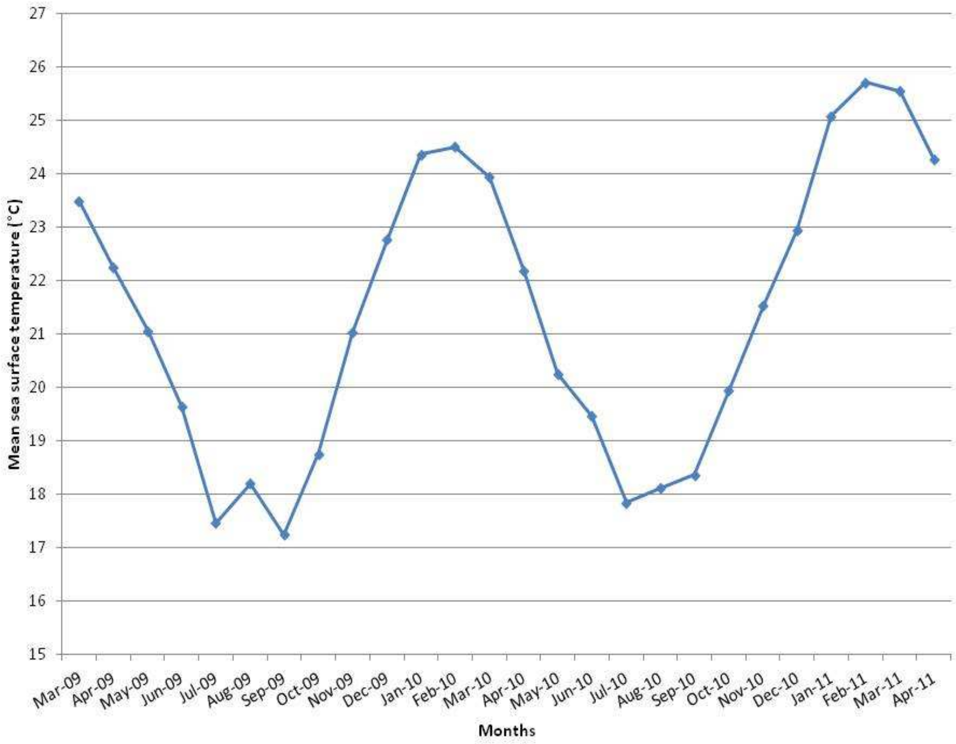

Figure 3.16 Monthly mean sea surface temperatures (SSTs) from Cockburn Sound, Western Australia (IMOS 2011).

Over the study period, the Perth Metropolitan area received a monthly mean of between 6.1 and 12.3 BSH (Figure 3.17). Since the increasing amount of light (and associated heat) can cause an increase in SST (Mercier and Hamel 2009), it is not surprising to see the cycle of SSTs mirror the cycle of BSH. BSH peaked in summer and reached lows in winter, mirroring the cycle of SSTs. As with SSTs, there was no significant difference between the two seasonal cycles of mean BSH during the study period (t=0.76, p=0.45). However, in 2010 – 2011, mean BSH started to increase earlier than the previous year (July instead of September). Over the summer of 2010 – 2011, mean BSH was also not as high as the previous year due to higher amounts of cloud cover.

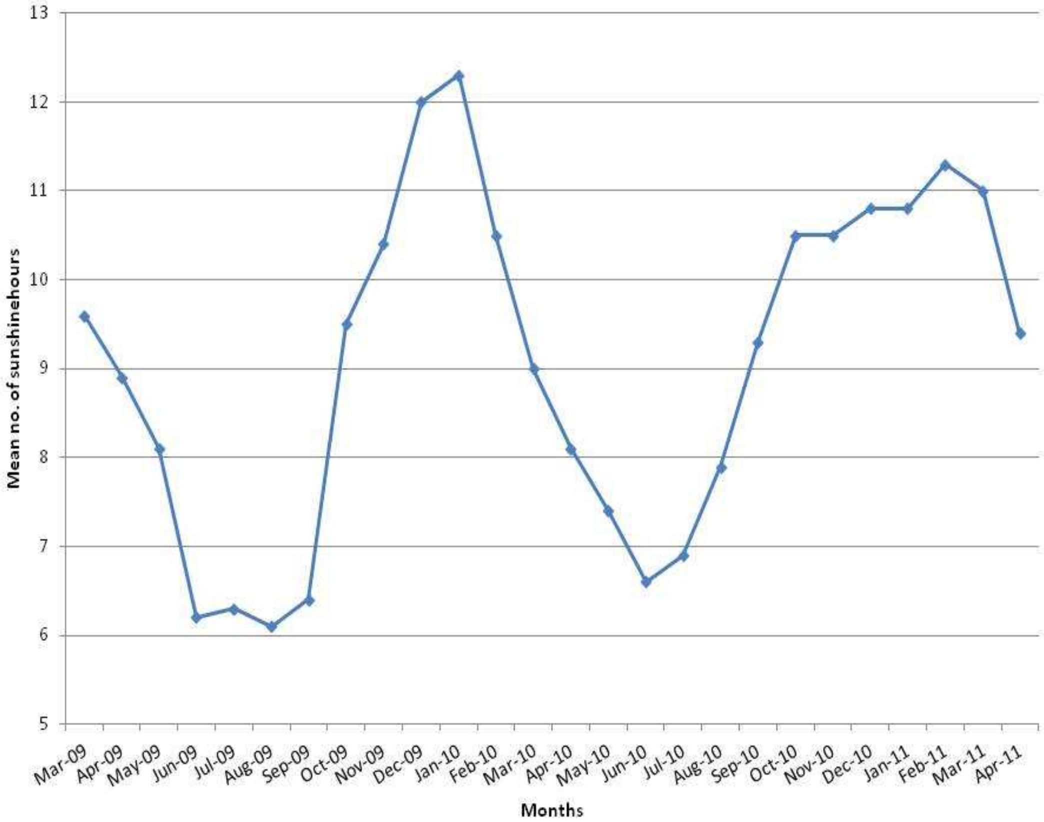

Figure 3.17 Monthly mean bright sunshine hours (BSH) for the Perth Metropolitan Area, Western Australia Bureau of Meteorology (BoM 2011).

The *Peronella lesueuri* reproductive cycle is seasonal; gametogenesis took place throughout spring and early summer as SSTs and BSH increased to peak levels. Spawning occurred from January to April, when SSTs and BSH were at their peak. Gonads were mostly spent and recovering over autumn and winter as SSTs and BSH decreased to minimum levels.

This was reflected in the annual changes in oocyte proportions of the different stages. Over the winter months from June to August, stage 1 oocytes made up between 80 to 100% of all oocytes in the gonad. The decline of stage 1 oocyte proportions around August and September signalled the initiation of vitellogenesis and corresponded to the increase in SSTs and BSHs from winter lows (Figure 3.18). As stage 1 oocyte proportions decreased, stage 2 oocyte proportions increased (Figure 3.19). Stage 2 oocyte proportions in both reproductive cycles peaked when mean SST was approximately 21°C (Figure 3.19B) and mean BSH was approximately 10.5 (Figure 3.19C).

In October both years, stage 3 oocyte proportions increased rapidly (Figure 3.20). Stage 3 oocyte continued to increase with SSTs and BSH, peaking in the period of highest mean SSTs and mean BSH between January and March. Stage 3 oocyte proportions were the highest around January and February, which was the start of the spawning period. As SSTs and BSHs started to decrease in March, proportions of stage 2 and stage 3 oocytes declined as gametogenesis slowed and mature gonads were spawned out. Stage 1 oocyte proportions started to increase to more than 90% between March and August, as spent gonads started to recover for the beginning of the next gametogenic cycle.

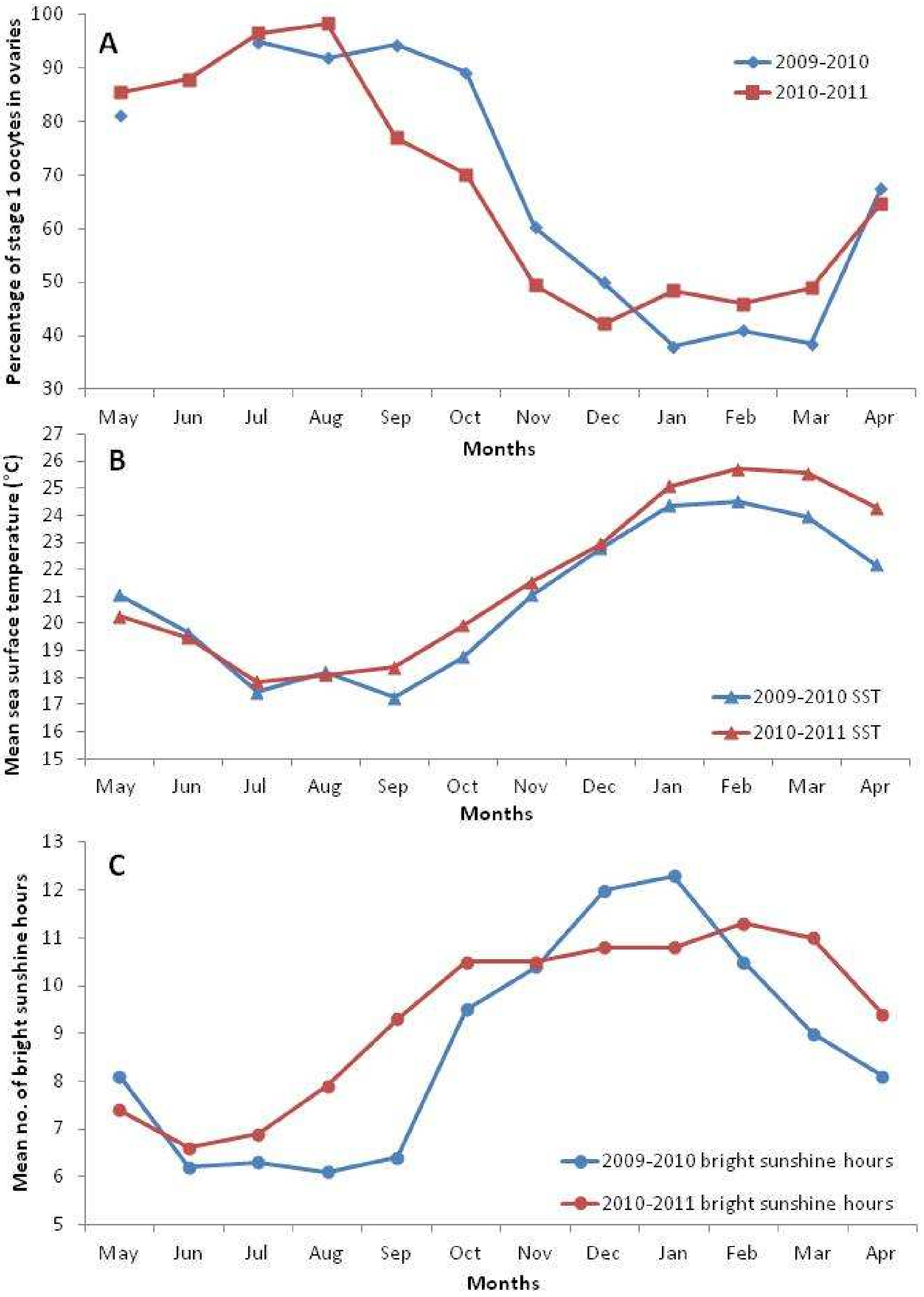

Figure 3.18 Comparisons of changes in A) Stage 1 oocyte proportions, B) SSTs and C) bright sunshine hours between the 2009-2010 and 2010-2011 reproductive cycles.

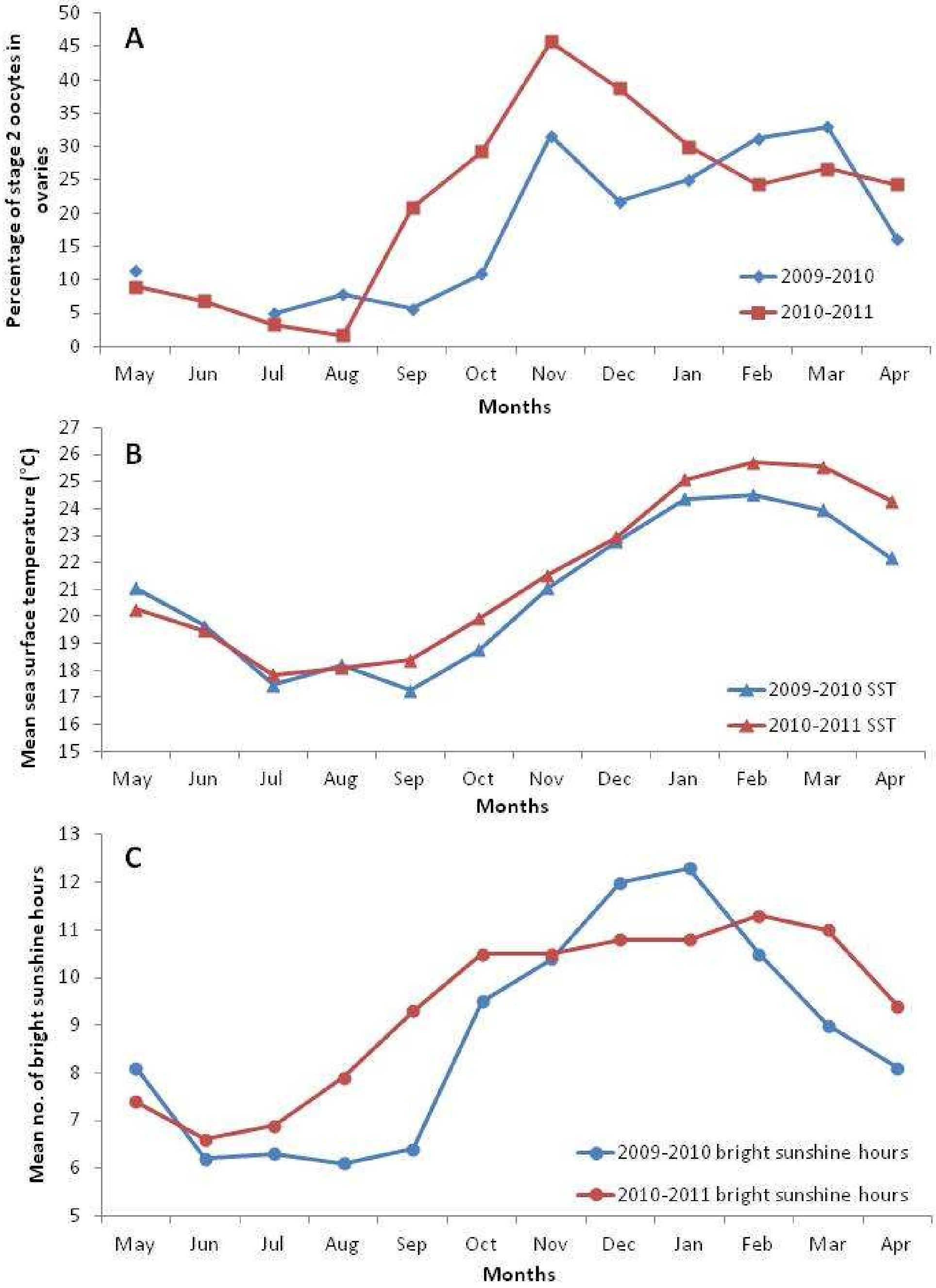

Figure 3.19 Comparisons of changes in A) Stage 2 oocyte proportions, B) SSTs and C) bright sunshine hours between the 2009-2010 and 2010-2011 reproductive cycles.

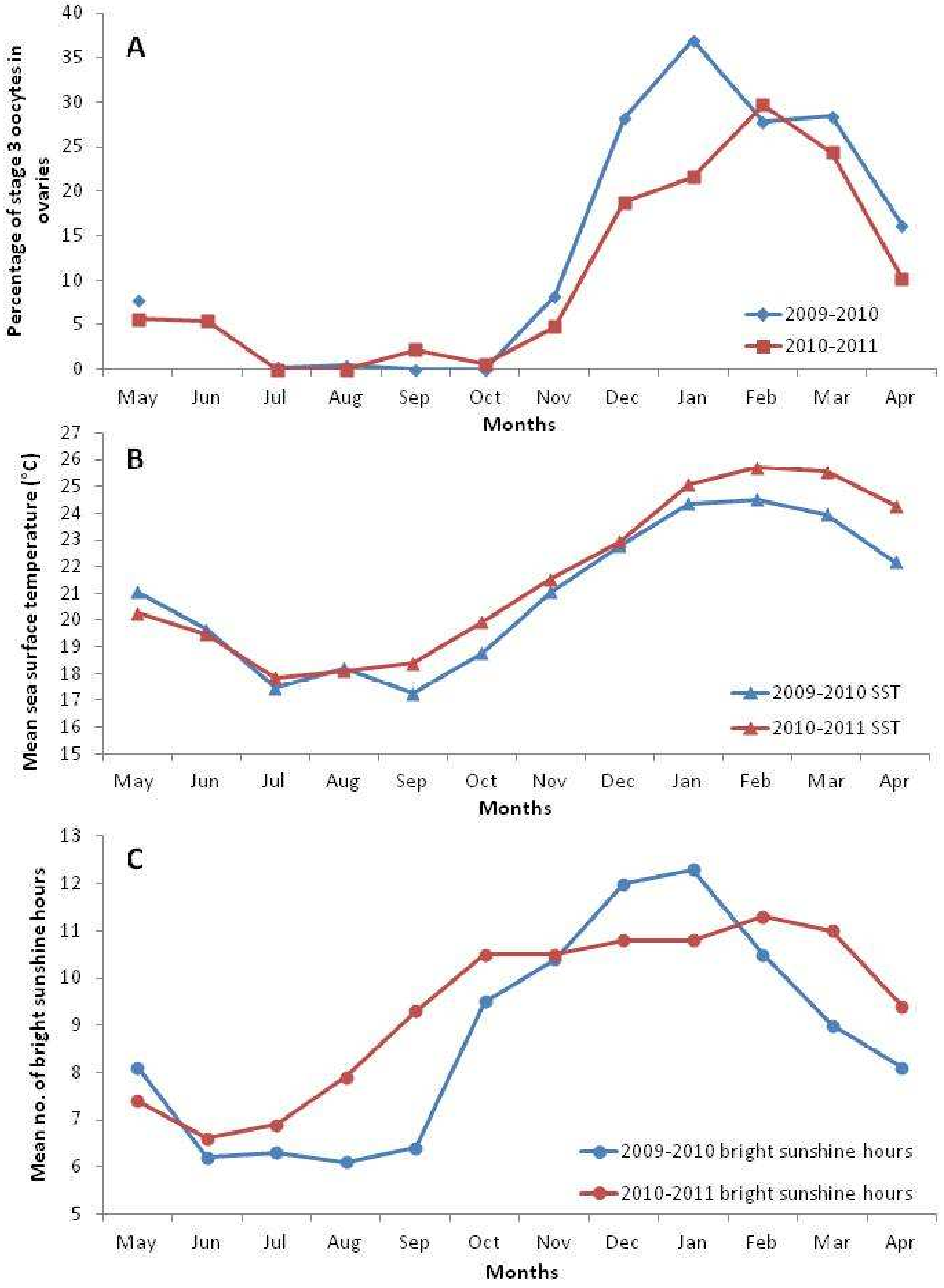

Figure 3.20 Comparisons of changes in A) Stage 3 oocyte proportions, B) SSTs and C) bright sunshine hours between the 2009-2010 and 2010-2011 reproductive cycles.

3.4 Discussion

Echinoid reproduction has been extensively studied. Reproductive cycles have been described for a large number of regular echinoids; however, reproduction in irregular echinoids appears to be less well-studied. Despite being a conspicuous component of the benthic community in Cockburn Sound, Western Australia and commonly found throughout an Indo-Pacific range, there was no biological or reproductive information on the sand dollar *Peronella lesueuri.*

The *Peronella lesueuri* population in Cockburn Sound was found to have a sex ratio of 1:1, which is common amongst echinoids (Pearse and Cameron 1991); e.g. *Mellita quinquiesperforata* in Brazil (Tavares and Borzone 2006) and *Dendraster excentricus* in San Diego, USA (Niesen 1977) were both determined to have sex ratios of 1:1.

External sexual dimorphism is not obvious in most echinoids (Pearse and Cameron 1991) and *Peronella lesueuri* was no different; there were no significant differences in the mean size and weight for adults of both sexes. In the field, the sand dollars would be impossible to sex visually as all individuals were a similar pink-orange colour. Upon dissection, the gonads were easy to sex under the dissecting microscope. However, during the months immediately post spawning, when gonads of both sexes were depleted of their mature gametes, they looked similar and some could only be sexed upon histological analysis

Peronella lesueuri individuals 115 mm and larger in size were deemed to be sexually mature as gonad tissue could be easily sexed at this size. 60% of juveniles between 80 mm and 109 mm were also thought to be sexually mature. Age at size indicated that *P. lesueuri* attained sexually maturity between 1.5 and 2 years of age. The sand dollar *Mellita quinquiesperforata* in the Gulf of Mexico attained sexual maturity at or near 1 year of age (Lane and Lawrence 1979).

Peronella lesueuri is known to have lecithotrophic larvae with ova 300 – 400 µm in diameter (Mortensen 1921). Another species of the *Peronella* genus, *Peronella japonica* also had large ova (~300 µm) and lecithotrophic larvae (Okazaki and Dan 1954). While the mean stage 3 oocyte/ova size in this study was not as large (210 µm), it was still much larger than the 100 – 152 µm ova size range of other sand dollar species with planktotrophic larvae (Chen and Chen 1993).

Reproductive cycles of several species of sand dollars have been described through histological analysis and/or gonad index studies; *Sinaechinocyamus mai* (Chen and Chen 1993), *Arachnoides placenta* (Haycock 2004), *Echinarachnius parma* (Cocanour and Allen 1967), *Mellita quinquiesperforata* (Lane and Lawrence 1979); *Dendraster excentricus* (Niesen 1977) and *Clypeaster ravenelii* (Vernon et al. 1993). Gonad index, commonly used in reproductive studies, was not utilised in this study primarily because the gonad structure of *Peronella lesueuri* was not suitable. It was not possible to excise accurate amounts of gonad tissue from *P. lesueuri* due to the extensive distribution of gonad tissue within the body cavity. The major assumption of gonad index is that animals of different body size have the same ratio of gonad to body size (Gonor 1972). This assumption did not hold up in echinoids *Strongylocentrotus purpuratus* (Gonor 1972), *Lytechinus variegatus* (Moore et al. 1963a), *Tripneustus esculentus* (Moore et al. 1963b) and sand dollar *Mellita quinquiesperforata* (Lane and Lawrence 1979). Thus gonad index should only be used to compare animals of the same size unless the relationship between the gonad size and body size has been established to be the same for individuals of all sizes. The sand dollars used in this study varied in size, and difficulty in excising precise amounts of gonad tissue meant that it was not possible to obtain an accurate gonad index or test for gonad size/body size relationship. Studies have also shown that changes in gonad index could be independent of gametogenesis; an increase could be due to accumulation of nutritive material without gamete production (Lane and Lawrence 1979) while a decrease may be due to utilization of the nutrient storage for energy requirements other than gametogenesis (Mercier and Hamel 2009). Thus, in this study, the *Peronella lesueuri* reproductive cycle was determined by histological analysis and was further supported by changes in proportion of oocyte stages and mean oocyte diameters over time.

The sequence of change in the gametogenic cells and nutritive phagocytes that make up the gametogenic cycle follows a regular pattern in echinoids that is remarkably similar between species (Pearse and Cameron 1991). The reproductive cycle of *Peronella lesueuri* presented morphological stages similar to those described in other echinoderm reproductive cycles (Chatlynne 1969; Byrne 1990; Falkner and Byrne 2003; MacCord and Ventura 2004). The cycle was annual and appeared to also be seasonal; vitellogenesis occurred in spring, spawning in summer, spent gonads in autumn and recovering gonads throughout the winter.

Vitellogenesis took place in spring and early summer (September – January), evidenced by increasing proportions of gonads in growth stages (Figure 3.6) and stage 2 and 3 oocytes in the females (Figure 3.7), as well as increasing mean oocyte diameters (Figure 3.8). As gametogenesis advanced, the quantity of nutritive phagocytes stored within the gonad decreased. This inverse relationship between the quantity of nutritive phagocytes and mature gametes is common in echinoids (Byrne 1990; Chen and Chen 1993) as the nutritive phagocytes provide the nutrients for the energy requirements of gametogenesis (Chatlynne 1969; Pearse 1969; Lane and Lawrence 1979).

Gonads that were in the mature stage, completely full of gametes, were not commonly observed. This suggested that gonads do not retain mature gametes for long. Fully mature gonads were also not commonly observed in the spatangoid *Echinocardium cordatum* in the North Sea; the authors also suggested that mature gametes were not stored in the gonad for long (Nunes and Jangoux 2004).

As a broadcast spawner, a synchronous spawning period for male and female *Peronella lesueuri* ensured viable gametes of both sexes were available in the water column during the same period, increasing fertilization success (Mercier and Hamel 2009). Analysis of gonad stages showed that spawning (when 50% or more of sand dollar gonad samples were in the partially spawned stage) occurred annually from January to April (Figure 3.7). Partially spawned gonads containing mature gametes were common all through the spawning period (Figure 3.7), which indicated that gametogenesis continued throughout the spawning period, allowing for individuals to continuously release gametes (Chatlynne 1969). Some gonads in the spent stages were, however, observed early on in the spawning season which indicated that spawning was a discrete event in some individuals and mature gametes were not continuously generated. Partially spawned males were observed in greater proportions and for a longer period than partially spawned females (Figure 3.7); a similar observation has been made in the sea urchin *Strongylocentrotus purpuratus* (Giese and Kanatani 1987). This "excess" of viable male gametes is common and helps to increase fertilization success. With population densities of between 0.3 to 2.5 individuals m^{-2} in Cockburn Sound, and no apparent aggregative behaviour for spawning, the ability to continually spawn throughout the spawning period as well as availability of "excess" male gametes enables *P. lesueuri* to maximise reproductive success.

After spawning, nutritive phagocytes started to appear as relict gametes underwent phagocytosis. Relict oocytes were observed in various stages of phagocytosis during histological examination. The phagocytes increased in number and size until they packed the lumen of the gonads. The quantity of nutritive phagocytes observed in gonads during the recovery stage suggested that the accumulated nutrient material had to come from more than just the breakdown of relict gametes, and likely came from outside the gonad and/or active synthesis (Pearse and Cameron 1991). *Peronella lesueuri* moved and fed throughout the winter (Yeo et al. 2013). The continued ingestion of food aided the build-up of nutritive phagocytes in the gonad throughout the winter. Therefore, the food availability during the recovery period may affect gametogenesis the from year to year by limiting the amount of nutrients available to gametogenic cells (Chatlynne 1969).

Annual reproductive cycles with seasonal periodicities are commonly observed in echinoids and have been documented in several species of sand dollars. Studies commonly used the spawning period to indicate peak reproductive activity. The occurrence of spawning was common in spring and summer, although some species have been documented to spawn in autumn and winter (Pearse and Cameron 1991). *Mellita quinquiesperforata* were observed to spawn in spring in the Gulf of Mexico (Lane and Lawrence 1979) and throughout spring and summer in Brazil (Tavares and Borzone 2006). *Dendraster excentricus* populations along the San Diego coast in California are most reproductive in spring, while in Bamfield, British Colombia, *Dendraster* populations spawned in summer (Burke and Bouland 1989). Autumn spawning was documented in *Arachnoides placenta* along the northern Queensland coast of Australia (Haycock 2004) as well as in *Sinaechinocymus mai* in Taiwan (Chen and Chen 1993). *Clypeaster ravenelii* was reported to spawn late in winter in the Gulf of Mexico (Vernon et al. 1993). The two annual reproductive cycles of *Peronella lesueuri* observed in this study exhibited seasonal periodicity, with vitellogenesis taking place in spring, spawning in summer, spent gonads observed in autumn and recovering gonads throughout the winter.

The seasonal periodicity of reproductive cycles in echinoids is controlled by seasonally varying exogenous factors and allows maximum fertilization success and/or ensures a high offspring survival rate in response to seasonal environmental changes (Mercier and Hamel 2009). In order to determine the individual effects of seasonally fluctuating factors, these factors have to be isolated and their effects on reproductive periodicities analysed independently (Morin et al. 1985). However, such studies are complex and cannot be carried out in the field. As such, the relationship between reproductive cycles and concurrently varying exogenous factors that come from field studies like this one remain speculative (Chia and Walker 1991).

Numerous studies have indicated that photoperiod and temperature were likely to be the exogenous factors that exerted most control over the echinoid reproductive cycle (Mercier and Hamel 2009). Oocyte proportions of both reproductive cycles in this study showed similar correlations with sea surface temperatures (SST) and mean bright sunshine hours (BSH). It was likely that the annual changes in SST and/or BSH had effects on the reproductive cycle of *Peronella lesueuri,* given the similar periodicity of proportion changes in all oocyte stages between both reproductive cycles.

Some studies have identified species-specific critical temperatures or photoperiods that could initiate or inhibit the various stages of gametogenesis in echinoids. Others have suggested that changes in temperature or photoperiod were the control. Vernon (1993) suggested that daylength may serve as an exogenous cue for the gametogenesis and spawning in sand dollar *Clypeaster ravenelii* in the Gulf of Mexico. Studies in Japan showed that temperature lows are often associated with the initiation of gametogenesis in the sea urchin *Hemicentrotus pulcherrimus* (Ito et al. 1989; Sakairi et al. 1989; Agatsuma and Nakata 2004), while in the North Sea gametogenesis in the spatangoid echinoid *Echinocardium cordatum* was initiated after minimum temperatures were reached (Nunes and Jangoux 2004). These authors suggested that the increase in temperature that followed was the initiating factor. Although it is unclear what the initiating factor for gametogenesis in *Peronella lesueuri* is, stage 1 oocyte proportions began to decline just as SSTs were increasing from winter minimums. It is possible that, like *E. cordatum*, SST increase following minimum temperatures is the trigger for gametogenesis in *P. lesueuri*.

In female sand dollars mature oocytes (stage 3 oocyte and ova) did not appear until November in both years, despite the presence of stage 2 oocytes in the gonads in the three months from onset of gametogenesis in August. This suggested that the development of mature oocytes was not just a consequence of gametogenesis, but possibly controlled by an external trigger. In November of both years, SST was approximately 21°C and BSH was approximately 10.5. It is possible that one or both factors were proximate cues for gonad maturation.

Other factors that have been found to affect echinoid reproduction include the timing, quantity and quality of food resources (Gonor 1973; Meidel and Scheibling 1998; Kelly 2000), lunar rhythms (Kennedy and Pearse 1975; Iliffe and Pearse 1982; Lessios 1991; Coppard and Campbell 2005; Muthiga 2005) and habitat conditions (Tavares and Borzone 2006). Further experiments need to be carried out in controlled conditions within aquaria in order to identify the factors that affect *Peronella lesueuri* reproduction and determine the role they play.

3.5 Conclusion

Prior to this study, there was no information in the literature on the reproductive biology of the sand dollar *Peronella lesueuri*. This study has provided basic information on *P. lesueuri* reproduction biology, determined the seasonality and periodicity of reproduction and provided the first histological description of the reproductive cycle of this species.

Peronella lesueuri is not sexually dimorphic and the population in Cockburn Sound has a sex ratio of 1:1. Juveniles attain sexual maturity between 1.5 and 2 years of age. Based on their histology, the male and female gonads of *P. lesueuri* follow a sequence of morphologically different stages, similar to those documented in the reproductive cycles of other echinoids (Chatlynne 1969; Gonor 1973; Byrne 1990; Nunes and Jangoux 2004). Growth, mature, partially spawned, spent and recovery stages was observed in both sexes and the stages were highly synchronous between sexes.

Giese and Pearse (1974) concluded that for populations with highly seasonal rhythms, it was highly likely that exogenous cues or regulators played a role. In Cockburn Sound, *Peronella lesueuri* has an annual reproductive cycle with strong seasonal correlations. Vitellogenesis occurred in spring, spawning took place over summer and gonads recovered over winter. As this was a field study, it was not possible to fully assess the effects of exogenous factors on the *P. lesueuri* reproductive cycle. Further research needs to be conducted in controlled conditions to identify the regulatory factors and to determine their effect on the reproductive cycle of *P. lesueuri*.

Chapter 4 Life history and Growth of *Peronella lesueuri*

4.1 Introduction

Research into the growth and longevity of echinoids has revealed that they possess a wide range of longevities. Maximum age estimates of between four and 75 years have been suggested for a variety of sea urchin species, with one estimate for the red sea urchin, *Strongylocentrotus franciscanus*, suggesting that they can live for more than 100 years (Ebert and Southon 2003). Longevity estimates for sand dollars suggest that in general, they are shorter. Several species of sand dollars, including *Encope grandis* and *Dendraster excentricus,* have been found to acquire their maximum sizes at 5-10 years (Ebert and Dexter 1975), which implies that possible maximum age was higher. The sand dollar *Encope stokesi*, however, has an estimated short life expectancy of less than one year (Dexter 1977).

Growth and mortality parameters can substantially affect the dynamics of a population. Many species of sea urchins and sand dollars, including *Dendraster excentricus* (Cameron and Rumrill 1982), *Mellita quinquiesperforata* (Lane and Lawrence 1980) *Echinarachnius parma* (Steimle 1990; Cabanac and Himmelman 1996) and *Arachnoides placenta* (Haycock 2004), recruit in cyclic pulses, following on from a seasonal reproductive cycle (Ebert et al. 1993). However the sand dollar *Encope stokesi* was found to recruit year round, although there were periods of peak recruitment (Dexter 1977).

Although most echinoids have annual reproductive cycles, the strength of recruitment varies from year to year. In the Gulf of St. Lawrence, Canada, the *Echinarachnius parma* population showed no recruitment between 1991 and 1993

(Cabanac and Himmelman 1996) despite having an annual reproductive cycle (Cocanour and Allen 1967). Annual recruitment strength can be affected by high pre- or post-settlement mortality of larvae and early recruits. Both mortality rates could be affected by a wide range of factors including current variations (Cameron and Rumrill 1982; Ebert et al. 1994), temperature (Hernández et al. 2010), predation (Highsmith 1982) and habitat differences (Tomas et al. 2004). Substrate conditioning by adults of the species (Pearce and Scheibling 1990) could affect settlement rates as well as post-settlement survival of echinoid larvae (Pearce and Scheibling 1990).

High rates of mortality have been recorded in the newly settled juveniles of several echinoid species. In Tampa bay, Florida, a cohort of recruits from a population of *Mellita quinquiesperforata* had a mortality rate of 95% in the first year post settlement (Lane and Lawrence 1980), while in Spain, juveniles of the sea urchin *Paracentrotus lividus* had a 90% mortality rate in the same period (López et al. 1998).

Growth in echinoids generally follows a sigmoidal growth curve, with the slowing or cessation of growth upon attaining their asymptotic size (Birkeland and Chia 1971; Steimle 1990; Cabanac and Himmelman 1996; Kang et al. 2007). For a variety of sand dollars the growth rate constant (K) was found to be between 0.29 and 0.59 (Ebert and Dexter 1975).   A suite of environment factors, such a seasonal temperature changes and weather events (Ebert 1968), food availability (Ebert 1968; Pearse and Pearse 1975; Ebert 1988), harsh site conditions (Birkeland and Chia 1971; Niesen 1977) and differences in substrate (Birkeland and Chia 1971), can have an effect on seasonal growth. Seasonal rates of “body growth”, attributed mainly to the reproductive cycle, were observed in the sand dollar *Mellita quinquiesperforata*, with the highest growth rate recorded in the summer and negative growth observed in some large individuals in the autumn (Lane and Lawrence 1980). Ebert (1967) also observed negative growth in large individuals of the purple sea urchin *Strongylocentrotus purpuratus*, which was attributed to the reabsorption of calcite from the test in response to environmental fluctuation.

As many echinoids attain their maximum size quickly, growth in echinoids is difficult to measure, especially in large individuals. Alternating light and dark zones, made up of calcium carbonate of different stereom microstructure, have long been observed in the spines, test plates and jaws of the echinoid skeleton (Pearse and Pearse 1975). These zones indicate changes in rates of growth (Pearse and Pearse 1975). In field conditions, these zones most likely are formed due to seasonal changes in echinoid growth rates and thus, have commonly been used as a method to estimate age and rates of growth of echinoids (Dix 1972; Crapp and Willis 1975; Gage 1991; Lumingas and Guillou 1994; Kang et al. 2007). Many echinoid studies have used growth zones as annual markers, with little verification of their annual nature (Lumingas and Guillou 1994; Cabanac and Himmelman 1996; Kang et al. 2007). Tetracycline is a skeletal growth marker and is commonly used to calibrate growth bands in the echinoid jaw and test (Taki 1972a; Taki 1972b; Ebert 1988; Gage 1992a; Gage 1992b; Zhang et al. 2008; Ellers and Johnson 2009). Using this method, Gage (1991; 1992b) concluded that growth bands were formed annually in echinoids in the British Isles.

Prior to this study, no growth, recruitment and juvenile mortality rates of *Peronella lesueuri* have been published. In this chapter, juvenile recruitment and growth were determined through size frequency distribution and in addition, growth rings on the test plates of large individuals were used to determine the life expectancy and growth rate of *P. lesueuri* in Cockburn Sound.

4.2 Sampling regime and laboratory procedures

4.2.1 Size frequency

Peronella lesueuri were sampled monthly from Jervoise Bay in Cockburn Sound for size frequency analysis from May 2009 to April 2011. Sampling was carried out by towing an epibenthic sled (Figure 4.1) behind a vessel. The mouth dimensions of the sled were 85 cm by 45 cm with a 5 cm cutting depth and the sled was surrounded with 1 cm mesh. Each tow was 200 m in length and tows were repeated until at least 100 sand dollars were collected.

Figure 4.1 The epibenthic sled and its contents after a 200 m tow.

Sand dollars were separated from the collected contents and length of all individuals was measured to the nearest millimetre. Maximum length was measured along the flat, oral surface of the animal, between the margins of the test in a line through the peristome (mouth) and periproct (anus). The measurements were then used to construct size frequency distributions for each sampling date.

Juveniles were considered to have recruited into the population when they were present in the dredge samples. Some individuals were badly broken during the collection process and length measurements were not possible. These individuals were excluded from the size frequency histograms.

4.2.2 Growth zones

Growth in echinoids is achieved through individual size increases of the small interlocking test plates that make up the complete echinoid test (Pearse and Pearse 1975). There are several variations on the age determination method, but upon removal of organic material from and charring the test plates, alternating “light” and “dark” zones become visible on the test plates (Figure 4.2). These zones are caused by the differences in the stereom microstructure varying the light transmission and reflection properties. Under reflected light, the denser microstructure of the stereom causes the “light” zone to reflect more light, hence appearing lighter in shade (Pearse and Pearse 1975). Conversely, the microstructure construct of “dark” zones in the test plate is less dense, allowing more light transmission through the plate, causing the area to appear darker in shade (Pearse and Pearse 1975). The growth bands in temperate zone echinoids are a result of a slowing or cessation of somatic growth due to low temperatures and limited food availability in the winter. Hence the best interpretation of the alternate growth banding is that the “light” zone is formed by the faster rate of plate growth in the summer while the “dark” zone corresponds to a slower rate of growth in the winter (Pearse and Pearse 1975). For the purposes of this study, one corresponding pair of “light” and dark” zones was known as a growth zone.

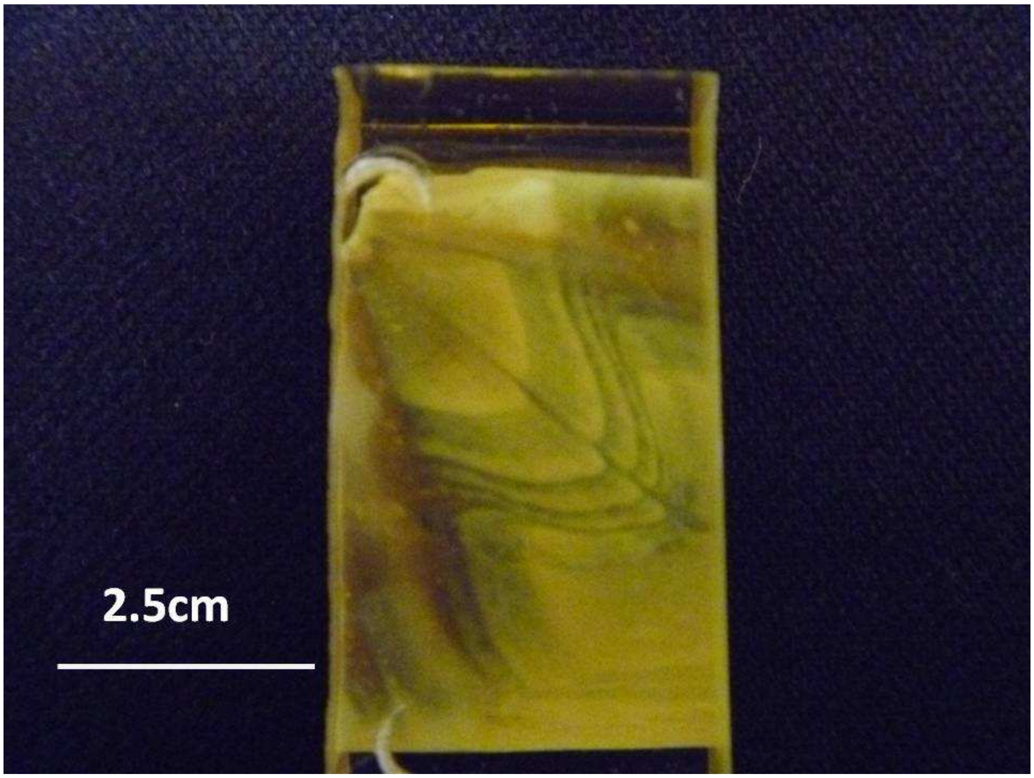

Figure 4.2 Light and dark zones visible on a sand dollar test plate embedded within resin.

Peronella lesueuri used in this study were collected from Jervoise Bank in August 2011 by divers using SCUBA. While this method of collection was biased against small individuals, which were not easily visible, sand dollars required for this part of the study needed to be of a medium to large size (110 -180 mm).

Sea urchins and sand dollars add coronal test plates to the interambulacrum throughout their life (Pearse and Pearse 1975). As a consequence, test plates may be of different ages and only the oral test plates are as old as the animal (Pearse and Pearse 1975). In view of this, the interambulacral test plates that formed the peristome were selected to be used in this study because they were large and the growth zones were highly visible. A preliminary study showed the following method to be the most effective way of revealing the contrasting “light” and “dark” growth zones present in the *P. lesueuri* test plates. Sand dollar tests were cleared of organic material in 50% commercial bleach. The cleaned tests were then rinsed and air-dried. A section of test adjacent to the peristome was cut from each sand dollar using a handheld rotating saw. Each section consisted of 4-8 test plates, allowing for comparisons of number of growth zones between plates. The sections were charred over a Bunsen flame. When cooled, the sections were embedded in clear epoxy resin. Test plates from 116 individuals were prepared for this study and sizes ranged from 62 mm to 165 mm. However, not all test plates processed showed growth zones distinct enough for analysis and eventually only 81 test plates from individuals sized 105 mm to 165 mm were used.

During counts and photographing, the resin blocks containing the test plates were placed on a dark surface. This increased the contrast of the light and dark zones for ease of counting. Only test plates which presented clear growth zones were taken into account. While it was unclear if the lack of growth zones was due to errors in the preparation process, it was interesting to note that three of the individuals with no visible growth zones were less than 100 mm.

4.2.3 Growth zone counts

The test plates embedded in resin were placed on a black background and the visible growth zones were counted for each test plate twice. Each pair of “light” and “dark” zones were counted as one growth zone and assumed to reflect annual growth. To minimise possible preparation error, only test plates with one or more growth zones were used. The growth zones were counted twice on separate occasions. Counts differed on three samples and in each case counts only differed by one growth zone. In the cases where counts differed, a third count was performed to resolve the discrepancies.

In order to eliminate reader bias, a subsample of 26 test plates was counted by a second reader (Peter Coulson) with experience in reading fish otolith growth zones. The second reader's counts were then compared with those recorded by the author. The level of agreement between the two readers was high. There was a single discrepancy in growth zone counts and it differed by one zone. In this case, both readers agreed to use the author's count as the second reader was reading sand dollar growth zones for the first time.

4.2.4 Width of growth zones

Images of test plates with clear growth zones in resin were taken using a SLR camera and used for measuring the widths of the growth zones. The zone widths on test plates were measured in 33 individuals. Each growth zone was measured to the nearest millimetre. The plates used to measure the growth zone widths were a subsample of the plates used in the growth zone counts.

The initial growth zone was measured from the edge of the test plate to the edge of the first "dark" zone. Generally, the initial growth zone at the oral end of the plate is the widest, with subsequent zones decreasing in width.

4.2.5 Trial validation of growth zones using tetracycline

In a preliminary trial, 25 sand dollars were then kept in two 1x1 metre holding tanks in a temperature controlled room at 20°C. Filtered seawater was used to supply the tanks. Surface sediment was obtained from the shallows at Point Walter to cover the bottom of the holding tanks to a depth of at least 5 cm. Both the water and sediment were partially replaced weekly for the duration of the trial to maintain clean water conditions and a food supply for the sand dollars. No additional food was provided to the sand dollars to avoid fouling of the water in the tanks. After two weeks, all the sand dollars were still alive and conditions in the holding tank were deemed suitable for maintaining the selected number of animals. Twenty large sand dollars were selected and injected with tetracycline (20 mg mL^{-1}) at a dose rate of 0.1 mL per 10 g of body weight (Gage 1992a). The five remaining sand dollars acted as control animals. The tetracycline-tagged sand dollars were then returned to the holding tanks.

After two weeks, eight tagged sand dollars and one control sand dollar were removed from the aquaria and their jaw segments and test plates viewed under UV light to locate the fluorescent tetracycline tag. After approximately five weeks, a further three tagged animals and one control animal were removed for analysis of jaw segments and test plates. After seven weeks, the jaw segments and test plates of the nine remaining tagged sand dollars and three controls were analysed.

In an attempt to tag juvenile sand dollars with tetracycline, 25 juvenile sand dollars were immersed in a solution of 2 g of tetracycline per 100 mL of seawater for five minutes as they were too small to inject. The juveniles were then returned to the holding tanks but none survived.

4.2.6 Observation of growth over time

In order to try to ascertain the growth rate of adult *Peronella lesueuri* in the natural environment, adult sand dollars were enclosed in large open-top holding cages constructed on the sea floor at Jervoise Bay and observed over approximately four months. The four circular cages were approximately six metres in diameter (circumference = 20 m, area = 31 m^2) and constructed with wire mesh. On 20 September 2010, 240 sand dollars between 88 and 164 mm were measured (the measurements were taken the same way as in section 4.2.1.1). Their tests were numbered with pencil and they were evenly distributed between the holding cages.

When divers returned on 4 November 2010, the cages were found to have been destroyed due to rough weather and sea conditions. Many of the sand dollars were free moving around the study area. However, divers were able to recover and measure 142 of the numbered sand dollars. Divers returned to the site on 3 February 2011 and measured 127 sand dollars.

4.2.7 Statistical Methods for Size Frequency and Growth Curve Analysis

The size frequency distributions indicated that *Peronella lesueuri* had a life expectancy longer than the study period making it impossible to follow a cohort from settlement through to senescence. *P. lesueuri* also appeared to achieve adult size quickly and form a stable size class of large individuals which comprised of several year classes, which meant that large sand dollars could not be aged by tracking a cohort through size frequency distributions. Conversely, growth zone counts could not be performed on juvenile test plates as they were too fragile to withstand the processing require to reveal the growth zones within the test plates.

The size frequency distributions at the beginning of the sampling period consisted of two groups of animals; a group of small individuals and a group of large individuals. However, from June 2010 onwards the size frequency distributions appeared to contain three groups, with the appearance of intermediate-sized animals. The numbers of groups in the distribution, however, were not distinct due to the small number of sand dollars of intermediate size. The group of intermediate-sized animals also overlapped with the group of smaller animals. In order to determine the likely number of groups present within each size frequency distribution, the data was analysed using mixture analysis in PAST version 2.12 (Paleontological Statistics). To determine the best fit, the size frequency data for each month in question was tested against models consisting of two or three groups. The fit of the models to the data was measured by the Akaike Information Criterion (AIC) value. The AIC value provides a mean of model selection as it measures the relative goodness of fit of a statistical model. The model which produced the smallest AIC value was deemed the best fit.

Density data obtained from the sled sampling was further used to corroborate the recruitment period indicated by the size frequency data and to determine recruitment strength and juvenile mortality.

An arbitrary birth date of 1 March was assigned to all the juveniles and pooled length-at-age data for the 2009 and 2010 cohort of recruits was obtained from the size frequency distributions. This was combined with length-at-age data for large “adult” sand dollars obtained from growth zone counts in order to construct a scatter plot to fit a growth curve for *Peronella lesueuri*.

In order to find the best descriptor of growth for *Peronella lesueuri,* the resulting scatter plot was tested against four commonly used growth functions: von

Bertalanffy, Brody-Bertalanffy, Gompertz and Richards’ growth curves using the nonlinear curve fitting function in JMP (SAS Institute).

4.3 Results

4.3.1 Size frequency distribution

Size frequency distributions were constructed for each sampling date. However, for ease of interpretation, histograms which best depicted the trends observed were used (Figure 4.3). The largest individual encountered during the study measured 182 mm. This suggests that this is the maximum size *Peronella lesueuri* can attain in Cockburn Sound. The mesh size of the dredge used to obtain the sand dollars was 10 mm square. The amount of sediment collected during the dredging process regularly clogged the mesh, allowing the dredge to retain contents much less than 10 mm in size. The three smallest individuals recorded were 8 mm in length. Two of the 8 mm individuals were encountered in August 2009 and one in July 2010. Both instances were early in the recruitment period and the scarcity of individuals 8 mm in size even at the commencement of recruitment suggest that at that early life stage growth is relatively rapid.

The monthly size distributions obtained throughout the study period showed the presence of two distinct groups of sand dollars from May 2009 (Figure 4.3) before increasing to three groups in June 2010. Akaike Information Criterion (AIC) values indicated that all distributions between June 2010 and April 2011 were likely to contain three groups of sand dollars of different size (Table 4.1).

Two of these groups show size progression over time and are likely to be made up of year classes. A single group encompassing the larger “adult” size classes (120-180 mm) appear to be stable in time and may be made up of a single year class, although it is more likely to consist of several year classes of individuals that had attained or were close to maximum size. Sand dollars of intermediate size were scarce and not observed in the first year of study.

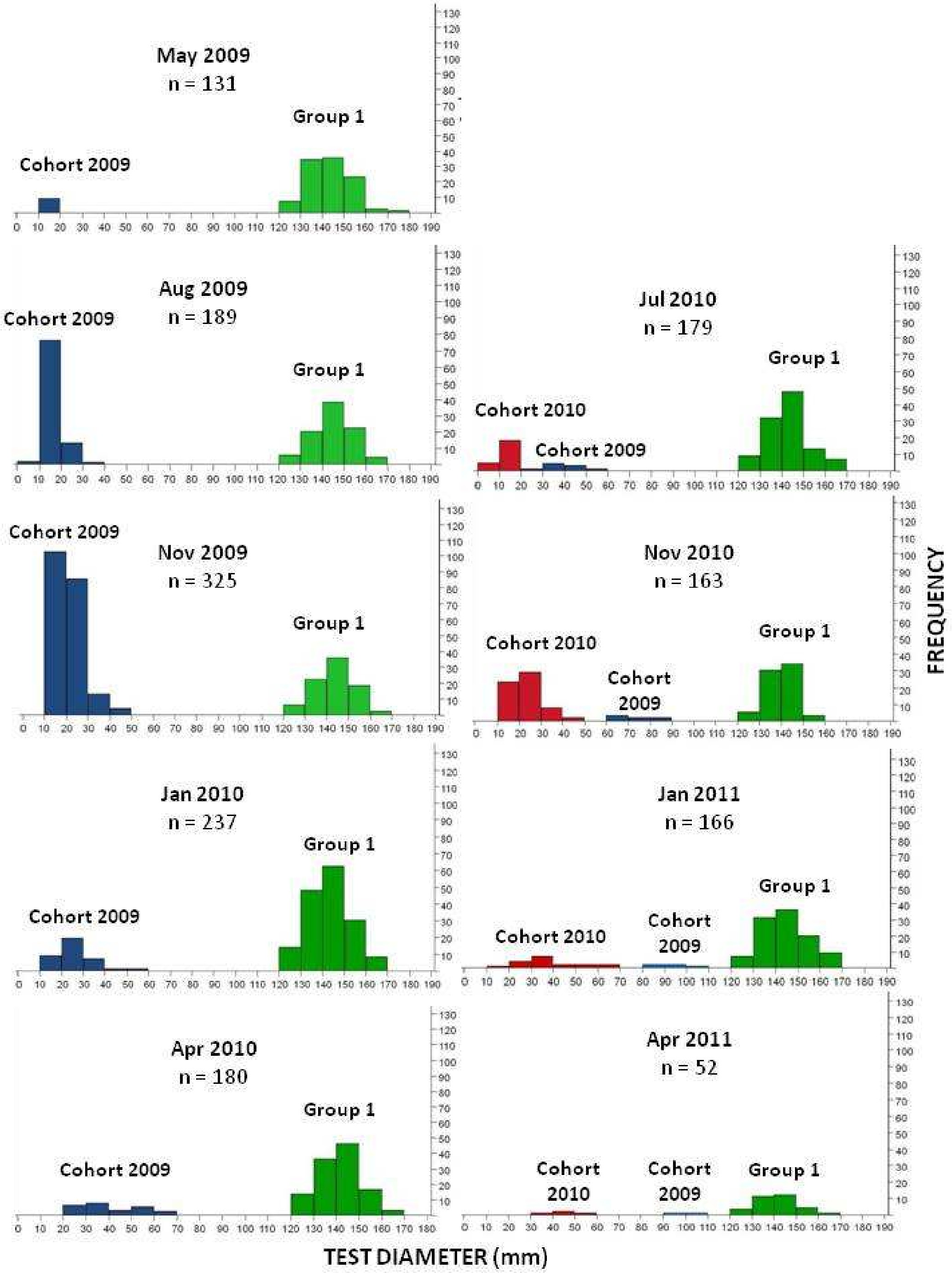

Figure 4.3 *Peronella lesueuri* size frequency distributions from Jervoise Bay in Cockburn Sound from May 2009 to April 2011. Group 1 consists of the stable adult population, likely to contain individuals from several cohorts. Cohorts 2009 and 2010 represent the juveniles that recruited into the population in 2009 and 2010.

Table 4.1 Akaike Information Criterion (AIC) values indicating the number of groups best fit to the data from June 2010 – April 2011. The smaller AIC value indicates a better fit.

Month	Number of Groups	Akaike IC value	Best fit number of groups
June 2010	2	621.7	3
	3	589.7	
July 2010	2	946.2	3
	3	920.6	
August 2010	2	696.8	3
	3	669.9	
September 2010	2	646.8	3
	3	603.7	
October 2010	2	745.4	3
	3	693.5	
November 2010	2	1017	3
	3	949	
December 2010	2	764.2	3
	3	724.6	
January 2011	2	880.5	3
	3	872.4	
February 2011	2	759.7	3
	3	751.4	
March 2011	2	573.6	3
	3	568.7	
April 2011	2	249.2	3
	3	246	

4.3.2 Timing of recruitment

At the beginning of the study in May 2009, two groups were seen in the size frequency distribution (Figure 4.3). The first and largest group consisted of individuals in the 120-180 mm size range. As this group of animals was likely to be made up of several cohorts of individuals, this will be referred to as Group 1. A second group was made up of nine individuals in the size class 10-20 mm. In August 2009, there was a large increase in the number of individuals in the second group. The second group was interpreted as a newly recruited cohort that settled before May 2009 (cohort 2009) (Figure 4.3). Between November 2009 and January 2010, the number of individuals from cohort 2009 decreased and remained low (<10 individuals) as the cohort increased in size throughout 2010 and 2011.

In June 2010, what appeared to be a third group was detected in the size frequency distribution (Table 4.3). This third group became more visually apparent in July 2010 (Figure 4.3) when 6 individuals were recorded in the 0-10 mm size class and 17 individuals were encountered in the 10-20 mm size class. This group of individuals continued to increase in size up to the end of sampling in April 2011 and was interpreted as a new cohort (cohort 2010) of individuals that settled after May 2010. The number of individuals encountered from cohort 2010 was lower than that of cohort 2009 (Figure 4.3).

4.3.3 Recruitment strength

There was marked variability in the density and juvenile percentage of two cohorts of recruits (2009 and 2010) during the study period (Figure 4.4). New cohorts were first detected in winter (May-July) of each year and continued to recruit into the population in subsequent months. This was reflected by the spike in densities of juvenile sand dollars and percentage of juveniles within samples of both cohorts between July and January in 2009 and August and January in 2010.

Between July and December 2009, density of cohort 2009 increased rapidly from 0.02 to 0.42 individuals m^{-2} (Figure 4.4A). Within the same period, the percentage of juveniles went from 2.6% of the total number of individuals sampled for the month to peak at 63.3% in November 2009 (Figure 4.4B). Between June and November 2010, the percentage of juveniles in the sample increased six-fold from 5.4% to 35.0%. Density increased from 0.007 to 0.07 individuals m^{-2}. Based on the percentages of juveniles encountered in samples at the peak of the recruitment period, the recruitment strength of cohort 2009 was approximately twice that of cohort 2010. This indicated that *Peronella lesueuri* juveniles recruited between winter and summer and recruitment strength varied annually. The variability of annual recruitment is reinforced by the absence of a 2008 cohort.

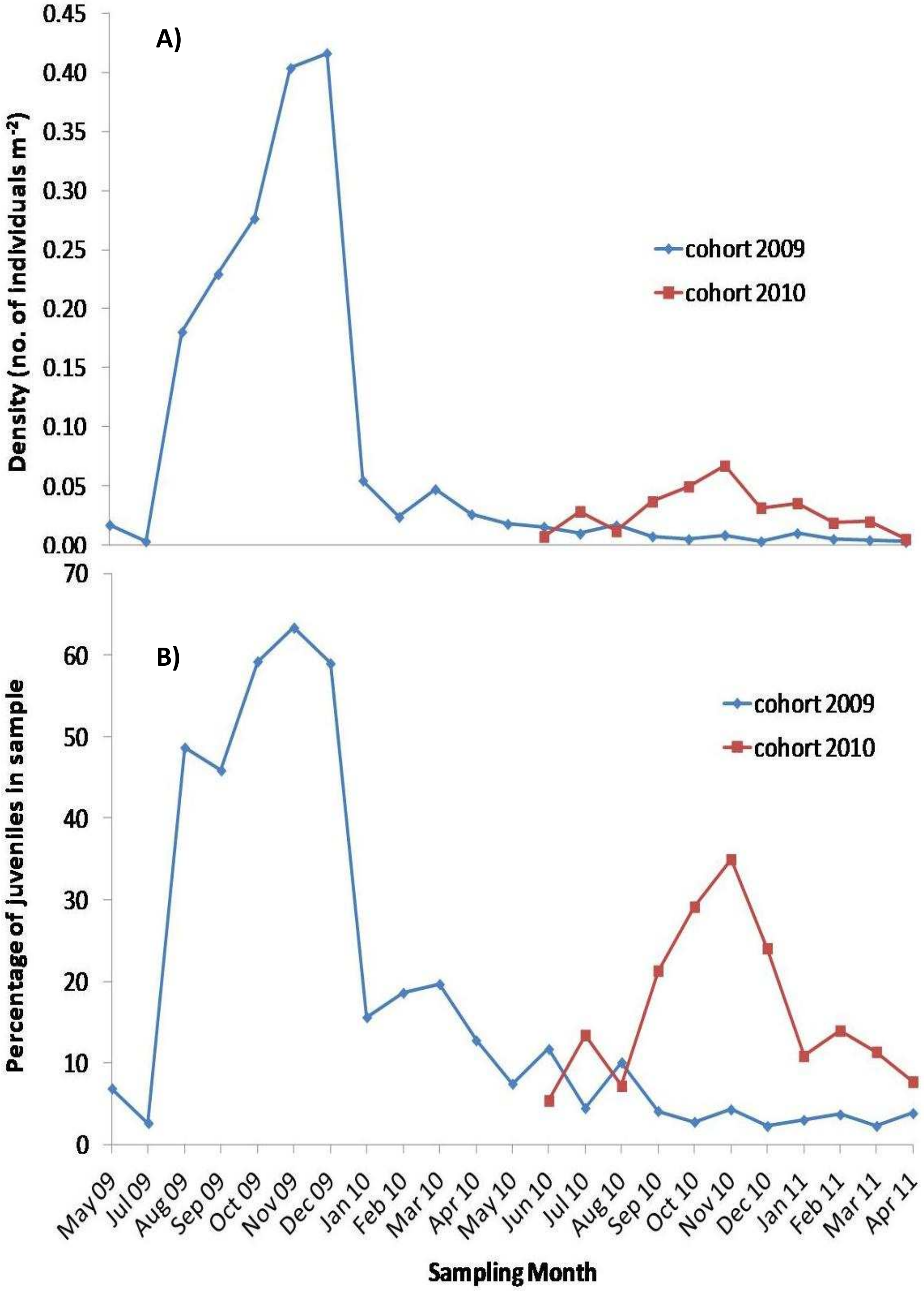

Figure 4.4 Changes in A) juvenile cohort density and B) juvenile cohort percentage over the sampling period.

4.3.4 Growth rates of recruited cohorts

The mean length of cohort 2009 increased from 17.78 mm (X ± SD = 17.78 ± 1.09, n = 9) in May 2009 to 39.17 mm (X ± SD = 39.17 ± 12.94, n = 23) in April 2010 (Figure 4.5). This translated into a mean growth rate of 21.39 mm yr^{-1} for cohort 2009 in the first year.

The size class of cohort 2009 continued to increase, attaining a mean length of 48.50 mm (X ± SD = 48.50 ± 16.89, n = 12) in May 2010 and 114.75 mm (X ± SD = 114.75 ± 6.70, n = 2) in March 2011 (Figure 4.5). There appeared to be a decrease in mean length of cohort 2009 between March and April 2011. This was due to a small sample size (2 individuals) in April 2011. Because of this, the change in mean length of cohort 2009 up to March 2011 was used to derive the mean growth rate of 71.45 mm yr^{-1} in the second year. In September 2010, at approximately 1.5 years of age, the growth rate of cohort 2009 appeared to increase rapidly (Figure 4.5). Between September 2010 and March 2011, juveniles from cohort 2009 grew at a rapid rate of 9.79 mm $month^{-1}$ or 117.5 mm yr^{-1}.

The mean length of cohort 2010 increased from 14 mm (X ± SD = 14 ± 0.37, n = 6) in June 2010 to 45 mm (X ± SD = 45 ± 3.94, n = 4) in April 2011 (Figure 4.5), which translated into an approximate growth rate of 37.2 mm yr^{-1} for cohort 2010 in the first year.

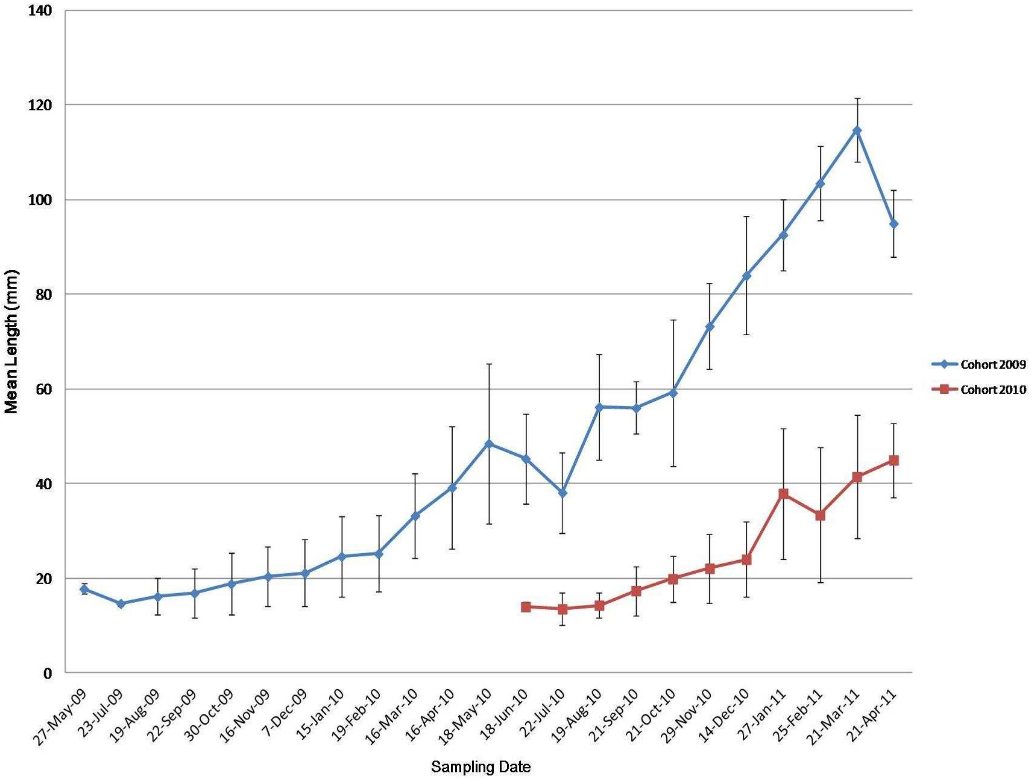

Figure 4.5 Increase in mean length ± SD of 2009 and 2010 cohorts between May 2009 and April 2011.

4.3.5 Mortality of recruited cohorts

Densities of juveniles and percentages of juveniles within samples recorded sharp decreases over the first summer (November to January period) for both cohorts (Figure 4.4). The low numbers of intermediate-sized sand dollars suggests the likely cause of this decrease is high juvenile mortality.

Between December 2009 and January 2010, juvenile density from cohort 2009 decreased sharply from 0.42 to 0.05 individuals m^{-2} (Figure 4.4A), with a corresponding decrease in percentage of juveniles in the dredge sample from 59.0% to 15.6% (Figure 4.4B), equivalent to a mortality rate of 73.6% $month^{-1}$. In the first year (May 2009 to May 2010), juvenile mortality was 88%. Second year (May 2010 to April 2011) juvenile mortality of cohort 2009 was approximately 48%. By the time the proportion of juveniles in the samples stabilised in September 2010, mortality of cohort 2009 was approximately 94%. Between September 2010 and April 2011, the percentage of juveniles for cohort 2009 fluctuated between 2.26 and 4.29% and mortality appeared to be extremely low. However, due to the small numbers of sand dollars of intermediate size encountered in each sample (between two and five individuals), the percentages of juveniles in cohort 2009 were unlikely to give a reliable mortality rate for the second year.

The 2010 cohort of juveniles showed a similar decrease in density between November and December 2010 from 0.07 to 0.03 individuals m^{-2} (Figure 4.4A). Percentage of juveniles also decreased between November 2010 and January 2011 from 35.0% to 10.8%, equivalent to a mortality rate of 34.6% $month^{-1}$(Figure 4.4B). By the end of the study period in April 2011, the mortality of cohort 2010 was 78%. The trend of the graph indicated that percentages and densities of juveniles were unlikely to increase if the study had been continued (Figure 4.4A and B).

The density and percentage data from juveniles of cohort 2009 and 2010 indicated an annual mortality of *Peronella lesueuri* recruits of up to 94%. The highest mortality rates were observed over a short period between November and January each year. For cohort 2009, the mortality rate then gradually decreased after January 2010 before the population stabilised in September 2010, after which mortality was low. The population of cohort 2010 had yet to stabilise at the end of the study period.

4.3.6 Verification of growth and growth zones

4.3.6.1 Tetracycline tagged sand dollars

The use of tetracycline to verify growth zones in *Peronella lesueuri* jaw segments and test plates was tested. All the juveniles immersed in tetracycline for five minutes died within two days of immersion.

Tetracycline tags did not show up in the jaw segments and test plates of the 20 tagged sand dollars, despite individuals being sampled at 2 weeks, 4 weeks, 6 weeks and 7.5 weeks. Ellers and Johnson (2009) reported that tetracycline tags were visible in jaw segments of the sea urchin *Strongylocentrotus droebachiensis* six to 13 days after tagging. Given that the tetracycline tags were not visible after several weeks, it was concluded that tetracycline tagging was not a suitable method of verifying growth zones in *Peronella lesueuri*.

4.3.6.2 Sand dollar growth

At the start of the observational period in September 2010, the mean length of the 240 sand dollars collected for the holding cages was 138.82 ± 10.45 mm (Table 4.2). Over the next 5 months, the sand dollars in the holding cages did not appear to grow in length. When re-measured in November 2010, the mean length was 139.03 ± 9.20 mm and in February 2011, the mean length was 140.33 ± 813 mm. Comparing the means using Tukey-Kramer HSD ($p<0.01$) showed no significant differences between all mean pairs, indicating that there was no measureable increase in length in adult sand dollars over time.

Table 4.2 Mean lengths of sand dollars measured on 20 September 2010, 30 November 2010 and 3 February 2011.

Date	N	Mean length ± standard deviation
20 Sep 2010	240	138.82 ± 10.45
30 Nov 2010	147	139.03 ± 9.20
3 February 2011	124	140.33 ± 8.13

4.3.7 Growth Zones

The initial growth in all sand dollar plates was represented by an indistinct grey zone at the oral edge of the test plate (Figure 4.6A). This initial zone was likely to be the slow winter growth of the newly recruited juveniles. This is supported by the size frequency histograms (Figure 4.3) that show initial recruitment of juveniles (≥8 mm) occurred in winter. Assuming a birth date of 1 March, which is the middle of the spawning season (Chapter 3), juvenile sand dollars (≥10 mm) are approximately 6 months old before the first light zone starts to form in spring (September).

Many test plates had distinct light zone between the last counted growth zone and the end of the test plate with no distinct formation of the subsequent dark zone (Figure 4.6B). As most of the tests examined for growth zones were from sand dollars collected in winter (August 2011), this light zone represented the growth from the previous spring and summer of at least 6 months.

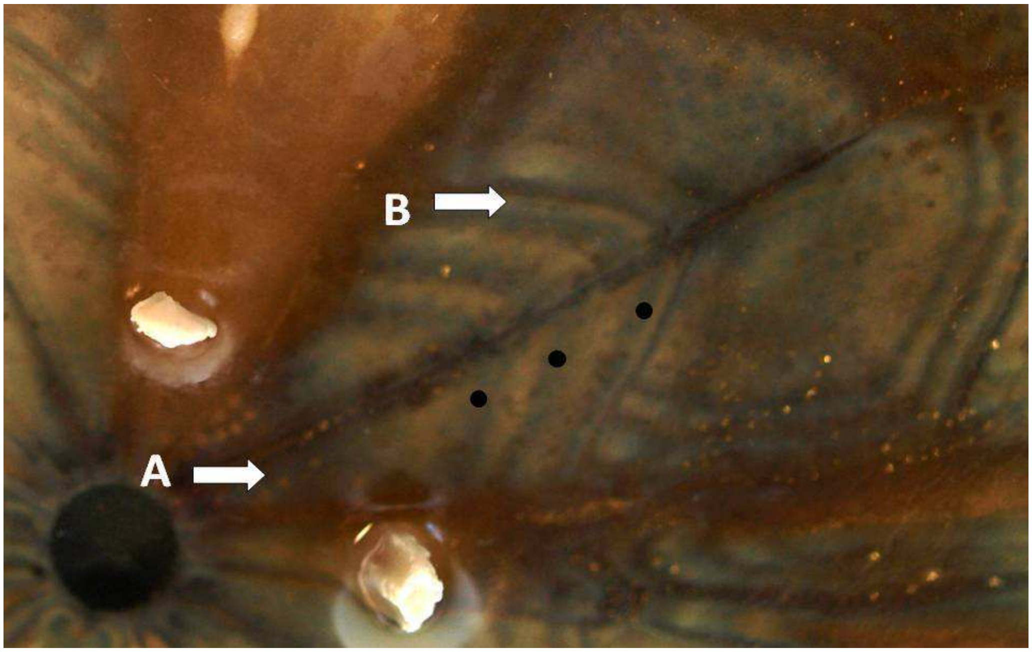

Figure 4.6 Sand dollar test plate. Black dots indicate each dark zone and arrows indicate: A) indistinct grey area of initial growth; and B) light area of growth after last counted ring.

Age of each sand dollar was therefore estimated as:

$$Age(years) = A + x + B$$

Where

$Age(years)$ = estimated age of sand dollar

A = time taken to form initial grey zone (year);

x = number of growth zones; and

B = time taken to form the last light zone to the edge of the test plate (year).

In this case, both A and B were 0.5 years. Therefore;

$$Age(years) = x + 1$$

A t-test with unequal variances was conducted in Microsoft Excel to determine if the mean length of sand dollars aged using zone counts was significantly different from the mean length of sand dollars of the same age obtained from size frequency data. The mean lengths of sand dollars at two years of age obtained using both methods were not significantly different (t test, $t_{10} = 2.2$, $p = 0.06$). This supports the hypothesis that the growth zones observed in the *Peronella lesueuri* test plates are annual and that large *P. lesueuri* individuals can be aged reliably up to 7 years using growth zone counts. Animals older than 7 years that have stopped growing, however, may not be able be aged with this method.

4.3.8 Growth zone width in *Peronella lesueuri* adults

Growth zones found within the oral test plates show general decreases in width with each subsequent zone toward the edge of the plate. The initial growth zone was measured from the edge of the test plate to the edge of the first “dark” zone. It consisted of an indistinct grey “initial” zone prior to a more distinct pair of light and dark zones. This “initial” zone was interpreted as the initial winter growth of newly recruited juveniles. Due to this “initial” zone, the first growth zone was the widest; however, it was most likely to have formed over 1.5 years.

On the oral test plate used for the measurement, the growth zone formation decreased from an initial width of 7.1 mm yr^{-1} to 1.3 mm yr^{-1} by the fifth zone (approximately age 6 years) (Figure 4.7). Only one individual in the sample, however, was recorded to have six growth zones. The decrease in the width of subsequent growth zones suggested a decrease in the rate of growth of the sand dollar as it ages. The first growth zone was the largest in all test plates examined and was deduced to have formed when the sand dollar was approximately 1.5 years of age.

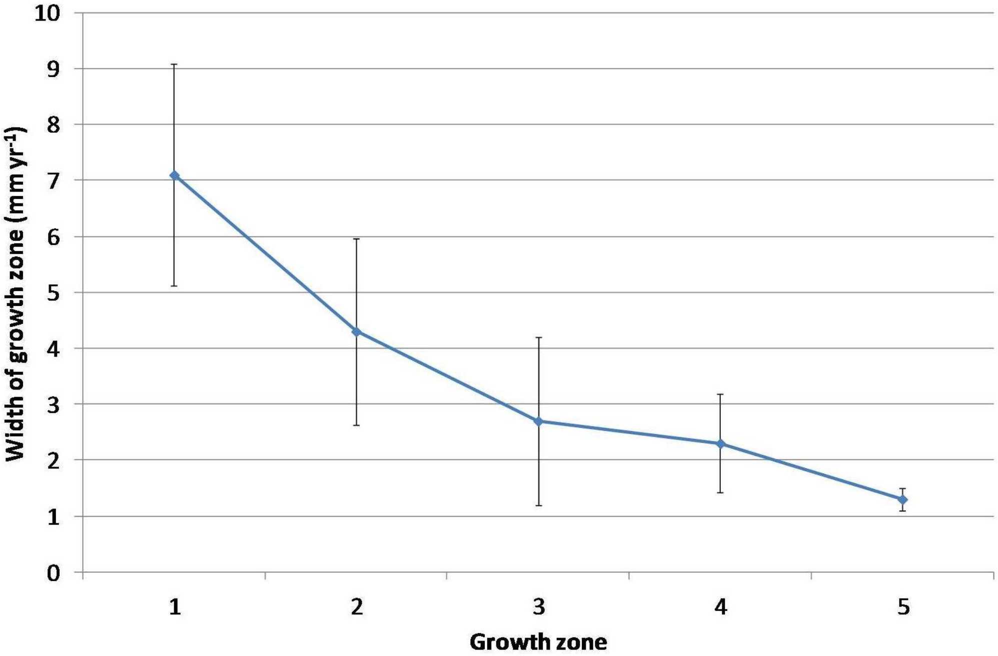

Figure 4.7 Width of growth zone formation per annum in the test plates of *P. lesueuri.*

4.3.9 Growth Curve

Both the Gompertz and the Richards' growth curves provided close fits. The Richards' (1987) growth curve, however, provided a slightly better fit to the data and was used to describe the relationship between test length and age (Table 4.3). Cabanac and Himmelman (1996) found that the Richard's growth curve was ideal for describing the growth of sand dollar *Echinarachnius parma* in the Gulf of St Lawrence, Canada.

Table 4.3 The sum of squares and R^2 values of the Gompertz and Richards' growth curves when fitted to length-at-age data for *Peronella lesueuri*. The higher R^2 value indicates that the Richards' growth curve provided a slightly better fit to the data.

Growth Curve	Sum of Squares	R^2 value
Gompertz	103471	0.9178
Richards'	84349	0.9324

The relationship of test diameter to age of *P. lesueuri* was best described by the Richards' (1987) Growth Function (Figure 4.8). The curve indicates that the rate of growth increases to a maximum of approximately 60 mm yr^{-1} at approximately two years of age. This is close to the 71.45 mm yr^{-1} juvenile growth observed in cohort 2009 in the second year (Section 4.3.4). The data from the size frequency distribution also shows that juveniles rapidly increase in size in the first two years of life and appear to join the "adult" size population towards the end of the second year of life (Figure 4.3). The graph shows the growth rate slowing rapidly at approximately 2.5 years of age (Figure 4.8). The Richards' growth curve predicts that the growth rate for sand dollars older than 2.5 years was almost zero.

While the growth rate appears to have stopped at 2.5 years, individuals with between two and four growth zones were common. This suggests that growth zones continue to be laid down despite the lack of major increase in test length of the sand dollars. Although subsequent growth zones decreased in width, the final zones in all test plates were wide enough to be distinct (Figure 4.7) when viewed under the microscope. There was no evidence of growth rings becoming indistinguishable or getting too close together. The growth rings can thus be used to accurately age the sand dollar population in Cockburn Sound.

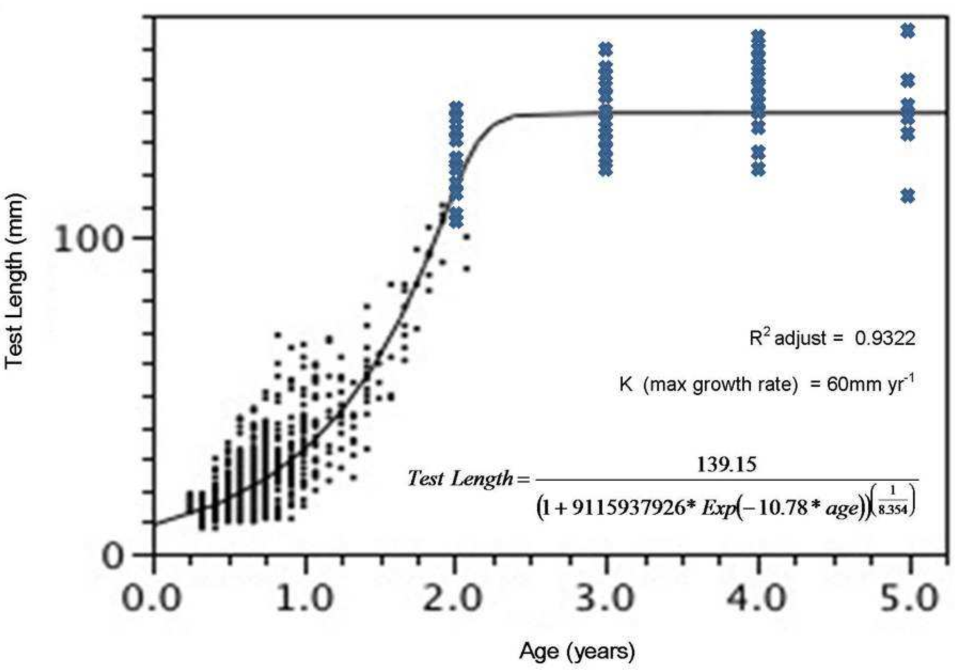

Figure 4.8 Growth curve for *Peronella lesueuri* based on the increase in test length of juveniles and growth ring counts from test plates for adults. The black dots represent age-at-length data obtained from size frequency distributions of 2009 and 2010 cohorts of recruits and the blue crosses represent age-at-length data obtained from growth zone counts.

Individuals with up to four growth zones, aged at five years, were commonly observed, however, one individual had six growth zones, putting its age at 7 years.

The close-to-zero growth rate of adult sand dollars is consistent with the observations of no significant growth in adult sand dollars over a period of five months (Section 4.3.6.2).

4.4 Discussion

Peronella lesueuri is common on the shallow, subtidal shelves of south-western Australia. The two-year study of a population in Jervoise Bay in Cockburn Sound revealed distinct trends and provides the first report of *Peronella lesueuri* life history and growth. Size frequency histograms revealed a predominantly bimodal structure (with intermediate-sized individuals less common) and annual recruitment. Juvenile densities and juvenile percentages in the monthly samples over time revealed high juvenile mortality in the first year. Growth zone counts from test plates allowed age estimates of individuals from the “standing” adult sand dollar population and combined with age-at-length data from juveniles measured for size frequency histograms, a growth curve was estimated for *Peronella lesueuri.*

4.4.1 Recruitment

Recruitment in most marine invertebrates is pulsed rather than continuous (Ebert et al. 1993). Pulsed recruitment has been observed in a range of echinoid species including *Strongylocentrotus franciscanus* and *S. purpuratus* (Ebert et al. 1993; Ebert et al. 1994), *Lytechinus variegatus* (Beddingfield and McClintock 2000), and sand dollars *Dendraster excentricus* (Cameron and Rumrill 1982), *Mellita quinquiesperforata* (Lane and Lawrence 1980), *Echinarachnius parma* (Steimle 1990; Cabanac and Himmelman 1996) and *Arachnoides placenta* (Haycock 2004), in keeping with cyclic reproduction. While annual recruitment is common, some species of echinoids showed two settlement peaks per year. For example, the sea urchin *Paracentrotus lividus* in the Mediterranean showed a main recruitment over spring-summer and a smaller recruitment event over autumn-winter (López et al.

1998; Tomas et al. 2004). Dexter (1977) found that recruitment of the sand dollar *Encope stokesi* in Panama occurred year round, with peak recruitment between January and April. This year round recruitment was likely due to the short life expectancy of *E. stokes*i of less than one year (Dexter 1977). Size frequency histograms indicated that annual recruitment was evident in *Peronella lesueuri* with peak recruitment in spring. Given that *P. lesueuri* spawned annually from midsummer to early autumn, the appearance of newly-settled juveniles (0-10 mm) in May 2009 and July 2010 suggested that juveniles required approximately six months to settle and grow to a post-settlement size that enabled them to be collected by the sampling methods used.

4.4.2 Recruitment variability

Despite the potential for regular pulsed recruitment, recruitment variability is common in echinoids, with strong recruitment in some years and little or none in others. Cabanac and Himmelman (1996) noted little or no recruitment in the three years they studied the *Echinarachnius parma* population at Havre-Saint-Pierre in the Gulf of St. Lawrence, Canada. The spatangoid *Echinocardium cordatum* in the Dutch North Sea recruited in five out of the 10 years of observation and successful cohorts were only developed in three years (Beukema 1985). In Cockburn Sound, it appears that *Peronella lesueuri* recruitment is an annual event. However, the recruitment strength of cohort 2009 was twice that of cohort 2010, indicating large variability in annual recruitment.

A variable recruitment could also cause the bimodal size frequency distributions encountered in the first year of the study. A hypothesis is that there was little or no recruitment in 2008 which resulted in an absence of an intermediate-sized group in the size frequency distributions of 2009. Causes for this could be low settlement rates or high post-settlement mortality of juveniles. The sampling method used in this study only detected juveniles 8 mm and larger, hence the size frequency distributions were unreliable for assessing settlement. Although this study did not explore the factors affecting settlement of *Peronella lesueuri*, other studies have indicated that a range of factors including current variations (Cameron and Rumrill 1982; Ebert et al. 1994), temperature (Hernández et al. 2010), presence of predators (Highsmith 1982), habitat differences (Tomas et al. 2004), as well as substrate conditioning by adults of the species (Pearce and Scheibling 1990), could affect settlement as well as post-settlement survival of echinoid larvae, consequently affecting the abundance of the adult population.

4.4.3 Cohort mortality and growth

An alternative hypothesis for the bimodal size frequency distributions was that settlement occurred but there was an extremely high juvenile mortality of cohort 2008 prior to the commencement of the study. The decrease in juvenile percentages could also be due to migration of juveniles out of the study area (Ebert and Dexter 1975). Adult *Peronella lesueuri* had directionally random movement rates of between 2.0 and 5.3 cm hr^{-1} (Yeo et al. 2013); juveniles which were much smaller were likely to move slower. The mass migration of juveniles away from the study area was therefore unlikely. In addition, the scarcity of sand dollars of intermediate size indicated that a high mortality rate was a more reasonable explanation. Data from the study showed extremely high juvenile mortality over one or two months in summer of up to 74%. The highest juvenile mortality occurred between November and January for both cohorts of recruits, at a time when sea surface temperatures peaked in Cockburn Sound (Figure 4.8). Although speculative, the warmer temperatures observed during this period may have contributed to a higher incidence of juvenile deaths in the new recruits.

In the first year of life, juvenile mortality for cohort 2009 was 88%. This decreased to approximately 48% mortality in the second year. A variety of echinoids have been observed to have high juvenile mortality in the first year with decreases in mortality rates in subsequent years. A mortality rate of 95% was observed in the first year for a cohort of recruits from a population of *Mellita quinquiesperforata* in Tampa Bay, Florida (Lane and Lawrence 1980). The mortality rate of the cohort subsequently decreased to 88% in the second year of life and 61% in the third year. In Spain, the sea urchin *Paracentrotus lividus* had a 90% mortality rate in the first year after settlement (López et al. 1998). The rate decreased to 60% in juveniles of 12 mm to adults of reproductive size.

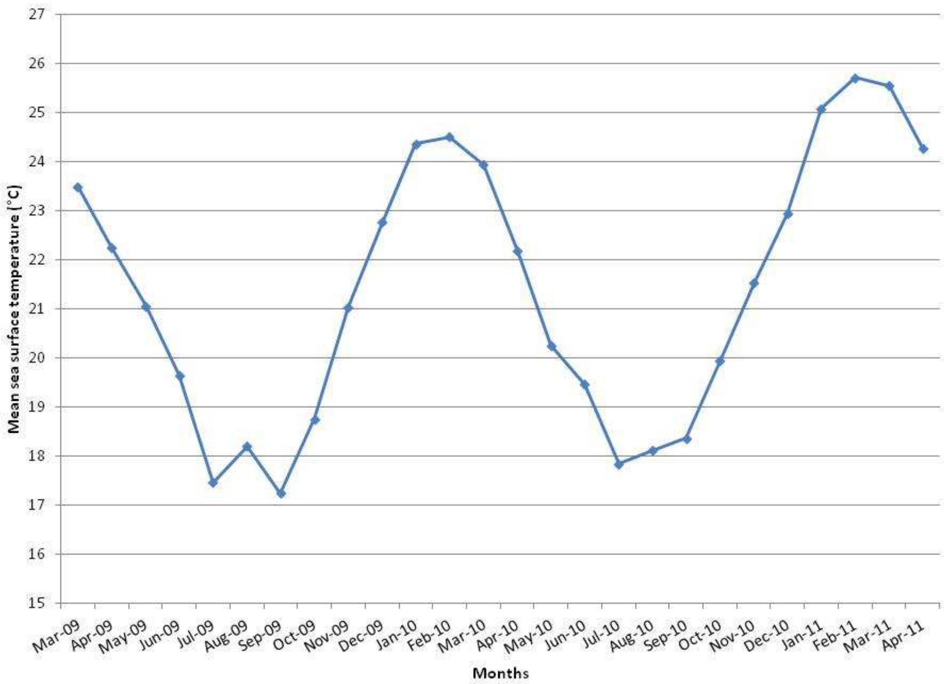

Figure 4.8 Mean sea surface temperatures in Cockburn Sound from March 2009 until April 2011 (IMOS 2011).

The decrease in the mortality rate as *Peronella lesueuri* juveniles grew larger indicated that there was a refuge in size. As juveniles increase in size, they become less susceptible to predators (Highsmith 1982) and transportation by water movement (Cabanac and Himmelman 1996) amongst other environmental stressors. The percentages for cohort 2009 appeared to stabilise in September 2010, when the mean length of juveniles for the cohort was 56 mm. The smaller juvenile sand dollars have a thin fragile test that is prone to breakage. At 56 mm, juveniles of *Peronella lesueuri* may be large enough and their tests are thick and heavy enough to be less susceptible to environmental factors, including water currents and shifting sand, that may transport, bury or crush them. From September 2010 until the end of the study in April 2011, there appeared to be an acceleration in the growth rate of cohort 2009 (Figure 4.5). Studies by Niesen (1977) and Birkeland and Chia (1971) indicated that growth rates of populations of the sand dollar *Dendraster excentricus* in habitats with differing conditions were not the same. Sand dollar populations in unprotected habitats, unsuitable substrates or areas of low food availability had lower growth rates because the energy cost of maintenance was high in these unfavourable conditions, likely at the expense of growth. A possible explanation for the accelerated growth in cohort 2009 after September 2010 was that larger juvenile sand dollars, without the high energy expense required to maintain growth in conditions unfavourable to smaller sand dollars, could direct the energy obtained from food into growth.

4.4.4 Growth Zones

The size frequency distributions in this study were unable to provide an accurate age estimate and growth rate of the larger sand dollars, so the growth zones observed within the test plates were used. Several species of sand dollars have been aged using growth ring counts, including *Echinarachnius parma* (Steimle 1990; Cabanac and Himmelman 1996), *Astriclypeus manni* (Kang et al. 2007), *Clypeaster japonicus* (Kang et al. 2007), *Dendraster excentricus* (Birkeland and Chia 1971) and *Mellita quinquiesperforata* (Lane and Lawrence 1980).

Several authors have, however, cautioned against using growth zones as echinoid age indicators without calibrating the periodicity of the growth zones (Ebert 1988; Russell and Meredith 2000; Tan and Lawrence 2001) because changes in environmental factors, for example, fluctuations in food availability, have been shown to cause additional growth zones (Pearse and Pearse 1975). An attempt to determine the periodicity of growth zones in the *Peronella lesueuri* test plates using tetracycline tags was unsuccessful when the fluorescent markers failed to show up under ultraviolet light in the jaw and test plates of all of the tagged sand dollars. However, the environmental conditions in Cockburn Sound have a pronounced seasonal cycle, which increases the likelihood of annual formation of growth zones in the sand dollar test. In addition, echinoids have been known to partition energy resources away from somatic growth to facilitate reproductive growth (Agatsuma and Nakata 2004). Agatsuma and Nakata (2004) found that the growth in sea urchin *Hemicentrotus pulcherrimus* in Oshoro Bay, Japan, ceased during winter, when gametogenesis was initiated. This cessation of growth caused the formation of a dark band in the growth zone. It is therefore reasonable to postulate that the distinct annual reproductive cycle of *Peronella lesueuri* (Chapter 4) would cause seasonal changes in energy allocated for test growth, which could cause annual growth bands to form. With the indistinct zone at the oral edge of the test plate hypothesized to be the result of the slow growth during the first winter of the newly settled recruits, it is proposed that the first growth zone is formed at approximately one and a half years of age. There also appeared to be a concurrence in the predicted length-at-age of sand dollars with one growth zone with that of juvenile cohorts observed over two years.

4.4.5 Growth curve

When the pooled length-at-age for the juvenile cohorts was combined with the estimated length-at-age of large sand dollars from growth zone counts, an S-shaped growth curve was obtained (Figure 4.8). Other sand dollar species also show similar S-shaped growth curves (Steimle 1990; Cabanac and Himmelman 1996). The growth rate of *Peronella lesueuri* increases with size to a maximum of 60 mm yr^{-1} when the sand dollars are approximately two years of age and 100 mm in length, then slows rapidly (Figure 4.8). The rapid growth rate of sand dollars of intermediate size predicted by the Richards' growth curve suggests that the time *P. lesueuri* juveniles spend at this size should be reduced. This, coupled with a high juvenile mortality and variable annual recruitment, provides an explanation for the scarcity of sand dollars between 50 and 100 mm.

The growth curve indicated that beyond approximately two and a half years of age, the growth rate of *Peronella lesueuri* is almost zero. This is supported by the observation of no change in mean length of a subsample of tagged sand dollars over time. The width of growth zones was observed to progressively decrease (Figure 4.7), also suggesting a slowing in growth over time. The cessation or slowing of growth has been observed in other species of sand dollars. Kang et al. (2007) concluded that in both *Astriclypeus manni* and *Clypeaster japonicus* most of the growth occurred in the first four years of life, with growth discontinued in the fifth and sixth year. Growth in *Echinarachnius parma*, which has a longer life expectancy, also appeared to cease or slow at approximately seven and a half years of age, when maturity was attained (Cabanac and Himmelman 1996). Reproductive data for *P. lesueuri* appeared to support the theory that the slowing of growth at two and a half years of age was due to attainment of sexual maturity. Sand dollars larger than 115 mm were deemed to be sexually mature (Chapter 4), at approximately two years of age (Figure 4.8). Plasticity in the allocation of energy resources away from somatic growth has been noted in *Mellita quinquiesperforata*; energy for growth was used on maintenance in sand dollars living in rough beach conditions (Tavares and Borzone 2006) and energy from somatic growth was channelled to support reproduction (Lane and Lawrence 1980). The attainment of sexual maturity in *P. lesueuri* concurrently with the slowing of growth strongly suggests that energy previously used to support somatic growth is channelled to fulfil the high energy demands of reproduction.

4.4.6 Predictions of maximum size and longevity

Peronella lesueuri is a large sand dollar; the largest individual recorded in this study was 182 mm in length. Individuals that large, however, were rare. The average maximum size of 139 mm predicted by the Richards' growth curve is consistent with the mean modal size of around 140-150 mm of large sand dollars (Group 1) from the size frequency distributions. Environmental conditions have been known to affect the maximum attainable size of *Dendraster excentricus* at Alki Point in Seattle, USA (Birkeland and Chia 1971), with the population in the harsher environment having a smaller mean adult size. The adult size of *P. lesueuri* indicated by the growth curve and size frequency distributions in this study, while useful as a general guide, may not reflect the sizes attainable in populations outside Cockburn Sound. Personal observations of *P. lesueuri* in other areas around Perth, e.g. C.Y. O'Connor Beach and Swan River, suggested that sand dollars in these areas were smaller than those observed in Cockburn Sound. Further research, however, is needed to confirm this.

Although working on different species of sand dollars, Steimle (1990) and Kang et al. (2007) both noted that due to reduced growth rates as sand dollars reached senescence, growths rings became either indistinguishable or too close together.

The consequence of this was that age could be underestimated by growth zone counts. Although the subsequent growth zones observed in the *Peronella lesueuri* test plates decreased in width, the final zones in all test plates were distinct (Figure 4.7) and with no evidence of growth rings becoming indistinguishable; while it is possible that age of larger sand dollars were underestimated by growth zone counts, this is unlikely.

In Cockburn Sound, although growth in the *Peronella lesueuri* population appears to stop after approximately three years, growth rings continue to form (Figure 4.8) Individuals with between two and four growth zones, aged between, three and five years were most common (Figure 4.8).). In this study, only one individual was encountered with the maximum number of six growth zones, putting its age at seven years. Senescence is not evident in the growth rings of this sand dollar population and therefore, it is likely that its average longevity of three to five years can be attributed to predation or other environmental factors and that maximum life span in an ideal environment without predators would likely be longer.

Chapter 5. Seasonal and diel variation in movement rhythms of sand dollar, *Peronella lesueuri* (Valenciennes, 1841), in Cockburn Sound, Western Australia

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I declare that the work contained within this paper is my own account of my research and the co-authors were my supervisors, who provided field and editorial help.

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Sharon Yeo Sue-Yee

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*The nomenclature of this species has been misidentified as *Peronella lesueuri* (Valenciennes, 1841) from the website, *Atlas of Living Australia*. The correct species name should be *Peronella lesueuri* (L. Agassiz, 1841) (Kroh 2012).

Seasonal and diel variation in movement rhythms of sand dollar, *Peronella lesueuri* (Valenciennes, 1841), in Cockburn Sound, Western Australia

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Abstract

Rates and direction of movement in the sand dollar *Peronella lesueuri* were measured in summer and winter in Cockburn Sound, a large coastal embayment in south-western Australia. *P. lesueuri* was found to have a diurnal activity pattern throughout the year and had a greater movement rate in the summer (mean of 5.3 cm hr^{-1}, day; 3.9 cm hr^{-1}, night) than in the winter (mean of 2.7 cm hr^{-1}, day; 2.0 cm hr^{-1}, night). Seasonal change in temperature and physiological requirements by the sand dollar are the most likely reason for the seasonal differences. Reasons for diurnal variation were not clear. Direction of movement was found to be random at both times of the year. Based on these movement rates, one sand dollar can bioturbate an approximate area of 0.1 m^2 day^{-1} and 36.4 m^2 $year^{-1}$. At a conservative density estimate of 0.5 sand dollars per m^2 it takes approximately 20

days for the sand dollars to rework the entire area of the sediments in the habitats they occupy.

Introduction

Sandy subtidal substrates form a vast proportion of the habitats along the world's coastlines with a range of faunal and floral assemblages, which show a diversity of patterns of distribution and abundances (Thrush 1991; Morrisey et al. 1992) often determined by gradients of physical disturbance of the seabed (Clark 1946). To best adjust to the shifting substrates, current and swell that often affect the soft-sediment communities at shallower depth, many organisms possess a means of locomotion (Tomas et al. 2004). Locomotion of benthic fauna can mix the sediments and cause bioturbation, which has major implications for biogeochemical cycling at the sea bed (Lohrer et al. 2004).

Irregular urchins, which include the sand dollars, are active burrowers in many benthic communities and are known to play a key role in bioturbation, influencing the biogeochemistry of surface sediments (Lohrer et al. 2004; Lohrer et al. 2005). To date, research on movement rates of echinoids has largely focused on regular echinoids (Tertschnig 1989; Domenici et al. 2003; Hereu 2005; Lauzon-Guay et al. 2006; Lauzon-Guay and Scheibling 2007; Ceccherelli et al. 2009), with few reports on movement of irregular echinoids, especially sand dollars.

Forming a dominant and conspicuous part of the soft sediment community in Cockburn Sound is the sand dollar, *Peronella lesueuri. P. lesueuri* is a large (15 cm diameter) sand dollar, with a wide Indo-Pacific distribution (Miskelly 2002). In Australia *P. lesueuri* is widely distributed and has been recorded from Queensland

(Clark 1938), the Kimberley region (Keesing et al. 2011), the Dampier Archipelago (Marsh and Morrison 2004), and down the west coast as far south as Albany (Clark 1938). In Cockburn Sound, *P. lesueuri* can occur at densities of up to 6 individuals m^{-2} (Forehead and Thompson 2010). *P. lesueuri* is a shallow burrower and occurs at densities which may influence surface sediment chemistry and community dynamics. Therefore knowledge of seasonal and diel movement rates and rhythms of this species are of key interest in understanding coastal sediment biogeochemical dynamics.

Sand dollars generally lie flat on the substrate and move forward along the anterior-posterior axis (Chia 1969; Merrill and Hobson 1970; Ghiold 1979) with occasional reversal or rotation upon encountering an obstacle (Chia 1969). *D. excentricus,* however, has been observed to be positioned on an incline to feed on suspended material (Merrill and Hobson 1970; Francisco and Herzka 2010) and was able to actively move in the inclined position. Movement rates for several species have been documented (Weihe and Gray 1968; Bell and Frey 1969; Ebert and Dexter 1975). In those studies, the seasonal and diurnal differences in movement rates were not investigated. As yet, there have been no published studies on movement patterns of the sand dollar, *P. lesueuri*. This study aimed to provide an insight into seasonal and diel movement rates and patterns of *P. lesueuri*.

Methods

The study was carried out on the *P. lesueuri* population at Jervoise Bank (32°09'S, 115°45'E) in Cockburn Sound, Western Australia. Cockburn Sound is a semi-enclosed embayment in the southwest of Western Australia, approximately 16 kilometres long and 9 kilometres wide (Steedman and Craig 1983). It consists of a main basin of mud and silt, 17-22 metres deep, and is edged with shallower sandy sills and beaches (Marsh and Devaney 1978; Pearce et al. 2011). Cockburn Sound has a mean maximum daily tidal range of 0.55 m and wind-driven currents with a mean of 0.05 m s^{-1} (Steedman and Craig 1983). Sea surface temperatures in the area range from a low of 16ºC in the winter to a high of 23ºC in the summer (Pearce et al. 1999). The experiment was first carried out in the winter (26 and 27 July 2010) and repeated in summer (1 and 2 February 2011).

The positions of the sand dollars (n = 262 in July, n = 185 in February) were marked in the morning (between 0900h and 1100h) by divers using SCUBA by inserting a numbered flag into the sediment 2 cm behind the animal in order not to disturb them or hamper direction of movement. The time of initial marking of each sand dollar was recorded. Divers returned in the late afternoon (between 1530 h and 1730 h) and the net distance each animal had moved from its flag was measured (Distance moved = Distance from flag – 2 cm) with a measuring tape. The net movement is the minimum absolute distance moved or total displacement as measured from the flag as not all sand dollars move in a straight line. The direction of movement was measured with a dive compass. When the measurements were made, the time was also recorded for an accurate calculation of movement rate.

The flag was then moved 2 cm behind the same sand dollar and the animals were left overnight. The next morning (between 0900 h and 1100 h) divers measured the direction and distance moved by the sand dollars and recorded the time. Occasionally, when divers returned to check the distance moved by the sand dollars, the paths of individuals had crossed. In these instances divers differentiated individuals by the direction of the tracks left in the sand from the flag to the sand dollar.

Analysis

Within each season (winter and summer) paired sample t-tests ($\alpha = 0.01$) were used to test the difference between day and night movement rates. Differences in day and night movement rates between seasons were tested using t-tests with unequal variances ($\alpha = 0.01$).

Frequency distributions of seasonal and diel movement rates were plotted and statistically tested to determine the best fitting distribution.

Directional readings were divided into eight categories (North, Northeast, East, Southeast, South, Southwest, West, Northwest) and movement rates and directional measurements were analysed with one-factorial analysis of variance (ANOVA).

Results

Peronella lesueuri was observed to be more active in the summer than in the winter and movement rates were generally higher in the day (Figure 1). In the summer, the rate of movement during the day ranged from 0 to 20.7 cm hr^{-1} ($X \pm SD = 5.3 \pm 3.5$ cm, $N = 207$). At night, the movement was less, with the rate between 0.3 and 11.4 cm hr^{-1} ($X \pm SD = 3.9 \pm 2.0$ cm, $N = 185$). The movement rates recorded in the winter were also higher in the day, ranging from 0 to 12.3 cm hr^{-1} ($X \pm SD = 2.7 \pm 2.3$ cm, $N = 283$) during the day and from 0.1 to 11.0 cm hr^{-1} ($X \pm SD = 2.0 \pm 1.4$ cm, $N = 262$) at night.

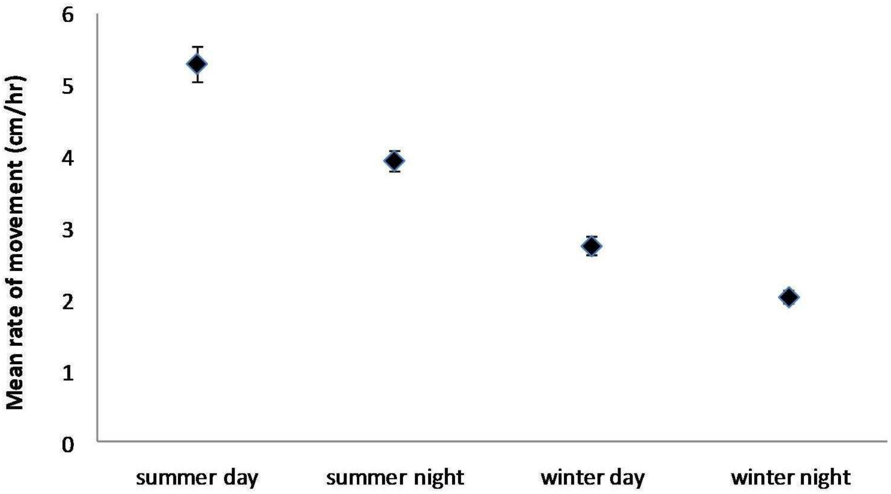

Figure 1. Seasonal and diel mean movement rates of *Peronella lesueuri* in Cockburn Sound with standard error bars.

Frequency distribution of the movement rates indicated that modal movement rates were higher in the summer than winter (Figure 2a-b). During the winter, modal movement rates were 0-0.5 cm hr^{-1} in the day and 1.01-1.5 and 2.01-2.5 cm hr^{-1} at night (Figure 2a). Summer modal movement rates were 3.01-3.5 and 3.51-4.0 cm hr^{-1} in the day and 3.01-3.5 cm hr^{-1} at night (Figure 2b). The range of movement rates in the day was larger in summer, with a maximum of 20.7 cm hr^{-1}. In the winter the maximum rate recorded in the day was 12.3 cm hr^{-1}. In both seasons, maximum rate of movement at night was around 11.0 cm hr^{-1}. All the distribution histograms were tested against a normal and a lognormal distribution. In all cases, the log-likelihood values indicated that the lognormal distribution was the best fit and that the movement distributions were skewed.

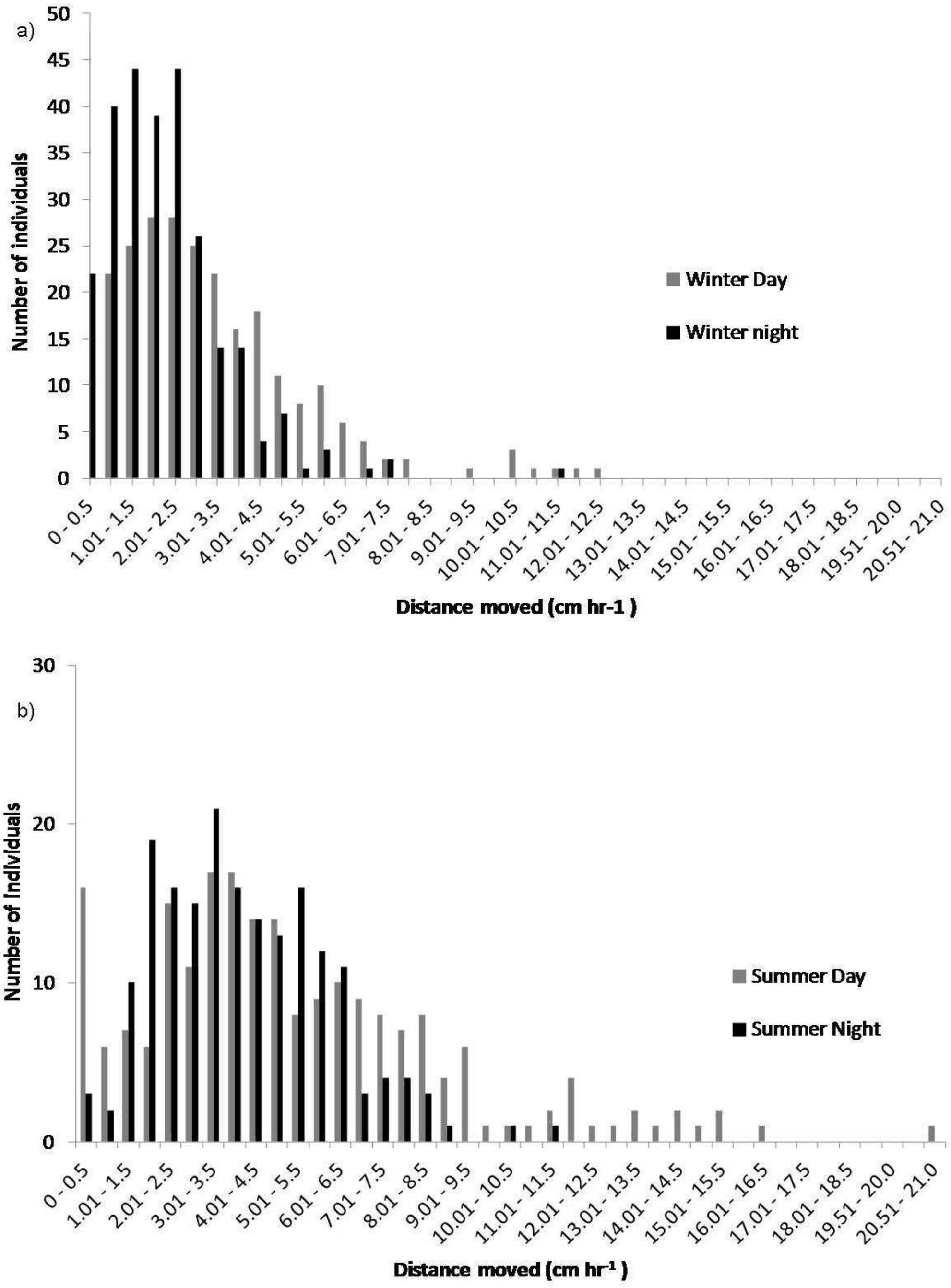

Figure 2a-b. Seasonal and diel frequency distribution of distance moved by *P. lesueuri* in a) winter and b) summer

The day and night rates of movement by *P. lesueuri* demonstrated diurnal activity, with a significantly higher rate of movement during the day, both during summer (t test, $t_{184} = 6.7$, $P < 0.01$) and winter (t test, $t_{261} = 3.8$, $P < 0.01$).

P. lesueuri also exhibited a seasonal activity rhythm, with both day and night rates of movement higher in summer (Day, Paired t-test, $t_{488} = 9.623$, $P < 0.01$; Night, Paired t-test, $t_{445} = 11.893$, $P = 0.01$) than in winter.

Irrespective of the season and time of day, *P. lesueuri* did not exhibit directionality in their movement (Summer day ANOVA: $F = 0.881$, $p = 0.523$; Summer night ANOVA: $F = 1.568$, $p = 0.148$; Winter day ANOVA: $F = 1.496$, $p = 0.176$; Winter night ANOVA: $F = 0.719$, $p = 0.656$) (Figure 3).

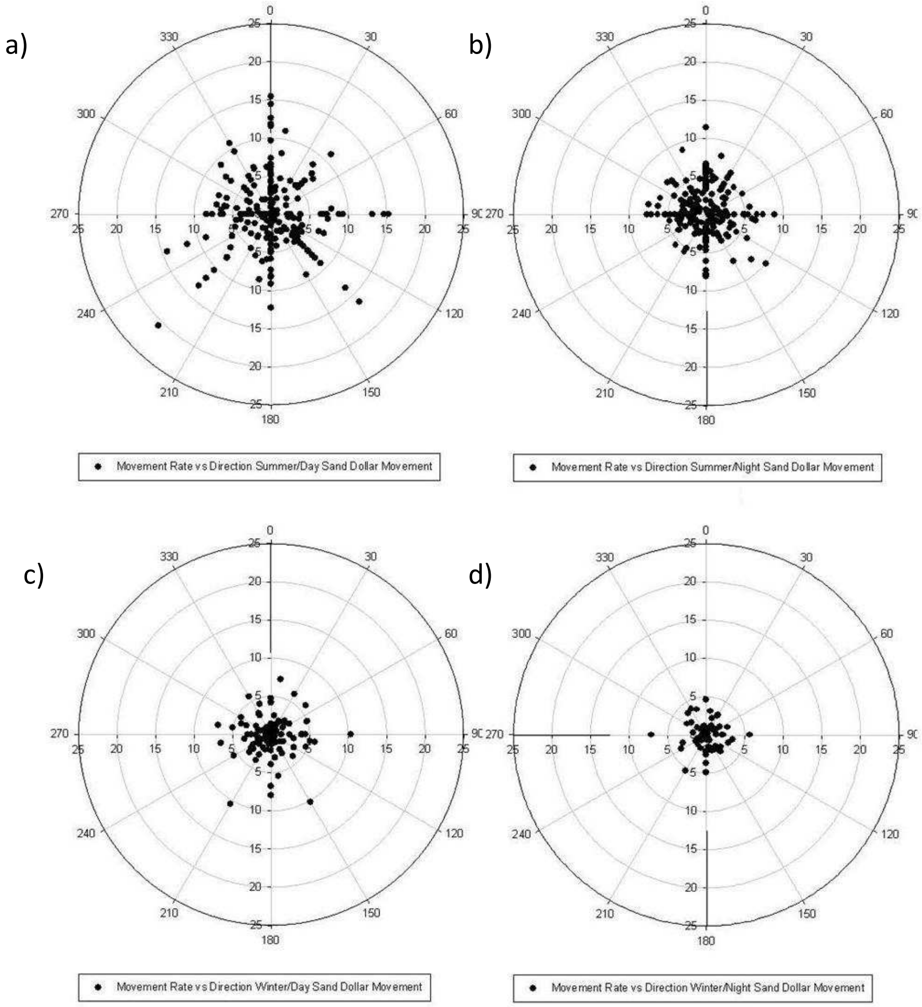

Figure 3a-d. Polar plots of sand dollar movement rate (cm/hr) vs direction (degrees) in a) Summer/Day, b) Summer/Night, c) Winter/Day and d) Winter/Night

Discussion

Rates of Movement

Peronella lesueuri was observed to have movement rates of up to 20.7 cm hr^{-1}, comparable to rates observed in other sand dollars. *Encope grandis* has been observed to move at least 12.5 cm hr^{-1} (3 m day^{-1}) (Ebert and Dexter 1975) while *Mellita quinquesperforata* is reported to move an average of 1.25 cm hr^{-1} (30 cm day^{-1}) although rates of 5-7.5 cm hr^{-1} were common (Bell and Frey 1969). Weihe and Gray (1968) also observed *M. quinquesperforata* to move at a rate of 1.25 cm hr^{-1} on sandy sediments, although the rate of movement dropped to 0.42 cm hr^{-1} when in sandy mud. Although *Dendraster excentricus* was observed to have average movement rates of 18.6 cm hr^{-1} (0.31 cm min^{-1}) (Merrill and Hobson 1970), active individuals of *Dendraster excentricus* have been reported to move at a rate of 120 cm hr^{-1} (2 cm min^{-1}) (Chia 1969); this high rate of movement was unlikely to have been sustained for any length of time however, as the sand dollars usually buried themselves within 15 minutes (Chia 1969).

Direction of movement

Studies on sand dollars show that while they have a predominantly forward movement (Weihe and Gray 1968; Chia 1969; Cabanac and Himmelman 1998), they also show randomness in direction of movement. In Cockburn Sound the tagged *P. lesueuri* showed random choice of movement direction at all times (p value range: 0.148 – 0.656). This corroborates an earlier study in which Weihe and Gray (1968) found that when initially placed oriented towards a particular direction, the subsequent directional choice of the sand dollar *Mellita quinquiesperforata* was random and unaffected by the prevailing current direction. Similarly, *Dendraster excentricus* was observed lying randomly on the sediment at Alki Point in Seattle, Washington, in no relation to the direction of the current (Chia 1969). Experiments conducted by Cabanac and Himmelman (1998) on *Echinarachnius parma* showed that slope and current affected directional movement of individuals of different sizes. Larger sand dollars had a tendency to move upslope and upstream to shallower waters, while smaller individuals preferred downslope movement and did not move far from the experiment release point. The authors suggested that in shallow water conditions juveniles are more vulnerable to predators and more likely to be affected by turbulent water. Adult *Echinarachnius parma* were less likely to be dislodged by water turbulence, therefore they could migrate to shallower waters to take advantage of the food resources (Cabanac and Himmelman 1996).

Hydrodynamics affect positions and movement rates of *D. excentricus*; the sand dollars lay flat and moved frequently in the calm, shallow water , were in an inclined position in moderate swell and were buried in the sediment in heavy seas (Merrill and Hobson 1970; Tomas et al. 2004; Francisco and Herzka 2010). During the course of this study, *P. lesueuri* was always observed either burrowed or flat on the substrate surface. With microtides and low tidal and wind-driven currents within Cockburn Sound, subtidal sediments are relatively stable and do not require *P. lesueuri* to exhibit adjustment behaviours. In addition, as the sand dollars are large and heavy, lying flat on the substrate and burrowing would provide optimum stability.

Seasonal movement patterns

Movement rates for *P. lesueuri* showed seasonal differences, with significantly higher summer movement rates. Such seasonal activity patterns are commonly observed amongst sub-tropical and temperate echinoderms, including echinoids, and are usually correlated to seasonal cycles in photoperiod, temperature and food availability. *P. lesueuri*, like most sand dollar species, either lies flat on or is partially buried in the substrate. The diet of *P. lesueuri* is not known but sand dollars are size-selective deposit feeders, feeding on diatoms from the microphytobenthos, foraminifera and other microeukaryotes, as well as the bacterial components coating the sedimentary particles (Mooi and Telford 1982; Findlay and White 1983; Ellers and Telford 1984; Telford et al. 1987). In echinoids, feeding, burrowing and locomotion all occur simultaneously (Ghiold 1979), with movement being an important part of food acquisition (Mooi and Telford 1982). Productivity of marine sediments is greater in summer as a result of higher temperatures, increased photoperiod and decreased turbidity (Miles and Sundbäck 2000; Boer et al. 2009) and an enhanced feeding rate by *P. lesueuri* in summer maybe in response to this.

Sea surface temperatures in areas around Cockburn Sound range from a maximum of 23°C in the summer to a low of 16°C in the winter (Pearce et al. 1999), thus higher movement rates in *P. lesueuri* could be associated with higher temperatures. A study on the sand dollar *Mellita quinquesperforata* showed that it fed almost continuously with rate of feeding being higher at warmer, summer water temperatures (Lane and Lawrence 1982). Heart urchins *Brissopsis lyrifera* and *Schizaster canaliferus* both show increased activity rates during the summer (Schinner 1993; Hollertz and Duchene 2001). Clear seasonal changes in rates of both movement and feeding have also been measured for other echinoderm classes; e.g. the crown-of-thorns starfish *Acanthaster planci* (Keesing and Lucas 1992; Keesing 1995) with rates of both movement and feeding greater in summer than in winter. Seasonal cycles of feeding intensity exhibited by sea cucumbers in Canada were related to the availability (Hamel and Mercier 1998) and quality (Singh et al. 1999) of food rather than temperature and photoperiod. Seasonal changes in water temperature play a role in increasing the metabolic rate of marine invertebrates, however, metabolic rate increase may also be caused by increasing physiological activity associated with feeding, growth and reproduction (Brockington and Clarke 2001). The reproductive periodicity of *P. lesueuri* is unknown. However, seasonal variation in movement may be co-incident with reproductive changes.

Morin et al.(1985) observed a seasonal migratory movement in the sand dollar (*D.excentricus)* beds in southern California. The sand dollars moved shoreward in summer and expanded seaward in winter in correlation with day length. While the factor controlling this shoreward migration was not identified, Morin et.al (1985) suggested that it was beneficial to sand dollar reproduction. No obvious migratory movement was observed in the population of *P. lesueuri* in Cockburn Sound, suggesting that the seasonal environmental conditions were not adverse enough to effect a migratory response.

Diurnal movement

P. lesueuri displayed a diurnal activity pattern throughout the year, moving at a greater rate during the day ($p < 0.01$ both in winter and summer). Diurnal patterns of behaviour are usually a response to food availability or predator avoidance. Diurnal movement in *P. lesueuri* may be in response to diurnal changes in the sediment. For example, microphytobenthos demonstrates diurnal migration within the sediment that is highly influenced by solar irradiation, with microphytobenthos migrating to the surface of the sediment during the day (Longphuirt et al. 2006). A heightened grazing intensity in tandem with microphytobenthos migration to the sediment surface could explain the higher rate of movement exhibited by *P. lesueuri* during the day. There has been no study on the diurnal changes in sediment biogeochemistry in our research area.

Laboratory studies have shown that sea urchins such as *Strongylocentrotus droebachiensis* and *Paracentrotus lividus* can detect light (Domenici et al. 2003; Ullrich-Luter et al. 2011), which can determine both direction and speed of movement (Domenici et al. 2003). This ability to detect light has not been studied in sand dollars, but could provide an explanation to the differing movement rates during the course of the day.

Nocturnal activity patterns are commonly observed in echinoids as a response to predation pressure during the day in habitats where their major predators have a diurnal rhythm activity (Nelson and Vance 1979; Bernstein et al. 1981; Hereu 2005; Miyamoto and Kohshima 2006; Young and Bellwood 2011). Some species of sea urchins appear to remain in their burrows during the day and emerge at night to forage (Nelson and Vance 1979; Hereu 2005). *P. lesueuri* are commonly found in open, sediment substrates with little or no shelter. Individuals are, however, usually found partially burrowed into the sediment, or camouflaged with a layer of sediment covering the aboral surface. Observations on the sand dollar *Dendraster excentricus* show that it can bury itself within 15 minutes (Chia 1969) and from personal observation, it takes an individual *P. lesueuri*, with all sediment shaken loose from its test, less than 30 minutes to re-bury itself. With this high level of camouflage ability, the diurnal pattern of activity of *P. lesueuri* suggests that predator avoidance is not a factor in its regulation of movement. Size is a further factor known to affect echinoderm movement rates. Keesing (1995) found diurnal patterns of activity of *Acanthaster planci* were strongest in smaller starfish which remained cryptic and were less mobile during the day, probably as an anti predator response. We were not able to compare movement patterns in small and large *P. lesueuri*, the former being either very uncommon or very cryptic. As was suggested for *A. planci* by Keesing (1995), large *P. lesueuri* probably achieve a refuge from predation in size and this may also help explain their diurnal behaviour pattern.

Ecological implications of movement behaviour

Feeding and locomotion occur simultaneously in burrowing echinoids (Ghiold 1979). When burrowing echinoids dominate a soft-bottomed habitat, they have the ability to turn over enough sediment to affect infaunal abundances (Dahlgren et al. 1999), lower levels of organic matter, inhibit eutrophication (Michio et al. 2003) and improve conditions for microphythobenthos production (Lohrer et al. 2004). *P. lesueuri* feeds and burrows on the surface sediment (top 1-2 cm) (Thompson and Riddle 2005), moving by manipulation of locomotory spines on their oral surface. As *P. lesueuri* burrows, the locomotory spines stir up food particles from the sediment below the oral surface (Mooi and Telford 1982). This action reworks the sediment, leaving a trail the width of its body. Based on the movement rates collected in this experiment, one sand dollar with an average width of 12 cm moving at an annualized rate of 83 cm day^{-1} (mean of day and night rates in winter and summer), will bioturbate an area of approximately 0.1 m^2 day^{-1} and 36.4 m^2 annually. This means that at a conservative density estimate of 0.5 sand dollars per m^2 it takes approximately 20 days for the sand dollars to rework the entire area of the sediments in the habitats they occupy. This implies that surface sediments in the sand dollar habitats are completely reworked approximately 18 times a year.

Through differences in movement rates, this study establishes that *Peronella lesueuri* in Cockburn Sound, Western Australia demonstrates diurnal and seasonal patterns of activity with random directionality of movement. In view of the burrowing and deposit feeding habits of *P. lesueuri*, there are strong implications for how its activity patterns can affect coastal soft sediment habitats.

Chapter 6 Conclusion

The pink sand dollar, *Peronella lesueuri*, has a wide Indo-Pacific distribution; it has been recorded in Hong Kong (Agassiz and Clark 1914; Clark 1921), Southern Japan (Agassiz and Clark 1914; Clark 1921), Singapore (Agassiz and Clark 1914) and the Philippines (Agassiz and Clark 1914). In Australia, its distribution extends from southern Queensland, northward and westward towards Darwin and Broome, and then along the west coast down to Fremantle and Albany (Clark 1938). In Cockburn Sound, southwest Australia, *P. lesueuri* is common in the sandy sediment substrates. A survey of a range of sites within Cockburn Sound showed that *Peronella lesueuri* was found in the shallower, sandy sills that surround the Sound but is notably absent from the deeper central basin, where the sediment is mainly composed of silt, suggesting that sediment composition is important in determining distribution. Silt has been shown to hinder movement, smother and cause death in several species of sand dollars (Weihe and Gray 1968; Merrill and Hobson 1970; Ghiold 1979; Ghiold 1982) by accumulating at the base of the spines and preventing spine and food movement (Ghiold 1979) and this may also be an important factor in *P. lesueuri*. Although there have been previous reports of sand dollar densities of up to 6 individuals m^{-2} in Cockburn Sound (Forehead and Thompson 2010), lower densities of between 0.3 to 2.5 individuals m^{-2} were recorded in this study. All the sites at which *P. lesueuri* occurred were at a depth of ten metres or less with sediment composed mainly of coarse to fine sands. Sand dollar densities were highest at sites less than five metres in depth with fine and medium sands, indicating that these were ideal environmental conditions for *P. lesueuri*.

Given the detrimental effects of silt on many species of sand dollars, the large-scale re-suspension and deposition of silt within sand dollar habitats may have effects on their population abundances. Extensive dredging activities at Bird Shoal, North Carolina deposited silt and mud on the sand flats, prevented settlement of larvae or smothered juveniles, decreasing the abundance of sand dollar *Mellita quinquiesperforata* (Weihe and Gray 1968). In Cockburn Sound, the effects of the sediment stirred up over the years by the dredging and rock wall creation of the Jervoise Bay harbour as well as the dredging of access channels to the Kwinana strip, and construction of the Garden Island causeway, on the recruitment and abundance of sand dollar populations in the Sound are unknown. Current recreational and commercial use of Cockburn Sound is extensive and as it continues to increase, new proposals for development such as the Mangles Bay Marina Tourist Precinct (Strategen 2012) and the Port Rockingham Marina (RPS Environment and Planning Pty RPS Environment and Planning Pty Ltd 2008) mean that more dredging and construction within the Sound will be likely. Therefore, there is a need to determine the effects sediment resuspension will have on the sand dollar population in Cockburn Sound.

Like many echinoids, *Peronella lesueuri* was observed to have a sex ratio of 1:1. There was no obvious external sexual dimorphism and the sand dollar could only be sexed upon dissection. All Individuals 115 mm and larger had sexable gonads and were deemed to be sexually mature. Age-at-length indicated that attainment of sexual maturity occurred between 1.5 and two years of age. Growth in *Peronella lesueuri* follows a sigmoidal growth curve and is best represented by the Richards' growth curve. Growth in newly recruited juveniles accelerated to a maximum growth rate of 60 mm yr^{-1} at two years of age, after which the growth rate decreased rapidly to almost zero at two and a half years of age. This corresponded to the age at which *P. lesueuri* attained sexual maturity, suggesting that energy previously used to support somatic growth was channelled to fulfil the high energy demands of reproduction. The maximum size in the population studied as 182 mm. The maximum life expectancy was approximately five years.

Histological analysis of gonads over time showed that *Peronella lesueuri* had an annual reproductive cycle with peak spawning in summer. *P. lesueuri* gonads underwent gametogenesis in spring, spawned in summer, were spent in autumn and recovered over winter. The sequence of change in the gametogenic cells and nutritive phagocytes in *P. lesueuri* gonads followed the pattern that had been described in other echinoids (Chatlynne 1969; Chen and Chen 1993; Byrne et al. 1998; Haycock 2004; Nunes and Jangoux 2004; Martinez-Pita et al. 2008). Growth, mature, partially spawned, spent and recovery stages of the gametogenic cycle were observed in both sexes and the stages were highly synchronised between sexes. As this was a field study, it was not possible to fully assess the effects of exogenous factors on the *P. lesueuri* reproductive cycle. However, correlations between oocyte proportions and sea surface temperature (SST) as well as mean bright sunshine hours (BSH) suggest that annual changes in one or both of these factors had effects on the reproductive cycle of *P. lesueuri*. Further research under controlled conditions need to be conducted to identify the regulatory factors and to determine their effects on the reproductive cycle of *P. lesueuri*.

Peronella lesueuri recruits annually. Spikes in the population densities of *P. lesueuri* at Jervoise Bay in December 2010 and January 2011 were clear indications of recruitment events. This is confirmed by size frequency distributions taken over the same period which recorded spikes in the number of juveniles. Although peak recruitment occurs in late spring and early summer, juveniles 8 mm and larger started appearing amongst the adult population around May/June, approximately five or six months after peak spawning. Despite the potential to recruit annually, there was annual variation in recruitment strength. The recruitment strength of juvenile cohort 2009 was twice that of cohort 2010. Although the factors affecting recruitment variability in *P. lesueuri* were not explored in this study, other studies have indicated that a whole suite of factors, including temperature (Hernández et al. 2010), current variations (Cameron and Rumrill 1982) and sediment suspension (Weihe and Gray 1968), could affect recruitment strength. Juvenile mortality was high, with up to 88% mortality in the first year. Juvenile mortality rates peaked at up to 74% mortality over one or two months in the summer, coinciding with maximum sea surface temperatures in Cockburn Sound. The high sea surface temperatures may have contributed to the higher incidence of juvenile deaths. Juvenile sand dollars appear to find refuge in size; juvenile number stabilised when the mean length of the cohort was 56 mm. At this size, juveniles may be less susceptible to environmental stressors, such as transportation by water movement (Cabanac and Himmelman 1996).

Peronella lesueuri was found at seven sites within Cockburn Sound, with the mean test lengths larger at three sites. These sites were shallow (< five metres) and mainly composed of medium sands. A study of the sediments at different depths at

Parmelia Bank in Cockburn Sound indicated that sediment at depth of four to eight metres were the most productive for epibenthic microphytobenthos (Forehead and Thompson 2010). This suggests that there may be some association between productive marine sediments and sand dollar growth rate, thus enabling faster growing juvenile sand dollars to achieve a larger size before growth slowed.

Peronella lesueuri was found to have a diurnal activity pattern throughout the year and had a greater movement rate in the summer than in the winter. Seasonal changes in water temperature play a role in increasing the metabolic rate of marine invertebrates; however, metabolic rate increase may also be caused by increasing physiological activity associated with feeding, growth and reproduction (Brockington and Clarke 2001). In summer, *P. lesueuri* is actively spawning and the increased movement in the summer may be due to enhanced feeding required to fulfil the energy requirements of reproduction. Another explanation for increased movement in summer is increased feeding in response to increased productivity of marine sediments as a result of higher temperatures, increased photoperiod and decreased turbidity (Miles and Sundbäck 2000; Boer et al. 2009). Reasons for diurnal variation in movement rates were not clear. Direction of movement was found to be random at both times of the year.

Peronella lesueuri feeds and burrows on the surface sediment (top 1-2 cm). When burrowing echinoids dominate a soft-bottomed habitat, they have the ability to turn over enough sediment to affect infaunal abundances (Dahlgren et al. 1999), lower levels of organic matter, inhibit eutrophication (Michio et al. 2003) and improve conditions for microphytobenthos production (Lohrer et al. 2004). Based on their movement rates, one sand dollar can bioturbate an approximate area of

0.1 m^2 day^{-1} and 36.4 m^2 $year^{-1}$. At a conservative density estimate of 0.5 sand dollars per m^2 it takes approximately 20 days for the sand dollars to rework the entire area of the sediments in the habitats they occupy. This makes *P. lesueuri* ecologically significant as a sediment bioturbator within their habitat and thus potentially an important agent in mediating biogeochemical processes in surface sediments. In Cockburn Sound, *P. lesueuri* occurs at the depths at which the sediment is the most productive (Forehead and Thompson 2010). The densities at which they occur suggest that the bioturbation effects produced by their movement through the sediment may affect sediment productivity by improving condition for microphytobenthos production such as sediment irrigation and nitrogen remineralisation.

Although the factors that affect reproduction, recruitment, growth and movement in *P. lesueuri* were not the focus of this study, it was apparent that temperature had the potential to significantly affect all the four biological aspects studied. The bathymetry of Cockburn Sound causes it to act mainly as a closed system (Steedman and Craig 1983). Therefore, irregular weather events can cause significant changes in temperature within the Sound as there is little water exchange with the open ocean. During the summer of 2010/2011, a “marine heat wave” was observed off the Western Australian coast, causing water temperatures within the Sound to rise up to 2° Celsius above the long-term seasonal mean. It is unclear if this affected *P. lesueuri* populations within Cockburn Sound, although it coincided with a lower percentage of ova in female gonads as well as a weaker recruitment. With climate change gradually increasing global temperatures (New et al. 2011), further research is required to identify temperature and the other factors that influence the biology of *P. lesueuri* and determine their effects.

In Cockburn Sound, it was established that the composition of sediment grain size was a determining factor in the distribution and density of *Peronella lesueuri.* This study is also the first to provide the histological description of the gametogenic cycle in *P. lesueuri* and ascertain that both the reproductive cycle and recruitment in *P. lesueuri* is annual. Like other species of sand dollars, there was annual variability in recruitment strength and juvenile mortality was high. While the bioturbation effects of burrowing heart urchins have been well studied (Widdicombe and Austen 1998; Lohrer et al. 2005; Vopel et al. 2007), the study of diurnal and seasonal patterns of activity in *P. lesueuri* is the first to indicate that sand dollars are ecologically significant sediment bioturbators. The densities in which *P. lesueuri* is found on the shallow sediment bottoms imply that they have a potentially important role as an agent in mediating biogeochemical processes in the surface sediments.

The diet of *Peronella lesueuri* remains unknown, along with the factors that affect reproduction, recruitment and growth. More research is required to fully assess the biological and ecological significance of *P. lesueuri*. While gaps remain in our knowledge of the biology and ecology of *Peronella lesueuri*, it is clear that this species is locally important in the ecology of Cockburn Sound.

The majority of the sand dollars studied thus far have been temperate species. This study of *Peronella lesueuri* biology and ecology adds to the knowledge of subtropical sand dollar species, which has so far been scant. *P. lesueuri,* like many other species of sand dollars, including *Echinarachnius parma* and *Mellita quinquiesperforata* (Telford et al. 1987), prefers fine to coarse sand sediments for burrowing and feeding. Despite its large size, *P. lesueuri* achieves average maximum size within two and a half years, much faster than the five to ten year range at which other sand dollar species take to reach maximum size (Ebert and Dexter 1975). This is due to their extremely high growth rate. The study also identified the significance of the burrowing action of sand dollars on the surface sediments, even at low densities of 0.5 individuals m^{-2}. Previously this had only been studied for heart urchins (Widdicombe and Austen 1998; Hollertz and Duchene 2001; Lohrer et al. 2004; Lohrer et al. 2005). Given that other sand dollar species have the propensity to aggregate, their movement through the surface sediments would have an even greater ecological impact on their habitats.

Chapter 7 Bibliography

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