Fisheries Research Report No. 193, 2009

Investigating Reproductive Biology Issues Relevant to Managing the Western Rock Lobster Broodstock

Final FRDC Report – Project 2003/005

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Correct citation:

Melville-Smith, R., de Lestang, S., Beale, N.E., Groth, D. and Thompson, A. Investigating Reproductive Biology Issues Relevant to Managing the Western Rock Lobster Broodstock. Final FRDC Report – Project 2003/005. Fisheries Research Report No. 193. Department of Fisheries, Western Australia. 122p.

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The Fisheries Research and Development Corporation plans, invests in, and manages fisheries research and development throughout Australia. It is a federal statutory authority jointly funded by the Australian Government and the fishing industry.

ISSN: 1035 - 4549 ISBN: 1 921258 58 6

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2003/005 Investigating reproductive biology issues relevant to managing the western rock lobster broodstock

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Objectives:

1. To investigate the implications of the setose and maximum size rules.
2. To undertake preliminary investigations into the implications of egg diameters being significantly smaller at the Abrolhos Islands than at the coast.
3. To quantify the extent of the breeding grounds, so as to weight the overall egg production index for the stock by the contribution of the management zones.

Non-Technical Summary

Increases in efficiency due to modern electronic equipment, improved fishing vessels and knowledge about the grounds and lobster behaviour, have all led to western rock lobster fishers becoming more efficient at catching western rock lobsters in Western Australia. One of the outcomes of these increases in efficiency has been an increase in pressure on the breeding stock. Managers have responded by introducing measures to protect the breeders. In 1993, this involved the imposition of measures to limit the exploitation of mature female lobsters by introducing a legal maximum size for females as well as the protection of setose (mature) females.

The combination of high exploitation rates and protection of large, mature females, but not males, has since resulted in very distorted sex ratios of mature animals across the fishery, particularly those over the maximum size limit. It is well known from laboratory studies on other species of lobsters, that highly distorted female-dominated sex ratios (such as has been caused in the wild by the setose rule), and large females being forced through lack of choice to mate with small males (such as has been caused in the wild by both the setose and maximum size measures) can lead to sperm limitation effects (fertilisation of eggs but reduced brood sizes). These same management measures could have more positive effects – for instance in some species, the older individuals produce larger eggs, that in turn produce larger larvae that have been shown to have better survival characteristics.

These, and other unknowns, led to the initiation of this research project aimed at investigating these biological issues. The outputs from this research, together with other data, provide an indication of the contribution to egg production of different management zones in the western rock lobster commercial fishery.

This study established that the sizes at which female and male western rock lobsters become mature is smaller in the northern part of the grounds than in the south. The study further showed that the size at first maturity has been decreasing in all areas of the grounds over the last 30 years. The reason for these changes may be due to warming of the water, which has occurred off the fishing grounds in the last century, or it could be due to selective fishing pressure favouring animals that mature at a small size.

The project also examined the moulting cycle of female lobsters. It showed that mature females are in breeding condition (setose) from June to February at which time the majority moult to a non-breeding condition. However, this is variable depending on the water temperature in January/February. When the water temperature is cool in those months, the proportion of females that stay in a breeding condition throughout the year is much higher (up to 40% of the samples). This research also showed that large lobsters carry two broods per season, whereas small lobsters only carry one brood. Whether or not a female carries one brood or two is not only dependent on the size of the animal, but also on the number of appendages that she has lost. The project has shown that the more appendages that have been lost, the less chance that the she will carry eggs.

This study also examined whether the size, biochemical composition and consequent likelihood of survival of western rock lobster larvae are different for those produced by small, average size, or large females in the three different management zones of the fishery. There was no apparent relationship between these variables and the conclusion was drawn that the individual female and her diet prior to spawning, may have a greater influence on the likely survival of the larvae than her size or location of capture.

Biological data were used to show the current contribution of broodstock to egg production in the different management regions of the fishery and historical length frequency data were used to show how egg production has changed in those regions over time. Results show that egg production is now more evenly distributed across management zones than in earlier years of the fishery. Egg production was high at the Abrolhos Islands in the 1990s owing to strong year classes of breeding animals entering the fishery over those years. Since 2000, egg production has fallen sharply at the Abrolhos Islands, but has increased in the coastal population compared with the 1980s and 1990s.

In the final research component of this study, paternity assignment, using genetic analyses of tissue, egg and spermatophore samples, was used in an attempt to establish mating choices of male and female western rock lobsters on an isolated deep water (50 m) reef, west of Dongara in the central part of the fishery. Depletion studies suggested that most of the resident lobsters on the reef were caught at least once on the separate sampling trips, but it became apparent that there was considerable emigration and immigration on and off the sampling site – an unexpected result. Accordingly, there were few matches of males caught, to the spermatophores or eggs sampled from the females at the site. The small sample of males that were matched to female mating partners did not show any relationship between either the size or number of females that the males mated.

The microsatellite (genetic) technique of being able to identify individual animals was also used to record intermoult increments. As with the paternity assignment work, few animals were recaptured, especially after moulting, but the method was used to record increments in nine males and three females.

Keywords: egg production; selective fishing; paternity assignment; behaviour; maturity; genetic

Acknowledgements

There have been a large number of people who have assisted on this project in one way or another and most of these people have been acknowledged in the particular chapters to which they made specific contributions.

We do have a few individual people or groups of people that we would like to specifically acknowledge:

- Many of the samples used in analyses in the report were collected during the course of the annual western rock lobster fishery Independent Breeding Stock Survey. All of the Department of Fisheries staff and industry skippers and crews that assisted in those surveys are thanked for their contribution.
- Our colleague, Nick Caputi, is thanked for his many useful comments and contributions in the form of ideas to many of the chapters.
- Finally, we thank the Fisheries Research and Development Corporation for financial support of this project.

1.0 Background

There is no doubt that western rock lobster fishers have become more efficient operators over time through advancements in gear technology, coupled with increased expertise in their ability to use this technology to target the stocks. One of the outcomes of this has been an increase in pressure on the breeding stock. Managers have responded to these pressures on the breeding stock by introducing measures to protect the breeders. In 1993, this involved the imposition measures to limit the exploitation of mature female lobsters by introducing a legal maximum size for females as well as the protection of setose (mature) females.

The combination of high exploitation rates and protection of large mature females, but not males, has since resulted in very distorted sex ratios of mature animals, particularly those over the maximum size limit. It is well known from laboratory studies on other species of lobsters, that highly distorted female-dominated sex ratios (such as has been caused in the wild by the setose rule), and large females being forced through lack of choice to mate with small males (such as has been caused in the wild by both the setose and maximum size measures) can lead to sperm limitation effects (fertilisation of eggs, but reduced brood sizes).

The western rock lobster fishery, through its unusual management measures of protecting the brood stock, probably has a more highly skewed sex ratio than most (perhaps all) other lobster fisheries in the world. This factor has been a strong motivator for research to be undertaken to evaluate this potential risk to mating success.

When this project was developed, there had been some evidence that the size of eggs at the Abrolhos Islands were 10-15% smaller in diameter than at the coast. It is known from studies on finfish, that eggs from first time spawners (and therefore probably small animals) have a lower hatching success compared to eggs from experienced spawners (and therefore probably older animals) (Solemdal et al., 1995; Trippel, 1998). It was therefore important to investigate the possibility that western rock lobster eggs spawned at the Abrolhos Islands might be less viable than those from the coast.

Finally, there was a need to quantify the extent of the breeding grounds in the western rock lobster fishery. Managers produce breeding stock indices for different management zones in the fishery and these are sometimes combined into a single breeding stock index for the fishing grounds. However, it has been identified that information on the proportion that the three management zones contribute to the overall index, is necessary.

This project, which commenced in July 2003, has attempted to provide answers to the above questions. Over the course of the study, it has frequently been found that addressing one issue has often led to interesting research results being achieved for other unanswered questions. Accordingly, this report has been prepared as a series of published (and unpublished) research papers. The way the objectives of this project have been met in these research papers is outlined in Chapter 1.3.

Outcomes Achieved To Date

The project has provided biological information to give confidence to managers and industry as to the effects various management measures, such as the setose rule and maximum size rule, might be having on the brood stock.

On the basis of the results, there has been reassurance to industry that there is no need for concern about distorted sex ratios and mismatches of size classes between the sexes of mature lobsters in the natural environment impacting on egg and larval production in the fishery.

The size or area in the fishery where breeders are resident has been shown to not affect the likely survival of the larvae they produce.

The proportion of egg production to the total production in the fishery has been estimated for the three management zones.

The microsatellite technique of being able to identify individual animals was shown to be useful for conducting mating choice studies for lobsters. In addition, the polymorphic loci developed in this project (supplemented with some additional polymorphic loci) will be used in the future to evaluate spatial and temporal population genetic structure in western rock lobsters.

1.1 Need

Setose and maximum size rules have severely distorted the sex ratios and may have affected the sizes of mature (breeding) animals in the population. Research is necessary to investigate current and future projection of these management measures on fertilisation success in the breeding population.

To effectively manage the brood stock, it is important to have a knowledge of whether eggs from different regions in the fishery differ in 'quality' (i.e. would be likely to produce first stage larvae with different survival characteristics). This is particularly important for the Abrolhos Islands, which is estimated to contribute between 45-65% of egg production, but where the sizes of the eggs have previously been recorded as 10 to 15% smaller than at the coast. This project will assess whether the smaller eggs result in lower survival of stage 1 larvae, as an indicator of overall survival.

At present, no weighting is given to the contribution made by the three zones to egg production in the fishery. It is necessary to more fully understand the spatial distribution of the breeding stock in the zones when assessing effects of management packages.

1.2 Objectives

1. To investigate the implications of the setose and maximum size rules.
2. To undertake preliminary investigations into the implications of egg diameters being significantly smaller at the Abrolhos Islands than at the coast.
3. To quantify the extent of the breeding grounds, so as to weight the overall egg production index for the stock by the contribution of the management zones.

1.3 Reporting Format

As noted in the Background to this report (see Chapter 1), much of the research in this document has either been, or is in the process of being submitted for publication in peer-reviewed scientific journals. Project objectives are outlined in Chapter 1.2 (above). The way that these objectives have been met in the following chapters is outlined below.

Chapter 3 is titled 'Visual Assessment of the Reproductive Condition of Female Western Rock Lobsters (*Panulirus cygnus*)'. This paper does not specifically address any of the objectives, but was necessary in order to be able to do the reproductive condition assessments that were used throughout this study.

Chapter 4 is titled 'Spatial and temporal variation in the size at maturity of the western rock lobster *Panulirus cygnus* George'. This paper, and the next in Chapter 5, 'Interannual variation in the moult cycle and size at double breeding of mature female western rock lobster *Panulirus cygnus*', together partially address Objective 1(to investigate the biological implications of the setose and maximum size rules), by examining: spatial and temporal changes in the size at first breeding in females; sizes responsible for multiple broods per season; and, year to year changes in the proportion of setose breeders. These data have all been used in Chapter 8 to calculate spatial and temporal changes in egg production.

Chapter 6 titled 'Changes in egg production of the western rock lobster (*Panulirus cygnus*) associated with appendage damage' was an indirect (and unforseen) benefit of the analysis that resulted from addressing Objective 1. It does however show that the unavoidable loss of appendages that would likely result from the continual sorting of setose and maximum sized females through the fishing season, will impact on egg production.

Chapter 7 titled 'Neither maternal size nor site of spawning influences larval competency in western rock lobster *Panulirus cygnus* George', completely addresses Objective 2 (to undertake preliminary investigations into the implications of egg diameters being significantly smaller at the Abrolhos Islands than at the coast).

Chapter 8 is titled 'Spatial and temporal changes in egg production in the western rock lobster (*Panulirus cygnus*) fishery' and has utilized much of the research in other chapters (e.g. changes in size at maturity over time; size at double breeding etc), to address Objective 3 in full, namely to quantify the extent of the breeding grounds, so as to establish the proportional contributions to egg production of the three management zones.

Chapter 9 is titled 'Characterization of polymorphic loci for the Western Rock Lobster (*Panulirus cygnus*)' and deals with the development of microsatellite loci for use in Chapter 10.

Chapter 10 is titled 'Use of microsatellite loci to establish mating choice and growth rates of western rock lobsters (*Panulirus cygnus*)'. This chapter also addressed aspects of Objective 1: to investigate the biological implications of the setose and maximum size rules, in this case by attempting to examine mating strategies in the wild as a means of identifying the implications of the distorted sex ratio that must result from the protection of females by the setose and maximum size rules.

2.0 Executive, non-technical summary of the project and its outcomes

2.1 Introduction

This final report differs from many other FRDC-funded projects, in that the subject matter has covered a wide range of research topics. The common thread that has joined these topics is their relevance to management of the broodstock of western rock lobsters. The research behind this project has been published in six well-respected scientific journals and a seventh chapter is in the process of being prepared for journal submission.

Each of the chapters in this report is in the format in which it was submitted for publication. The positive aspect of submitting a final report in this style is that those reading it can be assured that the work has been thoroughly peer reviewed. The downside however, is that the project outcomes have been geared towards a scientifically informed audience. The funder has therefore asked for this extended non-technical summary to be added, so as to encapsulate the intention, results and outcomes of the work for a wider non-scientific audience.

Back in 2003 when the project was funded, there were a number of unanswered reproductive biology questions being posed by stakeholders in relation to broodstock management of the western rock lobster fishery. Management measures requiring fishers to release setose (i.e. females with ovigerous setae, which are hairs on the swimmerets of mature females during the breeding season) and maximum size females (i.e. exceptionally large females above a particular size that differ in different parts of the fishery) had been introduced into the fishery in the 1992/93 fishing season. There was uncertainty as to how this might have distorted the sex ratios of the brood stock and what affect that might have had on fertilisation success.

There was also uncertainty as to the quality of eggs produced by different sized females and by females in different parts of the fishery. There is a considerable difference in the size ranges of breeding animals in different parts of the fishery and while it is well known that large females produce many more eggs than small females, three was no information on whether larvae produced by different sized females would differ in their likelihood to survive. This question had become more relevant given the protection to maximum sized females.

Finally, stakeholders were interested in the contributions made to egg production in the different management zones of the fishery. Estimates of egg production had been made for the different zones in earlier years, but since then exploitation rates had increased in a non-uniform way across the fishery and these earlier estimates were clearly no longer relevant.

2.2 Results

In order to undertake the objectives of this project, it was important to have very clear and reliable criteria for establishing the reproductive state of breeding females, i.e. whether the female is immature, or if it is a breeder, whether it is inactive, or likely to be a single or double breeder (the latter category being made up of females carrying more than one batch of eggs in a spawning season). A flow chart outlining the external characteristics used for determining the reproductive state of breeding females is provided in Chapter 3, Fig. 3.

Although the work outlining female breeding criteria in Chapter 3 is quite routine, it was critical to get these methods firmly established because much of the subsequent work in the report was based on these criteria. In the course of developing these we did establish that external features that had previously been employed to classify western rock lobsters as either single or double breeders were not as reliable as those outlined in this study.

With breeding criteria well established, the study moved to focussing on identifying the size at first maturity of both male and female western rock lobsters in different areas of the fishery, and whether there have been any changes in size at first maturity over time.

It is well known in the western rock lobster fishery that the average size of lobsters tends to be much larger in the south than the north and that this is also reflected in the size at first maturity, which is larger in the south than the north. Both the average size and size at first maturity are particularly small at the Abrolhos Islands. While various studies in the past have established size at first maturity for one or both sexes in different areas of the fishery, this study was the first to undertake a comprehensive overview of size at first maturity across a range of different areas in the fishery in a single year (2002). The results from that overview are summarised for females and males in Chapter 4, Figs. 2 and 3 respectively. The results confirmed the decrease in size at maturity as sampling progressed northward and showed that there is a consistent trend in that male western rock lobsters have a larger size at first maturity than females.

The study then examined changes in size at first maturity over time in six different areas in the fishery using more than one dataset. With the exception of the Abrolhos Islands where size at first maturity appears to have remained relatively constant over time, the other areas (Fremantle, Lancelin, Jurien Bay, Dongara and Kalbarri) showed substantial downward trends (Chapter 4, Figs. 6 and 7).

Female breeding patterns in relation to the moult cycle, were examined in detail. This work showed that mature females have ovigerous setae on their swimmerets from June through February-March. The proportion of females that had ovigerous setae in the period between February-March through June was variable. On average, about 80% of the females moulted into a non-setose phase between February to June. There were some years, notably when mean water temperatures in January and February were high, when nearly all females moulted into a non-setose state. In years when the mean water temperature in January and February was cold, up to 40% of females remained setose (Chapter 5, Fig. 6).

Most mature females produce a batch of eggs each breeding season, but large females frequently produce a second batch of eggs in the same breeding season. The mean size at which females produce a second batch of eggs in the same season was estimated for data collected in 2002 for six areas in the fishery (Chapter 5, Fig. 7). As with size at maturity, the size at double-breeding decreases in a northerly direction across the fishing grounds.

In the course of focussing on the likelihood of different sized females carrying one or two batches of eggs, it became clear that there was an association between appendage damage and egg production. Females that were missing one or more appendages (antennae/feelers or legs), were less likely to produce a batch of eggs than a similar sized female with a full batch of appendages. The greater the number of appendages missing, the less likely was it that the breeding female would produce eggs, or in the case of large females the less likely the female would produce a second batch of eggs in the season. To give an indication of the effect of appendage damage on egg production, females with one damaged appendage were 20% less likely to produce a batch of eggs, whereas those with five damaged appendages were around

85% less likely to produce a batch of eggs. Females with more than seven damaged appendages did not produce eggs.

There is published research, particularly on teleosts (fish), that shows that eggs from young spawners have a lower hatching success than those from older spawners. Also that because older females spawn longer and later into the breeding season, that this gives the early stage larvae from older females a greater possibility of coinciding with plankton production cycles and thereby increasing the possibility of those larvae flourishing due to greater food availability.

Based on this information, there was an unsubstantiated opinion that the eggs and larvae of large female lobsters might be more competent (i.e. more robust and 'healthy') and therefore more likely to survive than, eggs from small females. There was also a feeling that there may be differences in the survival characteristics of eggs taken from different parts of the fishery.

The detailed methods used to determine whether any of these differences occurred in western rock lobster eggs and larvae from different parts of the fishery and from different sized females are provided in Chapter 7. Results showed that there were no significant differences in the diameter of eggs, produced by females of different size or taken from different regions in the fishery. There was also no difference in the length of phyllosoma at hatching.

Lipid, fatty acid, protein and water compositions of egg samples taken from different sized females and from different part of the western rock lobster fishery were compared. Mostly there was no significant difference, but lipid classes of eggs differed significantly across the three size classes of females that were examined. Fatty acids differed between both eggs and phyllosoma grouped by different parts of the fishery and by the size classes of the females. There was no indication from the analyses of lipid classes of fatty acid composition in this study to suggest that they influenced larval competency or length-at-hatch.

The results suggest that neither the site at which the female lobster spawns, nor her size, nor the size of the larvae at hatch is a good indicator of larval competency. It seems more likely that larval competency depends on the condition of the female initially, and then presumably also the availability and abundance of food in the environment into which the phyllosoma hatch.

The results that have been outlined provided a better understanding of reproductive biological parameters such as size at first maturity (by location and over time), proportions of females breeding once or twice per season (again, by location and over time) than was previously understood. Furthermore, it is now apparent that there is no significant difference in larval competency based on size of female or location of spawning. The next step was therefore to utilise this knowledge to estimate changes in egg production in the fishery by the three management zones over two decades (1980-1989; 1990-1999) and the period 2000-2007.

The methods used and the many assumptions that have been made, are outlined in Chapter 8. The results show that because of the decrease in size at first maturity and size at double breeding, that there are now significantly more small females, many below the legal minimum size, contributing to the brood stock than at any time since the fishery was researched. This has led to an increase in egg production in the northern and southern coastal management zones. Egg production at the Abrolhos Islands has been more variable than at the coast. Changes in the contribution to total egg production of lobsters above and below legal size at the Abrolhos Islands in the 1990s, led to peak egg production in that management zone in the 1990s.

One of the biggest influences on egg production estimates is the application of efficiency increases. There is a high degree of uncertainty surrounding appropriate efficiency increases; this study has applied a single one-off increase of 18% at the Abrolhos Islands and annual increases of 6% and 2% in the northern and southern management zones respectively.

The final part of this study has attempted to gain insight into mate choice of western rock lobsters in the wild to assist in addressing the question of whether there are sufficient mature and particularly large sized males, to mate with the females in the population. This has been of concern in this fishery because current management rules protect all mature females whilst they are in a breeding state and large size females even in a non-breeding state, but there are no management measures to protect large males.

Sampling was conducted periodically on a deep (50 m) offshore reef near the centre of the fishery, between October 2004 and August 2006. Microsatellite paternity assignment tests were used to determine which males caught in pots on the site had mated with females at the sampling site. Only six of the 284 sampled male lobsters had mated with females caught over the same period. Four of these identified males had mated with only one sampled female, while the other two males had mated with three different females each. Multiple spermatophore samples matched each other, but did not match any tissue samples from males captured during the surveys. From the small number of male lobsters that were both sampled and that had mated females at the sampling site, choice of mating partner appeared to be random. Similarly, although the sample size was small, there appeared to be no relationship between size of the male lobster and number of females that he mated.

As a secondary output from this study, a total of nine male and three female lobsters were identified by the microsatellite analysis as being recaptured after moulting. These animals have provided growth data, but this method is expensive and would not be practical for obtaining large amounts of routine growth data.

2.3 Discussion

This study into the reproductive biology of the broodstock, has been very beneficial in terms of its outcomes. There has been a severe and unexplained downturn in puerulus settlement in 2008, which has led to an examination of factors leading to possible causes for that downturn. One factor which has been considered as a cause has been the state of the western rock lobster broodstock, and the outcomes of this study have been particularly timely in contributing to considerations of this possibility.

Prior to this study being undertaken, it had not been appreciated that the size at first maturity in the fishery had declined. Without taking into account that smaller animals are now contributing to egg production in the fishery than in the past, estimates of change in egg production over time were more pessimistic than since this has been incorporated into the calculations.

The reasons behind the change in size at maturity are still unclear. One possibility that has been considered, has been that mean water temperature has increased since the 1970s (the period over which size at maturity has been considered in this study) and there is evidence from more than one source to show that females mature earlier and at a smaller size in warmer water. A different possibility is that high exploitation rates combined with knife-edge size selection in the fishery, could be selecting for earlier maturation. Both factors may be playing a part in the changes that have been recorded.

That the proportion of setose females in the period February-March through June each year is so variable, is a finding that had not previously been identified in this fishery. It could have potentially important ramifications in the western rock lobster fishery where rules protecting the harvesting of setose lobsters are used as a management measure used to offer protection to a portion of the breeding stock. What this means is that unusually warm water conditions in January/February each year, such as might be anticipated under future global warming scenarios, could lead to higher exploitation rates of large breeding females.

The effect of appendage damage on egg production was an outcome of this study that had not been anticipated when the objectives were being formulated. As with much of this study, this information is particularly relevant given that the maximum size of western rock lobster females has been further reduced in the 2008/09 fishing season. Maximum size rules are potentially beneficial to improving overall egg production, but it is clear from this work there needs to be a strong educational element aimed at appealing to fishers to handle the above maximum sized females with care before returning them to the water, because any appendage damage could negatively impact the aim of the management measure.

The comparison of different sized females and different spawning locations on biochemical composition and larval competency, suggests that the quality of larvae produced by individual females may be more important in influencing larval competency in western rock lobster than the size of the female and the area in which she was caught. The factors responsible for individual females producing different quality larvae are unclear, but may be related the female's physiological condition at the time of spawning, which could be influenced by, for example, previous dietary intake and moulting or spawning history.

In terms of using this information, one would have to assume that there is no difference in the likelihood of survival of a larvae produced by a large compared to a small female or by a female in one management zone compared to another. Of course this excludes factors influencing survival after hatching, such as ocean currents and food availability for the hatched larvae.

This study has produced a different interpretation of the state of egg production in the western rock lobster fishery to previous results. One of the reasons for this difference is that this study has incorporated the decrease in size at maturity over time. The major unknown in estimating egg production is what annual efficiency increases to apply in the different management zones.

One final word of caution needs to be raised in utilising commercial fishing catch composition and catch rates to develop an index of egg production, as has been done in this and other similar studies. Some fisheries have recorded stocks contracting into optimal habitats at low population sizes, a response considered to be due to fish moving to more favourable habitat (or hotspots) from neighbouring areas of lower standard of habitat. It is possible that the western rock lobster breeding stock may be exhibiting density-dependent habitat selection and indeed some fishers acknowledge that this is now the case, as evidenced by having to set their gear on carefully chosen habitat to achieve catch rates that previously could have been obtained with less accurate placement. If this effect is occurring it would not necessarily be taken into account by the application of efficiency increases and could be distorting what appears to be in an historical context, acceptable level of egg production.

The utilisation of microsatellite loci to establish mating choice produced results which were less conclusive than had been anticipated. The reason for this was that there was a substantial turnover of lobsters on the small offshore reef that formed the study area, with many of the animals not being recorded beyond the month in which they were sampled. This unexpected result meant that the paternity assignment tests matched few males to egg or tar-spotted females, and furthermore, there were relatively few animals that were recaptured long enough after first being sampled to have moulted and grown.

Overall, this study has achieved all the objectives that it set out to achieve. Over and above that, it has provided insight into aspects of western rock lobster reproductive behaviour and physiology that were not part of the original project objectives. Decisions on the management of this fishery relating to reproductive issues can now be made with far more certainty than prior to this work being undertaken.

3.0 Visual assessment of the reproductive condition of female Western rock lobsters (*Panulirus cygnus*)

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Citation: Melville-Smith, R. and de Lestang, S. 2005. Visual assessment of the reproductive condition of female western rock lobsters (*Panulirus cygnus*). New Zealand Journal of Marine and Freshwater Research. 39, 557-562.

3.1 Abstract

An important issue when surveying the reproductive condition of large numbers of female *Panulirus cygnus* in the field is that each animal must be assessed quickly and accurately on the basis of only a few easily discernable criteria. The four biological criteria most commonly recorded for each lobster are (i) ovigerous setae condition (not present, partially developed and fully developed), (ii) the presence (unused or eroded) of a spermatophore mass, (iii) the presence and developmental stage of external ova, and (iv) the visual appearance of the ovaries through the dorso-thoracic musculature. Using the above criteria each female can then be assigned to a reproductive state, *i.e.* immature, inactive breeder, single breeder or double breeder. Researchers have used a combination of these criteria that, predominantly when assigning double breeders, relies very heavily on an accurate ovary assessment, a criterion that can be subjective.

This study compared external assessments with internal cellular development of the ovary (via histology) and elucidated the shortcomings of the external technique. This has resulted in the production of a set of criteria that, if sampled during the peak of the breeding season, enables the breeding state of a female western rock lobster to be more accurately determined in the field without causing it any damage. The height of the breeding season is the optimum time for stock egg production assessment.

Keywords: lobster; Panulirus cygnus; visual assessment; reproductive condition; double breeding

3.2 Introduction

The fishery for western rock lobster *Panulirus cygnus* is Australia's most valuable single-species fishery: the average harvest of 11,000 tonnes each year is worth about AUD$350 million. The sustainability of this valuable fishery has consequently been a focus of research since the 1970s, with the legal minimum size (76–77 mm carapace length) being below the size at first maturity in most regions of the fishery (Chittleborough, 1976; Grey, 1979). The western rock lobster is thus vulnerable to recruitment over-fishing. In recent years, egg production has been closely monitored during an annual survey to check if the fishery is sustainable (Melville-Smith *et al.*, 1998; Hall and Chubb, 2001; Caputi *et al.*, 2003). The annual egg production survey is conducted independently of commercial fishing over a ten-day period that spans the last new moon prior to the start of the commercial fishing season on the 15th November. Since collection of all females for dissection in the laboratory is not practical or very sustainable (over 5,000 females are sampled each year), the reproductive state of each female should be assessed quickly and accurately using external characteristics, thereby allowing them to be returned to the water unharmed.

Studies have generally assumed that female palinurids with ovigerous setae are sexually mature and thus likely to spawn within the breeding season (George, 1958; Fielder, 1964; Montgomery, 1992); however, Chittleborough (1976) showed that, in the laboratory, female *P. cygnus* did not necessarily breed once they developed ovigerous setae. More recently, Chubb (1991) used a combination of external characteristics, namely the stage of external ova, presence or absence of a spermatophoric mass, and ovary condition (assessed through the dorso-thoracic musculature), to determine whether a female is mature and if so, whether she will produce either one or two batches of eggs over that breeding season. However, the assessment of ovary condition employed by Chubb (1991) was not confirmed histologically and as such, has the potential to miss-assign maturity state.

The present study was initiated in order to confirm, at a cellular level using light microscopy, the developmental condition of an ovary and how this relates to the accuracy of the non-invasive visual stage as described by Chubb (1991) and currently employed in the analysis of the annual egg production surveys in this fishery.

3.3 Methods

3.3.1 Data sources

A total of 8,541 female *Panulirus cygnus* were collected by trapping during the western rock lobster fishery-independent egg production surveys at the Abrolhos Islands, Kalbarri, Dongara, Jurien Bay, Lancelin and Fremantle on the Western Australian coast (Fig. 1) in October/ November 2002, which corresponds with the peak of egg extrusion and fertilization by female *P. cygnus* (Chubb, 1991). All traps were set for a fixed period of time (24 h) in areas used by this species for breeding (Chubb, 1991).

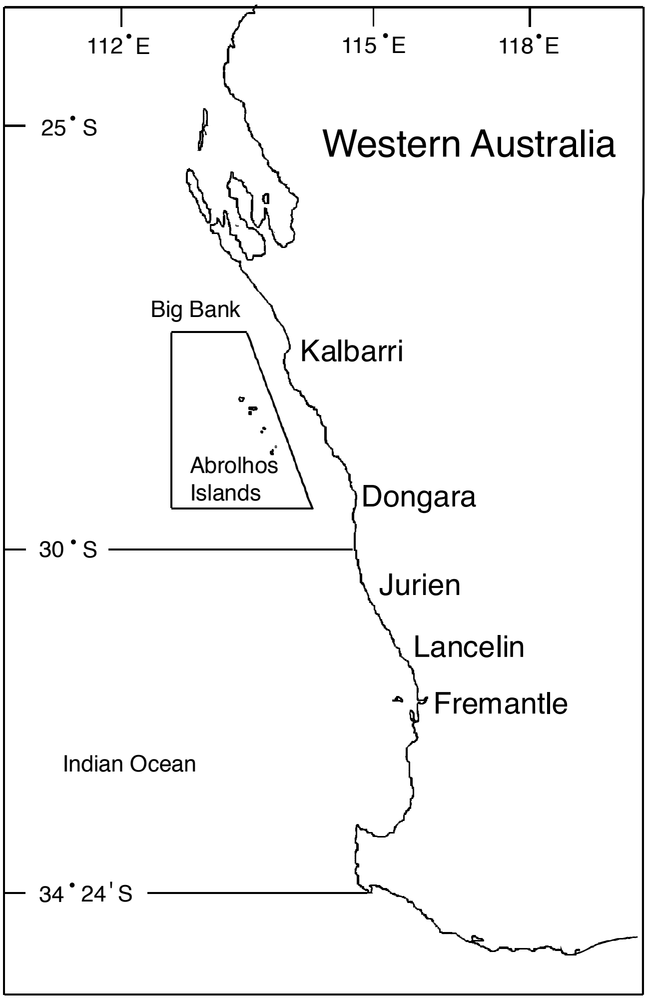

Figure 1. Map showing the locations of the fishery-independent egg production surveys in October/November 2002.

For every female lobster, the carapace length (*CL*), *i.e.* from the anterior edge of the carapace between the preorbital spines down the mid-dorsal line to the posterior edge of the carapace, was measured to the nearest mm. The state of ovigerous setae on the endopodites of the pleopods was recorded as either 'not present', 'immature setae' (partially developed) or 'mature setae' (fully developed) (Gregory and Labisky, 1981). The absence or presence and developmental stage of external ova attached to the setae were recorded as 'absent', 'early phase' (bright orange ova, no visible eye spots), 'mid-phase' (dark/dull orange ova, visible eye spots), 'late phase' (dark/grey ova, visible eye spots) or 'post hatching' (egg shells still attached). The absence or presence of a spermatophoric mass on the sternum was recorded as 'no spermatophoric mass present', 'unused spermatophoric mass present' or 'eroded/used spermatophoric mass present'. The colour of the ovaries, determined *in situ* through the dorso-thoracic musculature between the carapace and dorsal ridge of the first abdominal tergite, was used to assign the lobster's ovary to one of two stages: a transparent or white ovary was recorded as 'undeveloped', and a faint pink to orange ovary was recorded as 'developing'. Since it has been suggested that limb loss has the potential to influence the reproductive behaviour of female *P. cygnus*, all individuals that were missing limbs or possessed regenerated limbs were not included in the analysis.

On the basis of reproductive characteristics, females were classified as either a 'single breeder', a 'double breeder', an 'inactive breeder' or 'immature' using two sets of criteria, those developed during this study (see Fig. 3 in Results) and those described by Chubb *et al.* (1989); this latter criteria identified a double breeder as a female carrying eggs or bearing an eroded spermatophore, while possessing bright orange ripe ovaries.

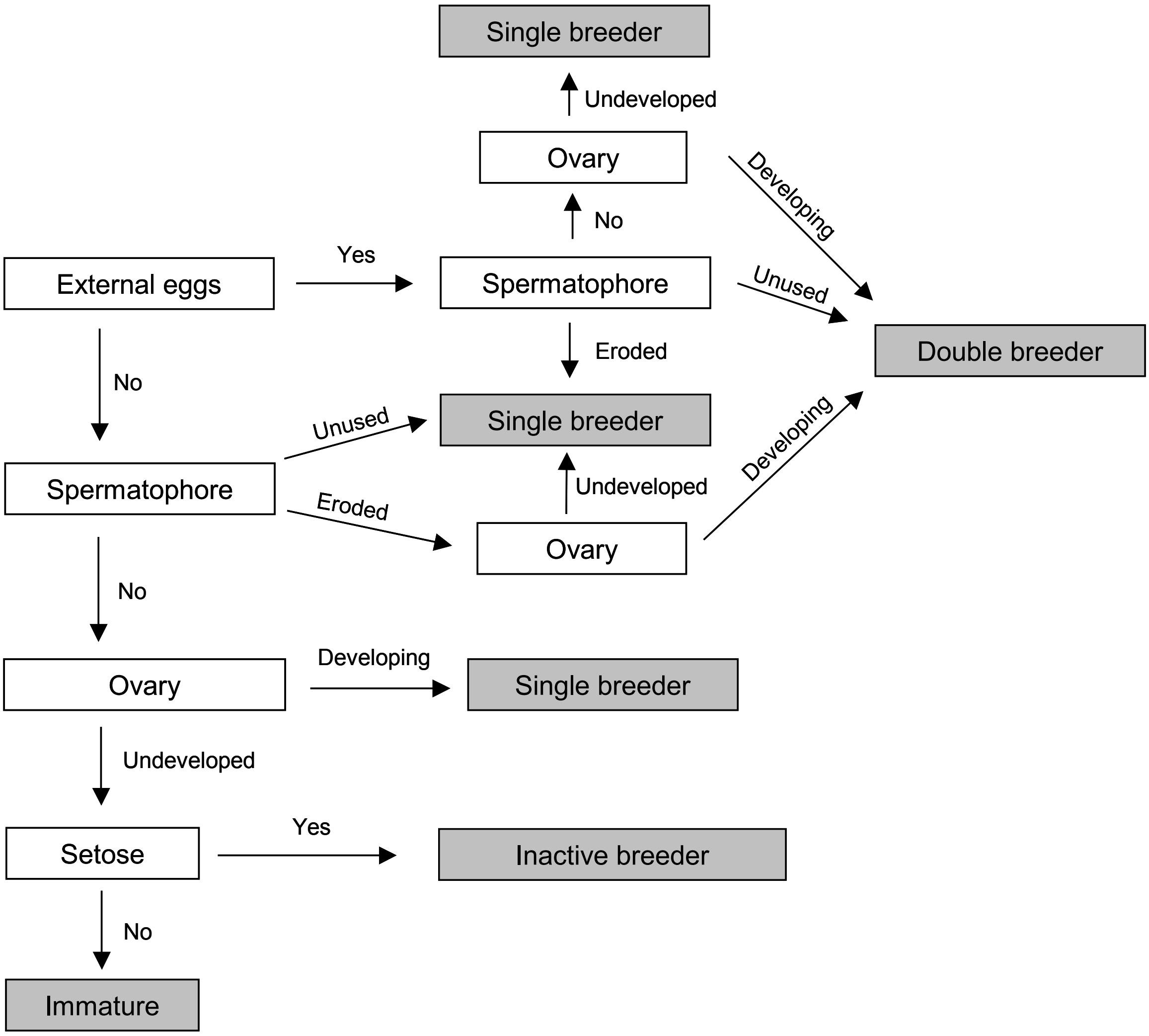

Figure 3. Flow chart showing the various combinations of external characteristics which, when combined, classify a female *Panulirus cygnus* as immature, inactive breeder, single breeder or double breeder.

3.3.2 Histological examination

Since almost all mature female *P. cygnus* had the distinctive reproductive characteristics that confidently indicated they were either single or double breeders, *i.e.* possessed both external eggs and an unused spermatophore or both late stage external eggs and obviously developing ovaries, only 10 lobsters in this condition, which covered the full size range, were selected for histological examination. Thirty-five females that proved difficult to assign an ovarian condition on the basis of an external examination were selected to determine histologically their true ovarian status. The ovaries of each *P. cygnus* selected for histological examination were assessed externally (through the dorso-thoracic musculature) before they were anaesthetised and had their ovaries removed and fixed in 4% glutaraldehyde in 0.025 M phosphate buffer (pH 7.0) for 24 h. Glutaraldehyde was used as it had previously been shown to be a good fixative for decapod ovarian tissue (de Lestang *et al.*, 2003). Once fixed, the ovaries were dehydrated in a series of increasing concentrations of ethanol and embedded in paraffin wax. Transverse sections (6 µm thick) of the gonad tissue were cut and stained with Mallory's trichrome and examined under an Olympus CH binocular microscope. The thickness of ovary walls and the diameters of 100 randomly selected oocytes from each of the developing ovaries (n=10), as well as the ovaries that were difficult to stage visually (n=35), were measured to the nearest µm using the imaging software Leica IM1000, which acquired the image via a Leica DC300 digital camera attached to a Leica MZ7.5 dissecting microscope.

3.4 Results

3.4.1 External and histological determination of ovarian development

A comparison of the ovaries from 45 female *Panulirus cygnus* that had been assessed both externally through the dorso-thoracic musculature and histologically in the laboratory, showed that the ovaries of all 14 lobsters classified through the dorso-thoracic musculature as 'developing' contained numerous small oocytes, as well as large proportions of oocytes > 170 µm in diameter (Fig. 2). Since early yolk granular oocytes, *i.e.* oocytes which are well on their way to developing, have a diameter of about 150 µm (data not shown), the visual assessment of the ovaries of *P. cygnus* through the dorso-thoracic musculature appears incapable of detecting the presence of any oocytes in the yolk vesicle and early yolk granule stages of development, and thus the early to mid stages of ovarian maturation. Thus, external assessment of the ovaries through the dorso-thoracic musculature underestimates the proportions of female *P. cygnus* that have 'developing' ovaries.

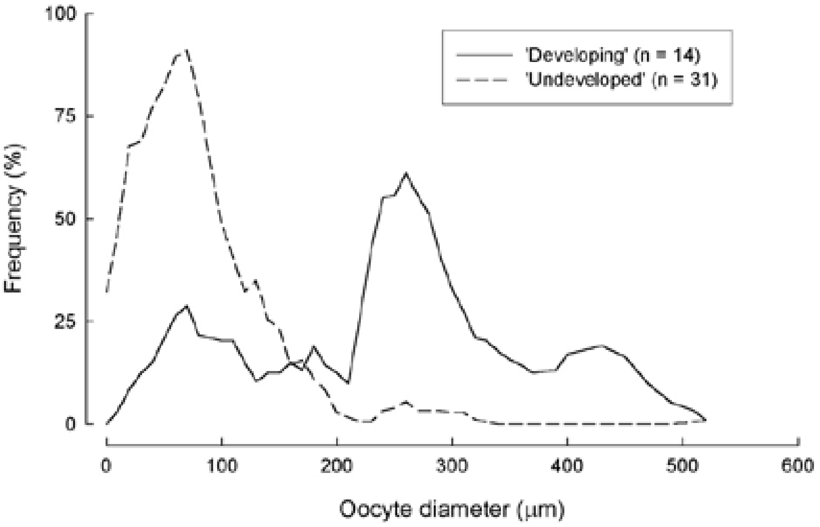

Figure 2. Oocyte diameters of *Panulirus cygnus* ovaries that were classified as 'developing' (yellow/orange) or 'undeveloped' (white/translucent) when viewed through the dorso-thoracic musculature.

Further histological examination showed that the ovaries of all females in our samples that had recently spawned (*i.e.* those that had early phase external ova) and that had their ovaries externally classified as 'developing', contained immature oocytes and remnant mature oocytes (late granular) undergoing atresia. Therefore, these ovaries were clearly not developing. Moreover, the ovary walls of all ovigerous *P. cygnus* in our samples were 2 to 8 times thicker than those of females that had not yet extruded their ova. A very thick ovary wall, which can remain thick until the ovary begins to mature a second time, can add a yellow tinge to the exterior of the ovary. As a result, these ovaries appear similar to 'developing' ovaries, which increases the likelihood of an externally assessed just spawned ovary being misclassified as 'developing'.

3.4.2 Assessment of reproductive stage

Although the visual assessment of an ovary's stage of development can be misleading, when this technique is combined with other external observations during the peak of the breeding season, *i.e.* when the maximum proportion of females are ovigerous, the results can more accurately determine the reproductive stage of a female *P. cygnus*. For example, the presence of an eroded spermatophore mass and early phase external ova suggest that an ovary classified as 'developing' is in fact undergoing atresia after histological examination. Under the criteria which are currently in use in this fishery these lobsters would have been designated as a double breeder, whereas, with the correct classification of it's ovary now known, it has been reclassified as a 'single breeder'.

The modified combinations of the external characteristics and their resultant reproductive classifications of an individual have been simplified by way of a flow diagram (Fig. 3) to enable researchers to easily and quickly classify a female *P. cygnus* as either 'immature', an 'inactive breeder', 'single breeder' or 'double breeder'.

3.4.3 Comparison between the classifications of single and double breeders using two different sets of criteria

During the 2002 survey, 8,541 female *P. cygnus* were collected and a record made of the condition of their setae, spermatophore mass, external ova and the stage of development of their ovaries (the last assessed through the dorso-thoracic musculature). From these data the lobsters were sorted twice, based on two sets of criteria, into immature, inactive, single or double breeders. The first sorting of lobsters was based on the modified set of criteria (Fig. 3) while the second was based on the unmodified criteria currently in use in the fishery.

Combinations of external characteristics that would result in a lobster being classified as a double breeder by the modified criteria and a single breeder by the unmodified criteria are either: an eroded spermatophore mass with a 'developing' ovary and no external eggs, or an unused spermatophore mass with external eggs and an 'undeveloped' ovary. A lobster would be classified as a single breeder by the modified criteria and a double breeder by the unmodified criteria if it had an eroded spermatophore mass with external eggs and a 'developing' ovary.

Although in many cases both sets of criteria classified each lobster as either a single or double breeder, 9.1 and 44.5% of lobsters classified by the unmodified criteria as single and double breeders, respectively, were classified by the modified criteria as double and single breeders, respectively.

Furthermore, comparisons between the proportions of female *P. cygnus* at six different sites that were classified as double breeders by both sets of criteria during the 2002 survey, showed that at five of the six sites, the unmodified criteria classified between 2 and 32% more lobsters as double breeders in that year than did the modified criteria (Fig. 4a). The modified criteria classified 4% more double breeders than the unmodified technique at Kalbarri (Fig. 4a). The main difference in the proportions classified as single/double breeders at the different sites, was due to the far greater proportions of small lobsters classified as double breeders by the unmodified criteria. For example, in the samples collected from Fremantle, where the females had the largest size range, both sets of criteria classified similar proportions of lobsters above 110 mm *CL* as double breeders. However the unmodified criteria classified a far greater proportion of the lobsters below 95 mm *CL* as double breeders than did the modified criteria (Fig. 4b).

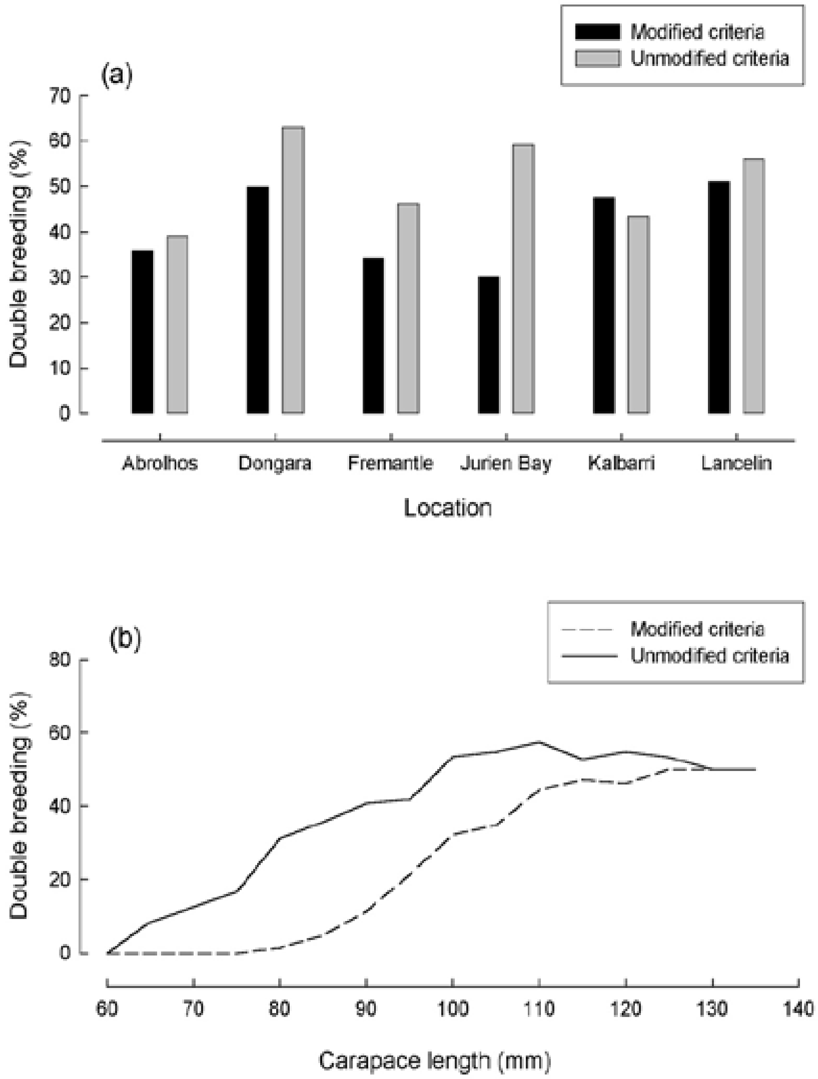

Figure 4. Comparison between the proportions of *Panulirus cygnus* classified as double breeders (a) at six locations and (b) in different carapace length classes collected at Fremantle, determined from modified criteria (see Fig. 3) and the unmodified criteria (see Chubb *et al.*, 1991). Carapace-length classes have been smoothed with a three-point moving average.

3.5 Discussion and Conclusions

The histological study confirmed that external features can be employed to classify female *P. cygnus* as either a single or double breeder (as well as an immature or inactive breeder), whether one uses modified or unmodified criteria. However, the visual ovarian assessment, is not, on its own, always reliable and when incorporated with other external characteristics in the format described by Chubb *et al.* (1989) has the potential to misclassify single and double breeders. Therefore, by identifying histologically the cases in which the ovary can be misclassified and modifying the relationships between the various criteria to account for this, this study has substantially improved the assessment of reproductive state without needing to sample each ‘doubtful’ ovary histologically.

The criteria used for assigning females into a particular reproductive state, are strongly influenced by the sampling period within the breeding season. In the early stages of the breeding season prior to egg extrusion, it is not possible to predict whether a female will carry more than one brood; neither is it possible to establish late in the season, whether a female carrying eggs but with criteria assigning it to be a single breeder, might have carried an earlier brood. The method described is therefore only effective during a period when the maximum proportion of the breeding females are ovigerous, *i.e.* the peak of the breeding season, and even then, cannot, by itself, be used as a definitive indicator of an animal’s recent spawning history or future spawning potential.

Newly matured females of spiny lobsters that repetitively spawn during a single season produce fewer broods than their larger conspecifics (Chubb, 2000), with newly matured *P. cygnus*, *P. longipes longipes* and *P. guttatus* all producing only one batch of eggs during a spawning season (Chubb *et al.*, 1994; Gomez and Bermas, 1994; Sharp *et al.*, 1997). Thus, the fact the criteria currently in use in this fishery classified a large proportion of small females, *i.e.* 18 % of those below the size at first maturity at Fremantle, as double breeders, indicates that this set of criteria is probably misclassifying a significant proportion of these lobsters. The modified criteria however did not classify any lobster below the size at maturity as double breeders, suggesting that, at least for smaller females, that the modified criteria produce more realistic results. The proportion of double breeders spawning by size is important when determining egg production indices, because the generally larger size of these animals makes the contribution of each brood substantial compared to the smaller single spawning females.

Because there is a short period between the extrusion of ova by breeding females and the redevelopment of external criteria indicating a presumptive second spawning (*i.e.* an unused spermatophore mass and developing ovaries) it is likely that the modified criteria will slightly underestimate the proportion of double breeding female *P. cygnus*. However, since MacFarlane and Moore (1986) reported that mating occurred shortly after ovulation in *P. ornatus*, and given that most of the females sampled in the survey that had early phase I external ova (< 3 weeks old) also had unused spermatophoric masses, it appears likely that only a few females would have been sampled between ovulation and mating.

3.6 Acknowledgements

We thank the skipper and crew of the *RV Naturaliste* and technical assistants in the Western Australian Department of Fishery for their assistance during the annual fishery-independent breeding stock survey when the animals used in this study were obtained. We also thank Mr Gordon Thomson from Murdoch University’s Centre of Fish and Fisheries Research, for processing the histological material. Funding was provided by the Australian Fisheries Research and Development Corporation.

4.0 Spatial and temporal variation in the size at maturity of the western rock lobster *Panulirus cygnus* George

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Citation: Melville-Smith, R. and de Lestang, S. 2006. Spatial and temporal variation in the size at maturity of the western rock lobster *Panulirus cygnus* George. Marine Biology. 150 (2), 183-195.

4.1 Abstract

The sizes at which female and male western rock lobster *Panulirus cygnus* become mature were examined over 32 years from records at six localities along the coast of Western Australia. The size of males at maturity was estimated from a subset of these data by a morphometric and a physiological method, with both producing statistically similar results. Males were larger at first maturity than females at the same sites. For both sexes, the sizes at first maturity at each location correlated ($P<0.05$) with the mean annual water temperature at that location, decreasing from south to north along the mainland coast and being smallest at the offshore Abrolhos Islands. Smaller sizes at maturity were recorded for both sexes than have been published previously. One certain explanation for these differences is that management measures protecting females with ovigerous setae, have distorted size compositions and the ratio of immature to mature females, thereby increasing the likelihood of capturing small mature females. However, these fishing effects cannot fully account for the progressive decline in CL_{50} observed over the past 20 years. Other possible hypotheses considered include increases in water temperature over this period, as well as whether this change could be consistent with a genotypic response caused by the selective removal of large lobsters combined with high exploitation rates.

Keywords: *Panulirus cygnus*; maturity; temperature; selection, exploitation rate

4.2 Introduction

The western rock lobster *Panulirus cygnus* George, which is endemic to Western Australia, forms the basis of a very significant fishery, worth about AUD$300 million annually. The legal minimum size (76 mm carapace length) of this valuable fishery has been shown by Chittleborough (1976), Grey (1979) and Chubb (1991), to be well below the size at first maturity in most regions of the fishery. As the western rock lobster is therefore vulnerable to recruitment over-fishing, management has focused much attention, particularly in recent years, on monitoring the brood stock to ensure it can sustain the fishing effort (Caputi et al., 1995a; Melville-Smith et al., 1998; Hall and Chubb, 2001).

Management measures have been introduced over the years to directly, or indirectly, protect the brood stock and enhance egg production. Some of these have relevance to this study, in particular the requirement that the number of escape gaps per pot be increased from one to three in 1986, to better allow undersize animals to escape. In the 1992-93 season, a regulation was introduced requiring fishers to release setose lobsters (females with ovigerous setae). Reproductively mature western rock lobsters generally have ovigerous setae from June to February/March, with most moulting into a non-setose condition between March and June each year (de Lestang and Melville-Smith, in press). Since the fishing season extends from November 15 to June 30, this regulation effectively protects most breeding females from exploitation in the first, but not the second half of the fishing season.

The state of egg production in the fishery is monitored using both fishery dependent, and fishery independent data (Caputi et al., 1995a; Melville-Smith et al., 1998). Egg production indices, expressed as number of eggs per pot lift, are estimated for each locality for which there are either fishery dependent or fishery independent data available, using a female size-fecundity within a season, relationship (Chubb, 1991). A key assumption in these models is that only females above the size at maturity contribute to egg production and more importantly, that this size has remained unchanged over time.

It is well known that size at maturity in decapods can vary significantly, both spatially and temporally, with water temperature, population density and habitat (e.g. Beyers and Goosen, 1987; Aiken and Waddy, 1989; Chubb, 1991; Arango and Marquez, 1995; Bianchini et al*.,* 1998; de Lestang et al*.,* 2003; Goni et al*.,* 2003). Previous work on *P. cygnus* has reported considerable spatial variation in the size at first maturity of both females and males, with both sexes maturing at a larger size in the southern region of the coastal distribution and, in the case of the females, at a smaller size in the offshore waters of the Abrolhos Islands (Chittleborough, 1976; Grey, 1979; Chubb, 1991). No attempt been made to examine temporal variations in the size at maturity of this species.

The aim of this study was twofold. Firstly, to produce consistent and accurate estimates of the sizes at which both female and male *P. cygnus* attain sexual maturity at six locations along about 600 km of the West Australian coast, a region that spans the main geographical distribution of the stock. Previous work either did not record size-at-maturity for males (Chittleborough, 1976; Chubb, 1991) or was geographically less widespread in its coverage (Grey, 1979). Secondly, to determine whether there have been changes in female size-at-maturity over time. Factors that may be responsible for variations in size at maturity, either spatially or temporally, are also discussed.

4.3 Methods

4.3.1 Sampling regime

Data used to determine the size at maturity of female and male *Panulirus cygnus* on the west coast of Australia was collected during two monitoring programmes: (i) the fishery-independent breeding stock survey (IBSS), which has been conducted annually at three localities (Lancelin, Dongara and Abrolhos Islands), and intermittently at three others (Fremantle, Jurien and Kalbarri) since 1992, and (ii) the fishery-dependent commercial catch monitoring survey (DCCM), which has been conducted annually at four localities (Fremantle, Lancelin, Jurien Bay and Dongara) since 1972 and at two others (the Abrolhos Islands and Kalbarri) since 1985 (for locations see Fig. 1).

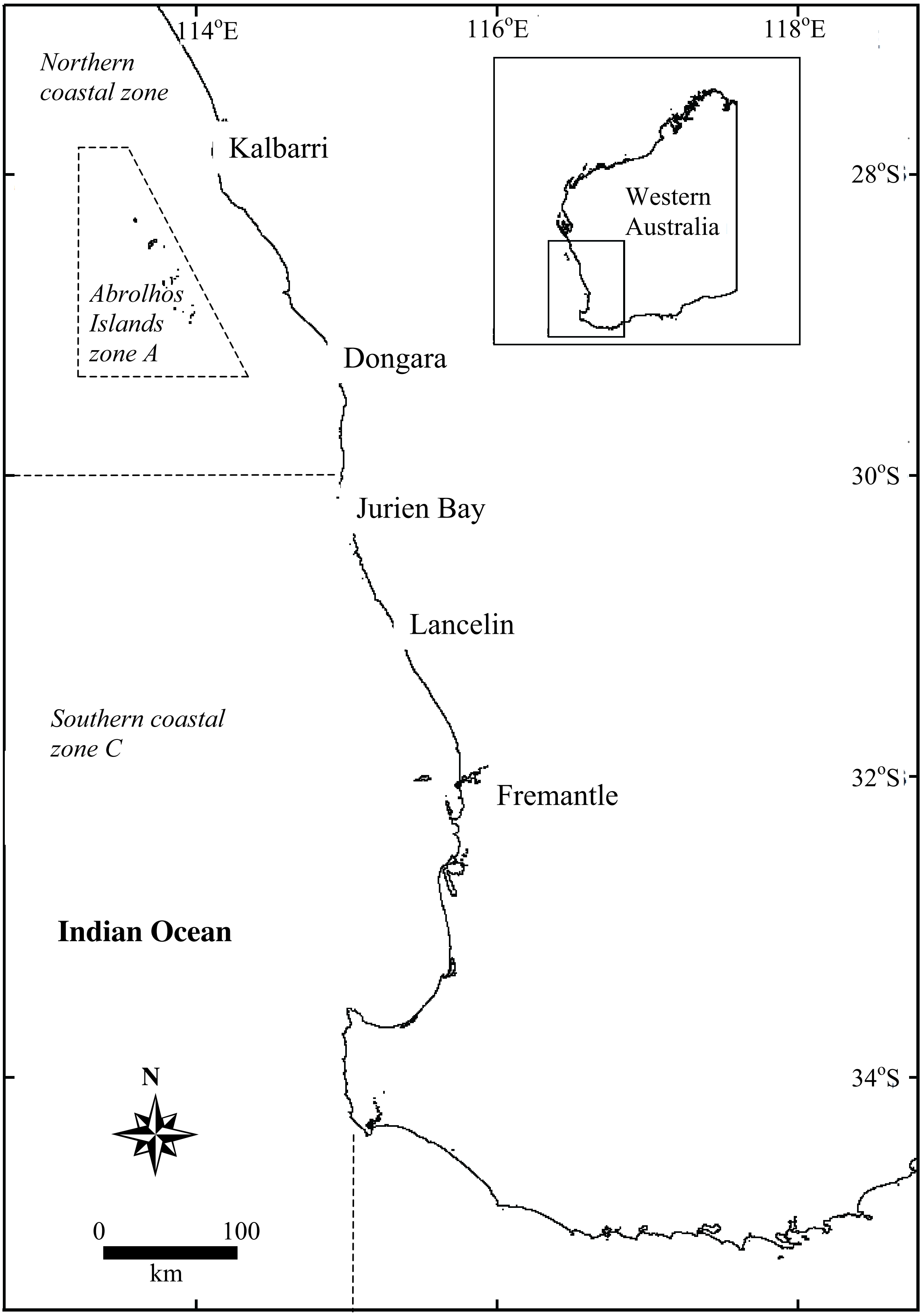

Figure 1. Locations at which the size-at-maturity data for western rock lobsters were collected.

The IBSS is undertaken over 10 days each year during the last new moon before the commercial lobster-fishing season starts on 15 November, which is close to the annual peak of egg-bearing (Chubb, 1991). The water depths surveyed range from 25 to 70 m at the five coastal locations and from 10 to 60 m at the Abrolhos Islands site and cover the depths at which the majority of breeding lobsters are found (Chubb, 1991). Since this survey was designed to be repeatable, with each survey using the same fishing gear (160 standard commercial-style pots, with wooden slats, a metal base and closed escape gaps, 80 of which are pulled every second day), bait (a combination of north sea herring and Australian salmon), and sites (same GPS coordinates), the results from each survey are directly comparable between years. This data set has therefore been used for most of the temporal and spatial comparisons in this study. For more details of the sampling regime see Chubb (2000).

The DCCM, which is conducted by research staff on board commercial vessels, is made each month during the fishing season (November – June) in a range of fishing depths and results in over 8000 pots being sampled annually; however, only data collected from water depths that contain predominately breeding *P. cygnus* (35 to 70 m) have been used in this study. Since this survey provides data representative of the commercial catch, it is impacted as a result of management changes and improvements in fishing technology, and some of the data are not directly comparable across years. We have thus limited the use of this data. For more details of the sampling regime see Caputi et al. (2000).

4.3.2 Measurements

In both surveys, the carapace length (CL) of each lobster (from the anterior edge of its carapace between the preorbital spines down the mid-dorsal line to the posterior edge of its carapace) was measured to the nearest 1 mm. For females, the presence and developmental stage of external ova attached to the endopodites and/or the presence of a spermatophoric mass attached to the fifth abdominal segment were also recorded (Melville-Smith and de Lestang, 2005). The developmental stage of female ovaries, as viewed through the dorso-thoracic musculature, was recorded during only the IBSS (Melville-Smith and de Lestang, 2005).

Additional data were collected during the 2002 IBSS: the length of the merus of the first and second pereiopod on the right hand side of at least 100 males (measured to the nearest 0.1 mm) from each of the six locations, and the weight of the vas deferentia from a wide size range of at least 50 males at each of the Abrolhos Islands, Dongara and Lancelin sites (measured to the nearest 0.01 g). Since the relationship between the length of the merus of the second pereiopod (SPL) and the carapace length (CL) showed the greatest change within the size range of animals examined, this structure was chosen for all subsequent allometric analyses to determine the morphometric size at which those lobster were undergoing a pubertal moult (Somerton, 1980).

Water temperatures (˚C) at each location in depths, which contain the majority of breeding females (35-70 m), were collected with a protected reversing thermometer during the DCCM. The mean water temperature, standardised for depth, month and year using ANCOVA, was then determined for each sampling site.

4.3.3 Assignment of maturity and analysis of data

Female *P. cygnus* were considered mature using both DCCM and IBSS data, if they had a spermatophore or an egg mass (Melville-Smith and de Lestang, 2005).

At each location each year of the IBSS, the proportion of female *P. cygnus* that were considered mature was examined by logistic regression to determine the size at which 50% of female lobster (CL_{50}) were mature. The data for each assemblage in each year were analysed by a nonlinear regression routine using the statistical package SPSS 11.5.0^{TM}, with the standard errors being derived from 100 bootstrap estimates. The equation used was

$$P_i = 1/[1+\exp(-\ln(19)\times(CL_i - CL_{50})/(CL_{95} - CL_{50}))]$$

where P_i is the proportion mature at CL_i, and CL_{50} and CL_{95} are the CLs at which 50 and 95% of the assemblage is mature, respectively.

Male *P. cygnus* were considered morphometrically mature (i.e. possessing morphometric characteristics distinct from those of immature individuals) on the basis of changes in the relationship between the natural logarithms of the length of the merus of the second pereiopod (SPL) and the CL as determined by log-log regression analysis (Somerton, 1980). Physiological maturity (i.e. possession of developed gonads) of male *P. cygnus* was assigned on the basis of a change in the relationship between the natural logarithm of the weight of the vas deferentia and the CL as determined by log-log regression analysis (Goni et al*.,* 2003). The above morphometric and physiological data sets were all better described by two rather than one regression line (Somerton, 1980).

At each location, the percentage of male *P. cygnus* with carapace lengths that were considered morphometrically or physiologically mature, was examined by logistic regression to determine the CL_{50}s for these two maturity assignments. The logistic regressions relating maturity and carapace length for either female or male *P. cygnus* in the different assemblages were compared using a likelihood ratio test, as described by Cerrato (1990) and using a Bonferroni correction.

Linear regression analyses of data from each location were made between size-at-maturity estimates for female and male *P. cygnus* and standardised mean annual water temperatures (10 year average from 1993 to 2003).

The mean CL of the smallest 10% of mature females sampled each year at Fremantle, Lancelin, Jurien Bay and Dongara by the DCCM between 1972 and 2003 and by the IBSS at the same localities since the early 1990s, was used to investigate possible long term changes in the size at first maturity (Jones and Simons, 1983). This measure was chosen as it was considered to provide a relatively unbiased indication of whether the size at onset of maturity of female *P. cygnus* has changed over the past three decades: traditional methods for determining size at maturity rely on ratios of immature to mature animals that, in this fishery, may be biased as current management arrangements allow only the taking of immature females. The mean size of the 10% smallest mature females is not a ratio and would thus not be biased by this fishing practice.

4.4 Results

4.4.1 Female maturity

The smallest mature female *P. cygnus* caught at each of the six locations sampled during the 2002 IBSS ranged from 36.7 mm at the Abrolhos Islands to 69.5 mm at Kalbarri. The size at which 50% of females were mature (CL_{50}) generally decreased from south to north along the coast, beginning with 87.5 mm at Fremantle, 82.2 mm at Lancelin, 81.4 mm at Jurien Bay, 77.2 at Kalbarri, 74.9 mm at Dongara, and ending with 65.0 mm at the Abrolhos Islands (Fig. 2). Lancelin and Jurien Bay were the only two locations for which the CL_{50}s did not differ significantly from each other (*P*>0.05).

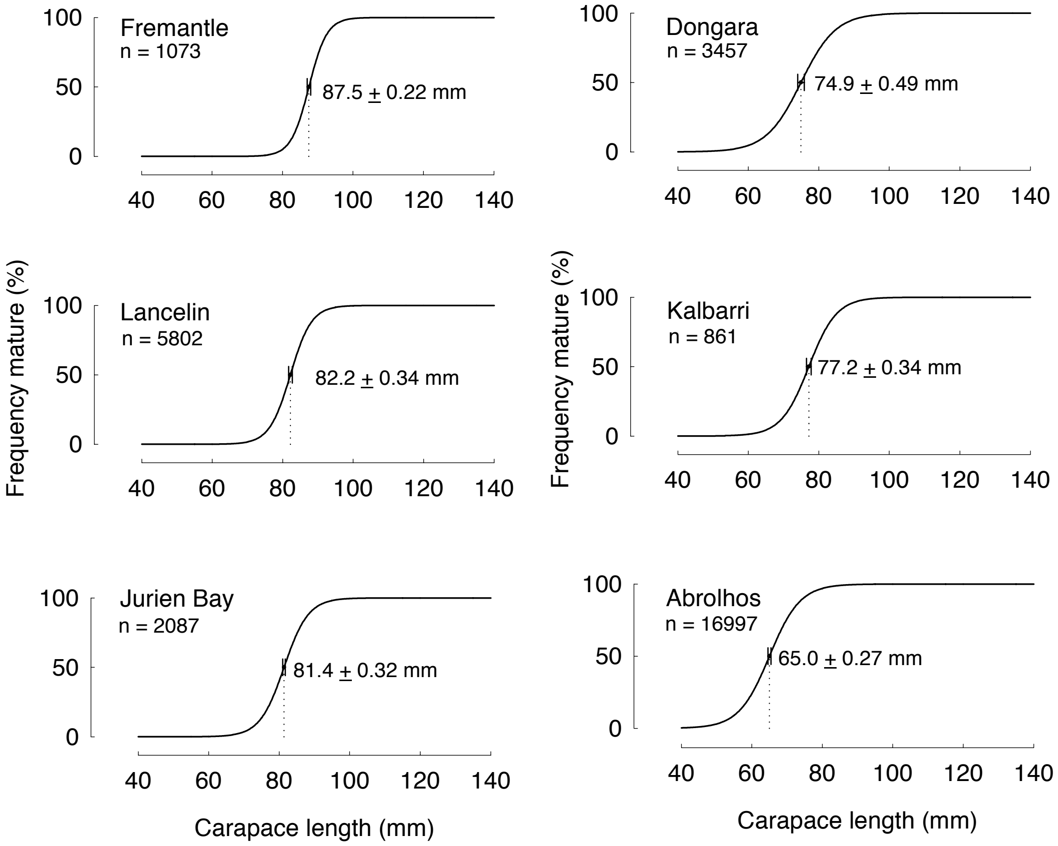

Figure 2. Logistic regressions fitted to the percentage of mature female *Panulirus cygnus* at different carapace lengths in six locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. CL_{50} ± 1 SE denotes the size at which 50% of the assemblage is mature and n the sample size.

4.4.2 Male maturity (Morphometric)

The smallest morphometrically mature male, based on log-log regressions at each location during the 2002 IBSS, ranged from 58.3 mm at the Abrolhos Islands to 83.7 mm at Kalbarri. The CL_{50}s determined for male *P. cygnus* by morphometric data showed a similar south to north trend to that of the females (Fig. 3). With a $P>0.05$, the morphometric CL_{50}s at Fremantle (95.3 mm) and Lancelin (92.5 mm) did not differ significantly from each other, but were both significantly larger ($P<0.05$) than that for Kalbarri (90.3 mm), which was in turn significantly larger ($P<0.05$) than the CL_{50}s recorded for Jurien Bay (85.1 mm) and Dongara (84.6 mm) (which did not differ significantly ($P>0.05$) from each other). The morphometric CL_{50} recorded for male *P. cygnus* in the Abrolhos Islands (72.2 mm) was significantly smaller ($P<0.05$) than those at all five coastal sites.

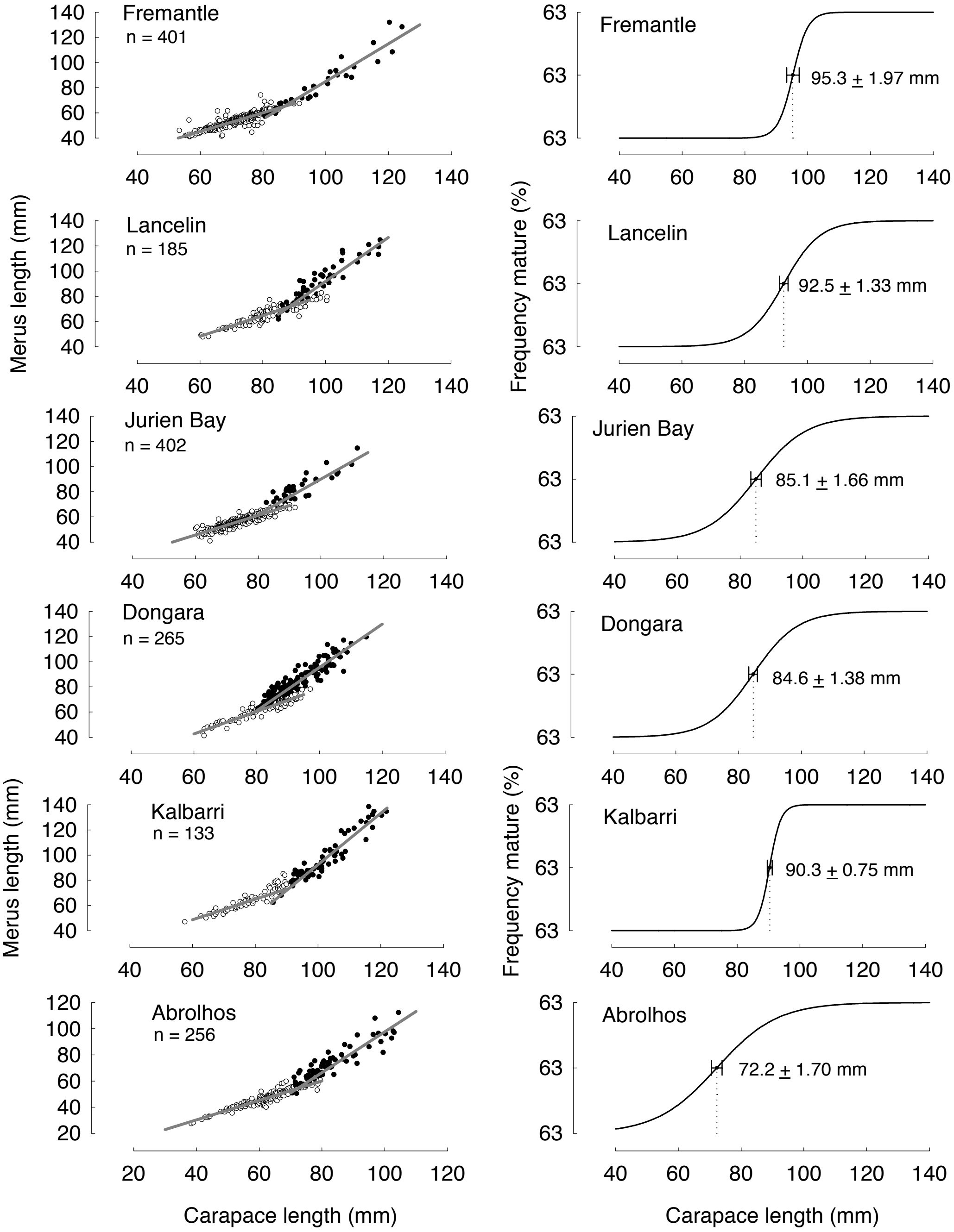

Figure 3. Relationship between the merus of the second pereiopod and carapace length of immature (○) and mature (●) male *Panulirus cygnus* (left) and logistic regressions fitted to the percentage of morphometrically mature males at different carapace lengths (right) in six locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. CL_{50} ± 1 SE denotes the size at which 50% of the assemblage is mature and n the sample size.

4.4.3 Male maturity (Physiological)

The physiological maturity of male *P. cygnus* in the 2002 IBSS was determined based on the log-log regressions for only three locations due to sampling constraints. The smallest mature individuals ranged from 42.7 mm at the Abrolhos Islands to 64.5 mm at Lancelin. The corresponding CL_{50} at the Abrolhos Islands (70.2 mm) was significantly smaller ($P<0.05$) than that recorded at Dongara (85.3 mm), which was in turn significantly smaller ($P<0.05$) than the CL_{50} at Lancelin, (92.9 mm) (Fig. 4). Since the physiological CL_{50}s for male *P. cygnus* at each of the three locations did not differ significantly ($P>0.05$) from the corresponding morphometric CL_{50} determined for that locality, all further analysis has used the CL_{50}s derived from morphometric analysis, as these data were available for all six locations.

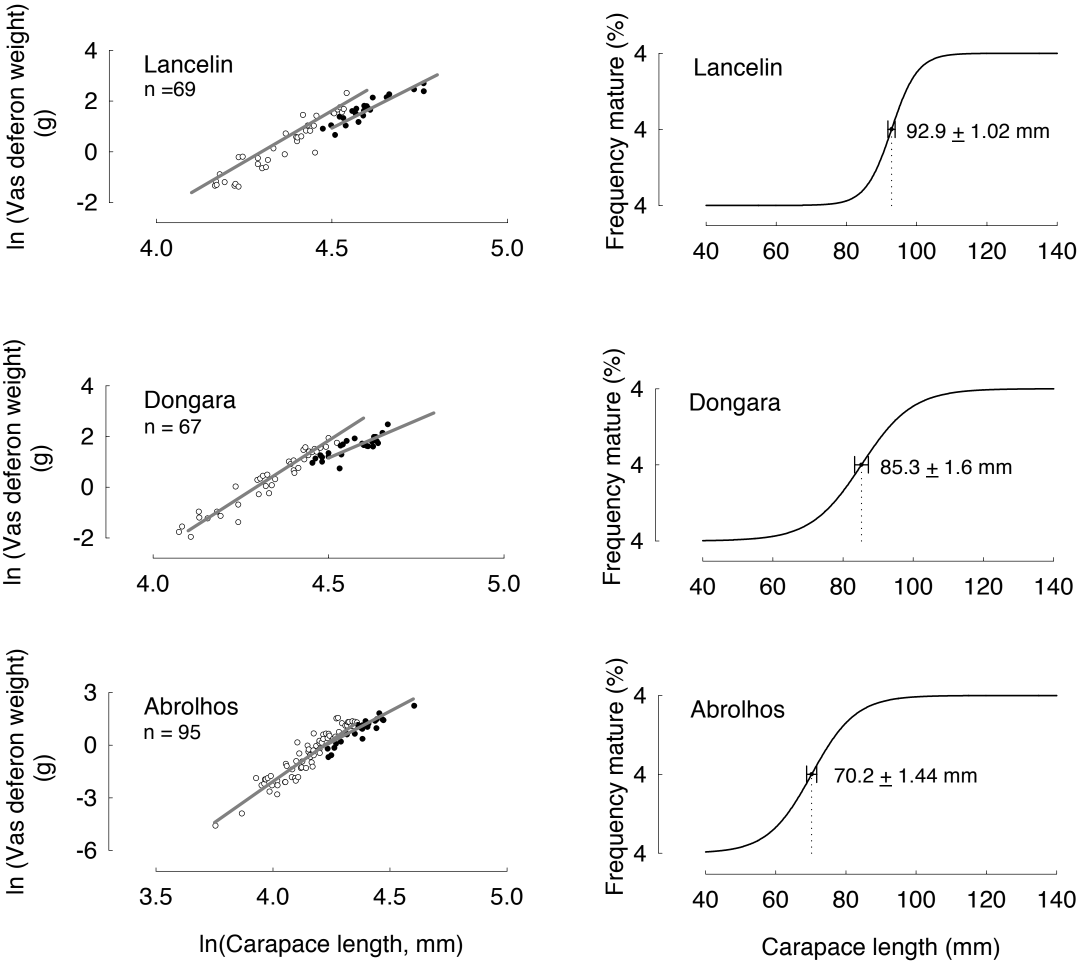

Figure 4. Relationship between the natural logarithms of the vas deferentia weight and carapace length of immature (○) and mature (●) male *Panulirus cygnus* (left) and logistic regressions fitted to the percentage of physiologically mature males at different carapace lengths (right) in three locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. CL_{50} ± 1 SE denotes the size at which 50% of the assemblage is mature and n the sample size.

4.4.4 Spatial variation in size-at-maturity

Although the CL_{50}s determined for male *P. cygnus* followed the same trend from location to location (declining in a northward direction up the coast from Fremantle to Kalbarri and then declining further at the Abrolhos Islands), the male CL_{50} was always 5 to 15 mm larger than the corresponding female CL_{50} at the same location (Fig. 5a).

Standardised mean (ten-year) water temperatures at each of the six locations had a negative relationship with female and male CL_{50}s: temperatures progressively increased from 20.9°C at Fremantle in the south to 21.6°C at Kalbarri in the north and then to 23.0°C off the coast at the Abrolhos Islands. There was a significant correlation between the CL_{50}s for both female and male *P. cygnus* and mean water temperature: $Female\mathrm{CL}_{50} = -10.75\times{}^{\circ}\mathrm{C}+311.64$ ($P<0.01$) and $Male\mathrm{CL}_{50} = -11.42\times{}^{\circ}\mathrm{C}+334$ ($P<0.01$), with the correlation coefficient for females (0.93) being the same as that for males (0.93) (Fig. 5b).

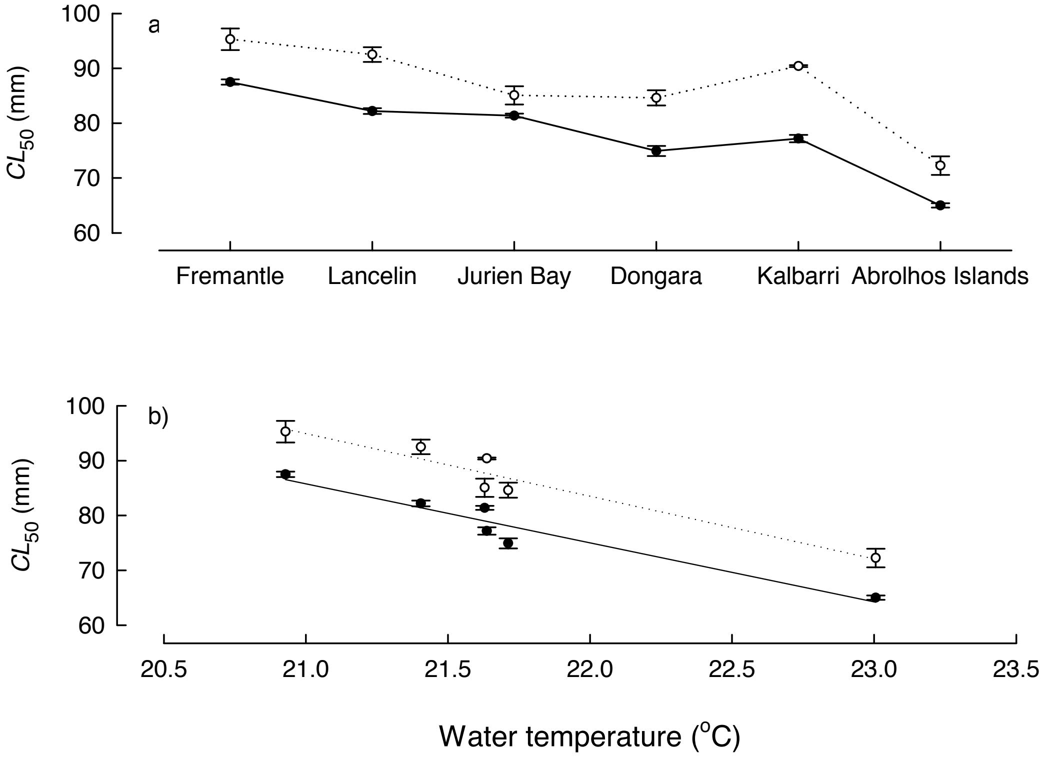

Figure 5. **(a)** Size at maturity (CL_{50}) ± 1 SE of female (●) and male (○) Panulirus cygnus at six locations and **(b)** linear regressions fitted to the relationships between female (●) or male (○) CL_{50}s at each location and the corresponding mean annual water temperature at that location.

4.4.5 Temporal variation in the size at maturity

Since CL_{50}s could be estimated for female *P. cygnus* from data collected during the IBSS at all six locations for most years between 1992 and 2005, these data were used to investigate whether size at maturity had changed significantly over the last fourteen years (Fig. 6). At four coastal locations (Fremantle, Jurien Bay, Dongara and Kalbarri), female CL_{50}s all started out high in 1992 and 1993, then declined in 1994 to lower values thereafter (Fig. 6). The CL_{50}s determined for female *P. cygnus* at the Abrolhos Islands remained at or close to the same value from 1991 through 2005.

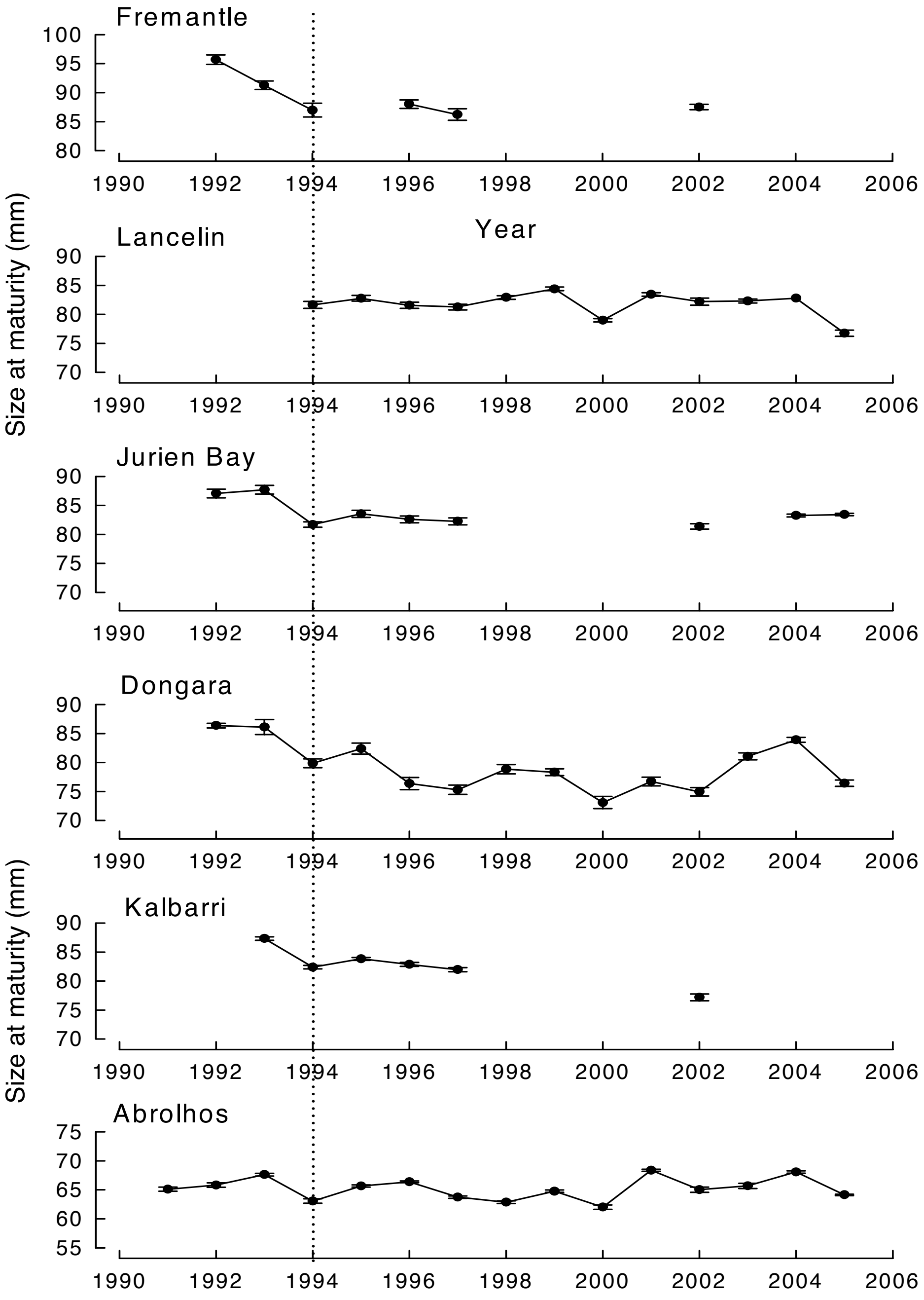

Figure 6. Size at maturity (CL_{50}) ± 1 SE of female *Panulirus cygnus* at six locations from the early 1990s to mid-2000s, based on data collected during the Independent Breeding Stock Surveys. Vertical dotted line represents 1994.

4.4.6 Temporal variation in size of the tenth percentile of mature females

During DCCM the mean CLs of the smallest 10% of mature females caught at Fremantle, Lancelin, Jurien Bay and Dongara in most years from 1972 to 2005 followed similar temporal trends: a progressive decline until the mean CL approached a CL range at or above the legal minimum size (76 mm), at which stage the decline became less marked. For example, in Dongara the mean CL of the smallest 10% of mature females caught each year declined from 82.0 mm in 1972 to 76.5 mm in 1981, before levelling out and remaining at or near this level through until 2005 (Fig. 7). The declining slopes, as determined by linear regression, of the progressive decline in mean CLs recorded in the years before the introduction into the fishery of three escape gaps (1986), did not differ significantly between locations ($P > 0.05$).

During the IBSS from 1992 to 2005, the mean CLs of the 10% smallest mature female *P. cygnus* caught each year in Fremantle, Lancelin, Jurien and Dongara followed the same downward trend as that displayed by the DCCM in the same years with the slope from the two datasets not differing significantly at all four locations ($P > 0.05$) (Fig. 7).

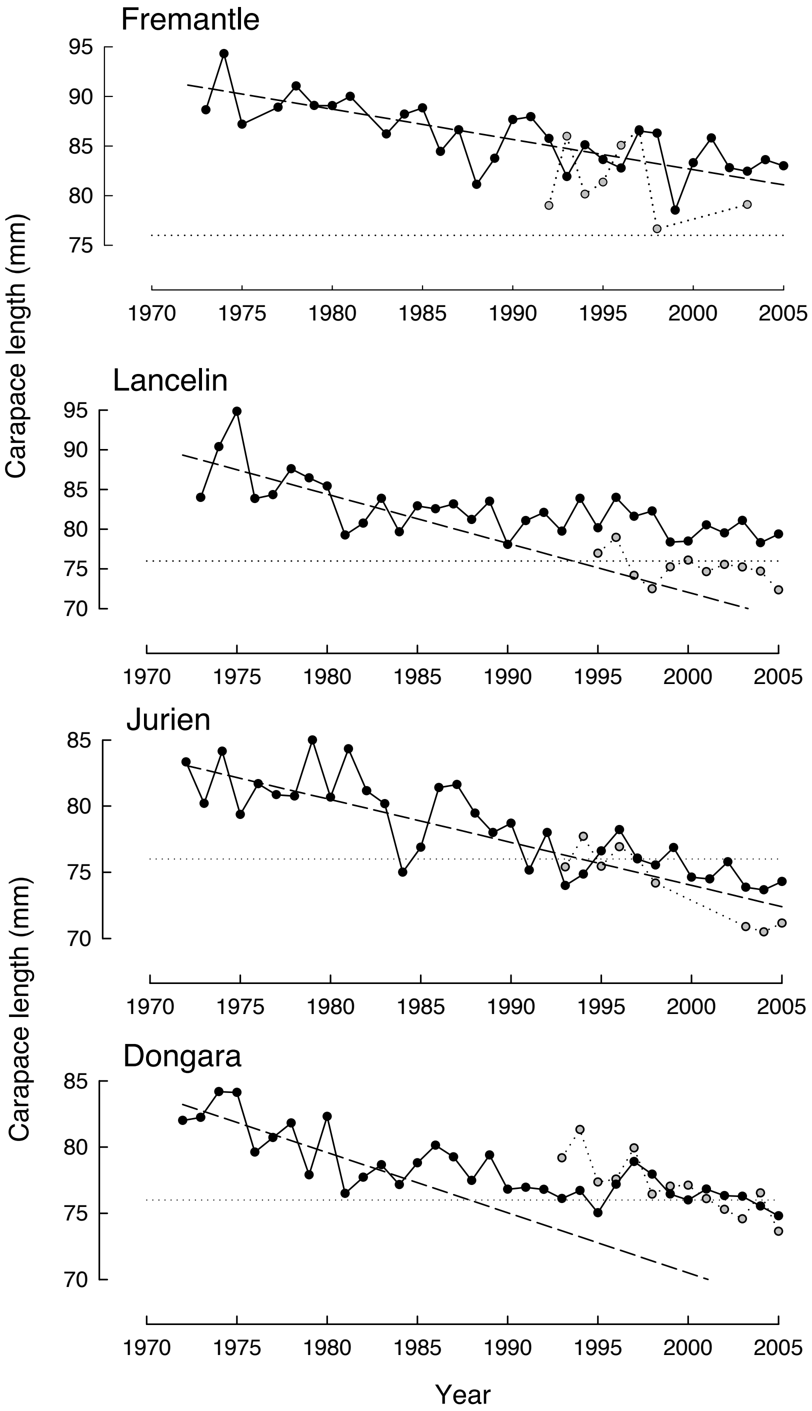

Figure 7. Mean carapace lengths of the smallest 10% of mature female *Panulirus cygnus* caught in each year at four locations from 1972 to 2005 based on data collected during Fishery Dependent Commercial Catch Monitoring (●) and from 1992 to 2005 based on data collected during Fishery Independent Breeding Stock Surveys (○). Horizontal dotted line represents 76 mm and dashed line represents a linear regression between mean carapace length and the years before 1986.

4.5 Discussion

4.5.1 Male size at maturity

Within each site, the size at which male *Panulirus cygnus* attained both morphological and physiological maturity were similar, indicating that either method can determine the potential size at male maturity in this species. However, being morphologically and physiologically capable of mating does not necessarily mean a male will mate successfully in the wild. Mating success also depends on the size composition of lobsters on a reef, as large males can out-compete small males for a mate (Fielder, 1965; Berry, 1970; MacDiarmid, 1989). Consequently, the size at which a male is functionally mature (will actually mate) can vary markedly across very small spatial scales, making large fishery-wide estimates impractical. Male functional maturity can be assessed on small spatial scales by determining which males are responsible for mating through DNA paternity assignment testing. To be totally successful, this approach does require that large numbers of males and females within the population be sampled. The method is currently being pursued in the western rock lobster fishery (Ireland and Melville-Smith, 2005). At the six locations sampled along the west coast of Australia, male *P. cygnus* always matured at a larger size than females in the same location (as Grey (1979) found at Fremantle and Geraldton). The larger CL_{50} for males is probably attributable to males growing faster than females, even before they mature in this species (Melville-Smith et al*.,* 1997) and other palinurids (Berry, 1970; Cockcroft and Goosen, 1995).

4.5.2 Spatial variation in the size at maturity

The CL_{50}s for both female and male *P. cygnus* varied between the sampled locations, generally decreasing in size with increasing latitude along the coast with the smallest CL_{50} at the offshore Abrolhos Islands. This trend was also recorded for either one or both sexes by Chittleborough (1976), Grey (1979) and Chubb (1991). Chittleborough (1976) attempted to relate spatial variation in the size at female maturity to differences in lobster density and mean annual water temperatures at the localities sampled. He described a relationship between CL_{50} and lobster density that was largely anecdotal and was not supported statistically, but in contrast with results from this study he showed no correlation between CL_{50} and water temperature. His inability to show this relationship was probably because the water temperature data he used were averaged from large areas (at least 10, 000 km^2) and were thus too coarse to provide sufficient contrast in temperature between sampling locations.

Inverse relationships between water temperature and CL_{50} have previously been recorded for other lobsters (Templeman, 1936; Sutcliffe, 1952; Street, 1969; Davis, 1975; Annala, 1980; Landers et al., 2001). Furthermore, temperature has been shown to directly increase growth rates of spiny lobsters by shortening intermoult periods, while not affecting moult increments (Oshima, 1941; Herrnkind et al., 1994; Dennis et al., 1997). It is therefore likely that lobsters maturing at smaller sizes in warmer waters are also maturing at a younger age. Aquaria studies (Johnston et al., unpub. data) conducted on *P. cygnus* at the Western Australian Marine Research Laboratories support this hypothesis. Two-year post-settlement animals held for a year at 23°C, matured precociously compared to controls held at ambient temperatures (14-24°C), despite food, habitat and density being constant in both treatments.

4.5.3 Temporal variation in the size at maturity

Despite being based on similar criteria and sampling regimes, all previous estimates of CL_{50}s for both female and male *P. cygnus* in Western Australia, were higher than the values recorded in this study for corresponding regions (Fremantle, Dongara and the Abrolhos Islands). For example, mean CL_{50}s of 95.8, 97.0 and 97.0 mm CL were recorded for females off Fremantle by Chittlebrough (1976), Grey (1979) and Chubb (1991). By comparison, in the same locality, we recorded a CL_{50} of 87.5 mm for 2002, which is a reduction in carapace length of about 10% and of about 25% in weight. The far smaller CL_{50} recorded in our study is due, in part, to the introduction of a regulation during the 1992/93 season that required rock lobster fishers to return all females in a "setose" condition to the water (Caputi et al., 2000). By having to return only setose females (which are likely to spawn that year), there has been an increase in any given size class of the proportions of females likely to spawn. This resulted in an apparent decline in the CL_{50} between 1993 and 1994 (see Figure 6). This decline was, however, not as apparent in the catches from the Abrolhos Islands, as the CL_{50} of female lobster at this location has always been below that of the minimum legal size and is thus not as susceptible to this bias.

Although the CL_{50}s estimated at each of the six locations for both female and male *P. cygnus*, based on data collected during the 2002 IBSS, do not accurately represent the size at which 50% of lobsters will mate in a natural situation, they do accurately describe the size at which 50% of the modified assemblages within the areas where spawning occurs are capable of mating. Since it is these modified assemblages that fisheries based models predict, the CL_{50} estimates produced in this study are the appropriate measures on which to base management decisions.

As for females, the CL_{50}s recorded for male *P. cygnus* in this study were also smaller than the corresponding estimates for this sex in 1978 Grey (1979). As male CL_{50} estimates would not have been directly distorted by the "setose rule", there must also have been biological and/or environmental reasons for the decline in the CL_{50}s through the 1980s and 1990s. This decline over time has been further validated by analysis showing a consistent long-term decrease in mean CL of the 10% smallest mature females sampled by the DCCM up to the early 1990s, and by the IBSS since that programme's inception. Although using the smallest of mature females is a crude measure for comparing size at first maturity, it does have the advantage of not being derived from ratios of immature and mature animals and as such, not being influenced by the "setose rule".

The mean CLs of the 10% smallest females sampled during the DCCM were, in the majority of cases, larger than corresponding values determined from IBSS data. This difference is due mainly to the IBSS being conducted over the peak of the breeding season (October to November) at the point when the majority of all mature females are mated or egg bearing. The DCCM on the other hand does not begin until after the IBSS and covers the subsequent decline in breeding activity when small single breeding females are no longer reproductively active and can even have moulted into their non-reproductive phase, while large double-breeding females can still be brooding eggs (de Lestang and Melville-Smith, in press). In fact, even though the IBSS is conducted over the same new moon period prior to the start of the fishing season (15th November), it does not always align with the peak of the breeding season. This variation between the timing of the IBSS and that of peak spawning is one factor contributing to the oscillating CL_{50} estimates for the various sites each year since 1994 (Fig. 6).

A decrease in size at maturity has been recorded in at least part of the clawed lobster (*Homarus americanus*) population (Landers et al., 2001). Over the period of change reported in that study, there has been a concomitant increase in exploitation rate in the fishery, as well as a significant decrease in growth rate and increase in sea temperature. Landers et al. (2001) emphasize one of the positive aspects resulting from the change in size at maturity of *H. americanus,* has been an increased egg production contribution from sub legal-size females; they suggest this may explain why lobsters in their study area may have been so resilient in the face of intense exploitation. This research has shown that the assumption that there has been no change in size at maturity over time, which has been made in calculations of egg production indices for this fishery (Phillips and Melville-Smith, 2005), needs to be revised. Failure to take into account the steady decline in size at maturity will have biased more recent egg production indices downwards. For example, using the CL_{50}s calculated in this study to calculate egg production indices for the northern western rock lobster fishing zone in 2004 instead of CL_{50}s calculated in the 1980s, would result in the index increasing by around 25%, from 0.18 to 0.23 million eggs per pot lift. This has allowed the adoption of a precautionary approach to the management of the fishery, as the relative quality of eggs from smaller compared to larger females was unknown. A study to determine whether there is any difference in their quality is currently underway (Melville-Smith, unpub. data).

This study has shown that size at maturity varies markedly between locations and is therefore a plastic response to extraneous factors such as possibly density and water temperature. The modelled residual biomass of legal sized lobsters, and therefore by implication their density at the end of each season, declined progressively through the 1980s. Management changes in the 1993/94 season brought about a sharp reversal in this trend, that has since the late 1990s once again begun to track downward in the northern locations (Wright et al., 2006). Since these trends in residual biomass differ from the unidirectional trends displayed by mean carapace lengths of the smallest 10% of mature females, it is unlikely that density and size at maturity in *P. cygnus* are related. Mean water temperatures on the other hand have generally increased along the coast of Western Australia since the early 1970s (de Lestang, unpub. data). Thus the inverse relationship between CL_{50} and water temperature reported in this study may help to explain the temporal decline in CL_{50}s.

A general temperature increase, together with the effect of selecting for maturity states in the proportional way that size at maturity is calculated, may provide part of the explanation for the observed temporal changes in size at maturity. However, it is possible that size selective fishing pressures, may have also played a role.

A reduction in adult survival is predicted by life-history theory to select for earlier maturation and increased reproductive effort (Reznick et al., 1990). Depending on the size of individuals being selected and the degree of size-selection, populations have been shown to respond in directions opposite to the size bias of whatever caused them to be culled (Edley and Law, 1988; Reznick et al., 1990; Conover and Munch, 2002; Walsh, 2006).

The western rock lobster fishery operates at high exploitation rates (currently ~75%, Wright et al. 2006). This, combined with knife-edge selection at a legal minimum size that for most of the fishery has been below the size at maturity, may be starting to produce an evolutionary response to harvesting. Similar changes in size and/or age at maturity have been noted in other species (*Homarus americanus*, Landers et al., 2001; *Gadus morhua*, Yoneda and Wright, 2004; *Pleuronectes platessa*, Rijnsdorp, 1993;), but inferring causation from what may be viewed as apparent fitness related response characteristics are acknowledged as being difficult to interpret because of confounding variables (Reznick, 1985; Roff, 1992).

Regardless of the reasons responsible for the spatial and temporal differences in size at maturity outlined in this study, the changes may have potentially important consequences for the management of this fishery. One outcome might have been anticipated to be a reduction in the productivity of the fishery, given the earlier allocation of energy to reproductive development rather than somatic growth. However there has been no sign of any such change (Phillips and Melville-Smith, 2005). A more positive outcome is that overall egg production in the fishery will benefit when the results of this study are incorporated into egg production indices which are one of the key performance triggers in this fishery.

4.6 Acknowledgements

We thank the skipper and crew of the RV *Naturaliste* and technical assistants in the Western Australian Department of Fisheries for their work during the IBSS and DCCM, and Adrian Thomson for his help with manipulating much of the data used in this study. Danielle Johnston, Brett Malony, Nick Caputi, Jim Penn, Rick Fletcher, Vivienne Mawson and two anonymous referees, provided useful comments on earlier drafts of the manuscript. The work was undertaken as part of Fisheries Research and Development Corporation Project 2003/005. All experiments were conducted in compliance with the current laws of Australia.

5.0 Interannual variation in the moult cycle and size at double breeding of mature female western rock lobster *Panulirus cygnus*

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Citation: Interannual Variation in the Moult Cycle and Size at Double Breeding of Mature Female Western Rock Lobster *Panulirus cygnus*. ICES Journal of Marine Science. 63 (9),1631-1639.

5.1 Abstract

The moult cycle of mature female *Panulirus cygnus* George was examined from data recorded over 32 years at four sites along the coast of Western Australia. A repeating trend for mature females was inferred from samples taken between 15 November and 15 August. Setae were present on the endopodites of all large females from June until February–March; between March and June the proportions of females with setae declined sharply as about 80% moulted into a non-setose phase. Females that did not undergo this moult in February remained setose throughout the "normal" non-setose period before moulting and producing a new set of setae by mid winter. New or eroded spermatophores were present on most large females between November and January, but their presence declined sharply during the February/March moult to be absent by May. The percentage of mature females that bypassed the non-setose moult varied markedly from 1 to 40%, and was well correlated with mean water temperatures in January/February. The proportion that did not undergo a moult increased when these months were cool and decreased when they were warm. The size (carapace width) at which females carry two batches of eggs in one breeding season (double breeder) declined progressively from 96.6 to 84.1 mm with decreasing latitude (32°S to 28°S) and further declined at the offshore Abrolhos Islands (29°S) to 78.7 mm. The implications of these findings are discussed in relation to management measures designed to protect females in a setose condition.

Keywords: *Panulirus cygnus*, double breeding, moult cycle, water temperature

5.2 Introduction

The western rock lobster, *Panulirus cygnus* George, constitutes Australia's most valuable single-species fishery (worth about AUD$ 250 – 350 million annually) with annual catches averaging 11 000 t. The species, which is endemic to Western Australia, is found predominantly in coastal waters from North West Cape (21°45 S) to Cape Leeuwin (34°22 S), in depths of less than 200 m (Gray, 1992).

Sustainability of this important fishery has been achieved by management regulations that include limited entry to the commercial fishery, effort controls, a closed fishing season from July to mid-November, a legal minimum size, and (for females) protection of all animals with either ovigerous setae or above a specified size (Caputi *et al*., 2000).

The single most important consideration in managing this fishery is that egg production be maintained at a safe level (Chubb, 2000; Hall and Chubb, 2001). Egg production is monitored from both fishery-dependent and fishery-independent data (Caputi *et al.*, 1995; Melville-Smith *et al.*, 1998). Indices, expressed as number of eggs per pot lift, are estimated for each locality using the relationship between female size, batch fecundity and number of batches produced within a season (Chubb, 1991). The size at which females start to carry two batches within a season is however not known with certainty and needs to be clarified for the production of more accurate egg production indices.

It is also important to clarify the moult cycle of mature female *P. cygnus*, and particularly to establish the proportion of females that remain setose throughout the fishing season (and are therefore protected from fishing). Females moult into a setose phase (June/July) prior to the spawning season. Following this moult copulation occurs resulting in a spermatophoric mass being attached to the sternums of receptive mature females. By September mated females begin to extrude their eggs, attaching them to ovigerous setae located on endopodites beneath their abdomens (Chittleborough, 1976; Chubb, 1991). The eggs are fertilized during this process with sperm released from the scratching of the spermatophoric mass by the female's fifth pair of legs. Remnants of the spermatophoric mass remain until they are either covered by a second mating or sloughed during moulting. The post-spawning moult results in females entering a non-setose phase during the fishing season (generally February), although the time of year at which this occurs and the proportions of females that moult vary between years, mainly as a result of variations in environmental conditions, e.g. water temperature (Chittleborough and Thomas, 1969; Chittleborough, 1976; Chubb *et al.*, 1989). Greater knowledge of this moult cycle is important, because the effectiveness of the legislation protecting females with ovigerous setae, in part depends on what proportion of mature females are fully protected from fishing throughout each part of the fishing season.

5.3 Methods

5.3.1 Sampling regime and measurements

To examine the moult cycle of mature female *Panulirus cygnus* on the west coast of Australia data were collected during fishery-dependent commercial catch monitoring surveys (*DCCM*) at four localities (Fremantle, Lancelin, Jurien Bay and Dongara; see Fig. 1) each year since 1972. Research staff on board commercial vessels conduct the *DCCM* each month during the fishing season (15 November–30 June) in a range of fishing depths from 5 to more than 80 m. The sampling regime is detailed in Caputi *et al.* (2000).

The data used to determine the size (carapace length) at which a female produces two broods of eggs per spawning season (*i.e.* becomes a "double breeder"), were collected during the 2002 fishery-independent breeding stock survey (*IBSS*). This survey has been made annually at three sites (Lancelin, Dongara and Abrolhos Islands), and intermittently at three others (Fremantle, Jurien Bay and Kalbarri) since 1992. All six locations were surveyed in 2002. This survey is undertaken over 10 days during the last new moon before the commercial lobster-fishing season starts (15 November), when the largest number of females bear eggs (Chubb, 1991). The water depths surveyed range from 25–70 m at the five coastal locations and from 10–60 m at the Abrolhos Islands. For details of the sampling regime see Chubb (2000).

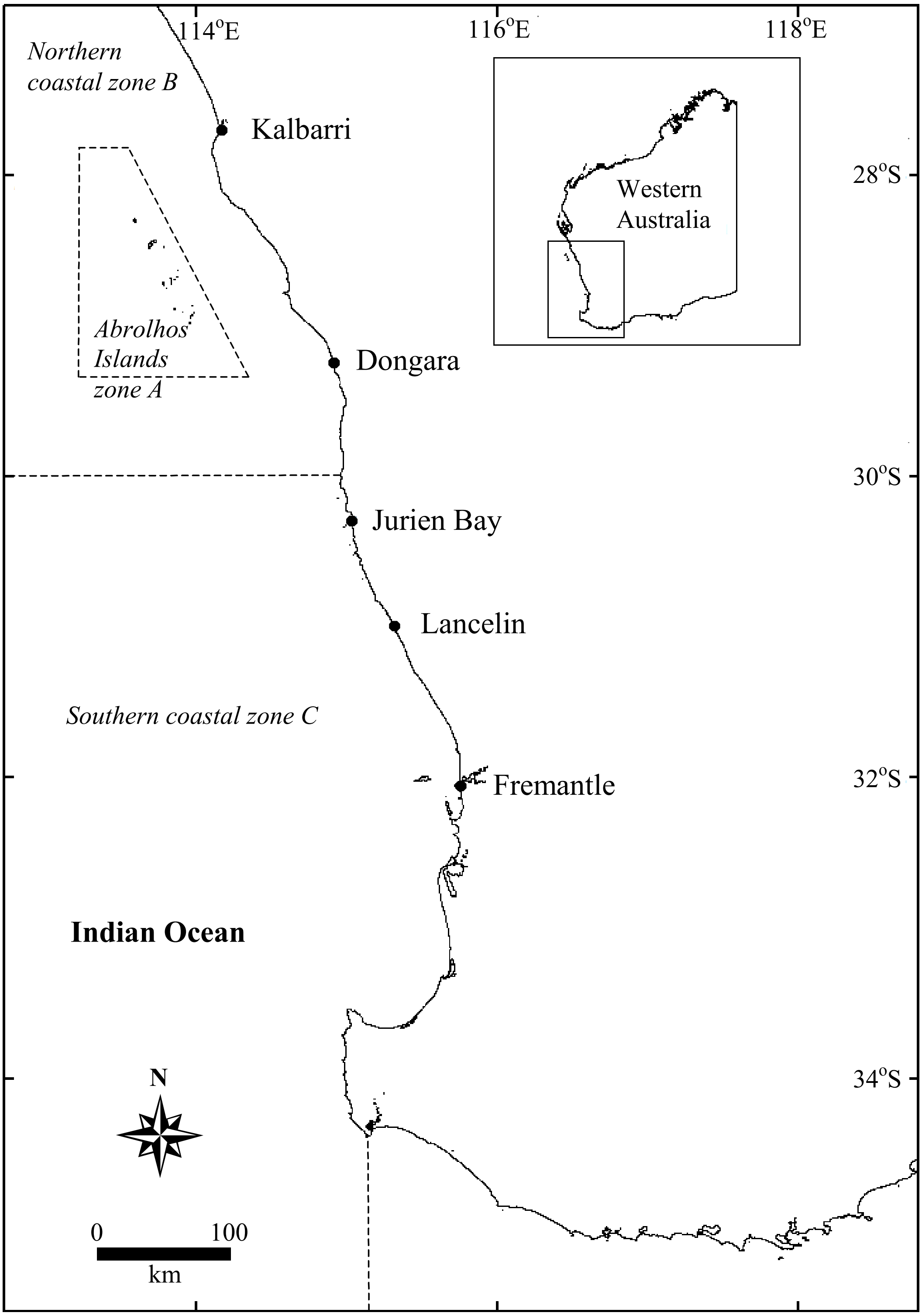

Figure 1. Management zones in the western rock lobster fishery and locations of fishery-independent breeding stock surveys and commercial monitoring of the catch.

In both surveys, the carapace length (*CL*) of each female lobster (from the anterior edge of its carapace between the preorbital spines down the mid-dorsal line to the posterior edge of its carapace) was measured to the nearest 1 mm. The presence of setae on the endopodites (Fig. 2), external ova attached to these setae and spermatophoric masses (new or eroded) attached to the fifth abdominal segment were recorded. During the *IBSS* the developmental stage of female ovaries, as viewed through the dorso-thoracic musculature, and whether a lobster had missing or newly regenerated appendages, were also recorded. For both surveys the presence of setae on the endopodites has been recorded since 1992.

Temperature measurements collected during the *DCCM* by a protected reversing thermometer at a maximum depth of 38 m were analysed by ANCOVA and standardised by location and month of capture and water depth (m) to produce mean monthly bottom water temperatures (*MWT*) (°C) for each year. Monthly mean sea level (*MSL*) data (cm) for the port of Fremantle in Western Australia were obtained from the Flinders University Tidal Institute in Adelaide, and used as a proxy for the strength of the Leeuwin Current.

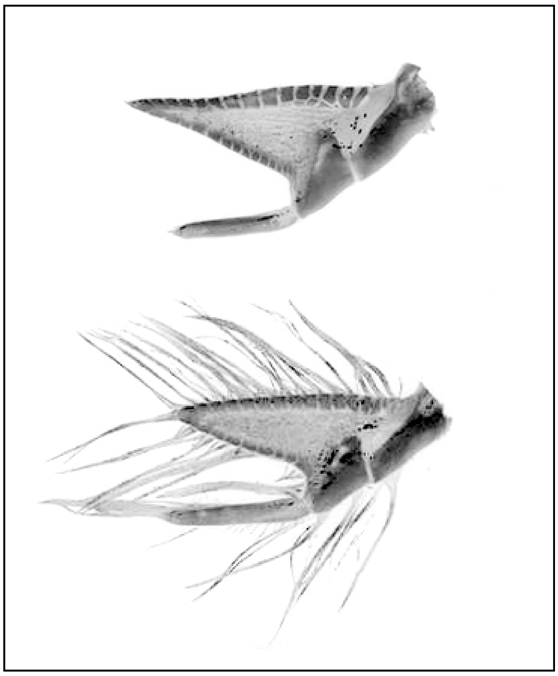

Figure 2. Non-setose (top) and setose (bottom) endopodites of female *Panulirus cygnus*.

5.3.2 Interannual variation in moulting of mature females

Data on the presence or absence of ovigerous setae on the endopodites has only been recorded since 1992. Therefore, to determine the moult cycle of females over the period 1972–2005, *DCCM* data on the presence of a spermatophoric mass deposit on females ≥ 95 mm *CL*, which is above the size at maturity (Chittleborough, 1976) were used to determine the proportions of mature female *P. cygnus* that moulted in February/March after mating and spawning; moulting results in the loss of all trace of spermatophoric mass deposits. The females were caught in water depths to 76 m in April each year since 1972, in the northern (Jurien Bay and Dongara) and southern (Fremantle and Lancelin) regions of the fishery. The standardised proportion of mature, unmoulted (and therefore setose) females, relative to all females in the same size range in April was determined by ANCOVA using location as a factor and depth (m) as a covariate.

The relationship between two sets of environmental measures (*MWT* and *MSL*) in months just prior to and during the non-setose moult (i.e. those that may affect participation in this moult) and the incidence of females that did not moult in February/March was examined using regression analysis. Thus, environmental measures recorded from November to March and combinations of these months were regressed against the proportions of large females with new or eroded spermatophores in April, using both an exponential decay (Leike, 2002) and a linear equation, with the resultant *R*-square values then being plotted. As both the exponential decay and linear models had the same number of parameters (two), resultant *R*-square values could be used to determine which model provided the better description.

5.3.3 Assignment of double breeding and analysis of data

Appendage damage significantly reduces the likelihood of mature female *P. cygnus* producing eggs (de Lestang unpub. data). Therefore only females with undamaged appendages were used to determine the size at double breeding. A female *P. cygnus* was considered to be a double-breeder (*DB*) if, at the peak of the breeding season (October/November), she had (a) external ova and an unused spermatophore; (b) late stage external ova, no spermatophore and a developing ovary; or (c) no external ova, a used spermatophore and a developing ovary (Melville-Smith and de Lestang, 2005).

At each location sampled during the 2002 *IBSS*, the data on all female *P. cygnus* classified as *DB*'s were analysed by logistic regression, to determine the size at which 50% of spawning female lobsters breed twice in a spawning season (DB_{50}). The data for each assemblage were analysed by a nonlinear regression subroutine in SPSS 11.5.0™, with the standard errors being derived from 100 bootstrap estimates. The equation used was:

$$P = P_{\max} / \left[1 + \exp\left(-\ln(19) \times \left(CL - DB_{50}\right) / \left(DB_{95} - DB_{50}\right)\right)\right];$$

where: P is the proportion of mature females at carapace length CL; P_{max}, which is constrained to be >0 and ≤1, is the maximum proportion of double breeders at CL_j, and DB_{50} and DB_{95}, respectively, are the *CL*s at which 50 and 95% of the assemblage breeds twice.

5.4 Results

5.4.1 Moulting cycle of mature females

The average moulting cycles of large (≥ 95 mm), and therefore presumed mature, female *P. cygnus* in the northern and southern regions of the western rock lobster fishery were very similar (Figs 3a, b). In both regions the frequency of ovigerous females declined progressively each month from a maximum of ~70% in November/December to zero in March, with no ovigerous females caught to the end of sampling in August (Figs 3a, b). The average proportions of setose and mated females to all large females (≥ 95 mm) in both regions remained at or close to 100% in all months from November until February, before both measures declined to ~ 20% in April. The contributions of mated females then declined further to essentially zero in May and June, while that of setose females increased in these months to 50 and 90% of all 'mature' females, respectively (Fig. 3a), due to a moult in May. In both regions the frequency of mated females began to increase again to ~40% in July and 60-80% in August (Figs 3a, b).

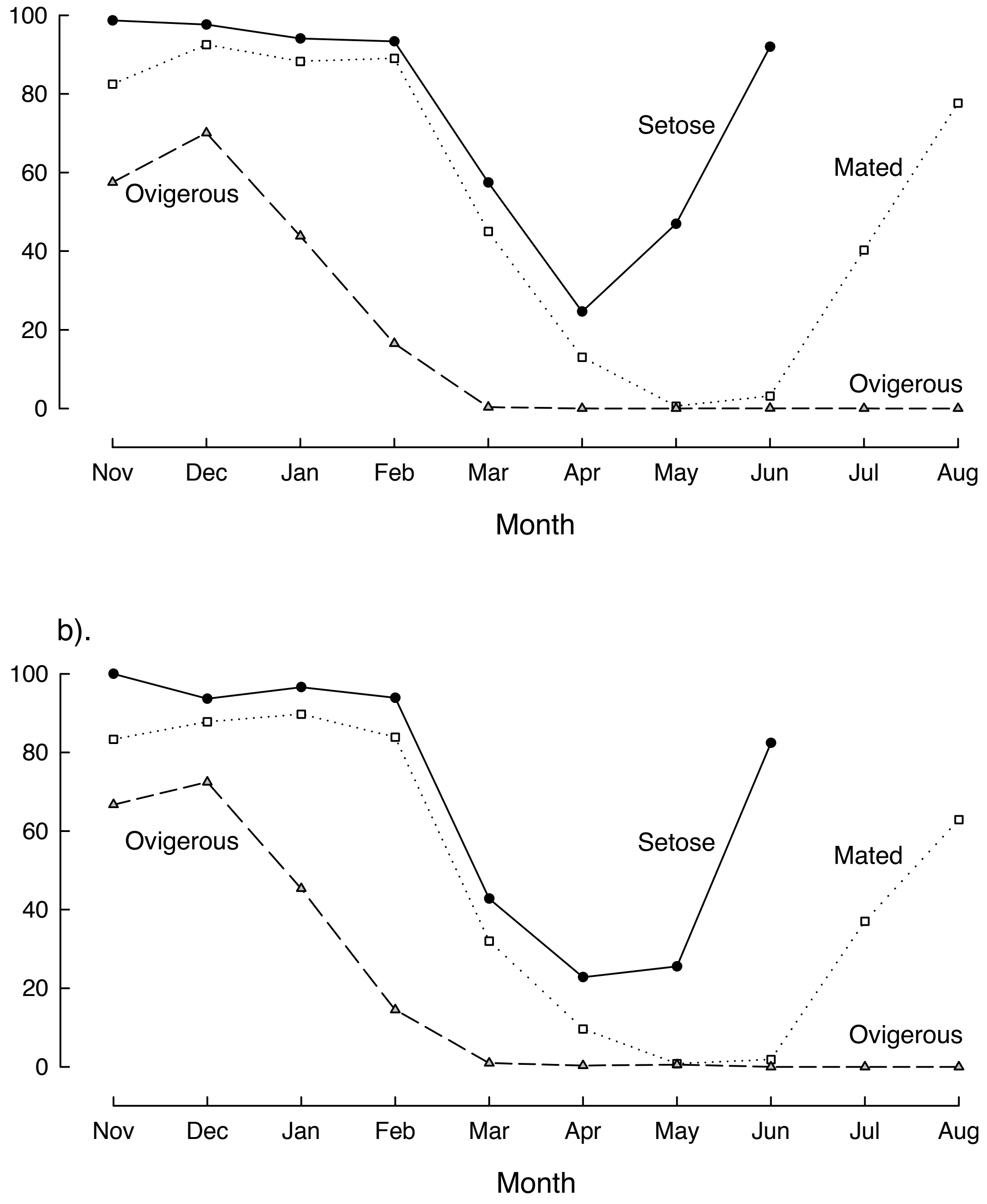

Figure 3. Mean monthly percentages of large (≥ 95 mm) setose, mated and ovigerous female *Panulirus cygnus* sampled during commercial monitoring surveys in the (a) northern and (b) southern zones of the western rock lobster fishery between 1972 and 2005.

5.4.2 Annual variation in the moulting of mature females

The proportions of large (≥ 95 mm *CL*) mated females in April ranged from 1 to 41% of the sampled population in different years (Fig. 4). Low incidences (< 5%) of mated females were recorded in 1973-75, 1978, 1989, 1990, 1992 and 1997, while high incidences (>20%) were recorded in 1977, 1991, 1993, 1998, 2002 and 2004.

For both the exponential decay and linear models, standardised mean water temperatures for the combined months of January and February produced the highest *R*-square values (0.67 and 0.48, respectively) with the proportions of mature females that had not moulted into a non-setose phase (Fig. 5a, b). The mean sea level correlations were too weak to be useful (Figs, 5a, b).

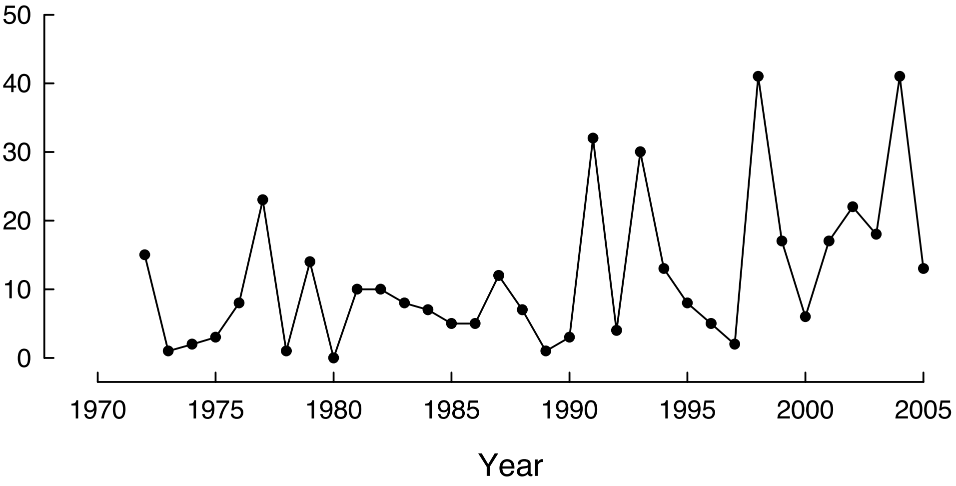

Figure 4. Percentage of large (≥ 95 mm) mated female *Panulirus cygnus* sampled throughout the fishery during April from 1972 to 2005.

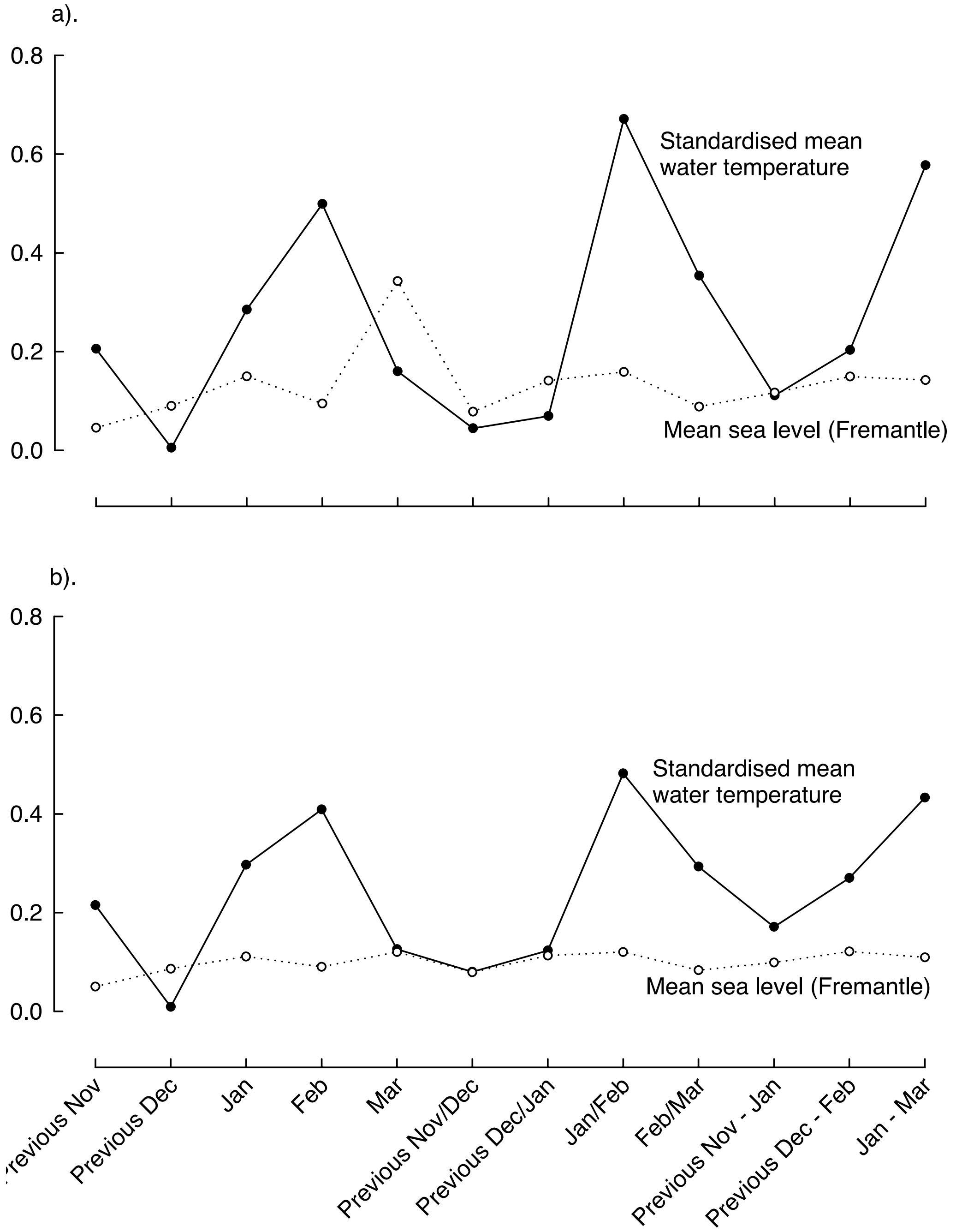

Figure 5. R-square values from (a) exponential decay and (b) linear regressions between the incidence of large (≥ 95 mm) mated female *Panulirus cygnus* in April and annual values, individual months and subsets of months of standardised mean water temperatures (°C) and mean monthly Fremantle sea levels (cm).

The best relationship between the proportions of large mated females in April (P_m) and standardised water temperature (T) averaged for the months of January and February is described by the exponential decay equation: $P_M = 1.37E + 12 \times \exp\left(-T/0.721\right)$ (Fig. 6). This model shows that, with lower water temperatures in mid-late summer, the proportion of female *P. cygnus* that moult into a non-setose phase after spawning decreases exponentially. This time period is towards the end to the egg-bearing season and just prior to the expected time of moulting.

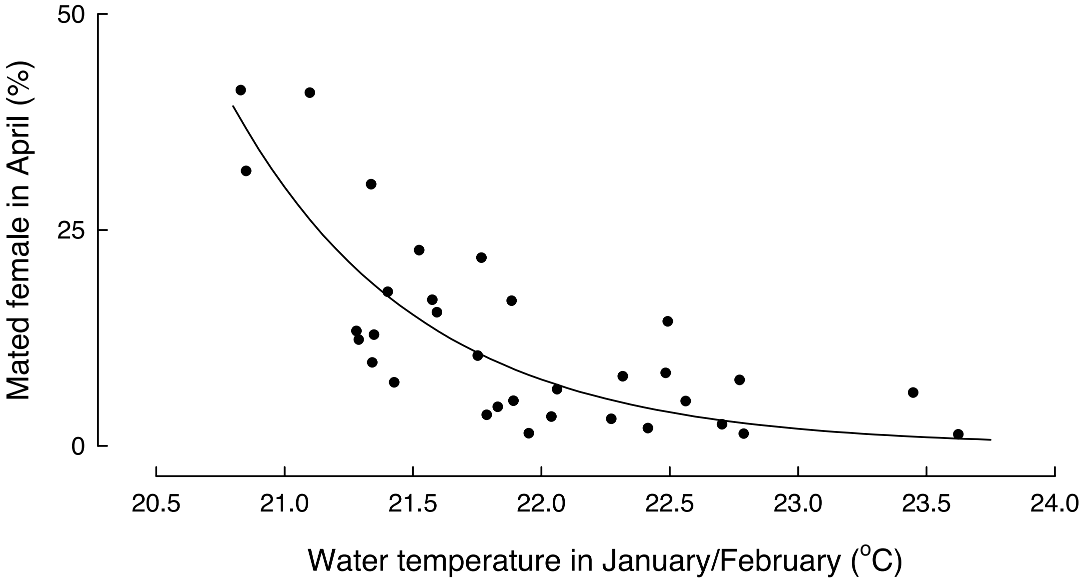

Figure 6. Exponential decay regression between the annual incidence of large (≥ 95 mm) mated female *Panulirus cygnus* in April and the annual standardised mean bottom water temperature for January and February (combined) of that year.

5.4.3 Size at double breeding and the setose moult

The carapace lengths at which 50% of multiple spawning female *P. cygnus* produce two broods of eggs within a breeding season (DB_{50}) were estimated from the 2002 *IBSS* (Fig. 7). The lengths declined progressively with decreasing latitude along the coast and then offshore to the Abrolhos Islands with the DB_{50} recorded at Fremantle (96.6 mm) being the largest, then Lancelin (90.4 mm), Jurien Bay, Dongara and Kalbarri (all about 85 mm) and finally the Abrolhos Islands (78.7 mm). This progressive declining trend along the coast and to the offshore islands, is similar to, but consistently greater (~10 mm), than the trend of size at first maturity recorded for this species (Table 1).

Table 1. The size at which 50 and 95% of female *Panulirus cygnus* produce one (SB) (Melville-Smith and de Lestang, in press) and two batches (DB) of eggs per spawning season at six locations along the west coast of Australia.

	Fremantle	Lancelin	Jurien	Dongara	Kalbarri	Abrolhos
SB_{50}	87.5 ± 0.22	82.2 ± 0.34	81.4 ± 0.32	74.9 ± 0.49	77.2 ± 0.34	65.0 ± 0.27
SB_{95}	92.8 ± 0.57	89.6 ± 0.87	90.6 ± 1.00	88.1 ± 1.26	87.5 ± 0.86	76.3 ± 0.51
DB_{50}	96.6 ± 1.52	90.4 ±0.74	84.6 ± 1.49	84.1 ± 1.46	86.5 ± 1.13	78.7 ± 1.58
DB_{95}	114.8 ± 3.82	100.8 ± 2.05	96.5 ± 3.50	98.6 ± 4.54	100.0 ± 3.48	96.8 ± 3.63

The maximum percentage of females assigned as double breeders never reached 100%, even in the largest size classes, and varied markedly between locations. The Abrolhos Islands recorded the highest percentage of lobsters assigned as double-breeders (79%), followed by Lancelin (70%), Kalbarri (57%), Dongara (52%), Fremantle (50%) and Jurien Bay (35%) (Fig. 7).

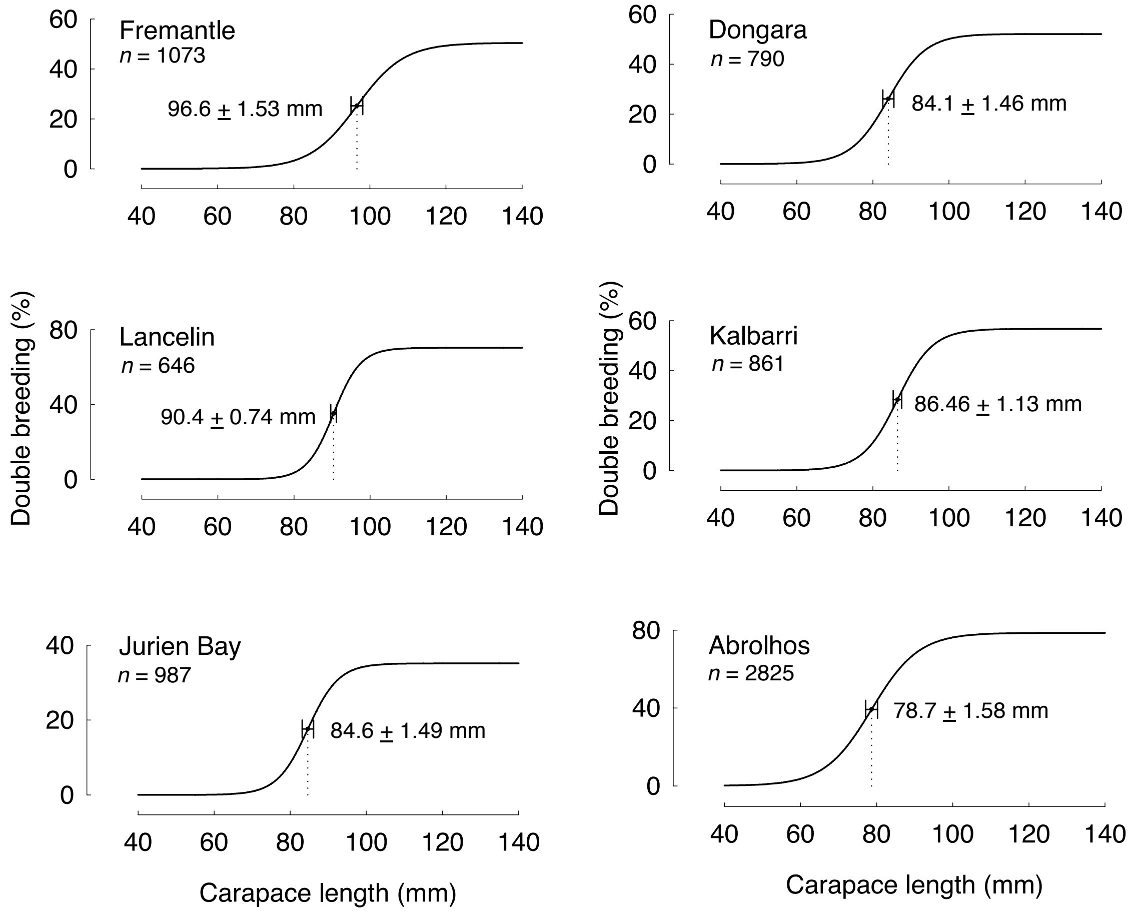

Figure 7. Logistic regressions fitted to the percentage of double-breeding female *Panulirus cygnus* at different carapace lengths in six locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. DB_{50} ± 1 SE denotes the size at which 50% of the assemblage is mature, and n the sample size.

The carapace lengths of those females that do not moult into a non-setose phase did not differ between years (Kolmogorov-Smirnoff, $p > 0.05$). Therefore, to ascertain whether these females breed once or twice a season the cumulative frequencies of females in 5 mm size classes that were classified as either being single- or double-breeders were plotted with the cumulative frequency of mated females in April pooled over the last 32 years (1972–2003) (Fig. 8 a, b).

In both the northern and southern zones, the cumulative frequency of mated females in April was, for corresponding size classes, significantly lower (Kolmogorov-Smirnoff, all $p < 0.01$) than that of either single- or double-breeding females in that size class. This indicates that lobster that are still mated in April, i.e. those that do not moult into the non-setose phase, are generally larger than double-breeding females (Fig. 8).

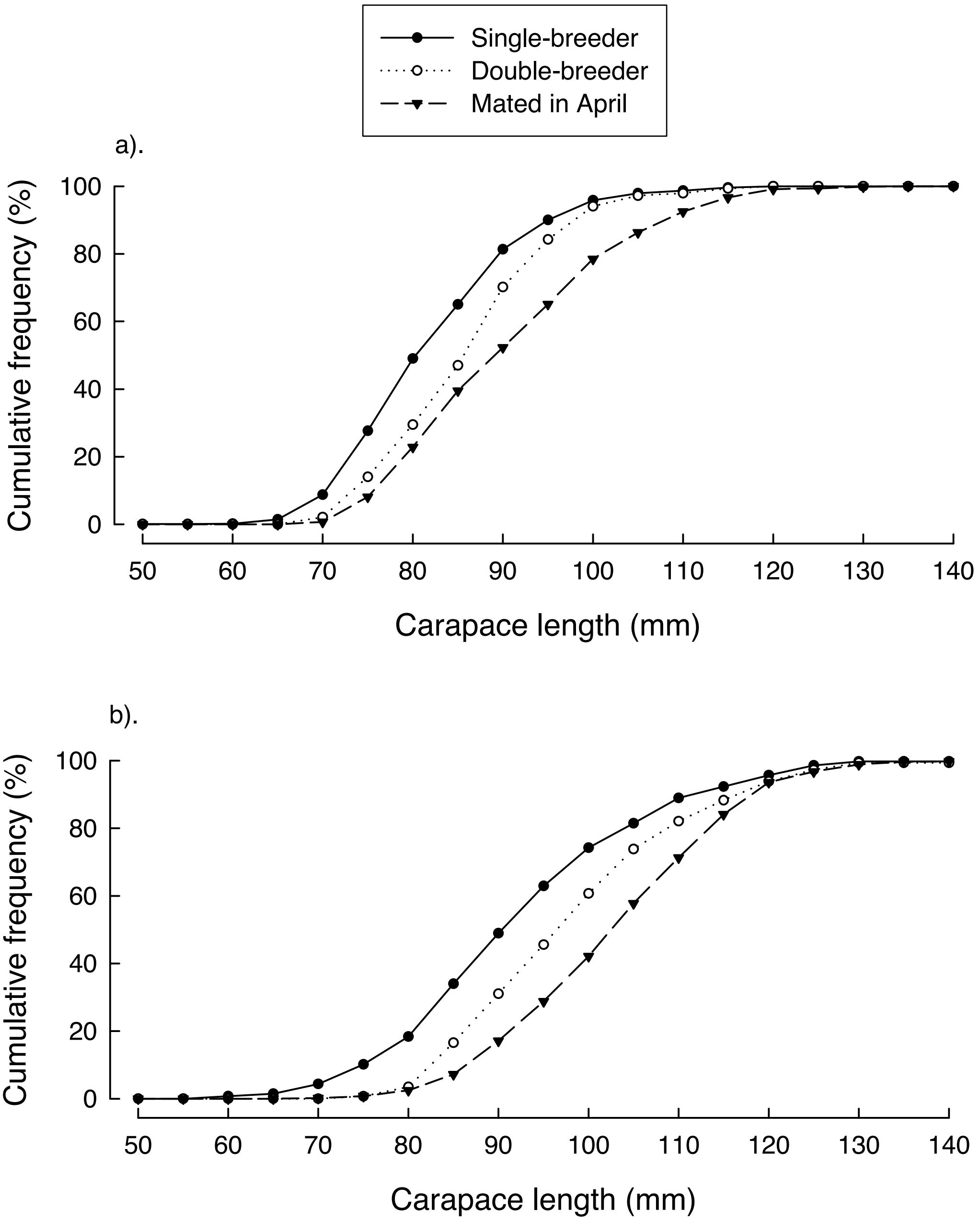

Figure 8. Cumulative frequencies (%) of single- and double-breeders and of mated female *Panulirus cygnus* recorded in April in sequential 5 mm *CL* classes in the (a) northern and (b) southern zones of the western rock lobster fishery.

5.5 Discussion

The different proportions of setose, ovigerous and mated females in commercial samples throughout the season provides a reliable indication of the moulting and mating cycles of mature female *P. cygnus* (Fig. 3). The sharp decrease in the proportion of setose and mated lobster in February/March indicates that most (~80%) breeding females moult into the non-setose phase at this time. The complete disappearance of females with new or eroded spermatophores in samples from May onwards indicates that those animals (~20%) that did not moult in February/March moulted by May. The high proportion of setose females in June indicates that those that moulted into a non-setose condition in February/March moult back into setose condition in preparation for the next breeding season by June. The increase in females with new spermatophores in July and August is a couple of months ahead of when external eggs are produced in mid spring (Chubb, 1991).

The ability for mature females to moult out of setose condition outside of the breeding season is common to numerous other spiny lobster species, for example *Jasus edwardsii*, MacDiarmid (1989); *Jasus lalandii*, Patterson (1969); *Panulirus japonicus*, Nakamura (1940) and *Panulirus argus*, Sutcliffe (1953).

The large numbers of reproductively active females not moulting into a non-setose phase in late summer appears linked to cool water temperatures towards the end of the egg-bearing season. Chittleborough (1976) showed that the development time of fertilised eggs of *P. cygnus* increased exponentially from about 26 days at 25°C to 70 days at 19°C. It is thus likely that females which become ovigerous late in the spawning season during a year when water temperatures are cool, may not have sufficient time to develop and release their eggs prior to the synchronous non-setose moult in February/March. Furthermore, females that produce two batches of eggs in a spawning season would be more likely to extrude their last (second) batch of eggs later in the season than would the single breeders that extrude only one batch of eggs. It therefore follows that the females most affected by cool water temperatures towards the end of the spawning period are the larger females, i.e. those that are more likely to produce two broods of eggs each season. It is not possible to directly measure this by examining the numbers of egg bearing females during this period since most will be bearing eggs in a late stage of development and females in this condition have an extremely low catchability (Waddington *et al*., 2005).

At each location the double spawning females were always the larger lobsters sampled in that area. This is consistent with previous findings for *P. cygnus* (Chittleborough, 1976; Chubb, 1991) and for other species of this genus (Berry, 1971; Briones-Fourzan *et al*., 1981; MacFarlane and Moore, 1986; Briones-Fourzan and Lozano-Alvarez, 1992; Gomez, 1994). However, the sizes recorded by Chubb (1991) at which 50% of *P. cygnus* became double-breeders (DB_{50}) at Two Rocks (between Fremantle and Lancelin) (~ 80 mm), Dongara (~ 93 mm) and Abrolhos Islands (~ 63 mm) were all markedly different from those recorded in the present study (see Table 1; Figure 7). These differences arise because Chubb (1991) visually classified lobsters with an eroded spermatophore mass, external eggs and a developing ovary as a multiple (i.e. double) breeders. Recently however, Melville-Smith and de Lestang (2005) showed that an ovary classified as “developing” in this situation is more likely a “spent” ovary containing residual atretic oocytes, which makes it appear yellow and developing and therefore is only a single breeder. Furthermore, the DB_{50} recorded by Chubb (1991) for Two Rocks, Dongara and the Abrolhos Islands are either the same as, or smaller than the size at first maturity recorded by the same author for the same regions (Chubb, 1991), which casts doubt on the appropriateness of the criteria used to assign double-breeding in this earlier study.

This study showed that large mature females were able to produce multiple batches of eggs within a spawning season, although whether this was limited to two broods was not able to be determined directly. Anecdotally however two broods does seem to be the maximum number *P. cygnus* is capable of producing by per season. Chittleborough (1976) showed that when kept at a constant temperature and fed to excess the maximum number of broods produced by female *P. cygnus* between moulting events was two.

The percentage of females classified as double-breeders not reaching 100% at any of the six locations, even in the very large size categories, was not unexpected. Clear identification of an individual female as a double-breeder is only possible when they are sampled reasonably close to the middle of their spawning season (Melville-Smith and de Lestang, 2005). Small variations are likely to exist in the stimuli that initiate spawning between and within each location resulting in spawning not being synchronous throughout the fishery. Consequentially, it was not possible to sample all double-breeding females when they were in the middle of their spawning season. As a result, some double-breeding females sampled will always be identified incorrectly as single-breeders. Since it is likely that miss classification results in percent of double breeders never reaching 100% is seems probably that, at each location, the size at DB_{50} recorded in this paper not only describes the size at which 50% of double spawning females are breeding twice but is also a good estimate of the size at which 50% of females within the sampled population will breed twice.

This study has found that double-breeding female lobster, sampled at six locations during the 2002 *IBSS*, are about 10 mm *CL* larger than females at first maturity recorded at the same locations (Melville-Smith and de Lestang, in press). Since 2–3 mm is the average moult increment achieved by newly matured female lobsters (Morgan, 1977), and females of this size are expected to moult twice a year (Chittleborough, 1976; Morgan, 1977), it seems likely that newly matured females require a further two years of growth before they increase in carapace length by ~10 mm and attain the size at which they start to produce two batches of eggs in a season.

While DB_{50} may correspond with females two years older than their size at first maturity (CL_{50}) at the same locality, it is unlikely that either size or age is directly responsible for this finding. It has been shown by Chittleborough (1974; 1976) that aquarium-held females kept at higher temperatures than in their natural environment (25°C) and fed to satiation can breed twice within an intermoult period from when they first mature, and thereafter to repetitively breed without moulting into a non-setose (resting) phase.

The size at maturity of females has declined at all coastal sites since the 1970s (Melville-Smith and de Lestang, in press). It has not been possible to investigate whether DB_{50} has decreased over the same period, because ovarian development has been recorded only since 1992. Furthermore, establishing whether a female is a single- or double-breeder is very sensitive to the time of year she is sampled (Melville-Smith and de Lestang, 2005) and suitable *IBSS* data are not available for this purpose in most years. However if, as we have suggested, DB_{50} occurs on average two years after CL_{50}, then it could be assumed that DB_{50} has followed a similar decline to CL_{50} since the 1970s. Incorporation of new DB_{50} estimates into the current egg production indices will swing the relative contribution of eggs towards the larger size classes of female lobsters, further highlighting their importance to the sustainability of this fishery. Furthermore, the addition of slowly declining DB_{50} estimates will increase recent egg production estimates relative to those determined for early periods, making the current position of these indices more positive.

There are important management implications resulting from identifying a relationship between water temperature and the likelihood of mature females moulting to the non-setose phase in February/March. A substantial portion of the commercially landed catch each year in March, April and May are mature-sized females that have moulted out of a setose condition. Cool water temperatures in January and February would increase the protection provided to the breeding stock by the "setose rule", which was introduced as one of several management measures in 1993/94 to limit fishing pressure on these females (Hall and Chubb, 2001). The Reynolds Sea Surface Temperature dataset (Reynolds, 1994) records a slight increase in water temperatures on the western rock lobster grounds over the last 30 years; this trend can be expected to dilute the effect of the setose rule over time, by allowing more females to moult into the non-setose condition and therefore becoming legal to retain. Conversely the more frequent occurrences of ENSO events since the 1990's have resulted in a higher frequency of weaker Leeuwin Current years and hence cooler water temperatures (de Lestang, unpub. data). This has resulted in the four highest percentages of setose females in April recorded since 1991.

5.6 Acknowledgements

Thanks are expressed to research staff and the crew of the RV *Naturalist* and to Drs Nick Caputi, Anthony Hart, Vivienne Mawson, James Penn, Rick Fletcher, Bruce Phillips and the two anonymous referees for editorial help. This work was undertaken as part of Fisheries Research and Development Corporation Project 2003/005.

6.0 Changes in Egg Production of the Western Rock Lobster (*Panulirus cygnus*) Associated with Appendage Damage

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Citation: Melville-Smith, R. and de Lestang, S. 2007. Changes in egg production of the western rock lobster (*Panulirus cygnus*) associated with appendage damage. Fishery Bulletin. 105 (3), 418-425.

6.1 Abstract

Data collected during fishery-independent sampling programs were used to examine the impact of appendage damage (indicated by lost or regenerated legs and antennae) on the reproductive output of female western rock lobster (*Panulirus cygnus*). Most of the damaged females sampled had one (53%), two (27%), or three (13%) appendages that had been lost or that were regenerating. Appendage damage was associated with the reduced probability of a female developing ovigerous setae; and if setae were produced, with the reduced probability that females would produce more than one batch of eggs within a season. These effects were more pronounced as the number of damaged appendages increased. From data collected in 2002, it was estimated that the total number of eggs produced by mature females caught in the fishery was significantly reduced ($P<0.001$) by 3–9% when the impact of appendage damage was included.

6.2 Introduction

Western rock lobster (*Panulirus cygnus*) are found only off Western Australia, where they form the basis of an intensive commercial fishery (Phillips and Melville-Smith, 2005). One result of the high exploitation rates experienced by western rock lobster (Brown and Caputi, 1985, 1986), and other decapod species (Krouse, 1976; Smith and Howell, 1987), is the damage sustained by the catch that is returned to the water. Damage, whether caused by aggression between conspecifics trapped in pots, desiccation on board boats before processing, or rough handling during sorting, is generally a combination of dehydration, broken body parts, and the loss of entire appendages. Apart from the mortality of animals due to processing, both the growth rate and fecundity of the surviving animals can be significantly reduced (Davis, 1981; Brouwer et al., 2006). Damaged animals appear to reallocate energy stores towards regenerating damaged appendages and away from growth and reproduction (Norman and Jones, 1992; Juanes and Smith, 1995; Mariappan and Balasundaram, 2001).

In the western rock lobster fishery, sustainability of the resource has been achieved by management regulations that include limited entry to the commercial fishery, a closed fishing season from July to mid-November, and return to the water of all lobsters that are outside the maximum and minimum legal size limits or that are in a breeding condition (i.e., bearing ovigerous setae) (Caputi et al., 2000; de Lestang and Melville-Smith, 2006). Anecdotally, these regulations are believed to result in 55% of the *P. cygnus* catch being returned to the sea. This species is especially susceptible to autotomizing (dropping) limbs (Brown and Caputi, 1983, 1985): 40–80 tonnes of legs are estimated to be lost from the landed catch of *P. cygnus* each year (Davidson and Hosking, 2002).

We used data from a variety of existing and new sources to examine the effect of appendage loss and regeneration (both antennae and legs) on the reproductive biology of female *P. cygnus*. We believe this study to be the first comprehensive assessment of the impact that appendage damage has on the reproductive output of a decapod species. This study assesses the impact of appendage damage on the proportion of females developing ovigerous setae, the proportion of females that will produce one or more batches of eggs within a breeding season, and the number of eggs in a batch.

Keywords: *Panulirus cygnus;* egg production; appendage damage; leg loss; autotomy

6.3 Methods

6.3.1 Sampling regime

Data were collected during a fishery-independent breeding stock survey (hereafter referred to as "the survey"), which has been conducted annually at three localities (Lancelin, Dongara, and Abrolhos Islands) and intermittently at three others (Fremantle, Jurien, and Kalbarri) since 1992. The commercial fishery in Western Australia is divided into three management zones: the Abrolhos Islands (zone A), north coastal (zone B), and south coastal (zone C) (Fig. 1). In some cases data were pooled into these zones for analysis.

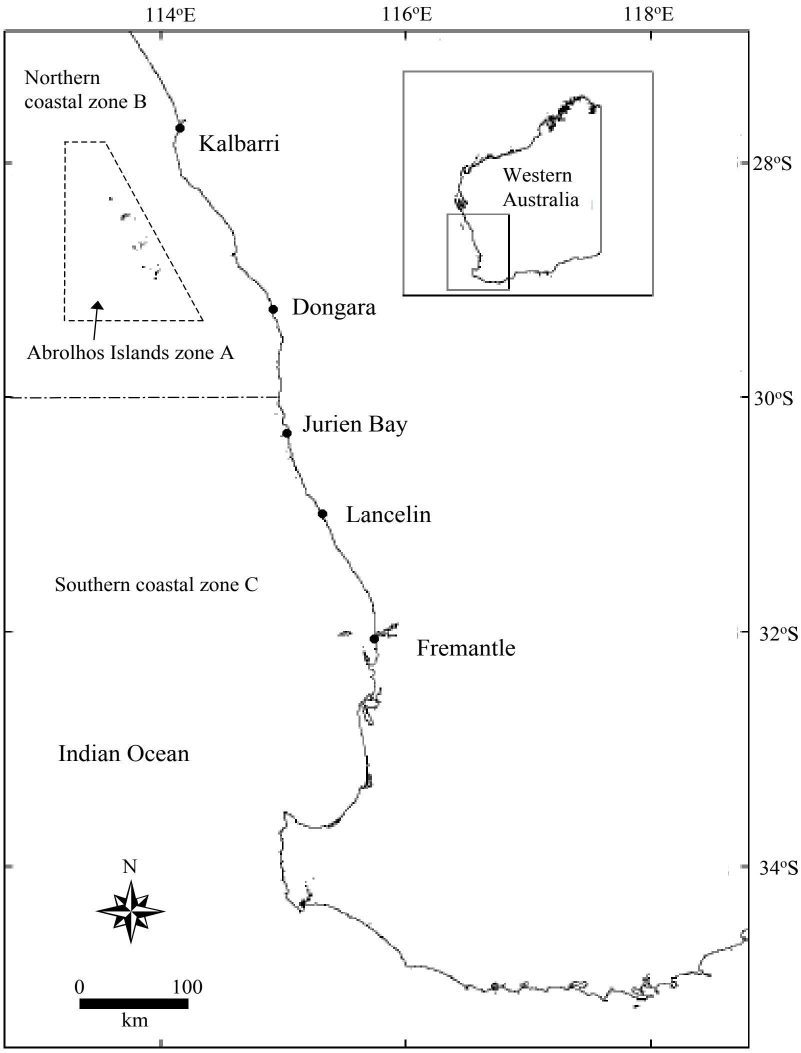

Figure 1. Management zones (zone A, Abrolhos Islands; zone B, northern coastal; zone C, southern coastal) in the western rock lobster (*Panulirus cygnus*) fishery, and six sites (five on the coast of Western Australia and one comprising the Abrolhos Islands) used for fishery-independent breeding stock surveys and commercial monitoring of the catch.

The surveys were undertaken over the course of ten days before the start of the commercial lobster fishing season on 15 November. This period is very close to the annual peak of the egg-bearing season, which is considered to occur in November of each year (Chubb, 1991). Because this survey was designed to be repeatable, the same fishing gear (batten pots with closed escape gaps), bait (a combination of north sea herring [*Clupea harengus*] and Australian salmon [*Arripis truttaceus*]), and locations (same GPS coordinates) were used. The results are therefore directly comparable between years. For more details on the survey sampling regime see Chubb (2000).

6.3.2 Measurements and records

During the surveys, the carapace length (CL) of each lobster was measured to the nearest 1 mm from midpoint between the preorbital spines down the mid-dorsal line to the posterior edge of its carapace. The presence of gonopores on the base of the fifth pair of pereiopods was used to identify males. For females, the presence of ovigerous setae attached to the endopodites, the visual appearance of the ovaries through the dorsothoracic musculature, the presence and developmental stage of external ova attached to the setae, and the presence of a spermatophoric mass attached to the fifth abdominal segment were recorded. These data have been used to predict whether a female would produce one or two batches of eggs in a spawning season (such females are known as "single breeders" and "double breeders, respectively)"–see Melville-Smith and de Lestang (2005) for a full description of this method).

Loss and regeneration of antennae and limbs were also recorded during the survey as either an old loss, new loss, or as a regenerated appendage and all three categories were grouped collectively and referred to as "appendage damage." Old loss was identified by dark melanization at the site of the lost appendage and new loss by exposed flesh without melanization. Although new leg loss was recorded, nearly all were considered to have resulted from capture and handling during the survey and therefore were excluded from our analysis of the impact of appendage damage on reproductive output. Regenerated limbs of *P. cygnus* were only easily identifiable in the first intermoult period after the limb was lost and were distinguished by being greenish in colour and noticeably smaller or thinner than existing limbs. Because old and new losses have been recorded since 1992 and regenerated limbs have been recorded since 2001, we used only the data collected since the 2001 survey. The incidence of old losses, new losses, and regenerated appendages between zones, sex, and carapace size was compared by using ANOVA.

6.3.3 Effect of appendage damage on fecundity estimates

Because most of the lobsters sampled in zone A (the Abrolhos Islands) during the 2001–03 surveys were larger than the size at maturity (Melville-Smith and de Lestang, 2006), data derived from sampling in this location were used to examine whether the incidence of old appendage-losses and regenerated appendages affects the reproductive state of female *P. cygnus*.

The batch fecundity (number of eggs in one batch) of 50 female *P. cygnus* with early-stage eggs that ranged in carapace length (CL) from 67.1 to 96.2 mm was determined. Twenty-three females had either one or two damaged appendages and 27 had no damaged appendages; females with early-stage eggs and more than two damaged appendages were seldom caught and therefore were not assessed. The endopodites with eggs were removed from the lobsters and dried in an oven for 24 hours. The eggs were then separated from the setae and weighed to the nearest 0.0001 g. Three subsamples of each brood (each of ~0.05 g) were taken and weighed. The number of eggs in each subsample was counted to determine the mean number of eggs per gram of dry egg weight, and the mean of these values was used to estimate the total number of eggs in the brood. The mean fecundity per spawning season was compared for females with and without appendage damage after standardizing for carapace length with ANCOVA.

The total number of eggs produced by all mature female *P. cygnus* caught during the 2002 survey in each of the three commercial fishing zones was estimated by using an equation that incorporates the number of broods of eggs produced each spawning season and the effects of appendage damage on the likelihood of spawning once or twice.

$$\left(TF = NB \times F \ \times PO_{DA}\right),$$

where TF = the total fecundity (number of eggs produced) by mature females;

NB = the probability of a female producing one or two broods each spawning season, on the basis of their CL;

F = the relationship of fecundity to carapace length; and

PO = the probability that females with damaged appendages DA will produce eggs.

$$P_1 = \left(1/1 + \exp\left(-\ln(19) \times \left(CL - SB_{50}\right)/\left(SB_{95} - SB_{50}\right)\right)\right),$$

$$P_2 = \left(1/1 + \exp\left(-\ln(19) \times \left(CL - DB_{50}\right)/\left(DB_{95} - DB_{50}\right)\right)\right),$$

where SB_{50} and SB_{95} = the CLs at which 50 and 95%, respectively, of the population at each location produced one brood of eggs (P_1); and

DB_{50} and DB_{95} = the CLs at which 50 and 95%, respectively, of the population at each location produced two broods of eggs (P_2) per spawning season (de Lestang and Melville-Smith, 2006).

6.4 Results

6.4.1 Frequency of appendage damage in 2001–05 surveys

The percentage of western rock lobster with damaged appendages in the 2001–05 survey catches decreased as the number of damaged appendages increased (Fig. 2, A–C). For example, in zone A, about 82% of all female and male *P. cygnus* in the catches had no appendage damage, whereas about 9%, 4%, and 2% of both sexes had one, two, and three damaged appendages, respectively. Only 1% of the catch of each sex had four damaged appendages and less than 0.5% of all lobsters had more than five damaged appendages.

The incidence of appendage damage was significantly different between zones ($P<0.001$) and both zones A and C had higher incidences than zone B (Table 1). In addition, within zones B and C, significantly ($P<0.001$) more females than males were caught with appendage damage. There was no significant difference ($P=0.14$) in the incidence of appendage damage for females and males caught at the Abrolhos Islands.

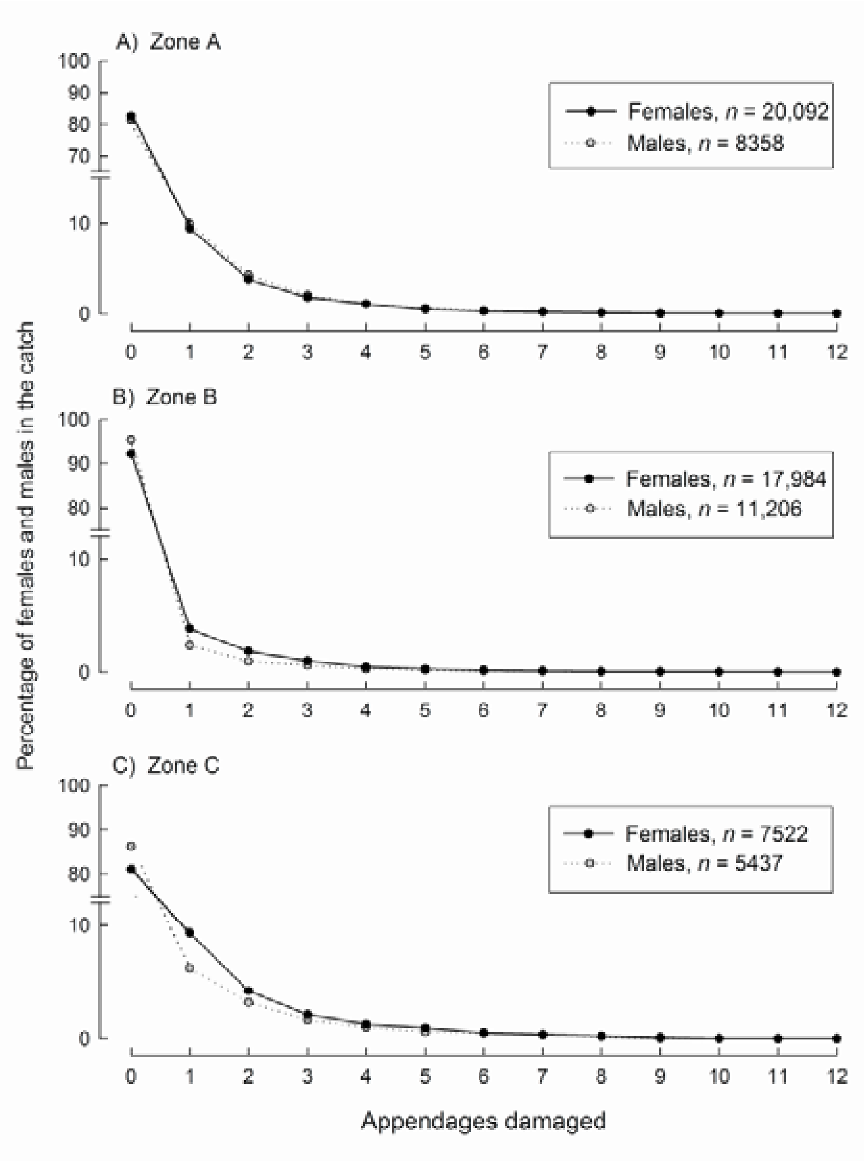

Figure 2. Percentage of female and male western rock lobster (*Panulirus cygnus*) with 0–12 damaged appendages in the catch at three management zones: (A) zone A, Abrolhos Islands; (B) zone B, northern coastal; and (C) zone C, southern coastal. Note that the *y*-axis is not continuous.

Table 1. Percentage of all female and male western rock lobster (*Panulirus cygnus*) with old damage, or regenerated appendages, in the three management zones of the fishery. Data are from the 2001–05 fishery-independent breeding stock survey.

	Fishing zone		
Sex	**Zone A**	**Zone B**	**Zone C**
Female	17.8%	12.4%	20.9%
Male	18.9%	8.7%	17.2%

6.4.2 Relationship between appendage damage and carapace length

The incidence of new appendage loss differed significantly (*P*<0.001) between the two sexes in the various size classes (Fig. 3A). New appendage loss in females remained at about 17% in all the size classes, whereas in males this loss decreased from 15% to 8% in the first four size classes, then increased substantially to 28% and 20% in the larger size classes (110–119 and 120–129 mm CL, respectively).

The incidence of old appendage loss also differed significantly (*P*<0.001) between sexes in different size classes (Fig. 3B). Old appendage damage was slightly more common as females increased in size, i.e., from 7% to 11% between the 60–69 and 100–109 mm CL size classes. This increase also occurred for males but to a much greater extent, i.e., from 4% to 23% between the 60–69 and 100–109 mm CL size classes. The incidence of old appendage damage in males then declined slightly over the two largest size classes (110–119 and 120–129 mm CL) 22 and 16%, respectively (Fig. 3B).

Regenerated appendages in the catches of lobster differed significantly (*P*<0.001) between sex and size classes (Fig. 3C). Regenerated appendages were more commonly recorded for females than for males, but regenerated appendages for each sex remained relatively constant at about 4% and 3%, respectively, in all size classes below 120 mm CL. Above this size class, the incidence of regenerated appendages increased markedly in females (10%) and declined to zero for males (Fig. 3C).

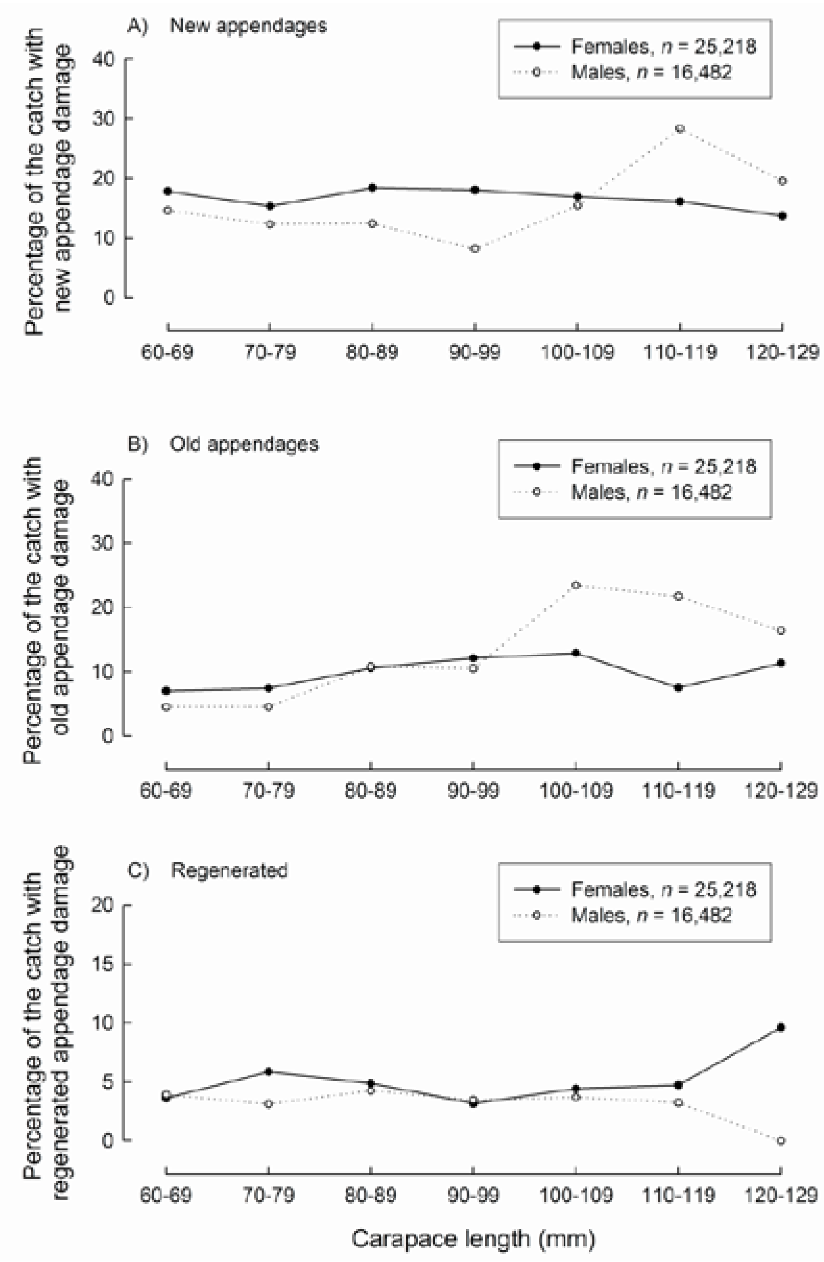

Figure 3. Percentage of female and male western rock lobster (*Panulirus cygnus*) in different size classes with **(A)** new or **(B)** old appendage loss, or **(C)** with regenerated appendages. The analysis uses 2001–05 fishery-independent breeding stock survey data from all five coastal sites.

6.4.3 The influence of appendage damage on egg production

The proportions of female *P. cygnus* (CL>65 mm) from the Abrolhos Islands that were classified as having ovigerous setae, as being single breeders, and as being double breeders, all declined with increasing appendage damage (Fig. 4, A–C). A consistent trend existed between the various reproductive states and the magnitude of their appendage damage. For females at the Abrolhos Islands above the size at maturity, the likelihood of developing ovigerous setae declined with the number of appendages damaged: 98% likelihood (one appendage damaged), 95% (two), 80% (five), and 58% (six). This likelihood continued to decline until it reached zero for all females with either 11 or 12 damaged appendages (Fig. 4A).

For ovigerous females with damaged appendages, the likelihood of producing either one or two batches of eggs each spawning season declined more rapidly than the likelihood of developing ovigerous setae. Females with one damaged appendage were 20% and 19% less likely to produce one or two batches of eggs, respectively, whereas those with five damaged appendages were around 85% and 65% less likely to produce one or two batches of eggs, respectively. Females with more than seven damaged appendages did not produce eggs (Fig. 4, B and C). Equations describing the relationships between appendage damage and the likelihood of spawning once for single breeders and twice for double breeders were not significantly (P=0.42) different from each other and were thus combined to produce a single equation to describe the likelihood of producing one or two broods of eggs:

$$PO_{DA} = \exp[-0.31 \times \ln(DA+1) + 0.742] - 1 .$$

The above relationship between appendage damage and the likelihood of lobsters developing ovigerous setae, or the likelihood of lobsters producing one or two broods of eggs at the Abrolhos Islands, was very similar in the other two coastal management zones.

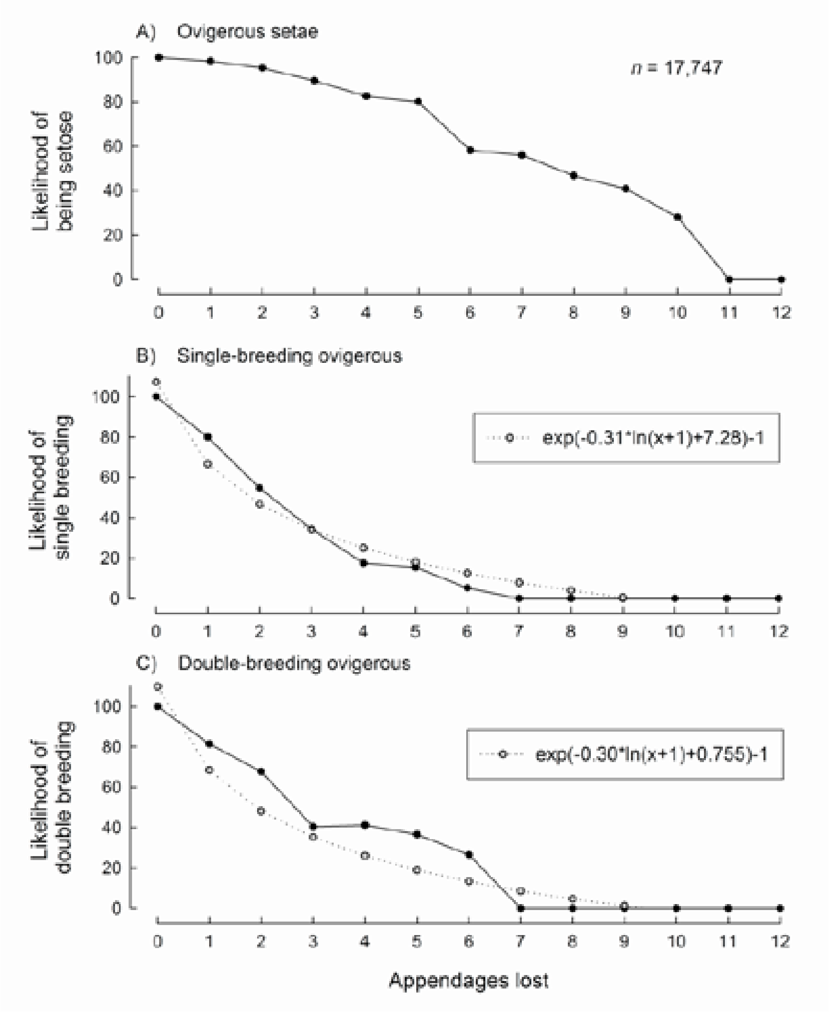

Figure 4. Likelihood of female western rock lobster (*Panulirus cygnus*) with **(A)** ovigerous setae, **(B)** or being single-breeding ovigerous individuals, and **(C)** double-breeding individuals, with 0–12 appendages damaged. Data are from the 2001–05 fishery-independent breeding stock surveys at the Abrolhos Islands (zone A) for all females > 65 mm CL combined. Equations in (B) and (C) refer to fitted relationships describing the likelihood of breeding with appendage loss.

6.4.4 The influence of appendage damage on fecundity

The mean fecundity per spawning season of female *P. cygnus* with a standardized CL of 77.0 mm did not differ significantly (*P*>0.05) between females with and without damaged appendages (i.e., 249,885 ±7873 eggs and 234,164 ±7094 eggs, respectively). Furthermore, regressions between fecundity (*F*) and carapace length (CL) of female *P. cygnus* with and without damaged appendages (Fig. 5) did not differ from each other (*P*>0.05) and were both very similar to the relation of carapace length to fecundity recorded for this species by Chubb (1991).

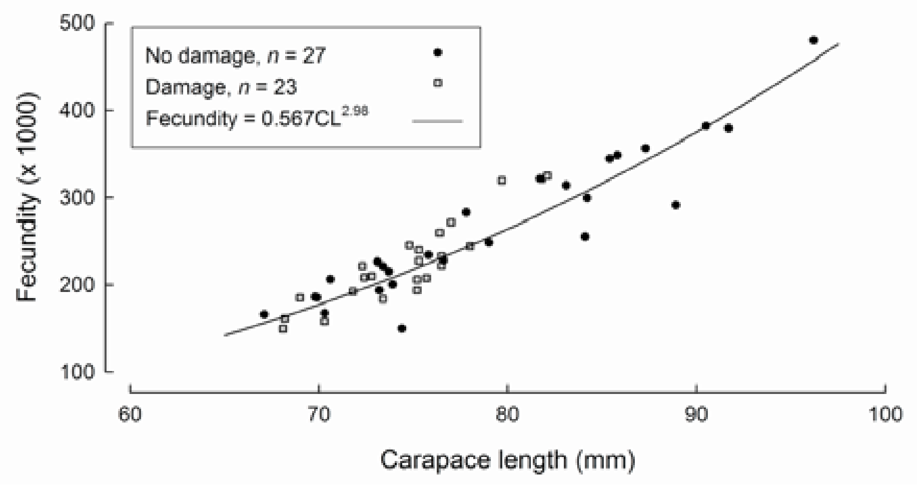

Figure 5. The relationship between fecundity and carapace length (mm) in western rock lobsters (Panulirus cygnus). The actual numbers of eggs carried by individuals with damaged appendages are shown by black circles and without damaged appendages, by open squares.

The effect of damaged appendages on the number of eggs produced per spawning season by female *P. cygnus* at the Abrolhos Islands was greater for large than for small females (Fig. 6). For example, two damaged appendages reduced the fecundity of a 70-mm-CL lobster by about 114,000 eggs, whereas the fecundity of a 120-mm-CL lobster was reduced by about 1,000,000 eggs (Fig. 6).

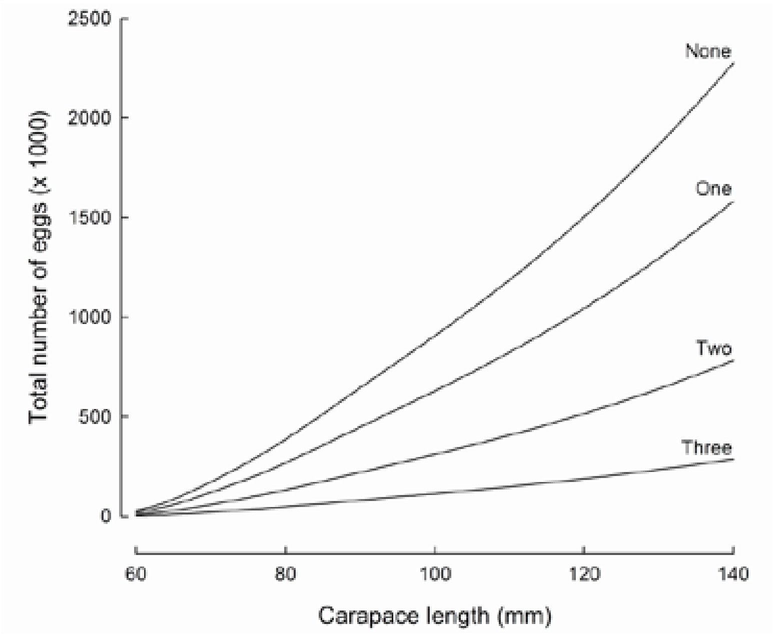

Figure 6. The relationship between total egg production (total fecundity) and carapace length (mm) of female western rock lobster (*Panulirus cygnus*) in one season at the Abrolhos Islands (zone A) with none, one, two, or three damaged appendages.

The total number of eggs produced in the 2002 survey was estimated for each of the three management zones separately for females with and without appendage damage. The inclusion of appendage damage significantly (all $P<0.001$, paired t-test) reduced egg production estimates by 8.5%, 3%, and 9% in zones A, B, and C, respectively.

6.5 Discussion

6.5.1 The incidence of appendage damage

The proportions of lobsters with damaged appendages varied markedly between sexes, sizes, and locations sampled. However, the timing and frequency within a year that moulting takes place for the sexes, and for different-size animals, plays only a relatively minor role in influencing these differences. Female western rock lobsters generally moult twice a year: February–March and again in May. A significant proportion of large breeding females occasionally skip the February–March moult, but all take part in the May moult (de Lestang and Melville-Smith, 2006).

New appendage damage occurred around the time of capture and could mostly be attributed to the survey sampling methods, either to capture in the pots or handling on deck. The slightly higher proportions of females than males recorded with new damage are possibly the result of the longer handling time needed to make additional observations, such as recording the presence or absence of eggs and spermatophores and visually assessing the condition of the ovary. In contrast to new appendage damage, the events that resulted in old damage and regenerated appendages occurred before the survey and, in the latter case (regenerated appendages), before the lobster's last moult, i.e., about May (de Lestang and Melville-Smith, 2006). It is therefore likely that much of this damage is inflicted during the commercial fishing season, possibly as a result of capture and handling. It is thus not surprising that the lobsters showing the greatest incidence of regenerated appendages are females above the maximum legal size (115 mm CL in zone C and 105 mm CL in zones A and B); many of these animals were likely handled and returned to the water many times during a season. Predators may be an additional cause of appendage damage. The fact that the incidence of old appendage damage increased in both sexes with size may indicate that larger individuals are more likely to survive the attack of a predator, although perhaps with the loss of appendages.

6.5.2 The effect of appendage damage on reproductive output

Appendage damage can lead to an associated reduction in the reproductive output of female *P. cygnus* directly, namely as reduced proportions of females that develop ovigerous setae, and as a reduction in the proportions of ovigerous females that will produce one or two batches of eggs within a season. Reproductive output is also affected indirectly when females with appendage damage do not moult into breeding condition (with ovigerous setae); a female above the legal minimum size without ovigerous setae can be legally retained by commercial and recreational fishermen and thus her contribution to the broodstock is removed.

The significant reduction in reproductive output of female *P. cygnus* with appendage damage is not surprising, because regeneration places large demands on energy reserves, often in the form of a reallocation of resources that were originally destined for reproduction and growth (Démeusy, 1965; Norman and Jones, 1992; Juanes and Smith, 1995). Moreover, if appendage damage is extensive, the process of regenerating multiple appendages may result in a long-term reallocation and an overall increase in energy demand (McVean, 1982).

Most *P. cygnus* caught during the survey were intact when examined; less than 15% of the entire catch had damaged appendages. This 15% was probably due, in part, to management measures based on previous work on the effects of appendage damage (Brown and Caputi, 1985, 1986) to initiate changes aimed at reducing limb loss. Methods for limiting appendage damage even further are being developed, i.e., cold stunning (Davidson and Hoskin, 2002). However, even with the best intentions, some appendage damage through handling is unavoidable.

This study has highlighted that management measures aimed at protecting the western rock lobster broodstock inevitably result in the animals being handled more than once (or multiple times) in the course of the fishing season, and the damage to appendages caused by handling produces a significant, and previously unrecognized, effect on the overall egg production of this resource. These effects need to be taken into account when considering the benefits of these management measures in this and other crustacean fisheries.

6.6 Acknowledgements

Thanks are expressed to research staff and crew of the RV *Naturaliste* for assistance with sampling. R. Campbell, N. Caputi, C. Lawrence, V. Mawson, and J. Penn provided constructive criticisms to earlier drafts of the paper. This work was undertaken as part of Fisheries Research and Development Corporation Project 2003/005. All experiments were conducted in compliance with the current laws of Australia.

7.0 Neither maternal size nor site of spawning influences larval competency in western rock lobster *Panulirus cygnus* George

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Citation: Melville-Smith, R., de Lestang, S., Chatfield, B., Nelson, M.M. and Nichols, P.D. 2007. Neither maternal size nor site of spawning influences larval competency in western rock lobster *Panulirus cygnus* George. Journal of Crustacean Biology 27 (3), 445–453.

7.1 Abstract

The size of the western rock lobster broodstock is variable over the range of the Western Australian fishery, due to regional differences in the density and length structure of the stock and the female's size at maturity. Management regulations do not currently discriminate in the protection afforded to the broodstock in the different regions. This study has examined whether regional and size differences in early egg diameter, phyllosoma length at hatch and phyllosoma competency, are related to lipid class and fatty acid composition, protein composition and water content in late stage eggs and early stage phyllosoma larvae. A positive relationship, particularly to phyllosoma competency, would have management implications for the protection of broodstock in different parts of the fishery. Females were sampled in three size groups captured in two sampling regions (Abrolhos Islands 60–70 and 80–90 mm CL; coastal 80–90 and >95 mm CL). Mostly, eggs and larvae from different maternal size classes and regions were not significantly different with respect to lipid class and fatty acid composition, protein composition and water content, and larval competency. The lipid classes of eggs differed significantly ($p < 0.02$) between the three maternal size-classes; fatty acids differed significantly between both eggs and phyllosoma grouped by maternal catch site and size-class ($p = 0.02$ and 0.003, respectively); and there was no correlation between biochemical differences and larval competency. These results imply that maternal identity and subsequent diet may have a greater influence on larval competency in *P. cygnus* than the maternal attributes (size and capture site) that were tested.

7.2 Introduction

Western rock lobster (*Panulirus cygnus*) is found only along the western seaboard of Australia, where it forms the basis of Australia's most valuable (AUD $300 million) single-species, wild-capture fishery. Its life cycle has been intensively researched over forty years (see reviews by Caputi et al., 2003; Phillips and Melville-Smith, 2005). The larvae hatch in spring and early summer and spend the next nine to eleven months in the plankton, with the mid-stages being found as far as 1,500 km offshore (Phillips et al., 1979). Late stage larvae metamorphose into pueruli and swim inshore to settle and start the juvenile phase of their life cycle (Phillips et al., 1979). With such a long larval life covering so vast an area, it is likely that larvae from different parts of the coast become mixed in a common larval pool. This has been substantiated by genetic investigations that showed no significant stock differences in larvae caught over a wide area of the fishery (Johnson and Wernham, 1999) and by larval transport modelling, which showed that, where a puerulus settled was independent of where it was spawned (Griffin et al., 2001). It is quite possible, therefore, that egg production from all regions of the population have an equal chance of contributing to this larval pool.

The western rock lobster fishery is heavily exploited, but management measures have ensured that egg production remains at a satisfactory level (Hall and Chubb, 2001; Phillips and Melville-Smith, 2005). About half of the eggs are produced by the breeding stock around the Abrolhos Islands (Chubb, 1991), a group of coral islands some 60 km offshore. The reason the Abrolhos Islands make this high contribution to egg production, despite contributing about 15% of the catch, is that the broodstock is well protected by having a size at first maturity that is well below the legal minimum size (Melville-Smith and de Lestang, 2006).

On the Western Australia coast, size at maturity has historically been well above the legal minimum size, but a recent analysis has shown significant changes in this index over time (Melville-Smith and de Lestang, 2006). The analysis shows that in the deep-water of many coastal areas where breeding occurs, apparent size at first maturity is now at, or below, the legal minimum size. Therefore, in addition to egg production being spatially concentrated, high exploitation rates combined with a shrinking size at first maturity, has led to smaller female size classes making a larger contribution to egg production in the fishery.

Past concerns about the state of egg production led management in 1993 to prohibit the take of female lobsters above particular size limits in the different regions of the fishery (Hall and Chubb, 2001). This maximum size limit is designed to increase the proportion of very large females in the population (Melville-Smith et al., 1998).

There are numerous studies in the literature showing that at least in fish, eggs from new spawners have a lower hatching success than those from older spawners (Solemdal et al., 1995; Trippel, 1998). Furthermore, in cod on the Grand Banks, older females spawn longer and later into the breeding season. Hutchings and Myers (1993) speculated that this extended period of spawning gives the early stage larvae from older females a greater possibility of coinciding with plankton production cycles. As in cod, large western rock lobster females carry more broods each season than do small females (Melville-Smith and de Lestang, 2005), and as a result are ovigerous for longer during the season. The same hypothesis that Hutchings and Myers (1993) suggested for cod larvae having access to plankton cycles longer into the year may therefore equally apply to rock lobster larvae.

Positive relationships between female size and egg size have also been reported in a range of crustacean species (Attard and Huddon, 1987; Annala, 1991; Gardner, 2001). Larger eggs have higher energetic values (Attard and Hudon, 1987), which would be advantageous to the survival of the hatching larvae. However, this female egg-size relationship is not universal (Fonseca-Larios and Briones-Fourzan, 1998; Briones-Fourzan and Contreraz-Ortiz, 1999; DeMartini et al., 2002).

The aim of this study was to examine whether western rock lobster eggs or early-stage larvae produced at different sites or by females of different sizes, exhibit different biochemical (lipid) compositions and whether these are associated with larval survival. The outcomes are important to the future management of broodstock in terms of identifying the possible need to improve protection of females in particular regions or size classes of the fishery.

7.3 Methods

7.3.1 Egg-size measurements

The diameters of the eggs of *Panulirus cygnus* increase disproportionately during their development; between extrusion and half-way to the time of hatching they increase by ~4%, but thereafter increase by a further ~21% before hatching (Tennyson, 2005). To compare the egg diameters of different sized lobsters caught at different sites, we used eggs that were, according to criteria of Tennyson (2005), less than one fifth along the path to hatching. They were therefore well below the stage at which the diameters increase dramatically.

The eggs were collected during October and November 2004 from 211 ovigerous female lobsters of a range of sizes at four sites (Abrolhos $n = 44$, 65.1–90.5 mm CL; Dongara $n = 48$, 74–124.1 mm CL; Jurien Bay $n = 52$, 74.2–121.1 mm CL; Lancelin $n = 67$, 72.7–126.1 mm CL; see Fig. 1). The eggs were kept in aerated seawater until they were taken to the laboratory (<24 h). Small numbers of eggs (50–100) were removed from the clutch of each experimental animal and placed into a Petri dish with sufficient seawater to keep the eggs moist. They were then teased apart and spread out so as not to touch each other, before being digitally photographed by a Leica DC300 camera attached to a Leica MZ7.5 dissecting microscope. The mean area of an egg was calculated from at least 25 eggs from each female. Since the eggs are generally spherical, the diameter of each egg was calculated by the formula: $Diameter = 2\sqrt{Area/\pi}$.

7.3.2 Broodstock

Early stage phyllosoma were required. For this purpose a total of 21 females with early-stage eggs and without missing or regenerated appendages, were collected from coastal sites at Lancelin ($n = 6$; 86.8–103.0 mm CL), Dongara ($n = 4$; 89.8–95.6 mm CL) and at the Abrolhos Islands ($n = 5$ large; 81.4–85.7 mm CL) and ($n = 6$ small; 64.1–69.8 mm CL) (Fig. 1).

The animals were held individually in 60 L aerated tanks with flow-through ambient seawater under a 12L:12D light cycle and were fed twice weekly with mussels (*Mytilus* spp.). The eggs on each female were inspected weekly for development. When they became brown they were examined daily under a dissecting microscope at 60× magnification. Eggs hatch shortly after three rows of chromatophores become visible on the appendages of the developing embryos (Tennyson, 2005). Accordingly, when this stage was reached, a sample of eggs (~ 4–6 g wet mass) was collected from each animal for biochemical analysis. If the remaining eggs on the female did not hatch within three days of the sample being taken, the sample was discarded and a new sample collected. After collection, the egg samples were rinsed three times with 0.5 M ammonium formate, filtered and then weighed onto tared aluminium foil (~ 2 g each for lipid and protein analysis). The samples were then individually wrapped in aluminium foil and snap frozen in liquid nitrogen for storage at –80°C.

7.3.3 Phyllosoma

Standpipes in each 60 L holding tank were covered with 1000 µm mesh to prevent the loss of phyllosoma between hatching and sampling. Upon hatching, phyllosoma were scooped from near the surface of the tank and concentrated by pouring over a submerged 1000 µm screen. When the hatching was partial, only phyllosoma from the second day of hatching were collected and used for subsequent trials. This was to ensure that the appearance of some larvae in the tanks was not simply a premature hatch by just a small part of the brood. The collected phyllosoma were rinsed with filtered (1 µm mesh), UV-sterilised seawater (UViVF-9, 30 W) and transferred to 10 L containers of seawater treated as before.

Samples of newly hatched larvae were collected shortly after hatching and fixed / stored in 5% formalin in seawater. The lengths of 25 larvae were measured from the anterior margin of the cephalic shield between the eyestalks, i.e., anterior tip of the cephalothorax, to the posterior point of the abdomen. Measurements to the nearest µm were made with the imaging software Leica IM1000, which acquired the image via a Leica DC300 digital camera attached to a Leica MZ7.5 dissecting microscope.

Approximately 3000 newly hatched (day 0) phyllosoma were collected from each of the 21 females for lipid and protein analysis. The samples of phyllosoma were treated as previously described for the eggs.

7.3.4 Larval competency

Triplicate samples of 20 phyllosoma from each spawning female were held in 200 mL of filtered (1-µm mesh), UV-sterilised seawater at 20°C without food. The larvae were checked daily by counting and transferring live individuals to a fresh container. Once 50% mortality was reached, the remaining live phyllosoma were collected and measured. Larval competency for each female, for each site, was taken as the mean number of days it took for half the phyllosoma to die.

7.3.5 Protein analysis

Samples of eggs and phyllosoma were lyophilized. Water content was determined gravimetrically. Samples of lyophilized aliquots were homogenized in 6% trichloroacetic acid using a Lowry et al. (1951) technique as modified by Clayton et al. (1988). The absorbance at 750 nm of the final supernatant was measured with a Beckman Coulter DU530 Life Science UV/Vis spectrophotometer and the protein content was determined by comparison with the accompanying Bovine Serum Albumin standard curve.

7.3.6 Lipid class and fatty acid analysis

Samples were extracted and analysed as described by Nelson et al. (2004). Briefly, eggs and larvae were quantitatively extracted overnight with a modified Bligh and Dyer (1959) one-phase methanol-chloroform-water extraction. Total lipid was determined gravimetrically. An aliquot of the total lipid was analysed with an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (Tokyo, Japan) to quantify individual lipid classes (Ackman, 1981; Volkman and Nichols, 1991).

An aliquot of the total lipid was *trans*-methylated with methanol-chloroform-hydrochloric acid to produce fatty acid methyl esters (Christie, 1982). Gas chromatographic (GC) analyses were made with a Hewlett Packard 5890A GC (Avondale, Pennsylvania, USA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.). Individual components were identified through mass spectral data and also by comparing retention times with those obtained for authentic and laboratory standards. GC-mass spectrometric analyses were made on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, Texas, USA) fitted with a capillary column similar to that described above.

7.3.7 Data analysis

ANCOVA was used to determine whether: (i) the relationships between carapace length and either, size of early-stage eggs, phyllosoma competency and phyllosoma length-at-hatch differed between maternal location of capture; and (ii) whether the relationships between phyllosoma competency and phyllosoma length-at-hatch differed between maternal location of capture.

The qualitative (%) and quantitative (mg g^{-1}) lipid class (LC) and fatty acid (FA) compositions of the eggs and newly hatched phyllosoma were aggregated by the maternal catch-site and size class as well as by three further groups: maternal catch-site/size class (coast medium [80–90 mm CL], coast large [>95 mm CL], islands small [60–70 mm CL] and islands medium [80–90 mm CL]); phyllosoma competency (2–3, 4–8 and 10–17 days); and phyllosoma length-at-hatch (1570–1615, 1620–1640 and 1650–1700 µm total length). All FA that contributed on average less than 0.5% or 1 mg g^{-1} to the qualitative and quantitative FA compositions of the eggs and newly hatched phyllosoma, were excluded from subsequent statistical analyses. The remaining suite of FA, which were well represented in the samples, comprised 20:4(n-6) (arachidonic acid, AA); 16:0; 18:1(n-9)c [with 18:3(n-3)]; 20:5(n-3) (eicosapentaenoic acid, EPA); 18:0; 22:6(n-3) (docosahexaenoic acid, DHA); 18:1(n-7)c; 16:1(n-7)c; 20:2(n-6); 22:4(n-6); 22:5(n-3); 20:1(n-9)c; 18:2(n-6); i17:0, 22:0; 20:0; 15:0; C_{22} (poly unsaturated fatty acid, PUFA); 14:0; 17:0; 20:1(n-11)c; 20:3(n-6); and 22:5(n-6).

The suite of FA was classified by hierarchical agglomerative cluster analysis using group-averaging linking, before being ordinated by non-metric multidimensional scaling (MDS). Both procedures used the PRIMER v5 package (Clarke and Gorley, 2001). Before these analyses, the qualitative and quantitative LC and FA compositions of both egg and phyllosoma samples were, respectively, square root and log-transformed, the Bray-Curtis similarity measure was used to construct the similarity matrix. Analysis of similarity (ANOSIM) was used to test whether the LC and FA compositions differed significantly among maternal size classes or maternal catch site. Similarity percentages (SIMPER) were used to determine which LC or FA contributed most to any dissimilarity between groups (Clarke, 1993).

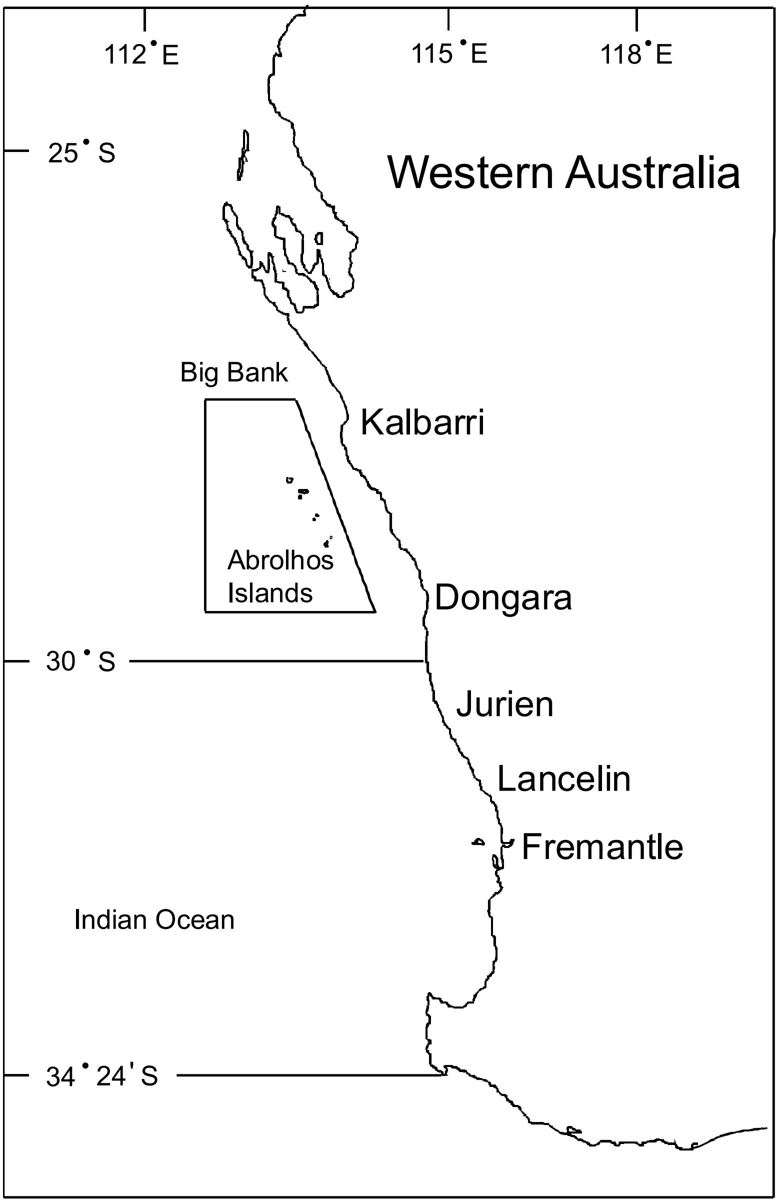

Figure 1. The areas on the Western Australian coast, from where pre-spawning female *P. cygnus* used in this study were collected.

7.4 Results

7.4.1 Egg and phyllosoma size measurements and phyllosoma competency

The diameters of the early-stage eggs and the lengths and competencies of the phyllosoma are presented by capture location and maternal size class in Figs. 2a, b c and d.

In all cases, egg diameter, phyllosoma competency and phyllosoma length-at-hatch did not differ significantly ($p > 0.05$) between site of maternal capture or maternal size class. The slight downward and upward trends in larval competency and larval length-at-hatch, respectively, with increasing maternal carapace length, are far from significant and are merely products of a small sample size, i.e., $n = 21$.

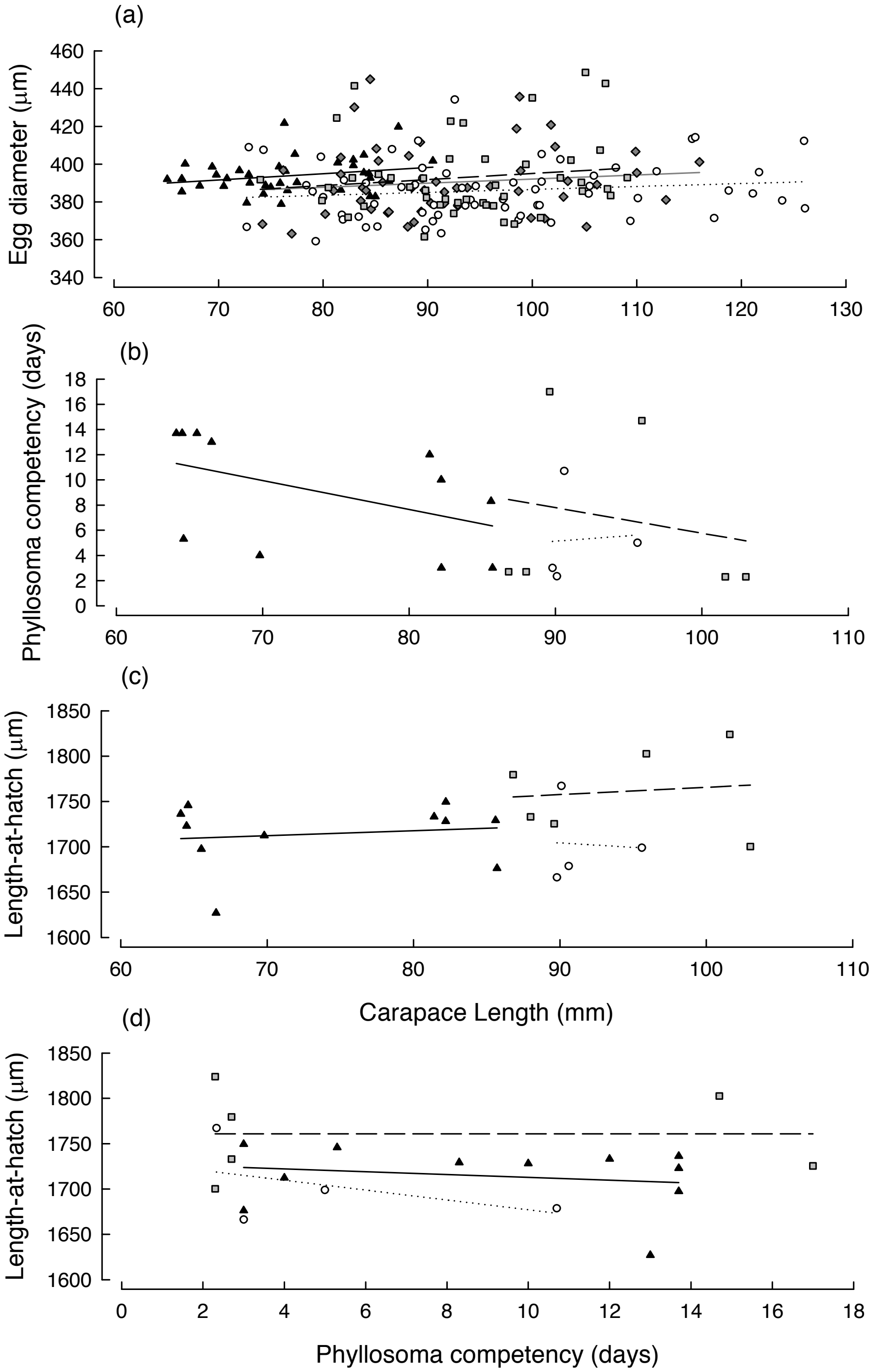

Figure 2. Relationships between the maternal carapace length *and* (a) egg diameter, (b) phyllosoma competency, and (c) phyllosoma length-at-hatch, for different maternal locations of capture. (d) Relationships between phyllosoma competency and phyllosoma length-at-hatch for different maternal locations of capture.

7.4.2 Lipid composition

The mean total lipid content of the eggs sampled at the three sites was 134 mg g^{-1} dry mass and this decreased in the day 0 phyllosoma larvae to 63% of the egg values (84 mg g^{-1} dry mass) (Table 1). The mean lipid value for day 0 phyllosoma in this study was nearly twice that previously reported for *P. cygnus* by Liddy et al. (2004) (43–54 mg g^{-1} dry mass), but close to that reported for *Jasus edwardsii* (87–155 mg g^{-1} dry mass) (Nelson et al., 2003; Ritar et al., 2003; Nelson et al., 2004).

The main LC in all eggs and phyllosoma was polar lipid (86% of mean total lipid), followed by sterol, which was slightly more abundant in day 0 phyllosoma (11%) than in eggs (8%) (Table 1). Triacylglycerol was more abundant in eggs (4%) than in phyllosoma (0.4%). Wax ester, free fatty acids and diacylglycerol were present in only minor proportions. The relative proportions of lipids in the different classes were consistent with those recorded for day 0 phyllosoma of *P. cygnus* (Liddy et al., 2004) and *J. edwardsii* (Phleger, 2000; Phleger et al., 2001; Nelson et al., 2003; Nelson et al., 2004).

Table 1. Lipid class composition (%) and total lipid content (mg g-1) of western rock lobster eggs and phyllosoma. Presented as mean±se.

		Percentage composition						Total lipid
	n	Wax ester	Triacylglycerol	Free fatty acid	Diacylglycerol	Sterol	Polar lipid	(mg g^{-1})
Mean								
Eggs	23	0.4 ± 0.1	4.2 ± 0.5	0.8 ± 0.1	0.8 ± 0.1	8.4 ± 0.3	85.5 ± 0.5	133.9 ± 5.4
Phyllosoma	21	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.9 ± 0.1	10.7 ± 0.2	87.3 ± 0.2	84.0 ± 1.9
Abrolhos Small								
Eggs	6	0.4 ± 0.1	3.9 ± 1.3	0.7 ± 0.1	0.8 ± 0.2	7.9 ± 0.8	86.2 ± 1.3	128.3 ± 11.3
Phyllosoma	6	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.0	0.8 ± 0.1	10.8 ± 0.4	87.2 ± 0.5	85.6 ± 4.7
Abrolhos Big								
Eggs	7	0.4 ± 0.1	4.7 ± 0.9	0.9 ± 0.1	0.7 ± 0.1	8.5 ± 0.7	84.9 ± 1.0	125.9 ± 11.9
Phyllosoma	5	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.8 ± 0.2	10.7 ± 0.5	87.4 ± 0.5	78.7 ± 3.5
Dongara								
Eggs	4	0.2 ± 0.0	4.1 ± 0.5	0.9 ± 0.1	0.8 ± 0.1	8.2 ± 0.4	85.9 ± 0.8	134.9 ± 10.4
Phyllosoma	4	0.4 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.9 ± 0.1	10.6 ± 0.4	87.4 ± 0.4	82.4 ± 3.6
Lancelin								
Eggs	6	0.3 ± 0.1	3.9 ± 0.9	0.7 ± 0.1	1.0 ± 0.1	9.0 ± 0.4	85.1 ± 0.6	148.3 ± 7.8
Phyllosoma	6	0.4 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	1.3 ± 0.1	10.7 ± 0.5	87.0 ± 0.4	87.9 ± 2.1

The qualitative (%) and quantitative (mg g^{-1}) LC compositions of eggs and newly hatched phyllosoma were shown by ANOSIM to differ significantly ($p < 0.02$) in only one of the six groupings: the quantitative composition of eggs from different maternal size classes (Table 2). The MDS plot of each maternal size-class grouped all points relating to the largest size-class below those of the medium size-class, with most of those in the smallest size-class lying above and to the left (Fig. 3). SIMPER showed that polar lipid contributed the most to the total concentrations of lipids in the eggs in the three maternal size-classes. Mean polar lipid and sterol concentrations increased progressively with increasing maternal size-class. The dissimilarities between the three maternal size-class groupings had similar magnitudes, ranging from 10.7–13.0% (Table 3). The LC that contributed most to the dissimilarity between each of the three size classes are shown in Table 3.

Table 2. *R*-statistics and significance levels from ANOSIM for the qualitative (%) and quantitative (mg g^{-1}) measures of lipid class and fatty acid compositions of *Panulirus cygnus* eggs and newly hatched phyllosoma from two maternal catch sites, three maternal size classes, four maternal capture site/size-class groupings, three phyllosoma competency levels and three phyllosoma length-at-hatch size-classes.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

	Mother			**Phyllosoma**	
	Capture sites (2 sites)	Size class (3 sites)	catch site / size class (4 groups)	Competency (3 levels)	Length-at-hatch (3 size-classes)
Lipids (n = 5)					
Egg %	0.04	0.12	0.07	-0.01	-0.17
Phyllosoma 0 %	0.10	0.05	0.01	-0.09	0.01
Egg mg g^{-1}	0.05	0.25 *	0.06	-0.04	-0.09
Phyllosoma 0 mg g^{-1}	0.10	0.04	0.07	-0.04	-0.03
Fatty acids					
Egg % (n = 24)	0.04	0.02	0.214 *	-0.01	-0.10
Phyllosoma 0 % (n = 20)	0.09	0.14	0.326 **	0.01	-0.12
Egg mg g^{-1} (n = 36)	0.08	-0.03	0.09	-0.01	0.01
Phyllosoma 0 mg g^{-1} (n = 33)	0.12	0.03	0.275 **	-0.06	-0.11

Table 3. Lipids that accounted most for the dissimilarities **(bold)** in lipid class composition of the newly-hatched *Panulirus cygnus* eggs of three maternal size-classes

Maternal size-class	Large	Medium	Small
Large	—	Sterol	Polar lipid
Medium	**10.69**	—	Triacylglycerol
Small	**13.03**	**11.84**	—

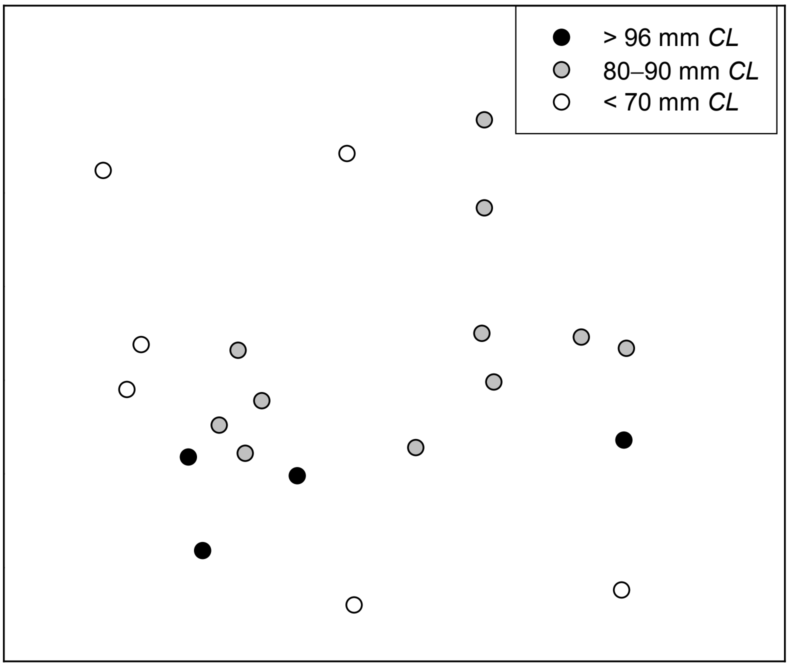

Figure 3. MDS ordination of the qualitative lipid class composition of *P. cygnus* eggs, grouped by size-class.

7.4.3 Fatty acid composition

The percentage FA composition of eggs and phyllosoma are shown in Table 4. Dominant FA in both egg and phyllosoma were: AA (15–20% of total FA); 16:0 (12–16%); EPA (10–13%); 18:1(n-9)c (8–14%); 18:0 (10–12%); DHA (6–10%); 16:1(n-7)c (2–5%) and 18:1(n-7)c (4%). Absolute FA content decreased from egg to phyllosoma.

The qualitative (%) composition of FA differed significantly between both eggs and newly hatched phyllosoma grouped by maternal catch site/size class ($p = 0.02$ and 0.003, respectively). The quantitative (mg g^{-1}) FA composition also differed significantly with maternal catch site/size class, but only in the case of newly hatched phyllosoma ($p = 0.01$) (Table 2). When grouped by the four other factors, i.e., maternal capture site, maternal size class, larval competency and larval length, neither the qualitative nor quantitative FA compositions differed significantly ($p > 0.05$) in either the eggs or newly hatched phyllosoma (Table 2).

MDS ordination of the qualitative FA composition of the eggs, grouped by maternal catch site/size class, aggregated the points representing large coastal females towards the middle to upper right of the plot, medium-sized coastal females in the centre, medium-sized island females to the centre–left and small-sized island females towards the bottom right (Fig. 4). The use of SIMPER showed that palmitic acid (16:0) typified the four maternal catch site/size class groups. The dissimilarities between each of the four maternal capture site/size class groupings had similar magnitudes, ranging from 5.9 to 7.6% (Table 5). The FA that contributed most to the dissimilarity between each of the four maternal capture site/size-classes are shown in Table 5.

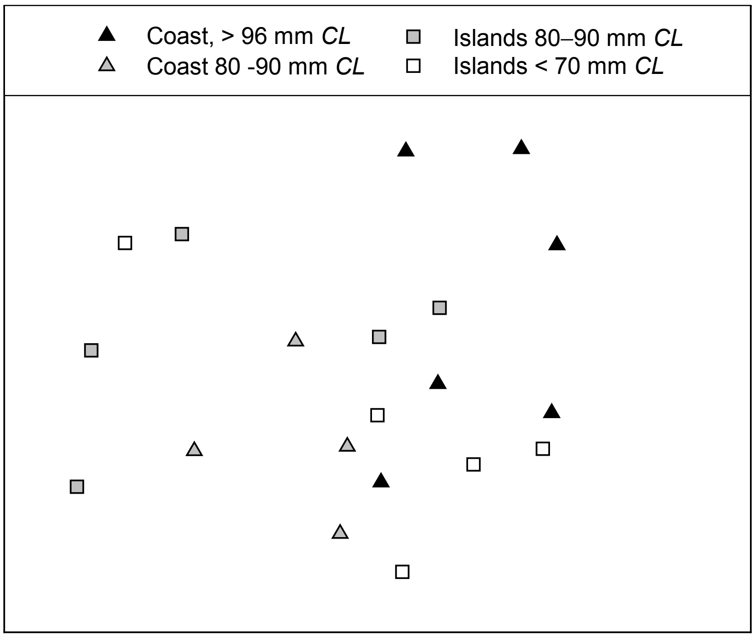

Figure 4. MDS ordination of the qualitative fatty acid composition of *P. cygnus* eggs, grouped by maternal capture site/size-class.

Table 5. The fatty acids that accounted most for the dissimilarities (bold) in fatty acid composition of *Panulirus cygnus* eggs from the four maternal capture site/size-class groups

Maternal capture site/size-class	Coast large	Coast medium	Islands medium	Islands small
Coast large	—	20:4(n-6) AA	18:2(n-6)	18:1(n-9)c (+18:3(n-3))
Coast medium	**6.68**	—	22:4(n-6)	20:1(n-9)c
Islands medium	**5.85**	**7.61**	—	18:2(n-6)
Islands small	**6.24**	**6.51**	**6.97**	—

The FA composition of the newly hatched phyllosoma also differed more substantially between maternal capture site/size class when using qualitative (%) rather than quantitative (mg g^{-1}) data. MDS ordination of the qualitative FA composition of newly hatched phyllosoma, grouped by maternal capture site/size-class, aggregated the points representing all coastal females from the top left (large-sized) to the bottom right (medium-sized). The points representing the small-sized maternal size classes from the islands were grouped below and to the left, while those of the medium-sized from the islands were spread across the centre of the plot (Fig. 5). SIMPER showed that the four maternal capture site/size-class groups were all typified by the same FA: 20:4(n-6) AA. The average dissimilarities between each of the four maternal capture site/size-class groupings were small, ranging from 4.6 to 5.7, and slightly lower than those recorded in the eggs (Table 6). The FA that contributed most to the dissimilarity between each of the four maternal capture site/size-classes is shown in Table 6.

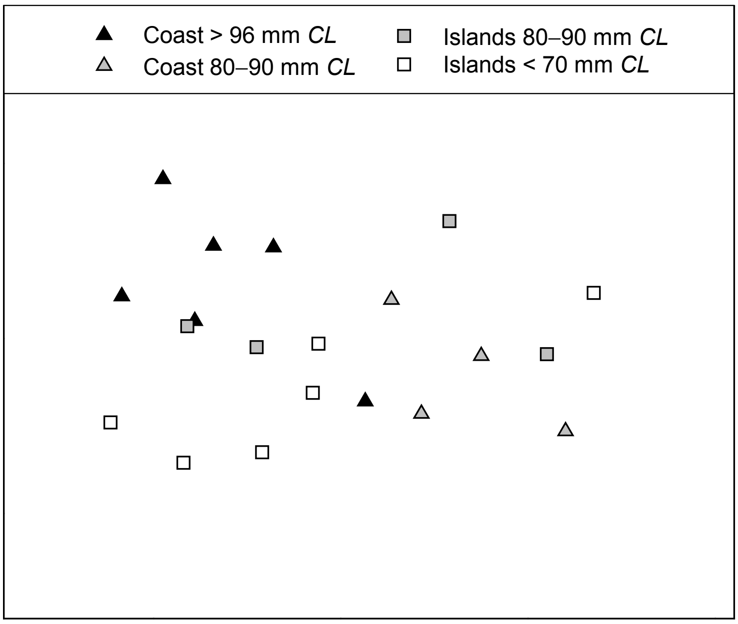

Figure 5. MDS ordination of the qualitative fatty acid composition of newly-hatched *P. cygnus* phyllosoma, grouped by maternal capture site/size-class.

Table 6. The fatty acids that accounted most for the dissimilarities (bold) in fatty acid composition of newly hatched *Panulirus cygnus* phyllosoma from the four maternal capture site/size-class groups

Maternal Capture site/size-class	Coast large	Coast medium	Islands medium	Islands small
Coast large	—	22:6(n-3) DHA	18:2(n-6)	16:1(n-7)c
Coast medium	**5.41**	—	22:6(n-3) DHA	20:1(n-9)c
Islands medium	**4.65**	**5.67**	—	18:0
Islands small	**5.17**	**4.55**	**5.58**	—

7.4.4 Protein content

The mean protein content of egg (224.8±9.2 mg g^{-1}) and day 0 phyllosoma (253.8±4.0 mg g^{-1}) samples collected from coastal sites did not differ significantly ($p > 0.05$) from those collected at the Abrolhos Islands (214.1±7.5 and 243.9±8.1 mg g^{-1}, respectively). The mean protein content in the eggs (218.7±5.8 mg g^{-1}) was significantly ($p < 0.001$) different than that in day 0 phyllosoma (248.6±2.8 mg g^{-1}).

7.4.5 Water content

There was no significant difference in water content between eggs or day 0 phyllosoma sampled at the coast and island sites, or the two maternal size classes. The mean water content of the eggs was 22%; it decreased to ~15% in early stage phyllosoma.

7.5 Discussion

Lipids, and to a lesser extent proteins, have been shown to be the major energy-rich fuel for larval development in a range of decapod species (Roustaian et al., 2001, Lovrich et al., 2003, Anger and Moreira, 2004).

Overall, there was limited indication from either the qualitative or quantitative composition of lipids in eggs and early-stage phyllosoma to suggest that the composition might have been influenced by maternal size or capture site. The significant ($p < 0.02$) differences in the qualitative composition of the LC in eggs from the three maternal size-classes were not apparent in the phyllosoma that hatched from the same batches of eggs. Furthermore, the LC responsible for the apparent dissimilarity between egg samples from the three maternal size-classes were not consistent: depending on the size class, triacylglycerol, polar lipid or sterol concentrations were responsible.

Differences in the qualitative and quantitative composition of FA in the eggs and phyllosoma were more consistent than the differences in LC; the interaction of capture site/size-class was a significant determinant of FA composition in eggs and phyllosoma. Also, there was some consistency in the FA responsible for the dissimilarities in the FA composition of eggs and phyllosoma in the four maternal capture site/size-class groups, with 18:2(n-6) and 20:1(n-9) c occurring in both (see Tables 5 and 6). However, as with the LC analysis, there was no consistency in the dissimilarities of FA composition when viewed across different capture site/size-class groups.

Our interpretation, therefore, is that *P. cygnus* generally can conserve egg and in particular larval LC and FA profiles, independent of the maternal size and catch site. A minor exception is observed with TAG (4.2% in eggs), with this storage lipid class rapidly depleted on hatching. For the other lipid classes, predominately PL in *P. cygnus*, this would involve using key PUFA sparingly and maintaining other FA proportions to ensure the essential PUFA profiles are maintained in the phyllosoma. This finding is consistent with the report by Smith et al. (2004) on the influence of diet on lipid and FA composition in *Jasus edwardsii* broodstock. Those authors showed that for broodstock fed either squid or beef-based diets for 5 months, the lipid and fatty acid compositions of ovary and hatched phyllosoma were both similar. There was no indication from quantitative or qualitative analyses of either LC or FA composition in this study to suggest that they influenced larval competency or length-at-hatch.

Mean protein content increased in the development from eggs to larvae, probably due to the development of protein-dominated tissues such as integument, muscle and nervous systems, in the larvae. It is well known that lipid reserves are utilised preferentially by decapod larvae (Anger, 2001). We consider that this would make protein content a less likely indicator than mean lipid content, of potential larval competency in *P. cygnus*.

The fact that overlapping sizes represented at both the coastal and island sites and also the maternal sizes represented only on the coast had similar phyllosoma competency suggests that neither the site at which the maternal lobster spawns, nor its size, nor the size of larvae at hatch is a good indicator of larval competency. It seems more likely that larval competency depends on the condition of the maternal parent initially, and then presumably also the availability and abundance of food in the environment into which the phyllosoma hatch. On the Abrolhos Islands, the food sources of the adult lobsters are typically those found in coral reef surroundings, in contrast with food available to the coastal animals that mostly inhabit cooler water habitats. The smaller size-at-maturity of Abrolhos lobsters has been noted above.

It is relevant here to consider the research by Smith et al. (2004). They showed that *J. edwardsii* broodstock caught in the wild or raised in captivity and fed very different diets (squid or beef-based), with markedly different FA profiles, produced larvae similar in size at hatching with similar FA profiles. However, the larvae grew at different rates. By stage IV, larvae from wild-caught broodstock were both significantly larger and had significantly greater survival rates than larvae from captive-held broodstock. Likewise, Liddy et al. (2004) showed that, in *P. cygnus*, different LC and FA assumed importance as the larvae progressed through developmental stages. The implication from that study, therefore, is that early-stage larvae showing differences in their biochemical makeup could have different responses to starvation at later stages in their larval development, although much would depend on the larva's diet as it developed.

A factor that has not been considered in this study is the paternal contribution to the genetic makeup of the larvae. Paternity affects early life-history traits in fish (Panagiotaki and Geffen, 1992; Reynolds and Gross, 1992; Rideout et al., 2004). The unknown paternal contribution to the parentage of the larvae used in this study may explain some of the variation in those factors examined.

In summary, all differences in biochemical composition and phyllosoma competency between maternal size and their catch sites were small and mostly non significant. Our results suggest that the quality of larvae produced by individual females may be more important in influencing larval competency in *P. cygnus* than the maternal attributes (size and catch site) that were tested. The factors responsible for producing differences in larval competency at the level of individual animals is unclear; the differences may be due to the female's physiological condition at the time of spawning, which could be influenced by, for example, previous dietary intake and moulting or spawning history. Additionally, the influence of parental genetic make-up cannot be ignored. Isolating these many possible influences will be a challenging prospect for future research.

7.6 Acknowledgements

We appreciate the assistance of M. Brock and M. Brown with protein analyses. G. Dunstan managed the GC and D. Holdsworth the GC-MS facilities at CSIRO Marine and Atmospheric Research. Mr. Dan French scanned and measured all the egg samples. Drs. Nick Caputi, Sabine Daume, Danielle Johnston, and Vivienne Mawson reviewed earlier drafts of this manuscript and provided many helpful comments. This study was part of Fisheries Research and Development Corporation Project 2003/005.

8.0 Spatial and temporal changes in egg production in the western rock lobster (*Panulirus cygnus*) fishery

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Citation: Melville-Smith, R., de Lestang, S. and Thomson, A.W. (in press). Spatial and temporal changes in egg production in the western rock lobster (*Panulirus cygnus*) fishery. *New Zealand Journal of Marine and Freshwater Research*.

8.1 Abstract

Size at maturity in the western rock lobster (*Panulirus cygnus*) fishery in Australia increases from north to south and female lobsters carry either one or two batches of eggs per season depending on their carapace length. There has been a steady decrease in the size at first maturity of western rock lobster over the last 30 years, and there are now significantly more small females, many below the legal minimum size, contributing to the brood stock than any time since the fishery has been researched. Biological data were used to show the current contribution to egg production in different management regions of the fishery and historical length frequency data were used to show how egg production has changed in those regions over time. Reasons for the change in size at maturity in this fishery are unclear, but the outcome has been that egg production is now more evenly distributed across management zones. Egg production was high at the Abrolhos Islands in the 1990s owing to strong year classes of breeding animals entering the fishery over those years. In the period since 2000, egg production has fallen sharply at the islands, but has increased in the coastal population compared with the 1980s and 1990s.

Keywords: *Panulirus cygnus*; egg production; size at maturity; Western Australia; efficiency increase

8.2 Introduction

Western rock lobster *Panulirus cygnus* (George, 1962) only occur in commercial quantities on the lower west coast of Australia, from east of Cape Leeuwin (34° 24'S) in the south to Shark Bay (24°45'S) in the north (Fig. 1) (Phillips et al., 2000). Juvenile animals tend to be found in shallow water (<40 m) and breeders tend to be in depths of 40–80 m. The fishery is managed in three separate zones, the offshore Abrolhos Islands (Zone A), a coastal region north of latitude 30°S (Zone B), and a southern coastal region (Zone C) (Fig. 1) (Phillips et al., 2000).

Western rock lobsters have particular economic importance, as Australia's most valuable single species wild capture fishery (Phillips et al., 2007). The fishery is recognised as being sustainably managed (see Phillips et al., 2007) and was the first fishery in the world to receive Marine Stewardship Council accreditation as a well-managed fishery (Phillips et al., 2007). Phillips et al. (2007) identified five principles that have guided the management of the western rock lobster fishery since the 1970s. These can be summarised as: (1) acquiring reliable catch, effort, and length-frequency data to monitor the effects of fishing pressure on the exploited population; (2) setting a legal minimum size, protection of females, and gear restrictions with adequate inspection and legislative backing; (3) controlling fishing effort to protect the breeding stock and maximize the benefit from the resource; (4) understanding the stock/recruitment/environment relationship; and (5) communicating effectively and regularly with both commercial and recreational fishers to ensure the objectives and methods of management are well understood.

A general theme that runs across these guiding principles relates to the need to maintain the breeding stock at a level that protects the fishery from recruitment overfishing and to have the appropriate data, management tools, compliance enforcement and research expertise, to achieve this goal. The way that these objectives have been achieved in the fishery has been discussed in recent reviews (see Caputi et al., 2003; Phillips and Melville-Smith, 2005; Phillips et al., 2007). Research published since these reviews has provided evidence that some life-history features of western rock lobster have progressively changed over time. The size at first maturity of both sexes has decreased across the fishery over the last 30 years (Melville-Smith and de Lestang, 2006) and it is believed that the size at which female lobsters produce more than one batch of eggs per season has decreased in a similar way over time (de Lestang and Melville-Smith, 2006a). There is also evidence that the size at which juvenile western rock lobster undertake their offshore migration to the deep-water breeding grounds has decreased (N. Caputi, pers. comm.).

The size at maturity of western rock lobster has consistently shown spatial differences across the fishery (Chittleborough, 1976; Grey, 1979; Chubb, 1991; Melville-Smith and de Lestang, 2006). Both sexes mature at a smaller size in the northern part of the fishery and the smallest mature animals are found at the offshore Abrolhos Island (Melville-Smith and de Lestang, 2006). These differences in size at maturity along the coast have led to the legal minimum size, which is the same across the whole fishery, offering different degrees of protection to breeding animals. As a consequence, and together with differences in harvest rate across the fishery, there have been very disparate regional contributions to egg production in the fishery which have varied over time. Morgan et al. (1982) estimated from their study conducted in 1979 that the breeding population at the Abrolhos Islands contributed 14% of the total egg production. Subsequently, Chubb (1991) estimated from research conducted between 1984 and 1989, that the Abrolhos Islands contributed between 45 and 65% of the total egg production of the fishery. In particular, he considered that the coastal population of female lobsters in the 1980s were being fished for 1 or 2 years before reaching maturity, compared to the Abrolhos Islands, where high densities of sexually mature sublegal sized lobsters were producing an estimated 35% of the total egg production of the fishery (Chubb 1991). The difference between the estimated contributions to total egg production made by the Abrolhos Islands in the 1970s compared with the 1980s, was considered by Chubb (2000) to be probably owing to rapid fishery-induced declines in egg production by the coastal breeding population.

The object of this study was twofold: first to use the best available empirical data to define the contributions to egg production from the different management zones in the fishery breeding grounds and, secondly, to use the contributions made by different size classes of lobsters over three decades, to estimate relative changes in egg production between the management zones and depths within these zones.

8.3 Methods

An onboard commercial catch length frequency-monitoring programme has been in existence for over 35 years, operating out of the ports of Fremantle, Lancelin, Jurien Bay and Dongara, and in more recent years Kalbarri, as well as the offshore Abrolhos Islands (Fig. 1) (Caputi et al., 2000). Monitoring the commercial catch occurs monthly throughout the season in four depth ranges measured in fathoms (0–10 fm, 11–20 fm, 21–30 fm, and >30 fm), which for practical purpose of this study were considered to be 0–20 m, 21–40 m, 41-60 m, and >60 m, to make them compatible with metric bathymetry depth intervals in ArcView GIS. For each depth sampled, the latitude and longitude of the pot sets are recorded, together with the number of pots sampled, the carapace length (CL), sex and other biological details of a minimum of 300 lobsters in the catch.

We used commercial catch length monitoring data for the coastal management zones (Zones B and C) in the months of February–June to obtain an annual index of coastal egg production. Only during these months does the commercial fishery target resident lobsters, many of which are mature, as opposed to the earlier part of the season when the fleet targets migrating immature lobsters (Caputi et al., 2000). This index, which is a catch rate measure of eggs per pot lift, was derived by estimating the batch fecundity of each female sampled above size at maturity, using a CL-fecundity relationship developed by Chubb (1991): $F = 1.92CL^{2.69}$, where F is the number of newly spawned eggs and taking into account spatial variations in size at maturity (Melville-Smith and de Lestang, 2006) and size at double breeding (de Lestang and Melville-Smith, 2006a). Even though commercial length frequency data were used to calculate egg production, it was necessary to assign maturity status based on CL (Table 1), because most breeding animals moult out of a breeding condition (setose) from February to May (de Lestang and Melville-Smith, 2006a) and without ovigerous setae, they are indistinguishable via externally visible characteristics, from immature lobsters (Melville-Smith and de Lestang, 2005).

Table 1. Carapace length (mm, mean ± 1SE) at which 50 and 95% of female *Panulirus cygnus* produce one (SB) and two batches (DB) of eggs per spawning season at six locations along the west coast of Australia (de Lestang and Melville-Smith 2006a).

	Fremantle	Lancelin	Jurien	Dongara	Kalbarri	Abrolhos
SB_{50}	87.5 ± 0.22	82.2 ± 0.34	81.4 ± 0.32	74.9 ± 0.49	77.2 ± 0.34	65.0 ± 0.27
SB_{95}	92.8 ± 0.57	89.6 ± 0.87	90.6 ± 1.00	88.1 ± 1.26	87.5 ± 0.86	76.3 ± 0.51
DB_{50}	96.6 ± 1.52	90.4 ±0.74	84.6 ± 1.49	84.1 ± 1.46	86.5 ± 1.13	78.7 ± 1.58
DB_{95}	114.8 ± 3.82	100.8 ± 2.05	96.5 ± 3.50	98.6 ± 4.54	100.0 ± 3.48	96.8 ± 3.63

Size at maturity and size at double breeding estimates have previously been determined for deep water locations (40-80 m) at five coastal sites spanning the fishery (Fremantle, Lancelin, Jurien Bay, Dongara, and Kalbarri) (Melville-Smith and de Lestang, 2006). These measures were assumed to change in a progressive linear way between neighbouring locations and this assumption was used to estimate the size at maturity and size at double breeding for lobsters in each 5 nautical miles (9.25 km) transect that did not contain estimates between 27°S and 34°S, in coastal management zones B and C (Fig. 1). A single estimate of size at maturity and size at double breeding was used for all of Zone A.

In addition to spatial differences in size at maturity, Melville-Smith and de Lestang (2006) have also shown temporal changes at coastal sites, but not at the Abrolhos Islands. Their results showed that size at the onset of maturity declined at a similar rate (average 0.42 mm/year) and this value was therefore used in this study to retrospectively estimate the size at maturity and size at double breeding for all years before 2002, when robust size at maturity estimates were produced for the five coastal locations from a comprehensive fishery-independent sampling regime during the peak of the breeding season (see below). These estimates were applied to the estimates of size at maturity and double breeding that were determined for each 5-min. latitudinal transect in the coastal data set. No temporal changes were made to size at maturity or size at double breeding for Zone A.

Western rock lobsters mature at a much smaller size at the Abrolhos Islands than at the coast (Melville-Smith and de Lestang, 2006), resulting in a substantial portion of the egg production being produced by animals below the 76 mm CL legal size limit. Commercial pots are fitted with escape gaps that allow most of the catch below the legal size limit to escape and therefore using those monitoring data would have grossly underestimated the egg production index for the Abrolhos Islands. Therefore, egg production below the legal size limit at the Abrolhos Islands was adjusted using Fishery Independent Breeding Stock survey (IBSS) data, which were derived from an annual 12-day survey which has been undertaken since 1990 during the height of the breeding season (Melville-Smith and de Lestang, 2006). The survey covers all of the major island groups, uses standard pots with closed escape gaps, sets the pots on the same GPS positions each season, and covers a range of depths to 40 m (Melville-Smith and de Lestang, 2006).

IBSS data were used to determine the annual proportion of egg production attributable to females above and below legal size, in two depth categories (0–20 m and >20 m). This ratio was used to adjust upwards, biases in the egg production calculated using commercial monitoring length frequency data. No IBSS data were available for years before 1990, so the mean egg production ratio between lobsters above and below legal size for the 1990s, was used to adjust commercial monitoring data at the Abrolhos Islands in the 1980s.

In considering decadal shifts in egg production, it was necessary to account for efficiency increases resulting from improvements in gear technology and industry experience, otherwise greater fishing efficiency would have the effect of biasing egg production upwards over time. Estimates of efficiency increases in the western rock lobster fishery vary (Caputi et al., 2000) and the ones applied in this study were 6% and 2% per year in zones B and C since the 1980s, and in Zone A a single one-off increase in the 1993/94 season of 18% (Wright et al., 2006).

Weighting observations by the number of pot lifts used to calculate that particular catch rate, decadal egg production indices (eggs/potlift) were modelled for the fishery as:

$$Y_{ijklmon}^{0.25} = z_i + y_o(d_j) + a_k + f_l(z_i) + fd_{lj}(z_i) + gf_{ml}(z_i) + zd_{ij} + \varepsilon_n$$

where $Y_{ijklmon}$ is the catch of eggs/potlift for zone i, decade j, month k, depth l and latitudinal block m (nested in zone i) for observation n; z_i is zone $i \in$ (Zones A, B, C); d_j is decade $j \in$ (1980–89,1990–99, 2000–07); a_k is month $k \in$ (February, March, April, May, June); f_l is depth $l \in$ (0–20, 21–40, 41–60, 61–80 m); g_m is latitudinal block m (each g_m belongs to a specific zone); and y_o is year o.

Least-squares means (SAS Institute Inc. 1989) were calculated for different factors and back-transformed to obtain standardised eggs per pot lift for each factor level.

Egg production indices were calculated for each of the three decades (1980–89, 1990–99 and 2000–2006) in each management zone, by taking the standardised estimate of egg production (eggs/potlift) for each zone over each decadal period and multiplying by the approximate area (in km^2) for each zone (calculated using Arcview GIS). One scenario considered decadal changes in zonal egg production without accounting for efficiency increases associated with improvements in gear technology, whereas a second scenario incorporated the efficiency estimates of Wright et al. (2006). Egg production indices, standardised by month, latitudinal block and year, were calculated in four depth ranges (0–20 m, 21–40 m, 41–60 m, 61–80 m) in the three management zones in the fishery.

8.4 Results

The spread of western rock lobster sampling between February and June 1981/82– 2006/07 shows that the bulk of sampling was close to the ports that are monitored, but there was also good representation over a much wider area of the grounds (Fig. 1). Standardised egg production averaged over time for the western rock lobster fishery in the same depth and latitudinal blocks showed that the bulk of egg production was centred around the Abrolhos Island region of the fishery, whereas it occurred mostly in offshore, deeper water regions along the coast (e.g. 40-80 m) (Fig. 2).

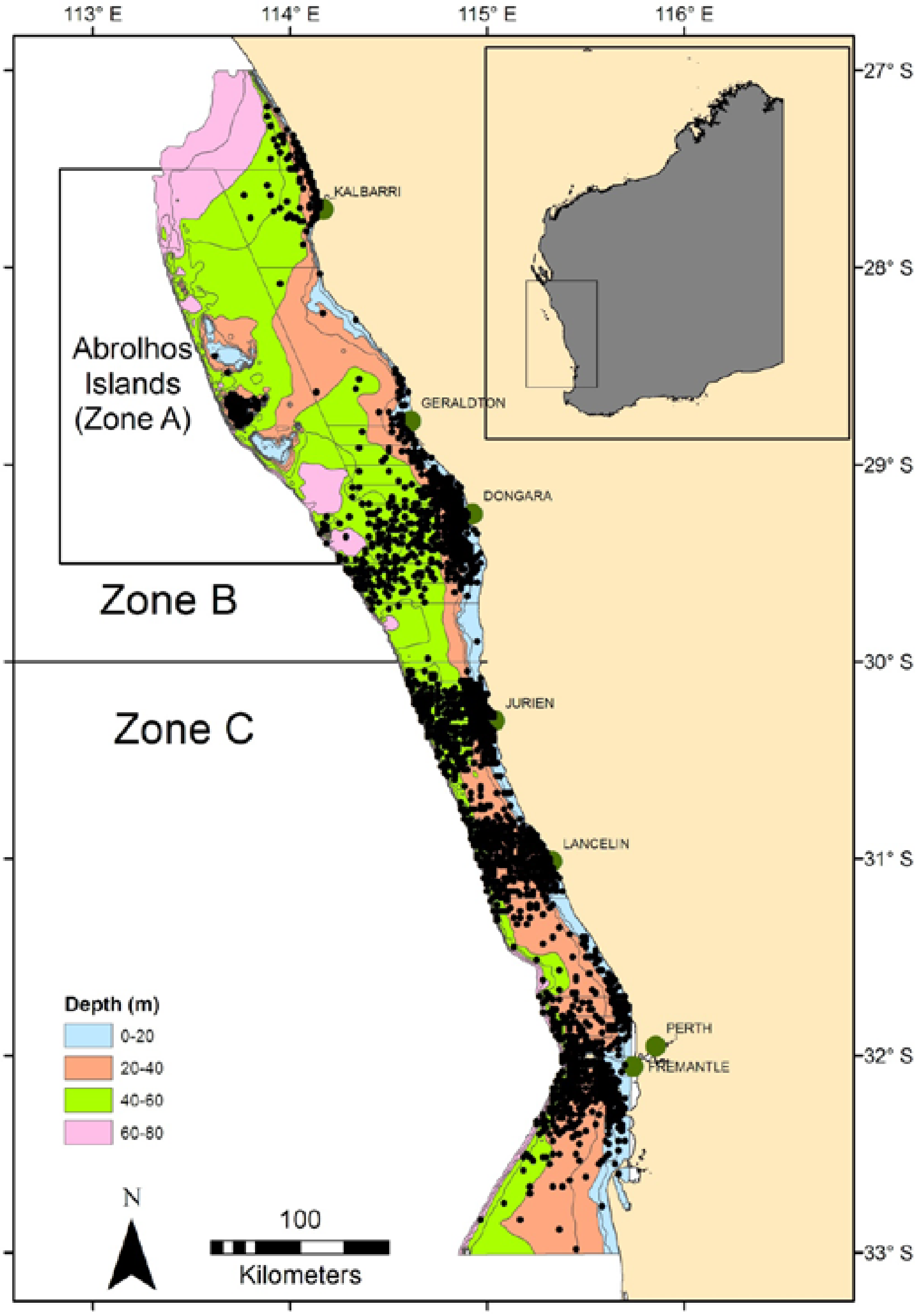

Figure 1. Management zones and 20, 40, 60, and 80 m bathymetry lines in the *Panulirus cygnus* fishery and locations sampled between March and June 1981/82–2006/07, Western Australia.

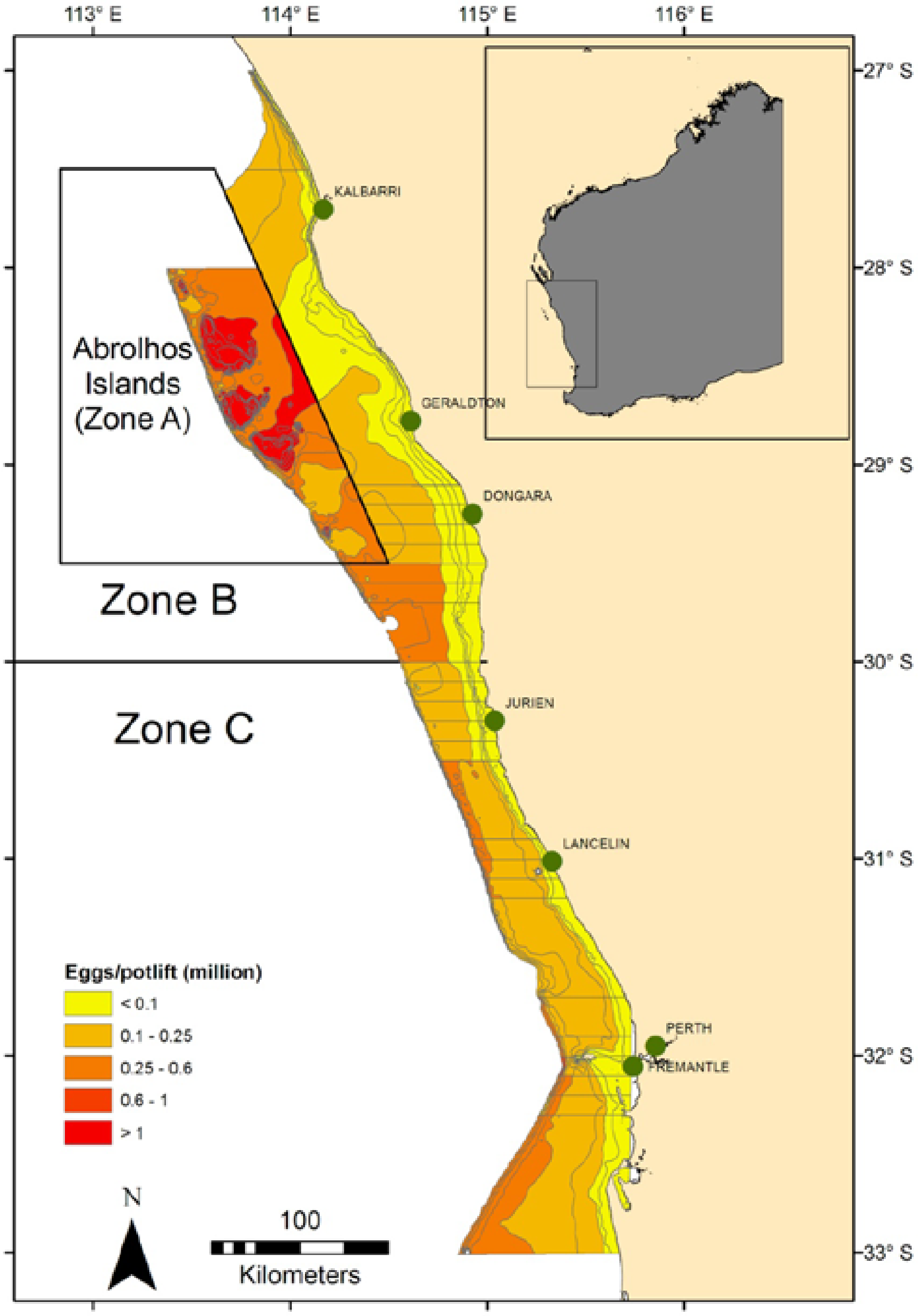

Figure 2. Mean (±1SE) annual percentage egg production of *Panulirus cygnus* lobsters above legal size sampled in the Independent Breeding Stock Survey at the Abrolhos Islands in the 0–20 and 20–40 m depth intervals, as a percentage of total egg production.

There was a general decrease in the contribution to total egg production by legal sized females at the Abrolhos Islands in the mid to late-1990s in both shallow and deep waters (Fig. 3). This was followed by a steady increase, particularly in shallow water, to the highest ratio over the 15-year dataset being recorded in the 2006/07 season. A greater proportional contribution to the total egg production index was made by legal sized lobsters in the 20–40 m range, than in the 0–20 m range.

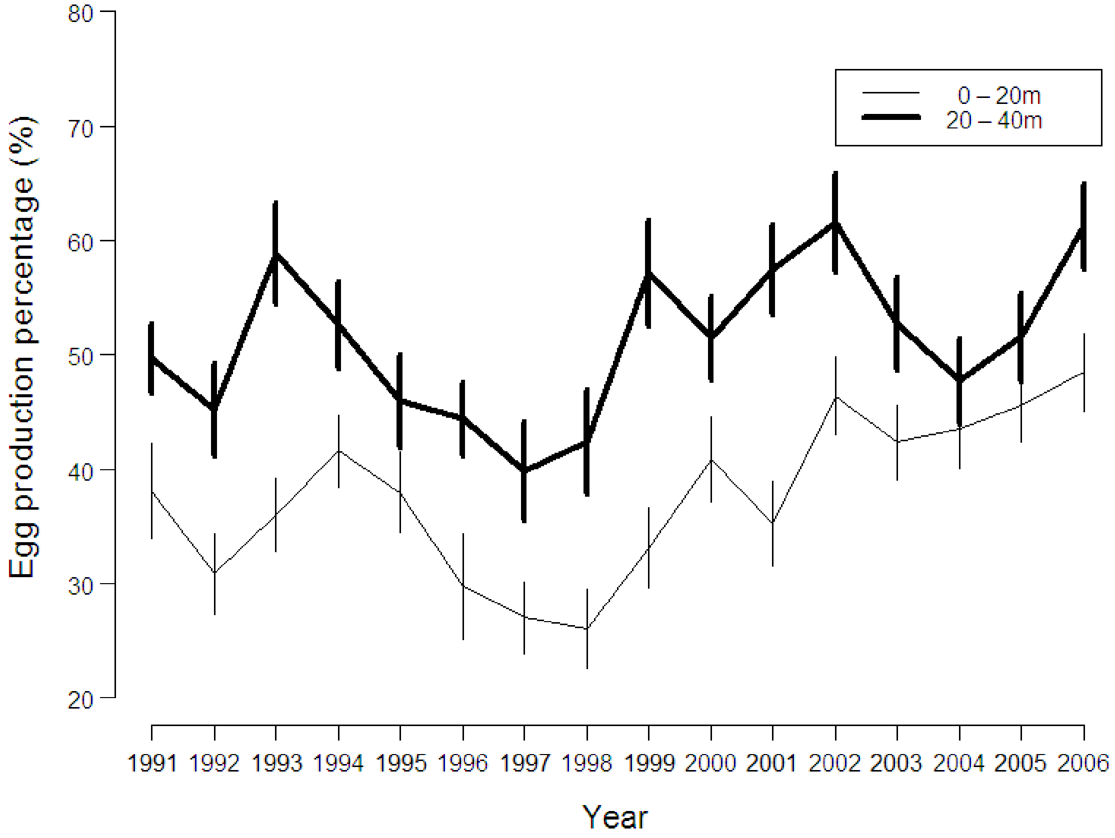

Figure 3. Standardised egg production for *Panulirus cygnus* (millions of eggs/potlift) in grid-depth areas, using all monitoring data 1980/81–2006/07.

Egg production indices over three decades have been calculated for each management zone in the fishery, assuming no efficiency increases (Fig. 4A). The Abrolhos Islands had a high index, with zones B and C showing similar indices, but generally lower ones than Zone A. When accounting for a single one-off increase in fishing efficiency of 18% in the 1993/94 season and annual increases of 6% and 2% in zones B and C, respectively, there was only a slight difference in the index for the Abrolhos Islands, but substantial changes to the proportional contributions to egg production of Zones B and C (Fig. 4B).

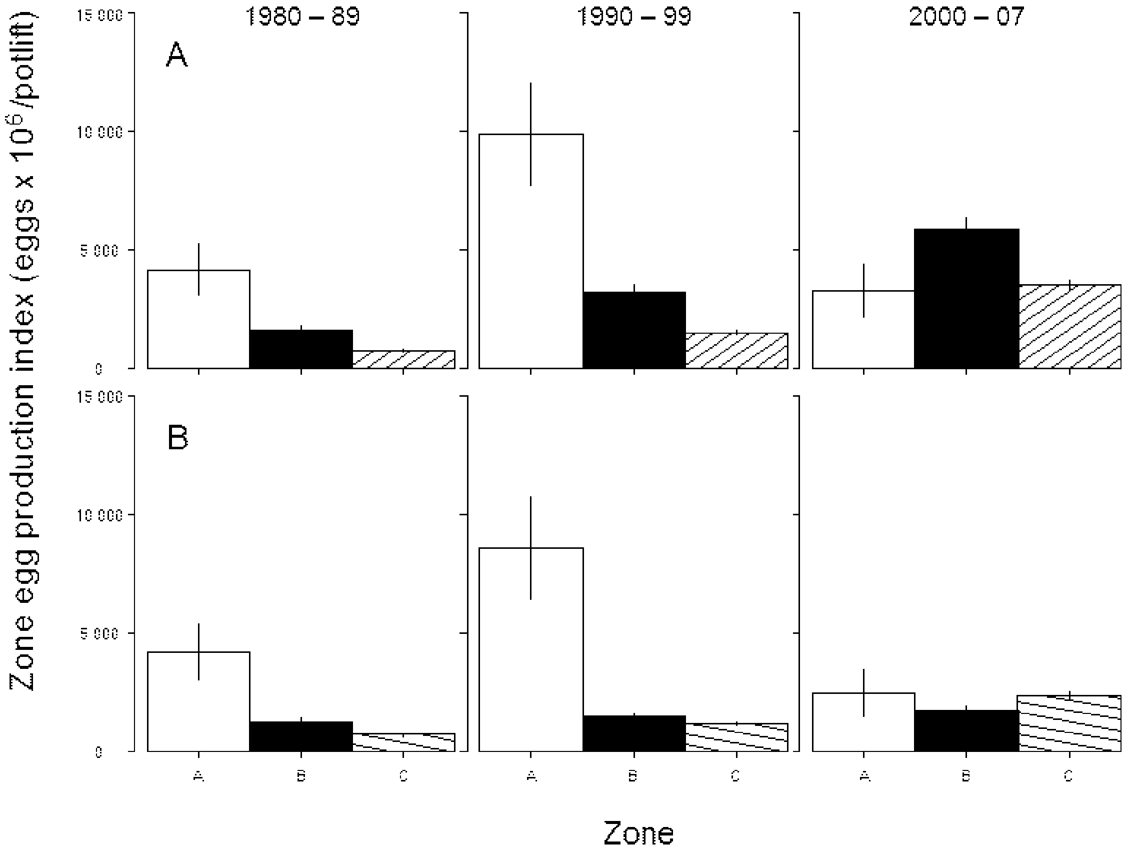

Figure 4. Total egg production indices (no. of eggsx10^6/potlift per management zone) (mean±1SE) for *Panulirus cygnus* for three decades with **A**, no increase in fishing efficiency and **B**, with a single one-off increase in fishing efficiency of 18% applied to Zone A in the 1993/94 season and increases of 6% and 2% applied annually in zones B and C, respectively.

Zone A consistently dominated in terms of the proportional contribution to total egg production, and during the 1980s, produced an estimated 70% of the egg production for the fishery. Between 2000-2006, contribution to overall egg production by Zone A fell to 38%, with Zone B producing 27% and Zone C 36%.

Standardised egg production indices showed that the highest index per unit area in Zone A was consistently measured in the shallows in all decades (Fig. 5A). By comparison, over the same three decades, egg production indices have dominated the deeper, offshore depths >40 m in zones B and C (Fig. 5B, 5C).

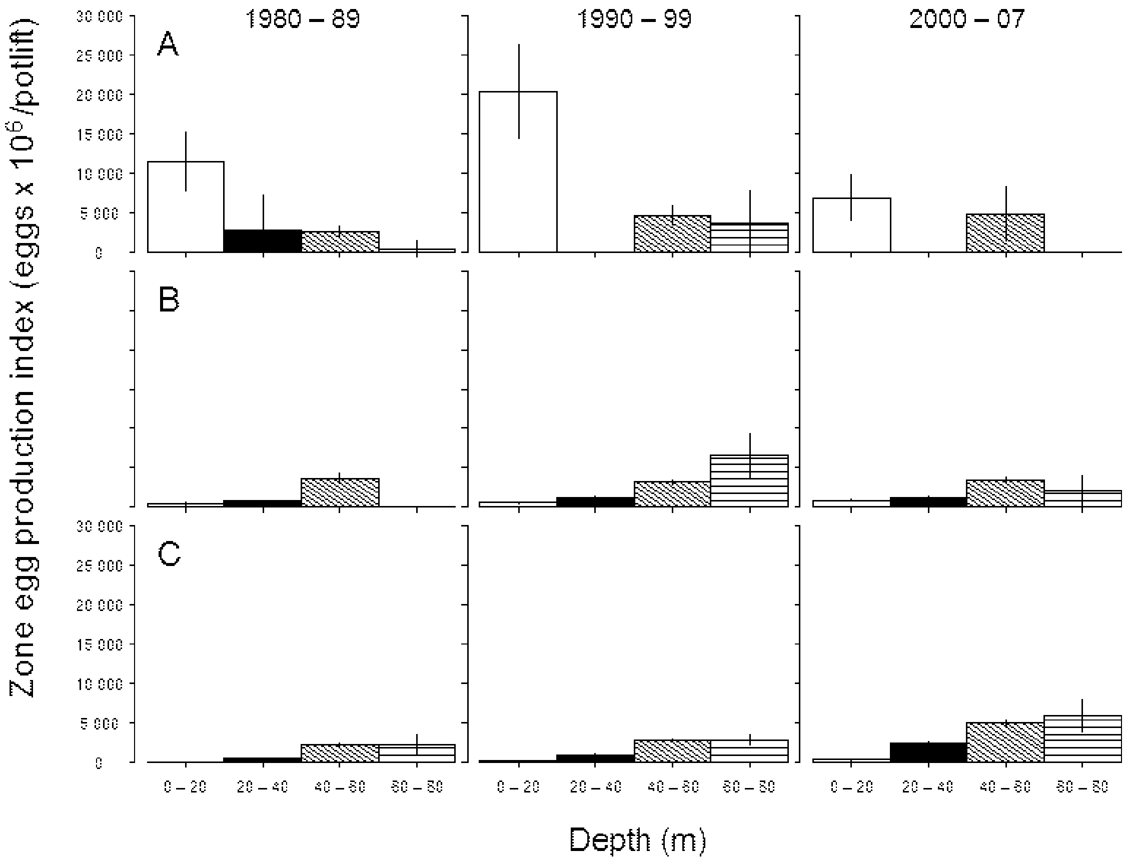

Figure 5. Standardised egg production indices (no. of eggsx106/potlift) (mean±1SE) for *Panulirus cygnus* by depth in A, Zone A, B, Zone B, and C, Zone C.

8.5 Discussion

8.5.1 Spatial changes

The depth distribution of egg production indices in this study confirm Chubb's (1991) assertion that the majority of the western rock lobster breeders occur in deeper offshore waters (36–73 m) at the coast. It is unclear whether there has always been a scarcity of breeding animals in shallow coastal waters, or whether this distribution is a result of greater fishing pressure in the shallows compared with the deep. A series of interviews recording the recollections of fishers and divers who were active as far back as the 1930s, reported large lobsters to have been abundant in the shallows in the Perth metropolitan area, particularly before 1965 (Ottaway et al., 1985). That large lobsters were abundant in shallow waters (< 40 m) at the coast in earlier years, suggests these depths probably made a greater contribution to egg production than currently.

There is a trend for breeding females at the Abrolhos Islands to be larger at depths >20 m. Since both inshore and offshore fishing grounds at the Abrolhos Islands have high exploitation rates, the presence of larger breeders in deeper waters may indicate that females mature in the shallows where the pueruli settle, and gradually disperse into greater depths over time. Alternatively, it could be that the larger deeper water breeders may have recruited to the islands from the coast during the offshore migration phase. Regardless of their origin, egg production indices showed that the index in Zone A was generally higher in the shallows (<20 m) than at other depths.

It has been shown from simulated larval tracks from release to settlement (Griffin et al., 2001), that western rock lobster larvae spawned at the northern and southern extreme ends of the fishery are no less likely to return to settle inshore than those released in the centre of the fishery. Although the species has a coastal distribution that extends from north of Carnarvon (24°S) to south and east of Albany (35°S), we have restricted this estimate of egg production indices to a considerably smaller area, but which covers c.95% of the commercial fishery. Given that the bulk of the lobster population is within this area, we do not consider the omission of data outside of this area to significantly change the conclusions of this study.

Although the Abrolhos Islands only produce about 15-20% of the catch in the western rock lobster fishery, our data suggest that the island group is responsible for a disproportionately large contribution to total egg production in the fishery. High egg production in this zone is owing to large numbers of mature animals that occur at the islands, because of the relatively small size at maturity there compared with the coast (Melville-Smith and de Lestang, 2006), allowing much of the brood stock to be protected by the legal minimum size.

This study assumed that indices of egg production within a grid area can be applied across all grounds of similar depth range within that grid. For this scenario to be valid, there would have to be a similar proportion of suitable breeding ground habitat across each grid area of similar depth range. This assumption will be examined in the future as data on habitat abundance are obtained.

8.5.2 Temporal changes

This study produced a different interpretation of the state of egg production in the western rock lobster fishery to previous results (Caputi et al., 2003; Phillips et al., 2007). Downward changes in size at onset of maturity documented by Melville-Smith and de Lestang (2006), which appear to be owing to a general temperature increase in the marine environment off Western Australia (Melville-Smith and de Lestang, 2006), or a genotypic response to size-selective fishing pressure (Allendorf et al., in press; Melville-Smith and de Lestang, 2006). were not taken into account in previous estimates of egg production. These changes have resulted in substantially more mature females at the coast now being protected by the legal minimum size, which has been the primary driver increasing egg production in the coastal zones of the fishery (Fig. 4A, 4B). In contrast to other studies, which have suggested declines in egg production through the 1980s to the early 1990s and again from the late 1990s onwards, the present study suggests that egg production in coastal zones has increased.

The significant temporal changes since the 1980s in the relative importance to egg production of the three management zones in the western rock lobster fishery, has resulted from a combination of changes to the size at maturity over time on the coast, changes in management measures that have been introduced to protect the brood stock, changes in the proportional contribution to egg production of females below legal minimum size at the Abrolhos Islands, and to estimations that have been used to take increases in efficiency in the fishery into account.

Estimates of egg production indices for the 1980s used the proportional indices for above and below legal size from the 1990s. It is unknown whether this assumption is justified given that there were few large spikes in puerulus settlement at the Abrolhos Islands over the 1980s (de Lestang and Melville-Smith, 2006b). Changes in the contribution to total egg production of lobsters above and below legal size at the Abrolhos Islands since 1991, appear to be related to strong year classes resulting from peaks of puerulus settlement that were recorded at the Abrolhos Islands in the mid-1990s (de Lestang and Melville-Smith, 2006b).

Efficiency increases in the fishery that have occurred over time as a result of improved technology (e.g., larger and faster boats, echo sounders, GPS, plotters, bait) has also placed uncertainty on temporal changes to the egg production index. Management has, by reducing effort in the fishery over time, contained increases in effective fishing effort that would have led to increased exploitation rates and greater pressure on the brood stock (Caputi et al., 2000). These effort reductions have decreased the number of allowable pots in the fishery (Caputi et al., 2000), as well as the number of allowable fishing days in the season (de Lestang and Melville-Smith, 2006b).

Calculations of increases in effective fishing effort in the fishery (i.e., effort taking into account efficiency increases), have previously considered that fishing effort expended in the shallows (<40 m) and deep water (>40 m) expanded at a rate of about 1–1.5% and 2–3% per year, respectively (Caputi et al., 2000). However, there is uncertainty surrounding these adjustments, which are considered by many to be conservative (S. Gill, quoted in Department of Fisheries, 2008).

The efficiency increases that were used in this study were based on depletion of commercial catch rates over the fishing season (Wright et al., 2006) and in Zone B, were considerably greater than the constant adjustment of 1-2% per year that has been applied to previous egg production indices for the western rock lobster fishery (Chubb, 2000). The compensation figure that has been applied is unlikely to be constant over time and over depth, but a potentially far larger bias is the possibility that the efficiency adjustment for one or all of the zones is incorrect.

Another assumption that might have distorted temporal patterns of egg production is the question of whether fishing targeting high rates of legally retainable lobsters necessarily provides a catch composition that is a reliable index of egg production in the area and depth being considered. Some fisheries for example, have recorded stocks contracting into optimal habitats at low population sizes (Marshall and Frank, 1995; Swain and Sinclair, 1994), a response considered to be owing to fish moving to more favourable habitat (or hotspots) from neighbouring areas of lower standard of habitat. It is possible that the distribution of western rock lobsters on the fishing grounds may be in response to the high exploitation rates that have been recorded in the fishery (Wright et al., 2006), by becoming more patchy as the residual animals become more selective in colonising available habitat. Some western rock lobster commercial fishers acknowledge that they now need to set their gear on carefully chosen habitat to achieve catch rates that previously could have been obtained with less accurate placement (T. Lissiman, pers. comm.). An increased concentration of effort in a reduced spatial area of fishing would result in an increase in catchability and harvest rate, as has been observed in the depletion analysis by Wright et al. (2006). Although the 6% increase in ”fishing efficiency” appears to be high, it may reflect the combined effect of increasing fishing efficiency and habitat contraction.

The possibility that the breeding stock may be exhibiting density-dependent habitat selection responses is of concern, because the egg production index used in this study is an indirect form of catch rate. The potential to overestimate changes in stock abundance, or in this instance egg production, by using commercial catch per effort as a proxy for stock abundance is well known (Walters and Maguire, 1996). The risk is that estimates of egg production indices in recent years could be under estimating those from earlier decades when the stock is likely to have been more widely dispersed over the grounds.

8.5.3 Effects of the changes

Compared with the 1980s, a greater proportion of egg production in this fishery is now being produced by small breeders and from the coast rather than the Abrolhos Islands, which was previously the dominant centre of egg production. There have been concerns that there may be differences in the quality of eggs and larvae produced by females of different size and from different areas in the fishery and that these might impact the survival of early stage larvae. This possibility has been rejected by Melville-Smith et al. (2007), with their study concluding that the maternal identity and subsequent diet of western rock lobster possibly have a greater influence on larval competency than the size of breeding female and site of capture.

8.5.4 Comparisons with other exploited fish stocks

Spatial and temporal differences in reproductive output have been recorded in other species of spiny lobsters for which long term fecundity datasets are available (e.g., *Jasus lalandii*, Beyers and Goosen, 1987; *P. marginatus*, DeMartini et al., 2003). The reasons for these differences are unclear, but the apparent widespread variability of lobster fecundity at size and size at maturity, shows the potential for plasticity of these parameters.

Rideout and Morgan (2007) compared estimates of population egg production in three species of northwest Atlantic flatfishes using new and historic fecundity data extrapolated to the small breeders recorded in more recent years. In the most extreme example, the comparison resulted in egg production in yellowtail flounder *Limanda ferruginea* being overestimated by 41%. In their study the application of historic fecundity data to recent length frequency samples led to the erroneous conclusion that the stock is better able to sustain fishing pressure than what it would have been had recent fecundity at length data been used. Although this is the opposite result to the one reported here, the cause of the error was similar, namely the estimation of egg production in a stock by assuming an invariant size at maturity.

8.5.5 Future research

Future investigations into the state of egg production in the fishery will follow a number of different research directions. The biological emphasis will be to isolate the causes of changes in size at maturity. Some hypotheses have been developed (Melville-Smith and de Lestang, 2006; N. Caputi et al., pers. comm.) and these will need to be refined. The stock management research emphasis will be directed at addressing some of the uncertainties in the egg production index, in particular by attempting to improve estimates of the annual efficiency increases. This will be aided by a newly developed model for the fishery and improved biological parameters that will go into the model (de Lestang et al., 2008).

The contribution to egg production by shallow water unfished areas needs to be assessed, rock lobster populations in newly proclaimed unfished areas on the coast are currently being monitored (Hyndes and MacArthur, 2006) and in time these areas will show whether there is an increase in breeding animals in these depths.

Finally, the question of density-dependent changes in habitat colonisation needs to be addressed. As a way of dealing with this issue, a new project has recently been initiated, which has as one of its objectives, to quantify habitat usage by western rock lobsters inside unfished reference areas. Areas in the deep water (40-80 m) suitable for closure to fishing are currently being negotiated with commercial fishers.

8.6 Acknowledgements

We acknowledge the many Department of Fisheries Western Australia employees who have been responsible over the years for collecting the data used in this study. We are also grateful to Nick Caputi and two anonymous referees for their comments and suggestions to earlier drafts of this manuscript.

9.0 Characterization of polymorphic loci for the western rock lobster (*Panulirus cygnus*)

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Citation: Groth, D.M., Lim, F., de Lestang, S., Beale, N.E. and Melville-Smith, R. (in press). Characterization of polymorphic loci for the western rock lobster (*Panulirus cygnus*). *Conservation Genetics Resources*.

Keywords: *Panulirus cygnus*, microsatellite, SSR

Running title: Microsatellite loci in Western Rock Lobster

9.1 Abstract

Nine microsatellite loci were identified in the western rock lobster (*Panulirus cygnus*) using two different methods. The first method involved the screening of a small fragment partial genomic library with a radioactive $(CA)_6$ probe. The second method was based upon a capture enrichment system and used biotinylated tetranucleotide microsatellite probes. The nine loci described are all very polymorphic, with 11 to 34 alleles observed for each locus and heterozygosities ranging from 0.58 to 0.86. These microsatellite loci will be useful in analysing both the population structure and the mating systems used by this species and will add important information for the management of the wild stocks of this economically important species.

9.2 Discussion

The western rock lobster is Western Australia's most important single species fishery with annual harvests over the last decade ranging between approximately 9 and 14.5 thousand tons per annum. The elucidation and understanding of population and mating structures for this species will allow the information obtained to be incorporated into an improved management strategy for the brood stock. In order to understand the population structure and mating systems of the western rock lobster, polymorphic, microsatellite genetic markers have been isolated and characterised. Two strategies were used to develop these microsatellites, the first consisting of the construction of a Hae III small fragment library which was screened with a (CA)n probe and the second library was made using an enrichment technique followed by screening with tetranucleotide microsatellite motifs. Nine microsatellite loci have been fully characterised and these are described in this report.

A DNA genomic library was made using Qiagen Tissue Kit (Qiagen) to isolate DNA extracted from tail muscle tissue. The DNA was digested with HaeIII restriction endonuclease (Promega, CA, USA) followed by excision of the 100-500bp region after agarose (1%) gel electrophoresis. Fragments were ligated into Sma I (Promega) digested, dephosphorylased pUC18 plasmid vector and transformed into competent cells (DH5α Invitrogen). Colonies were transferred to Hybond N+ membranes and screened with radiolabelled (CA)10 probe. Thirty clones were identified after the first round of screening. Following successive screening rounds to further isolate the clones, twenty of these were sequenced. Plasmid DNA was isolated from the positive clones and sequenced using cycle-sequencing combined with IRD_{800} labelled primers on a Li-Cor gene sequencer. Primers were designed using Mac Vector (Eastman-Kodak, USA) or PRIMER 3 software (Rozen and Skaletsky, 2000). Many of the loci identified had insufficient flanking DNA sequences or produced microsatellites containing few repeat units. Three loci (WRL 1, WRL 2 and WRL 3) identified by using this method were deemed to be useful.

A second library enriched for tetranucleotide microsatellites was prepared using genomic DNA. Briefly, rock lobster DNA (20ug) was digested with either Hae III or RsaI restriction endonuclease and ligated to super SNX linkers (Glenn and Schable, 2005). The ligated DNA was amplified by polymerase chain reaction (PCR) using the super SNX 24 primer. The PCR product was heat denatured and hybridised to a pool of 5' biotinylated microsatellite motif ($GATA_5$, $GGAT_5$, $GAAA_5$ and $GACA_5$) oligonucleotides at 45°C. The hybridising DNA was captured on magnetic streptavidin beads and unbound or weakly bound amplified lobster DNA washed away using a series of stringency washes (final wash at 1 x X SSC at 50°C). The remaining captured DNA was recovered by heating the beads to 95°C for 5 mins in 0.1X SSC buffer and removal of the magnetic beads using a magnetic tube station. The eluted DNA was then subjected to an additional round of PCR amplification using super SNX 24 primer as described by Glen and Schable, (2005). Following PCR amplification, the product was ligated into the plasmid (pGEM) using the pGEM T easy cloning kit (Promega) and used to transform *TOPO10 E. coli* by electroporation. *E. coli* colonies were grided onto 82 mm petri plates, lifted and screened using 5' end labelled (^{32}P) oligonucleotides. The genomic library enriched for microsatellites was screened using radiolabelled oligonucleotides identical to those used during the capture process. The result of this enrichment and screening process was the identification of over 70 clones containing identifiable microsatellite motifs. All four of the repeat motifs used in the enrichment process were identified in *P. cygnus*. However, a relatively high proportion of the loci identified showed little or no flanking sequences and hence were unsuitable for further primer design. Furthermore, several clones had very large-sized repeat elements, which were not amenable to PCR amplification and so were not developed any further.

The PCR primers used in this study were made by Geneworks (Adelaide, Australia). PCR reactions were performed in an Eppendorf thermal cycler and comprised the following reaction mixture; approximately 20 ng of lobster DNA, 1 x PCR buffer (Invitrogen), 0.5 U Platinum Taq (Invitrogen), 1.5 mM $MgCl_2$, 2 mM each dNTP, xxug of BSA (Roche Molecular Systems) and 1 pmol of labelled forward primer (IRD_{800} LiCor, USA or Beckman), 5 pmol of forward and reverse primers as described in Table 1. The PCR cycling parameters were as follows; denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 20 sec, T_{ann} for 30 sec and 72°C for 1 min, followed by a final incubation at $72^{O}C$ for 5 mins. The cycling protocol was as follows: 5 min at 95°C, 10 sec at annealing temperature, followed by 72°C for 30 sec, next 8 cycles at 94°C for 30 sec, annealing temperature for 10 sec and $72^{0}C$ for 30 sec, then 24 cycles of 94°C for 20 sec, annealing temperature for 10 sec and $72^{0}C$ for 30 sec. Next, $92^{0}C$ for 20 sec, annealing temperature for 10 sec and a final extension of 72°C for 5 min completed the run. Fragment sizes were determined with reference to standard base pair ladder or an in-house generated allelic ladder. GENEPOP 4.0 (Raymond and Rousset 1995) was used to calculate heterozygosities, Fis and observed deviations from Hardy-Weinberg equilibrium.

Primers were designed for twelve loci, for which nine loci gave consistently reliable amplification and displayed a relatively high degree of polymorphism. The three loci identified from the screening of the small insert library with the (CA)10 oligonucleotide probe (WRL 1, WRL 2 and WRL 3) and six tetranucleotide motif containing loci identified through specific sequence capture showed robust amplification reactions. The primer sequences for these nine loci (WRL 1, WRL 2, WRL 3, S3, S8, S28, S36, S50 and W25) are described in Table 1 together with their associated GenBank accession details. The number of observed alleles for each locus ranged from 11 to 34 and the observed heterozygosity varied from 0.58 to 0.86. Deviations from Hardy-Weinberg equilibrium were detected in 8/9 loci tested. The majority of these deviations were also observed in three other sample collections from various locations (data not shown). Locus WRL 1 showed a significant non-amplification in 7/43 samples implying a "null" allele at high frequency in this population. When the frequency of the "null" allele was incorporated into the HW calculation there was still a significant deviation from HW observed for this locus. These nine loci when used on DNA extracted from eggs and parental tissue derived from a tank based mating experiment comprising four families, confirmed a simple autosomal Mendelian inheritance for all loci (data not shown).

Deficiency of heterozygotes in marine invertebrates is not uncommon (Maggioni and Rogers, 2001; Puebla, Parent and Sevigny, 2003; Divu, Karunasagar and Karunasagar, 2008; Feng and Li, 2008). The reason for the observed departure from HWE seen in this study could include systematic errors in the genotyping of these loci, such as unrecognised polymorphisms in one or both of the primer sequences leading to the presence of an apparent "null" allele. Alternatively, a lack of a microsatellite motif could also lead to the presence of an apparent "null" as the fragment size would be very small and hence be outside the normal allele size range. Another possibility is that the population studied is not randomly mating or is under some form of selective pressure, such as zygote viability selection, resulting in an apparent increase in homozygotes, as has been observed in some other marine species (Reece *et al*., 2004; Launey and Hedgecock, 2001; Malmquist *et al*., 2002). However, no homozygotes were consistently observed for a "null" allele in these populations except for the WRL 1 locus described above. As these microsatellites will be used to identify spermatophores deposited on females during the mating process we have calculated that the probability of two samples being identical at all nine loci is less than 1 in 10,000 and in most cases is considerably lower. No significant linkage disequilibrium was observed between the loci in the population studied.

9.3 Acknowledgments

The work described was funded by a grant from the Fisheries Research and Development Corporation, Department of Fisheries Western Australia and the School of Biomedical Sciences at Curtin University.

Table 1. Panulirus cygnus microsatellite loci showing name of locus, GENBANK accession number, sequences of forward (F) and reverse (R) primers with labled primer indicated *; the repeat motif, the annealing temperature (°C), size of the cloned PCR product in base pairs, the number of individuals genotyped (N), and the number of alleles detected (Na). Observed (H_o) and expected heterozygosities (He) and probablility of deviation from Hardy-Weinberg equilibrium (HW) using Weir and Cockerham (1984) are presented, Fis Nei's (1987) *F*-statistics.

Locus ID	GenBank Accession number	Primer sequence (5'-3')	Repeat motif	Annealing Temp (°C)	Size (bp)	N	Na	Ho	He	HW	Fis
WRL 1	FJ001813	F- CCACAGTCACACGTGCATGG R- GAACCCAAACCTGGGGTGTT	(CA)	55	143	41	15	0.61	0.82	<0.01	0.273
WRL 2	FJ001814	F- CAACTTCCGACATTACGTCT R - GGATACCTGGCTCAGGTTGG	(CA)	55	116	41	21	0.58	0.93	<0.01	0.335
WRL 3	FJ001815	F – GCTCACAACAGAAAATGAATATCGG R-GAAAGAGTTGAACGTTGGTGAGTATG	(GT)	55	125	41	21	0.73	0.93	<0.01	0.138
S3	EU152080	F:TTGCGTCGAGAAGCATTAGA R:CATCCACCTAGCCAACCAAA	(GGAT)	58	248	64	22	0.78	0.89	0.01	0.246
S8	EU152081	F:CATTCTTCGAACAGTCCATATAA R: TGGAAGGAGCAACTGGAAGT	(GATA)	50	226	64	34	0.77	0.97	0.02	0.391
S28	EU152082	F:GGAAGGGGTGGGTTTACTGTAATG R: CATCCGCTCTGTTATGAGTCATC	(GACA)	55	251	64	12	0.78	0.81	ns	0.071
S36	EU152083	F: GGTCTGTCGACCTCTTTTCCT R: TCGAACACGGGACCTTATGT	(GATA)	50	165	64	14	0.79	0.91	<0.01	0.333
S50	EU152084	F: GAGGTGTGCGTCATGACAAG R: TCTTCGGTTACACCCGTCTCT	(GACA)	48	217	64	30	0.86	0.97	<0.01	0.213
W25	EU152085	F: TCGTCCGTCACCGCCACCGA R: CCAGTGAGCAATTACCAG	(GAAA)	53	205	64	11	0.64	0.72	<0.01	0.294

10.0 Use of microsatellite loci to establish mating choice and growth rates of western rock lobsters (*Panulirus cygnus*)

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10.1 Abstract

This study was primarily aimed at gaining insight into mate choice of western rock lobsters in the wild to assist in addressing the question of whether there are sufficient mature and particularly large sized males, to mate with the females in the population. This has been of concern in this fishery because current management rules protect all mature females whilst they are in a breeding state and large size females even in a non-breeding state, but there are no management measures to protect large males. Sampling was conducted periodically on a deep (50 m) offshore reef near the centre of the fishery, between October 2004 and August 2006. Microsatellite paternity assignment tests were used to determine which males caught in pots on the site had mated with females (either deposited a spermatophore, or fertilised eggs) at the sampling site. Only six of the 284 sampled male lobster had mated with females caught over the same period. Four of these identified males had mated with only one sampled female, while the other two males had mated with three different females each. Multiple spermatophore samples matched each other but did not match any tissue samples from males captured during the surveys. From the small number of male lobsters that were both sampled and that had fertilised females at the sampling site, choice of mating partner appeared to be random. Similarly, although the sample size was small, there appeared to be no relationship between size of the male lobster and number of females that he mated. As a secondary output from this study, a total of nine male and three female lobsters were identified by the microsatellite analysis as being recaptured after moulting. These animals have provided intermoult increment (growth) data.

10.2 Introduction

There has been increasing interest as to what affect human exploitation rates might have on mating systems in fished crustacean populations (Butler and MacDiarmid, 2005). Many fished species of lobster and crabs in particular, are considered to be vulnerable to fishing pressure because of high exploitation rates and knife-edge size selection, which results in the removal of the large individuals from populations. Studies have shown for example, that this can lead to some females not mating due to low mate-encounter rates (Ennis, 1980), or recording low fertility rates due to males becoming sperm-depleted as a result of frequent mating (Rondeau and Sainte Marie, 2001).

The western rock lobster fishery, which is input-controlled, is fished at very high exploitation rates of up to 75% (Wright et al., 2006). The principal means of ensuring the sustainability of the fishery is by monitoring the size of the breeding stock (Chubb, 2000). Over the years, the breeding stock has fallen below target levels and various measures have been used to improve egg production (Phillips et al., 2007). Measures used to reduce fishing pressure on breeding females has included: the introduction of the 'setose' rule, which requires fishers to return females with ovigerous setae or an external spermatophore to the sea; and, the introduction of a maximum size rule, which requires fishers to return large females (over 115 mm carapace length (CL) south of 30°S and over 105 mm CL north of latitude 30°S) to the sea (Phillips et al., 2007).

High exploitation rates, combined with management measures to protect breeding females but not mature males, have raised concerns as to whether there are sufficient males to mate with the female brood stock (Chubb, 2000). Chubb (2000) has noted this to be of particular concern given Chittleborough's (1974) finding that females are considered to require males of approximately equal or larger size for successful mating. However,it should be noted that Chittleborough's (1974) work was done in an aquarium, using a limited number of animals across a narrow size range.

This study was initiated to gain insight into mate choice of western rock lobsters in the wild and so to address the question of whether there are sufficient mature males to mate with the females in the population. In particular, we were interested in determining whether it is only large males that mate with the large protected females, as if so, managers would have to be especially concerned about possible distortions in the sex ratios of these size classes caused by this management measure.

Previous research has provided some information on mate choice and the number of broods carried per season (Berry, 1970; Chittleborough, 1974), but those studies were both limited by the number and sizes of the animals and the uncertainties that surround studies conducted in a laboratory setting. This study attempted to establish mating strategies in the wild by regularly sampling lobsters on an offshore reef. Identification of individual male-female mating partnerships was established by matching DNA 'signatures' from spermatophores or eggs carried on the females with DNA 'signatures' from male tissue samples., A by-product of this study was to use the DNA 'signatures' of individual animals, to establish moult increments for any animals that moulted between recapture events.

10.3 Methods

Western rock lobsters were sampled by potting at a site off Dongara, Western Australia (29° 22.5' S and 114°33.2' E) (Figure 1). The site was chosen because it was productive lobster habitat that is regularly fished by a small number of commercial fishers each season and is surrounded by an extensive sandy patch. The sandy patch was considered important in limiting movement by resident lobsters, although there was no evidence to confirm or refute the possibility that adult lobsters might cross sand to move between hard grounds. Potting on the site was carried out by a licensed commercial fisher. Approximately 30 slat pots, as used in the western rock lobster commercial fishery, were used (with closed escape gaps) on the site over a five-day period (one-day soaks) in October 2004. This intensity of pot usage is far in excess of what is generally used in the commercial fishery for a small, deepwater reef area, but the object was to define the extent of lobster distribution on the site and secondly, to use the daily catches to estimate the population size through a depletion experiment.

It is recognised that because of moulting patterns and responses to environmental conditions, that lobsters are not always catchable. Therefore, the sample site was re-visited and repeatedly sampled over 3-5 day trips in November 2004, August 2005, September 2005, October 2005, November 2005, February 2006 and August 2006 to sample as many of the lobsters resident at the site as possible and to gauge the extent of movement on and off the experiment site.

For each lobster caught, details were recorded on their: sex, carapace length, presence of spermatophore (tarspot), stage of egg development, and, merus length (males only). Prior to being released, each lobster was tagged with a numbered cable tag around one of its antennae and a sample identification number was also assigned.

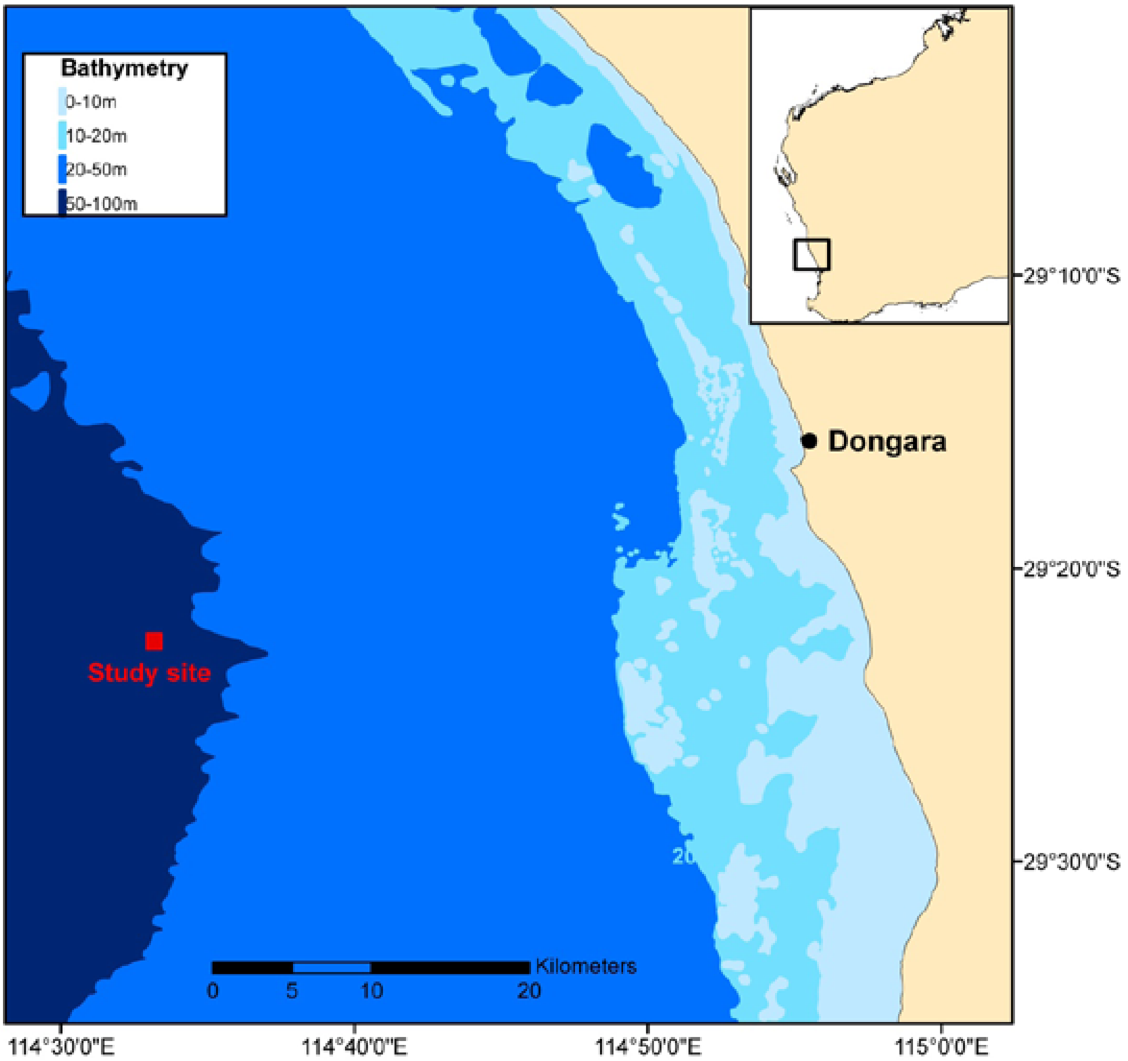

Figure 1. The study site for collection of lobsters for the genetic analysis was west of Dongara town, Western Australia at 29° 22.5' S and 114° 33.2' E in a depth of approximately 50 m.

A small piece of muscle tissue was sampled from each male lobster and female lobsters that bore eggs, by using a leather hole-punch to punch a 5 mm diameter tissue sample from the upper, fleshy region of the telson. A small sample of eggs were taken and spermatophores were completely removed and kept from female lobsters. All samples were immediately stored and labelled in 70% ethanol. Lobsters were returned to the sea in approximately the same location as they were caught.

Any lobsters that were re-caught were recorded as a 'tag-recapture' and assigned another identification number, if a new spermatophore or eggs were sampled. Lobsters that showed signs of recently moulting and that had a scar on their telson were recorded as 'moult-recaptures' and were also assigned another identification number for a tissue sample and any new spermatophore or egg samples.

Male and female tissue samples were genetically analysed at three loci (S50, S3 and W25, detail provided in Chapter 8 - Paper 7) to produce a ‘signature’ for each animal sampled, that formed the basis of a DNA ‘library’. All spermatophores and egg samples were genetically analysed to identify the male lobster that had mated with the particular female lobster being sampled.

Macro-code was used in Microsoft Excel to create a program (from herein referred to as the ‘matching program’) that compared the genetic results for: spermatophores and egg samples with male tissue samples in order to assign paternity; spermatophores with spermatophores, to identify multiple matings (i.e. a spermatophore-spermatophore match indicates that the same male mated with both females from which spermatophores were removed); male tissue with male tissue, and female tissue with female tissue, to identify individual lobsters that had been assigned more than one identification number (i.e. they were initially sampled and then re-caught as a ‘moult-recapture’). These latter matches intermoult growth increments for males and females to be collected.

10.4 Results

10.4.1 Depletion study

Based on catches of lobsters resulting from extensive potting on the reef area as well as on sand surrounding the reef habitat, the approximate size of the site was 200 m by 200 m (Figure 2).

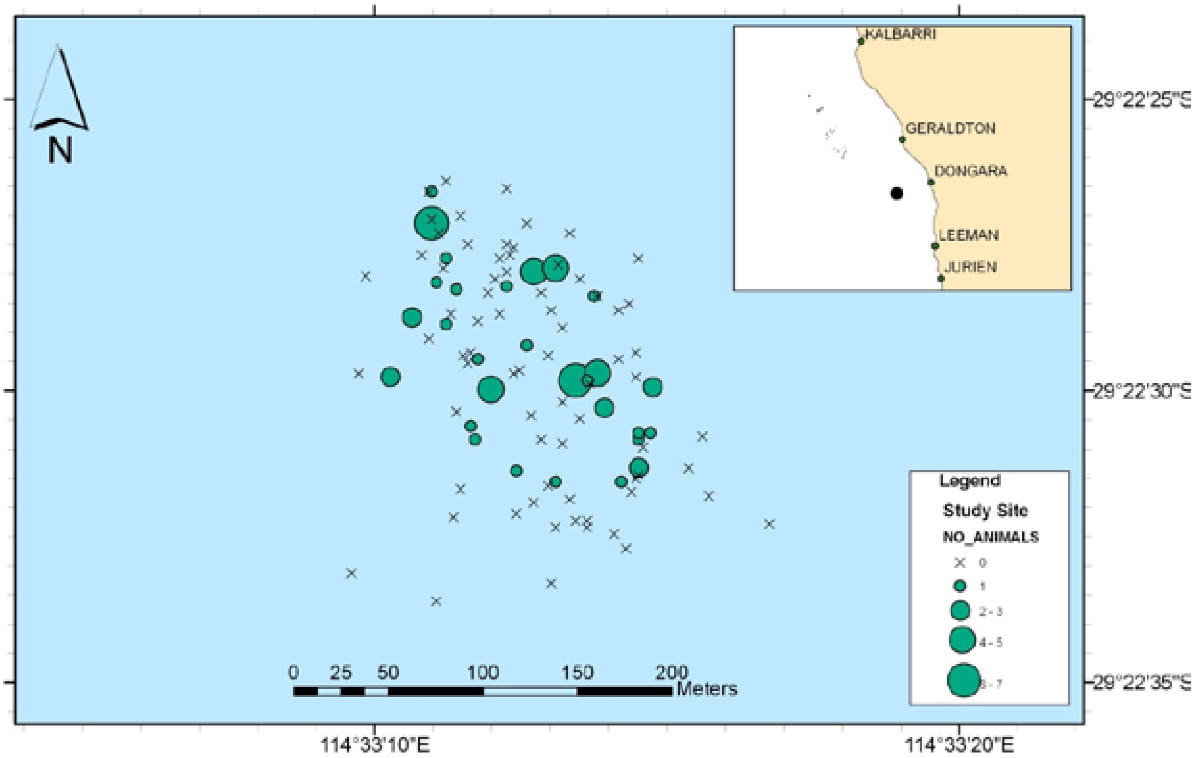

Figure 2. The number and distribution of western rock lobsters caught, tagged and processed for genetic samples in October 2004. This plot was used to determine the extent of the site.

Lobsters were collected from the site over five days in October 2004 and four days in November 2004. The depletion study indicated that by the end of the ninth day, assuming no immigration or emigration, around 90% of the lobsters had been sampled from the site (Figure 3).

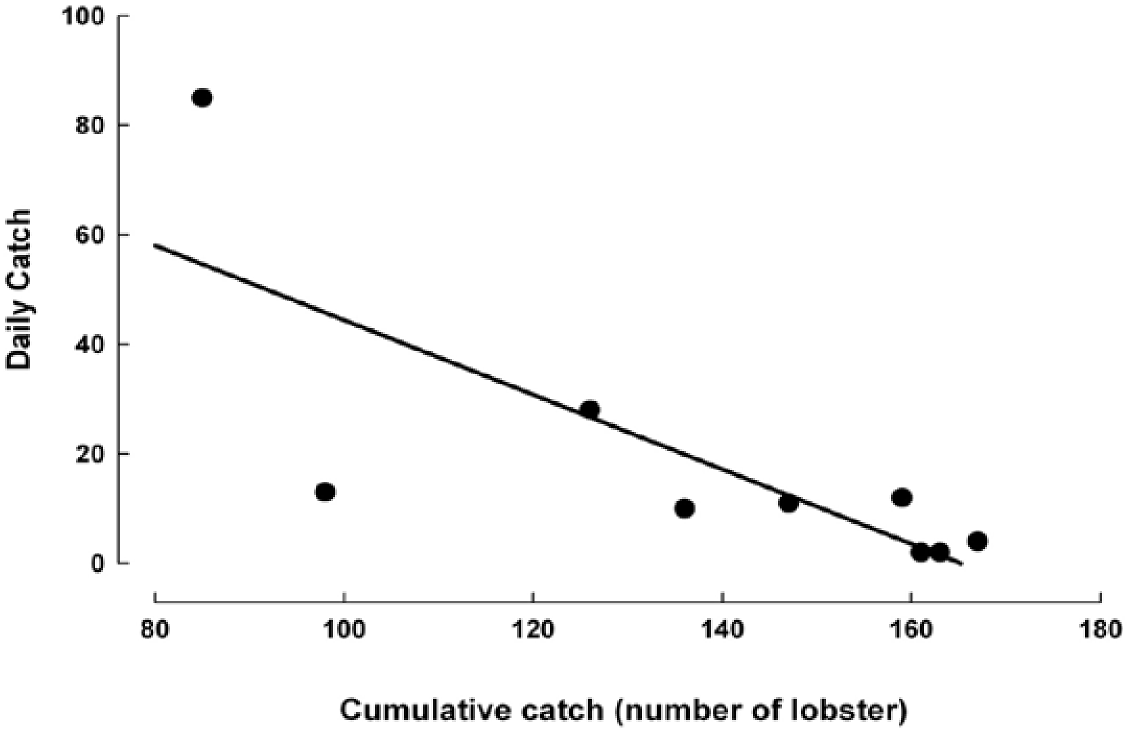

Figure 3. The daily cumulative catch of western rock lobsters, that had not been previously sampled on the site (x axis), plotted against the daily catch of lobsters that had not previously been sampled (y axis) over consecutive days during the sampling periods of October and November 2004.

10.4.2 Sample collection

A total of 850 female tissue samples were collected over the course of the study. Of this total, genetic tissue was only processed for those females that had eggs, or had not been previously recaptured (and only for the 2004/05 season), or were a moult recapture in 2005/06 season. These limitations reduced the sample size to 246 and of this total, 235 were successfully genetically analysed (Table 1). All captured males were sampled for tissue and of the 293 tissue samples collected, 284 were successfully analysed (Table 1). Numbers of spermatophore and egg samples collected and analysed are also provided in Table 1.

Table 1. Number of samples collected and analysed over the sampling period (October 2004 to August 2006).

Sample type	Sex	No. sampled	No. analysed
Tissue	Male	293	284
Tissue	Female	246	235
Spermatophores	Female	295	276
Eggs	Female	98	71

10.4.3 Paternity assignment of spermatophores

The matching program used to match spermatophore samples with the tissue samples taken from all mates caught over the duration of the survey, showed that only six of the 284 sampled male lobsters had mated with female lobsters that had been caught over the same period. Four of these identified males had mated with only one sampled female lobster each, while the other two male lobsters had mated with three different female lobsters each (Figure 4). While the females involved in the single matings were sampled for these spermatophores in different sampling periods ranging from November 2004 to September 2006, the females involved in the multiple matings were all sampled in November 2005.

The matching program which was used to match genetic results from all spermatophores against each other, indicated that there were multiple spermatophore samples that matched each other but that did not match any tissue samples from males captured during the surveys. That is, those spermatophores were the result of individual males that had mated with more than one female captured at the study site, but had not been sampled themselves. There were seven cases of double matings by individual male lobsters and five cases of triple matings (Figure 4). The results suggest that the remaining 243 spermatophore samples were produced by 243 individual males, which were not sampled (Figure 4).

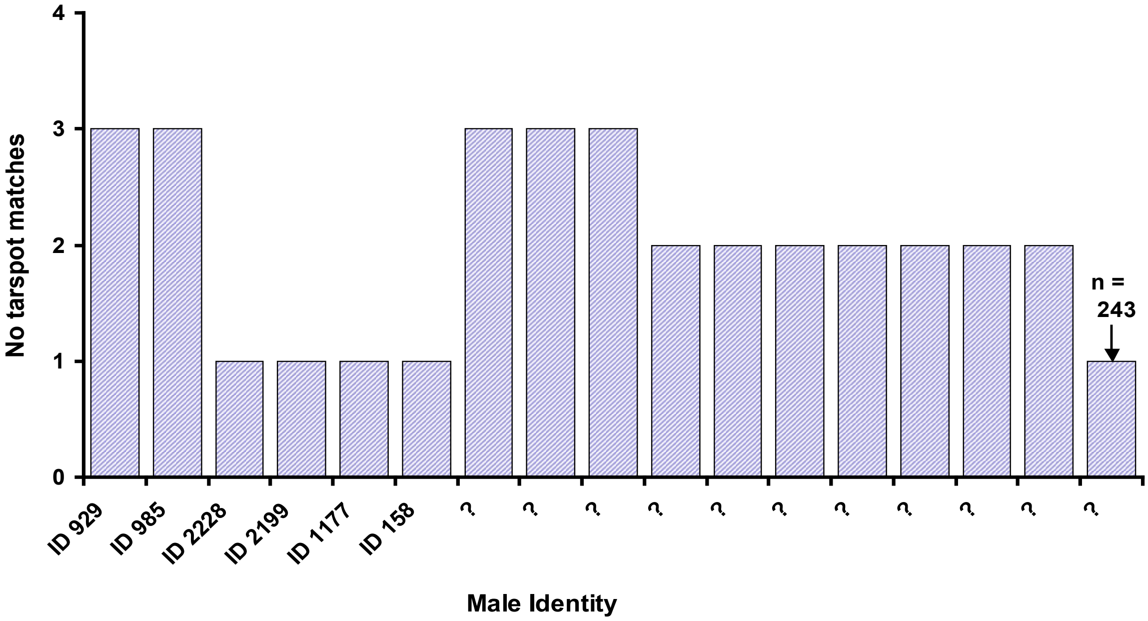

Figure 4. The number of western rock lobster spermatophore matches with identified (i.e. sampled (ID number shown) and unidentified (shown as '?')) male lobsters.

The six male lobsters identified as having produced spermatophores ranged in size from 81.6 mm to 100.7 mm carapace length (CL) and were either sampled in October 2004 or August 2005. Their female mating partners were of a larger size range (76.6 mm to 125.2 mm CL). Choice of a mating partner appears to be random, at least in terms of size (Figure 5).

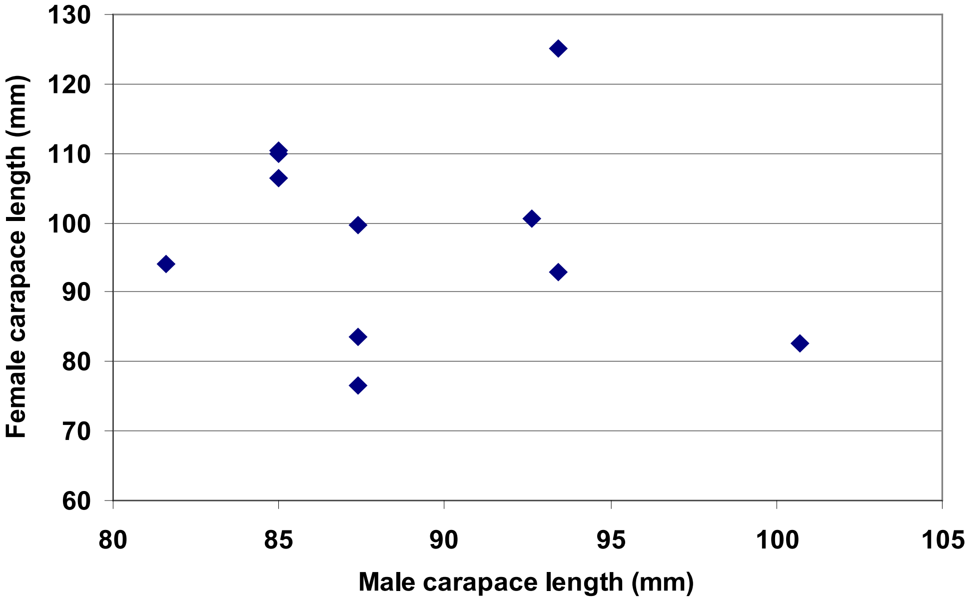

Figure 5. Relationship between the size of female and male western rock lobsters that were identified as mating partners.

Similarly, although the sample size is small, there appears to be no relationship between size of the male lobster and the number of females that he had mated in our samples (Figure 6).

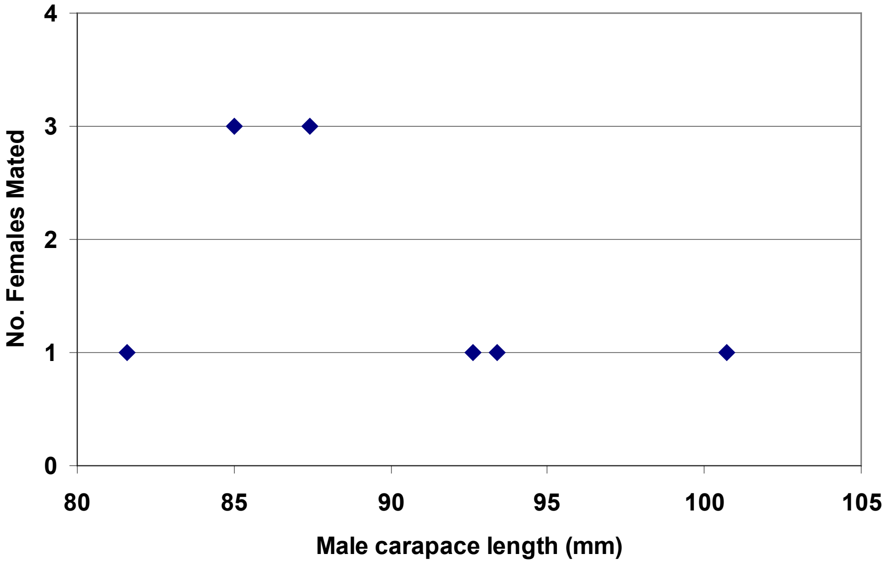

Figure 6. Relationship between size of male western rock lobster and the number of identified matings with females sampled during this study.

10.4.4 Paternity assignment of eggs

The matching program was used to match the genetic results from egg samples taken from ovigerous females with the genetic results from all male tissue samples and indicated that only two of the egg samples were fathered by male lobsters that had been sampled in our surveys. One female was 92.9 mm CL and her mate was 93.4 mm CL. The potential mates of the second female were more difficult to identify; the allele results from the genetic analysis of this female's eggs showed multiple allele peaks.

Consequently, there were five males identified as possible mating partners and their lengths ranged from 61.4 to 90.8 mm CL, compared to the 90.1 mm CL female.

Male ID 2228 (93.4 mm CL), sampled in August 2005, was identified through the genetic analyses of egg samples as mating in October 2004 with a female lobster of length 92.9 mm CL and through the spermatophore analyses as having mated with a larger, female lobster of length 125.2 mm CL in November 2004. This was the only sampled male that was identified through spermatophore and egg samples as having mated different female western rock lobsters.

10.4.5 Growth

The matching program used to match the genetic identity of tissue samples against each other produced nine male matches. Those animals grew between 1.5 and 5.9 mm after being at large for between 64 and 175 days, while another cluster grew between 6.9 and 15.3 mm after being at large for 300 to 358 before recapture (Figure 7).

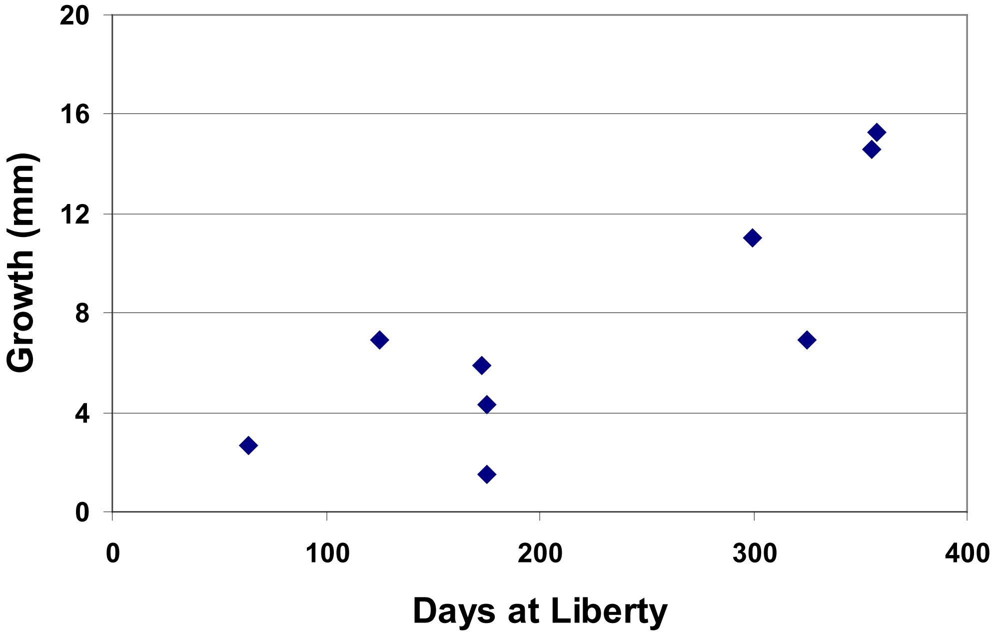

Figure 7. Growth of male western rock lobsters that were recaptured (as identified by their genetic tissue match) and that recorded growth.

A similar search for genetic tissue sample matches for females resulted in three matches with increments of 4.1 to 10.8 mm after 304 to 403 days at large between sampling (Figure 8).

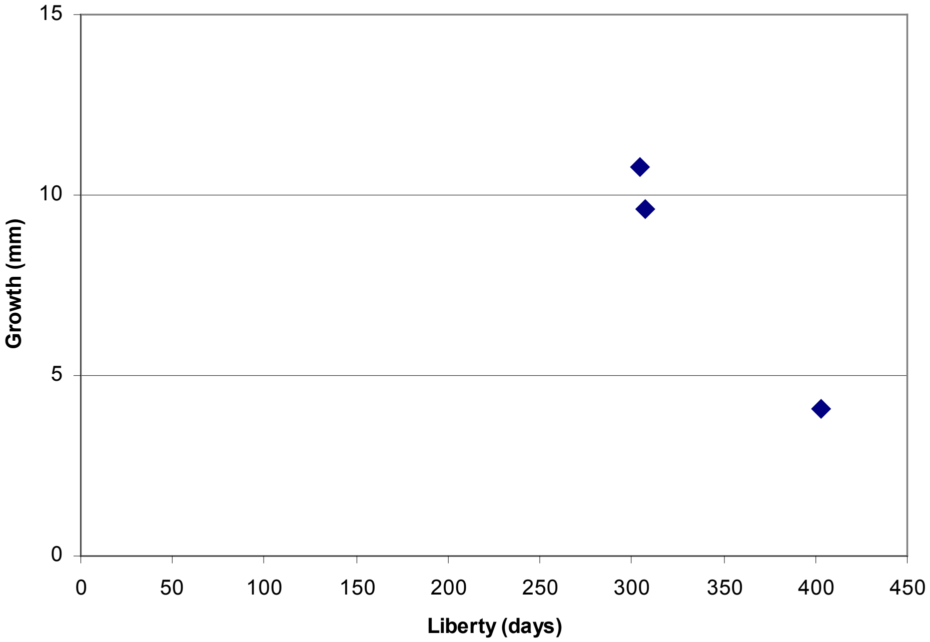

Figure 8. Growth of female lobsters that were recaptured (as identified by their genetic tissue match) and that recorded growth.

10.5 Discussion

The results from this study were not as conclusive as had been anticipated. We had hoped that because the offshore lobster habitat of the study site was surrounded by sand, that besides an annual influx of white migrating lobsters settling on the reef there would be minimal immigration and emigration to and from the resident population. This turned out not to be the case and it is now clear that there was a substantial turnover of the population, with many of the animals not being recorded beyond the month in which they were sampled. Unfortunately, this unexpected result meant that the paternity assignment tests matched few males to egg or spermatophore-bearing females and furthermore, there were relatively few animals that were recaptured after a period of time that was long enough to show a moult increment.

It is known from tagging studies that western rock lobster breeders generally do not move large distances. Chubb and Cheng (unpub. data) showed from their extensive analysis of over 18,500 lobsters tagged between 1988-2000, that 98.6% of breeders were recaptured within 20 km of where they had been tagged. Results from this study were therefore notable in showing that while western rock lobsters do not necessarily move large distances, they do move frequently between outcrops of good habitat – a fact that was probably not previously appreciated. Frisch (2008) showed in a study on the social organisation and den utilisation of another Palinurid, *Panulirus versicolor*, that species moved frequently to and from nearby dens and the composition of the groups regularly changed. This behaviour might therefore not be uncommon in spiny lobsters, but the movements may be wider in *P. cygnus* than some other Palinurids, in that few animals were recaptured on the same isolated sampling site after more than one or two months.

That few males were identified as being the mating partners of breeding females on the study site is certainly the result of frequent movement by both sexes on and off the site, but it may also be that the large males were less catchable – a possibility supported by the fact that sex ratios during sampling at the study site were highly skewed towards females.

Of the six males that were identified as being mating partners of females in the sampled catches, there appeared to be little relationship between the size and sex of partners. Apart from a multitude of studies on *Jasus edwardsii* (e.g. MacDiarmid et al., 2000; Buxton, 2004; MacDiarmid and Stewart, 2005a,b), there is surprisingly little published information on mate size selection in spiny lobsters.

Berry (1970) noted that *Panulirus homarus* males are polygamous and that dominant males prevent subordinate males from mating. MacDiarmid and Sainte-Marie (2006) state that mate selection is much more precise in *Jasus edwardsii* than it is for *Panulirus argus.* MacDiarmid and Butler (1999) speculate that the reason for the difference between these latter two species, probably lies in the fact that there can be a 28 day lapse between mating and egg laying in *P. argus* (Lipcius, 1985), but in the genus *Jasus*, the spermatophore must be used by the female immediately after being deposited. The longer time period between mating and egg laying provides *P. argus* females with greater opportunity to mate a number of times, thereby increasing the potential for sperm competition (MacDiarmid and Butler, 1999).

As with *P. argus*, *P. cygnus* also has a lengthy period between mating and egg laying and the lack of relationship between size and sex of mating partners in this study might be explained by the same reasoning provided by MacDiarmid and Butler (1999), above. In this regard, it was notable that we did record at least one instance where there was evidence from microsatellite analyses of individual eggs, of a brood having been fertilised by more than one male – presumably due to multiple mating. Evidence for multiple mating in spiny lobsters is rare (the only reference that we could find was that of Mota-Alves and Paiva (1976) to this occurring in *Panulirus laevicauda*). It is conceivable that, had there been more analyses of individual eggs per brood, we might have found more instances of multiple mating in *P. cygnus*.

As with the lack of relationship between the mating size of partners in this study, so too was there no relationship between the size of the male and the number of identified matings with females sampled. This was not unexpected, given the apparent transitory nature of residency at the sampling site by both sexes. It is relevant to mention that, Frisch (2008) found no significant relationship between male *Panulirus versicolor* body size and the number of females co-habiting in their dens. The inference from this is that if the females co-habiting in the dens had been the only mating partners of those males, that as with this study there would have been no relationship between size of male and the number of females that they mated.

There is evidence from other studies to support the fact that large spiny lobster males are capable of mating with substantially more females than small males. For example, MacDiarmid and Stewart (2005) have shown in tank studies, that small (500 g) *J. edwardsii* males can mate with 1-5 females in a singe mating season, while large males (3000 g) can mate with up to 18 females, however this has still to be confirmed in field based studies.

A small amount of growth data were obtained during the course of the study. The increments show the typical double annual moult that is expected for individuals of the sizes that were recaptured. The growth increments were unusually variable compared to tag-recapture data, but this observation may be a reflection of the small sample size (12 individuals of both sexes, some of which had moulted more than once).

10.6 Acknowledgements

The assistance of Mr Terry Lissiman in providing us with such a suitable sampling site for this work and the assistance of him and his crew with the sampling are much appreciated. Our thanks also to a number of our colleagues who assisted with field work on the different surveys. A number of different people assisted with the microsatellite analysis of the samples, in particular we thank Florence Lim, Farhanah Ibrahim and Justine Thompson.

11.0 Benefits

At the time that funds were granted for this project there was a lot of uncertainty surrounding the effects that the setose and maximum size rules might be having by distorting sex ratios in the population and thereby possibly impacting mate choice and egg production. Questions were also being asked about whether eggs from different sized females and from different areas of the fishery were of similar quality. Also, what the contribution to egg production was in different management zones in the fishery.

This project has succeeded in answering those questions – and at the same time has provided insight into aspects of other western rock lobster reproductive behaviour and physiology that were not part of the project objectives.

The benefit to fishers, administrators and researchers, is that decisions on the management of this fishery relating to reproductive issues, can now be made with far more certainty about likely impacts than prior to this work being undertaken.

12.0 Further Development

Further development of the results of this research will include:

(i) Continued monitoring into the future of changes in size at maturity. Further monitoring will be important to stock assessment model inputs. Furthermore, there are more than one possible causes for these changes in size at maturity – future monitoring may help to narrow the cause of these changes.

(ii) Development of additional polymorphic loci to enable microsatellite techniques to be used to evaluate spatial and temporal population genetic structure in western rock lobsters.

13.0 Planned Outcomes

There were a number of planned outcomes identified in the funding application:

(i) For this project to investigate to what extent the setose and maximum size rules have distorted sex ratios and affected the sizes of breeding animals in the population and then depending on that result, to modify management rules accordingly.

The project showed that while sex ratios are inevitably going to differ throughout the fishery, mate selection is not necessarily size dependent. This means that small males are quite capable of mating with much larger females. Also, that one male can fertilise several females. Distorted sex and size ratios do not therefore appear to pose a particular management issue to the western rock lobster fishery at current ratio levels.

(ii) For this project to establish whether eggs from different regions in the fishery differ in 'quality' so that if there were differences these could be taken into account in the way that the brood stock is managed. The project outcome was that eggs from different regions do not differ significantly in terms of 'quality' and therefore there is no need to manage the stock differently in different regions.

(iii) For this project to establish whether eggs from different sized females differ in 'quality' so that if there were differences, these could be taken into account in the way that the brood stock is managed. The project outcome was that eggs from different sized females do not differ significantly in terms of 'quality' and therefore there is no need to manage the stock differently according to the size of breeding females.

(iv) For this project to examine the proportion of egg production emanating out of the three different management zones in the fishery, so that egg production indices for the fishery could be adjusted by values for production from the three management zones.

The project outcome delivered more than just this objective, by examining changes in the proportion of egg production in the three management zones by decade from the 1980s to 2007. In future, total egg production indices for the fishery will be able to be adjusted by the proportional contribution from different management zones.

14.0 Conclusions

This project has provided knowledge on reproductive biology issues relevant to managing the western rock lobster brood stock that will provide the scientific basis for future brood stock management decision-making capabilities for the fishery. Much of this research, as well as being of value to the fishery, has also been scientifically of interest, resulting in the project producing several peer reviewed journal articles.

Under the performance indicators of this project, it was stated that the project would be considered successful if it achieved the following three objectives:

(i) Provides an evaluation of the biological risks that might result from the setose and maximum size management measures that are currently in place to protect the brood stock.

The research has shown that the biological risks appear to be low, based on the fact that small males in the wild would appear to be equally capable as are large males, of fertilising large females. It is also apparent that males in the wild can and do, fertilise several females. Skewed sex ratios would therefore not appear to be of concern in this fishery.

The work did show that when water temperature is high in January and February, that high proportions of mature females moult into non-setose condition and therefore become available to the fishery. This previously unknown fact while not necessarily a risk, is useful for management to bear in mind because with high exploitation rates and probably higher average water temperatures under a future climate change scenario, there will be a need to monitor the effectiveness of the setose rule in protecting breeders.

(ii) Results in an understanding of the likely survival characteristics of larvae hatching from the smaller Abrolhos Island eggs compared to those from the coast.

Research has shown that the survival characteristics of larvae from small Abrolhos Island females are not necessarily any different to the larvae from small or large females at the coast.

(iii) Quantifies the extent of the western rock lobster breeding grounds.

The extent of the breeding grounds so as to weight the overall egg production index for the stock by the contribution of the management zones has been outlined in detail both spatially and temporally (across three decades).

Given that the project achieved the above performance goals (and much more), it would seem fair to conclude that it has been highly successful.

15.0 References

Ackman, R.G., 1981. Lipids Part D, In: Lowenstein, J.M. (Ed.). Methods in enzymology. Academic Press, New York. pp. 205–252.

Aiken, D.E., Waddy, S.L., 1989. Allometric growth and onset of maturity in male American lobsters *Homarus americanus*, the crusher propodite index. J. Shellfish Res. 1, 7-12.

Allendorf, F.W., England, P.R., Luikart, G., Richie, P.A., Ryman, N., 2008. Genetic effects of harvest on wild animal populations. Trends in Ecology and Evolution 23, 327-337.

Annala, J.H., McKoy, J.L., Booth, J.D., Pike, R.B., 1980. Size at the onset of sexual maturity in female *Jasus edwardsii* (Decapoda: Palinuridae) in New Zealand. N.Z. J. Mar. Freshwat. Res. 14, 217-227.

Annala, J.H., 1991. Factors influencing fecundity and population egg production of *Jasus* species. In: Wenner, A., Kuris, A. (Eds.), Crustacean Egg Production, Crustacean Issues 7, A.A. Balkema, Rotterdam. pp. 301-315.

Anger, K., 2001. The biology of decapod crustacean larvae. Crustacean Issues 14, 141-420.

Anger, K., Moreira, G.S., 2004. Biomass and elemental composition of eggs and larvae of a mangrove crab, *Sesarma rectum* Randall (Decapoda: Sesarmidae) and comparison to a related species with abbreviated larval development. Sci. Mar. (Barc.) 68, 117-126.

Arango, L., Marquez, E., 1995. Evaluation of spiny lobster (*Panulirus argus*) stock in Islas Providencia and Santa Catalina, Colombian Caribbean. Rev. Cub. Invest. Pesq. 19, 88-94.

Attard, J., Hudon, C., 1987. Embryonic development and energetic investment in egg production in relation to size of female lobster (*Homarus americanus*). Can. J. Fish. Aquat. Sci. 44, 1157-1164.

Berry, P.F., 1970. Mating behaviour, oviposition and fertilization in the spiny lobster *Panulirus homarus* (L.). Investigational Report. Oceanographic Research Institute South Africa 24, 1-16.

Berry, P.F., 1971. The biology of the spiny lobster *Panulirus homarus* (Linnaeus) off the east coast of southern Africa. Oceanographic Research Institute Investigative Report 28, 1-75.

Beyers, C.J. de B., Goosen, P.C., 1987. Variations in fecundity and size at sexual maturity of female rock lobster *Jasus lalandii* in the Benguela ecosystem. S. Afr. J. Mar. Sci. 5, 513-521.

Bianchini, M.L., Di Stefano, L., Ragonese, S., 1998. Size and age at onset of sexual maturity of female Norway lobster *Nephrops norvegicus* (Crustacea; Nephropidae) in the strait of Sicily (central Mediterranean Sea). Sci. Mar. (Barc.) 62, 151-159.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Briones-Fourzan, P., Lozano-Alvarez, E., Martinez-Guerreo, A., Cortes, A.S., 1981. Aspectos generales de la biologia y pesca de las langostas en Zihuatanejo, Gro, Mexico (Crustacea, Palinuridae). An. Inst. Cienc. Mar Limnol. Univ. Nac. Auton. Mex. 8, 79-102.

Briones-Fourzan, P., Lozano-Alvarez, E., 1992. Aspects of the reproduction of *Panulirus-inflatus* (Bouvier) and *Panulirus gracilis* Streets (Decapoda, Palinuridae) from the Pacific coast of Mexico. J. Crust. Biol. 12, 41-50.

Briones-Fourzan, P., Contreras-Ortiz, G., 1999. Reproduction of the spiny lobster *Panulirus guttatus* (Decapoda: Palinuridae) on the Caribbean coast of Mexico. J. Crust. Biol. 19, 171-179.

Brouwer, S.L., Groeneveld, J.C., Blows, B., 2006. The effects of appendage loss on growth of South African west coast rock lobster *Jasus lalandii*. Fish. Res. 78, 236-242.

Brown, R.S., Caputi, N., 1983. Factors affecting the recapture of undersize western rock lobster *Panulirus cygnus* George returned by fishermen to the sea. Fish. Res. 2, 103-128.

Brown, R.S., Caputi, N., 1985. Factors affecting the growth of undersize western rock lobster *Panulirus cygnus* George returned by fishermen to the sea. Fish. Bull. 83, 567-574.

Brown, R.S., Caputi, N., 1986. Conservation and recruitment of the western rock lobster (*Panulirus cygnus*) by improving survival and growth of undersize rock lobsters captured and returned by fishermen to the sea. Can. J. Fish. Aquat. Sci. 43, 2236-2242.

Butler, M., MacDiarmid, A., 2005. Sixth International Crustacean Congress, Glasgow, U.K., Symposium 5: The effects of human exploitation on decapod mating systems. On line at http://www.gla.ac.uk/icc6/symp5.html.

Buxton, C., 2004. TAFI News. Fishing Today 16(6), 16.

Caputi, N., Chubb, C.F., Brown, R.S., 1995. Relationships between spawning stock, environment, recruitment and fishing effort for the western rock lobster, *Panulirus cygnus*, fishery in Western Australia. Crustaceana 68, 213-226.

Caputi, N., Chubb, C.F., Hall, N.G., Brown, R.S., 2000. Measurement of catch and fishing effort in the western rock lobster fishery. In: Phillips, B.F., Kittaka, J. (Eds.), Spiny lobsters fisheries and culture. Fishing News Books, Oxford, pp. 334-356.

Caputi, N., Chubb, C., Melville-Smith, R., Pearce, A., Griffin, D., 2003. Review of relationships between life history stages of the western rock lobster, *Panulirus cygnus*, in Western Australia. Fish. Res. 65, 47-61.

Cerrato, R.M., 1990. Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. Can. J. Fish. Aquat. Sci. 47, 1416-1426.

Chittleborough, R.G., Thomas, L.R., 1969. Larval ecology of the Western Australian marine crayfish, with notes upon other palinurid larvae from the eastern Indian Ocean. Aust. J. Mar. Freshwat. Res. 20, 199-223.

Chittleborough, R.G., 1974. Western rock lobster reared to maturity. Aust. J. Mar. Freshwat. Res. 25, 221-225.

Chittleborough, R.G., 1976. Breeding of *Panulirus longipes cygnus* George under natural and controlled conditions. Aust. J. Mar. Freshwat. Res. 27, 499-516.

Christie, W.W., 1982. Lipid Analysis. Pergamon Press, Oxford. 325pp.

Chubb, C.F., Dibden, C., Ellard, K., 1989. Studies on the breeding stock of the western rock lobster, *Panulirus cygnus*, in relation to stock and recruitment. Fisheries Department of Western Australia, FIRTA Project No. 85/87, 37p.

Chubb, C.F., 1991. Measurement of spawning stock levels for the western rock lobster *Panulirus cygnus*. Rev. Invest. Mar. 12, 223-233.

Clark, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117-143.

Chubb, C.F., 2000. Reproductive biology: issues for management. In: Phillips, B. F., Kittaka, J. (Eds.), Spiny lobsters fisheries and culture. Fishing News Books, Oxford. pp. 245-275.

Clark, K.R., Gorley, R.N., 2001. Primer Version 5. Primer-E, Plymouth, United Kingdom. 91p.

Clayton, J.R., Dortch, Q., Thoresen, S.S., Ahmed, S.I., 1988. Evaluation of methods for the separation and analysis of proteins and free amino acids in phytoplankton samples. J. Plankton Res. 10, 341-358.

Cockcroft, A.C., Goosen, P.C., 1995. Shrinkage at moulting in the rock lobster *Jasus lalandii* and associated changes in reproductive parameters. S. Afr. J. Mar. Sci. 16, 195-201.

Conover, D.O., Munch, S.B., 2002. Sustaining fisheries yields over evolutionary time scales. Science 297, 94-96.

Davidson, G., Hosking, W., 2002. Development of a method for alleviating leg loss during post-harvest handling of rock lobsters. In: van Barneveld, R., Phillips, B. (Eds.), Proceedings of the fourth annual rock lobster enhancement and aquaculture subprogram/rock lobster post-harvest subprogram, Rock Lobster Enhancement and Aquaculture Subprogram Publication No. 7, Barneveld Nutrition Pty Ltd. Springwood, Queensland, Australia, pp. 85-89.

Davis, G.E., 1975. Minimum size of mature spiny lobsters, *Panulirus argus*, at Dry Tortugas, Florida. Trans. Am. Fish. Soc. 104, 675-676.

Davis, G.E., 1981. Effects of injuries on spiny lobsters, *Panulirus argus*, and implications for fishery management. Fish. Bull. 78, 979-984.

de Lestang, S., Hall, N., Potter, I.C., 2003. Reproductive biology of the blue swimmer crab, *Portunus pelagicus* (Decapoda: Portunidae) in five water bodies on the west coast of Australia. Fish. Bull. 101, 745-757.

de Lestang, S., Melville-Smith, R., 2006. Interannual variation in the moult cycle and size at double breeding of mature female western rock lobster *Panulirus cygnus.* ICES J. Mar. Sci. 63, 1631-1639.

de Lestang, S., Melville-Smith, R., 2006b. West coast rock lobster managed fishery. In: Fletcher, W.J., Head, F., (Eds.), State of the fisheries report 2005/06, Western Australia, Department of Fisheries. Pp. 14–21.

de Lestang, S., Caputi, N., Melville-Smith, R., 2008. Using fine scale catch predictions to examine spatial variation in growth and catchability of *Panulirus cygnus* along the west coast of Australia. N. Z. J. Mar. Freshwat. Res. (In Press).

DeMartini, E.E., DiNardo, G.T., Williams, H.A., 2003. Temporal changes in population density, fecundity, and egg size of the Hawaiian spiny lobster (*Panulirus marginatus*) at Necker Bank, northwestern Hawaiian Islands. Fish. Bull. 101, 22-31.

Démeusy, N., 1965. Somatic growth and reproductive function in the female brachyuran decapod *Carcinus maenas* Linné [In French]. Arch. Zool. Exp. Gen. 106, 625-663.

Dennis, D.M., Skewes, T.D., Pitcher, C.R., 1997. Habitat use and growth of ornate rock lobsters, *Panulirus ornatus* (Fabricius, 1798), in Torres Strait, Australia. Mar. Freshwat. Res. 48, 663-670.

Department of Fisheries, 2008. Western rock lobster stock assessment and harvest strategy workshop, 16–20 July 2007. Department of Fisheries, Western Australia, Fisheries Occasional Publication 50. 52 p.

Divu, D., Karunasagar, I., Karunasagar, I., 2008. Microsatellite DNA markers in the giant freshwater prawn, *Macrobrachium rosenbergii*: a tool for genetic analysis. Molecular Ecology Resources 8, 1040-1042.

Edley, M.T., Law, R., 1988. Evolution of life histories and yields in experimental populations of *Daphnia magna*. Biol. J. Linn. Soc. 34, 309-326.

Ennis, G.P., 1980. Size-maturity relationships and related observations in Newfoundland populations of the lobster (*Homarus americanus*). Can. J. Fish Aquat. Sci. 37, 945-956.

Feng, J.B., Li, J.L., 2008. Twelve polymorphic microsatellites in Oriental river prawn, *Macrobrachium nipponense*. Molecular Ecology Resources 8, 986-988.

Fielder, D.R., 1964. The spiny lobster, *Jasus lalandei* (H. Milne Edwards), in South Australia, II. Reproduction. Aust. J. Mar. Freshwat. Res. 15, 133-144.

Fielder, D.R., 1965. A dominance order for shelter in the spiny lobster *Jasus lalandii* (H. Milne Edwards). Behaviour 24, 236-245.

Fonseca-Larios, M., Briones-Fourzan, P., 1998. Fecundity of the spiny lobster *Panulirus argus* (Latreille, 1804) in the Caribbean coast of Mexico. Bull. Mar. Sci. 63, 21-32.

Frisch, A.J., 2008. Social organisation and den utilisation of painted spiny lobster (*Panulirus versicolor*) on a coral reef at Northwest Island, Australia. Mar Freshwat. Res. 59, 521-528.

Gardner, C., 2001. Composition of eggs in relation to embryonic development and female size in giant crabs [*Pseudocarcinus gigas* (Lamarck)]. Mar. Freshwat. Res. 52, 333-338.

George, R.W., 1958. The status of the "white" crayfish in Western Australia. Aust. J. Mar. Freshwat. Res. 8, 476-490.

Glenn, T.C., Schable, N.A., 2005. Isolating microsatellite DNA loci. Methods in Enzymology 395, 202-222.

Godø, O.R., Moksness, E., 1987. Growth and maturation of Norwegian coastal cod and northeast Arctic cod under different conditions. Fish. Res. 5, 235-242.

Gomez, E.D., Junio, M.A.R., Bermas, N.A., 1994. Reproduction of *Panulirus longipes* in Calatagan, Batangas, Philippines. Crustaceana 67, 110-120.

Goni, R., Quetglas, A., Renones, O., 2003. Size at maturity, fecundity and reproductive potential of a protracted population of the spiny lobster *Panulirus elephas* (Fabricius, 1787) from the western Mediterranean. Mar. Biol. 143, 583-592.

Gray, H., 1992. The western rock lobster *Panulirus cygnus*. Book 1: A Natural History. Westralian Books, Geraldton, Western Australia. 112p.

Gregory, D.R., Labisky, R.F., 1981. Ovigerous setae as an indicator of reproductive maturity in the spiny lobster, *Panulirus argus* (Latreille). Northeast Gulf Science 4, 109-113.

Grey, K.A., 1979. Estimates of the size at sexual maturity of the western rock lobster, *Panulirus cygnus*, using secondary sexual characteristics. Aust. J. Mar. Freshwat. Res. 30, 785-791.

Griffin, D.A., Wilkin, J.L., Chubb, C.F., Pearce, A.F., Caputi, N., 2001. Ocean currents and the larval phase of Australian western rock lobster, *Panulirus cygnus*. Mar. Freshwat. Res. 52, 1187-1199.

Hall, N., Chubb, C.F., 2001. The status of the western rock lobster, *Panulirus cygnus*, fishery and the effectiveness of management controls in increasing the egg production of the stock. Mar. Freshwat. Res. 52, 1657-1667.

Herrnkind, W.F., Jernakoff, P., Butler, M.J., 1994. Puerulus and post-puerulus ecology. In: Phillips, B.F., Cobb, J.S., Kittaka, J. (Eds.), Spiny Lobster management. Blackwell Scientific, London, pp. 213-229.

Hutchings, J.A., Myers, R.A., 1993. The effect of age on the seasonality of maturation and spawning of Atlantic cod, *Gadus morhua,* in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 50, 2468-2474.

Hyndes, C., MacArthur, L., 2006. Ecological interactions in coastal marine ecosystems: rock lobster. In: Keesing, J.K., Heine, J.N. (Eds.), Strategic research fund for the marine environment final report. Volume 1: the SRFME initiative and collaborative linkages program. Strategic Research Fund for the Marine Environment, Council for Scientific and Indusrial Research Office, Australia. pp. 143–154.

Ireland, S., Melville-Smith, R., 2005. The strange and secret army: Western Australia's rock lobsters. Western Fisheries December 2005, pp.14-19.

Johnson, M.S., Wernham, J., 1999. Temporal variation of recruits as a basis of ephemeral genetic heterogeneity in the western rock lobster *Panulirus cygnus*. Mar. Biol. 135, 133-139.

Jones, M.B., Simons, M.J., 1983. Latitudinal variation in reproductive characteristics of a mud crab, *Helice crassa* (Grapsidae). Bull. Mar. Sci. 33, 656-670.

Juanes, F., Smith, L.D., 1995. The ecological consequences of limb damage and loss in decapod crustaceans: a review and prospectus. J. Exp. Mar. Biol. Ecol. 193, 197-223.

Krouse, J.S., 1976. Incidence of cull lobsters, *Homarus americanus*, in commercial and research catches off the Maine coast. Fish. Bull. 74, 717-724.

Landers, D.F., Keser, M., Saila, S.B., 2001. Changes in female lobster (*Homarus americanus*) size at maturity and implications for the lobster resource in Long Island Sound, Connecticut. Mar. Freshwat. Res. 52, 1283-1290.

Launey, S., Hedgecock, D., 2001. High genetic load in the Pacific Oyster *Crassostrea gigas*. Genetics 159, 255-265.

Leike, A., 2002. Demonstration of the exponential decay law using beer froth. Eur. J. Phys. 23, 21-26.

Liddy, G.C., Nelson, M.M., Nichols, P.D., Phillips, B.F., Maguire, G.B., 2004. The lipid composition of early stage western rock lobster (*Panulirus cygnus*) phyllosoma: importance of polar lipid and essential fatty acids. J. Shellfish Res. 23, 265-273.

Lipcius, R.N., 1985. Size-dependent reproduction and moulting in spiny lobsters and other long –lived decapods. In: Wenner, A.M. (Ed) 'Crustacean Issues 3. Factors in adult growth'. pp. 129-148. Balkema, Rotterdam.

Lovrich, G.A., Thatje, S., Calcagno, J.A., Anger, K., Kaffenberger, A., 2003. Changes in biomass and chemical composition during lecithotrophic larval development of the southern king crab, *Lithodes santolla* (Molina). J. Exp. Mar. Biol. Ecol. 288, 65-79.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

MacFarlane, J.W., Moore, R., 1986. Reproduction of the ornate rock lobster, *Panulirus ornatus* (Fabricius), in Papua New Guinea. Aust. J. Mar. Freshwat. Res. 7, 55-65.

MacDiarmid, A.B., 1989. Moulting and reproduction of the spiny lobster *Jasus edwardsii* (Decapoda: Palinuridae) in northern New Zealand. Mar. Biol. 103, 303-310.

MacDiarmid, A.B., 1989. Size at onset of maturity and size-dependent reproductive output of female and male spiny lobsters *Jasus edwardsii* (Hutton) (Decapoda, Palinuridae) in northern New Zealand. J. Exp. Mar. Biol. Ecol. 127, 229-243.

MacDiarmid, A. B., Butler, M. J., IV, 1999. Sperm economy and limitation in spiny lobsters. Behav. Ecol. Sociobiol 46, 14-24.

MacDiarmid, A., Stewart, R., Oliver, M., 2000. Mate choice in rock lobsters. Seafood N.Z. 8 (7), 38-39.

MacDiarmid, A., Stewart, R., 2005a. Male mating capacity in the New Zealand red rock lobster. The Lobster Newsletter 18(1), 7.

MacDiarmid, A., Stewart, R., 2005b. Male – but not female – mate choice by the red rock lobster is influenced by numbers of potential mates in a den. The Lobster Newsletter 18(1), 8-9.

MacDiarmid, A., Sainte-Marie, B., 2006. Reproduction. In: Phillips, B. (Ed) 'Lobsters: Biology, Management, Aquaculture and Fisheries'. pp. 45-77. Blackwell Publishing, Oxford.

Maggioni, R., Rogers, A.D., 2001. Microsatellite primers for three Western Atlantic *Farfantepenaeus* prawn species. Molecular Ecology Notes 100, 8-9.

Malmquist, H.J., Inginarsson, F., Johannsdottir, E.E., Gislason, D., Snorrason, S.S., 2002. Biology of Brown Trout (Salmo trutta) and Artic Char (Salvelinus alphinus) in four Faroese Lakes. Ann. Soc. Scient. Faeroensis Suppl. 36, 94-113.

Mariappan, P., Balasundaram, C., 2001. Limb autotomy along with exuvium in freshwater prawn, *Macrobrachium malcolmsonii*. Indian J. Anim. Sci. 71, 1001-1002.

Marshall, C.T., Frank, K.T., 1995. Density-dependent habitat selection by juvenile haddock (*Melanogrammus aeglefinus*) on the southwestern Scotian Shelf. Can. J. Fish. Aquat. Sci. 52, 1007–1017.

McVean, A., 1982. Autotomy. In: Sandeman, D.C., Atwood, H.L. (Eds.), The biology of crustaceans, neural integration and behaviour. Academic Press Inc., New York, pp. 107-246.

Melville-Smith, R., de Lestang, S., 2006. Spatial and temporal variation in the size at maturity of the western rock lobster *Panulirus cygnus*. Mar. Biol. 150, 183-195.

Melville-Smith, R., Jones, J.B., Brown, R.S., 1997. Biological tags as moult indicators in *Panulirus cygnus* (George). J. Mar. Freshwat. Res. 48, 959-965.

Melville-Smith, R., Chubb, C.F., Caputi, N., Cheng, Y.W., Christianopoulos, D., Rossbach, M., 1998. Fishery-independent survey of the breeding stock and migration of the western rock lobster (*Panulirus cygnus*). Fisheries Research and Development Project No. 96/108, Final Report. 47pp.

Melville-Smith, R., de Lestang, S., 2005. Visual assessment of the reproductive condition of female western rock lobsters (*Panulirus cygnus*). N. Z. J. Mar. Freshwat. Res. 39, 557-567.

Melville-Smith, R., de Lestang, S., Chatfield, B., Nelson, M.M., Nichols, P.D., 2007. Neither maternal size nor site of spawning influences larval competency in western rock lobster *Panulirus cygnus* George. J. Crust. Biol. **27(3), 445–453**.

Montgomery, S.S., 1992. Sizes at onset of breeding in female *Jasus verreauxi* (Decapoda: Palinuridae) from New South Wales, Australia. Aust. J. Mar. Freshwat. Res. 43, 1373-1379.

Morgan, G.R., 1977. Aspects of the population dynamics of the western rock lobster and their role in management. PhD thesis, University of Western Australia. 341pp.

Morgan, G.R., Phillips, B.F., Joll, L.M., 1982. Stock and recruitment relationships in *Panulirus cygnus*, the commercial rock (spiny) lobster of Western Australia. Fish. Bull. 80, 475–486.

Mota-Alves M.I., Paiva, M.P., 1976. Frequencia de acasalamentos em lagostas do genero Panulirus White (Decapoda, Palinuridae). Arq. Cien. Mar. 16, 61-63.

Nakamura, S., 1940. Ecological studies of the spiny lobster *Panulirus japonicus* (v. Siebold), with special reference to its conservation. J. Imp. Fish. Inst. (Japan) 34, 101-113.

Nei, M. 1987. Molecular evolutionary genetics. Columbia Press, New York.

Nelson, M.M., Crear, B.J., Nichols, P.D., Ritz, D.A., 2003. Feeding southern rock lobster, *Jasus edwardsii* Hutton, 1875, phyllosomata in culture: recent progress with lipid-enriched *Artemia*. J. Shellfish Res. 22, 225-233.

Nelson, M.M., Crear, B.J., Nichols, P.D., Ritz, D.A., 2004. Growth and lipid composition of phyllosomata of the southern rock lobster, *Jasus edwardsii*, fed enriched *Artemia*. Aquacult. Nutr. 10, 237-246.

Norman, C.P., Jones, M.B., 1993. Reproductive ecology of the velvet swimming crab, *Necora puber* (Brachyura: Portunidae), at Plymouth. J. Mar. Biol. Assoc. U.K. 73, 379-389.

Oshima, Y., 1941. A note on the ecology of Japanese spiny lobster *Panulirus japonicus*. Suisan Gakkaiho 8, 231-238.

Ottaway, J.R., Cary, J.L., Robinson, J.E., 1985. Population changes, deduced from anecdotal information, of certain molluscs, crustaceans and fish in the Marmion Marine Park. Environmental Protection Agency Technical Series 19, 163–199.

Panagiotaki, P., Geffen, A.J., 1992. Parental effects on size variation in fish larvae. J. Fish Biol. 41 (Supplement B), 37-42.

Paterson, N.F., 1969. The behaviour of captive Cape rock lobsters, *Jasus lalandii* (H. Milne-Edwards). Ann. S. Afr. Mus. 52, 225-264.

Phillips, B.F., Chubb, C.F., Melville-Smith, R., 2000. The status of Australia's rock lobster fisheries. In: Phillips, B.F., Kittaka, J. (Eds.), Spiny lobsters: fisheries and culture, Blackwell Science, Oxford. pp. 45–77.

Phillips, B.F., Melville-Smith, R., 2005. Sustainability of the western rock lobster fishery: a review of past progress and future challenges. Bull. Mar. Sci. 76 (2), 485-500.

Phillips, B.F., Melville-Smith, R., Caputi, N., 2007. The western rock lobster fishery in Western Australia. In: McClanahan, T.R., Castilla, J.C. (Eds.), Fisheries Management: progress towards sustainability. Blackwell Press, Oxford. pp. 231–252.

Phleger, C.F., 2000. Biochemical characterisation of the nutritional status of wild Australian southern rock lobster: final report. CSIRO Marine Research, Hobart, Tasmania, Australia.

Phleger, C.F., Nelson, M.M., Mooney, B.D., Nichols, P.D., Ritar, A.J., Smith, G.G., Hart, P.R., Jeffs, A.G., 2001. Lipids and nutrition of the southern rock lobster, *Jasus edwardsii*, from hatch to puerulus. Mar. Freshwat. Res. 52, 1475-1486.

Puebla, O., Parent, E., Sevigny, J.M., 2003. New microsatellite markers for the snow crab *Chionoecetes opilio* (Brachyura: Majidae). Molecular Ecology Notes 3, 644-646.

Raymond, M., Rousset, F., 1995. GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86, 248–249.

Reece, K.S., Ribeiro, W.L., Gaffney, P.M., Carnegie, R.B., Allen, S.K., 2004. Microsatellite marker development and analysis in the eastern oyster (*Crassostrea virginica*): Confirmation of null alleles and non-Mendelian segregation ratios. Journal of Heredity 95, 346-352.

Reynolds, J.D., Gross, M.R., 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. Proc. R. Soc. London B 250, 57-62.

Reynolds, R.W., Smith, T.M., 1994. Improved global sea surface temperature analyses using optimum interpolation. J. Climate 7, 929-948.

Reznick, D., 1985. Costs of reproduction: an evaluation of the empirical evidence. Oikos 44, 257-267.

Reznick, D.A., Bryga, H., Endler, J.A., 1990. Experimentally induced life-history evolution in a natural population. Nature 346, 357-359.

Rideout, R.M., Trippel, E.A., Litvak, M.K., 2004. Paternal effects on haddock early life history traits. J. Fish Biol. 64, 695-701.

Rideout, R.M., Morgan, M.J., 2007. Major changes in fecundity and the effect on population egg production for three species of north-west Atlantic flatfishes. J. Fish Biol. 70, 1759-1779.

Rijnsdorp, A.D., 1993. Fisheries as a large-scale experiment on life-history evolution – disentangling phenotypic and genetic effects in changes in maturation and reproduction of North Sea plaice, *Pleuronectes platessa* L. Oecologia 96, 391-401.

Ritar, A.J., Dunstan, G.A., Crear, B.J., Brown, M.R., 2003. Biochemical composition during growth and starvation of early larval stages of cultured spiny lobster (*Jasus edwardsii*) phyllosoma. Comp. Biochem. Physiol. A 136, 353-370.

Roff, D.A., 1992. The evolution of life histories: theory and analysis. Chapman and Hall, New York, 535 p.

Rondeau, A., Sainte-Marie, B., 2001. Variable mate-guarding time and sperm allocation by male snow crabs (*Chionoecetes opilio*) in response to sexual competition, and their impact on the mating success of females. Biol. Bull. 201, 204-217.

Roustaian, P., Kamarudin, M.S., Omar, H.B., Saad, C.R., Ahmad, M.H., 2001. Biochemical changes in freshwater prawn *Macrobrachium rosenbergii* during larval development. J. World Aquacult. Soc. 32, 53-59.

Rozen, S., Skaletsky, H., 2000. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz, S., Misener, S. (Eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology Series). Humana Press, Totowa, NJ, pp. 365-386.

SAS Institute, Inc. 1989. SAS/STAT user's guide, Vol 2, version 6, 4th ed. SAS Institute, Inc., Cary, North Carolina.

Sharp, W.C., Hunt, J. H., Lyons, W.G., 1997. Life history of the spotted spiny lobster, *Panulirus guttatus*, an obligate reef dweller. Mar. Freshwat. Res. 48, 687-698.

Smith, E.M., Howell, P.T., 1987. The effects of bottom trawling on American lobsters *Homarus americanus* in Long Island Sound. Fish. Bull. 85, 737-744.

Smith, G.G., Ritar, A.J., Johnston, D., Dunstan, G.A., 2004. Influence of diet on broodstock lipid and fatty acid composition and larval competency in spiny lobster, *Jasus edwardsii*. Aquaculture 233, 451-475.

Solemdal, P., Kjesbu, O.S., Fonn, M., 1995. Egg mortality in recruit-and repeat- spawning cod - an experimental study. ICES CM/G35, Mimeo. 10 pp.

Somerton, D.A., 1980. A computer technique for estimating the size of sexual maturity in crabs. Can. J. Fish. Aquat. Sci. 37, 1488-1494.

Street, R.J., 1969. The New Zealand crayfish *Jasus edwardsii* (Hutton 1875). New Zealand Marine Department Fisheries Technical Report 30, 1-53.

Sutcliffe, W.H., 1952. Some observations of the breeding and migration of the Bermuda spiny lobster, *Panulirus argus*. Gulf and Caribbean Fisheries Institute, University of Miami Proceedings 4, 64-69.

Sutcliffe, W.H., 1953. Some observations on the breeding and migration of the Bermuda spiny lobster, *Panulirus argus*. J. Mar. Res. 12, 173-183.

Templeman W., 1936. Local differences in the life-history of the lobster (*Homarus americanus*) on the coast of the maritime provinces of Canada. J. Biol. Bd Canada 2, 41-87.

Swain, D.P., Sinclair, A.F., 1994. Fish distribution and catchability: what is the appropriate measure of distribution? Can. J. Fish. Aquat. Sci. 51, 1046–1054.

Tennyson, A., 2005. Post gastrula development of the western rock lobster, *Panulirus cygnus* (George), with a guide for aging eggs and a model for predicting the time of egg hatch. Honours Thesis, University of Western Australia, Australia. 77 pp.

Trippel, E.A., 1998. Egg size and viability and seasonal offspring production of young Atlantic cod. Trans. Am. Fish. Soc. 127, 339-359.

Volkman, J.K., Nichols, P.D., 1991. Application of thin layer chromatography–flame ionization detection to the analysis of lipids and pollutants in marine and environmental samples. J. Planar Chromatogr. 4, 19-26.

Waddington, K., Melville-Smith, R., Walker, D., Knott, B., 2005. Effects of reproductive state and sex on movement and food consumption of western rock lobster (*Panulirus cygnus*) in a tank experiment. N. Z. J. Mar. Freshwat. Res. 39, 365-372.

Walsh, M.R., Munch, S.B., Chiba, S., Conover, D.O., 2006. Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecol. Lett. 9, 142-148.

Walters, C., Maguire, J.J., 1996. Lessons for stock assessment from the northern cod collapse. Rev. Fish Biol. Fish. 6, 125–137.

Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38, 1358-1370.

Wright, I., Caputi, N., Penn, J., 2006. Depletion-based population estimates for western rock lobster (*Panulirus cygnus*) fishery in Western Australia. N. Z. J. Mar. Freshwat. Res. 40, 107-122.

Yoneda M., Wright, P.J., 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Mar. Ecol. Prog. Ser. 276, 237-248.

16.0 Appendices

Appendix I

INTELLECTUAL PROPERTY:

There is no identifiable intellectual property arising from the project.

Appendix II

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