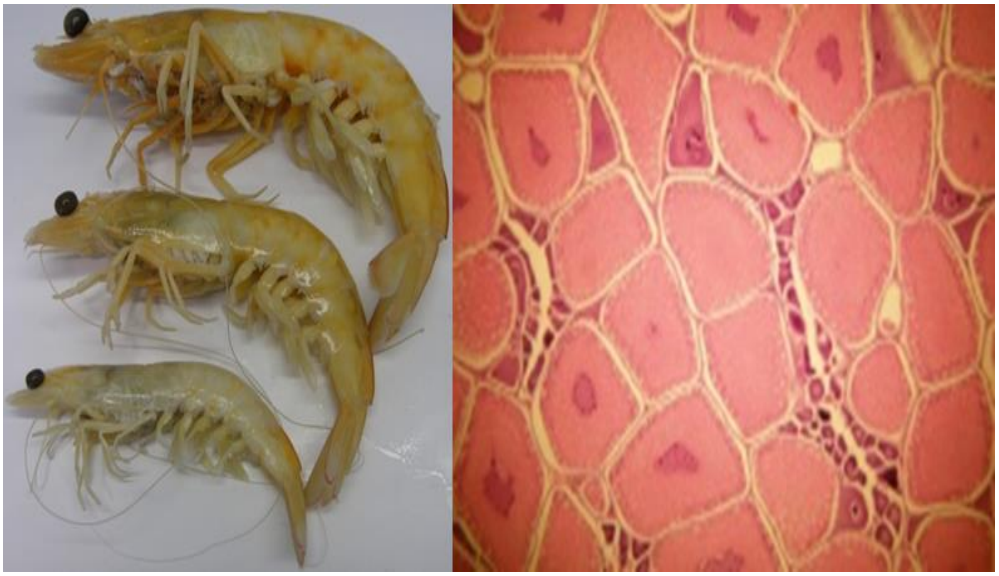
Reproductive biology of the western king prawn *Penaeus (Melicertus) latisulcatus* (Kishinouye 1896) in Spencer Gulf and Gulf St Vincent, South Australia



Nadine Emily Hackett B. Science., Hons. Marine Biology

A thesis submitted in fulfilment for the degree of

Doctor of Philosophy

School of Biological Sciences

Faculty of Science and Engineering

Flinders University of South Australia

March 2017

Table of Contents

List of Figures

List of Tables

Summary

The western king prawn *Penaeus (Melicertus) latisulcatus* (Decapoda, Penaeidae) occurs within coastal waters of South Australia (Gulf St Vincent, Spencer Gulf and West Coast), but is also distributed in areas of northern and western Australia. There are considerable knowledge gaps in the fundamental reproductive biology of *P. latisulcatus* in southern Australia. The aim of this study was to examine the reproductive traits of *P. latisulcatus* by: i) improving the knowledge on the reproductive biology of western king prawns in South Australia and provide a better understanding for maturity indices and ovarian classification methods; and ii) identifying and assessing spawning occurrence in Spencer Gulf, South Australia.

Macroscopic staging is widely used in penaeid fisheries to identify ovarian stage. This research developed an improved microscopic assessment criterion for *P. latisulcatus*, by reviewing microscopic criteria in the literature, and assessing cell composition of ovary tissue from 97 females. The accuracy and precision of macroscopic staging was also determined. Macroscopic staging can be used as a rapid measure of spawning occurrence for penaeids; however, changes to the process of data collection should be incorporated into assessments to improve its accuracy.

Assessing the reproductive biology of penaeids is important in understanding the relationship between a species and its environment. The reproductive biology of *P. latisulcatus* in South Australia's gulfs was described for females with a size range from 21 to 1 mm carapace length (CL), with the smallest ripe female sampled at 30 mm CL. Physical maturity of females was determined by the appearance, shape and colour of the thelycum. Gonadal somatic index (GSI) increased with CL and fecundity varied largely between individuals, ranging from 9,500 to 567,000 eggs counted per female. The thelycum of physically mature females were white and fully formed in shape while the thelycum of immature females were transparent and not fully formed. Size at 50 % physiological maturity was at 21.5 mm CL with the proportion of physically mature females increasing

rapidly at larger sizes. A peak in the reproductive period in November was evident with a change in the number of females reaching reproductive maturity (increase from 9 to 27 %).

Detection of postovulatory follicles (POF) and their degeneration is commonly used to examine spawning incidence and batch fecundity in teleost species. Whilst efficient for fishes, this technique has never been applied to a penaeid prawn. POF were detected in histological examinations of *P. latisulcatus* ovaries and degeneration of POF was experimentally evaluated at 0 to 6, 12, 24, 48, 72 and 96 h post spawning. POF were then histologically classified which allowed clear detection of spawning events from 3 d post spawning. This experimentally derived classification can be used to improve the assessment of spawning incidences in prawns and the estimation of their spawning occurrence. Once histologically classified this assessment was used to identify the occurrence of POF in female *P. latisulcatus* and identify spawning occurrence among *P. latisulcatus* from females caught within a single spawning season in Spencer Gulf South Australia.

The findings presented here provide invaluable knowledge on the reproductive biology of female *P. latisulcatus* in southern Australia. The information can also be used for comparative studies with other penaeid species. The study demonstrates for the first time that POF can be used to detect spawning in a prawn species, which can lead to similar advancements in other prawns. This novel approach can thus improve the understanding of the reproductive biology of other commercially valuable prawn species.

Declaration

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

Authority of access

I consent to this thesis being made available for photocopying, scanning and loan under the Copyright Act, 1968.

Signed

Nadine Emily Hackett

Acknowledgements

This PhD research was conducted as part of a project established between Flinders University and the South Australia Research and Development Institute (SARDI), supported by the Fisheries Research and Development Corporation (FRDC) grant number 2000801.

During the course of my project I have had many supervisors all of whom have contributed to the final thesis some way in another. Dr Toby Bolton and Dr Shane Roberts were supervisors at the beginning of this project and have both helped to shape not only this thesis but myself into becoming a more confident and diligent scientist. Dr Cameron Dixon gave me much guidance and advice in helping to shape this research also. He has displayed tremendous patience on many occasions as I have tried to juggle an often challenging work/life balance. Most importantly, I would like to thank the two greatest supervisors I could have had, who have gotten me over the line, thanks to Professor Sabine Dittmann and Dr Erin Bubner both from Flinders University of South Australia.

Research conducted within this project relied heavily on the support and co-operation of the Spencer Gulf and West Coast Prawn Association, SARDI Ngerin crew and those involved with the prawn subprogram at SARDI. This project would not have been a success without the support of the Spencer Gulf prawn fisherman and their crews who put in an enormous amount of time and energy. The field and laboratory components of this research required a large amount of time and effort and there were a large number of people that assisted in a voluntary or paid capacity. Particular thanks go to Graham Hooper for his assistance not only in the field but also with data collection, maintenance and many other tasks too numerous to mention.

To the staff and students at Lincoln Marine Science Centre, I thank you for your friendship and continued support over the past 8 years. Working at the LMSC has been a wonderful experience and a truly unique environment to work in where I have made lifelong friendships.

I am forever grateful to my parents, Michael and Francesca Hackett, who have always supported and encouraged me to be independent and to follow my dreams. I save the biggest thanks to my husband Richard and my children Rylan and Estelle. This journey has been long and seemingly endless but we have finally made it! Without your love and I would never have gotten this far and I thank you.

I dedicate this thesis in memory of my Mum who passed away during the final stages of its completion. Mum, you are the most inspirational person I have ever met and if I have learnt, anything from you it is to never give up, where there is a will there is a way. One of your most memorable sayings was “if it is meant to be it will be” and your faith in me was always strong. Your fight was long and hard but you never gave in right to the end. Not a day will go by that you will not be in my heart.

Chapter 1: General Introduction

Predicting the responses of organisms and communities to changes in environmental parameters is extremely difficult (Bhaud *et al.* 1995). A potential means of inferring how organisms and communities may respond to such changes is to make comparisons between populations of species that are widely distributed. For example, species of some temperate regions of South Australia (Gulf St Vincent and Spencer Gulf) are also distributed in tropical areas of northern Australia (Dall *et al.* 1990). Those that exist in the temperate areas are subject to highly variable sea temperatures and wide seasonal fluctuations in food resources, while those distributed in tropical waters experience varying wet and dry seasonal conditions (Roberts *et al.* 2012). Sources of variation in maternal investment in offspring include differences in the temperature and salinity of the water that they inhabit (Ives *et al.* 2009). The environmental conditions experienced by females during maturation can have an effect on larval tolerance (Preston 1985a; Preston 1985b).

1.1 Diversity of life history strategies among marine invertebrates

Marine invertebrates display highly diverse reproductive strategies, ranging from broadcast spawning and pelagic offspring (larval) development to direct development of offspring within the adult (e.g. Hossain *et al.* 2012; Jackson *et al.* 2001; Levin and Bridges 1995; Moran and McAlister 2009; Rothlisberg *et al.* 1985). These reproductive modes can be divided into two major patterns: (1) planktotrophy and (2) lecithotrophy. Lecithotrophy can be divided into the non-feeding categories of i) pelagic non-feeding, ii) benthic non-feeding, and iii) intragonadal non-feeding (Levin and Bridges 1995). Planktotrophic larvae are dependent on planktonic food (micro-algae, bacteria) for growth, whereas lecithotrophic larvae utilize maternally supplied energy reserves for development and metamorphosis into the juvenile form. The reproductive strategy employed has profound implications for population genetic structuring, population dynamics and the ecology of marine communities (Pechenik 1999). Consequently, the ecology of marine invertebrate reproductive strategies has been a major focus of marine organismal biology for more than a century.

Broadcast spawning is the predominant method of reproduction among marine invertebrates, and in conjunction with the development of planktotrophic larvae, is widely considered to be the ancestral reproductive mode (Dall *et al.* 1990; Vance 1973). Broadcast spawning involves the release of gametes into the water column (Christiansen and Fenchel 1979; Emlet *et al.* 1987; Havenhand 1995; Pechenik 1999; Thorson 1950; Vance 1973). Evolutionary changes in the reproductive modes of marine invertebrates have led to morphological changes in larvae that involve life-history trade-offs in fecundity, mortality and dispersal potentials (Christiansen and Fenchel 1979). For example, the loss of feeding structures in the evolutionary transition from feeding to non-feeding larval development leads to the need for increased maternal energy investment in offspring to support development and a concomitant reduction in fecundity, planktonic development period, dispersal potential and gene flow (Dall *et al.* 1990). These factors are thought to be "traded-off" in the evolutionary sense by a reduction in mortality rates, although there is little empirical data to support this (Dall *et al.* 1990). Penaeid prawns are unique amongst decapods in that they are broadcast spawners with fertilisation occurring externally (Dall *et al.* 1990). Once hatched the first larval stage called a nauplius emerges and goes through several moults, getting larger with each moult, until changing to a protozoea. The prawn similarly moults and grows through several protozoea then mysis stages before developing into a prawn (Dall *et al.* 1990). The selective pressures driving evolutionary transitions between reproductive strategies remain poorly understood despite much research.

Spawning individuals play an important role within a population as relationships between spawning and recruitment are evident among some penaeid species (Garcia 1996; Penn and Caputi 1986), but can be more complex due to environmental variability affecting larvae and juveniles (Preston 1985a; Preston 1985b). Methods used to identify spawning females by the presence of ripe eggs include: macroscopic staging observations; gonadosomatic index (GSI); histological staging (West 1990) and the population fecundity index (PFI) (Crocos 1987). GSI is the calculation of the gonad mass as a proportion of the total body mass, which increases as the ovary ripens (Penn 1980). Cellular types present within the ovary are identified during histological staging (Courtney *et al.* 1996) and the PFI

developed by Crocos (1987) is an index of population egg production. Each of these techniques have benefits and downfalls with a combination of all three methods used to obtain the most accurate determination of reproductive development (Booth and Weyl 2000).

Macroscopic observations are typically the cheapest and quickest option, while histological staging can be the most time consuming and expensive method (Die *et al.* 1995; Humason 1962). Macroscopic observations are based upon ovary colour, density and shape (King 1948; Tuma 1967). This type of staging can be done in the field with live penaeids where no samples need to be retained.

1.2 Reproduction and general biology of penaeids

There are four types of penaeid life cycles including type 1 which is entirely estuarine, types 2 and 3 having both an estuarine and oceanic phase and type 4 which is entirely oceanic (Dall *et al.* 1990; Marshall and Keough 2006). Generally penaeids prefer substrate with a high proportion of mud content and vegetative cover and change colour to blend in with their environment (Dall *et al.* 1990). Adult females will extrude eggs into the water column and fertilisation occurs as eggs pass through a sperm package contained within the thelycum, with hatching of a pelagic non-feeding nauplius stage occurring within 1 to 2 d after spawning (Dall *et al.* 1990). Over the next 2 to 3 weeks, the young develop through a protozoea stage, followed by a mysis stage and a post larval stage that settles out of the water column into a benthic phase (Dall *et al.* 1990). Post larval penaeids settle in a number of locations including mangrove-lined, muddy estuaries; seagrass or algal beds and over the following 2 to 3 months grow into juveniles. Juveniles are tolerant of a broad range of salinities and may ascend several kilometres upstream to almost freshwater. When they are about half the length of adult size, they leave the estuary and continue to grow, mature, mate and spawn in open offshore waters. *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus*) falls into the type 3 life cycle, preferring high salinity and sheltered inshore waters (Figure 1.1). Seagrasses are the preferred nursery grounds of *P. latisulcatus,*

however, in Western Australia they occur on shallow sandy areas and are also known to inhabit sandy mud or coralline rubble (Dall *et al.* 1990; Kangas and Jackson 1998; Marshall and Keough 2006). This preference for intertidal sand/mud flats by juvenile *P. latisulcatus* differs from that of many other species that are found on vegetated habitats (Dall *et al.* 1990).

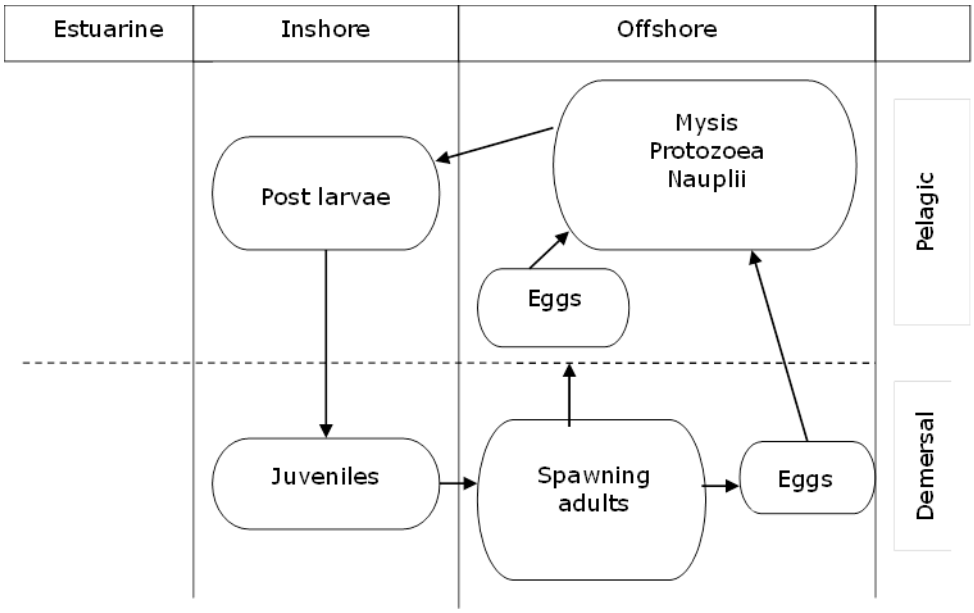


Figure 1.1. Type 3 life cycle of *Penaeus (Melicertus) latisulcatus* from Dall et al. (1990).

The sex of a penaeid is distinguished externally by the appearance of the genitalia. The male petasma is formed by the development of the endopodites of the first pleopod and in mature males they join to form a single organ (Figure 1.2). The role of the petasma is to transfer spermatophores to the ventral surface of the female. Female penaeids have a more complex reproductive system than males, with a pair of ovaries and oviducts that run along the entire length of the female and finish at the thelycum (Figure 1.2) (Dall *et al.* 1990).

Penaeids form two broad groups classified by either having an open or closed thelycum (Dall *et al.* 1990). Those with an open thelycum are found within the waters of the Atlantic coast or North, Central and South America, while those with a closed thelycum are found

within the coastal waters of Australia, Asia, East Africa and the Mediterranean Sea (Yano 1995). Life history strategies among penaeids differ between open and closed thelycum species. At the beginning of the maturation cycle, female penaeids are soft from recently moulting and thus this is when closed thelycum species mate. Open thelycum species, however, mate at the end of the maturation cycle during the intermoult stage either just before or during spawning (Dall *et al.* 1990).

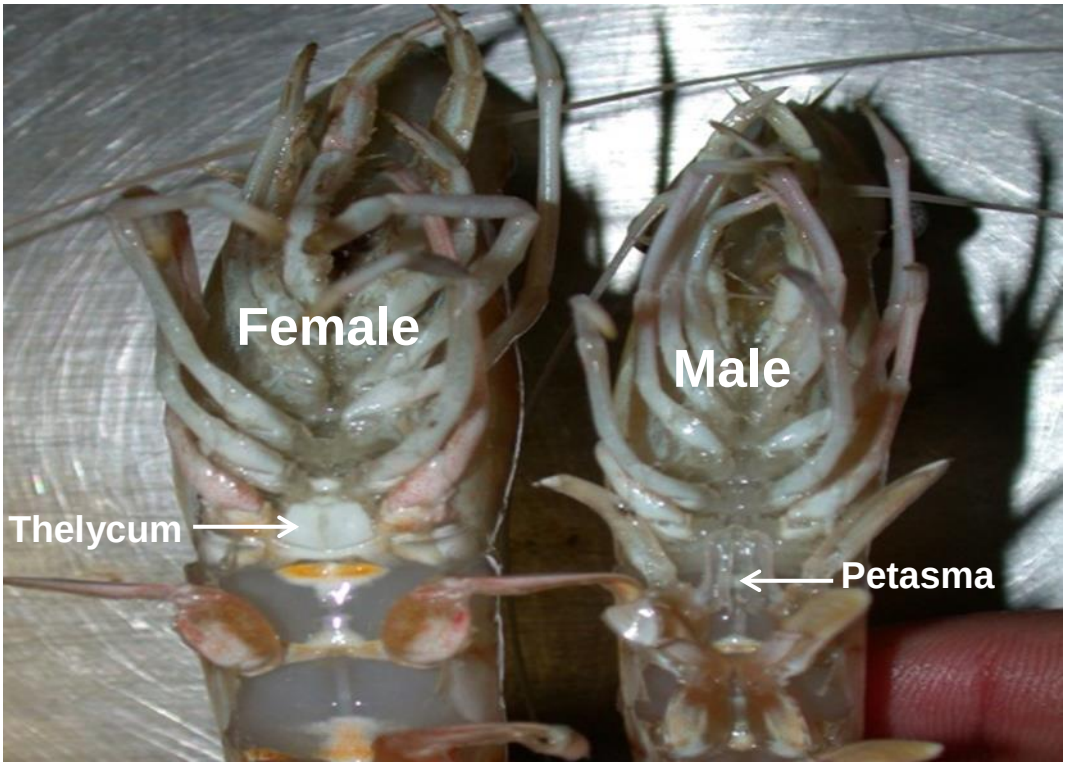


Figure 1.2. Reproductive organs of female and male *Penaeus (Melicertus) latisulcatus*. Displaying the matured thelycum of the female and the clear petasma of the male.

Female penaeid ovaries have development stages that can be observed both macroscopically (visual appearance of the ovary through the external carapace) and microscopically (visual appearance of the ovary viewed under a microscope after histological preparations have been carried out). Ovaries progress through a series of changes involving size and colour during development. For *P. latisulcatus*, these stages have been observed and grouped into five stages beginning with an undeveloped ovary and finishing with a spent (recently spawned) ovary. The five stages of development

classified by Tuma (1967) (Table 1.1) identify ovarian development for both microscopic and macroscopic techniques within female *P. latisulcatus*.

Table 1.1. Stages of development for female *Penaeus (Melicertus) latisulcatus* adapted from Tuma (1967).

	Microscopic	Macroscopic
Undeveloped	Stage 1	Stage 0
Developing	Stage 2	Stage I and II
Early Maturity	Stage 3	Stage III
Ripe	Stage 4	Stage IV
Spent	Stage 5	-

1.3 Biology of *Penaeus (Melicertus) latisulcatus* (Kishinouye 1896)

The western king prawn is distributed throughout the Indo-West Pacific (Grey *et al.* 1983). Its distribution in South Australia is at its lowest temperature range, restricted to waters of Spencer Gulf, Gulf St Vincent and the West Coast (Dixon *et al.* 2006; Kangas and Dixon 2008). They are an easily accessible species inhabiting both tropical and temperate environments (Dall *et al.* 1990; Holthuis 1980). Published aspects of the biology and ecology of *P. latisulcatus* are generally limited to studies conducted in warmer waters of Western Australia (e.g. Hall and Penn 1979; Penn 1975; Penn 1976; Penn 1980; Rothlisberg and Jackson 1987; Rothlisberg *et al.* 1987) and Queensland (Courtney and Dredge 1988). Few studies relating to population dynamics have been published for South Australian populations (Kangas and Dixon 2008).

Populations of *P. latisulcatus* in South Australia are geographically isolated from their northern counterparts and there is evidence of differences in the life history strategies of these organisms between temperate and tropical regions (Dall *et al.* 1990). Specifically, spawning and fecundity are affected by water temperature: in tropical waters of the Gulf of Carpentaria, *P. latisulcatus* showed peaks of early larval abundance in September and

January, with spawning occurring over a temperature range of 10 C° (Penn 1980; Rothlisberg and Jackson 1987). Courtney and Dredge (1988), showed that *P. latisulcatus* had a peak in reproductive periods at 25 °C in June and July, while those in temperate waters appear to reproduce only a few times during the austral summer months (Dixon *et al.* 2012). Unpublished data also suggest that a number of reproductive traits differ markedly in South Australia compared to tropical regions, including size at first maturity, the proportion mature and insemination rates (Carrick 2003). Genetic differences have been detected between populations of *P. latisulcatus* from the Gulf of Carpentaria and Western Australia (Mulley and Latter 1981). The measures of genetic divergence between these populations does exist, however, there is currently no evidence to suggest that genetic divergence exists between the two stocks of *P. latisulcatus* in Southern Australia.

Mating behaviour is triggered by moult cycle in *P. latisulcatus* as they have a closed thelycum and mate soon after the female has moulted while the cuticle is still soft (Dall *et al.* 1990). As moulting occurs at night, most mating is thought to be nocturnal. A male will follow a female along the bottom and attempt to get underneath her. The female will leave the bottom and be chased by the male who will then grasp the female from underneath so that the spermatophore may be transferred to the thelycum (Dall *et al.* 1990). In *P. latisulcatus* the orientation of the male is not known nor how long the position is maintained. Mating behaviour ascribed for *P. latisulcatus* here is inferred from limited observations on other species, as it has not been directly observed.

1.4 Environmental variation and reproductive strategies

Predicting the responses of organisms and communities to changes in environmental parameters is extremely difficult. A possible means of inferring how organisms and communities may respond to such changes is to make comparisons between populations of species that are distributed across environments that experience different environmental conditions. While comparisons of maternal investment have been made between populations of species inhabiting locations that have different conditions (George 1994;

George 1996) few studies have been undertaken on *P. latisulcatus* within its distribution range.

Temperature is correlated strongly with reproduction and often drives the onset and completion of each breeding cycle. Generally, warmer temperatures mark the onset of the breeding season with the colder weather bringing it to a close (Sakai and Harada 2001). Yet, in tropical locations spawning can occur throughout the entire year showing that individuals may spawn at a constant continual rate (Emlet *et al.* 1987). The relationship between moulting and the onset of spawning is thought to be strongly affected by local environmental conditions experienced by both female and males. Courtney *et al.* (1996) and Smith and Sainte-Marie (2004) have both shown that moulting in females is initiated by the lunar phase, which has a strong effect on reproduction as generally a female is inseminated directly after moulting while she is still soft.

Penn (1980), Carrick (2003) and Courtney and Dredge (1988) have shown that there has been little research history on *P. latisulcatus* despite its economic importance. Pivotal questions regarding spawning periodicity and spawning occurrence have not been resolved for this species.

1.5 Thesis scope and outline

Many factors can influence a species population dynamics, with environmental stressors such as temperature change becoming recognised as a key factor. Despite the fact that *P. latisulcatus* is the basis of a major commercial fishery, little is known about its population dynamics in southern Australia compared with sub-tropical and tropical populations of the species. Of particular interest is the understanding of reproductive biology and how reproductive traits vary across temperate and tropical environments specifically in comparison to studies from Penn (1980), Rothlisberg et al. (1987) and Rothlisberg and Jackson (1987).

An understanding of these questions can be gained by examining the reproductive strategies of *P. latisulcatus* in different environmental conditions and determine what effect they have on reproductive output. The primary objective of the research was to increase the knowledge base for *P. latisulcatus* in temperate South Australian waters. Studies were conducted in two parts: Part I: Ovarian stages (1) microscopic analyses of ovarian stages of *P. latisulcatus*, (2) Postovulatory follicles (POF) in a penaeid prawn: detection and histological classification of degeneration up to 96 h post spawning and (3) validation of visual determinations of ovary development stages. Part II: Reproductive biology of *P. latisulcatus* (1) reproductive parameters of *P. latisulcatus* including size at 50 % maturity, percent mature and GSI and fecundity (2) assessment of maturity periods through POF. This thesis concludes with a summary of the main results from each area investigated, how the research will contribute to the knowledge base for *P. latisulcatus* and directions of future research from the findings presented here.

Part 1: Ovarian stages

Chapter 2: Microscopic analysis of ovarian stages of the western king prawn *Penaeus (Melicertus) latisulcatus* (Kishinouye 1896)

2.1 Abstract

The determination of ovarian stages is essential for an understanding of reproductive biology and for the sustainable management of commercially caught marine organisms. This research developed improved microscopic assessment criteria for the prawn, *Penaeus (Melicertus) latisulcatus*, by reviewing microscopic criteria used in the literature, and assessing cell composition of ovary tissue from 97 females. Whilst histological staging is not a new technique, this research outlines new development criteria for assessing ovary development stages in *Penaeus (Melicertus) latisulcatus*. A principal component analysis (PCA) was used with the microscopic classification system, and showed distinct groups for each stage. Based on the proportion of cell types, distinct groupings were apparent, which corresponded to the main microscopic stages, in particular for stage 3 ovaries.

2.2 Introduction

Penaeids represent an important resource worldwide. In particular, the western king prawn *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus*) is an easily harvestable species inhabiting both tropical and temperate environments (Dall *et al.* 1990; Holthuis 1980). *Penaeus latisulcatus* is distributed through the Indo-West Pacific (Grey *et al.* 1983). In South Australia, its distribution is at its lowest temperature range, restricted to waters of Spencer Gulf, Gulf St Vincent and the West Coast (Dixon *et al.* 2006; Kangas and Dixon 2008). Data on maturity and fecundity, as well as the timing, frequency and location of spawning events, are central to the understanding of reproductive biology (Garcia 1996; Watson and Sumner 1999). However, there are substantial knowledge gaps in reproductive biology for many species that may be further compounded by inaccuracies or uncertainties surrounding available data, or the methods used to generate them.

Species-specific changes in the characteristics of ovary development may explain disparities in the accuracy of macroscopic staging techniques among species (e.g. Peixoto *et al.* 2003; Quintero and Garcia 1998). Olney *et al*. (2001) demonstrated that macroscopic and microscopic staging are not interchangeable, with the greatest disagreement observed in the macroscopic staging of spent and partially spent American Shad (*Alosa sapidissima*). Maturing and partially spent ovaries were often confused when using macroscopic methods, and more accurate counts were obtained through histological examination (Olney *et al.* 2001). Irrespective of whether there are differences in the accuracy of assigning ovary development stages using macroscopic methods among species, establishing the accuracy of the technique through accompanying microscopic analysis of stages should be made in all cases. At present, the use of ovary stage descriptions varies widely in the literature.

The aim of this study was to describe each of the six histological stages of ovary development of *P. latisulcatus* and combine them into a classification table with data available from previous research (Tuma 1967). Past literature and cell composition data were examined in the current study to develop an improved classification scheme based on microscopic criteria.

2.3 Materials and methods

2.3.1 Sample collection

Female *P. latisulcatus* were collected using a demersal otter trawl in Gulf St Vincent, South Australia, within the range of 34°45'S, 138°15'E and 34°47'S, 138°15'E on the 23rd November 2009. This area lies within the main fishing grounds for *P. latisulcatus* in Gulf St Vincent. Trawls were undertaken at night using the South Australian Research and Development Institute's (SARDI) research vessel R.V. Ngerin. The double-rig trawl gear had a maximum total headline length of 29.26 m and a minimum mesh size of 4.5 cm. Each net was fitted with a codend consisting of a PVC protective mat and 44 mm diamond mesh. A random sample of female prawns was obtained from the trawl catch and 100 individuals tagged for identification. Of these 100 females 98 were used for histological analysis, with 1

stage 4 female later excluded from the analysis due to insufficient samples for this stage. Therefore a total of 97 females were used in this analysis.

2.3.2 Fixation and histological analysis of specimens

Specimens were fixed in a solution of formalin, glacial acetic acid and calcium chloride (FAACC) (Humason 1962; Kaplan and Amadeo 1996) within 2 h of capture and held in this solution for 48 h, and then transferred to 70 % ethanol. Ovaries were removed following the methods given by Montgomery *et al.* (2007b) and weighed (± 0.01 g) using an electronic balance. For consistency, sections of tissue from the mid-region of ovaries were then dissected for histological analyses. Previous studies indicate that there are no differences in the distribution of oocyte stages among areas of the ovary (Montgomery *et al.* 2007b). Dissected ovary tissue was embedded in paraffin and sectioned (6 µm thickness) using a microtome. Two sections of ovary tissue from each specimen were placed onto microscope slides and stained with haematoxylin and eosin.

Sections were digitally imaged through a compound microscope (Olympus BX51) fitted with an Olympus C-7070 camera at 100 × magnification. Images were examined using Image J software. Three lines (1000 µm length) were drawn randomly across scaled images of each section of ovarian tissue using the software, and the proportions of oocytes and other cell types falling along these lines that were indicative of different stages of ovary development were recorded. Criteria given by Tuma (1967) and Courtney (1995b) were used to identify ovarian cell types and included oogonia, peri nucleolus, vitellogenic, cortical specialisation, elongated cortical specialisation, follicle cells, post-ovulatory follicle cells, tissue and vacant space. Once each cell type had been recorded, the proportion within each individual was calculated and used to assign an ovarian development stage.

A principal component analysis (PCA) was carried out on non-transformed data and Euclidian distance using PRIMER (v6) to assess the criteria set for determining microscopic oocyte stages.

2.4 Results

2.4.1 Histological stages

After examining criteria by King (1948), Tuma (1967), King (1979), Courtney (1995b) and Montgomery *et al.* (2007b), it was apparent that a more species specific set of criteria was needed to define the microscopic stages of penaeids for *P. latisulcatus*. Consequently, a specific set of criteria for *P. latisulcatus* was designed for this study. Microscopic ovarian stage was determined for each specimen by the proportion of the derived percentages of the cell type present (Table 2.1).These newly developed objective criteria for *P. latisulcatus* were added to a comparative table (Table 2.1).

Six ovary development stages were recorded based on microscopic criteria (Table 2.1, Figure 2.1 – 2.5). Oogonia and perinucleolus stages represent developing ovaries with cell types typically more than 50 % in the perinucleolus stage and less than 20 % vitellogenic and equal percentages of perinucleolus and vitellogenic cell types respectively (Table 2.1). Oogonia stage ovaries were classified by a high presence of perinucleolus cell types staining basophilic with visible nucleoli and follicle cells while the perinucleolus stage also have oil globules and yolk granules present (Figure 2.1). Vitellogenic stage oocytes were characterised by more than 40 % of the cells being vitellogenic and less than 40 % post vitellogenic, with the oocytes acidophilic (Figure 2.2). Cortical specialisation is derived by the presence of tubular cortical crypts in more than 40 % of the vitellogenic cells (Table 2.1, Figure 2.3). Germinal vesicle break down occurs when the cortical crypts become elongated in shape with the oocytes and there are more than 50 % present within the oocyte (Table 2.1, Figure 2.4). The fifth and final stage of ovary development is the spent stage, which is characterised by collapsed or distended oocytes and the presence of postovulatory follicle cells (Table 2.1, Figure 2.5).

Based on the proportion of cell types derived, distinct groupings were apparent for *P. latisulcatus* among the cell types of perinucleolus, vitellogenic and cortical specialisation

(Figure 2.6). These cell types corresponded to the main microscopic stages, in particular for cortical specialisation type cells which showed the most distinct grouping.

Table 2.1. Comparison of macroscopic and microscopic staging of ovary development used by King (1948), Tuma (1967), King (1979), Courtney *et al.*, (1995b) and Montgomery *et al.*, (2007b) used to develop objective assessment criteria for *Penaeus (Melicertus) latisulcatus* in this study (S is stage, Oo is Oogonia, PERI is perinucleolus, VIT is vitellogenic, CS is cortical specialisation, GVBD is germinal vesicle break down, Sp is spent, postVIT is postvitellogenic cells, FC is follicle cells, Ep early primary oocytes, Lp late primary oocytes, Ev early vitellogenic oocytes, Lv late vitellogenic ooctyes) Percentage refers to current percentage of a cell type.

Macroscopic staging						Microscopic staging					
	King, J.E., 1948	Tuma, D.J., 1967	King, M.J., 1979	Montgomery *et al*., 2007	Newly developed objective criteria		King J.E., 1948	Tuma D.J., 1967	King M.J., 1979	Courtney *et al*., 1995	Montgomery *et al*., 2007	Newly developed objective criteria
Stage	*P. setiferus*	*P. merguiensis*	*P. latisulcatus*	*P. plebejus*	*P. latisulcatus*	Stage	*P. setiferus*	*P. merguiensis*	*P. latisulcatus*	*P. plebejus*	*P. plebejus*	*P. latisulcatus*
0	Small in size Transparent in nature Undeveloped	Diameter of ovarian lobes much smaller than diameter of adjacent gut	Ovary not evident through the carapace Gut and muscle are visible through carapace	Ovary is transparent and no thicker in diameter than the adjacent gut	Ovary cannot be seen through the carapace Gut and muscle tissue is visible through the carapace	Oo	Oocytes are uniformly small and stain blue (basophilic) with haematoxylin-eosin (H&E)		Scattered basophilic oocytes Oviduct visible Lots vacant space		Primarily oocytes of PERI stage, FC and tissue < 2 % VIT cells <1 % CS	Oocytes stain basophilic with visible nucleoli and follicle cells More than 50 % of oocytes are perinucleolus Less than 20 % of oocytes are vitellogenic or Less than 10 % are postVIT
1	Glands increase in size Ovaries become opaque Developing	Diameter of ovarian lobes similar to diameter of adjacent gut	Ovary translucent and not visible through carapace Gut and muscle visible	Ovary appears as two light yellow lines along the abdomen no thicker in diameter than the adjacent gut	Ovary is translucent to milky white in colour Ovary cannot be seen through the carapace Gut and muscle are still visible	PERI	Oocytes are uniformly small and stain blue (basophilic) with H&E		^cell size of 30 to 60µm Oocytes stain basophilic, Nucleoli are visible FC present		25 % PERI and VIT Less FC, tissue and space than in stage 0 <1 % CS	Oocytes stain basophilic Oil globules and yolk granules are visible Ovary contains equal percentage of perinucleolus and vitellogenic cells or There is less than 40 % vitellogenic and less than 10 % postVIT

Macroscopic staging						Microscopic staging						
	King, J.E., 1948	Tuma, D.J., 1967	King, M.J., 1979	Montgomery *et al*., 2007	Newly developed objective criteria		King J.E., 1948	Tuma D.J., 1967	King M.J., 1979	Courtney *et al*., 1995	Montgomery *et al*., 2007	Newly developed objective criteria
Stage	*P. setiferus*	*P. merguiensis*	*P. latisulcatus*	*P. plebejus*	*P. latisulcatus*	Stage	*P. setiferus*	*P. merguiensis*	*P. latisulcatus*	*P. plebejus*	*P. plebejus*	*P. latisulcatus*
2	Yellowish which deepens to a yellowish-orange	Diameter of ovarian lobes larger than diameter of adjacent gut Two distinct size groups of ova	Ovary pale yellow and not visible through the carapace Gut and muscle visible	Ovary is yellow to orange in colour, thicker in diameter than the adjacent gut Two distinct lobes occupy a majority of the space in the cephalothorax	Ovary is pale yellow in colour Ovary cannot be seen through the carapace Gut and muscle are still visible	VIT	Large oocytes which stain red (acidophilic) Some smaller oocytes which stain blue (basophilic)		^cell size to 210µm with nucleus basophilic Oil globules and yolk granules acidophilic Less space between cells Small groups PERI		Predominantly oocytes of VIT stage Follicle cells, tissue and space are less than stage 0 and I 4 % CS	Nucleus is basophilic Oil globules and yolk granules are acidophilic There are greater than 40 % of cells in the vitellogenic stage There are less than 40 % of cells in the postVIT stage
3	Olive-brown colour Ovary distended and fills all available space of the body cavity Clearly visible through abdomen	Diameter of ovarian lobes is much larger than diameter of adjacent gut	Ovary yellow and visible through the carapace with red chromatophores Muscle partially obscured	Ovary is brown to olive in colour Granular in appearance Thicker in diameter than the adjacent gut Two distinct lobes fill space in the cephalothorax	Ovary is yellow and is visible through the carapace Muscles are becoming partially obscured within the carapace due to increased size of ovary	CS	Most oocytes are fully matured and stain red (acidophilic) Possess rod-shaped bodies arranged radially in the cytoplasm which stain red Few or no oocytes stain blue with H&E		Oocyte diameter increase to 228µm Presence of cortical crypts Nucleus stain basophilic cytoplasm and cortical crypts stain acidophilic		Predominantly oocytes in CS stage Proportions of oocytes in PERI FC, tissue and space are less than in stage II 6 % VIT	Nucleus is basophilic Cytoplasm and cortical crypts are acidophilic Greater than 40 % of vitellogenic cells contain predominant tubular cortical crypts within oocytes

Macroscopic staging						Microscopic staging						
	King, J.E., 1948	Tuma, D.J., 1967	King, M.J., 1979	Montgomery *et al*., 2007	Newly developed objective criteria		King J.E., 1948	Tuma D.J., 1967	King M.J., 1979	Courtney *et al*., 1995	Montgomery *et al*., 2007	Newly developed objective criteria
Stage	*P. setiferus*	*P. merguiensis*	*P. latisulcatus*	*P. plebejus*	*P. latisulcatus*	Stage	*P. setiferus*	*P. merguiensis*	*P. latisulcatus*	*P. plebejus*	*P. plebejus*	*P. latisulcatus*
4			Ovary orange and covers most of the internal organs with red chromatophores prominent Muscle obscured		Ovary is orange in colour Covers a large area within the carapace Muscles are not visible	GVBD			Oo are basophilic Reabsorbing oocytes acidophilic Empty cysts Some FC Cortical crypts become elongate			Greater than 50 % of cells are vitellogenic Cortical crypts are elongated within oocytes Any oogonia within the cells are basophilic
5	Collapsed distended condition Not as deeply coloured as ripe phase Colour disappears structure remains opaque	Ovarian lobe are flaccid and very much convoluted	Not distinguishable macroscopically	Ovary is translucent, convoluted and thicker in diameter than adjacent gut Two translucent lobes are still evident in the cephalothorax	Cannot be distinguished macro-scopically as very similar to a stage 0 ovary	Sp	Variable quantity of matured oocytes undergoing resorption Wholly constituted oocytes are uniformly small as in stage 0/1 Stain blue (basophilic)				Oocytes in CS stage < in stage IV Proportions of oocytes at the PERI stage and tissue are greater than at stage IV	Collapsed or distended oocytes Presence of postovulatory follicles The proportion of cells in the perinucleolus stage has increased from the previous stage

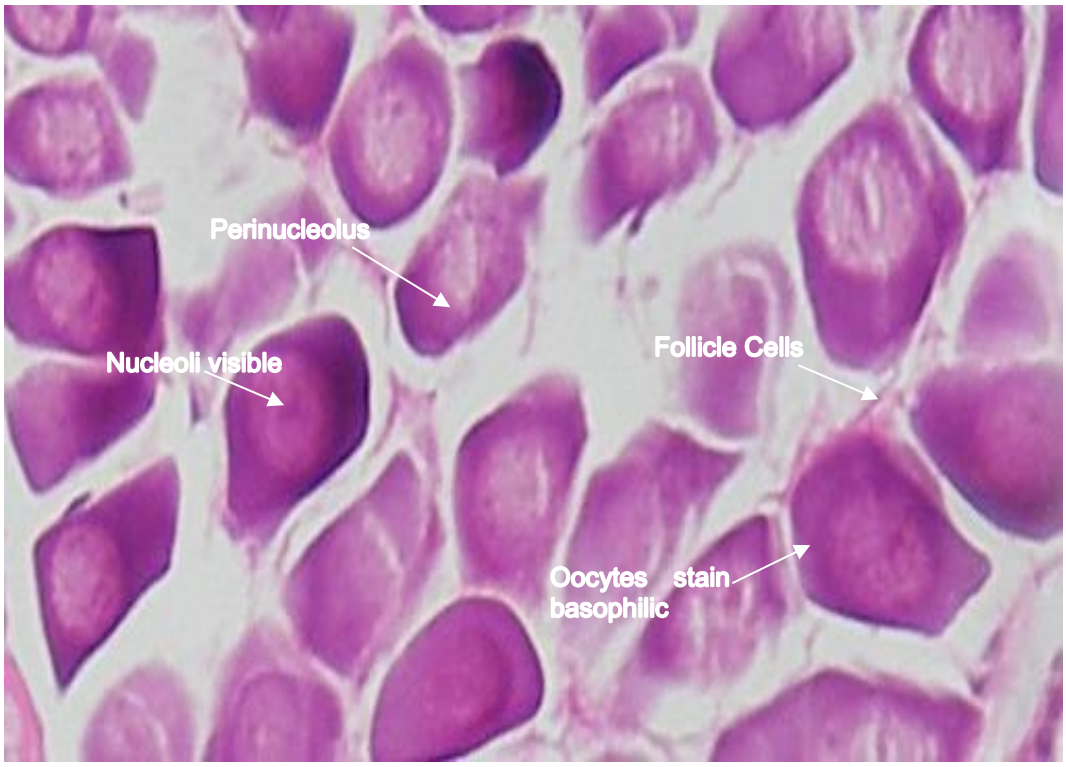


Figure 2.1. Characteristics of ovary development visible under microscopic examination (100 x) in histologically prepared sections of ovarian tissue of *Penaeus (Melicertus) latisulcatus*: for stage 0 and 1 with perinucleolus type cells (developing).

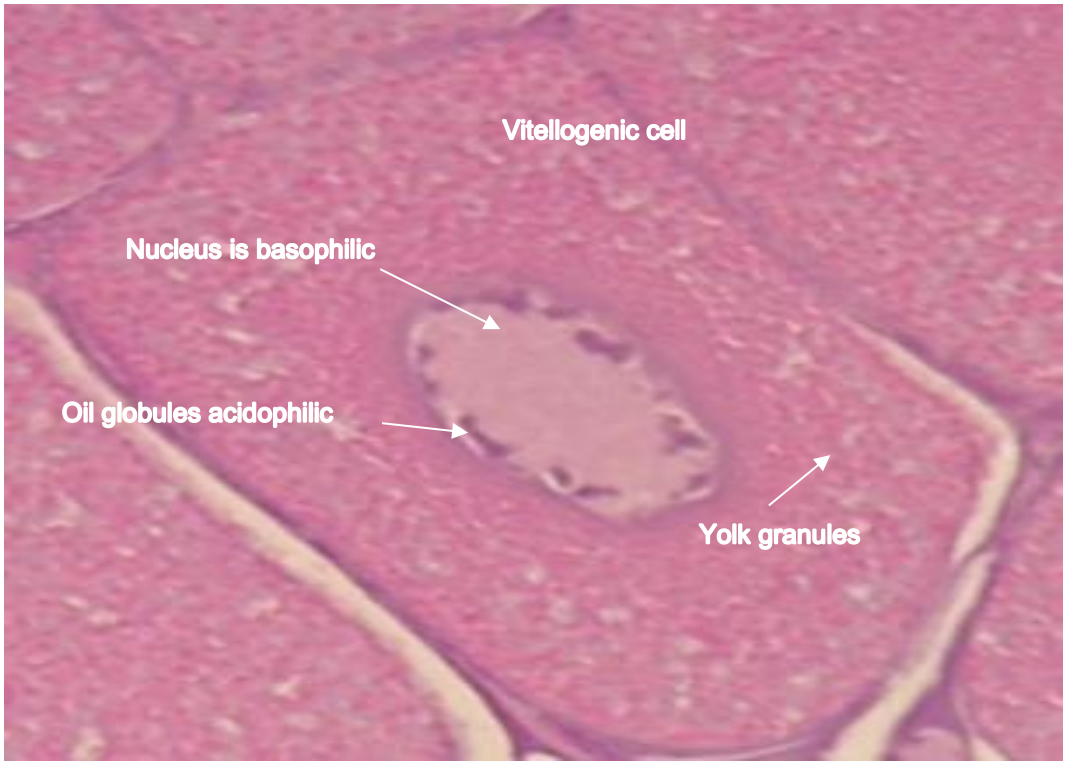


Figure 2.2. Characteristics of ovary development visible under microscopic examination (100 x) in histologically prepared sections of ovarian tissue of *Penaeus (Melicertus) latisulcatus*: for stage 2 with vitellogenic type cells (early maturity).

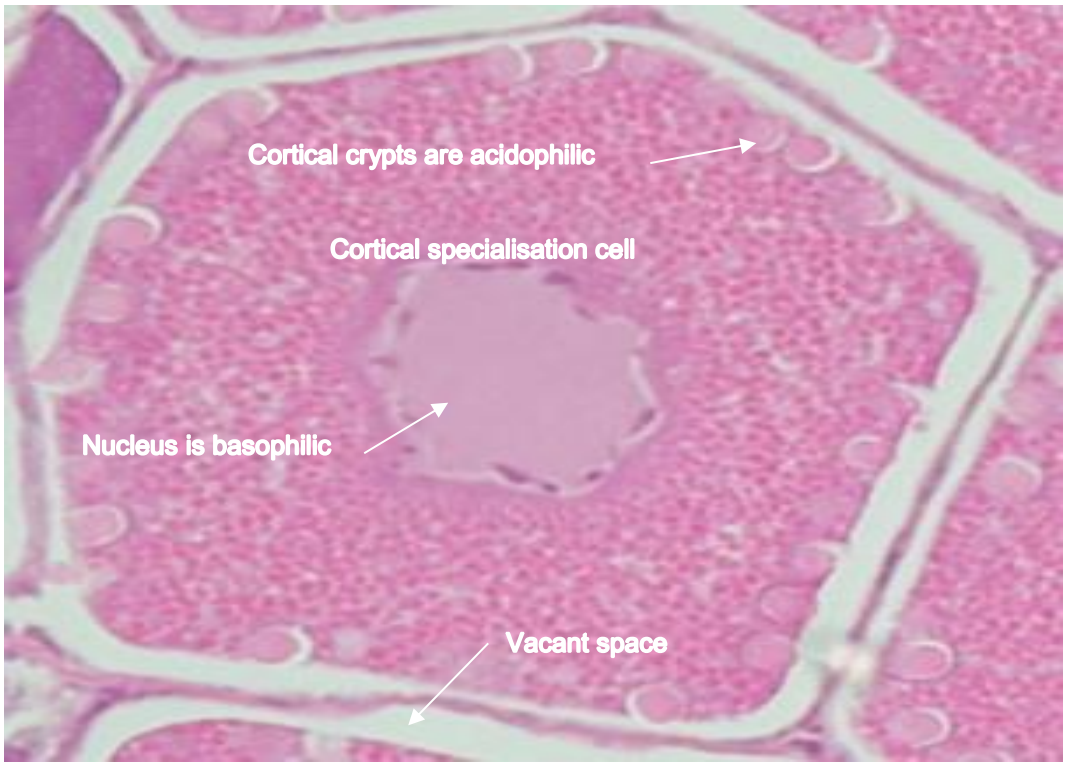


Figure 2.3. Characteristics of ovary development visible under microscopic examination (100 x) in histologically prepared sections of ovarian tissue of *Penaeus (Melicertus) latisulcatus*: for stage 3 or cortical specialisations (early ripe).

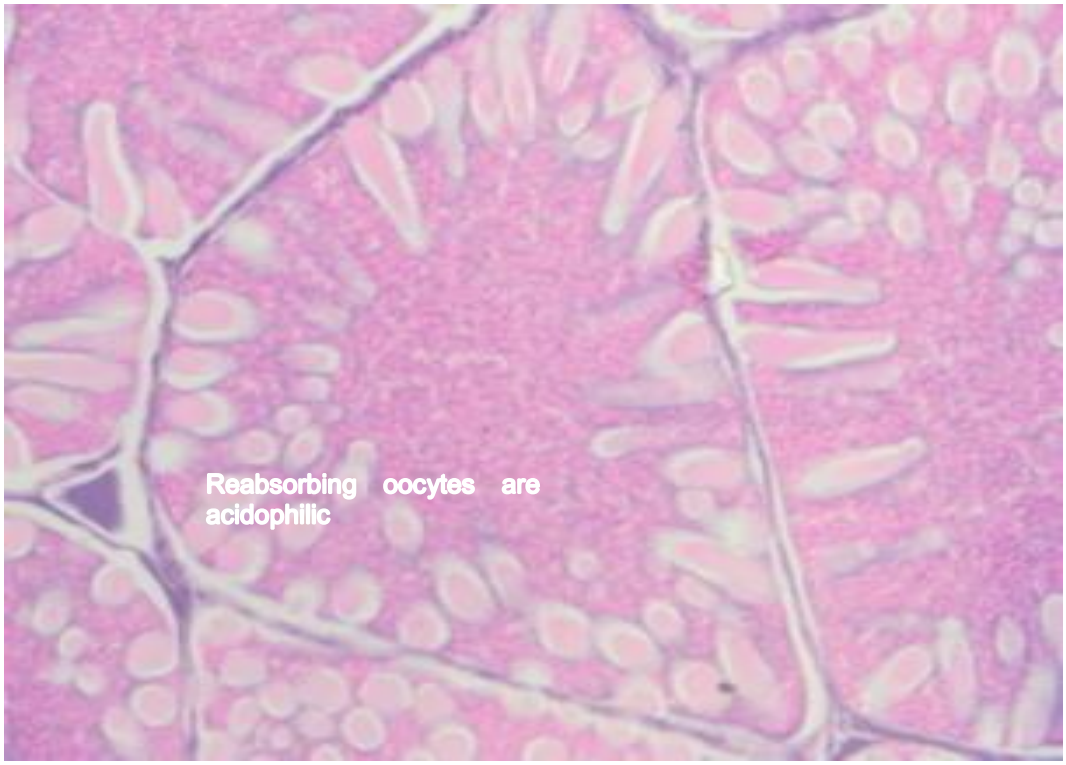


Figure 2.4. Characteristics of ovary development visible under microscopic examination (100 x) in histologically prepared sections of ovarian tissue of *Penaeus (Melicertus) latisulcatus*: for stage 4 or germinal vesicle break down (ripe with elongated cortical crypts).

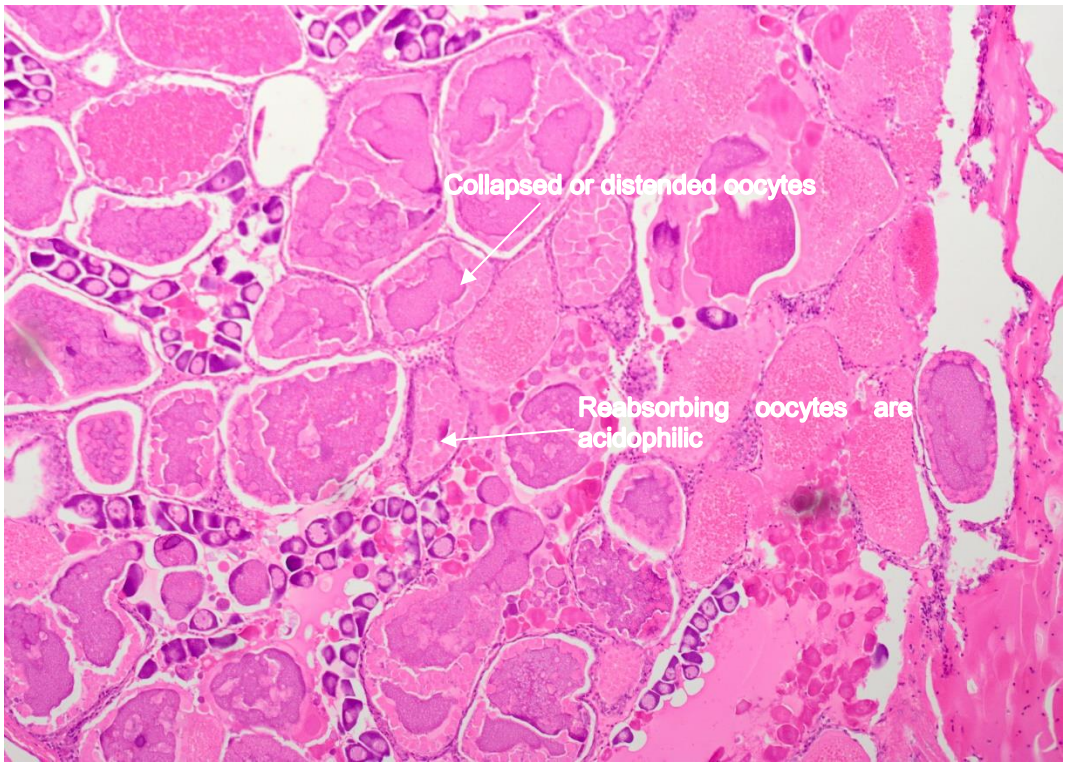


Figure 2.5. Characteristics of a stage 5 spent ovary visible under microscopic examination (100 x) in histologically prepared sections of ovarian tissue of *Penaeus (Melicertus) latisulcatus*.

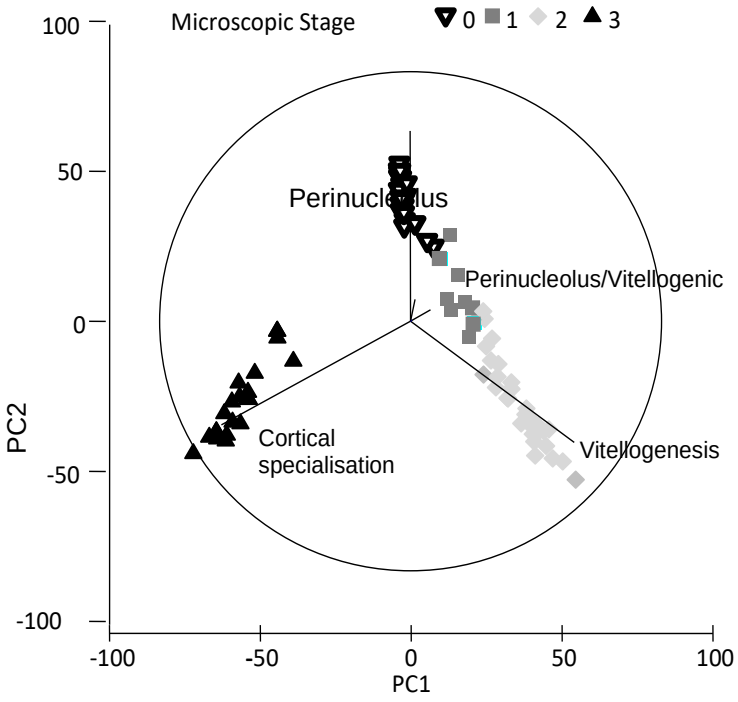


Figure 2.6. PCA plot of cell types, corresponding with the microscopic stages of *Penaeus (Melicertus) latisulcatus* using the newly developed criteria within this study. Oocyte development stages are perinucleolus (developing) for stage 0, perinucleolus/vitellogenic for stage 1, vitellogenesis for stage 2 and cortical specialisation for stage 3.

2.5 Discussion

The microscopic staging criteria developed in this study effectively discriminated each of the six stages of oocyte development. Histological analysis of *P. latisulcatus* ovaries demonstrated a gradual process of development within the oocytes. Six types of cells were distinguished including: oogonia, perinucleolus, vitellogenic, cortical specialisation, elongated cortical specialisation and postovulatory follicles. With this classification it was possible to determine the percentage of each cell type within the six stages and hence assign a percentage of cell types to each.

P. latisulcatus females have previously been described to have six stages of gonad development (King 1979; Penn 1980), however, all of these previous classifications give little information regarding the cell types associated with each of the stages (Table 2.1). Montgomery et al. (2007b) gave a brief classification of the cell types and percentages found with each stage for *Melicertus plebejus*, however, more comprehensive criteria were necessary for *P. latisulcatus*. Data presented here showed that a distinct grouping occurs between the proportions of cell types within each stage. The development of oocytes for *P. latisulcatus* was similar to that described by King (1979) for microscopic staging. Whilst microscopic staging descriptions were common amongst the species compared, none of the previous studies were specific enough to identify the microscopic stages for *P. latisulcatus*.

Overall, this study demonstrated that a more comprehensive histological classification of *P. latisulcatus* was necessary to evaluate oocyte development and puts forward a new process for the evaluation of microscopic stages with the addition of the newly developed objective criteria (Table 2.1). The success of identifying prominent cell types within each stage suggests that this method may be applied to other penaeids.

Chapter 3: Postovulatory follicles in a penaeid prawn: detection and histological classification of degeneration

3.1 Abstract

Detection of postovulatory follicles (POF) and their degeneration is commonly used to examine the frequency and timing of spawning in teleost species. Whilst efficient for fishes, this technique has never been applied to a penaeid prawn species. POF were detected in histological examination of ovaries of the western king prawn *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus*) and criteria developed to age POF. Female prawns were held in an experimental system under constant observation, and after spawning occurred, they were kept for set treatment times. The presence and degeneration of POF were then evaluated from prawns that had recently spawned (0 to 6 h) and at several subsequent intervals up to 96 h post spawning. Females from 0 to 24 h post-spawning were categorised as early spawners with POF having a clearly defined cell wall containing oval shaped follicles and a large amount of space within the cell. After 24 h this space shrank and the degeneration of POF became more visible. Histological classification of POF presented here allows clear detection of spawning events for up to 4 d post spawning. This method can be used to improve the assessment of spawning in prawns.

3.2 Introduction

Postovulatory follicles (POF) detection is a reliable indicator for estimating the frequency and timing of spawning in a population for many vertebrate species such as fish (Alday *et al.* 2008; Drummond *et al.* 2000; Goldberg *et al.* 1984; Hunter and Goldberg 1980). POF are produced after ovulation and consist of follicular layers that remain in the ovary, with the formation and degeneration process varying over time and between species (Saidapur 1982). Following ovulation, the POF initially has a distinct structure; however, this rapidly deteriorates and becomes undetectable within days (Hunter and Goldberg 1980). In teleosts, postovulatory follicles are rapidly reabsorbed and degenerate as the follicular cells develop phagocytic activity (Hunter and Goldberg 1980).

The technique of identifying and classifying POF has been well documented among vertebrate species, however, no research has been carried out on the identification and degeneration of POF in crustaceans, such as penaeids, with the exception of oocyte development of the freshwater prawn *Macrobrachium amazonicum* where POF morphology has been described (da C. Chaves and Magalhaes 1993). The classification of POF in penaeid prawns to identify spawning period has yet to be shown.

If applicable to Crustacea, the use of POF will allow the identification of the time of spawning within individual prawns as well as among populations. This study aimed to detect and age POF and assign a spawning time so that future assessment of spawning may be made from histological examination. The prawn species used was the western king prawn, *Penaeus (Melicertus) latisulcatus* which is distributed throughout the Indo-West Pacific and is the basis of valuable fisheries in northern and southern Australia (Grey *et al.* 1983; Penn 1980; Xiao 2004). Throughout its tropical range, *P. latisulcatus* exhibits peak reproductive outputs over a temperature range of around 10 °C. Rather than being a serial spawner, *P. latisulcatus* had peaks of early larval abundance in September and January (Courtney and Dredge 1988; Rothlisberg and Jackson 1987). In contrast, temperate South Australian populations of *P. latisulcatus* only reproduce when water temperatures rise above 17 °C resulting in only one reproductive period during the year (Dixon *et al.* 2009; Roberts *et al.* 2012). The reproductive activity of temperate *P. latisulcatus* occurs from October to February, with a spawning peak in November (Dixon *et al.* 2009) while larval growth and survival is greatest from December spawning (Rodgers *et al.* 2013). Consequently, current management arrangements for the fishery protect the spawning biomass during November/December fishing (Dixon *et al.* 2010; Rodgers *et al.* 2013). Populations of *P. latisulcatus* in temperate latitudes reach reproductive maturity at a smaller size than tropical populations (Courtney and Dredge 1988; Dixon *et al.* 2009; Roberts *et al.* 2009), with a postulated greater size-specific fecundity (Dixon *et al.* 2009).

Recording the occurrence spawning in *P. latisulcatus* is important for establishing the timing of spawning. Spawning occurrence has been related to moult frequency, based on the fact

that no recently moulted females have been found with well-developed (stage 3 or 4) ovaries, (Courtney and Dredge 1988; Crocos 1987; Crocos and Kerr 1983; Penn 1980); females lose their spermatophores with the exuvae at moult (Crocos and Kerr 1983; Penn 1980) and; the average interval for both moulting and spawning has been shown to be the same (Penn 1980). Crocos and Kerr (1983) describe the sequence of events between the moult and maturation cycles for *P. merguiensis* with insemination occurring immediately after moulting. The average moult interval for mature females and hence spawning interval, during the spawning season in Western Australia was estimated at 30 to 40 d (Penn 1980). As yet, spawning occurrence within the reproductive period as well as size-specific variation of spawning occurrence is also unknown for populations of *P. latisulcatus* in South Australia. This experimental investigation therefore aimed at identifying the presence of POF in *P. latisulcatus* and to classify histological change (degeneration) for up to 96 h post spawning, adopting methods used by Hunter and Goldberg (1980). The process of identifying and classifying POF in a penaeid species constitutes a new technique for penaeid research, which will advance the assessment of spawning occurrence among individuals.

3.3 Materials and methods

3.3.1 Collection of *P. latisulcatus*

Female *P. latisulcatus* were collected on 6th November 2010 during a 1 d survey near Ceduna, west coast of South Australia and from a 2 d survey on 27th and 28th October 2011 near Wallaroo, Spencer Gulf, South Australia (Figure 3.1). All sampling was carried out at depths ranging between 10 to 45 m. The geographic range of sampling covered the main fishing grounds for *P. latisulcatus* within South Australia, with the exception of the Gulf St Vincent fishery. Samples were taken using a demersal otter trawl after dusk on a commercial fishing vessel. The double-rig gear had a maximum total headline length of 26 m and a minimum mesh size of 4.5 cm. Each net was also fitted with a codend consisting of a PVC protective mat and a mesh bag with 150 mm mesh to keep crabs out of the main catch.

Sixty mature female *P. latisulcatus* were retained during each sampling trip according to the relative size and shape of their ovaries that were visible through the dorsal exoskeleton. These prawns were staged according to criteria given by King (1979). Specifically, female prawns were deemed physiologically mature if they possessed gonads at stage 2, 3 or 4. Those determined to be at stage 4 of maturity were retained and placed into one of six plastic baskets (approximately ten prawns per basket measuring 45 mm in length, 30 mm width and 15 mm height). Plastic baskets were held within a 300 L insulated container filled with seawater with air flow provided to each basket via a battery operated pump with a tube and air stone. Seawater was exchanged regularly throughout the duration of the survey to maintain a constant water temperature and prevent the water from fouling.

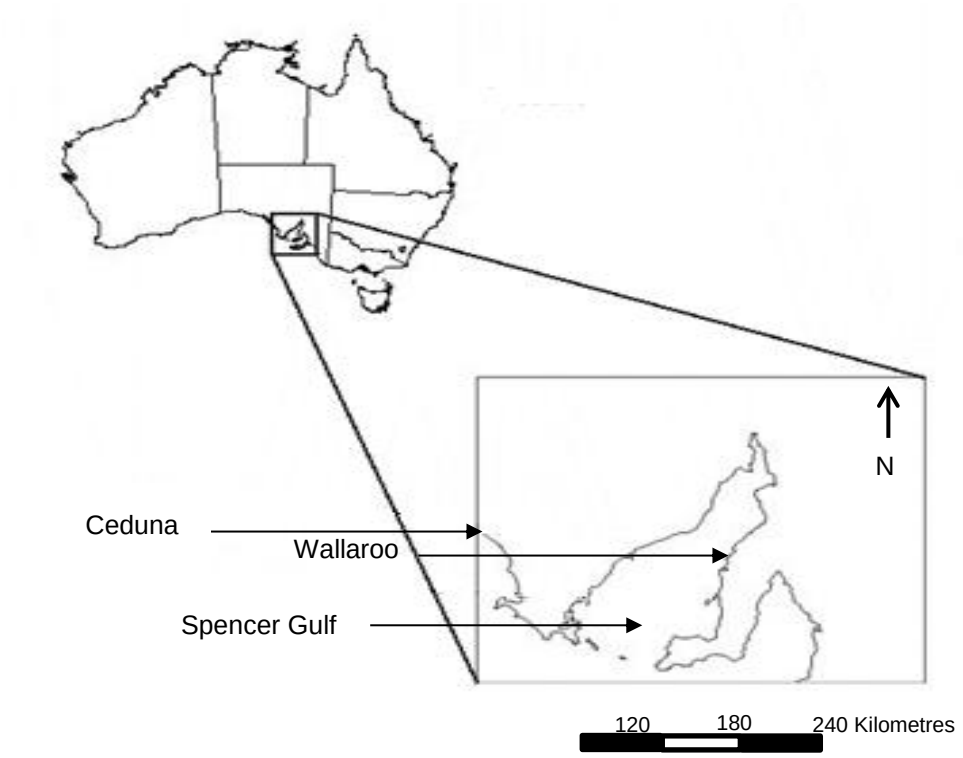


Figure 3.1. Main offshore fishing grounds for *Penaeus (Melicertus) latisulcatus* of Ceduna in West Coast (32.1267 °S, 133.6738 °E), and Wallaroo Spencer Gulf (34.3036 °S, 136.9805 °E), where samples for this study were collected.

3.3.2 Laboratory based experiments

Within 1 d of capture, female specimens were transferred in 300 L insulated containers by vehicle into individual 10 L buckets in the laboratory. Each bucket had 4, 60 mm holes within their sides, which were covered with a nylon mesh to allow adequate water exchange. In experiments 1 and 2, the mesh size was 500 and 150 µm, respectively (Figure 3.2 A to B). The mesh size was changed to 150 µm in experiment 2 to prevent spawned eggs from escaping out of the buckets and cross-contaminating the other buckets, which was observed in experiment 1. A lid was placed on each bucket to prevent females from escaping and to reduce the amount of light and thus potential stress on the specimens.

Six 10 L buckets per 1000 L flow-through holding tank and three 10 L buckets per 150 L flow-through holding tank were used for experiments 1 and 2, respectively (Figure 3.3 A to D). For logistic reasons, different sized holding tanks were used within each experiment as

they were conducted at different locations. Each bucket had a foam ring secured around the top to make it float within the holding tank (Figure 3.2 A) and was fitted with an air stone to supply supplementary air. Water conditions were monitored continuously throughout experiments 1 and 2 with temperature, salinity, dissolved oxygen (TPS dissolved oxygen and temperature electrode WP - 82), pH (Aqua One marine pH test kit), ammonia, nitrite and nitrate (Aqua One test kits) measured upon stocking and then daily during the experimental period. Water temperature was slightly warmer in experiment 2 with a range from 16.4 to 17.8 °C and 18.5 to 18.7 °C for experiment 1 and 2 respectively (Table 3.1). Dissolved oxygen varied slightly between experiments with experiment 2 having the lowest oxygen level at day four. Dissolved oxygen saturation ranged between 82.8 to 95.9 % for experiment 1 and 79.5 to 94.9 % for experiment 2 (Table 3.1). Ammonia and pH remained stable over the experimental period for both experiments 1 and 2. Average water conditions were similar to those experienced by prawns within the wild at the time of this study (Table 3.1).

As spawning is most likely to occur at night (Dall *et al.* 1990), all observations were carried out overnight. Upon dusk, each female was monitored on an hourly basis throughout the night using a torch covered in red cellophane to prevent disturbance. Immediately following the detection of eggs, the respective bucket was marked with time and date. All buckets had been randomly assigned post-spawning fixation times prior to the experiment, and spawned females were left undisturbed for the assigned time post-spawning until harvest and ovary fixation (i.e. between 0 to 6 h, 6 to 12 h, 24 h, 48 h and 72 h). An additional post-spawning fixation time of 96 h was included in the second experiment in order to extend the detection of POF degeneration. At the completion of the allocated time period, the specimens were removed from their bucket and fixed for histological analysis as described below. Ten females (total of 50 in experiment 1 and 60 in experiment 2) were assigned to each of the post-spawning fixation times in experiments 1 and 2 respectively (grand total of 110 specimens).

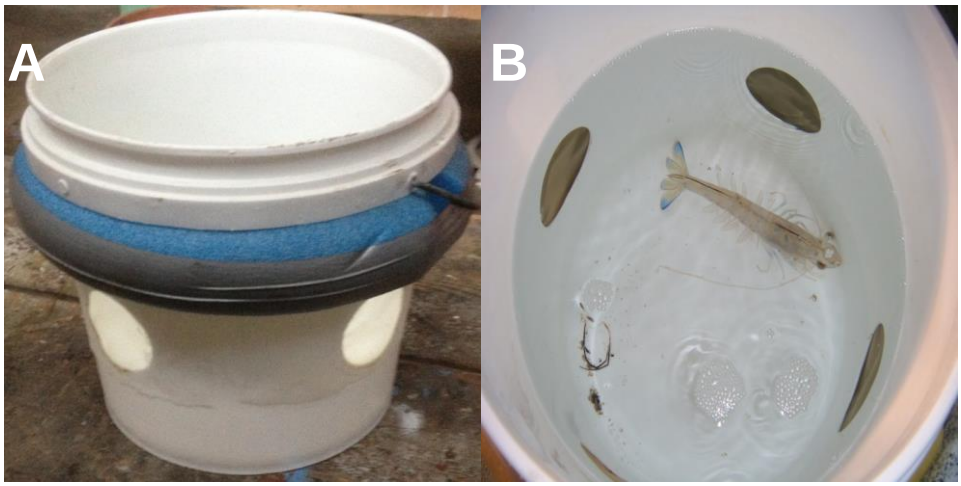


Figure 3.2. A) 10 L buckets from experiments 1 and 2. Holes covered with 500 and 150 µm mesh respectively for the experiments. The foam ring supported stability of buckets when placed in tanks B) View inside of bucket showing positioning of holes and female *Penaeus (Melicertus) latisulcatus*.

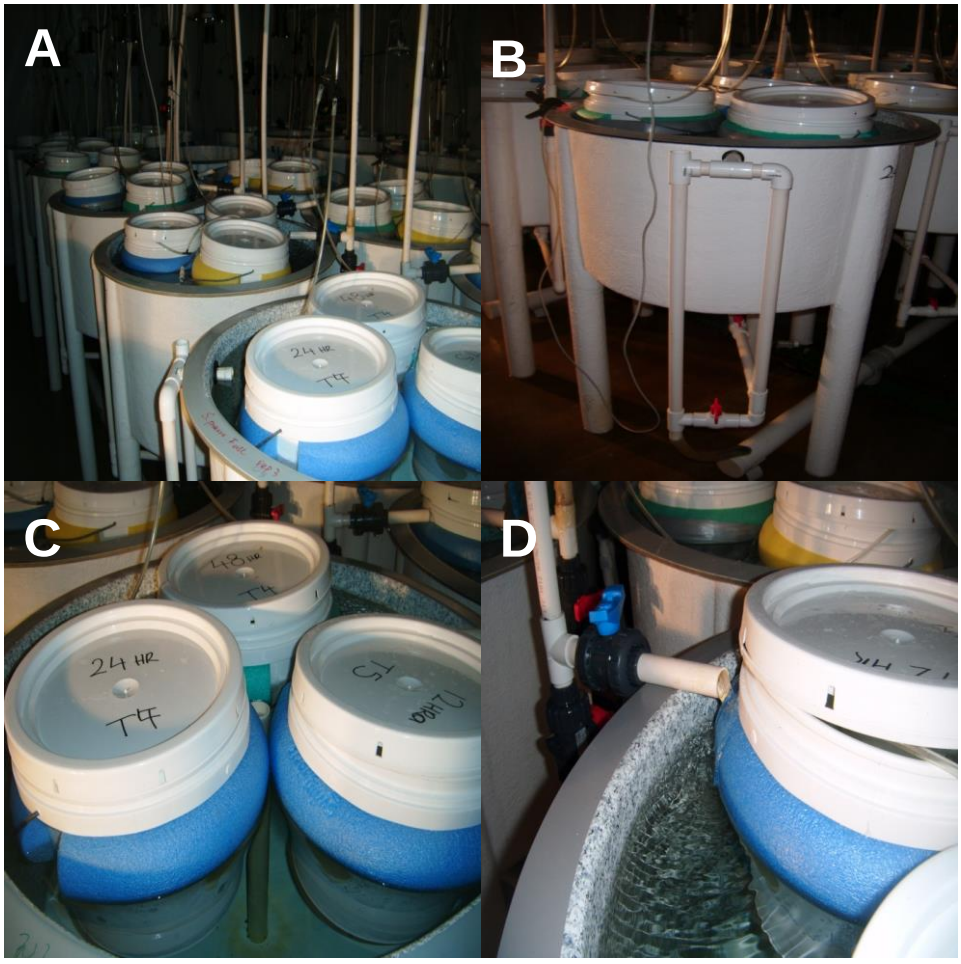


Figure 3.3. Spawning experiment 2 aquaria set up with A) Three 10 L buckets positioned within each tank B) Individual aquaria tank showing water outflow and air filtration lines C) Positioning of three buckets within each tank with bucket number and treatment times displayed on lid and D) Water inflow into each aquaria.

Table 3.1. Water conditions in experimental tanks during the study period for the western king prawn Penaeus (Melicertus) latisulcatus, from Day 0 (stocking) to Day 4. Experiment 1 was carried out in November 2010, and Experiment 2 a year later in October 2011.

	Experiment 1					Experiment 2				
	Day					Day				
	0	1	2	3	4	0	1	2	3	4
Temperature (ºC)	17.3	16.4	17.7	17.8	17.5	18.5	18.5	18.5	18.7	18.6
Salinity	35.5	35.7	35.6	36.2	36.2	35.7	35.9	36.1	36.5	36.6
DO (%)	94.6	95.9	83.9	87.6	82.8	94.6	94.9	85.2	80.2	79.5
pH	8.6	8.6	8.6	8.6	8.6	8.4	8.4	8.7	8.6	8.6
Ammonia (mg/L)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nitrite (mg/L)	0	0	0	0	0	0	0	0	0	0
Nitrate (mg/L)	0	0	0	0	0	0	0	0	0	0

3.3.3 Histological analysis of ovaries

Prior to fixation females were weighed (total wet weight ± 0.1 g) and measured (CL ± 1 mm). Females were then fixed in FAACC (Formalin 10 %, Acetic Acid Glacial and Calcium Chloride) solution for 48 h before being transferred to 70 % ethanol for storage and subsequent processing. Once dissected total ovary weight was recorded (± 0.1 g) with tissue from the mid-section of the ovary used for histological analysis. The distribution of oocyte stages does not differ significantly across areas of the ovary, so samples were taken from anywhere along the ovary for analysis (Montgomery *et al.* 2007b). The sampled ovary was embedded in paraffin, subsequently sectioned (6 µm thick), placed onto a slide and stained with haematoxylin and eosin. Sections were digitally imaged through a compound microscope (Olympus BX51) fitted with an Olympus camera C-7070 at 400x magnification. Two sections per specimen were examined.

The maturity stage of cells within each ovary was described according to the colour, size and shape of the ovary originally described by Tuma (1967) and as further classified in

chapter 2. (Figures 2.2, A and B, 2.3 A and B and 2.4, Table 2.1) Each slide was analysed for the presence of POF with their degeneration noted up to 72 h (96 h in experiment 2) post spawning to provide a reference to spawning time.

3.3.4 Data analysis

Relationships between carapace length and ovary weights were examined using Spearman's rank correlation with a regression analysis to test the relationship between mature ovary weight (g) and carapace length (mm).

Data were analysed using a non-parametric Kruskal-Wallis H-test (IBM SPSS version 20) to explore whether an increase in time since spawning, has an effect on the overall ovary weight of the individual (e.g. the loss of ovary weight may be greater for those at 96 h post spawning than those at 0 to 6 h). Data exploration included potential variance in the size (CL) of prawns spawning (~ages) as well as the percentage of those females which actually spawned between the two experiments.

3.4 Results

3.4.1 Relationship between carapace length and ovary weight

Carapace length (CL) and associated ovary weight were available for the 38 individual females that spawned in experiments 1 and 2. Ovaries could be discerned in all individuals ranging from 31 to 61 mm (± 0.1 mm) CL. A maximum ovary weight of 2.58 g was recorded for a female of 51 mm CL. On average, ovary weight was positively related to CL (R^2 = 0.2264, $P < 0.05$) (Figure 3.4). Body weight varied ranging between 15.97 to 66.97 g (± 0.1 g).

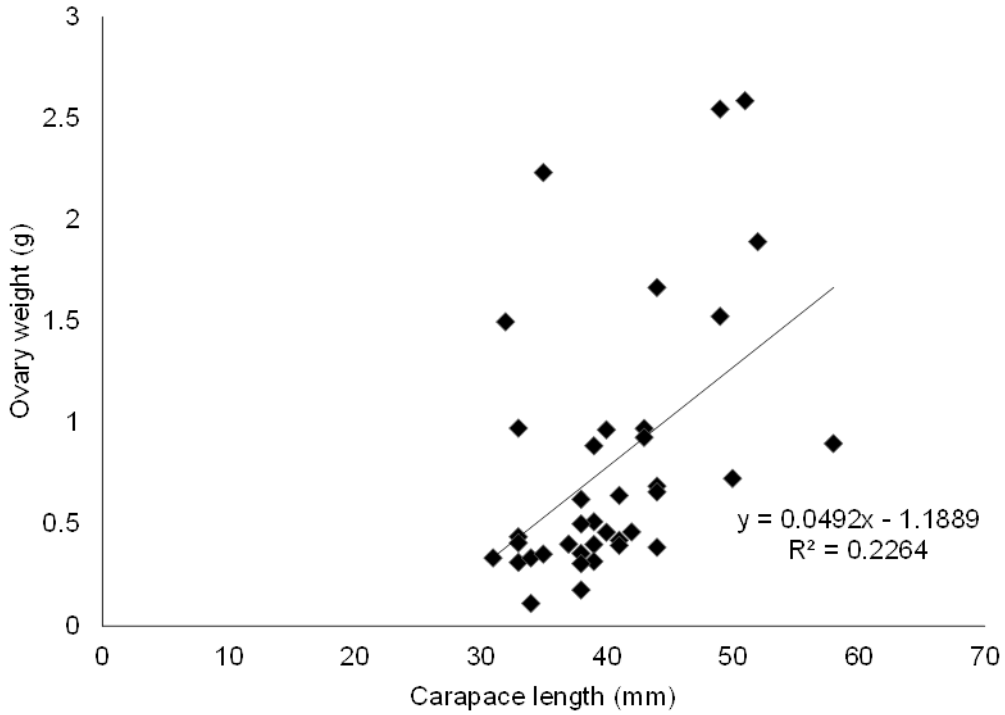


Figure 3.4. Post-spawn ovary weight and carapace length relationship for female *Penaeus (Melicertus) latisulcatus* spawned within the laboratory.

3.4.2 Description of histological change in POF degeneration

To measure the rate of spawning, the identification and description of POF degeneration from 0 to 6 h, 6 to 12 h, and after 24, 48, 72 and 96 h (experiment 2 only) were categorised. POF in the ovaries of *P. latisulcatus* were assigned an age (i.e. time after spawning) using cell characteristics as a measure such as follicle shape, amount of space, presence of connective tissue, degeneration signs as well as Alpha's stage atresia and the presence or absence of atretic oocytes (Table 3.2). Treatment time was also used in this classification (Table 3.2). POF shape changed throughout degeneration for *P. latisulcatus*.

Ovaries of matured females that had spawned within the laboratory contained POF in all cases (23 and 42 % of prawns in experiment 1 and 2 respectively). POF were similar in appearance to those described for teleost species (Hunter and Goldberg 1980; Saidapur 1982). POF were present in varying numbers within the ovary of each specimen. In females of the 0 to 6 h and 6 to 12 h treatment times, POF consisted of irregularly shaped structures composed of oval shaped follicle cells (Figures 3.5 A to B and 3.6 A to B). Connective

tissue was also present around the edges of these POF. After 12 h the space within these cells became smaller with the sidewalls of the POF becoming more squashed in appearance (Figure 3.6 B). Between treatment times of 24 and 48 h the overall shape of the POF changed from oval to more convoluted (Figures 3.7 A to B and 3.8 A to B respectively). At 24 and 48 h POF were collapsed, although the walls of the follicle cells were still visible. Alpha stage atresia (AA) was also distinguished at 24 h by the disintegration of the nucleus within a cell (Figure 3.7 A). By 48 h AA was at the intermediate phase as the cell walls were beginning to break down (Figure 3.8 A).

Results from experiment 1 showed that POF at treatment times of 72 h were degenerating and beginning to collapse, however, they were not fully degenerated and still visible. Upon this basis further assessment at 96 h post spawning was carried out in experiment 2. By 96 h, the POF had become thin in shape and almost completely collapsed (Figures 3.9 A to B and 3.10 A to B respectively). It was also evident that the oocytes at 72 and 96 h were becoming atretic (AO), whereby ovarian follicles begin to degenerate and subsequent resorption of one or more immature ovarian follicles occurs. Based on these findings, a classification system for the ageing of POFs was established up to 96 h post spawning in order to identify and describe POF degeneration in *P. latisulcatus* (Table 3.2).

Table 3.2. Identification and description of postovulatory follicle (POF) degeneration in western king prawn *Penaeus (Melicertus) latisulcatus* from 0 to 96 h post spawning within the laboratory.

Treatment Time	Description of POF
0 to 6 h	POF oval in shape
	Degeneration of the follicle is not evident
	Large amount of space within the centre of POF
	Connective tissue visible (stains pink with H&E)
6 to 12 h	POF is similar in shape and appearance to 0 to 6 h
	Degeneration evident as the nuclei of the granulose cells are dense
	Cell wall consists of oval shaped follicles
	Space within centre of the POF
	Connective tissue visible (stains pink with H&E)
24 h	Additional signs of degeneration to the POF are evident
	POF smaller than at 0 to 6 h
	Cell membrane is becoming less distinct
	Granulosa layer comprised of numerous cells
	Thecal layer (TL) appears thicker due to follicle compacting slightly
	Alpha stage atresia (AA) distinguished by disintegration of the nucleus
48 h	POF is about half the size from 0 to 6 h
	Shape is more convoluted with a greater number of folds
	Intermediate phase of Alpha stage atresia (AA) as the cells begin to break down
	Few intact cell membranes
	Thecal layer (TL) remains a distinct thick band of cells
72 h	Thecal layer (TL) appears thick
	POF is about one third the size at 48 h
	POF irregular and flattened (collapsed) in shape
	Appearance of atretic oocytes (AO)
96 h	POF irregular and flattened (collapsed) in shape
	Presence of atretic oocytes (AO)

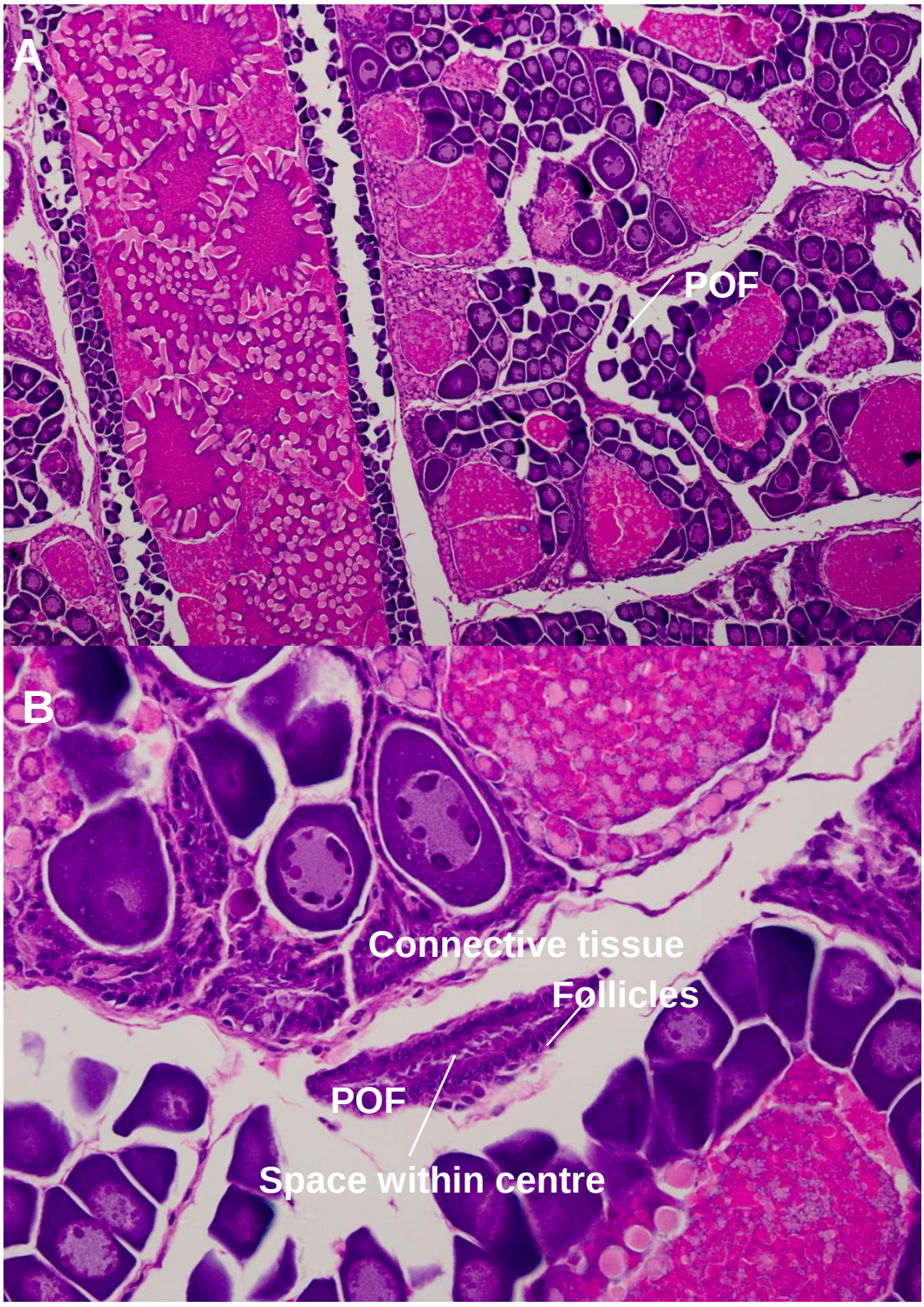


Figure 3.5. Histological sections (100 x and 400 x respectively) of *Penaeus (Melicertus) latisulcatus* at 0 to 6 h post spawning. A) Section of recently spawned ovary with no degeneration evident B) Same section of ovary at 400 x showing oval shaped POF at 0 to 6 h post spawning with no degeneration evident.

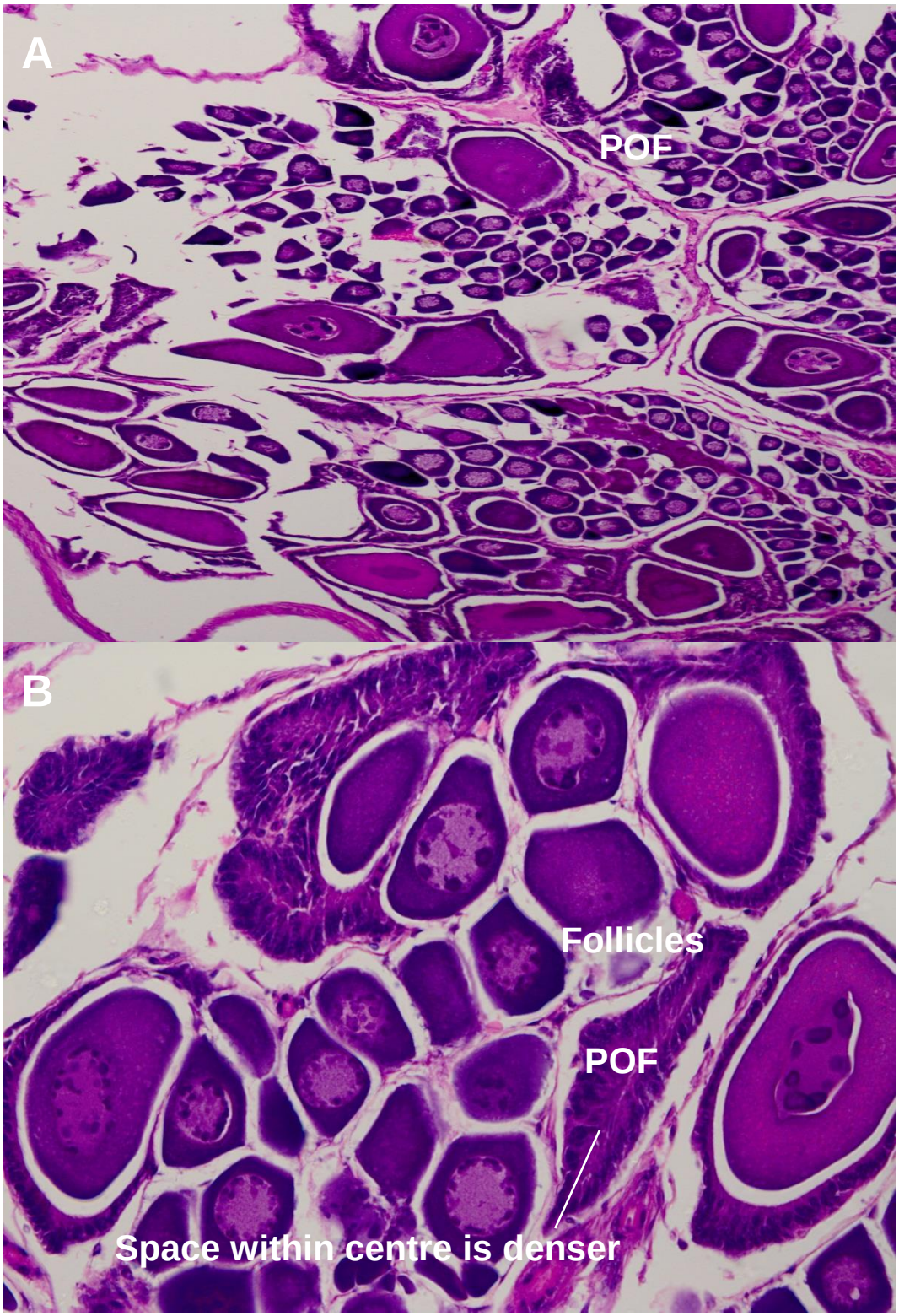


Figure 3.6. Histological sections (100 x and 400 x respectively) of *Penaeus (Melicertus) latisulcatus* at 6 to 12 h post spawning. A) Section showing degeneration of ovary B) POF with a cell wall consisting of oval shaped follicles.

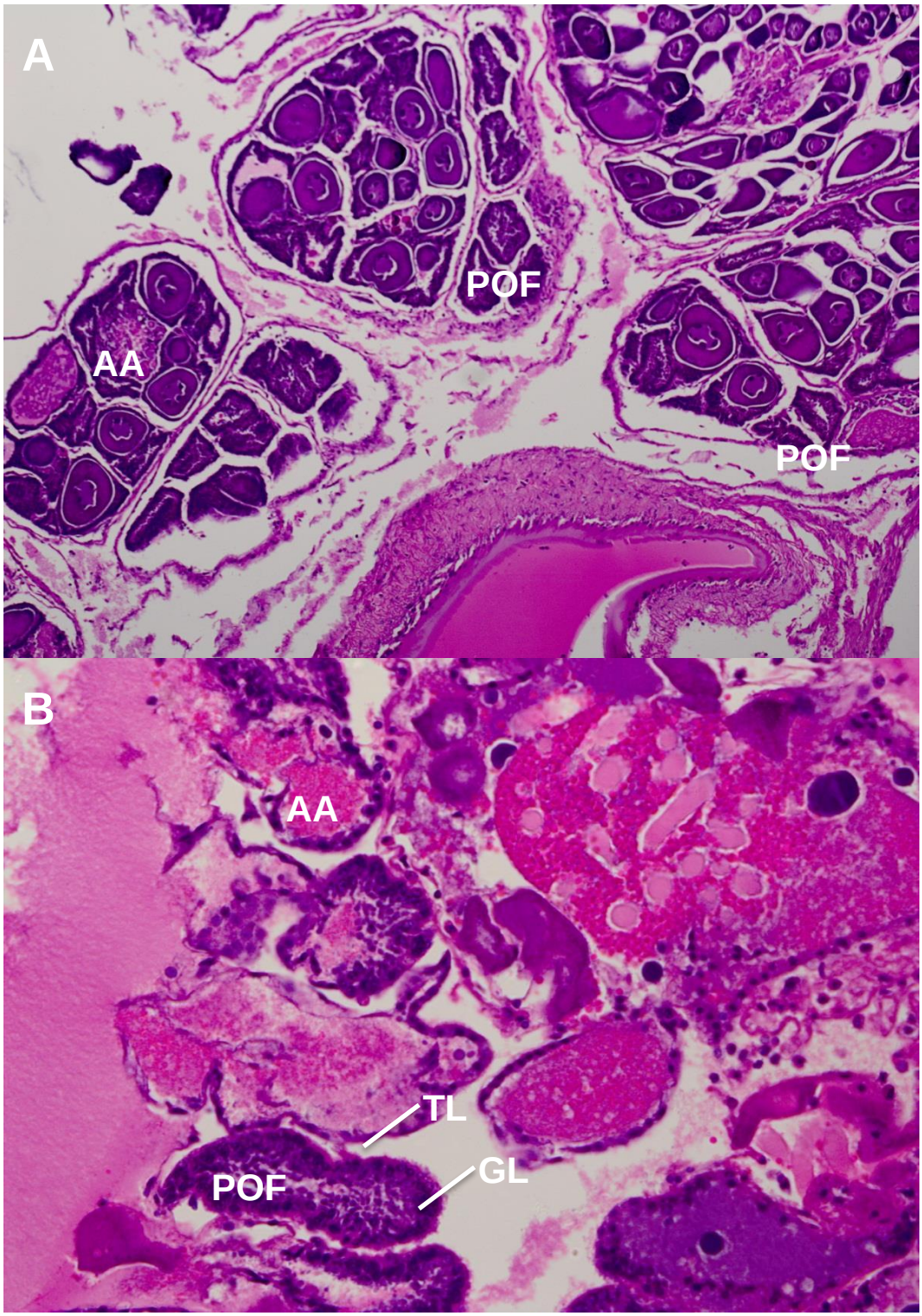


Figure 3.7. Histological sections (100 x and 400 x respectively) of *Penaeus (Melicertus) latisulcatus* at 24 h post spawning A) Section showing degeneration of ovary with initial phase of alpha-stage atresia (AA) distinguished by disintegration of the nucleus B) POF with further degeneration of the nuclei and thicker thecal layer (TL) due to the compacting of the follicle cells. Granulosa Layer (GL) comprised of numerous cells.

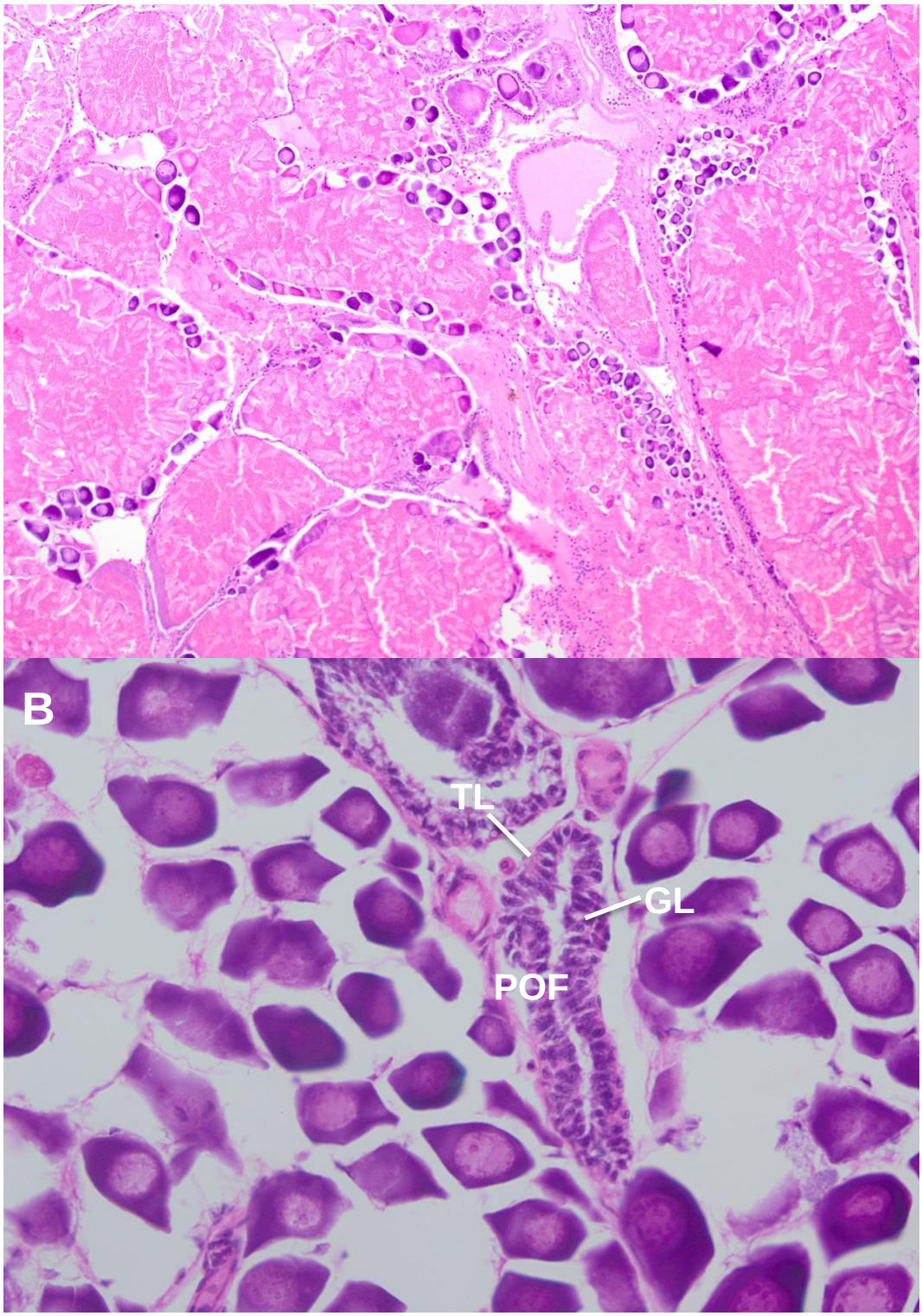


Figure 3.8. Histological sections (100 x and 400 x respectively) of *Penaeus (Melicertus) latisulcatus* at 48 h post spawning. A) Section showing degeneration of the ovary with intermediate phase of alpha-stage atresia B) POF that is convoluted and flattened in shape with further degeneration. Thecal layer (TL) remains a distinct band of cells and granulosa layer (GL) has degenerated further.

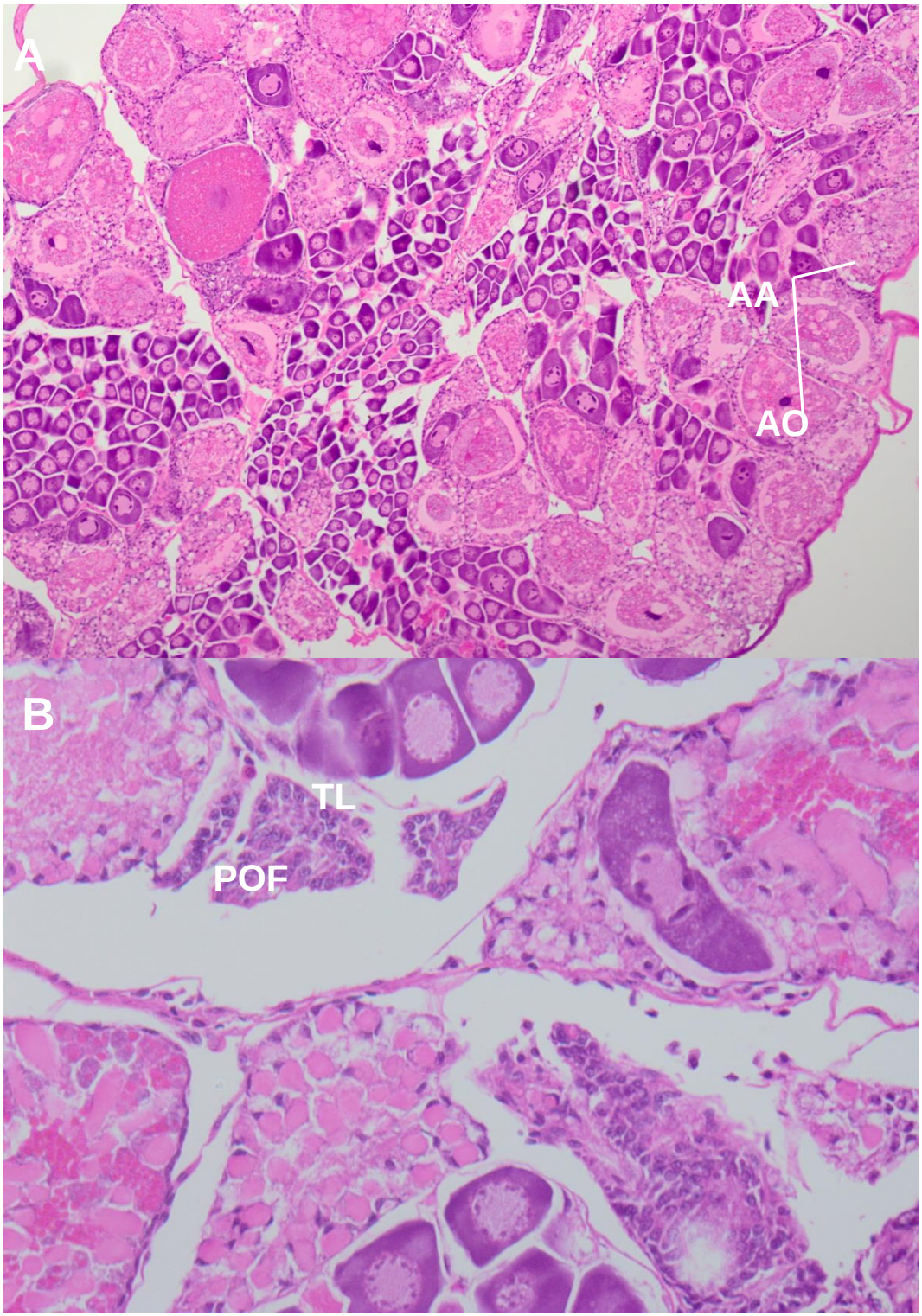


Figure 3.9. Histological sections (100 x and 400 x respectively) of *Penaeus (Melicertus) latisulcatus* at 72 h post spawning A) Section showing degeneration of the ovary with advanced phase of alpha-stage atresia (AA) and appearance of atretic oocytes (AO) B) Thecal layer (TL) appears very thick and POF is flattened or collapsed in shape.

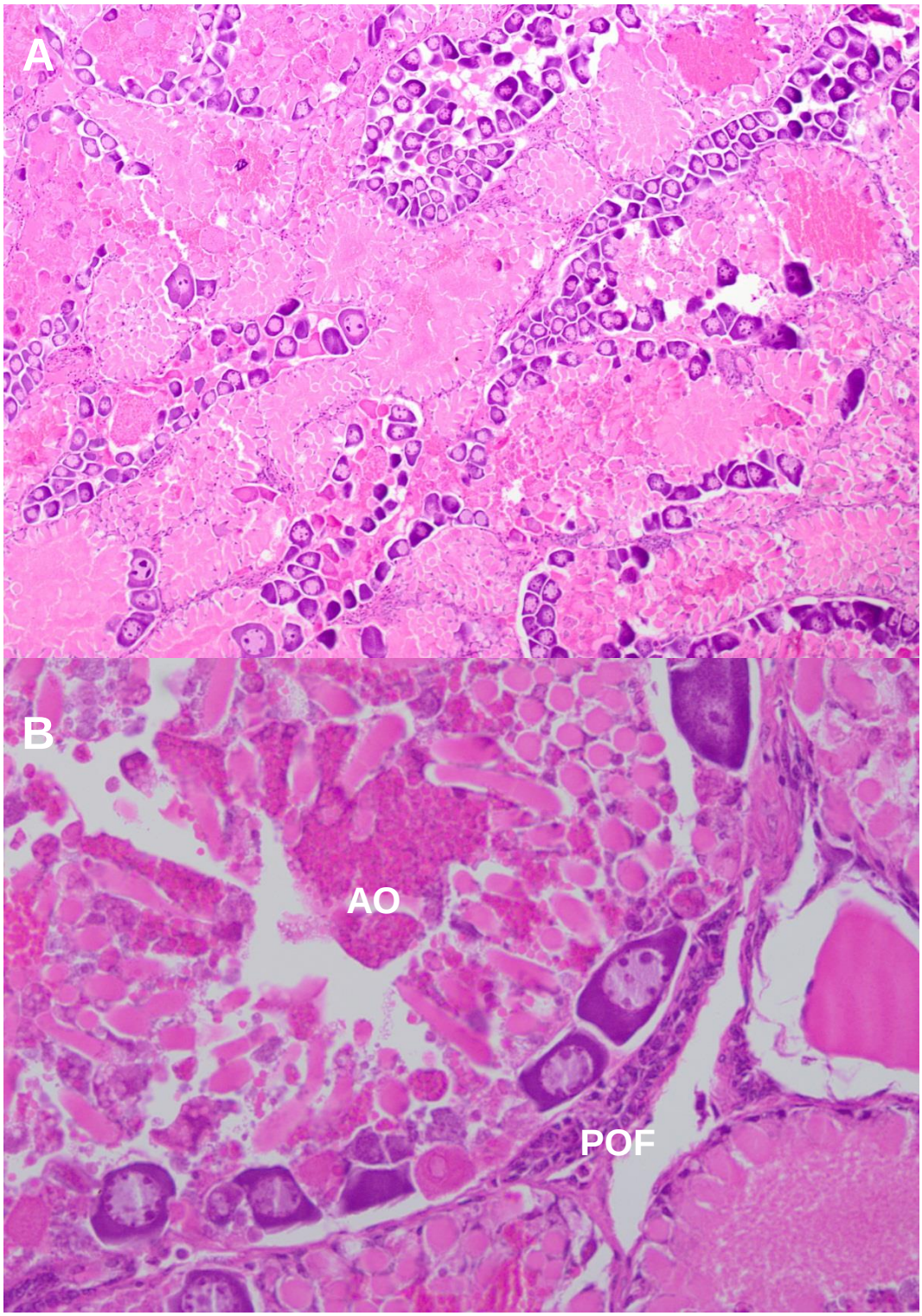


Figure 3.10. Histological sections (100 x and 400 x respectively) of *Penaeus (Melicertus) latisulcatus* at 96 h post spawning. A) Section showing degeneration of the ovary with characteristics of beta-atresia (AO) B) POF irregular in shape and completely flattened in appearance.

3.5 Discussion

The results of this study provide evidence that POF occur in *P. latisulcatus* and their degeneration can be classified from 0 to 96 h post spawning. An accurate assessment of reproductive variables, such as spawning occurrence is crucial for the understanding of the population dynamics of many species (Niamaimandi *et al.* 2007). In terms of reproductive strategy, species often show asynchronous oocyte development, where they release several batches of eggs during the reproductive season and exhibit indeterminate fecundity (Penn 1980). In species with indeterminate fecundity, the annual fecundity needs to be calculated from the combination of data about batch fecundity, spawning occurrence and the duration of the spawning season (Fitzhugh *et al.* 2012). The classification of POF presented here will allow improvements to such calculations for prawns.

To obtain spawning occurrence data, a method is needed to identify recently spawned females. POF are remnants of the ovulated follicle that remain in the ovary (Hunter and Goldberg 1980). It is often difficult to measure the longevity of POF as they are short lived, but the time of spawning is needed for classification. The use of POF to estimate spawning occurrence needs to occur soon after spawning (Yamamoto and Yoshioka 1964). Hunter and Goldberg (1980) were able to induce spawning in the Northern anchovy *Engraulis mordax* in the laboratory, which allowed them to categorize the degeneration of POF over time. The classification method outlined here, based on a similar experimental approach, will allow the identification of recently spawned female *P. latisulcatus* for up to 96 h post spawning.

To my knowledge, this paper presents the first classification for the use of POF to detect spawning incidence in crustaceans. The spawning of *P. latisulcatus* in temperate South Australian waters occurs at SSTs above 17 °C. POFs are assumed to be short lived and reabsorb quickly after spawning (Hunter and Goldberg 1980). This is consistent with other POF studies including those that suggest differing water temperatures may have an effect on the rates of POF degeneration (Fitzhugh and Hettler 1995). Alday *et al*. (2008) found that at temperatures between 17 °C and 21 °C POF degeneration was consistent among

experimental treatments and those within the wild, with no noticeable delay in degeneration could be shown in relation to temperature. Water temperature within this study averaged at 17.5 °C and 18.5 °C respectively in experiments 1 and 2, corresponding with SSTs between 17.5 °C and 24 °C for Spencer Gulf and the West Coast during those periods (Carrick 2003; Roberts *et al.* 2012). SSTs therefore suggest that POF degeneration of recently spawned *P. latisulcatus* held within the aquaria over the experimental period was similar to *P. latisulcatus* within the wild. Although SSTs and other parameters measured (Table 4.1) were consistent with those SSTs experienced by *P. latisulcatus* in the wild, it is unknown what the effects of stress and food consumption were within this experiment.

Six stages of POF have been distinguished based on the POF method used in this study. The histological changes in POF degeneration in actively spawning *P. latisulcatus* were determined for periods of 0 to 6 h, 6 to 12 h, 24, 48, 72 and 96 h. Although no degeneration was evident until after 12 h post spawning, the shape of the POF changed throughout degeneration. This is similar to that of the Iberian sardine *Sardina pilchardus*, in which the POFs were seen to shrink by up to 50 % daily, giving each stage a distinct shape (Ganias *et al.* 2007). Throughout degeneration, POF could be seen to change from an irregular oval shape to a more rectangular and finally a flattened or squashed like appearance. This change in shape provides some useful morphological criterion to aid in determining the age of the POF.

To create a detection period for POF in *P. latisulcatus* Table 3.2 was developed in accordance with all relevant information available on the classification of cells. The greatest degeneration in POF was noted 24 h post spawning, with little difference in the shape and structure of the cell wall up to 12 h post spawning. These results differ greatly to the degeneration of teleost species which show rapid degeneration and full resorption by day 2 (up to 53 h post spawning) (Hunter and Goldberg 1980). POF degeneration of the Biscay anchovy *Engraulis encrasicolus L.,* however, is spread over seven stages with full resorption of POF occurring between 55 to 60 h with lower SSTs of between 13 and 19 °C. Alday *et al.* (2008) describes the degeneration of the Biscay anchovy to be longer than that

of other small pelagic fishes within similar waters which can achieve full degeneration with 24 h. With *P. latisulcatus* still displaying degeneration at 96 h it is likely that this species has a much slower rate of degeneration than most teleost species. Crocos (1985) found that the duration of the spent stage for *P. esculentus* accounted for only 6 % of the intermoult period, spending an average of 2 out of 29 d in the spent stage.

This study demonstrates that POF can be identified in penaeids via histological analysis of their ovaries so that their degeneration may be used as a measure for post spawning time and for determining egg production of a fishery to underpin fisheries management. This study puts forward a visually documented six stage process for the identification of POF in *P. latisulcatus* up to 96 h post spawning. The success of identifying POF within penaeids using the classification presented in this study suggests that other penaeid fisheries should be able to employ it to improve calculations of spawning occurrence. While the aim of this study was to provide a classification for the determination of POF in penaeid prawns post spawning and directly estimate spawning occurrence, further study is needed to test the wider applicability of this classification scheme for conspecifics and the effects of size, temperature, food availability and stress on other prawns.

Chapter 4: Validity of visual determination of ovary development stages of the western king prawn *Penaeus (Melicertus) latisulcatus* (Kishinouye 1896)

4.1 Abstract

An understanding of reproductive biology is essential for the sustainable management of exploited marine organisms. Macroscopic examination of gonad condition is a common tool used for rapid assessment of reproductive status of many exploited marine organisms to assist with their sustainable management. Whilst this method is efficient, inaccurate or imprecise determination of reproductive status may result in poor management advice. This one off study on *Penaeus (Melicertus) latisulcatus* reviews the accuracy and precision of macroscopic staging determined by nine observers. Prawns at stage three of ovary development (where the ovary is visibly yellow and the gut and muscle are partially visible) were categorised with the greatest accuracy (72 %), while prawns at the two earliest stages of ovary development (stage 0 and 1) were only categorised correctly 26 % and 31 % of the time, respectively. A generalised linear mixed model analysis (GLMM) and weighted Cohen's Kappa's suggested that macroscopic assessment was unable to distinguish between the early stages of reproduction (stages 0, 1 and 2), but was useful in distinguishing these from females close to spawning (stage 3).

4.2 Introduction

Maturity indices based on macroscopic gonad assessment (i.e. visual assessment) are widely used in fisheries science as a means of identifying reproductive status in fish and crustaceans (Ferreri *et al.* 2009). Macroscopic staging on its own is a quick and relatively easy method to apply; however, this method is also subject to variation in the accuracy of assessment made by different observers. Complementing macroscopic assessments with microscopic analyses of histologically prepared sections of gonad tissue can be used to

validate and refine macroscopic maturity determinations (Acuna *et al.* 2000; Kaunda-Arara and Ntiba 1997).

Although macroscopic staging of ovary development is widely used in penaeid fisheries (e.g. Ayub and Ahmed 2002; Baelde 1992; Montgomery *et al.* 2007b), there have been no previous studies specifically examining the accuracy of macroscopic staging using microscopic validation, with the exception of Penn (1980). Examination of egg assemblages in the penaeid *Melicertus plebejus*, revealed good agreement between ovary development stages determined from macroscopic and microscopic techniques (Montgomery *et al.* 2007b). Although no quantitative measure of accuracy was provided, Montgomery *et al*. (2007b) suggested that macroscopic staging may have great utility in assessments of the reproductive development of other penaeid species, but that its accuracy may differ among species. Species-specific changes in the characteristics of ovaries as they develop may explain disparities in the accuracy of macroscopic staging techniques among species (e.g. Peixoto *et al.* 2003; Quintero and Garcia 1998). Olney *et al*. (2001) demonstrated that macroscopic and microscopic staging are not interchangeable, with the greatest disagreement observed in the macroscopic staging of spent and partially spent American Shad (*Alosa sapidissima*). Maturing and partially spent ovaries were often confused when using macroscopic methods, and more accurate counts were obtained through histological examination (Olney *et al.* 2001). Irrespective of whether there are differences in the accuracy of assigning ovary development stage using macroscopic methods among species, establishing the accuracy of the technique should be made in all cases.

Stock assessment surveys of the western king prawn; *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus)* are conducted annually throughout South Australia's Spencer Gulf and Gulf St Vincent. These surveys occur during November and December using fishery-independent observer's on-board commercial vessels to provide a snapshot of the relative biomass prior to fishing. Spencer Gulf and Gulf St Vincent prawn trawl fisheries harvest approximately 2,200 tonne of *P. latisulcatus* annually (Dixon *et al.* 2012). Around 20 % of this catch is harvested during the November and December fishing periods when domestic demand

peaks prior to Christmas (Dixon *et al.* 2009). As this coincides with the onset of spawning, management measures to protect the spawning biomass are critical at this time, with recent studies identifying that the total catch during the pre-Christmas period was the key factor influencing the strength of subsequent recruitment (Dixon *et al.* 2009). The spawning occurrence of female prawns has also been used as a tool to determine closed areas during these fishing periods. To generate information on the distribution and reproductive status of female *P. latisulcatus*, macroscopic determinations of ovary development have been routinely made since 1998 in Spencer Gulf and 2004 in Gulf St Vincent. Observers on survey vessels categorise the ovary development stages of haphazardly sampled female prawns from the catch according to macroscopic criteria that were originally defined by King (1979) (Figure 4.1 A to D). Ovaries, which can be observed through the dorsal surface of the carapace, are categorised into stages, and the scheme given observers ranges from small and transparent when in non-vitellogenic stages (stage 0), to large and orange immediately before spawning occurs (stage 4). Historically, this information was used to minimise the impact of fishing on egg production by directing commercial trawlers away from areas of prawns that are ready to spawn (> 50 % stage 3 and 4). South Australian populations of *P. latisulcatus* spawn annually when water temperatures rise above 17^{o}C, which generally occurs from November to March each year (Dixon *et al.* 2009).

The aim of this study was to determine the accuracy of macroscopic categorisations of ovary development of *P. latisulcatus* by observers as used for stock assessment surveys. Past literature and cell composition data was examined in the current study to develop an improved classification based on microscopic criteria (Chapter 2.0). Agreement was then assessed between macroscopic determinations of ovary development stages made by the observers on board the survey vessel to the microscopic examinations of histological sections of ovary tissue made on the same prawns. A generalised linear mixed model analysis and weighted Cohen's Kappa's was used to determine observer accuracy and precision by stage, and to determine differences among individual observers.

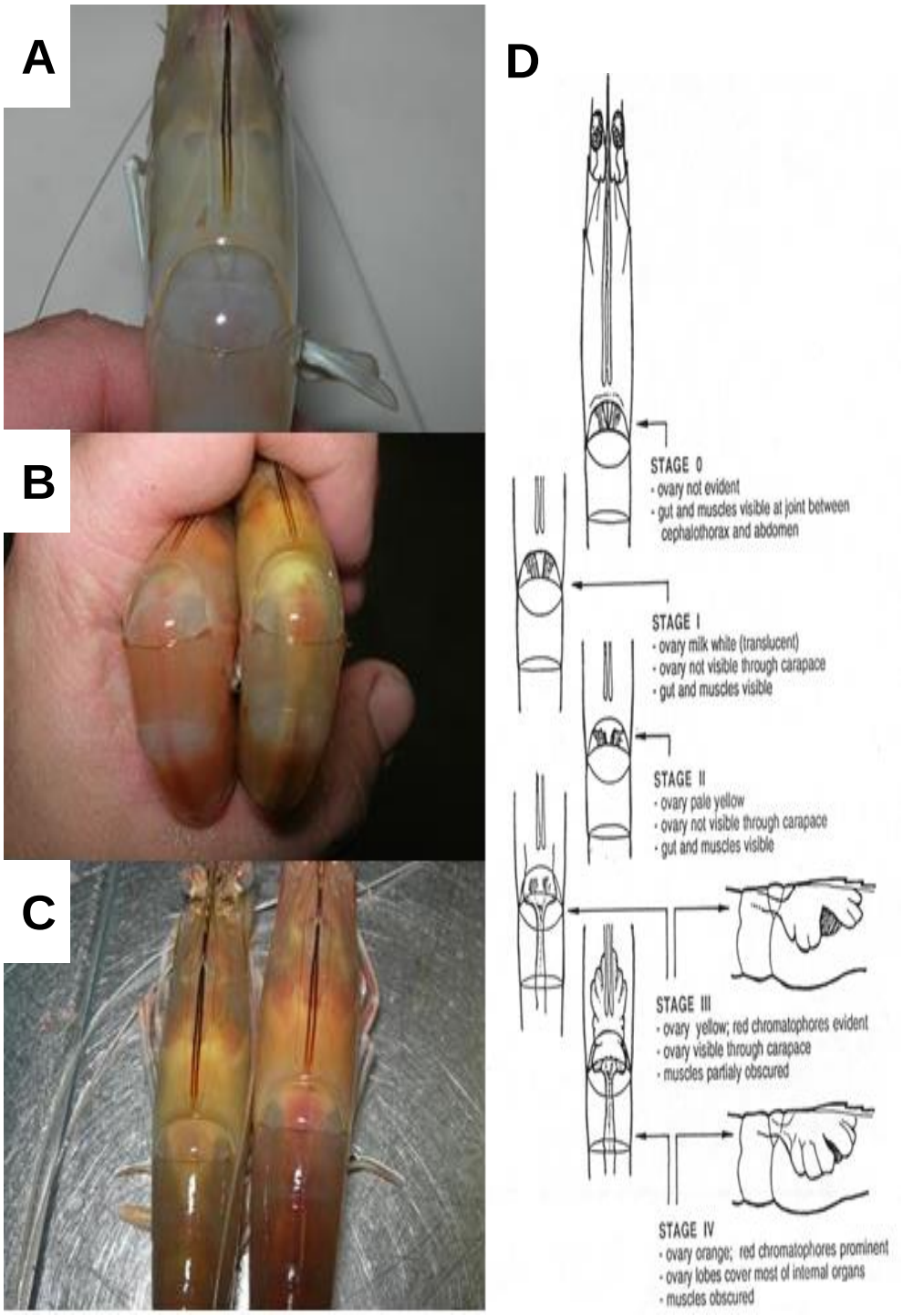


Figure 4.1. Visual reference guide and explanations provided to observers on survey vessels for macroscopic determinations of ovary development stages of *Penaeus (Melicertus) latisulcatus* in South Australia (Dixon and Hooper 2008). A) Digital images of stage 0, B) differences between stages 2 and 3, C) stages 3 and 4 and D) Diagrammatic representations and explanations of ovary development taken from King (1979).

4.3 Materials and methods

4.3.1 Sample collection and macroscopic staging

Female *P. latisulcatus* were collected using a demersal otter trawl in Gulf St Vincent, South Australia, within the range of 34°45'S, 138°15'E and 34°47'S, 138°15'E on the 23rd November 2009. This area lies within the main fishing grounds for *P. latisulcatus* in Gulf St Vincent. Trawls were undertaken at night using the South Australian Research and Development Institute's (SARDI) research vessel R.V. Ngerin. The double-rig trawl gear had a maximum total headline length of 29.26 m and a minimum mesh size of 4.5 cm. Each net was fitted with a codend consisting of a PVC protective mat and 44 mm diamond mesh. A random sample of female prawns was obtained from the trawl catch and 100 individuals tagged for identification. Of these 100 females 98 were used for histological analysis, with one stage 4 female later excluded from the analysis due to insufficient samples for this stage. The ovary development stage of each specimen was macroscopically categorised by nine observers within approximately 2 h of capture using criteria given by King (1979) and the reference guide Figure 4.1 A to D. Specimens were fixed and histologically staged as outlined in the methods of chapter 2.3.2.

4.3.2 Comparison of macroscopic and microscopic staging

Descriptive patterns of agreements between macroscopic and microscopic staging techniques were assessed across all observers combined. For each prawn and observer, the correctness of staging was determined as a binary variable (1 - correct staging, 0 – incorrect staging) using a generalised linear mixed model analysis (GLMM) (Bolker *et al.* 2009). Individual observers and oocyte stage, as well as their interaction, were modelled as fixed factors (within and between respectively) as it was of interest to explore specific differences between observers. GLMM procedures implemented in IBM SPSS 20 were used with Satterthwaite approximation for degrees of freedom and robust estimations being accommodated to address the small sample size. Sequential Bonferroni correction for multiple comparisons was applied where appropriate.

An assessment of systematic effects and quantifying the degree of agreement was done by calculating weighted Cohen's Kappa's (Watson and Petrie 2010). Analyses were carried out for each observer separately using ComKappa2 software (Hadzi-Pavlovic 2010; Robinson and Bakeman 1998).

4.4 Results

4.4.1 Comparison between macroscopic and microscopic staging

Out of 97 females, 32 were microscopically determined to be stage 0, 12 as stage 1, 31 as stage 2 and 22 as stage 3 (Table 4.1). One microscopic determination for a stage 4 ovary was excluded from the data due to insufficient sample size and no determinations were made for stage 5 ovaries. A total of 873 macroscopic observations were made by 9 observers on the 97 females, with a total of 356 correct observations (75 stage 0, 33 stage 1, 106 stage 2 and 142 stage 3). Although many assessments were made incorrectly, the majority of incorrect assessments made by individual observers were an overestimation of stage.

Observers correctly determined prawns at stage 3 of ovary development more frequently (mean 72 %) than earlier stages of ovary development, with stage 0 having the least agreement (mean 26 %). Pairwise comparisons identify that stage 3 was identified significantly ($p<0.05$) better than all other stages.

Table 4.1. The number of Penaeus (Melicertus) latisulcatus microscopically staged in categories 0, 1, 2 and 3 and the total number and percentage of macroscopic stage estimates provided by 9 observers. Underlined numbers correspond to the number of correct observations within each group.

Micro Stage	Sample (n)	Total observations	Macroscopically Staged				
			0	1	2	3	4
0	32	288	<u>75</u> (26 %)	170 (59 %)	34 (11.8 %)	5 (1.7 %)	4 (1.4 %)
1	12	108	4 (3.7 %)	<u>33</u> (30.5 %)	59 (54.6 %)	11 (10.2 %)	1 (0.93 %)
2	31	279	2 (0.7%)	35 (12.5 %)	<u>106</u> (38 %)	122 (43.7 %)	14 (5 %)
3	22	198	0	0	13 (6.6 %)	<u>142</u> (71.7 %)	43 (21.7 %)

4.4.2 Individual observer-validation

K values from weighted Cohen's Kappa ranged from 0.46 to 0.54, suggesting that the rate of agreement for observers was moderate (Hadzi-Pavlovic 2010) (Table 4.2). Generally a k value of zero suggests that the agreement is no better than that which would be obtained by chance (Watson and Petrie 2010). Percentage agreement between observers ranged from 32 to 48 %. Chance agreement ranged between 19 and 23 % for observers suggesting a relatively low probability for an individual to guess the correct stage. Overall observers demonstrated a tendency to overestimate staging. On average only nine assessments were underestimated, 45 were overestimated by one, four overestimated by two, one by three and one by four stage values (Figure 4.2).

There was considerable variability in the proportion of correct estimates among observers and grades (Figure 4.3 A to D). In general, stage 3 was well assessed by all observers (range 0.50 to 0.90; mean 0.74), while stage 0 was poorly assessed (range 0.08 to 0.40; mean 0.19). Assessments of stage 1 (range 0.03 to 0.70; mean 0.27) and stage 2 (range 0.10 to 0.65; mean 0.35) were highly variable.

Table 4.2 Statistics associated with weighted Cohen's Kappa, including the percentage and chance agreement for each individual observer when macroscopically staging Penaeus (Melicertus) latisulcatus.

Observer	Kappa Maximum (k)	Weighted Kappa	Percentage agreement	Chance agreement
1	0.66	0.46	37	22
2	0.56	0.48	39	19
3	0.60	0.49	41	21
4	0.58	0.54	44	22
5	0.68	0.48	40	21
6	0.60	0.49	44	23
7	0.63	0.51	48	23
8	0.62	0.52	42	23
9	0.48	0.46	32	19

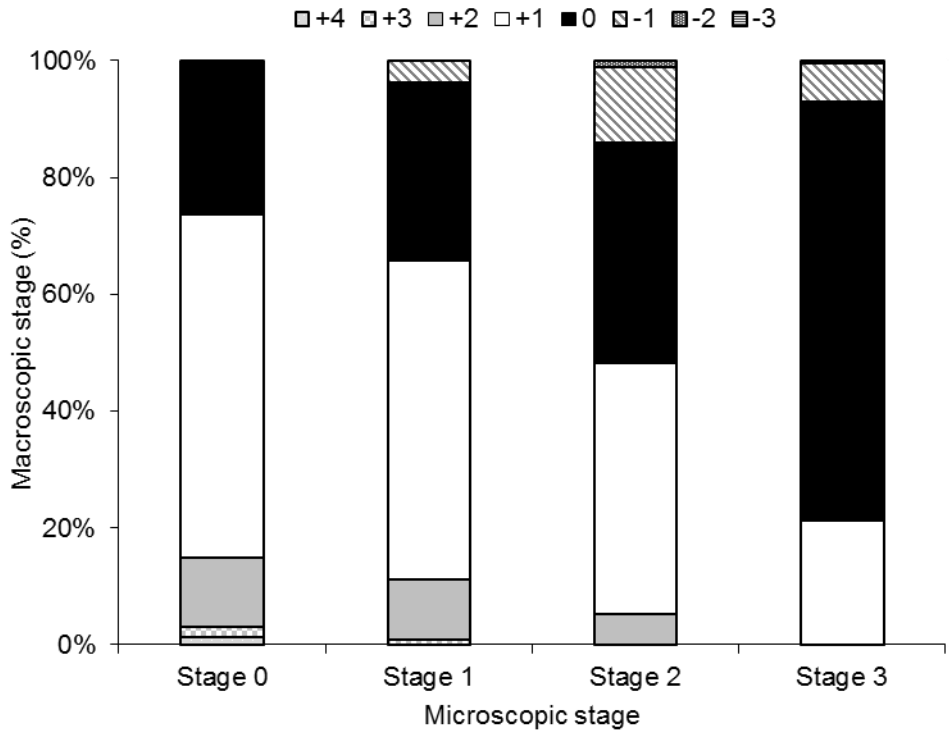


Figure 4.2. Average number of correct (0), over staged (+) and under staged (-) ovaries for microscopically staged female *Penaeus (Melicertus) latisulcatus* for all observers across stages 0, 1, 2 and 3.Symbols explain the sequence from bottom to top of the stacked bars.

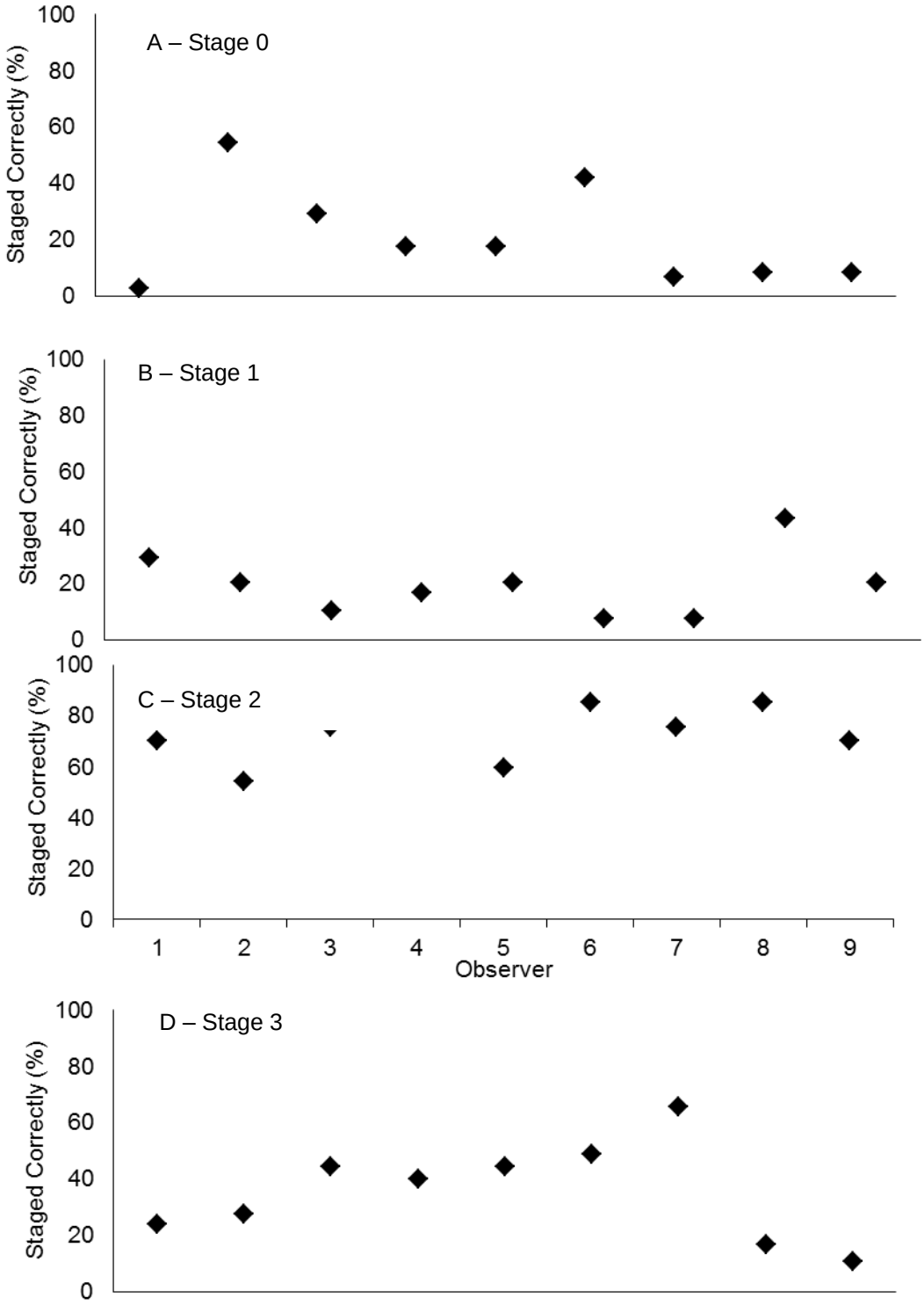


Figure 4.3. Percentage correct of female *Penaeus (Melicertus) latisulcatus* for individual observers (1 to 9) for A) stage 0, B) stage 1, C) stage 2 and D) stage 3.

4.5 Discussion

When paired with histological techniques, macroscopic staging forms a strong set of criteria to aid in the classification of ovary development (Ferreri *et al.* 2009). Comparisons between macroscopic versus microscopic techniques in the current study suggest that the macroscopic staging criteria that are currently used are not accurately assessing oocyte development of *P. latisulcatus*. Specifically, the accuracy of macroscopic assessments by observers was highly variable among stages. The stage that was assigned correctly most often was stage 3 (72 %, Table 4.2), while stages 0, 1 and 2 were each accurately assessed on < 40 % of occasions, with stage 0 having the poorest accuracy (26 %). The microscopic staging criteria developed in this study (Chapter 2) effectively discriminated each stage of oocyte development. To improve the accuracy of macroscopic staging for *P. latisulcatus,* a refined set of criteria was developed based on findings in this study and those of previous studies (Montgomery *et al.* 2007b).

A potential contributing factor to the observed inaccuracy when using macroscopic staging may be due to the visual reference images provided to the observers. The images did not clearly depict the full range of variation in the shape, colour and size of the ovaries apparent among *P. latisulcatus* within each of the stages of ovary development. In this study, observers were provided with photographic colour images of stages 0, 2, 3 and 4 of ovary development, and black and white line diagrams of five stages (spent stage excluded) with written explanations of visual criteria taken directly from King (1979) and as shown in Figure 4.1 A to D. The greatest degree of inaccuracy was apparent in stages 0 and 1, yet King (1979) notes that the area underlying the carapace and the first abdominal segment that the ovary occupies is clear at stage 0 and transitions to a milky-white colour at stage 1. Despite this clear distinction, observers only categorised *P. latisulcatus* at stage 0 correctly 26 % of the time and incorrectly categorised them as stage 1 approximately 60 % of the time. Although this transition should be readily apparent based on the description by King (1979), these inaccuracies suggest that visual aids such as colour photographs are likely to be an important tool for macroscopic staging (Figure 4.1 A to D).

Analysis of incorrect assessments identified that > 80 % of mistakes were overestimates. This could be explained by the subtle changes in visually observable characteristics between some stages of ovary development exacerbated by colour changes post capture (Ferreri *et al.* 2009). The colour of the ovary tends to fade soon after capture (Hackett pers. obs.) making visual discriminations of ovary development stages more difficult. Thus, while the visual reference guide is likely to improve staging ability compared to a written description only, it is also likely that subtle changes in the appearance of the ovary following capture contribute to inaccuracy. These problems are also likely to be worsened by unfavourable working conditions that observers can experience on-board survey vessels. Time constraints in processing samples, combined with adverse sea conditions, and fatigue associated with working during night trawling operations, are all likely to contribute to inaccurate macroscopic staging of ovary development. In addition, factors affecting the reproduction of penaeids may also influence macroscopic assessment, such as moult stage, bopyrid parasites and lunar cycles: moult cycles are synchronised with reproduction and lunar cycles, with spawning occurring towards the end of intermoult (Courtney 1991; Dall *et al.* 1990; Owens and Glazebrook 1985); ovaries are always in a spent or re-absorbed condition in early post-moult (Dall *et al.* 1990) and; bopyrid parasites sterilise female prawns preventing ovary development with the incidence of parasitism exceeding 30 % at times in some fisheries (Courtney 1991). While these factors affect both macroscopic and microscopic staging, an awareness of such factors should help reduce variation and uncertainty in staging.

A large database of macroscopic determinations of ovary development has been developed for *P. latisulcatus* in South Australia over the past 45 y. The inaccuracy in the determinations of ovary development reported in this current study raise the question of how best to utilise this data for the management of *P. latisulcatus* in the future. Since most incorrect staging was due largely to miss identifying stages 0, 1 and 2, grouping stages into categories of mature (stages 3 and above) versus immature (stages 0, 1 and 2) might improve the correctness of the proportion of mature specimens. The application of such

categorisation to historic data may provide useful trends for assessment of the reproductive status for this species.

A problem associated with analysing method agreement in binary data is that it can be affected by chance (Hadzi-Pavlovic 2010). To address this problem, a chance-corrected measure of agreement can be used. Within this study Cohen's Kappa was used with the rate of agreement for observers emerging as moderate (Hadzi-Pavlovic 2010). Individual observers within this study would be unlikely to correctly assign ovarian stage to a female prawn if they were to guess. This is due to a K value of 0, suggesting that the agreement is no better than that which could be obtained by chance (Watson and Petrie 2010).

Based on the findings from the current study, it is suggested that increasing the level of accuracy of macroscopic staging will require multiple strategies. First, observers need to be trained to distinguish stages of ovary development and the effectiveness of the training needs to be validated by determining the accuracy of their categorisations. Variation in accuracy of macroscopic determinations was apparent among observers, although the level of prior experience of observers was not a factor in determining their accuracy. The accuracy of staging also needs to be validated to account for the mitigating influences of working on-board survey vessels. Second, the visual reference that observer are provided with needs to include all six stages of ovary development (see chapter 2), and may also need to include examples of the variation of ovary characteristics within each stage. In addition, the written and line drawing descriptions of ovary development given by King (1979) need to be adapted for use by observers on-board vessels rather than being reproductions of detailed figures. Finally, determinations of ovary development need to be made immediately after capture to eliminate the potential of confounding characteristics of colour change post-capture some of which has been highlighted by Penn (1980).

Overall, this study demonstrated that macroscopic staging of ovary development can be used as a rapid measure of spawning occurrence for penaeids; however, changes to the process of data collection should be incorporated into future stock assessment surveys to

improve its accuracy. Furthermore, the recording of females as “immature” and “mature” may provide a useful basis upon which to utilise the substantial historic datasets on macroscopic staging for this species to improve their accuracy. The validation of macroscopic staging with microscopic techniques is a valuable resource used throughout fisheries worldwide and the variation observed in the current study suggests that other fisheries that utilise macroscopic staging techniques should employ similar validity studies to those presented here to improve management decisions.

Part II Reproductive biology

Chapter 5 Reproductive biology of the western king prawn (*Penaeus (Melicertus) latisulcatus* Kishinouye, 1896) in a temperate environment

5.1 Abstract

Despite a history of sustainable fishery, there are considerable knowledge gaps in the fundamental reproductive biology of *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus*) in temperate regions. The present study investigated the reproductive biology of female *P. latisulcatus*. Samples were obtained from Spencer Gulf on a fortnightly basis for a period of 5 m over the main spawning season (October to February) from areas representative of the commercial fishing grounds. Female size ranged from 21 to 61 mm carapace length (CL) with the smallest ripe female sampled at 30 mm CL. Physiological maturity of females was determined by the appearance, shape and colour of the thelycum. Gonadosomatic index (GSI) increased with CL and fecundity varied largely between individuals, ranging from 9,500 to 567,000 eggs counted per female. The thelycum of physically mature females were white and fully formed in shape while the thelycum of immature females were transparent and not fully formed. Size at 50 % physiologically mature was 21.5 mm CL with the proportion of physically mature females increasing rapidly at larger sizes. A peak in the reproductive period in November was evident with an increase from 9 % mature to 27 % mature. This study provides invaluable information on the reproductive biology of female *P. latisulcatus* in a temperate environment.

5.2 Introduction

For species which occur in tropical and temperate environments, assessments of reproductive biology can provide insight to environmental effects on population biology (Penn 1980). Females of the same penaeid species are known to have differing reproductive patterns in their temperate and tropical distribution ranges, with a shorter spawning season limited to summer months in temperate environments (Courtney and

Dredge 1988; Dixon *et al.* 2012). Effects of environmental factors such as temperature, moon phase and moult cycle on the spawning periods and size at sexual maturity in penaeids is little understood. Water temperature affects the reproductive development in penaeids and often drives the onset and completion of each breeding cycle (Bhaud *et al.* 1995). Generally, warmer temperatures mark the onset of the breeding season, with colder weather bringing it to a close (Sakai and Harada 2001). Yet, in tropical locations spawning can occur throughout the entire year due to the continual high water temperature (Emlet *et al.* 1987; Rothlisberg and Jackson 1987; Rothlisberg *et al.* 1987), however, many tropical species of penaeids exhibit peaks in reproductive activity throughout the cooler months when water temperature drops to 18 °C to 23 °C (de Croos *et al.* 2011; Penn 1980).

In Australian waters, populations of the western king prawn *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus*) occur in hypersaline marine embayment's including Exmouth Gulf, Shark Bay and Cockburn Sound in Western Australia, around Northern Australia extending from Western Australia to Queensland as well as Spencer Gulf and Gulf St Vincent of South Australia (Grey *et al.* 1983; Penn 1980). While the reproductive development of penaeids is well understood in tropical environments (e.g. Peixoto *et al.* 2003; Peixoto *et al.* 2005a; Sakaji 2001), there are substantial knowledge gaps in the reproductive patterns of penaeids in temperate South Australian waters. Given that *P. latisulcatus* is at the southern extent of its geographic distribution in temperate South Australia, it could be expected that their reproductive biology differs to their tropical counterparts. Information on the reproductive biology of *P. latisulcatus* in temperate South Australia has so far only been provided in the unpublished thesis of King (1979) and fishery assessment reports by Carrick (2003). King's (1979) research efforts concentrate on the Spencer Gulf and Gulf St Vincent regions with his reproductive research focussing on size at sexual maturity, spawning cycle, sex ratios and spawner recruit relationships. Carrick's (2003) research is an assessment report on the Spencer Gulf prawn fishery which includes information in regards to the fecundity and main spawning periods of *P. latisulcatus*. Other research includes growth models for *P. latisulcatus* within Gulf St Vincent (Xiao and McShane 2000a; Xiao and McShane 2000b) and aspects of reproduction such as carapace

length and weight relationships, GSI and macroscopic ovarian stage in the temperate waters of Peel-Harvey estuary (Potter *et al.* 1991).

In tropical Gulf of Carpentaria, *P. latisulcatus* was found to have peaks in reproductive periods in September and January (Rothlisberg and Jackson 1987; Rothlisberg *et al.* 1987). A relationship between reproduction and season between locations has also been suggested by Montgomery *et al. (2007a)* for *Melicertus plebejus,* which highlights how reproductive patterns differ between environments. Furthermore, the relationship between moulting and the onset of spawning is thought to be strongly affected by local environmental conditions experienced by both female and males (Courtney *et al.* 1995b; Smith and Sainte-Marie 2004). Moulting in females is thought to be initiated by the lunar phase (Courtney *et al.* 1995a). In thelycum species, a female is inseminated directly before the moult cycle begins when the body is still soft (Courtney *et al.* 1995b; Smith and Sainte-Marie 2004). Multiple spawning of *P. latisulcatus* in two northern Western Australian populations occurred all year round although the main spawning period was between May and October as water temperature remained above the threshold temperature of 17 ˚C for spawning (Penn 1980).

South Australia's Spencer Gulf and Gulf St Vincent prawn trawl fisheries harvest ~2,200 t of *P. latisulcatus* annually. Around 20 % of this catch is harvested during the November and December fishing periods when domestic demand peaks prior to Christmas (Dixon *et al.* 2009). This coincides with the onset of spawning and thus management measures are critical to protect the spawning biomass at this time. A recent study identified that the total catch during the pre-Christmas period is the key factor influencing the strength of subsequent recruitment within a population (James *et al.* 2010). Currently, the visual assessment of reproductive staging of female prawns developed by King (1979) is being used to determine closed areas during these fishing periods. A survey is conducted over two nights within Spencer Gulf South Australia. Based on the catch and visual assessment within each area (Figure 5.1) the fishing grounds for commercial catch are determined. Further research has shown (Chapter 4) that staging female *P. latisulcatus* with King's (1979) guide as a reference has caused consistent over estimating of the ovarian stages.

This over estimation has implications on pre-Christmas harvest strategies as the total allowable catch is based on the visual assessment. Increasing our knowledge of *P. latisulcatus* reproductive biology, may guide better management decisions.

Indices based on the assessment of individual specimens and ovaries are widely used in fisheries science to identify reproductive status in fish and crustaceans (Ferreri *et al.* 2009). Common techniques include data collection for the histological determination of ovarian maturity stage, gonadosomatic index, fecundity, size at 50 % physiological maturity and percent ripe. These measures require additional assessment of ovary stage by microscopic validation.

Morphometric relationships are useful measures for understanding reproduction or population biology in general. For penaeid species, length frequency data can be converted to weight frequency data for stock assessment models (Dall *et al.* 1990; Primavera *et al.* 1998). Measurements generally include carapace length (CL), body length, total length and wet weight. Length-weight relationships have been published in detail in both the field (Bishara 1976; Penn and Hall 1974; Yamada *et al.* 2007) and laboratory based growth studies (Cheng and Chen 1990; Saldanha and Chatterji 1997). Growth and maturation in penaeids has been associated with differences in larval salinity and temperature tolerance (Preston 1985a; Preston 1985b). Temperature and salinity are two of the main factors that affect growth in Penaeids with research showing that larval temperature and salinity tolerance can be linked to environmental conditions of females during maturation (Preston 1985a).

The majority of penaeid reproductive studies focus on the maturation of ovaries. Specifically many of these studies are based on the colour of the ovary or the histological staging of the ovary (Ayub and Ahmed 2002; King 1948; Tuma 1967). Histological maturation of ovarian developmental stages has been described for many penaeid species (e.g. King 1948; Peixoto *et al.* 2005b; Quinitio *et al.* 1993; Yano 1995). Other studies have also demonstrated that oocyte size, frequency and diameter are good indicators of ovarian

development (Baelde 1992; Tan-Fermin 1991). The oocyte composition of ovaries in the eastern king prawn (*M. plebejus*) varies depending on location (Montgomery *et al.* 2007b), which is useful for identifying when and where spawning has taken place. Montgomery *et al.* (2007b) found that the proportions of oocytes in the vitellogenic and cortical specialisation stages were greater in the northern locations of their study, suggesting that the proportions of mature oocytes increased with decreasing latitude. On the other hand, two species of penaeids within one location can reach maturity and spawn at different sizes (Courtney and Dredge 1988), indicating that reproductive variation can also be species specific. Factors attributing to reproductive maturity in different species within the same location may include size at first maturity in relation to growth and moult frequency (Montgomery 1992; Prangnell and Fotedar 2006).

Examinations of histological ovarian tissue and GSI have been used for estimating size at sexual maturity and spawning seasons of penaeids (Ohtomi *et al.* 2003). The kuruma prawn *M. japonicus* in Japan differs in size at sexual maturity between two regions (Yatsushiro Sea and Shibushi Bay) along with the spawning season (Ohtomi *et al.* 2003). Ohtomi *et al.* (2003) suggests that reproductive activity of *M. japonicus* is continuous throughout the year within Shibushi Bay, as the water temperature remained above 18 °C compared to Yatsushiro Sea where reproductive activity is limited from April to September.

The purpose of this study was to examine fundamental aspects of the reproductive biology of *P. latisulcatus* in temperate Spencer Gulf, South Australia. A histological description of ovarian stages is provided in chapter 2 with a focus on developing more comprehensive criteria for histological ovarian staging in *P. latisulcatus*. Chapter 4 within this study also shows that a consistent overestimation of staging is occurring macroscopically for *P. latisulcatus*. This chapter describes some reproductive characteristics of *P. latisulcatus* such as physiological maturity by maturity stage and size, GSI and fecundity estimates for ripe females and the percent of ripe females over the sampling period. The information provided within this chapter contributes to the understanding of current reproductive biology of *P. latisulcatus* also advances the findings of chapters 2 and 4 by enhancing the

understanding of ovarian staging, given insufficiencies and errors emerging from previous work, and providing a more comprehensive set of data for reproduction in *P. latisulcatus*.

5.3 Materials and methods

5.3.1 Sample collection

Sampling was conducted fortnightly in Spencer Gulf, South Australia, from October 2008 until February 2009. The geographic range of sampling covered one of the three main offshore fishing grounds for *P. latisulcatus* in South Australia (Figure 5.1). Samples were taken using a demersal otter trawl after dusk on commercial fishing vessels and the RV Ngerin, at depths ranging from 10 to 45 m. All of the trawls were made at night and lasted from 10 to 30 min. The double-rig gear had a maximum total headline length of 29.26 m and a minimum mesh size of 4.5 cm. Each net was fitted with a codend consisting of a PVC protective mat and a mesh bag with 150 mm mesh. This design is used to keep crabs out of the main catch and is often referred to as a crab bag. Sea surface water temperature was also recorded using a digital thermometer. *P. latisulcatus* were sampled from the catch by taking a 7 L bucket of prawns with a total number of 340 prawns collected over the 9 sampling trips (Table 5.1). Prawns within the bucket sample were sexed and after each trawl, the females retained for ovary dissection were macroscopically assessed for maturity stage. Sampling trips were scheduled to co-inside with a full moon or new moon to explore the relationship between maturity stage and lunar phase. The full moon cycle in December was missed, however, due to Christmas when no crew was available for sampling.

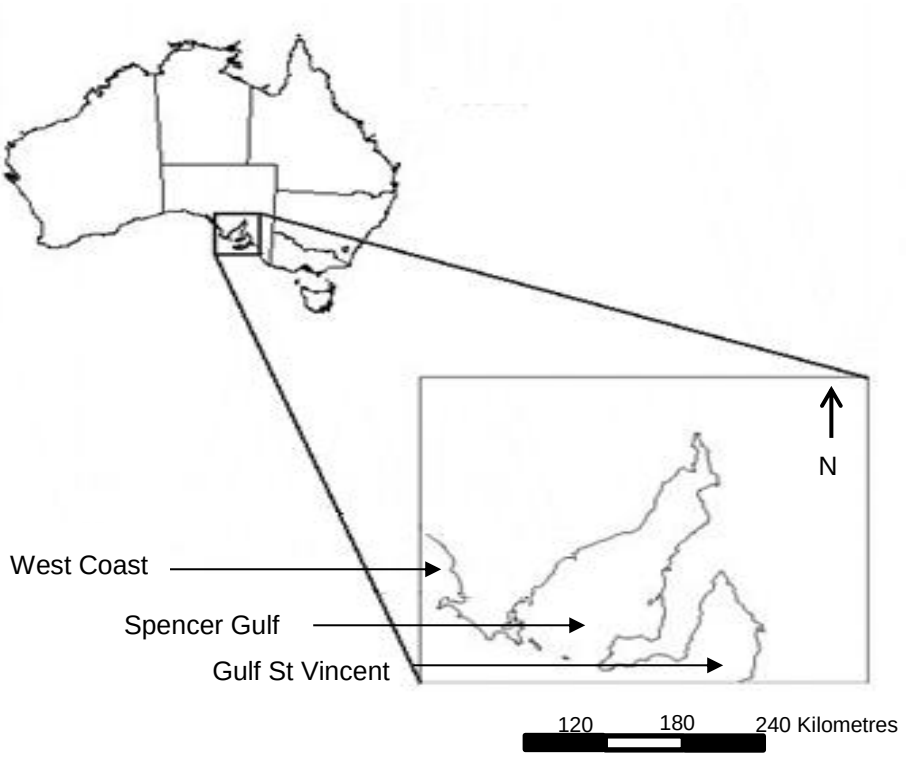


Figure 5.1. Main offshore fishing grounds for *Penaeus (Melicertus) latisulcatus* of West Coast, Spencer Gulf (34.3036 °S, 136.9805 °E) and Gulf St Vincent (35.2167° S, 138.2500° E) in South Australia.

Carapace length (CL) was measured from the posterior margin of the orbit to the middorsal posterior edge of the carapace using manual vernier callipers (± 1 mm). Total weight and ovary weight of each specimen was measured using an electronic balance (± 0.01 g). Six reproductive stages were determined according to criteria given by King (1979), based on the colour and fullness of the ovary (Chapter 2, Table 2.1). Three size classes of prawns were selected: small (≤ 35 mm), medium (36 to 45 mm) and large (≥ 46 mm), on the basis of commercial prawn survey length-frequency data from the South Australian Research and Development Institute and its historical database (Dixon *et al.* 2012). Measurements for each female included carapace length (CL), total body weight (g), ovary weight (g) and macroscopic gonad stage. Female prawns were deemed physiologically 'mature' if they possessed a thelycum that was well formed and white, while physiologically 'immature' females possessed a thelycum which was poorly developed and transparent. The thelycum of each female was also examined for the presence of a spermatophore. Ovary development stages 0, 1 and 2 are referred to here as 'not ripe' and ovary development

stages 3 and 4 as ‘ripe’. Ovary development stage 5 is referred to as ‘spent’ although no stage 5 females were recorded.

A minimum of nine females per reproductive stage and over the three size classes were collected where possible. Due to weather conditions, collecting this number of samples was not always possible (Table 5.1). All 340 collected females were retained for microscopic assessment.

Table 5.1. Samples collected from the nine commercial and research trips including the number of prawns, size of prawns (S, M, and L) and macroscopic stage (0, 1, 2, 3, 4 and 5) of female *Penaeus (Melicertus) latisulcatus* collected (S = small ≤ 35 mm, M = medium 36 to 45 mm and L = large ≥ 46 mm).

	Macroscopic stage and Size Class																		
Stage	0			1			2			3			4			5			Total
Field Trip Date	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L	S	M	L	
17th October	3	3	3	3	3	3	1	3	3	0	3	3	0	0	0	0	0	0	31
29th October	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0	0	0	0	36
15th November	3	3	3	3	3	3	3	3	3	2	3	3	0	0	0	0	0	0	35
28th November	1	3	3	3	3	3	2	3	3	2	3	3	0	1	2	0	0	0	35
15th December	3	3	3	3	3	4	3	3	4	3	4	5	0	3	3	0	0	0	47
11th January	3	3	3	4	3	1	3	3	3	2	4	3	1	2	3	0	0	0	41
27th January	3	3	1	2	3	3	3	3	3	3	3	3	0	0	0	0	0	0	33
9th February	3	3	3	2	3	3	3	2	3	3	3	3	3	2	1	0	0	0	40
25th February	3	3	3	3	3	3	3	3	3	2	4	3	0	2	4	0	0	0	42
																			340

5.3.2 Histological determination of ovarian maturity stage

The total of 340 females collected consisted of 99 small, 120 medium and 121 large specimens, within the six stages of macroscopic ovary development (Table 5.1). Samples were fixed in a solution of formalin, glacial acetic acid and calcium chloride (FAACC) (Humason 1962; Kaplan and Amadeo 1996) for 48 h before being transferred to 70 % ethanol. Subsequent histological analysis was carried out using techniques previously given in chapter 2.3.2.

5.3.3 Reproductive analyses

Analyses of biological data included length-weight relationships, gonadosomatic index (GSI), fecundity, physiological maturity and percent ripe. GSI is the calculation of the gonad mass as a proportion of the total body mass and was calculated as follows: GSI (%) = 100 x OW/BW where, OW is ovarian weight (g) and BW is the total body weight (g) (Barber and Blake 2006). Coefficients were calculated among the three size classes of female prawns for each stage of ovary development that were independently determined from histological examinations of ovary tissue.

The fecundity of *P. latisulcatus* with ripe ovaries was determined from counts of the number of eggs in 0.001 g sample of ovary tissue. Females were cut along the dorsal surface to expose the ovarian tissue and then preserved in FAACC. Counts were made on three samples of ovarian tissue from each specimen. Ovarian tissue was teased apart using dissecting needles under a microscope to release eggs from other tissues. Once the eggs were separated from connective tissues they were transferred to a microscope slide with a grid of 1 mm^2 divisions and enumerated under a compound microscope. The mean number of ripe ova per gram of ovary and the total weight of the ovary were used to calculate fecundity, based on ovaries from a total of 97 mature females from which enough ovary could be retained for analysis. This method has been adapted from Penn (1980).

Physiological maturity was classified by examining the structure, colour and development of the thelycum, which is an external pocket on the ventral side of the thorax in penaeid

females functioning as a seminal receptacle (James 1977; King 1948; Motoh and Buri 1980). 50 females ranging from 13 to 29 mm CL were examined under a dissecting microscope. The size at which 50 % of the sampled female population has reached physiological maturity was measured. The cumulative percent of individuals was plotted against size and then fitted with a logistic curve using equation:

$$P_m = \frac{1}{1 + e^{a(CL-B)}}$$

Where P_m is the proportion of mature animals, CL is the carapace length, e is the inflexion point of the curve and ^{a} and ^{b} are constants.

5.3.4 Data analysis

The statistical software IBM SPSS V20 was used for all statistical analyses. A non-parametric Kruskal Wallis test was used to assess significant differences in ovary weight and carapace length and GSI and carapace length (Robinson and Bakeman 1998).

Relationships between carapace length and all ovary weights, mature ovary weights and GSI were examined using Spearman's rank correlation. Regression analyses were used to test the relationship between mature ovary weight (g) and carapace length (mm) (Field 2009).

5.4 Results

5.4.1 Length – weight relationship

Carapace length and associated weight were available for 340 individual females collected from Spencer Gulf during October 2008 to February 2009. Ovaries could be discerned in individuals as small as 21 mm in CL with 'not ripe' females ranging from 21 mm to 61 mm (± 0.1 mm) and 'ripe' females from 30 to 52 mm (± 0.1 mm). The smallest ripe female observed was 30 mm CL. Body weight varied from 3.77 to 98.83 g (± 0.1 g) (Figure 5.2). Fewer than 12 % of females were histologically classed as ripe, although more than 31 %

were originally macroscopically classed as ripe. At 42 mm carapace length 50 % of female *P. latisulcatus* were found to be mature.

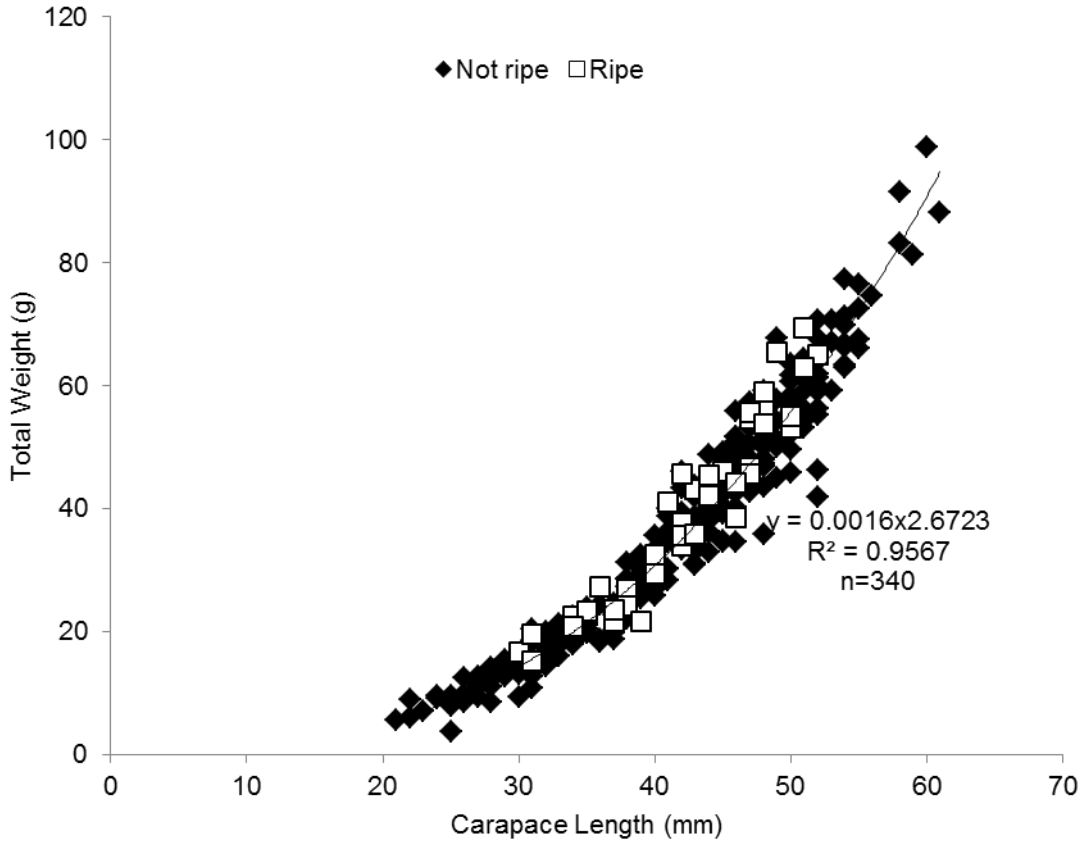


Figure 5.2. Total length-weight relationship for ripe and not ripe female *Penaeus (Melicertus) latisulcatus* caught over summer 2008 to 2009 in Spencer Gulf, South Australia.

A maximum ovary weight of 5.72 g was recorded for a female of 58 mm in carapace length, with ovary weight positively correlated to carapace length ($P < 0.05$). A Kruskal-Wallis test revealed a statistically significant difference in ovary weight (g) across the three different size classes ($P < 0.001$), with females of the large size class having heavier ovaries than those from the small and medium size classes ($P < 0.017$). A regression of the relationship between ripe ovary weight (g) and carapace length (mm) showed that carapace length can account for up to 30 % of the variation in ovary weight (Figure 5.3). Examination of the relationship between ripe ovary weight and the base weight (total weight less the weight of the ovary) for the same sample indicated that ripe ovary weight makes up an average of 5.5

% of the total weight, however, this can be as high as 8.8 % of the total body weight within the size range examined (30 - 52 mm).

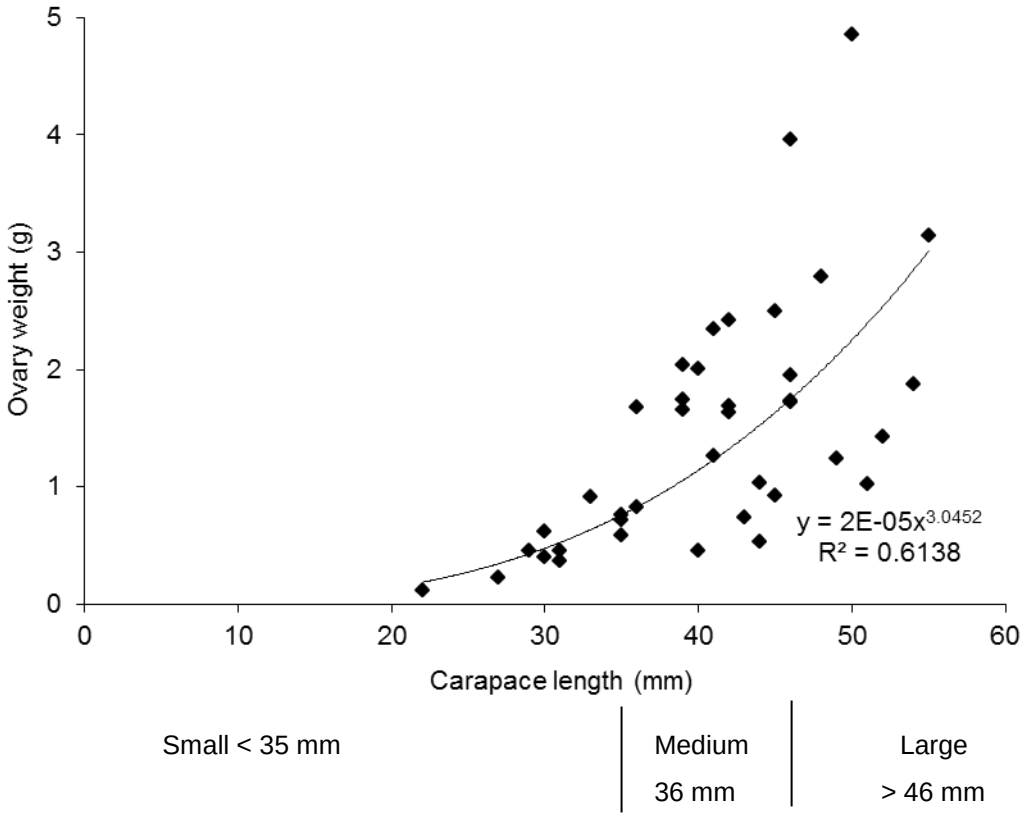


Figure 5.3. Relationship between ripe (stage 3 and 4) ovaries and carapace length for female *Penaeus (Melicertus) latisulcatus* caught from Gulf St Vincent in December 2007 and Spencer Gulf in November 2007 and February 2008. N = 39.

5.4.2 Gonadosomatic index and fecundity

Overall GSI ranged from 0.04 to 8.33 for individuals between 21 - 61 mm CL. A maximum GSI of 8.33 was recorded for a female of 38 mm in CL and deemed physiologically mature at stage 3 of oocyte development. GSI was positively correlated to carapace length ($P< 0.05$). A regression of the relationship between GSI and CL (mm) showed that carapace length can account for up to 6 % of the variation in ovary weight ($r^2 = 0.067$). All females recording a GSI > 0.06 were found to be mature compared to almost 70 % of females with a GSI 0.03.

Fecundity of *P. latisulcatus* varied between 40,000 to 207,000 eggs per gram (body weight) for individuals between 21 - 61 mm CL. To relate the ovary weight data to fecundity, the relationship between the size of the individual and the number of ova per gram of ovary were investigated. On average across all specimens, the mean number of oocytes per gram of ovary tissue in ripe specimens was 100,992 (± 5073). Using this value, fecundity ranged from 9,423 to 567,600 eggs over a size range of 21 - 61 mm CL.

5.4.3 Physiological maturity

Size at 50 % physiological maturity (meaning females are physically capable of spawning but may not necessarily have mature ovaries present) was 21.5 mm CL (Figure 5.4) with the proportion of mature females increasing rapidly at larger sizes.

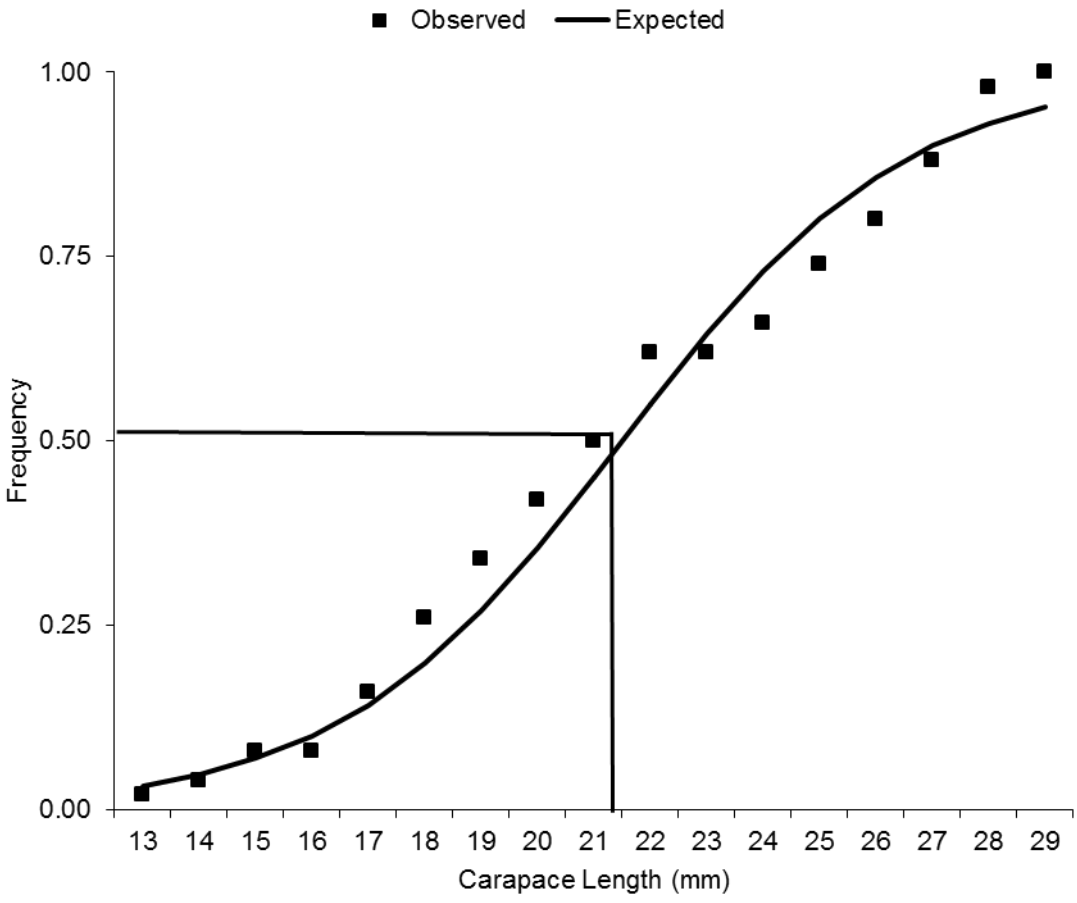


Figure 5.4. Logistic curve to determine 50 % physiological maturity (■) of female *Penaeus (Melicertus) latisulcatus*.

5.4.4 Insemination rates

Over the sampling period, 340 *P. latisulcatus* females were examined of which 304 were found to be inseminated. The smallest *P. latisulcatus* found inseminated was 23 mm CL (Figure 5.5). The frequency of insemination increased sharply around 24 mm CL. 100 % of female *P. latisulcatus* over 50 mm CL were found to be inseminated.

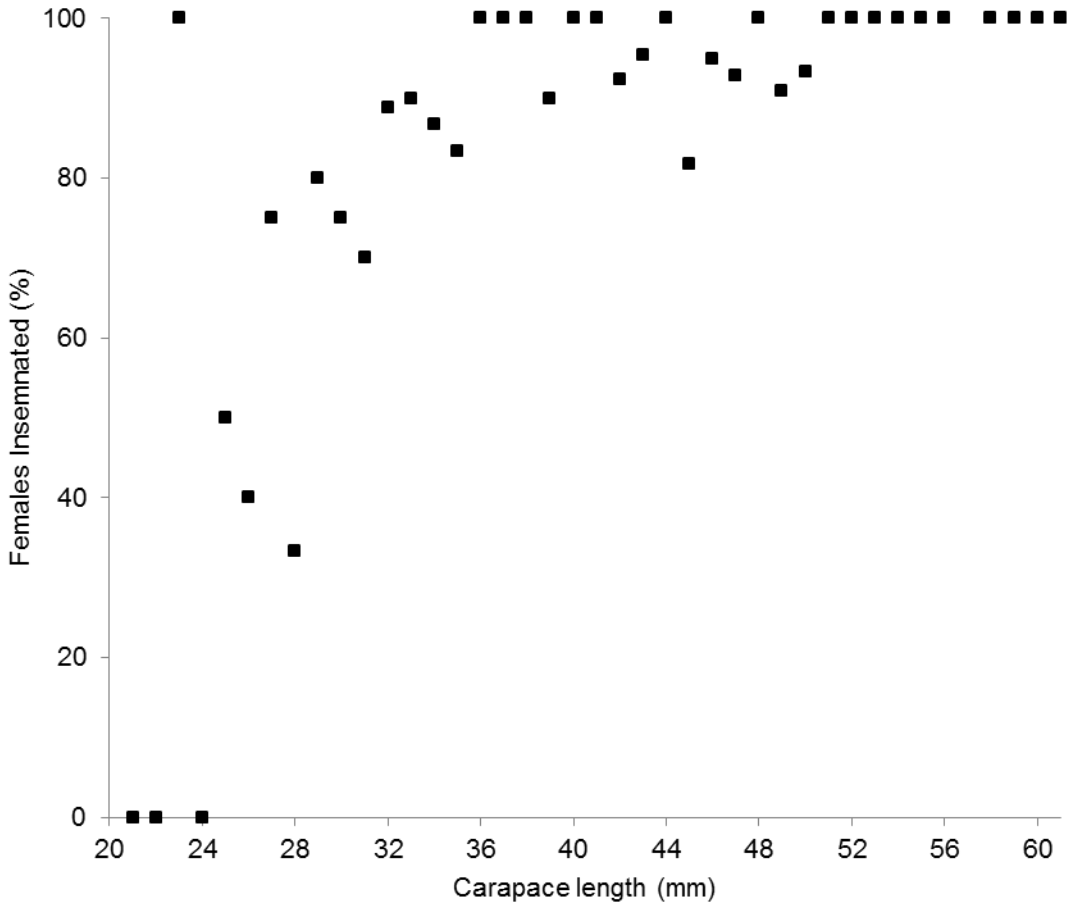


Figure 5.5. Percentage of inseminated females in different size classes for *Penaeus (Melicertus) latisulcatus* in Spencer Gulf South Australia.

5.4.5 Percent ripe

The proportion of stage 0, 1 and 2 ovaries increased with increasing size from 30 % in the small size class (≤ 35 mm) to 35 % for the large size class (≥ 46 mm) (Figure 5.6). The percentage of stage 3 and 4 (ripe) females ranged from 7 to 16 % with the proportion of females with stage 4 ovaries never exceeding 12 % in any month sampled.

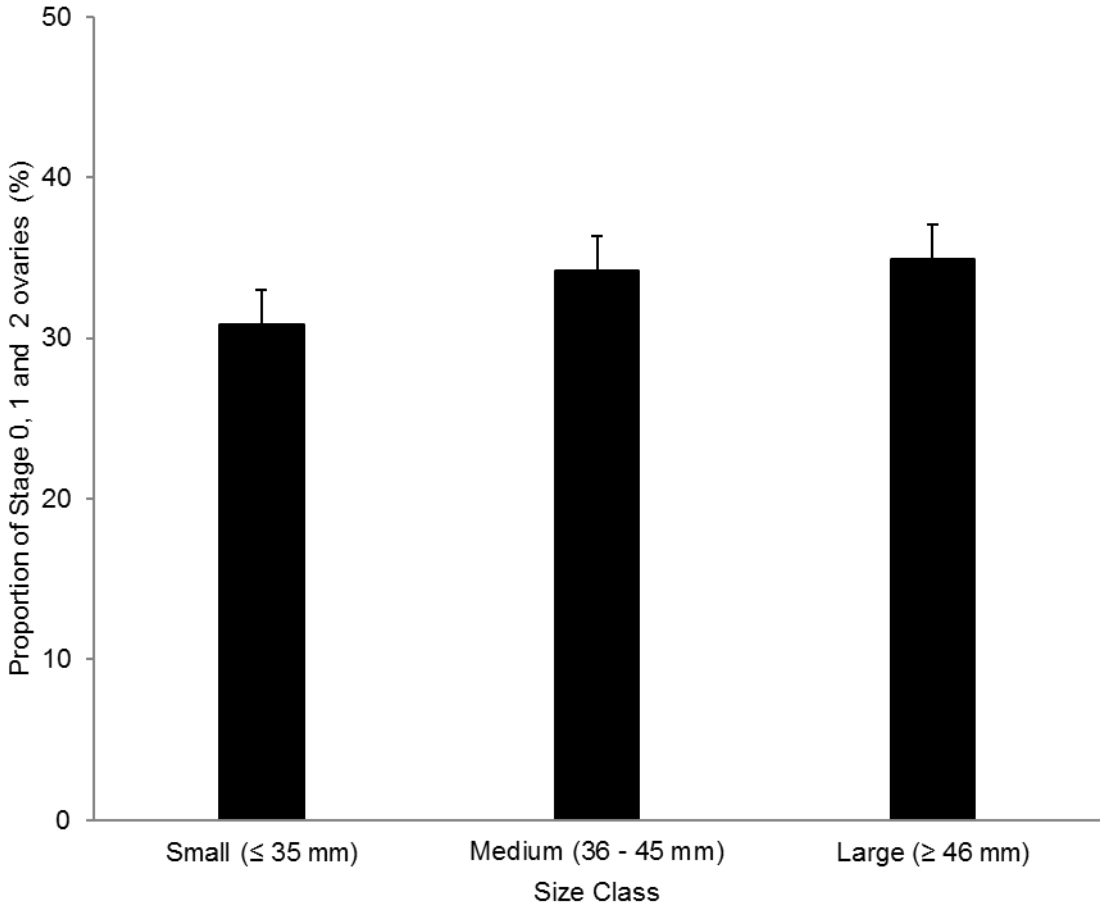


Figure 5.6. The proportion of stage 0, 1 and 2 ovaries for size classes of small (≤ 36 mm) medium (37 to 45 mm) and large (≥ 46 mm) female *Penaeus (Melicertus) latisulcatus* sampled from October 2008 to February 2009 from commercial surveys in Spencer Gulf South Australia (±SD).

Catches of stage 0, 1 and 2 females in October/November were lower than in January/February whilst catches of stage 3 and 4 prawns were greatest in November for medium and large size classes. This is consistent with a large proportion of stage 3 and 4 females occurring in November followed by a steady decline (Figure 5.7).

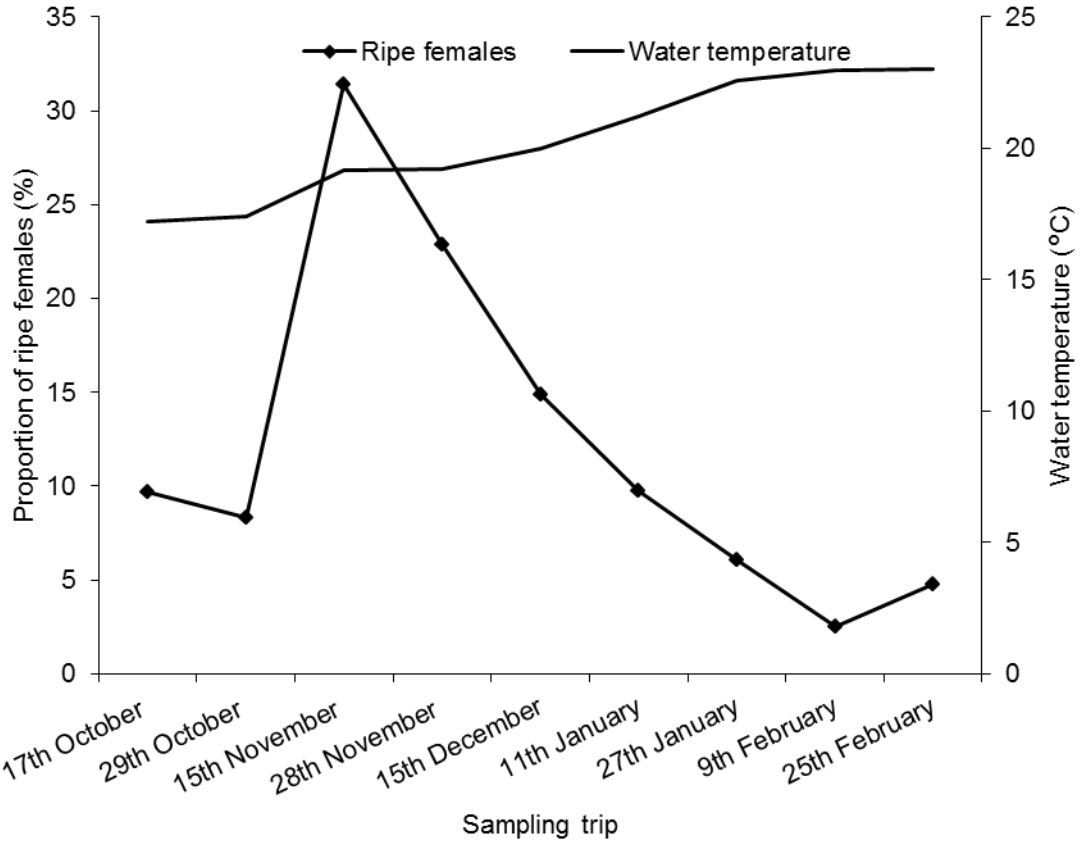


Figure 5.7. The proportion of stage 3 and 4 (ripe) female *Penaeus (Melicertus) latisulcatus* and water temperature for each month from October 2008 until February 2009 over a 6 m sampling period, in Spencer Gulf, South Australia.

Water temperature increased steadily over the sampling period from 17.2 °C in October 2008 to 23 °C in February 2009 (Figure 5.8 A to C). Few females spawned before November (Figure 5.8 A to C). Over all size classes, the greatest proportion of spawning females was seen during November (Figure 5.8 A to C), however, the percentages are quite low with less than 30 %. For smaller females the proportion of ripe individuals occurred only over a short time in November (Figure 5.8 A).In the medium size class, proportions of spawning females were highest over the November and December sampling period, while in the large size class, the proportion of ripe females stayed between 20 and 30 through to the end of January (Figure 5.8 B to C).

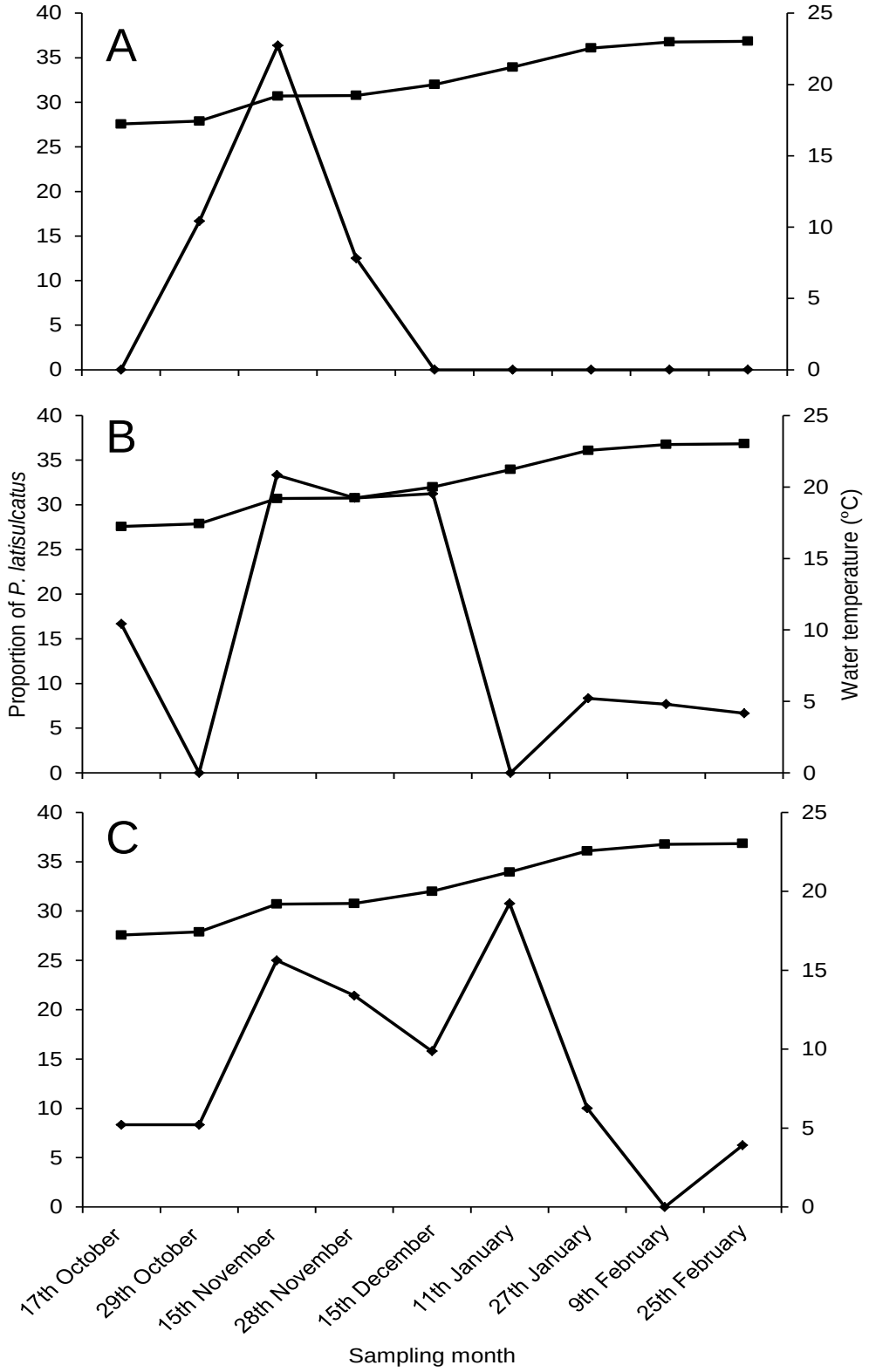


Figure 5.8. Proportion of ripe (stage 3 and 4) female *Penaeus (Melicertus) latisulcatus* and average sea surface water temperatures for each sampling occasion between October 2008 and February 2009 for Spencer Gulf South Australia. A) Small females <36 mm B) Medium females 37 to 45 mm C) Large females of >46 mm.

5.4.6 Histological validation of ovarian stage

The histological validation of ovarian stage developed in Chapter 2 was used to validate macroscopic assessments of the 340 female P. latisulcatus collected within the field. From the 340 females assigned macroscopically to stages 0 to 5 (yet no stage 5 found), overall 49 % were staged correctly. During October and November stage 3 ovaries were overestimated with macroscopic staging compared to microscopic validation (Figure 5.9). Stage 0 was consistently underestimated with the exception of November. The greatest underestimation occurred in February (Figure 5.9). Stage 2 was also underestimated from November to February (Figure 5.9). The number of females with stage 4 ovaries was found to be greatest in November and not February as the macroscopic data suggested (Figure 5.9).

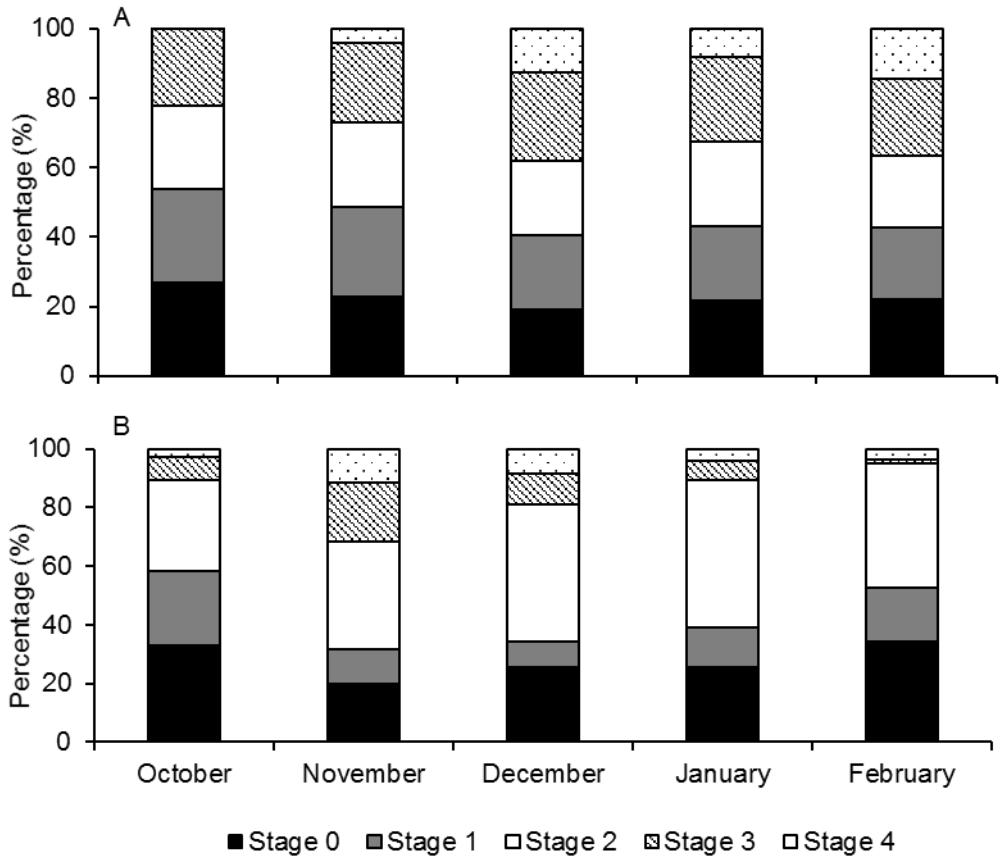


Figure 5.9. The percentage (%) of female *Penaeus (Melicertus) latisulcatus* a) macroscopically staged and then b) microscopically validated using the validation criteria derived in Chapter 2 for sampling months of October 2008 to February 2009 in Spencer Gulf, South Australia.

5.5 Discussion

Reproductive studies on *P. latisulcatus* have been largely carried out in tropical environments (e.g. Abdel Razek *et al.* 1996; Courtney and Dredge 1988; Penn 1975; Penn 1976; Penn 1980; Roongratri 1993; Rothlisberg and Jackson 1987; Rothlisberg *et al.* 1987) with little research focussing on *P. latisulcatus* in temperate South Australian regions (Carrick 2003; Kangas 1999; King 1979; Potter *et al.* 1991). This research here broadens the knowledge with a focus on total length-weight relationships, GSI and fecundity, physiological maturity and percent ripe, comparing the reproductive cycle of *P. latisulcatus* for Spencer Gulf, South Australia with *P. latisulcatus* of tropical waters.

5.5.1 Total length - weight relationship

In the present study, female *P. latisulcatus* were caught as small as 13 mm CL with the smallest ripe female at 30 mm CL. These results are similar to Penn (1980) for tropical *P. latisulcatus* in Western Australia who found smallest ripe females at 28 mm CL, however, Courtney and Dredge (1988) found the smallest ripe *P. latisulcatus* in tropical Queensland at 43.1 mm CL. This comparison suggests that *P. latisulcatus* of temperate South Australian waters produces ripe ovaries at a similar size to those in Western Australia, but differs to those in Queensland. With maturity closely related to CL in penaeid prawns, size at maturity, shown as the CL at which 50 % of females are mature, provides a good indication of spawning within a population (Crocos 1987). Size at maturity of *P. latisulcatus* for Spencer Gulf is at 42 mm CL. The length-weight relationship for Spencer Gulf prawns has previously been determined from a sample of over 2000 prawns in a tag-recapture study (Carrick 2003). Juvenile *P. latisulcatus* length weight relationships have also been studied by Kangas (1999) in Gulf St Vincent. Kangas (1999) also found that the length weight relationship for juvenile *P. latisulcatus* increased with increasing weight, with a size range of individuals from 2.4 to 20.4 mm CL. Carrick (2003) showed that the power relationship between male and female *P. latisulcatus* in Spencer Gulf varied between males and females, and was described by the equation “Weight = a x carapace length^ b”, where a and b are constants (males a = 0.00124, b = 2.76 and females a = 0.00175, b = 2.66).

Carrick (2003) used a modified von Bertalanffy growth model to research sex-specific growth parameters within male and female *P. latisulcatus* in Spencer Gulf South Australia. Carrick's (2003) research showed that male prawns are generally smaller in size and grow slower than females with maximum growth rates occurring from February to April. The von Bertalanffy limited growth model is $dL/dt = k (L\infty - L)$, where k is a function of temperature. The formula for growth is usually re-written as $L (t) = L\infty (1\text{-er } (t\text{-}t0))$, where r is the specific growth rate, t is time, and k = r. The constant r is gender and species specific. Based on this relationship for female *P. latisulcatus* in Spencer Gulf Carrick (2003), found that 50 % of female *P. latisulcatus* have spawned or reached maturity are approximately 1.75 y old.

5.5.2 GSI and fecundity

GSI was positively related to CL in female prawns and was found to increase with ovarian maturity stage. Ohtomi *et al.* (1998) found that female deep-water mud shrimp *Solenocera melantho* with a GSI greater than 12 were mature. Although GSI was lower for *P. latisulcatus* within this study (range of 0.04 to 8.33) it increased with maturity. Potter *et al*. (1991) found that the mean GSI for *P. latisulcatus* in the temperate Peel-Harvey estuary ranged from 0.78 in immature females to 10.5 in mature females, which suggests that *P. latisulcatus* of temperate environments show similar increases in GSI with maturity.

Maximum reported fecundity for *P. latisulcatus* ranges from 105,000 to 650,000 ova within a CL range of 29 to 53 mm (Dall *et al.* 1990; Penn 1980), with the maximum fecundity of *P. latisulcatus* from this study fitting within this range (maximum estimated at about 317,000 ova). A significant relationship between CL (mm) and ovary weight (g) showed that ovary weight increases with CL (mm), meaning that larger prawns are more fecund and therefore more likely to produce large quantities of eggs. Recorded estimates of fecundity ranging from 44,000 to 534,000 ova for *P. duorarum* (Martosubroto 1974) to as high as 800,000 to 1,300000 ova for *P. trisulcatus* [= *P. kerathurus*] (Heldt 1938 cit. in Penn 1980) show that estimates of fecundity for penaeids vary between species and with size.

5.5.3 Insemination and Physiological maturity

Evidence of insemination within *P. latisulcatus* started at approximately 23 mm CL. The results shown here for spawning *P. latisulcatus* are much smaller than those by Penn (1980) and Courtney and Dredge (1988) who found it was rare for *P. latisulcatus* to mate when less than 28 mm CL and 27 mm CL respectively.

Physiologically mature females were recorded at 13 mm CL with 50 % reaching physiological maturity at 21.5 mm CL. *Penaeus latisulcatus* in Spencer Gulf has thus a smaller size at physiological maturity than *P. latisulcatus* in Western Australia. Penn (1980) described the size at first maturity of *P. latisulcatus* in Western Australia as 25 mm. This slight difference may be due to the warmer waters in Western Australia creating an environment that is favourable of increased growth allowing *P. latisulcatus* to reach physiological maturity at a larger size. Carrick (2003) showed seasonal growth, as very little growth occurs for *P. latisulcatus* in Spencer Gulf from July to December whereas during the warmer months of February to April growth rates were quite high.

The number of females that were physiologically mature in Spencer Gulf was high between October and November with a peak in numbers during November, followed by a gradual decline from December to February. This peak in maturity corresponds with increased water temperature (rise above 17 °C) seen to increase steadily from October into late summer. Water temperature alone, however, cannot explain the peak in the reproductive period during November, as temperature increases at a constant rate. Water temperature is thought to play a vital role in the onset of spawning in female *P. latisulcatus*, once risen above 17 °C (Rodgers *et al.* 2013). Courtney and Dredge (1988) reported a steady decline in ovary weight for *P. latisulcatus* from July to March with the minimum recorded ovary weight in March each year over the sampling period. Courtney and Dredge (1988) saw a peak in stage 3 and 4 ovaries in June to August (winter) when temperatures were at their lowest (around 24 °C). Similarly, Rothlisberg et al. (1987) and Rothlisberg and Jackson (1987) saw that two seasonal peaks in spawning occurred for *P. latisulcatus* within the Gulf of Carpentaria in Australia. These peaks in reproductive maturity of *P. latisulcatus* within

Australian waters coincide with a rise or fall in water temperatures, with an optimum spawning temperature range for *P. latisulcatus* between 17 to 30 °C (Penn 1980; Rothlisberg and Jackson 1987; Rothlisberg *et al.* 1987). Similarly, research by Cha *et al. (*2002) on *Penaeus chinensis* has shown that seasonal reproductive patterns occur due to changes in ovary maturation and insemination with a rise in temperature triggering the onset of spawning.

5.5.4 Percent ripe

Few small ripe females were found, suggesting that small females may be reabsorbing and re-investing their energy in growth. For prawns in related families (*Solenocera melantho*, *Solenoceridae*), Ohtomi *et al.* (1998) found that the spawning season runs from June until December, with a peak in spawning during October and November. Larger females of 2 and 3 y in age were found to ripen earlier in the season, while smaller females were seen to ripen for most of the spawning period (Ohtomi *et al.* 1998). When data from October 2008 to February 2009 surveys were compared with commercial survey data, small (< 36 mm) ripe female *P. latisulcatus* showed similarities in the percentages of ripe females (Dixon and Hooper 2008; Dixon *et al.* 2009), inferring that the percentage of small ripe females from this study are consistent with those from commercial surveys in Spencer Gulf. Alternatively, Crocos and Kerr (1983) have suggested that the distribution of female prawns with different ovarian stages varies with water depth. *P. merguiensis* has been described as having a spawning cycle with seasonal variation in relation to water depth, which coincides with migration (Crocos and Kerr 1983). Seasonal egg production has also been described for *P. semisulcatus* within the North-western Gulf of Carpentaria (Crocos 1987). If sampling has been restricted to locations of commercial catches, it is possible that the detection of all ovarian stages may be biased.

The decline in reproductive activity over time may be explained in part by the effect of size on spawning occurrence. Smaller individuals were seen to mature during October to February but were not producing ripe ovaries. It appears that these individuals may be reabsorbing oocytes, investing their energy into growth during the later months of the

spawning period while larger females spawn again (personal observation). Ceballos-Vázquez *et al.* (2010) have shown that multiple spawning and higher spawning occurrence in female penaeids is directly related to age and size.

Data presented here shows one peak during the spawning season for *P. latisulcatus* in Spencer Gulf. To determine if this peak is a continual occurrence, subsequent sampling would need to be completed. Studies by Carrick (2003) showed spawning to be seasonal for *P. latisulcatus* in Spencer Gulf with the proportion of reproductively mature females increasing with size. During November and December in GSV, female numbers were seen to increase leading towards a female biased population (Svane and Roberts 2005). Crocos (1987) found that the proportion of female *P. semisulcatus* within a population actually spawning varied with season. The highest proportion was between August to November/December (>40 %) and the lowest was between February and June (<30 %). Similarly Crocos and Van der Velde (1995) found that egg production was seasonal with a major spawning peak in August/September and a minor one in February. A determination of the number of spawning events for individual prawns of different sizes would require tracking individual specimens over the spawning season. Tag and recapture studies have previously been carried out for *P. latisulcatus* within Cockburn Sound, Western Australia (Penn 1975) and for growth models in Gulf St Vincent, South Australia (Xiao and McShane 2000a; Xiao and McShane 2000b). Over a period of 30 to 45 d, Penn (1975) recaptured 44 females from the original 2723 tagged, of which 55 % had spawned with 29 % showing ovary development towards a second spawning. Penn's (1975) research has shown multiple spawning with a single year occurs within *P. latisulcatus* populations. As no published tagging data is available for *P. latisulcatus* in Spencer Gulf a study of this type would prove useful in determining the number of spawns per female.

5.5.5 Histological Validation of ovarian stage

In this study, it was demonstrated how staging techniques can be used to quantify ovarian development and that macroscopic staging is suitable for differentiating between immature and mature eggs of western king prawn *Penaeus (Melicertus) latisulcatus* within Spencer

Gulf, South Australia. With the exception of Montgomery et al. (2007b) few studies exist where macroscopic staging has been compared to microscopic staging. The use of this technique has the advantage of describing the development of the egg assemblage and by doing so considers eggs at different stages of development within the ovary. It also avoids the bias associated with classifying ovaries by the dominant oocyte type and errors associated with measuring the size of oocytes.

Whilst classification of penaeid gonads by macroscopic examination is subjective, I have demonstrated an association between maturation stages determined by macroscopic and histological observations for *P. latisulcatus*. Thus, this study, along with Quintero and Gracia (1998) , Peixoto et al. (2003) and Montgomery et al. (2007b), is one of few that links macroscopic observations to oocyte development. The descriptions of changes in gonad colour particularly in the stages III and IV seem to differ between penaeids (Dall *et al.* 1990). For example, Quintero and Gracia (1998) and Peixoto et al. (2003) described a “light green” colour for an early mature stage in *Farfantepenaeus brasilliensis* and *Farfantepenaeus paulensis*, respectively. In the present study, the gonad colour of *P. latisulcatus* was yellow to orange, similar to that found by Montgomery et al. (2007b) in M. *plebejus*.

While this study has improved the knowledge for the reproductive biology of the western king prawn *P. latisulcatus*, research into spawning occurrence could be improved. This study has provided a greater understanding of the relationships between size and reproduction, which will greatly improve the knowledge base of *P. latisulcatus* in Southern Australia.

Chapter 6: Assessment of postovulatory follicle degeneration in wild caught western king prawns, *Penaeus (Melicertus) latisulcatus* (Kishinouye 1896), in Spencer Gulf, South Australia

6.1 Abstract

The postovulatory follicle (POF) method commonly used in fish was applied to assess the occurrence of spawning in the western king prawn *Penaeus (Melicertus) latisulcatus (P. latisulcatus*) in Spencer Gulf, South Australia. Using a recently developed classification for POF degeneration, it was possible to identify POF in *P. latisulcatus* for up to 96 h post spawning. Spawning occurrence was estimated for females using samples collected over the six month spawning period from October 2008 until February 2009 and a classification based on five categories of morphology including, germinal vesicle migration (GVM), POF classifications of ≤ 24 h, 48 h, ≥ 72 h and non-spawning ovaries. Out of 340 females, 34 % (114 females in total) contained identifiable POF. The earliest detectable POF was observed at 12 h post spawning, whilst the latest detectable POF was observed at 96 h. These POFs were found in the months of November and February respectively. Spawning occurrence values ranged from 1 to 25 % (11.55 % on average). Although multiple spawning events during a single spawning season have been reported for tropical *P. latisulcatus*, no female *P. latisulcatus* were found to contain more than a single age group of POF. Yet, the occurrence of early or mid and late yolk stage oocytes together with POF at all stages of degeneration give some indication that multiple spawning can occur. This is the first study to demonstrate the usefulness of POF in detecting spawning occurrence of prawns.

6.2 Introduction

Many species of fish are indeterminate spawners, meaning they produce multiple batches of eggs over a single spawning period (Ganias *et al.* 2007). Spawning occurrence amongst fishes is an important measure that helps in determining the daily egg production, which is widely used to assess spawning biomass amongst fishes (Ganias *et al.* 2007). Not only are

spawning occurrence values important for spawning biomass estimation, but also for providing information on the reproductive biology of species when comparing among habitats and seasons (Ganias *et al.* 2007).

The use of postovulatory follicles (POF) as a means of estimating the incidence of spawning has been well studied among fish and was first developed by Hunter and Goldberg (1980) for the northern anchovy *Engraulis mordax*. Other fish species for which the POF method is used to estimate spawning incidence include the albacore tuna, the southern bluefin whiting *Micromesistius australis* and the chub mackerel *Scomber japonicus* (Farley and Clear 2008; Macchi *et al.* 2005; Shiraishi *et al.* 2009). The method has not yet been adapted for crustaceans, including penaeids.

The standard methods for estimating biomass and frequency of spawning in penaeid prawn populations are commercial logbook data and trawling surveys, along with tag and recapture studies (e.g. Crocos 1985; Crocos and Kerr 1983; Montgomery 1981; Penn 1975; Penn 1976; Xiao and McShane 2000a). These methods are employed throughout penaeid fisheries to identify areas of high density catches and potential spawning aggregations (Courtney 1995; Penn 1980). However, no adequate method exists for estimating the number of spawning events per year or the number of eggs released per spawning event for the penaeid prawn *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus*).

Penaeid prawns, such as *P. latisulcatus* occur within both tropical and temperate latitudes (Dall *et al.* 1990). Female prawns of tropical origin are suspected to undergo multiple spawning within a single season (Crocos and Van der Velde 1995; Ibarra *et al.* 2007; Rothlisberg 1998). Multiple spawning in penaeids has been proven by the use of eyestalk ablation in the induced spawning of *Penaeus orientalis* [= *Penaeus chinensis*] in the laboratory (Arnstein and Beard 1975). *P. latisulcatus* females are known to lose their spermatophore when moulting, with no ripe soft-shelled female ever being recorded (Penn 1980). This suggests that spawning of fertile eggs following ovary development must occur during a single intermoult period and that moult frequency during a spawning season may be a governing factor in spawning occurrence.

Penaeid prawns are one of the most important economic resources in crustacean fishing industries throughout the world, accounting for more than half of the gross prawn production in tropical and temperate fisheries (Dall *et al.* 1990; Holthuis 1980). The economic importance of these resources has driven extensive studies of penaeid stocks in many areas of the world, aimed towards their sustainable management (e.g. Baelde 1992; Courtney and Dredge 1988; Courtney *et al.* 1995b; Crocos 1990; Crocos and Van der Velde 1995; Hossain and Ohtomi 2008; Somers 1990).

The western king prawn, *P. latisulcatus*, is the basis of valuable fisheries in northern and southern Australia (Penn 1980; Xiao 2004). Throughout its tropical range, *P. latisulcatus* has been known to have peaks in reproductive periods during September and January (Rothlisberg and Jackson 1987; Rothlisberg *et al.* 1987). In contrast, temperate South Australian populations of *P. latisulcatus* spawn when water temperatures rise above 17 °C, which is generally from November to March each year (Dixon *et al.* 2009).

These stocks collectively support an intensive commercial fishery with an annual yield of approximately 2000 tonnes (Dixon and Sloan 2007). Three commercial prawn fisheries occur within South Australia: Spencer and St Vincent Gulfs and the West Coast (See Figure 5.1). This research is based on the Spencer Gulf Prawn Fishery. With the introduction of stock assessment surveys it is possible to measure the relative biomass or mean survey catch rate of the fishery. At present the relative biomass of the fishery is calculated based on mean catch rates and compared over annual measures (Dixon *et al.* 2012). Annually surveys are conducted during the months of November, February and April with all trawling carried out at night (Dixon *et al.* 2012).

Studies on fecundity in *P. latisulcatus* are limited to counting the number of ripe eggs within an individual and assume determinate fecundity (Abdel Razek *et al.* 1996; Courtney and Dredge 1988; Penn 1980). Tropical *P. latisulcatus* are suggested as having indeterminate fecundity, meaning they produce multiple batches of eggs over a single spawning period (Ganias *et al.* 2007). No present studies, however, have considered the spawning

occurrence of *P. latisulcatus* in temperate waters and whether or it has determinate or indeterminate fecundity. The purpose of this research is to describe the spawning pattern of *P. latisulcatus* in Spencer Gulf South Australia and to determine reproductive parameters of spawning occurrence, using the presence or absence of POF.

6.3 Materials and methods

6.3.1 Collection of *P. latisulcatus*

Female *P. latisulcatus* were collected in the main offshore fishing grounds of *P. latisulcatus* in Spencer Gulf, South Australia. Sampling was performed from trawlers of the commercial fleet during the fishing season of October 2008 until February 2009. Surveys were conducted twice a month except for December 2008 due to Christmas. Sampling occurred from October until February as this period has been shown to be the main spawning period for *P. latisulcatus* in Spencer Gulf (Dixon *et al.* 2012). During the 2008/2009 season, sampling was also carried out on the RV Ngerin, conducted on a fortnightly basis. All sampling was from depths ranging between 10 to 45 m. All vessels used demersal, otter-trawl, double-rig gear with a maximum total headline length of 29.26 m and a minimum mesh size of 4.5 cm. Each net was also fitted with a codend consisting of a PVC protective mat and a mesh bag with 150 mm mesh. All of the trawls were made at night and lasted from 10 to 30 minutes.

Penaeus latisulcatus were sampled from the catch by taking a 7 L bucket of prawns from the catch. After each trawl, the prawns were sexed and females retained for ovary dissection. Each female was macroscopically assessed for maturity stage and measured to the nearest millimetre. Carapace length (CL) was measured from the posterior margin of the orbit to the mid-dorsal posterior edge of the carapace using manual vernier callipers (± 1mm). Females were fixed in FAACC (Formalin 10%, Acetic Acid Glacial, Calcium Chloride) solution for 48 h immediately after identification to preserve ovary condition for histological analysis. Samples were then transferred to 70 % ethanol for storage and

subsequent examination within the laboratory. From a total of 549 female prawns sampled, ovary dissections were carried out on 340 female prawns.

6.3.2 Histological classification

Preserved female *P. latisulcatus* ovaries were removed, with a piece from the mid-section removed for histological analysis. The mid-section was used as the distribution of oocyte stages is uniform between different areas of ovary along the body of a prawn (Montgomery *et al.* 2007b). Sections of the ovary were dehydrated, embedded in paraffin and then cut at 6 µm, where they were placed onto one slide and stained with haematoxylin and eosin stain.

Sections were digitally imaged through a compound microscope (Olympus BX51) fitted with an Olympus C-7070 camera at 100 x and 400 x magnification. Each ovary was classified histologically using the methods from chapter 3. In each ovary, the presence or absence of the following characters was recorded; oocytes that had not begun vitellogenesis (oogonium stage / stage 0); oocytes in the early vitellogenic stages (perinucleolus stage/early or mid yolked / stage 1); advanced yolked oocytes (vitellogenic stage/late yolked / stage 2) and hydrated (cortical specialisation / stage 3 and 4) (Table 2.1, Figure 2.1 – 2.5). Ovaries were then reviewed based on the presence or absence of germinal vesicle migration (GVM), POF at periods of 0 to 6 h, 6 to 12 h, 24, 48, 72 and 96 h and atretic oocytes (AO).

For the purpose of this study, germinal vesicle migration (GVM) is classified as a ripe ready to spawn ovary. Ovarian atresia is the process in which immature ovarian follicles degenerate and are subsequently re-absorbed. In penaeids, ovarian atresia follows a sequence of development stages recently categorised by histological sections and macroscopic analysis (Chapter 2). Here, ovarian sections were analysed for the presence of AO, as the identification of AO may indicate the end of the spawning season (Hunter and

Macewicz 1980). AO were grouped as; none, < 50 % (1 to 49 % of the yolked oocytes were in AO), or ≥ 50 % (50 % or more of the yolked oocytes were in AO).

6.3.3 Spawning Occurrence

To measure spawning occurrence from POF, it is essential to determine the age of the POF in relation to their deterioration (Hunter and Goldberg 1980). To estimate spawning of *P. latisulcatus*, the reproductive status of females during the main spawning period (October to February) was calculated on the basis of histological evidence collected over 96 h (Chapter 3). As *P. latisulcatus* spawn only at night (Dall *et al.* 1990; Penn 1980), it was possible to estimate the elapsed time between spawning and capture through POF degeneration.

POF presence and degeneration within each individual were recorded for a total of 340 female *P. latisulcatus*. Spawning occurrence was calculated using the 6 categories from chapter 4. This was reduced to five categories as only two of the females contained POF of 96 h post spawn; hence these were pooled with those of 72 h to create a category of ≥ 72 h. The morphology of POF and stage of the oocytes were recorded as; i) GVM oocytes, ii) POF up to 24 h old, iii) POF 48 h old, iv) POF ≥ 72 h old, v) non-spawning ovary which contain many yolked oocytes, but no POF. This approach was adapted from methods by Shiraishi *et al.* (2009).

6.3.4 Data analysis

Data were analysed using a Chi-square test carried out in Excel to explore differences between the frequencies of particular ovarian stages over time. Exploration of the data included potential size differences among spawning prawns (~ages) as well as the percentage of females which contained POF. A one-way between groups analysis of variance was conducted in IBM SPSS 20 with a Tukey post hoc test to explore POF degeneration between sampling months. For the purpose of this test *P. latisulcatus* were divided into three groups according to the age of POF (≤ 24 h, 48 h and ≥ 72 h) and GVM were excluded as these individuals had not yet spawned. The average number of spawning

females per sampling trip was calculated from the four categories of GVM and POF at ≤ 24 h, 48 h and ≥ 72 h.

6.4 Results

Of the 340 female *P. latisulcatus* sampled and dissected for further analysis of ovaries (Table 6.1), 180 were histologically classed as mature due to the presence of yolked oocytes. Peak numbers of mature females were caught in late November and early January (Table 6.1).

Table 6.1. Number and size range of *Penaeus (Melicertus) latisulcatus* sampled during surveys carried out from October 2008 until February 2009, in Spencer Gulf, South Australia.

Month (date)	No. of ovaries sampled	No. of mature females	Length of sampled females (CL, mm)	Percentage of mature oocytes
17th October	31	10	23 to 58	32
29th October	36	17	25 to 60	47
15th November	35	23	24 to 53	66
28th November	35	22	26 to 54	63
15th December	47	27	21 to 56	57
11th January	41	24	24 to 58	59
27th January	33	18	26 to 52	55
9th February	40	22	27 to 54	55
25th February	42	17	26 to 52	40
Total	340	180		

An increase in mature oocytes was seen from late October to November (Table 6.1), followed by relatively stable numbers during December and January (Table 6.1). A decrease in mature oocytes was evident in late February (55 %, to 40 %, Table 6.1). Females from the November surveys had the highest percentage of mature oocytes (63 %, Table 6.1).

6.4.1 Postovulatory follicle presence in female *P. latisulcatus*

The ovaries of 33.5 % (114 individuals) of females contained POF (≥ 72 h) indicating that these females had spawned within the last 96 h. Fifteen of these females had mature oocytes (stage 2, 3 or 4) which also contained POF (≥ 72 h).

The presence of POF in females over the sampling period varied being lowest in December (9 %) (Figure 6.1). POF presence was greatest in February (34 %) (Figure 6.1). POF presence in January was similar to October and November (Figure 6.1). The proportion of female prawns with POF varied with size, as females of the medium size class (17 to 45 mm) had the highest presence of POF (Figure 6.2).

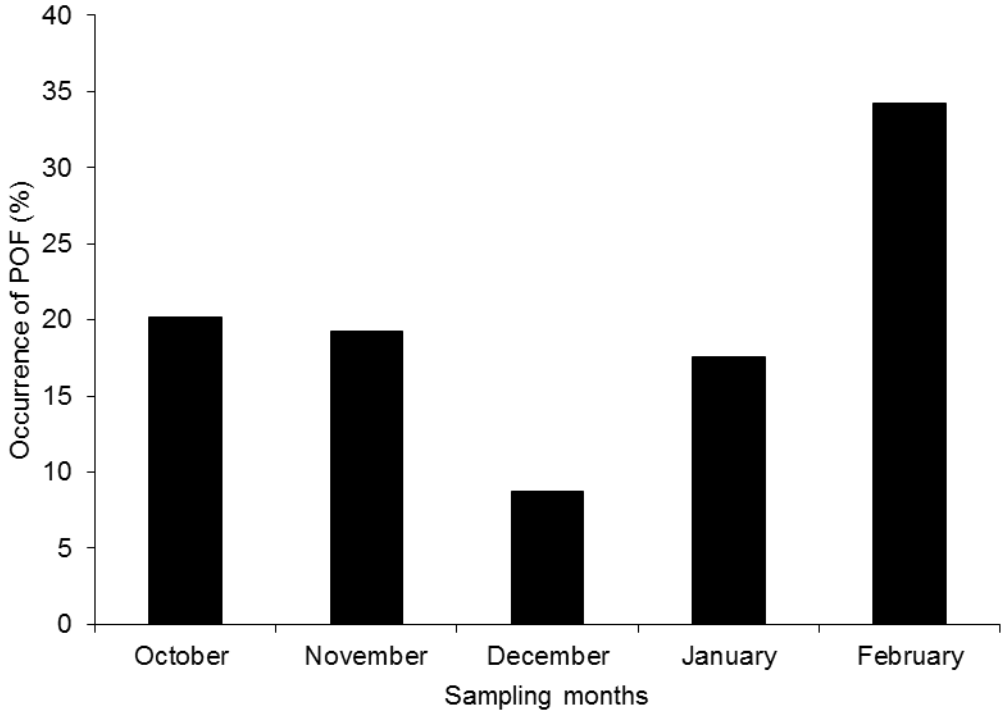


Figure 6.1. Percentage occurrence of postovulatory follicles (POF) in *Penaeus (Melicertus) latisulcatus* from October to February (n = 23, 22, 10, 20 and 39 respectively).

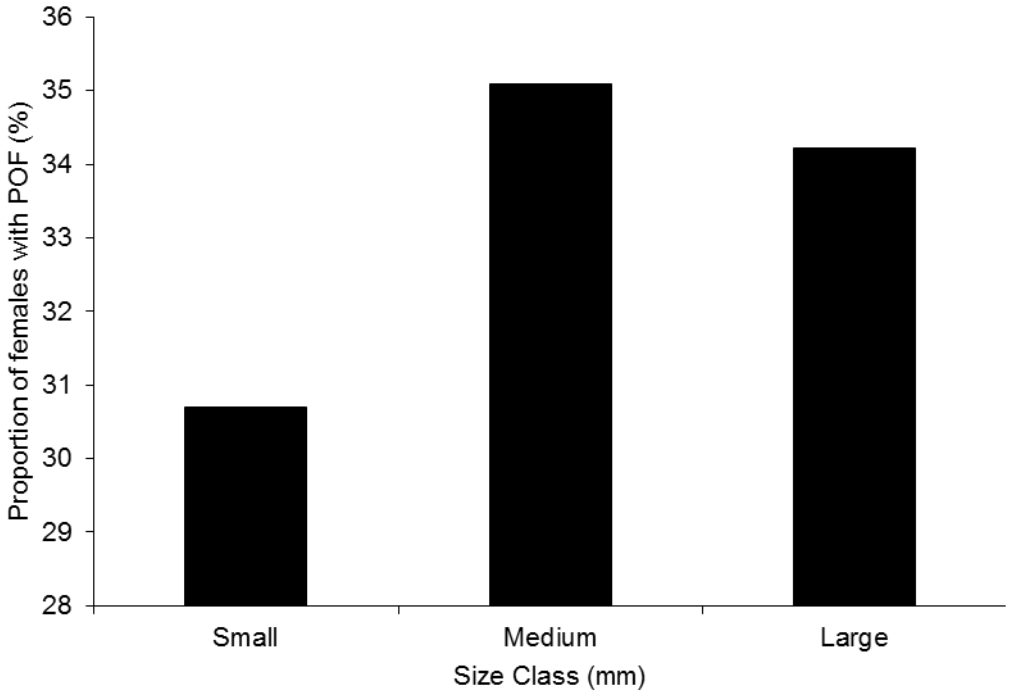


Figure 6.2. Proportion of *Penaeus (Melicertus) latisulcatus* females with postovulatory follicles (POF) per size class of small (< 36 mm), medium (37 to 45 mm) and large (> 46 mm) prawns.

A one-way between groups analysis of variance was conducted to explore POF degeneration between sampling months. There was no statistically significant difference in the type of POF between months ($F_{(2, 9)} = 0.823$, $p = 0.470$). Post-hoc comparisons of sampling month with type of POF using Tukey HSD indicated that there were no significant differences between any of the groups of POF at ≤ 24 h, 48 h and ≥ 72 h ($p > 0.05$) in prawns for *P. latisulcatus*.

6.4.2 Spawning Occurrence

Generally, ovaries of females with evidence of spawning showed POF in different stages of degeneration. The percentage occurrence of prawns based on the five categories of staging (POF degeneration + GVM) was calculated (Table 6.2). Total spawning values (percentage females spawning per survey) which were calculated using the reproductive stages (except the non-spawning stage) ranged from 1 % to 25 % (11.5 % on average) (Table 6.2). Total spawning varied between surveys at 8 % to 16 % of prawns that had spawned per survey, being highest in November and early February (Table 6.2).

The greatest percentage of ripe, ready to spawn females occurred in early November (31 %), and the lowest rate in early February (2.5 %) (Table 6.2, Figure 6.3). When sampling trips were combined for each month, (two sampling trips per month except for December due to Christmas), the greatest percentage of ready to spawn females was in November (GVM 27 %) (Figure 6.3). The spawning intervals of female *P. latisulcatus* could not be estimated as a combination of oocyte and POF within a single individual stage is needed.

Table 6.2. Spawning percent frequency of *Penaeus (Melicertus) latisulcatus* from October 2008 to February 2009 and the presence of histological characters indicating spawning, in Spencer Gulf, South Australia (GVM germinal vesicle migration with spawning imminent, POF postovulatory follicles).Values of 12.3 %, 1 %, 7.5 %, 25 % represent the total spawning with 11.5 % representing the average of spawning over the sampling period.

Date	N	Non spawning prawns %	Spawning per survey (%) (SPS)				
			GVM	POF 24 h	POF 48 h	POF ≥72 h	Average SPS (%)
17th October	31	45.16	9.68	3.23	3.23	38.70	13.71
29th October	36	66.67	8.33	0.00	0.00	25.00	8.33
15th November	35	37.14	31.43	0.00	5.71	25.71	15.71
28th November	35	45.71	22.86	5.71	8.57	17.14	13.57
15th December	47	63.83	14.89	0.00	4.26	17.02	9.04
11th January	41	68.29	9.76	0.00	7.32	14.63	7.93
27th January	33	60.61	6.06	0.00	18.18	15.15	9.85
9th February	40	35.00	2.50	0.00	15.00	47.50	16.25
25th February	42	61.90	4.76	0.00	4.76	28.57	9.52
			12.3 %	1.0 %	7.5 %	25 %	11.5 %

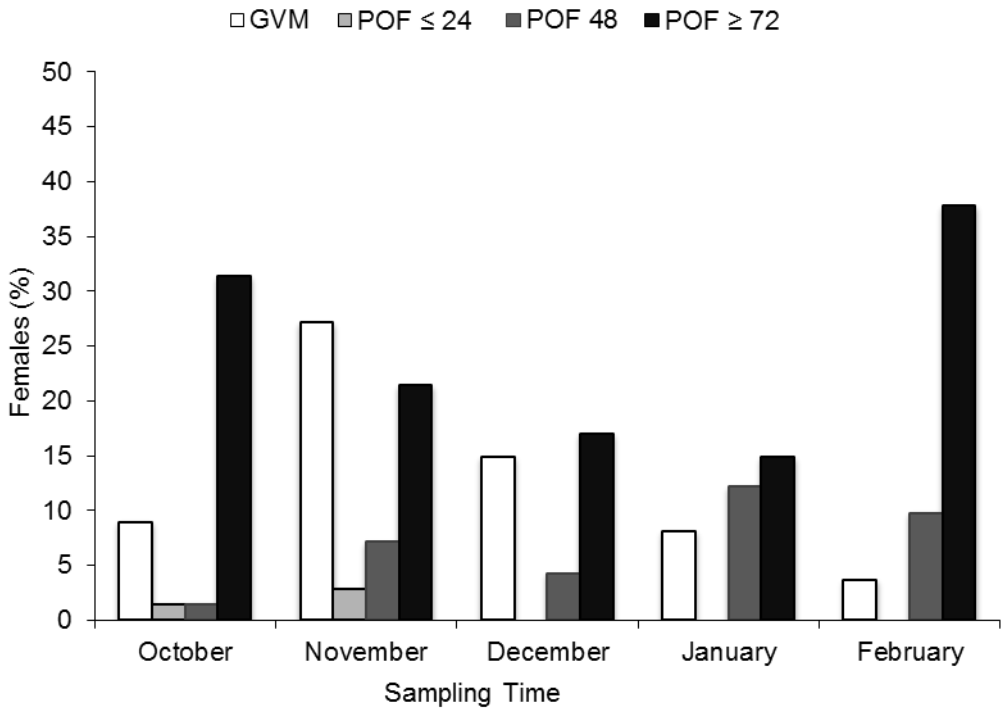


Figure 6.3. Percentage of female *Penaeus (Melicertus) latisulcatus* (%) ready to spawn and post spawning for sampling trips from October to February. Germinal vesicle migration (GVM) represents ready to spawn individuals, post spawning individuals are POF at ≤ 24, 48 and ≥ 72 h.

It is possible to determine if female *P. latisulcatus* have spawned more than once over a period of a few days by examination of the ovarian tissue for certain cell types. This is evident in ovaries that contain germinal vesicle migration (GVM) and POF up to 24 h old, as well as ovaries containing late yolk stages with POF up to 6 h old and POF of 48 h old. The data presented here highlights that POF can be found within wild caught female *P. latisulcatus* at differing stages of ovarian development. Although Chapter 5 highlights the potential for tracking multiple spawning events within a single female over the spawning season, the data here do not indicate this. The most commonly detected POF among females over the 6 m sampling period were at 48 and ≥ 72 h post spawning (October to February) (Figure 6.3). The earliest detectable POF was observed in a female from late November at 12 h post spawning whilst the latest detectable POF was observed in late February at 96 h.

6.5 Discussion

This study indicates that POF in wild caught western king prawns (*P. latisulcatus*) are detectable through histological analysis at up to 96 h post spawning. The reproductive status of females could be classified according to six criteria based on the morphology of the POF and the development stage of the oocyte; however, as only two prawns were found to contain POF at 96 h, this data was pooled with those of 72 h to create five classifications. The spawning occurrence of *P. latisulcatus* in Spencer Gulf, South Australia was analysed using information provided from previous research on *P. latisulcatus* (Chapter 5). The estimates presented here are limited due to the small sample size and inability to repeat the sampling over successive seasons, as well as a higher frequency of sampling over the main spawning period (October to February), providing scope for future research.

The frequency of *P. latisulcatus* females that were ready to spawn ranged from 2.5 to 31 %. *P. latisulcatus* is thought to spawn more than once during the spawning season (October to February) in temperate waters of Southern Australia (Dixon *et al.* 2012), like its conspecifics inhabiting tropical waters (Penn 1980; Rothlisberg 1998). As spawning is directly related to

moulting, a female must shed her exuvae more than once within a single season for multiple spawning events to occur (Crocos 1985; Crocos and Kerr 1983; Lindner and Anderson 1956; Penn 1980).

Data presented here show that partial spawning events are occurring within individuals in temperate waters (Alday *et al.* 2008; Ganias *et al.* 2007). The 15 female *P. latisulcatus* containing mature oocytes as well as the presence of POF suggest that only partial spawning has occurred. This may also indicate that a long interval occurs between successive spawning, given that no POF of different ages was present in the same prawn. Further analysis is, however, needed to identify individuals, which have degenerated POF at more than one age as this would allow to estimate the interval between spawning.

Increased water temperature can accelerate POF degeneration among teleost species, such as the southern blue whiting (*Micromesistius australis*) (Macchi *et al.* 1992) and chub mackerel (*Scomber japonicus*) (Dickerson *et al.* 1992). Different rates of POF degeneration occur according to water temperature, as species which spawn in warmer waters have faster rates of degeneration than those occurring/spawning in cooler waters (Macchi *et al.* 2005). Water temperature in Spencer Gulf over the period of October to February increased steadily from 17 to 23 °C respectively (Chapter 5) and this increase in water temperature may therefore accelerate POF degeneration. Fitzhugh and Hettler (1995) showed that observations on POF duration vary among species that spawn at different temperatures. In the Atlantic menhaden, POF could be observed from 36 to 60 h post spawning in temperatures of 15 to 20 °C, whilst the Northern anchovy, Peruvian anchovy and Pacific sardine had POF lasting only 48 h in similar temperatures (13 to 19 °C) (Fitzhugh and Hettler 1995). It is clear from these previous observations that an increase in temperature causes a decrease in POF detection among teleosts. From the data presented in this study, no significant difference was found between the types of POF detected with sampling month. As more recently spawned females were detected during February when the water temperature was at its highest, (Chapter 5, Figure 5.6) POF degeneration in *P. latisulcatus* may not be affected by changes in water temperature. Thus POF in prawns (and other

crustaceans) may be a previously underutilised approach in assessing spawning in these commercially important species.

Following methodology used to estimate spawning occurrence in teleosts, it was possible to identify spawning among female *P. latisulcatus*. We chose a similar method to Shiraishi *et al.* (2009) using the morphology of POF and the developmental stages of oocytes to estimate spawning occurrence (GVM and POF at ≤ 24, 48 and ≥ 72 hour), which gave an overall estimation of 11.55 %. Shiraishi et al. (2009) found that when using GVM ooctyes with POF zero and POF one the estimation of spawning occurrence was similar to that for the overall estimation (17 % and 19 % respectively). However, they also found that the estimation of spawning occurrence varied greatly among research (Dickerson *et al.* 1992; Wantanabe *et al.* 1999; Yamada *et al.* 1998) as different criteria are often used for the estimates, making it difficult to compare spawning occurrence among studies. Prawns within Spencer Gulf clearly indicated that females which were about to spawn (those with GVM oocytes) were more abundant in November, which is consistent with a peak in the reproductive period for this fishery (Dixon *et al.* 2009).

Overall, this study demonstrated that POF can be detected in wild caught *P. latisulcatus* and used to detect spawning occurrence as a measure of spawning occurrence for *P. latisulcatus*. This study puts forward a process for the estimation of spawning in *P. latisulcatus*. The success of identifying POF within penaeids using the classification presented in this study suggests that other penaeid fisheries should be able to employ it to improve calculations of spawning occurrence.

Chapter 7: General Discussion

The overall aim of this study was to identify and describe the reproductive biology of *Penaeus (Melicertus) latisulcatus* (*P. latisulcatus*) by: i) improving the knowledge on the reproductive biology of western king prawns in South Australia and provide a better understanding for maturity indices and ovarian classification methods; and ii) identifying and assessing spawning occurrence in Spencer Gulf, South Australia. In addressing these aims, western king prawn populations in relation to reproduction were assessed. The following discussion addresses the knowledge gained on reproduction, specific to western king prawns in South Australian waters, and how this data may be used for future stock assessments. I highlight the main findings discussed in each chapter and discuss directions for further research.

The microscopic analysis of ovarian oocyte development of *P. latisulcatus* was described using previously determined techniques by Tuma (1967) and King (1948). Characteristics of ovarian cell type, colour and fullness were used to determine ovarian stage. Five cell types were identified (Chapter 2, Table 2.1) and described: oogonia, perinucleolus, vitellogenic, cortical specialisation and germinal vesicle break down, all of which appear as distinct cohorts in oocyte distribution and thereby closely correspond to the oocyte classifications given by Montgomery *et al.* (2007b).

Histological observations of oocytes are considered as one of the most accurate methods for the definition of mature females (Dall *et al.* 1990). Reproductively mature individuals are capable of producing eggs or sperm of a developed stage ready for fertilisation (Dall *et al.* 1990). Development of oocytes in *P. latisulcatus* was similar to that described for other penaeid prawns (e.g. Courtney *et al.* 1995b; Penn 1980; Tuma 1967). Some stages of oocyte development were rare, whilst others were more common. Variation in the incidence of different ovarian stages most likely reflects the duration of each stage. The low presence of oogonia stage ovaries was therefore likely due to the short duration of this stage. Many females were of the perinucleolus stage, ready to mature and spawn. Low numbers of females in the cortical specialisation phase and none in the germinal vesicle break down

(GVBD) phase indicate that a short period exists between cortical specialisation and spawning. This short period between the cortical specialisation phase and spawning in *P. latisulcatus* contrasts other closely related species such as *Sicyonia ingentis*, where this process occurs over an extended period of up to 150 h from the initial stage of cortical specialisation to spawning (Anderson *et al.* 1984). Although many studies have been conducted on the maturation of ovarian stages in penaeid prawns (e.g. Amanat and Qureshi 2011; Ayub and Ahmed 2002; Crocos 1985; King 1948; Tuma 1967; Yano 1988), little research has been conducted on the timing between each stage (Anderson *et al.* 1984). The present study is the first to describe the occurrence of ovarian histological development and develop microscopic assessment criteria (Chapter 2, Table 2.1) for *P. latisulcatus* in South Australia.

Spawning of prawns in the laboratory is thought to be similar to that of wild females, and can thus be used as an indicator of spawning activity (Anderson *et al.* 1984; Crocos 1985; Peixoto *et al.* 2005a). Crocos (1985) showed that successful maturation of penaeids within the laboratory is not straightforward and requires particular conditions which may vary among species. Crocos (1985) describes 4 stages of maturation (immature, early ripe, ripe and spent) within his study. Many maturation studies have shown that between four and six ovarian stages exist among penaeids (Amanat and Qureshi 2011) with Penn (1980) first quantifying *P. latisulcatus* as having five stages; undeveloped, developing, early maturity, ripe and spent. As no spent individuals were identified within the present study, only four stages were used to quantify oocyte development. Overall, this study demonstrated that the maturation stages of *P. latisulcatus* and the development of macroscopic staging techniques can be used as a valuable measure of spawning occurrence. The development of criteria (Chapter 2, Table 2.1) for identifying and assigning ovarian stages for *P. latisulcatus* will be an invaluable piece of information for the development of maturation studies on penaeids worldwide.

The reproductive biology of *P. latisulcatus* from Spencer Gulf, South Australia, was described using an approach of microscopic examination of ovaries to determine, length-weight relationships, gonadosomatic index, physiological maturity, insemination rates and

percent ripe (Chapter 5). Extensive reproductive studies have been carried out around the world on penaeids. Although some of the reproductive data presented here, including ovarian examination, spawning occurrence and fecundity is similar to that of Penn (1980) for *P. latisulcatus* in Western Australia and Shlagman *et al.* (1986) for *P. semisulcatus,* this study complements previous research.

Physically mature females were identified at 13 mm CL within this study which is smaller than other penaeid species found within Australia (Courtney and Masel 1997; Penn 1980). The average CL of a mature female *P. latisulcatus* in Western Australia is 41.6 mm CL (Penn 1980), much larger than the average size of females in South Australia. Courtney and Masel (1997) found *P. esculentus* matured at 30 mm CL in Moreton Bay Queensland which is also much larger than the 13 mm CL of *P. latisulcatus* within this study. In Southern Australia, *P. latisulcatus* reproduce over the warmest months of the year with a spawning season from October to February, as indicated by the presence of ripe ovaries within this study and stock assessment survey data collected throughout the season on an annual basis by SARDI (Dixon and Sloan 2007). When compared, *P. esculentus* showed similar reproductive patterns with major spawning occurring from October to December in Western Australia and Moreton Bay Queensland (Courtney and Masel 1997). These differences provide insight into how reproductive dynamics can vary between species and locations. Courtney and Masel (1997) showed that recruitment overfishing was possible for *P. esculentus* during periods of peak egg production. The incidence of peak spawning for *P. esculentus* coincided with lower numbers meaning fewer eggs were being produced. As spawning biomass of *P. latisulcatus* is currently protected during the November/December peak in egg production, *P. latisulcatus* should not be vulnerable to recruitment overfishing.

The presence of postovulatory follicles (POF) within an ovary suggests that ovulation of the oocyte has occurred and that spawning is imminent or has recently occurred in relation to the stage of its deterioration or resorption. POF detection in penaeids applies a well know technique used for teleosts to examine the degeneration over time. A laboratory experiment (Chapter 3) showed that POF were indeed present within recently spawned *P. latisulcatus*

and therefore a degeneration description could be identified over time up to 96 h post spawn. POF were identified and assigned an age within ovarian sections of *P. latisulcatus* as a means of assigning spawning time. Whilst the technique of ageing POF is common amongst teleosts (Hunter and Goldberg 1980) it is a new approach for penaeid prawns. It was possible to identify POF in recently spawned female *P. latisulcatus* and assess spawning occurrence in an individual as well as the reproductive status in the population. It was difficult to determine more than one type of POF within an individual. However, it was possible to estimate the spawning occurrence of *P. latisulcatus* with the categories devised in Chapter 3 (Hunter and Macewicz 1985). Dickerson *et al.* (1992) and Macewicz and Hunter (1993) showed that the number of oocytes per spawn (batch fecundity) for Chub and Jack Mackerel could be measured from the total number of oocytes within the ovary containing migratory nuclei or hydrated oocytes. This study demonstrates that POF can be identified in penaeids via histological analysis of their ovaries so that their degeneration may be used as a measure for post spawning time and for determining egg production of a fishery to underpin fisheries management. More importantly, the development of this technique is a valuable contribution to penaeid biology and its potential application to the fishery as a measure of spawning occurrence.

This study on the reproductive biology of the western king prawn provides further insight into *P. latisulcatus* within Spencer Gulf South Australia. Future research based on this study could include more frequent sampling of female *P. latisulcatus* over subsequent spawning periods (October to February annually) and thus provide more direct evidence for multiple spawnings during a single season. At present, it remains unknown whether *P. latisulcatus* spawn more than once within a single spawning season. Furthermore, the importance of postovulatory follicle cell detection in *P. latisulcatus* holds the potential for further research into batch fecundity and total number of spawns per individual within a season.

References

8.1 References

Abdel Razek, F.A., Abdel Rahman, S.H., and Zaglol, S.S. (1996) Aspects of the reproduction of the prawn *Penaeus latisulcatus* Kishinouya in the Gulf of Suez, Egypt. *Journal of King Abdulaziz University (Marine Science) Special Issue* **7**, 153 - 159.

Acuna, A., Viana, F., Vizziano, D., and Danulat, E. (2000) Reproductive cycle of female Brazilian codling, *Urophycis brasiliensis* (Kaup 1858), caught off the Uruguayan coast. *Journal of Applied Ichthyology-Zeitschrift Fur Angewandte Ichthyologie* **16**(2), 48 - 55.

Alday, A., Uriarte, A., Santos, M., Martin, I., Martinez De Murguia, A., and Motos, L. (2008) Degeneration of postovulatory follicles of the Bay of Biscay anchovy (*Engraulis encrasicolus L.)*. *Scientia Marina* **72**(3), 565 - 575.

Amanat, Z., and Qureshi, N.A. (2011) Ovarian Maturation Stages and Size at Sexual Maturity of *Penaeus indicus* (H. Milne Edwards, 1937) in the Lagoon Water of Sonmiani Bay, Balochistan. *Pakistan Journal of Zoology* **43**(3), 447 - 459.

Anderson, S.L., Chang, E.S., and Clark, W.H. (1984) Timing of postvitellogenic ovarian changes in the ridgeback prawn *Sicyonia ingentis* (Penaeidae) determined by ovarian biopsy. *Aquaculture* **42**, 257 - 271.

Arnstein, D.R., and Beard, T.W. (1975) Induced maturation of the prawn, *Penaeus orientalis* Kishinouye in the laboratory by means of eyestalk removal. *Aquaculture* **5**, 411 - 412.

Ayub, Z., and Ahmed, M. (2002) A description of the ovarian development stages of penaeid shrimps from the coast of Pakistan. *Aquaculture Research* **33**, 767 - 776.

Baelde, P. (1992) Reproductive biology of commercially exploited deep-water royal red prawns (*Haliporoides-sibogae, Solenceridae*) in south-east Australia. *Marine Biology* **113**(3), 447 - 456.

Barber, B.J., and Blake, N.J. (2006) Chapter 6 Reproductive physiology. In Development in aquaculture and fisheries science. Vol. 35. (Eds. SE Shumway and GJ Pasrsons) pp. 357 - 416. (Elsevier)

Bhaud, M., Cha, J.H., Duchene, J.C., and Nozais, C. (1995) Influence of temperature on the marine fauna: What can be expected from a climatic change. *Journal of Thermal Biology* **20**(1 - 2), 91 - 104.

Bishara, N.F. (1976) Contributions to the biology of penaeid prawns in lake Manzalah, Eygpt. I. Growth and length-weight relationship. *Aquaculture* **8**, 337 - 349.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., and White, J.S.S. (2009) Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**(3), 127 - 135.

Booth, A.J., and Weyl, O.L.F. (2000) Histological validation of gonadal macroscopic staging criteria for *Labeo cylindricus* (Pisces : Cyprinidae). *African Zoology* **35**(2), 223 - 231.

Carrick, N.A. (2003) Spencer Gulf Prawn (*Melicertus latisulcatus*) Fishery. Fishery Assessment Report to PIRSA. SARDI Aquatic Sciences RDO3 / 0079-2.

Ceballos-Vázquez, B.P., Palacios, E., Aguilar-Villavicencio, J., and Racotta, I.S. (2010) Gonadal development in male and female domesticated whiteleg shrimp, *Litopenaeus vannamei*, in relation to age and weight. *Aquaculture* **308**, 116 - 123.

Cha, H.K., Oh, C.W., Hong, S.Y., and Park, K.Y. (2002) Reproduction and population dynamics of *Penaeus chinensis* (Decapoda: Penaeidae) on the western coast of Korea, Yellow sea. *Fisheries Research* **56**, 25 - 36.

Cheng, C.S., and Chen, L. (1990) Growth characteristics and relationships among body length, body weight and tail weight of *Penaeus monodon* from a culture environment in Taiwan. *Aquaculture* **91**, 253 - 263.

Christiansen, F.B., and Fenchel, T.M. (1979) Evolution of marine invertebrate reproductive patterns. *Theoretical Population Biology* **16**, 267 - 282.

Courtney, A.J. (1991) Occurrence and influence of Bopyrid parasites on female penaeid prawns from coastal waters of central Queensland (Australia). *Australian Journal of Marine and Freshwater Research* **42**(6), 615 - 623.

Courtney, A.J. (1995) 'Trends in catches of eastern king prawns, *Penaeus plebejus*, from Queensland coastal waters -- considerations for the spawning stock recruitment relationship.' (Department of Primary Industries, Brisbane, Queensland Australia) 5 - 61.

Courtney, A.J., Die, D.J., and McGilvray, J.G. (1996) Lunar periodicity in catch rate and reproductive condition of adult eastern king prawns, *Penaeus plebejus*, in coastal waters of south-eastern Queensland, Australia. *Marine & Freshwater Research* **47**, 67 - 76.

Courtney, A.J., and Dredge, M.C.L. (1988) Female reproductive biology and spawning periodicity of two species of king prawns, *Penaeus longistylus* Kubo and *Penaeus latisulcatus* Kishinouye, from Queensland's east coast fishery. *Australian Journal of Marine and Freshwater Research* **39**(6), 729 - 741.

Courtney, A.J., and Masel, J.M. (1997) Spawning stock dynamics of two penaeid prawns, Metapenaeus bennettae and Penaeus esculentus, in Moreton Bay, Queensland, Australia. *Marine Ecology-Progress Series* **148**(1-3), 37-47.

Courtney, A.J., McGilvray, J.G., and Die, D.J. (1995a) 'Lunar variation in population structure and reproductive activity in Penaeus plebejus.' (DEPARTMENT OF PRIMARY INDUSTRIES, BRISBANE, QLD. (AUSTRALIA)) 2-94

Courtney, A.J., Montgomery, S.S., Die, D.J., Andrew, N.L., Cosgrove, M.G., and Blount, C. (1995b) Maturation in the female eastern king prawn *Penaeus plebejus* from coastal waters of eastern Australia, and considerations for quantifying egg production in penaeid prawns. *Marine Biology* **122**(4), 547 - 556.

Crocos, P.J. (1985) Appraisal of some factors relevant to the development of penaeid prawn population reproductive models. In 'Second Australian National Prawn Seminar'. (Eds. PC Rothlisberg, BJ Hill and DJ Staples) pp. 159 - 164. (Cleveland, Australia)

Crocos, P.J. (1987) Reproductive dynamics of the grooved tiger prawn *Penaeus semisulcatus* in the north-western Gulf of Carpentaria, Australia. *Australian Journal of Marine and Freshwater Research* **38**, 79 - 90.

Crocos, P.J. (1990) Reproductive dynamics of three species of Penaeidae in tropical Australia, and the role of reproductive studies in fisheries management. *Crustacean Issues* **7**, 317 - 331.

Crocos, P.J., and Kerr, J.D. (1983) Maturation and spawning of the banana prawn *Penaeus merguensis* in Albatross Bay, Gulf of Carpentaria, Australia. *Journal of Experimental Marine Biology and Ecology* **69**, 37-59.

Crocos, P.J., and Van der Velde, T.D. (1995) Seasonal, spatial and interannual variability in the reproductive dynamics of the grooved tiger prawn *Penaeus semisulcatus* in Albatross Bay, Gulf of Carpentaria, Australia: The concept of effective spawning. *Marine Biology Research* **122**, 557 - 570.

da C. Chaves, P.T., and Magalhaes, C. (1993) The oocyte development of *Macrobrachium amazonicum* (Heller, 1862), a freshwater shrimp of the Amazon River (Crustacea: Decapoda: Palaemonidae). *Acta Amazonica* **23**(1), 17 - 23.

Dall, W., Hill, B.J., Rothlisberg, P.C., and Staples, D.J. (1990) The Biology of Penaeidae. In Advances in Marine Biology Vol. 27. (Eds. JHS Blaxter and AJ Southward) pp. 1 - 489. (Academic Press Limited New York)

de Croos, M.D.S.T., Palsson, S., and Thilakarathna, R.M.G.N. (2011) Sex ratios, sexual maturity, fecundity, and spawning seasonality of *Metapenaeus dobsoni* off the western coastal waters of Sri Lanka. *Invertebrate Reproduction and Development* **55**(2), 110 - 123.

Dickerson, T.L., Macewicz, B.J., and Hunter, J.R. (1992) Spawning frequency and batch fecundity of Chub Mackerel, *Scomber japonicus*, during 1985. *CalCOFI* **33**, 130 - 140.

Die, D.J., McGilvray, J.G., Courtney, A.J., and Cosgrove, M.G. (1995) A quantitative method for staging penaeid prawn ovaries using image analysis. *Invertebrate Reproduction and Development* **28**(3), 177 - 183.

Dixon, C.D., and Hooper, G.E. (2008) Spencer Gulf Prawn (*Melicertus latisulcatus*) Fishery 2006/2007. SARDI Aquatic Sciences, No. 322, Adelaide.

Dixon, C.D., Hooper, G.E., and Burch, P. (2012) Spencer Gulf prawn *Penaeus (Melicertus) latisulcatus* fishery 2010/2011. Fishery assessment report to PIRSA Fisheries and Aquaculture. South Australian Research and Development Insitute (Aquatic Sciences), Adelaide. SARDI publication number F2007/000770-5. SARDI Research Report Series No. 603.

Dixon, C.D., Hooper, G.E., and Roberts, S.D. (2009) Spencer Gulf prawn *Penaeus (Melicertus) latisulcatus* fishery 2007/2008. Fishery assessment report to PIRSA Fisheries. South Australian Research and Development Insitute (Aquatic Sciences), Adelaide. SARDI publication number F2007/000770-3. No. 387.

Dixon, C.D., Hooper, G.E., and Roberts, S.D. (2010) Spencer Gulf Prawn *Penaeus (Melicertus) latisulcatus* Fishery 2008/09. Fishery Assessment Report to PIRSA Fisheries. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. F2007/000770-4. SARDI Research Report Series No. 511.

Dixon, C.D., Roberts, S.D., Hooper, G.E., Steer, M.A., and Ward, T.M. (2006) Gulf St Vincent Prawn (*Melicertus latisulcatus*) Fishery. Fishery Assessment Report to PIRSA. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI Publication No. RD03/0063-4. SARDI Research Report Series No. 159.

Dixon, C.D., and Sloan, S. (2007) Management plan for the South Australian Spencer Gulf prawn fishery. The South Australian fisheries management series. Paper No. 54.

Drummond, C.D., Bazzoli, N., Rizzo, E., and Sato, Y. (2000) Postovulatory follicle: A model for experimental studies of programmed cell death or apoptosis in teleosts. *Journal of Experimental Zoology* **287**(2), 176 - 182.

Emlet, R., McEdward, L., and Strathmann, R. (1987) Echinoderm larval ecology viewed from the egg. *Echinoderm Studies* **2**, 55 - 136.

Farley, J., and Clear, N. (2008) Albacore tuna: investigation of size monitoring, age composition, and spawning activity in the ETBF. Final Report to AFMA.

Ferreri, R., Basilone, G., D'Elia, M., Traina, A., Saborido-Rey, F., and Mazzola, S. (2009) Validation of macroscopic maturity stages according to microscopic histological examination for European anchovy. *Marine Ecology* **30**(1), 181 - 187.

Field, A. (2009) 'Discovering statistics using SPSS.' Third edn. (SAGE Publications Ltd: London) 821

Fitzhugh, G.R., and Hettler, W.F. (1995) Temperature influence on postovulatory follicle degeneration in Atlantic menhaden, *Brevoortia tyrannus Fishery Bulletin* **93**(3), 568 - 572.

Fitzhugh, G.R., Shertzer, K.W., Kellison, G.T., and Wyanski, D.M. (2012) Review of size- and age-dependence in batch spawning: implications for stock assessment of fish species exhibiting indeterminate fecundity. *Fishery Bulletin* **110**(4), 413 - 425.

Ganias, K., Nunes, C., and Stratoudakis, Y. (2007) Degeneration of postovulatory follicles in the Iberian sardine *Sardina pilchardus*: structural changes and factors affecting resorption. *Fishery Bulletin* **105**, 131 - 139.

Garcia, A. (1996) White shrimp *(Penaeus setiferus)* recruitment overfishing. *Marine and Freshwater Research* **47**, 59 - 65.

George, S.B. (1994) Population differences in maternal size and offspring quality for *Leptasterias-epichlora* (Brandt) (Echinodermata, Asteroidea). *Journal of Experimental Marine Biology and Ecology* **175**, 121 - 131.

George, S.B. (1996) Echinoderm egg and larval quality as a function of adult nutritional state. *Oceanologica Acta* **19**, 297 - 308.

Goldberg, S.R., Alarcon, V.H., and Alheit, J. (1984) Postovulatory follicle histology of the pacific sardine, *Sardinops-Sagax*, from Peru. *Fishery Bulletin* **82**(2), 443 - 445.

Grey, D.L., Dall, W., and Baker, A. (1983) “A guide to the Australian penaeid Prawns”. Department of Primary Production, Northern Territory, Australia.

Hadzi-Pavlovic, D. (2010) Agreement II: Categorical measures. *Acta Neuropsychiatrica* **22**, 155 - 157.

Hall, N.G., and Penn, J.W. (1979) Preliminary assessment of effective effort in a two species trawl fishery for penaeid prawns in Shark Bay, Western Australia. In Population assessments of shellfish stocks. (Ed. HJ Thomas) pp. 147 - 154.

Havenhand, J.N. (1995) ' Evolutionary ecology of larval types. In: L. McEdward (ed.) Ecology of marine invertebrate larvae.' (CRC Press Inc: Boca Raton, Florida) 79 - 122.

Heldt, J. (1938) La reproduction chez les crustaces decapodes de la famille des penaeids. *Annual Institution of Oceanography* **18**, 1 - 206.

Holthuis, L.B. (1980) Shrimp and prawns of the world. An annotated catalogue of species of interest to fisheries. FAO species catalogue. FAO Fisheries Synopsis. (125) Volume 1. pp. 271.

Hossain, M.Y., and Ohtomi, J. (2008) Reproductive biology of the southern rough shrimp *Trachysalambria curvirostris* (Penaeidae) in Kagoshima bay, southern Japan. *Journal of Crustacean Biology* **28**(4), 607 - 612.

Hossain, M.Y., Ohtomi, J., Jaman, A., Jasmine, S., and Vadas, R.L., Jr. (2012) Life history traits of the Monsoon River prawn *Macrobrachium malcolmsonii* (Milne-Edwards, 1844) (Palaemonidae) in the Ganges (Padma) River, northwestern Bangladesh. *Journal of Freshwater Ecology* **27**(1), 131 - 142.

Humason, G., L. (1962) 'Animal tissue techniques.' Third edn. (W.H. Freeman and Company: United States of America)

Hunter, J.R., and Goldberg, S.R. (1980) Spawning incidence and batch fecundity in Northern anchovy, *Engraulis mordax*. *Fishery Bulletin* **77**(3), 641 - 652.

Hunter, J.R., and Macewicz, B.J. (1980) Sexual maturity, batch fecundity, spawning frequency, and temporal pattern of spawning for the northern anchovy, *Engraulis Mordax,* during the 1979 spawning season. *CalCOFI* **21**, 139 - 149.

Hunter, J.R., and Macewicz, B.J. (1985) Measurement of spawning frequency in multiple spawning fishes. In An egg production method for estimating spawning biomass of Pelagic fish: Applicaton to the Northern Anchovy, *Engraulis mordax* (Lasker, R. ed.). *U.S. Department of Commerce, NOAA Technical Report NMFS* **36**, 79 - 94.

Ibarra, A.M., Racotta, I.S., Arcos, F.G., and Palacios, E. (2007) Progress on the genetics of reproductive performance in penaeid shrimp. *Aquaculture* **268**(1 - 4), 23 - 43.

Ives, M.C., Scandol, J.P., Montgomery, S.S., and Suthers, I.M. (2009) Modelling the possible effects of climate change on an Australian multi-fleet prawn fishery. *Marine & Freshwater Research* **60**(12), 1211 - 1222.

Jackson, C.J., Rothlisberg, P.C., and Pendrey, R.C. (2001) Role of larval distribution and abundance in overall life-history dynamics: a study of the prawn *Penaeus semisulcatus* in Albatross Bay, Gulf of Carpentaria, Australia. *Marine Ecology Progress Series* **213**, 241 - 252.

James, C.E., Dixon, C.D., Fowler, A.J., Linnane, A., Luick, J.L., McClatchie, S., McGarvey, R., Middleton, J.F., Steer, M.A., and Wu, Y. (2010) Effects of environmental variability on recruitment to South Australian fisheries: A preliminary investigation. South Australian Research and Development Institute, Aquatic Sciences, No. 2006/046, Adelaide.

James, P.T. (1977) The comparative morphology of the thelycum in the *Euphausia gibba* species group. *Deep-Sea Research* **24**, 1037 - 1047.

Kangas, M. (1999) Postlarval and juvenile western king prawns *Penaeus latisulcatus* Kishinouye studies in Gulf St Vincent, South Australia with referene to the commercial fishery. PhD Thesis, University of Adelaide, Adelaide

Kangas, M., and Dixon, C.D. (2008) Prawns of Gulf St Vincent. In Natural history of Gulf St Vincent. 1st Edition edn. (Eds. SA Shepherd and S Bryars) pp. 456 - 63. (Royal Society of South Australia: Adelaide)

Kangas, M.I., and Jackson, W.B. (1998) Sampling juvenile Penaeus latisulcatus Kishinouye with a water-jet net compared with a beam-trawl: spatial and temporal variation and nursery areas in Gulf St Vincent, south Australia. *Marine and Freshwater Research* **49**(6), 517-523.

Kaplan, L.A., and Amadeo, J.P. (1996) 'Clinical chemistry theory, analysis, and correlation.' Third edn. (Mosby-Year Book Incorporation: United States of America)

Kaunda-Arara, B., and Ntiba, M.J. (1997) The reproductive biology of *Lutjanus fulviflamma* (Forsskal,1775) (Pisces : Lutjanidae) in Kenyan inshore marine waters. *Hydrobiologia* **353**, 153 - 160.

King, J.E. (1948) A study of the reproductive organs of the common marine shrimp, *Penaeus setiferus* (Linnaeus). *Biological Bulletin* **94**, 244 - 262.

King, M.G. (1979) The biology of the western king prawn *Penaeus latisulcatus* Kishinouye and aspects of the fishery in South Australia. PhD Thesis, Adelaide University, Adelaide

Levin, L.A., and Bridges, T.S. (1995) Pattern and Diversity in Reproduction and Development. In Ecology of Marine Invertebrate Larvae. (Ed. LR Mc Edward) pp. 1 - 48. (CRC Press: Florida)

Lindner, M.J., and Anderson, W.W. (1956) Growth, migrations, spawning and size distribution of shrimp, *Penaeus setiferus*. *Bulletin of U.S Fisheries and Wildlife Service* **56**, 554 - 645.

Macchi, G.J., Iorio, M.I., and Christiansen, H. (1992) Aspects of spawning and fecundity of the shrimp *Pleoticus muelleri* (Bate, 1888) (Crustacea, Decapoda, Solenoceridae). *Revista de Biologia Marina* **27**(1), 43 - 58. [In Spanish]

Macchi, G.J., Pajaro, M., Wohler, O.C., Acevedo, M.J., Centurion, R.L., and Urteaga, D.G. (2005) Batch fecundity and spawning frequency of southern blue whiting (*Micromesistius australis*) in the southwest Atlantic Ocean. *New Zealand Journal of Marine and Freshwater Research* **39**(5), 993 - 1000.

Macewicz, B.J., and Hunter, J.R. (1993) Spawning frequency and batch fecundity of Jack Mackerel, *Trachurus symmetricus* off California during 1991. *California Cooperative Oceanic Fisheries Investigations* **34**, 112 - 121.

Marshall, D.J., and Keough, M.J. (2006) Complex life cycles and offspring provisioning in marine invertebrates. *Integrative and Comparative Biology* **46**, 643 - 651.

Martosubroto, P. (1974) Fecundity of the pink shrimp, *Penaeus duorarum* Burkenroad. *Bulletin of Marine Science* **24**, 606 - 627.

Montgomery, S.S. (1981) Tagging studies on juvenile eastern king prawns reveal record migration. *Australian Fisheries* **40**(9), 13 - 18.

Montgomery, S.S. (1992) Sizes at first maturity and at onset of breeding in female Jasus verreauxi (Decapoda: Palinuridae) from New South Wales waters, Australia. *Australian journal of marine and freshwater research. Melbourne* **43**(6), 1373. [In English]

Montgomery, S.S., Courtney, A.J., Blount, C., Stewart, J., Die, D.J., Cosgrove, M., and O'Neill, M.F. (2007a) Patterns in the distribution and abundance of female eastern king prawns, *Melicertus plebejus* (Hess, 1865), capable of spawning and reproductive potential in waters off eastern Australia. *Fisheries Research* **88**(1 - 3), 80 - 87.

Montgomery, S.S., Stewart, J., and Blount, C. (2007b) The use of a point-count technique to quantify the egg assemblage in *Melicertus plebejus* (Hess, 1865) ovaries. *Fisheries Research* **83**(2 - 3), 228 - 237.

Moran, A.L., and McAlister, J.S. (2009) Egg Size as a Life History Character of Marine Invertebrates: Is It All It's Cracked Up to Be? *Biological Bulletin* **216**(3), 226 - 242.

Motoh, H., and Buri, P. (1980) Development of the external genitalia of the giant prawn, *Penaeus monodon*. *Bulletin of the Japanese Society of Scientific Fisheries* **46**(2), 149 - 155.

Mulley, J.C., and Latter, B.D.H. (1981) Geographic Differentiation of Tropical Australian Penaeid Prawn Population. *Australian Journal of Marine and Freshwater Research* **32**(6), 897 - 906. [In English]

Niamaimandi, N., Arshad, A.B., Daud, S.K., Saed, R.C., and Kiabi, B. (2007) Population dynamic of green tiger prawn, *Penaeus semisulcatus* (De Haan) in Bushehr coastal waters, Persian Gulf. *Fisheries Research* **86**(2 - 3), 105 - 112.

Ohtomi, J., Tashiro, T., Atsuchi, S., and Kohno, N. (2003) Comparison of spatiotemporal patterns in reproduction of the kuruma prawn *Marsupenaeus japonicus* between two regions having different geographic conditions in Kyushu, southern Japan. *Fisheries Science* **69**, 505 - 519.

Ohtomi, J., Yamamoto, S., and Koshio, S. (1998) Ovarian maturation and spawning of the deep-water mud shrimp *Solenocera melantho* de Man, 1907 (Decapoda, Penaeoidea, Solenoceridae) in Kagoshima Bay, Southern Japan. *Crustaceana* **71**, 672 - 685.

Olney, J.E., Denny, S.C., and Hoenig, J.M. (2001) Criteria for determining maturity stage in female American shad, *Alosa sapidissima*, and a proposed reproductive cycle. *Bulletin Francais De La Peche Et De La Pisciculture* **362**(63), 881 - 901.

Owens, L., and Glazebrook, J.S. (1985) 'The biology of bopyrid isopods parasitic on commercial penaeid prawns in northern Australia.'

Pechenik, J.A. (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. *Marine Ecology Progress Series* **177**, 269 - 297.

Peixoto, S., Cavalli, R.O., D'Incao, F., Milach, A.M., and Wasielesky, W. (2003) Ovarian maturation of wild *Farfantepenaeus paulensis* in relation to histological and visual changes. *Aquaculture Research* **34**, 1255 - 1260.

Peixoto, S., Cavalli, R.O., and Wasielesky, W. (2005a) Recent developments on broodstock maturation and reproduction of *Farfantepenaeus paulensis*. *Brazilian Archives of Biology and Technology* **48**(6), 997 - 1006.

Peixoto, S., Coman, G., Arnold, S., Crocos, P., and Preston, N. (2005b) Histological examination of final oocyte maturation and atresia in wild and domesticated *Penaeus monodon* (Fabricius) broodstock. *Aquaculture Research* **36**(7), 666 - 673.

Penn, J.W. (1975) Tagging Experiments with western king prawn *Penaeus latisulcatus* Kishinouye. I. Survival, growth and reproduction of tagged prawns. *Australian Journal Marine Freshwater Research* **26**, 197 - 211.

Penn, J.W. (1976) Tagging Experiments wilth western king prawn, *Penaeus latisulcatus* Kishinouye II. Estimation of population parameters. *Australian Journal Marine and Freshwater Research* **27**(2), 239 - 250.

Penn, J.W. (1980) Spawning and fecundity of the western king prawn, *Penaeus latisulcatus* Kishinouye, in Western Australian waters. *Australian Journal Marine Freshwater Research* **31**, 21 - 35.

Penn, J.W., and Caputi, N. (1986) Spawning stock-recruitment relationships and environmental influences on the tiger prawn (Penaeus esculentus) fishery in Exmouth Gulf, Western Australia. *AUST. J. MAR. FRESHWAT. RES.* **37**(4), 491-505. [In English]

Penn, J.W., and Hall, N.G. (1974) Morphometric data relating to the western king prawn Penaeus latisulcatus (Kishinouye, 1896) and the brown tiger prawn Penaeus esculentus (Haswell 1879) from Shark Bay, Western Australia. *Fisheries Bulletin of Western Australia* **15**, 1 - 12.

Potter, I.C., Manning, R.J.G., and Loneragan, N.R. (1991) Size, movements, distribution and gonadal stage of the western king prawn (*Penaeus latisulcatus*) in a temperate estuary and local marine waters *Journal of Zoology* **223**, 419 - 445.

Prangnell, D.I., and Fotedar, R. (2006) The growth and survival of western king prawns, Penaeus latisulcatus Kishinouye, in potassium-fortified inland saline water. *Aquaculture* **259**(1-4), 234-242.

Preston, N. (1985a) The combined effects of temperature and salinity on hatching success and the survival, growth, and development of the larval stages of *Metapenaeus bennettae* (Racek and Dall). *Journal of Experimental Marine Biology and Ecology* **85**, 57 - 74.

Preston, N. (1985b) The effects of temperature and salinity on survival and growth of larval *Penaeus plebejus*, *Metapenaeus macleayi* and *M. bennettae*. In Second Australian National Prawn Seminar. (Eds. PC Rothlisberg, BJ Hill and DJ Staples) pp. 31 - 40. (NPS2: Cleveland, Australia)

Primavera, J.H., Parado-Estepa, F.D., and Lebata, J.L. (1998) Morphometric relationship of length and weight of giant tiger prawn *Penaeus monodon* according to life stage, sex and source. *Aquaculture* **167**, 67 - 75.

Quinitio, E.T., Calallero, R.M., and Gustilo, L. (1993) Ovarian development in relation to changes in the external genitalia in captive *Penaeus monodon*. *Aquaculture* **114**, 71 - 81.

Quintero, M.E.S., and Garcia, A. (1998) Stages of gonadal development in the spotted pink shrimp *Penaeus brasiliensis*. *Journal of Crustacean Biology* **18**(4), 680 - 685.

Roberts, S.D., Dixon, C.D., and Andreacchio, L. (2012) Temperature dependent larval duration and survival of the western king prawn, *Penaeus (Melicertus) latisulcatus* Kishinouye, from Spencer Gulf, South Australia. *Journal of Experimental Marine Biology and Ecology* **411**, 14 - 22.

Roberts, S.D., Dixon, C.D., and Hooper, G.E. (2009) Gulf St Vincent Prawn, *Penaeus (Melicertus) latisulcatus*, Fishery 2007/08, Fishery assessment report to PIRSA Fisheries and Aquaculture. South Australian Research and Development Institute (Aquatic Sciences), Adelaide. SARDI publication number F2007/000782-3. SARDI research Report Series No. 382.

Robinson, B.F., and Bakeman, R. (1998) ComKappa: A Windows '95 program for calculating kappa and related statistics. *Behavior Research Methods, Instruments and Computers* **30**(4), 731 - 734.

Rodgers, G.G., Roberts S.D., and Dixon, C.D. (2013) The effects of temperature on larval size in the western king prawn, *Penaeus (Melicertus) latisulcatus* Kishinouye, from Spencer Gulf, South Australia: implications for fishery management. *Marine and Freshwater Research* **64**(10), 976 - 985.

Roongratri, M. (1993) Spawning seasons and some biological aspects of green tiger prawn, *Penaeus semisulcatus*, western king prawn, *P. latisulcatus*, and banana prawn, *P. merguiensis*, along the eastern coast of the Gulf of Thailand. *Thai marine fisheries research bulletin* **4**, 37 - 43.

Rothlisberg, P.C. (1998) Aspects of penaeid biology and ecology of relevance to aquaculture: a review *Aquaculture* **164**, 49 - 65.

Rothlisberg, P.C., and Jackson, C.J. (1987) Larval ecology of penaeids of the Gulf of Carpentaria, Australia. II. Hyrdographic environment of *Penaeus merguiensis*, *P. esculentus*, *P. semisulcatus* and *P. latisuclatus* zoeae. *Australian Journal of Marine and Freshwater Research* **38**(1), 19 - 28.

Rothlisberg, P.C., Jackson, C.J., and Pendrey, R.C. (1987) Larval ecology of penaeids of the Gulf of Carpentaria, Australia. 1. Assessing the reproductive activity of five species of Penaeus from the distribution and abundance of the zoeal stages. *Australian Journal of Marine and Freshwater Research* **38**(1), 1 - 17.

Rothlisberg, P.C., Staples, D.J., and Crocos, P.J. (1985) A review of the life history of the banana prawn, *Penaeus merguiensis* , in the Gulf of Carpentaria. In Second Australian National Prawn Seminar. (Eds. PC Rothlisberg, BJ Hill and DJ Staples) pp. 125 - 136. (NPS2, Cleveland, Queensland, Australia: Brisbane)

Saidapur, K.S. (1982) Structure and function of postovulatory follicles (corpora lutea) in the ovaries of nonmammalian vertebrates. *International Review Of Cytology* **75**, 243 - 285.

Sakai, S., and Harada, Y. (2001) Why do large mothers produce large offspring? Theory and a test. *American Naturalist* **157**, 348 - 359.

Sakaji, H. (2001) Maturation and spawning of the small penaeid shrimp *Metapenaeopsis dalei* in Tosa Bay, Pacific coast of southern Japan. *Fisheries Science* **67**(3), 444 - 448.

Saldanha, C.M., and Chatterji, A. (1997) Lenght and weight relationship of laboratory reared penaeid prawn *Penaeus monodon* (Fabricius) (Crustacea: Penaeidae). *Indian Journal of Marine Sciences* **26**, 389 - 391.

Shiraishi, T., Ketkar, S.D., Katoh, Y., Nyuji, M., Yamaguchi, A., and Matsuyama, M. (2009) Spawning frequency of the Tsushima current subpopulation of chub mackerel *Scomber japonicus* off Kyushu, Japan. *Fisheries Science* **75**, 649 - 655.

Shlagman, A., Lewinsohn, C., and Tom, M. (1986) Aspects of the reproductive acitvity of Penaeus semisulcatus De Haan along the Southeastern coast of the mediterranean. *Marine Ecology* **7**(1), 15 - 22.

Smith, S.J., and Sainte-Marie, B. (2004) Biological reference points for invertebrate fisheries: Introduction. *Canadian Journal of Fisheries and Aquatic Sciences* **61**(8), 1303 - 1306.

Somers, I.F. (1990) Manipulation of fishing effort in Australia's penaeid prawn fisheries. *Australian Journal Marine and Freshwater Research* **41**, 1 - 12.

Steer, M.A., Moltschaniwskyj, M.A., Nichols, D.S., and Miller, M. (2004) The role of temperature and maternal ration in embryo survival: using the dumpling squid *Euprymna tasmanica* as a model. *Journal of Experimental Marine Biology and Ecology* **307**, 73 - 89.

Svane, I., and Roberts, S.D. (2005) Gulf St Vincent Prawn Fishery (*Melicertus latisulcatus)*. Fishery assessment report to PIRSA. South Australian Research and Development Insitute (Aquatic Sciences), Adelaide. SARDI publication number RD03/0063-3

Tan-Fermin, J.D. (1991) Effects of unilateral eyestalk ablation on ovarian histology and oocyte size frequency of wild and pond-reared *Penaeus monodon* (Fabricius) broodstock. *Aquaculture* **93**, 77 - 86.

Thorson, G. (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biolgical Reviews* **25**, 1 - 45.

Tuma, D.J. (1967) A description of the development of primary and secondary sexual characters in the banana prawn, *Penaeus merguiensis* De Man (Crustacea: Decapoda: Penaeinae). *Australian Journal Marine Freshwater Research* **18**, 73 - 88.

Vance, R.R. (1973) On reproductive strategies in marine benthic invertebrates. *The American Naturalist* **107**, 399 - 352.

Wantanabe, C., Hanai, T., Meguro, K., Ogino, R., and Kimura, R. (1999) Spawning biomass estimates of chub mackerel *Scomber japonicus* of Pacific subpopulation off central Japan by a daily egg production method. *Nippon Suisan Gakkaishi* **65**, 695 - 702.

Watson, P.F., and Petrie, A. (2010) Method agreement analysis: A review of correct methodology. *Theriogenology* **73**, 1167 - 1179.

Watson, R.A., and Sumner, N.R. (1999) Uncertainty and risk associated with optimised fishing patterns in a tropical penaeid fishery. *Environment International* **25**(6 - 7), 735 - 744.

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

Xiao, Y., and McShane, P. (2000a) Estimation of instantaneous rates of fishing and natural mortalities from mark-recapture data on the western king prawn *Penaeus latisulcatus* in the Gulf St. Vincent, Australia, by conditional likelihood. *Transactions of the American Fisheries Society* **129**(4), 1005 - 1017.

Xiao, Y., and McShane, P. (2000b) Use of age and time-dependent seasonal growth models in analysis of tag/recapture data on the western king prawn *Penaeus latisulcatus* in the Gulf St. Vincent, Australia. *Fisheries Research* **49**(1), 85 - 92.

Xiao, Y.S. (2004) Use of generalized linear models in analyzing the catch and effort data on the western king prawn *Penaeus latisulcatus* Kishinouye in the Gulf St. Vincent, Australia. *Fisheries Research* **68**(1 - 3), 67 - 82.

Yamada, R., Kodama, K., Yamakawa, T., Horiguchi, T., and Aoki, I. (2007) Growth and reproductive biology of the small penaeid shrimp *Trachysalambria curvirostris* in Tokyo Bay. *Marine Biology* **151**(3), 961 - 971.

Yamada, T., Aoki, I., and Mitani, I. (1998) Spawning time, spawning frequency and fecundity of Japanese chub mackerel, *Scomber japonicus* in waters around the Izu islands, Japan. *Japan Fisheries Research* **38**, 83 - 89.

Yamamoto, K., and Yoshioka, H. (1964) Rhythm of development in the oocyte of the medaka, *Oryzias latipes*. *Bulletin of Faculty Fisheries Hokkaido University* **15**, 5 - 19.

Yano, I. (1988) Oocyte development in the kuruma prawn *Penaeus japonicus*. *Marine biology* **99**, 547 - 553.

Yano, I. (1995) Final oocyte maturation, spawning and mating in penaeid shrimp. *Journal of Experimental Marine Biology and Ecology* **193**(1 - 2), 113 - 118.