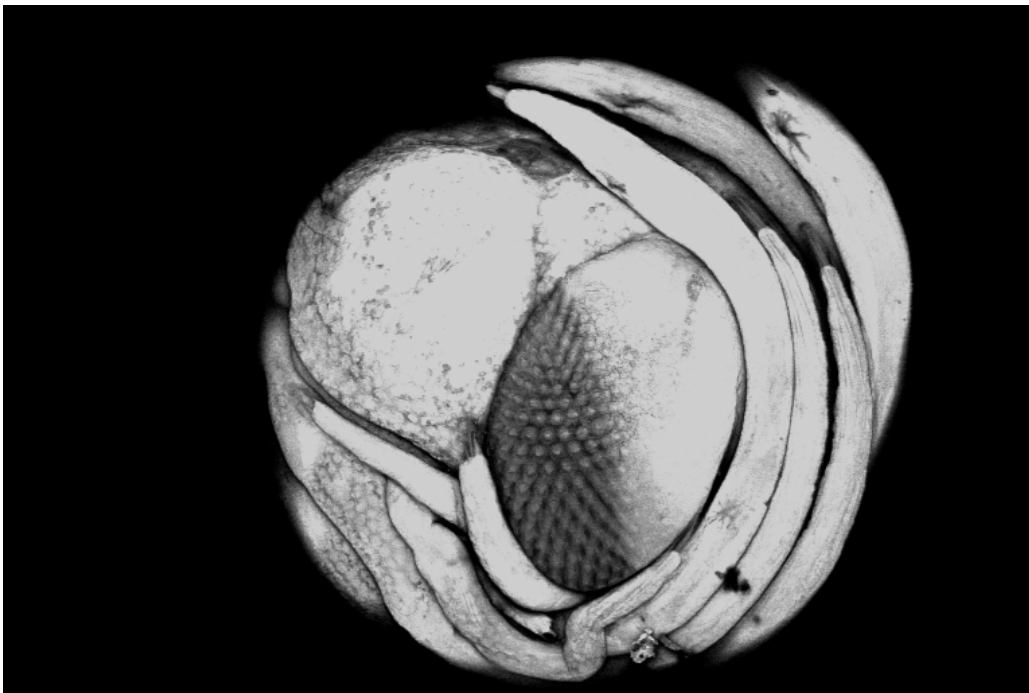# Investigating Reproductive Biology Issues Relevant to Managing the Western Rock Lobster Broodstock

Source: Andrew Tennyson

**Melville-Smith, R., de Lestang, S., Beale, N.E.,
Groth, D. and Thompson, A.**

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Department of Fisheries

Fisheries Research and Development Corporation Report

FRDC Project 2003/005

FINAL REPORT

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

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Date: January 2009

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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 lobsters. One of the outcomes of these increases in efficiency has been an increase in pressure on the breeding stock. Managers have had to respond by introducing measures to protect the breeders. In 1993 this involved the imposition of imposed 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). 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 and using those outputs, together with other data, to 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 lobster 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 a 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 batches of eggs.

This study has examined whether the size, biochemical composition and consequent likelihood of survival of larvae of western rock lobster are different for those produced by small, average size and 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 area of capture.

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. 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. 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.

In the final research component undertaken by this study, paternity assignment tests were used in an attempt to establish mating choices of male and female western rock lobsters on an isolated deep water (50 m) reef. Depletion studies suggested that most of the lobsters resident on the reef were caught on the separate sampling trips, but it became apparent that there was considerable emigration and emigration on and off the sampling site – an unexpected result. Accordingly, there were few matches of males caught, to the spermatophores or eggs sampled on 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, but the method was used to record increments in nine males and three females.

KEYWORDS: egg production; selective fishing; paternity assignment; behaviour; maturity

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 particular people or groups of people that we would like to specifically acknowledge:

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 catch. One of the outcomes of these increases in their efficiency has been an increase in pressure on the breeding stock. Managers have had to respond to these pressures on the breeding stock by introducing measures to protect the breeders. In 1993 this involved the imposition of imposed 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 protecting the brood stock, probably has a more highly skewed sex ratio than most (perhaps all) other lobster fisheries in the world, which has been a strong motivator for the 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 fish, that eggs from new spawners (and therefore probably small animals) have a lower hatching success compared to eggs from old 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. The fishery produces breeding stock indices for different zones in the fishery

and these are sometimes combined into a single breeding stock index for the fishery, but information on the proportion that the three zones contribute to the overall index have been missing.

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 question. 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 of various management measures such as the setose rule and maximum size rules might be having on the brood stock.

On the basis of these results there has been reassurance to industry that there would seem not to be cause for concern that distorted sex ratios and mismatches of size classes between the sexes of mature lobsters in the natural environment, is impacting 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 our outlined in 1.2 (above). The way that these objectives have been met in the following chapters is outlined below.

Chapter 2 is titled 'Visual Assessment of the Reproductive Condition of Female Western Rock Lobsters (*Panulirus cygnus*)'. This paper does not address any of the objectives specifically, but was necessary in order to be able to do the reproductive condition assessments that were used throughout this study.

Chapter 3 is titled 'Spatial and temporal variation in the size at maturity of the western rock lobster *Panulirus cygnus* George'. This paper, and the next one in Chapter 4, '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 and 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 7 to calculate spatial and temporal changes in egg production.

Chapter 5 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 came out of 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 egg production.

Chapter 6 titled 'Neither maternal size nor site of spawning influences larval competency in western rock lobster *Panulirus cygnus* George addresses in full, Objective 2: to undertake preliminary investigations into the implications of egg diameters being significantly smaller at the Abrolhos Islands than at the coast.

Chapter 7 is titled 'Spatial and temporal changes in egg production in the western rock lobster (*Panulirus cygnus*) fishery' has utilized the 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 weight the overall egg production index for the stock by the contribution of the management zones.

Chapter 8 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 9.

Chapter 9 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 Visual assessment of the reproductive condition of female Western rock lobsters (Panulirus cygnus)

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2.1 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

2.2 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.

2.3 Methods

2.3.1 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).
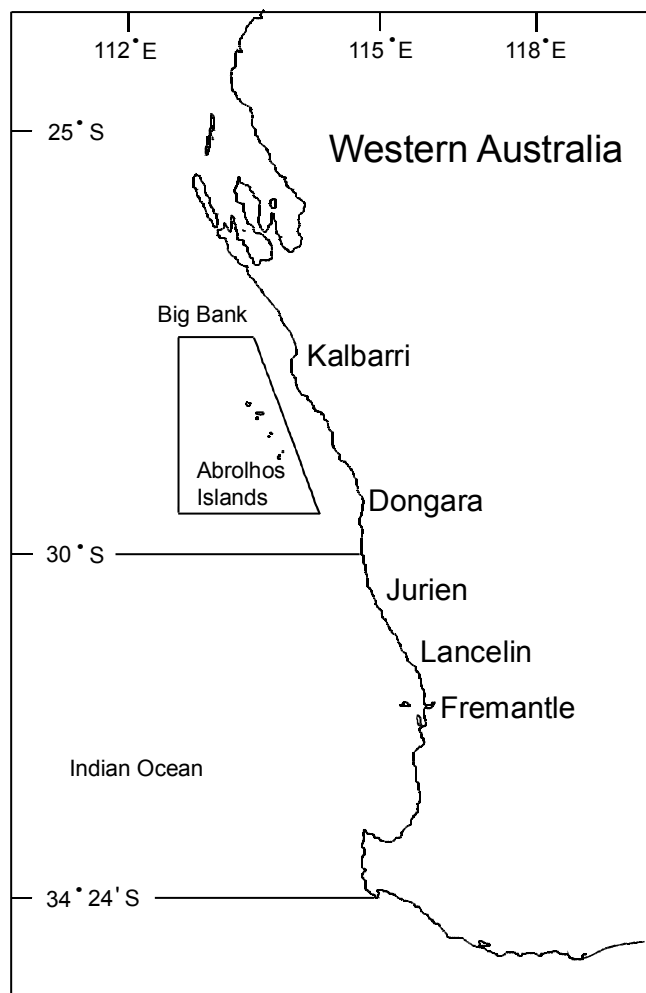
Figure 1. Map showing the locations of the fishery-independent egg production surveys in October/November 2002.

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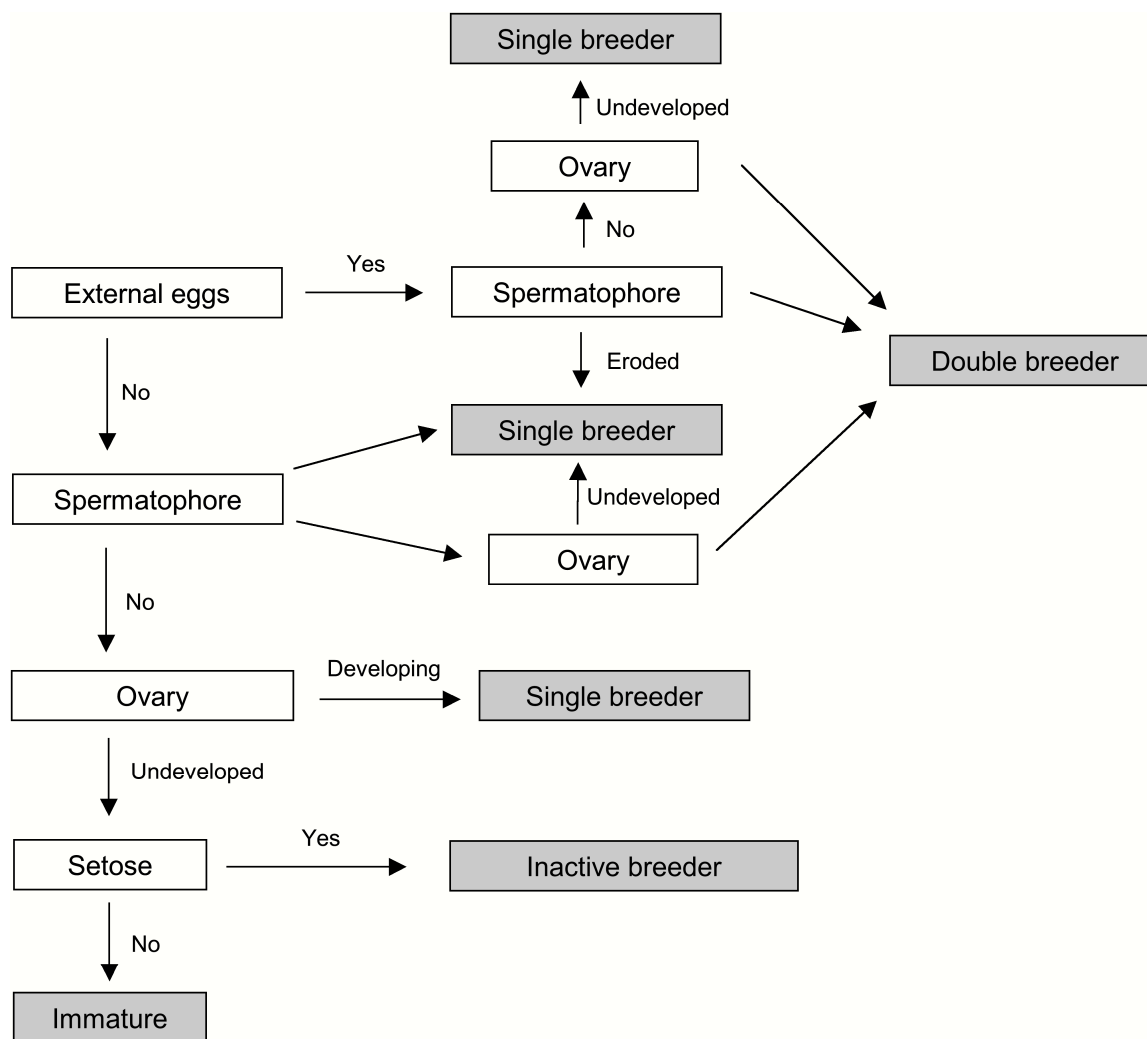
Figure 3. Flow chart showing the various combinations of external characteristics which, when combined, classify a female *Panulirus cygnus* as immature, inactive breeder, single breeder or double breeder.

2.3.2 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.

2.4 Results

2.4.1 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.
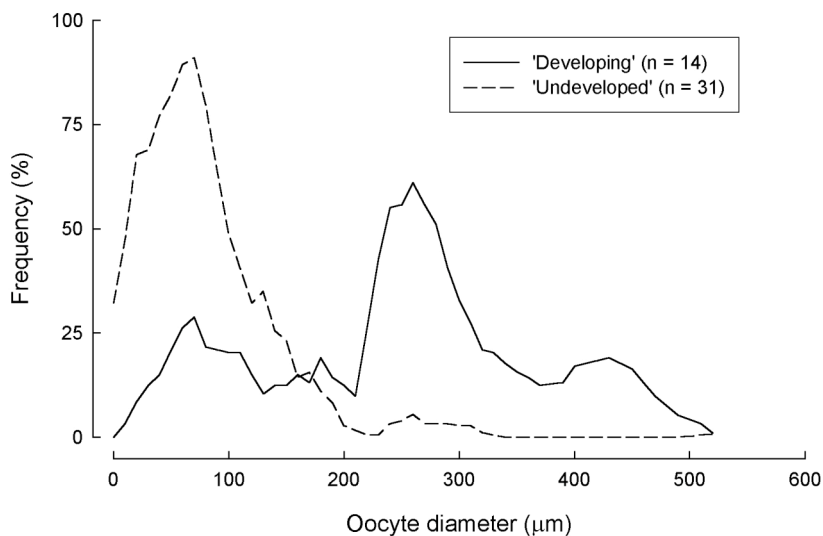
Figure 2. Oocyte diameters of *Panulirus cygnus* ovaries that were classified as 'developing' (yellow/orange) or 'undeveloped' (white/translucent) when viewed through the dorso-thoracic musculature.

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'.

2.4.2 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'.

2.4.3 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).
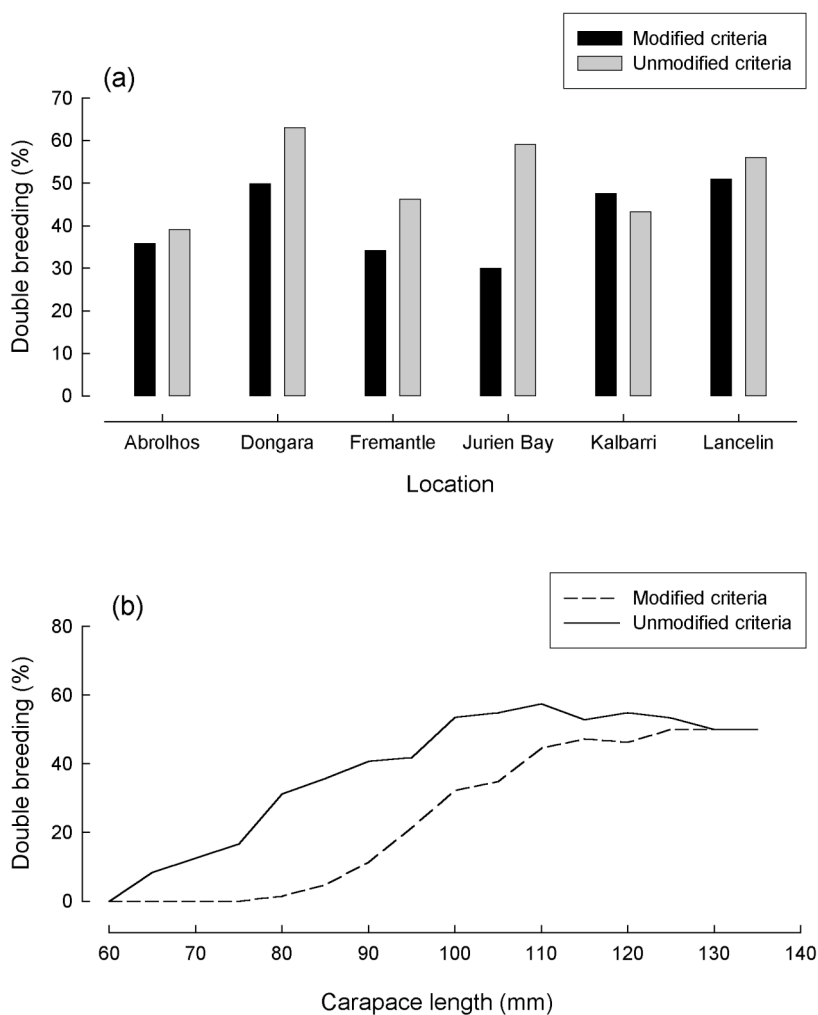
Figure 4. Comparison between the proportions of *Panulirus cygnus* classified as double breeders (a) at six locations and (b) in different carapace length classes collected at Fremantle, determined from modified criteria (see Fig. 3) and the unmodified criteria (see Chubb *et al.*, 1991). Carapace-length classes have been smoothed with a three-point moving average.

2.5 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.

2.6 Acknowledgements

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

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3.1 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

3.2 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.

3.2 Methods

3.2.1 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).
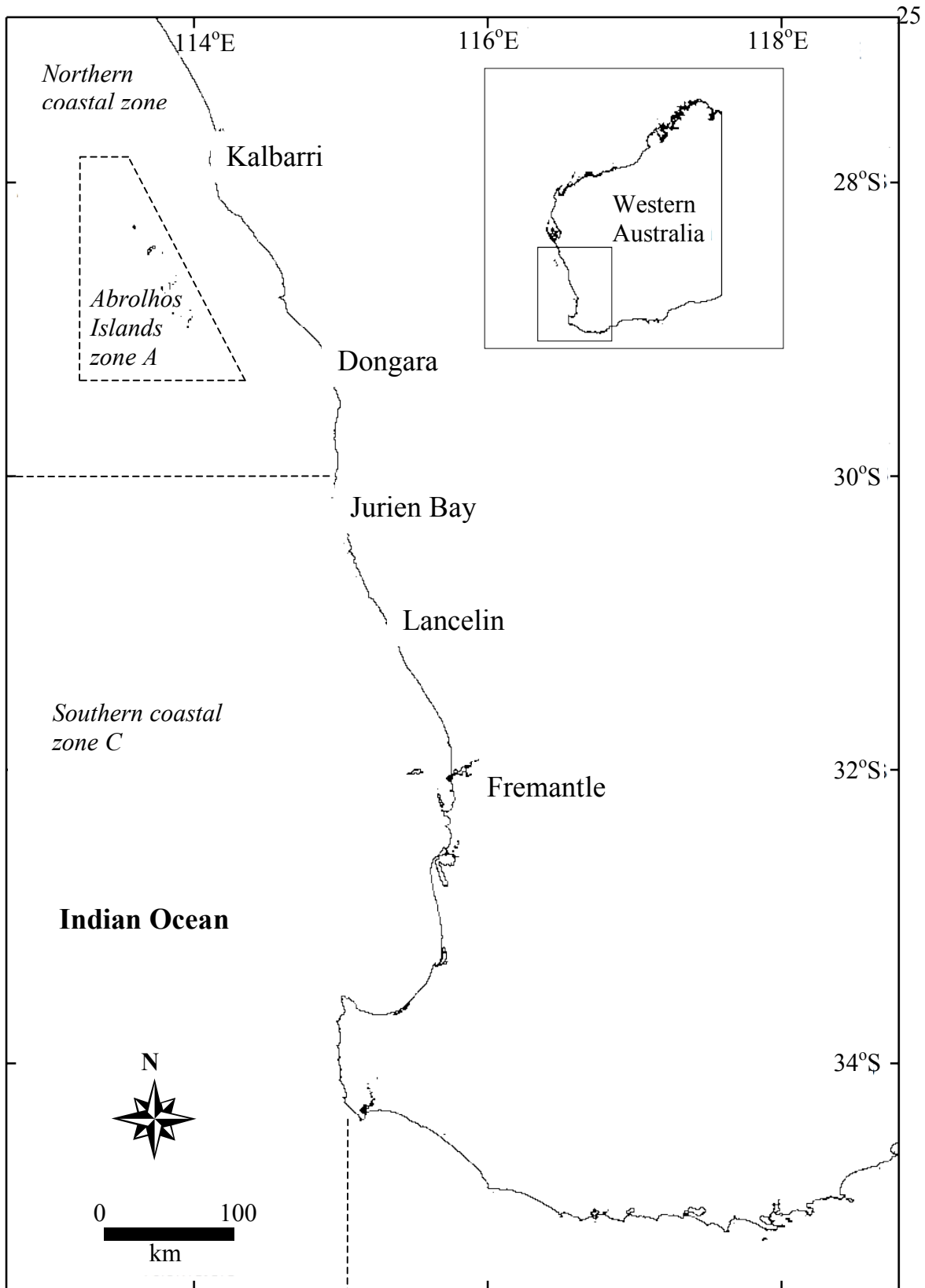
Figure 1. Locations at which the size-at-maturity data for western rock lobsters were collected.

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).

3.2.2 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 (^{o}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.

3.2.3 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

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

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.

3.3 Results

3.3.1 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 ($P>0.05$).
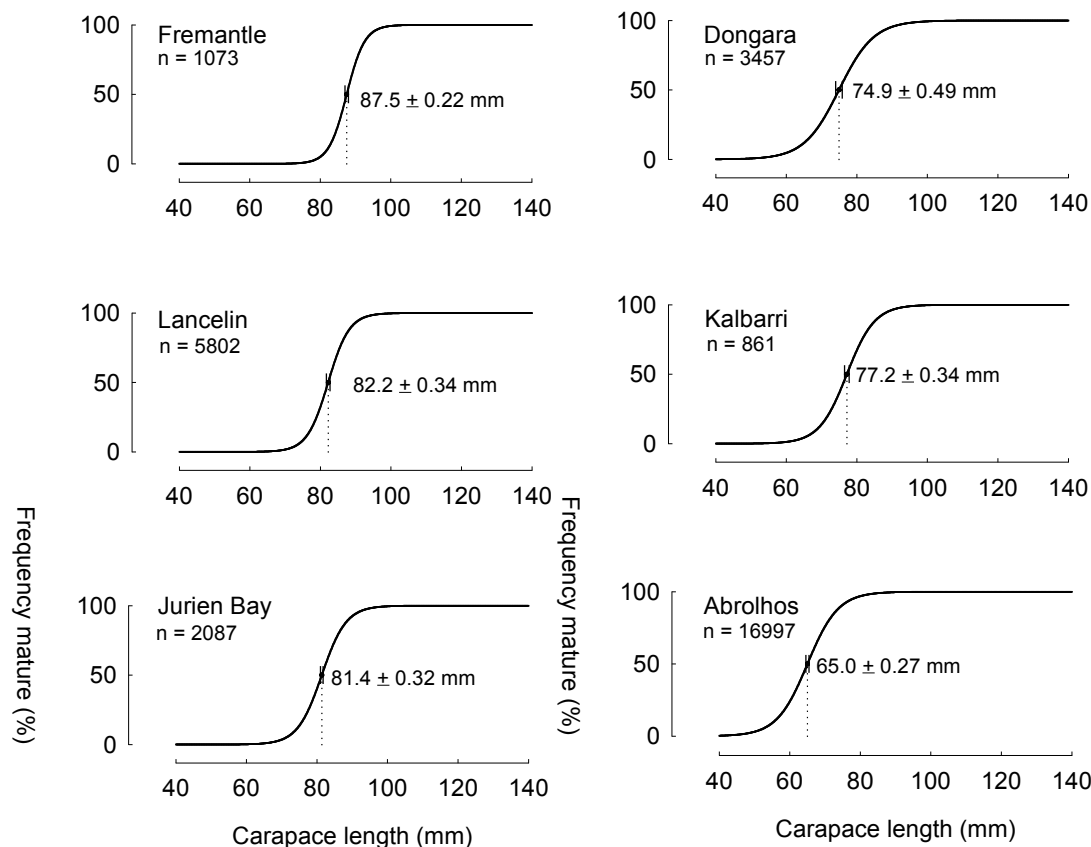
Figure 2. Logistic regressions fitted to the percentage of mature female *Panulirus cygnus* at different carapace lengths in six locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. $CL_{50} \pm 1$ SE denotes the size at which 50% of the assemblage is mature and *n* the sample size.

3.3.2 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.
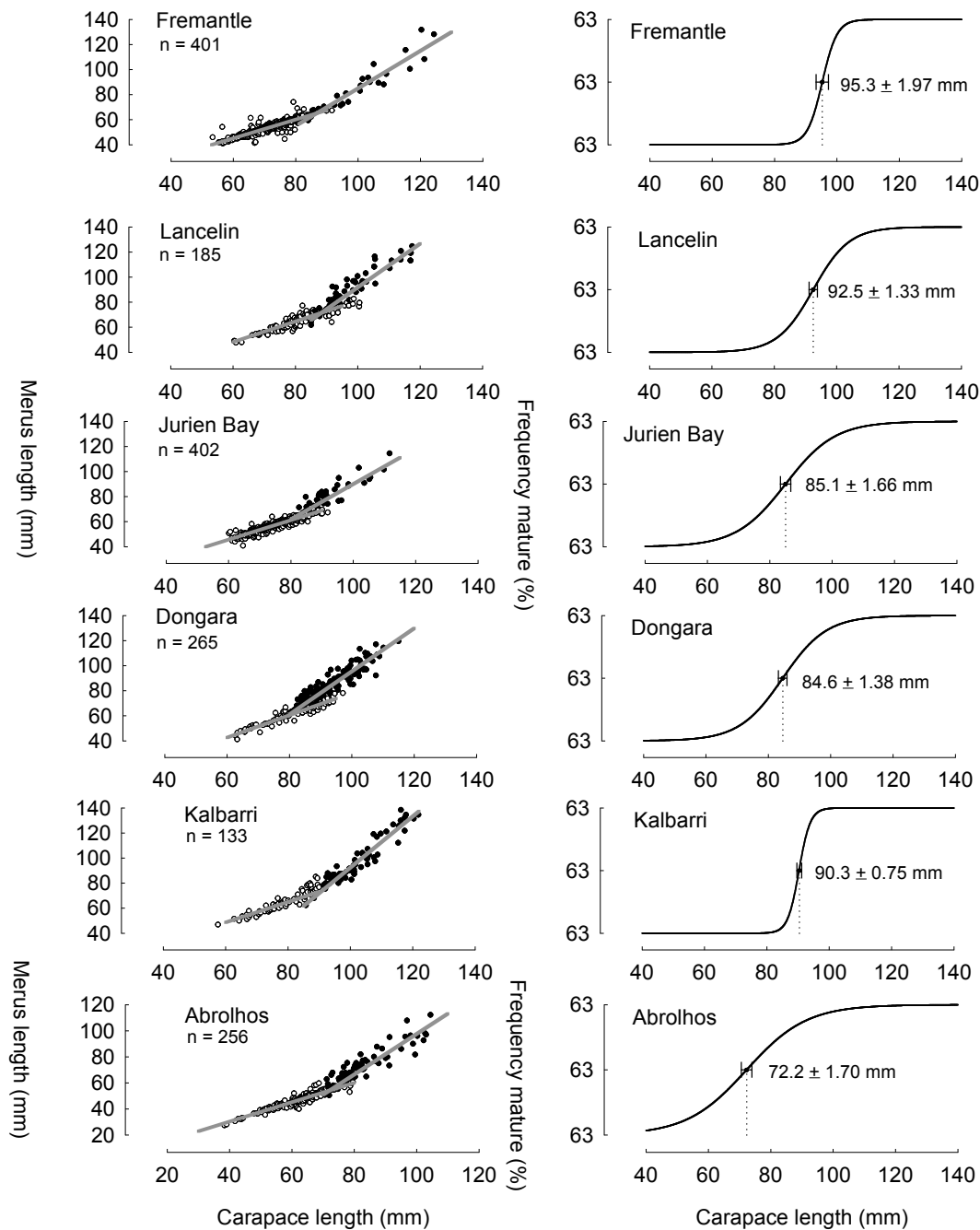
Figure 3. Relationship between the merus of the second pereiopod and carapace length of immature (○) and mature (●) male *Panulirus cygnus* (left) and logistic regressions fitted to the percentage of morphometrically mature males at different carapace lengths (right) in six locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. $CL_{50} \pm 1$ SE denotes the size at which 50% of the assemblage is mature and n the sample size**.**

3.3.3 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.
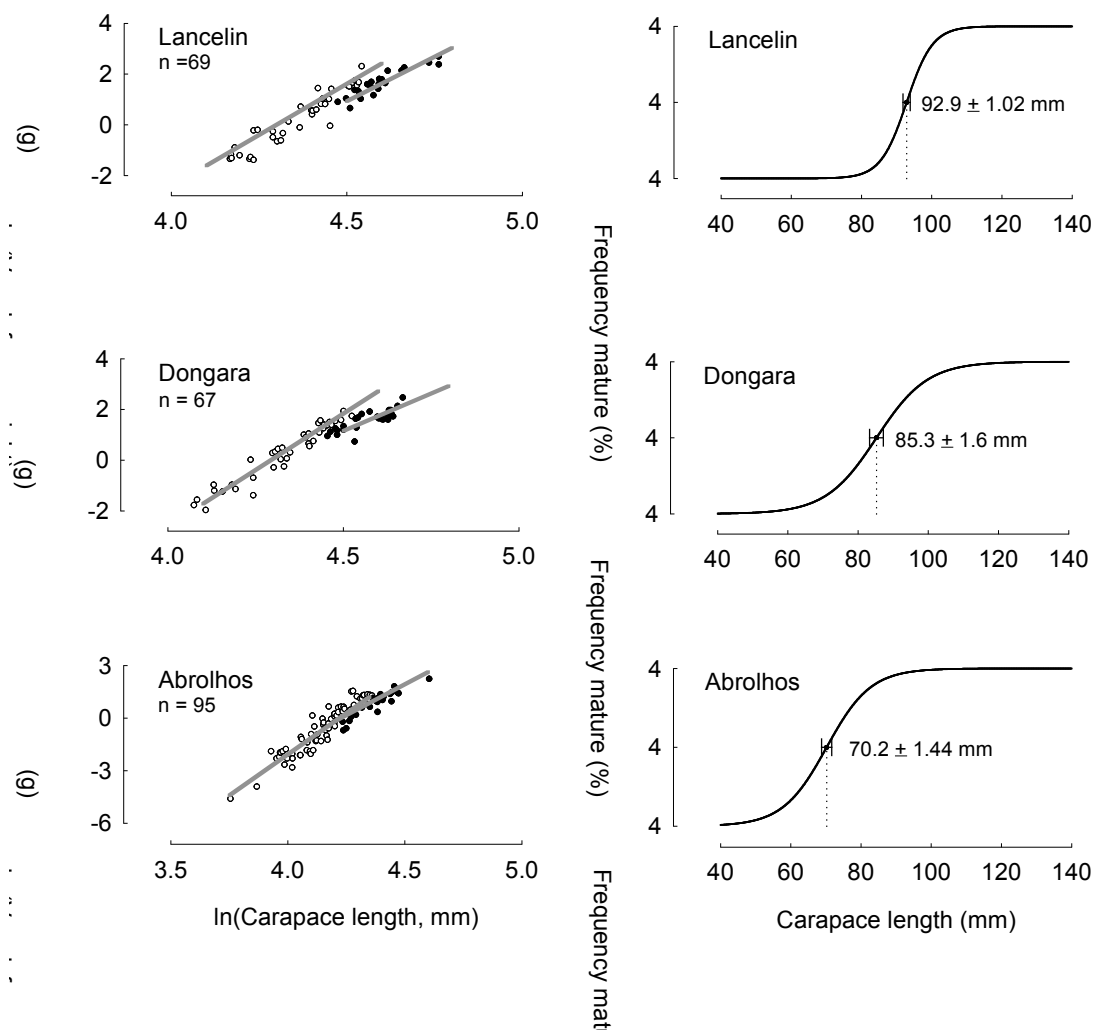
Figure 4. Relationship between the natural logarithms of the vas deferentia weight and carapace length of immature (○) and mature (●) male *Panulirus cygnus* (left) and logistic regressions fitted to the percentage of physiologically mature males at different carapace lengths (right) in three locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. $CL_{50} \pm 1$ SE denotes the size at which 50% of the assemblage is mature and n the sample size**.**

3.3.4 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: $FemaleCL_{50} = -10.75x°C + 311.64$ ($P<0.01$) and $MaleCL_{50} = -11.42x°C + 334$ ($P<0.01$), with the correlation coefficient for females (0.93) being the same as that for males (0.93) (Fig. 5b).
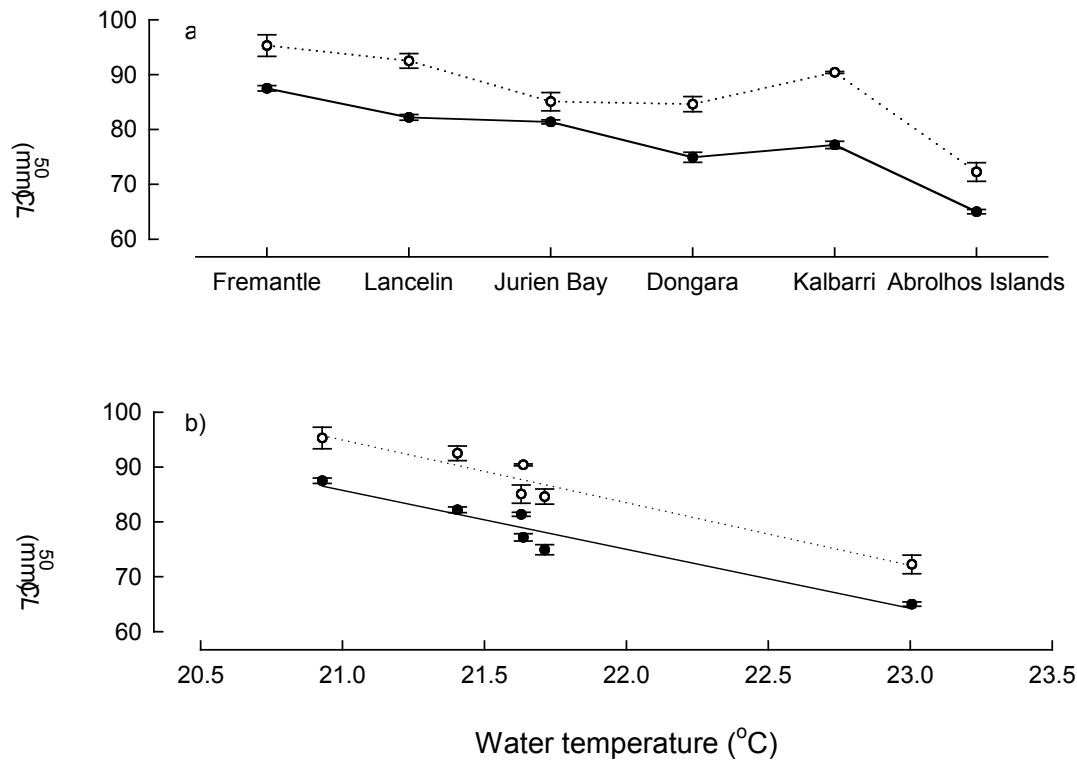
Figure 5. (a) Size at maturity (CL_{50}) ± 1 SE of female (●) and male (○) *Panulirus cygnus* at six locations and **(b)** linear regressions fitted to the relationships between female (●) or male (○) CL_{50}s at each location and the corresponding mean annual water temperature at that location.

3.3.5 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.
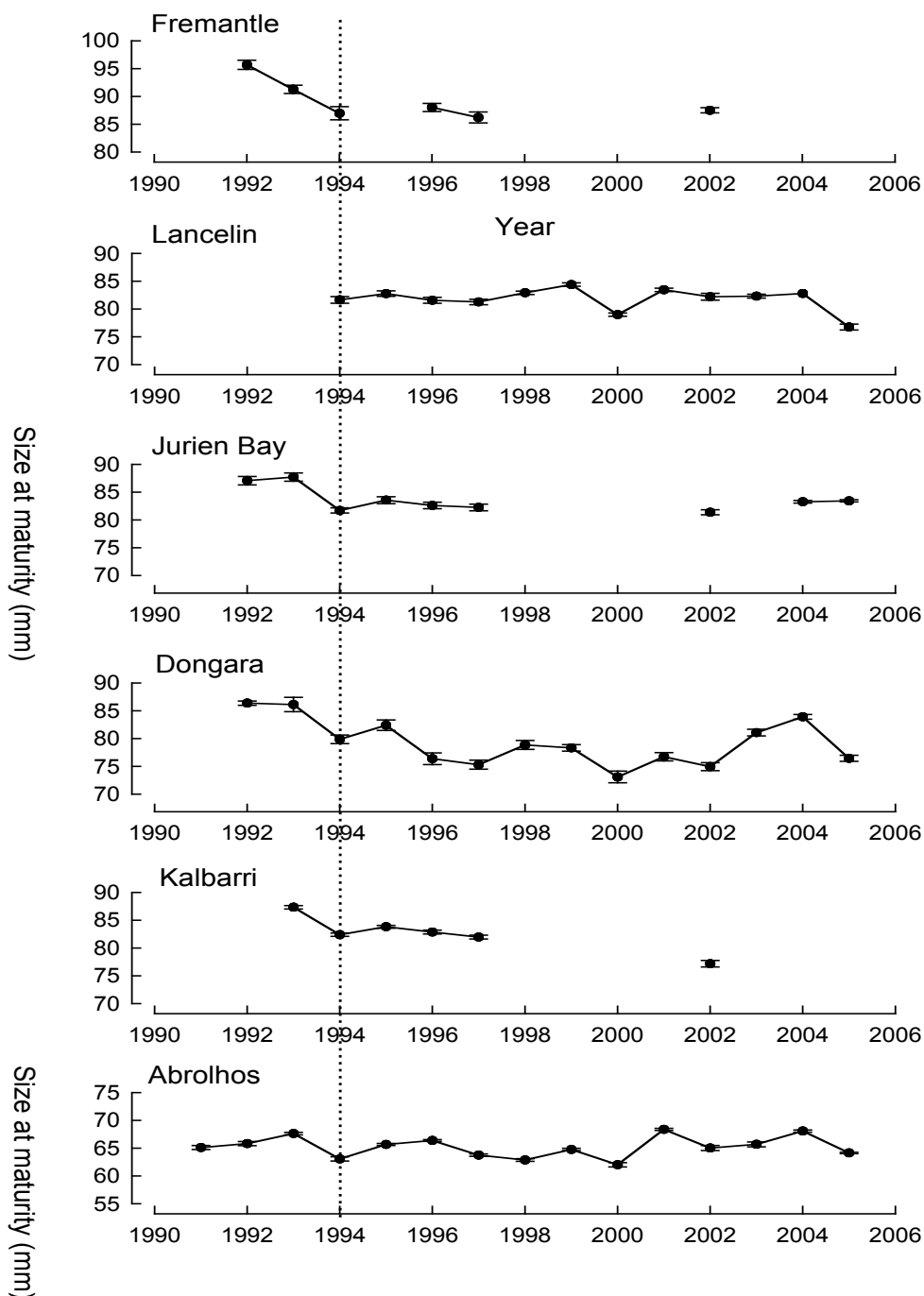
Figure 6. Size at maturity (CL$_{50}$) ± 1 SE of female *Panulirus cygnus* at six locations from the early 1990s to mid-2000s, based on data collected during the Independent Breeding Stock Surveys. Vertical dotted line represents 1994**.**

3.3.6 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).
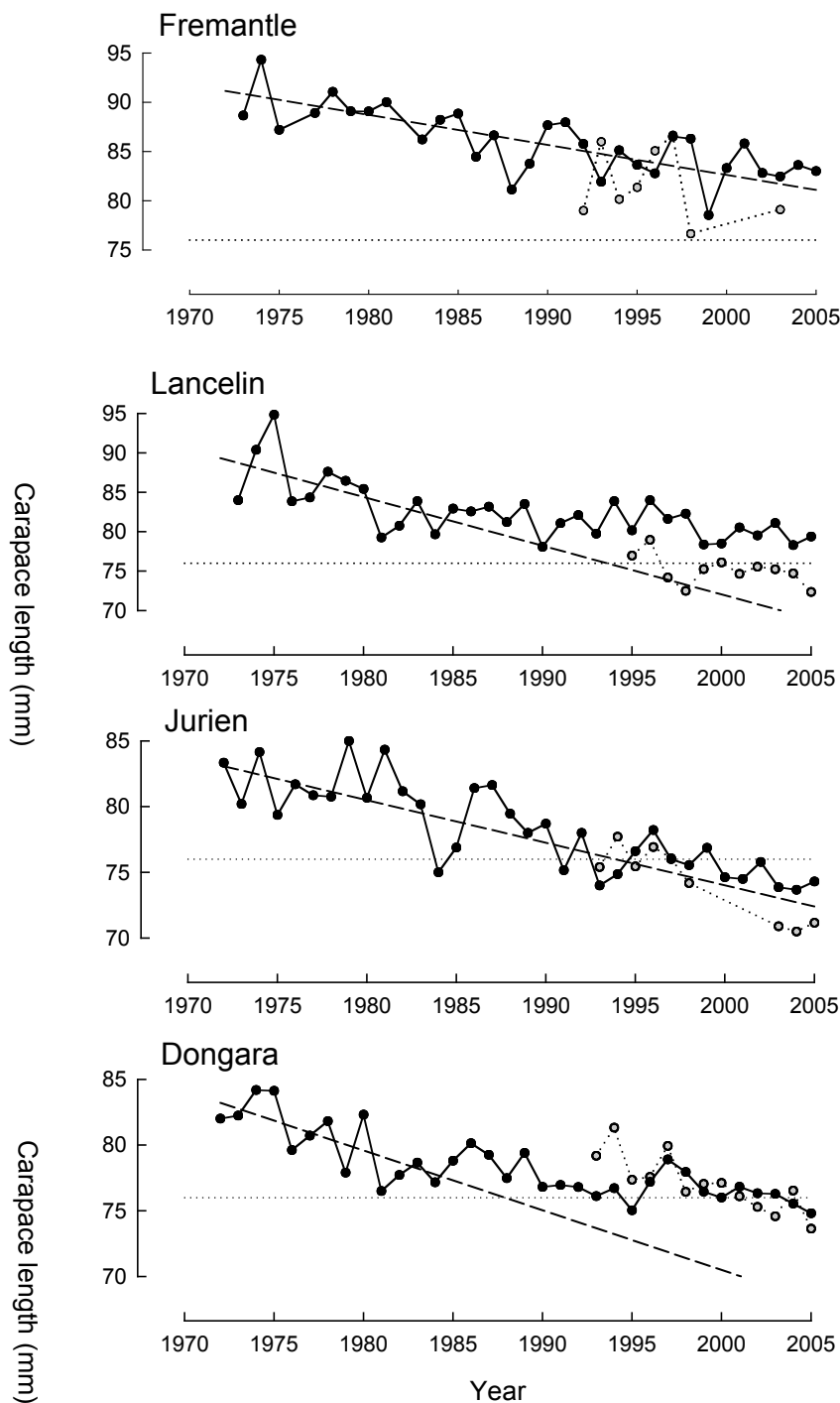
Figure 7. Mean carapace lengths of the smallest 10% of mature female *Panulirus cygnus* caught in each year at four locations from 1972 to 2005 based on data collected during Fishery Dependent Commercial Catch Monitoring (●) and from 1992 to 2005 based on data collected during Fishery Independent Breeding Stock Surveys (○). Horizontal dotted line represents 76 mm and dashed line represents a linear regression between mean carapace length and the years before 1986.

3.4 Discussion

3.4.1 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).

3.4.2 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.

3.4.3 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.

3.5 Acknowledgements

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

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4.1 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

4.2 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.

4.3 Methods

4.3.1 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).
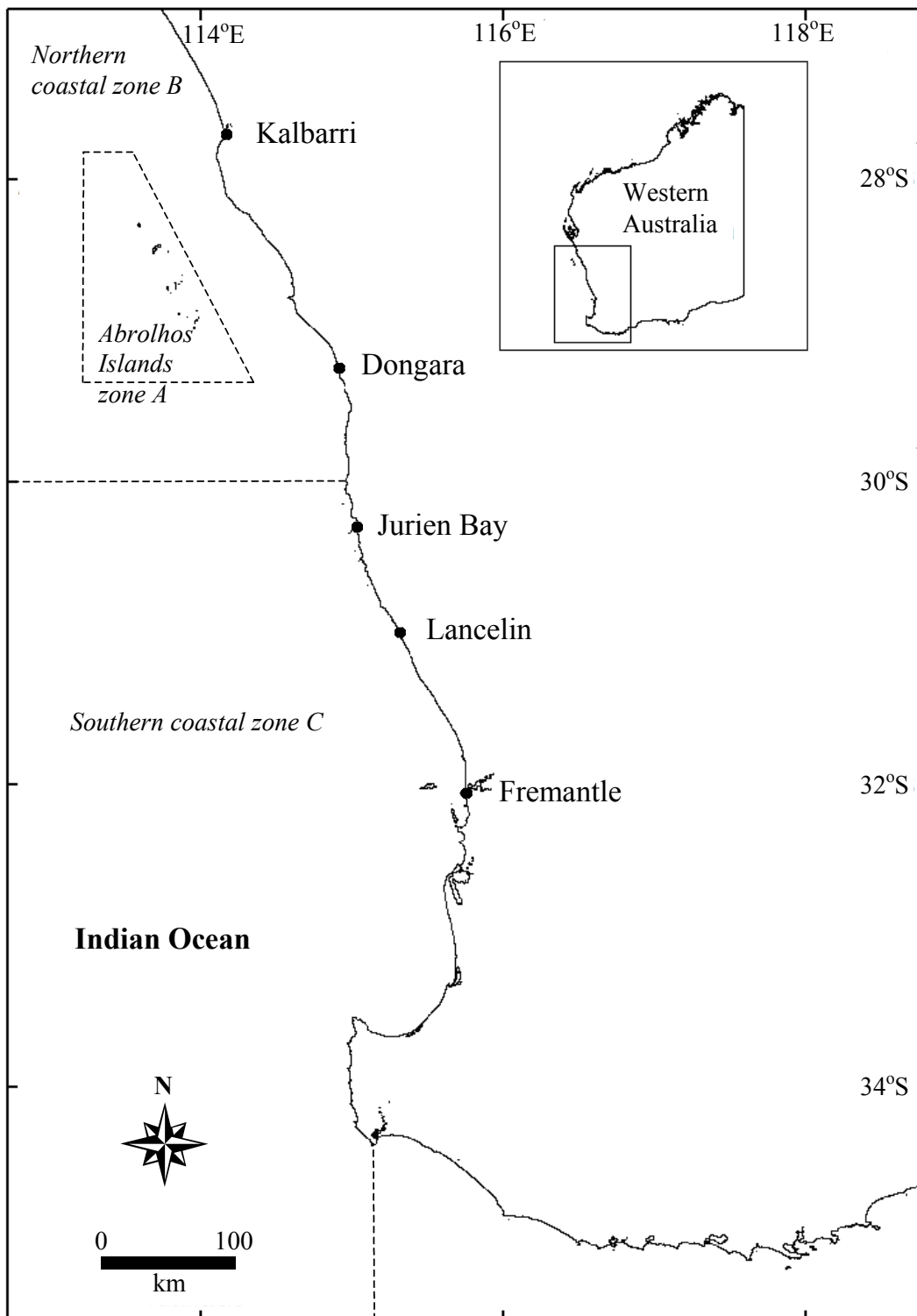
Figure 1. Management zones in the western rock lobster fishery and locations of fishery-independent breeding stock surveys and commercial monitoring of the catch.

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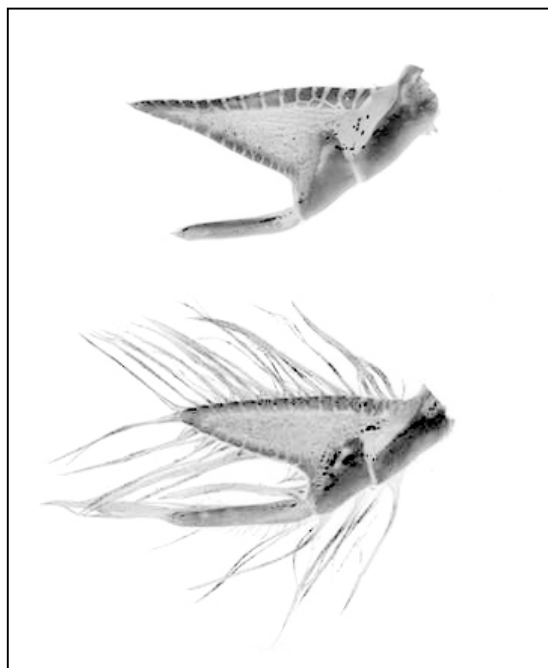
Figure 2. Non-setose (top) and setose (bottom) endopodites of female *Panulirus cygnus*.

4.3.2 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.

4.3.3 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^{TM}, with the standard errors being derived from 100 bootstrap estimates. The equation used was:

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

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 CLs at which 50 and 95% of the assemblage breeds twice.

4.4 Results

4.4.1 Moulting cycle of mature females

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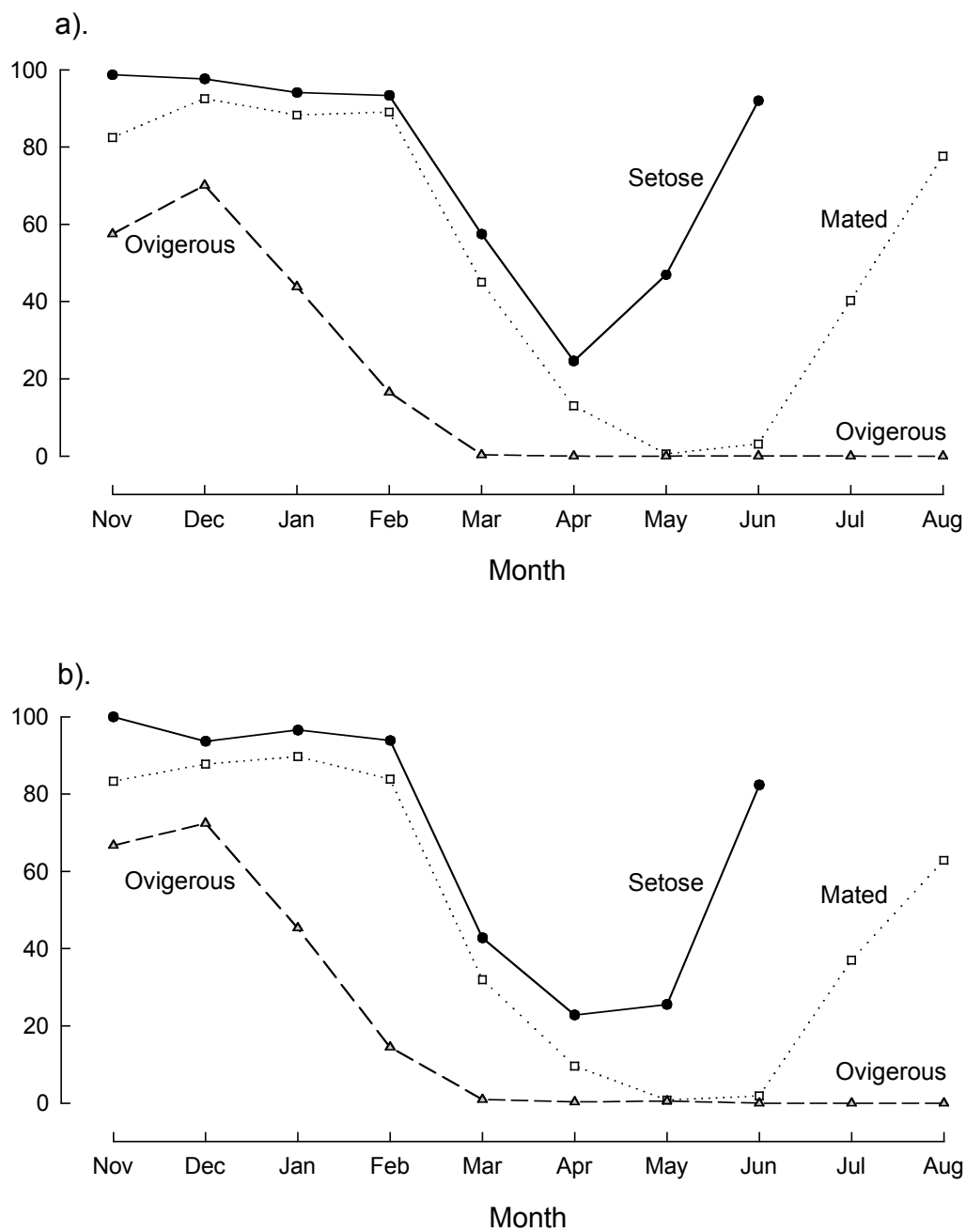
Figure 3. Mean monthly percentages of large (≥ 95 mm) setose, mated and ovigerous female *Panulirus cygnus* sampled during commercial monitoring surveys in the (a) northern and (b) southern zones of the western rock lobster fishery between 1972 and 2005.

4.4.2 Annual variation in the moulting of mature females

The proportions of large ($\geq$ 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).
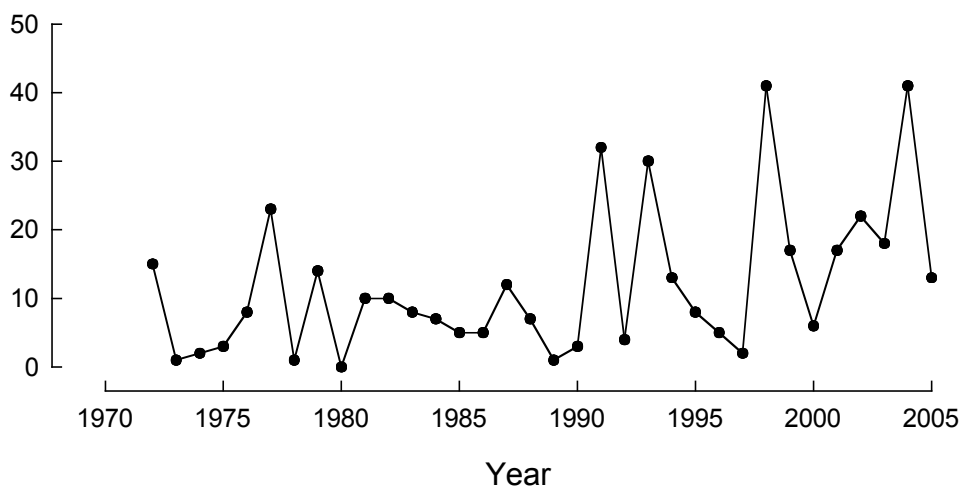
Figure 4. Percentage of large ($\geq$ 95 mm) mated female *Panulirus cygnus* sampled throughout the fishery during April from 1972 to 2005.
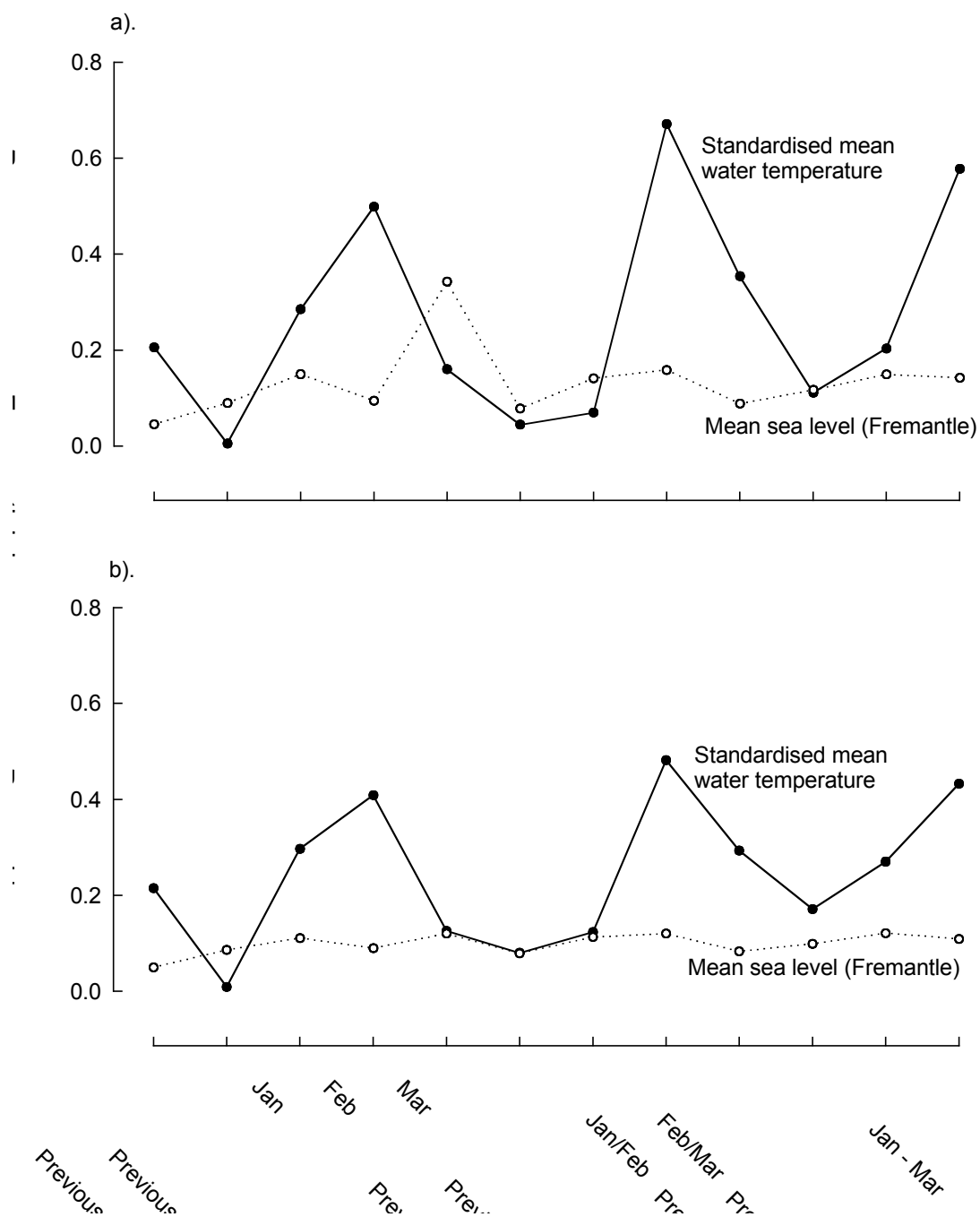
Figure 5. *R*-square values from (a) exponential decay and (b) linear regressions between the incidence of large ($\geq$ 95 mm) mated female *Panulirus cygnus* in April and annual values, individual months and subsets of months of standardised mean water temperatures (^{o}C) and mean monthly Fremantle 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: $P_M = 1.37E + 12 \times \exp(-T/0.721)$ (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.
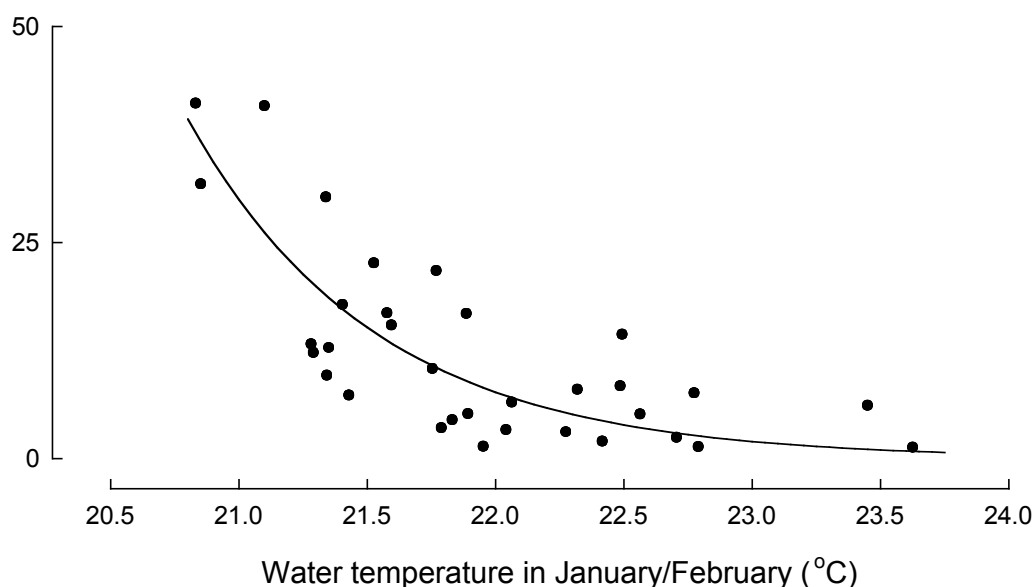
Figure 6. Exponential decay regression between the annual incidence of large (≥ 95 mm) mated female *Panulirus cygnus* in April and the annual standardised mean bottom water temperature for January and February (combined) of that year.

4.4.3 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).
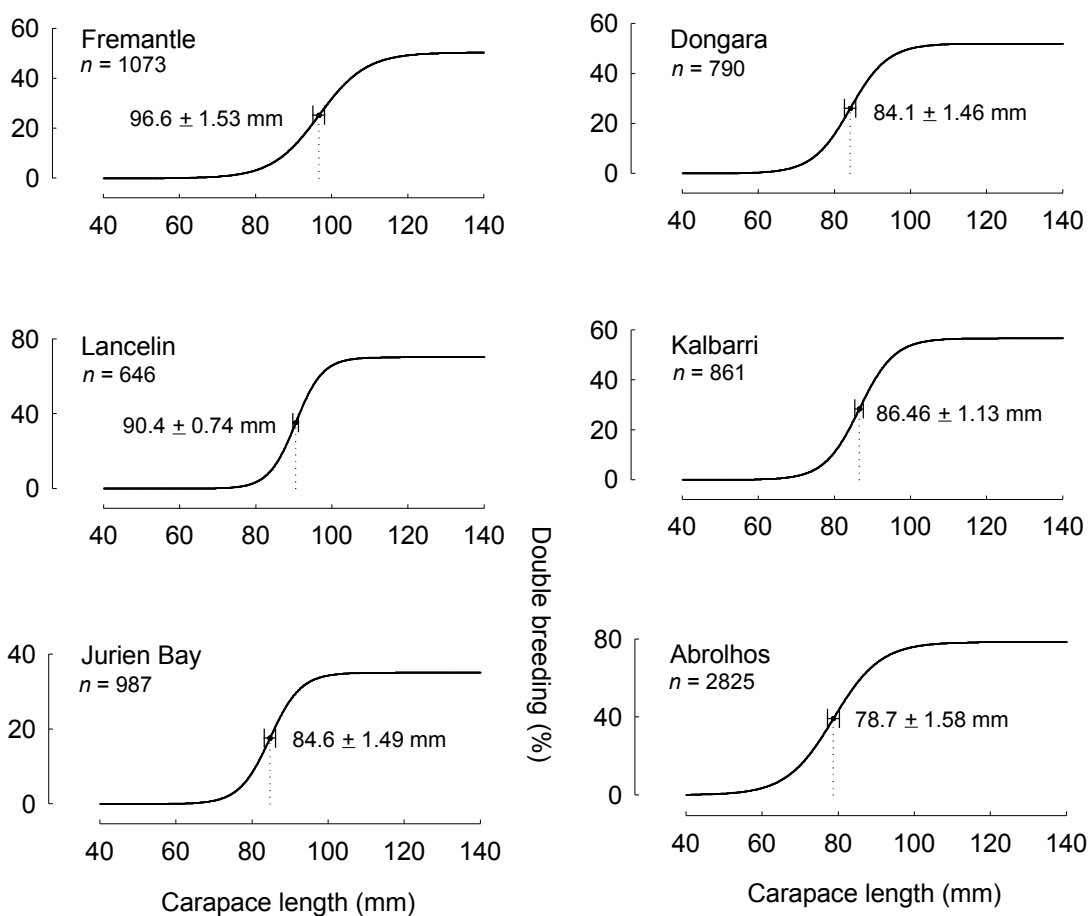
Figure 7. Logistic regressions fitted to the percentage of double-breeding female *Panulirus cygnus* at different carapace lengths in six locations in Western Australia, based on data collected during the 2002 Independent Breeding Stock Survey. $DB_{50} \pm 1$ SE denotes the size at which 50% of the 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).
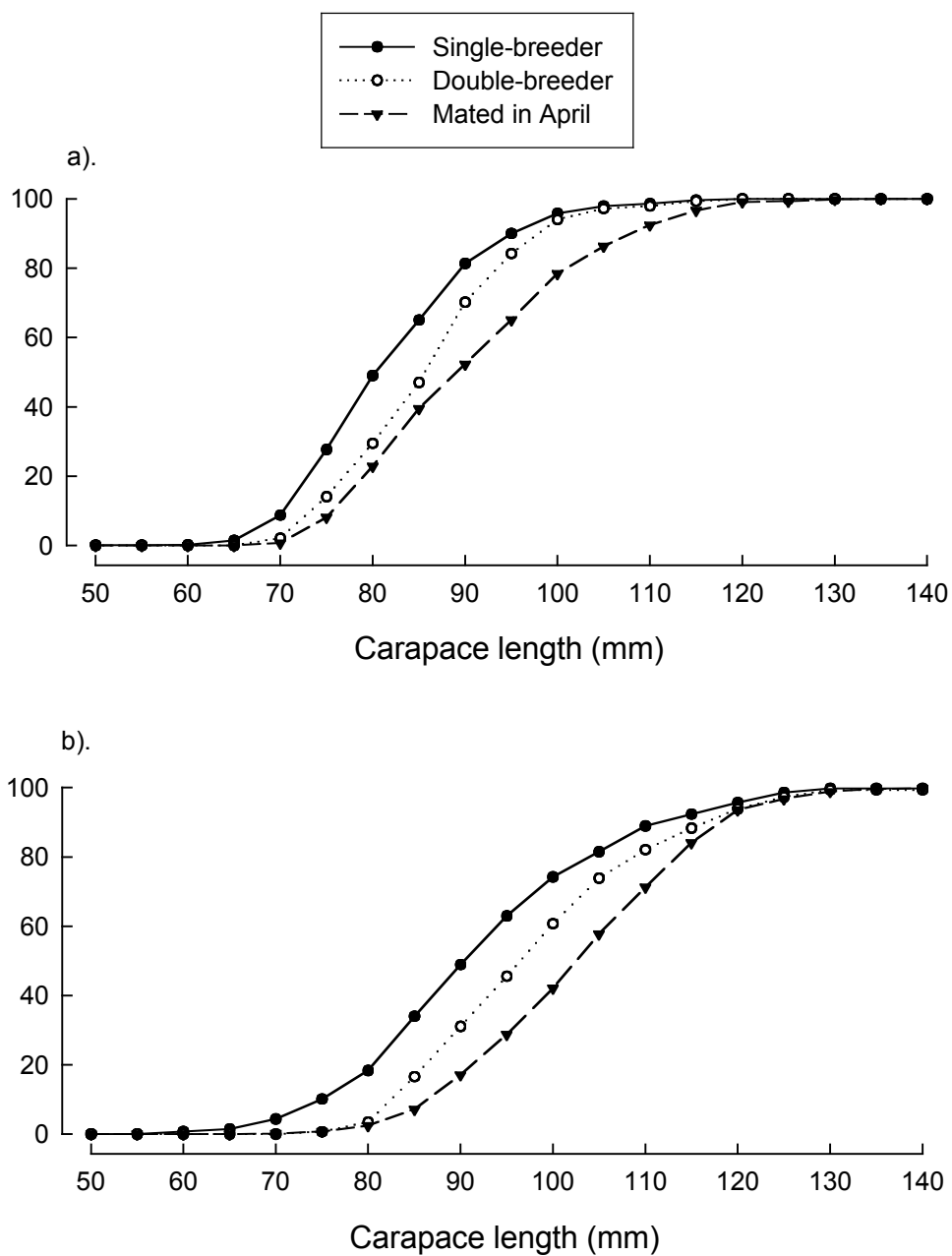
Figure 8. Cumulative frequencies (%) of single- and double-breeders and of mated female *Panulirus cygnus* recorded in April in sequential 5 mm *CL* classes in the (a) northern and (b) southern zones of the western rock lobster fishery.

4.5 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 ($25^{o}C$) 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.

4.6 Acknowledgements

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

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5.1 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.

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

5.2 Methods

5.2.1 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.
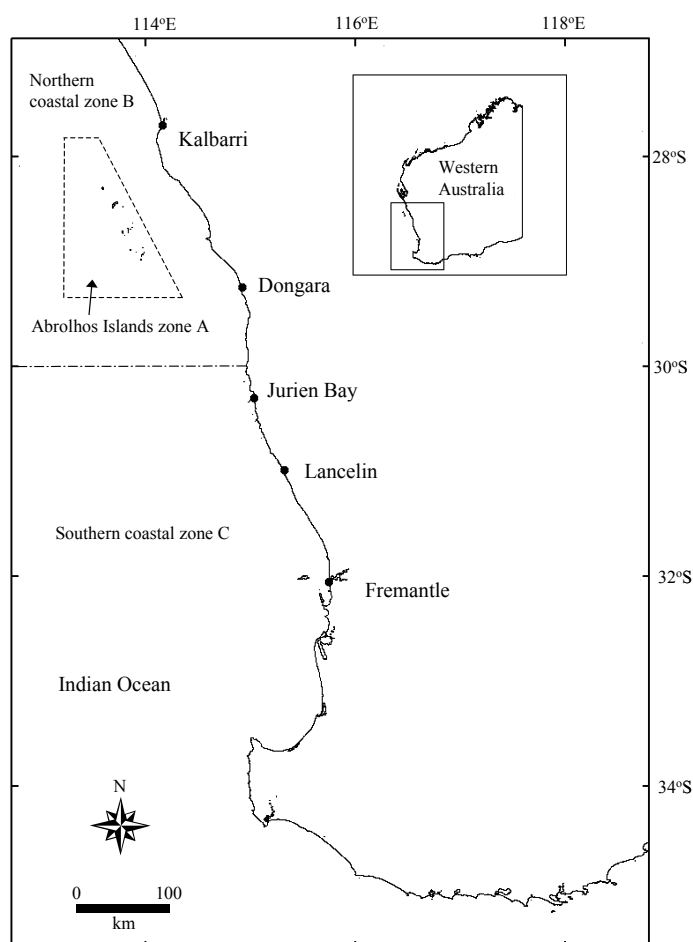

Figure 1. Management zones (zone A, Abrolhos Islands; zone B, northern coastal; zone C, southern coastal) in the western rock lobster (*Panulirus cygnus*) fishery, and six sites (five on the coast of Western Australia and one comprising the Abrolhos Islands) used for fishery-independent breeding stock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

5.2.2 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.

5.2.3 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.

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

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

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).

5.3 Results

5.3.1 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.
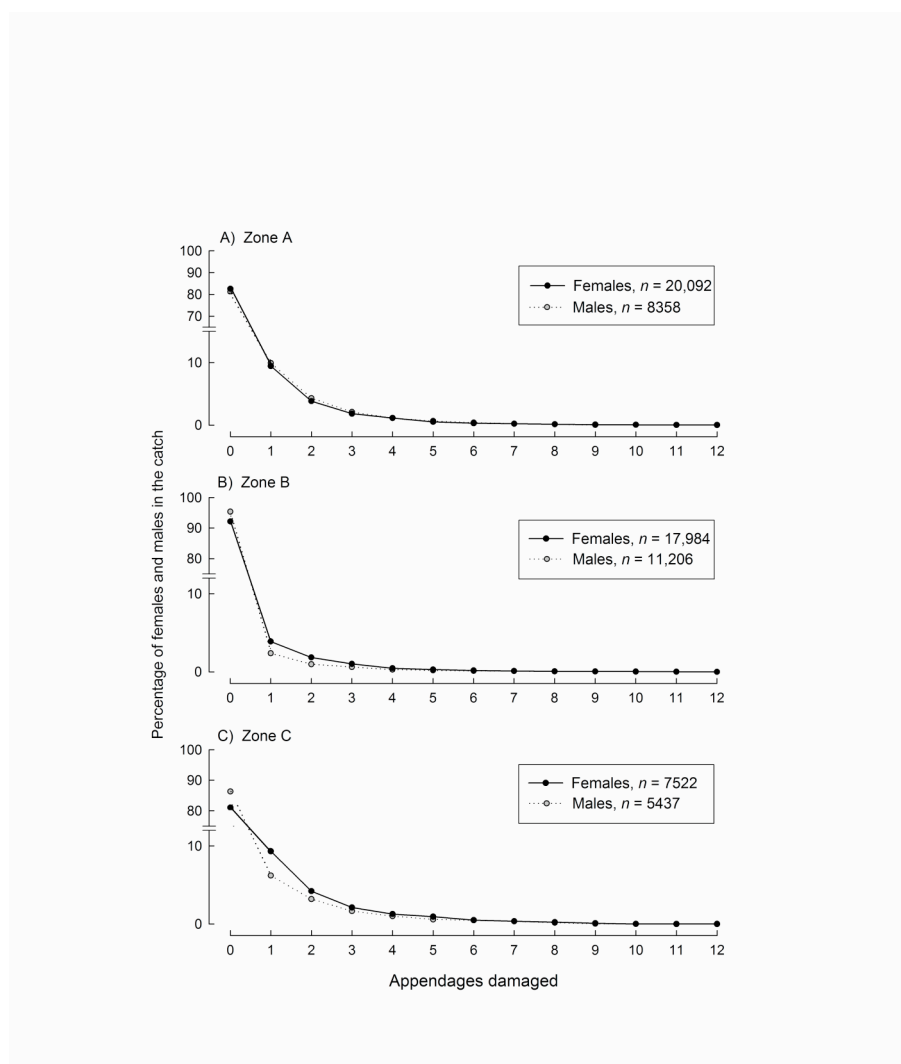
Figure 2. Percentage of female and male western rock lobster (*Panulirus cygnus*) with 0–12 damaged appendages in the catch at three management zones: (**A**) zone A, Abrolhos Islands; (**B**) zone B, northern coastal; and (C) zone C, southern coastal. Note that the *y*-axis is not continuous.

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.

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

5.3.2 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).
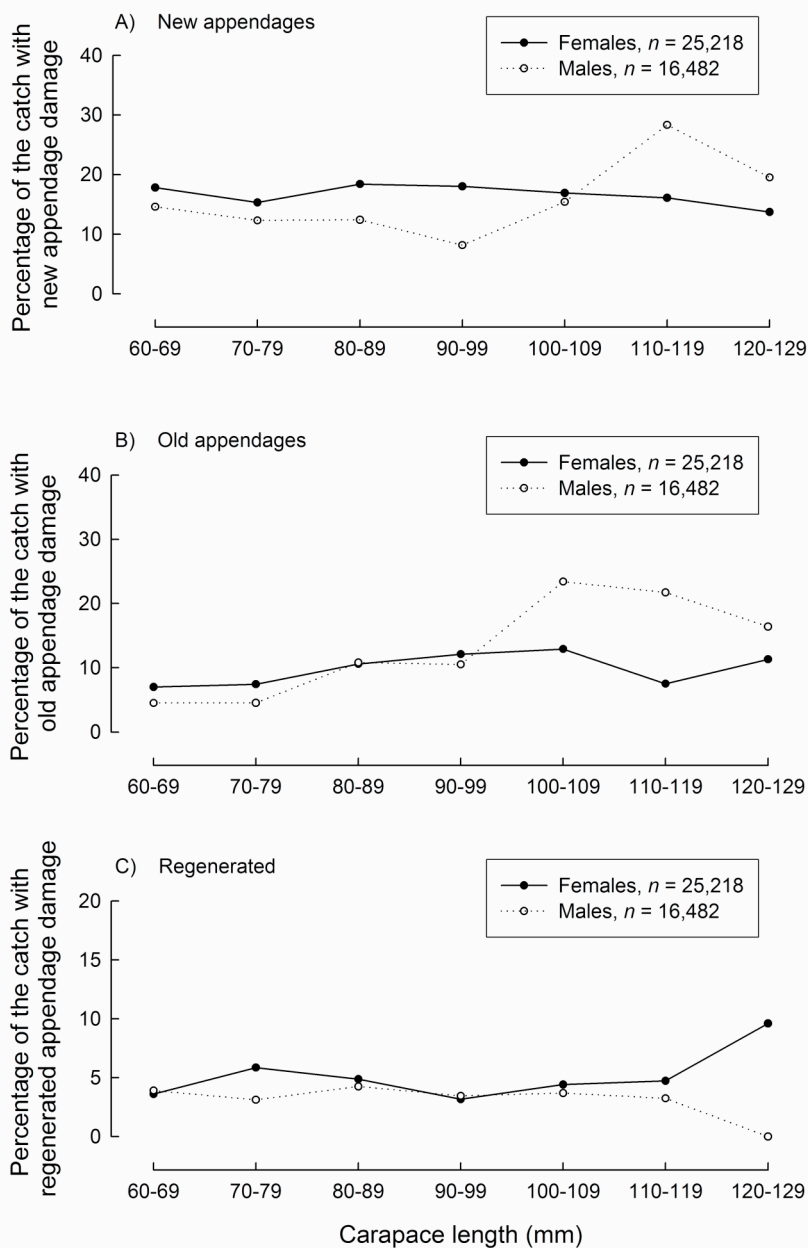
Figure 3. Percentage of female and male western rock lobster (*Panulirus cygnus*) in different size classes with (**A**) new or (**B**) old appendage loss, or (**C**) with regenerated appendages. The analysis uses 2001−05 fishery-independent breeding stock survey data from all five coastal sites.

5.3.3 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:

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

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.
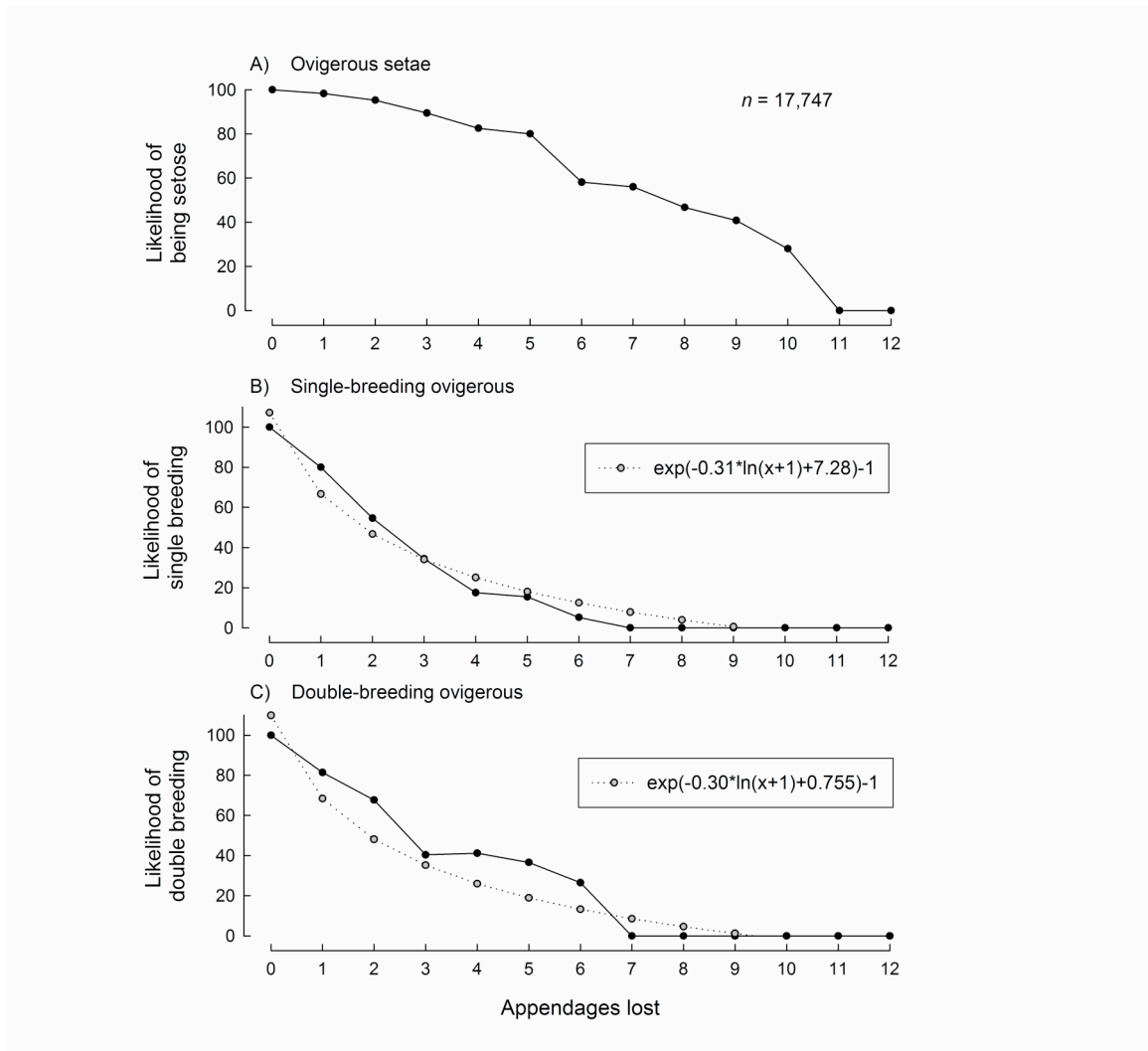
Figure 4. Likelihood of female western rock lobster (*Panulirus cygnus*) with (**A**) ovigerous setae, (**B**) or being single-breeding ovigerous individuals, and (**C**) double-breeding individuals, with 0−12 appendages damaged. Data are from the 2001−05 fishery-independent breeding stock surveys at 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.

5.3.4 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).
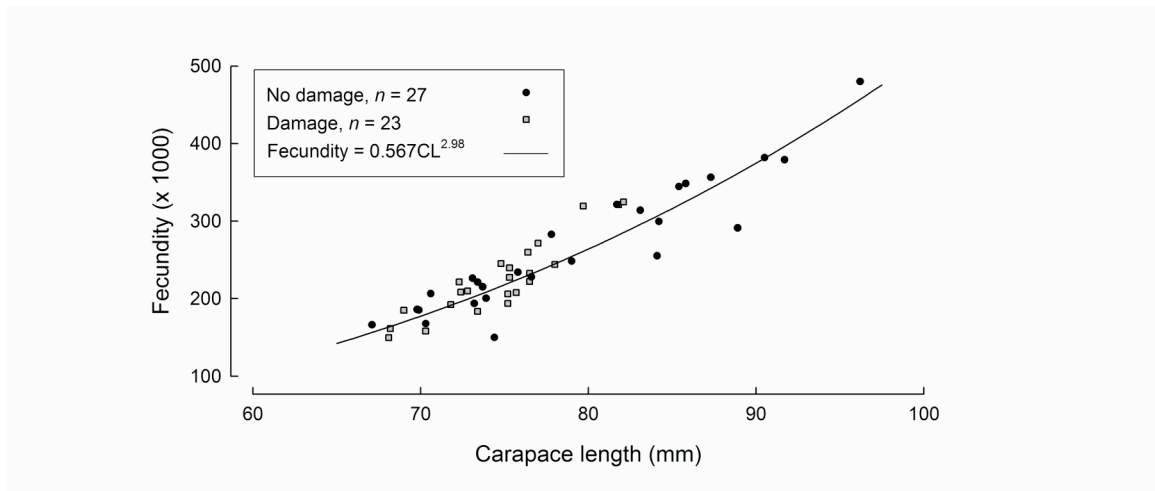
Figure 5. The relationship between fecundity and carapace length (mm) in western rock lobsters (*Panulirus cygnus*). The actual numbers of eggs carried by individuals with damaged appendages are shown by black circles and without damaged appendages, by open squares.

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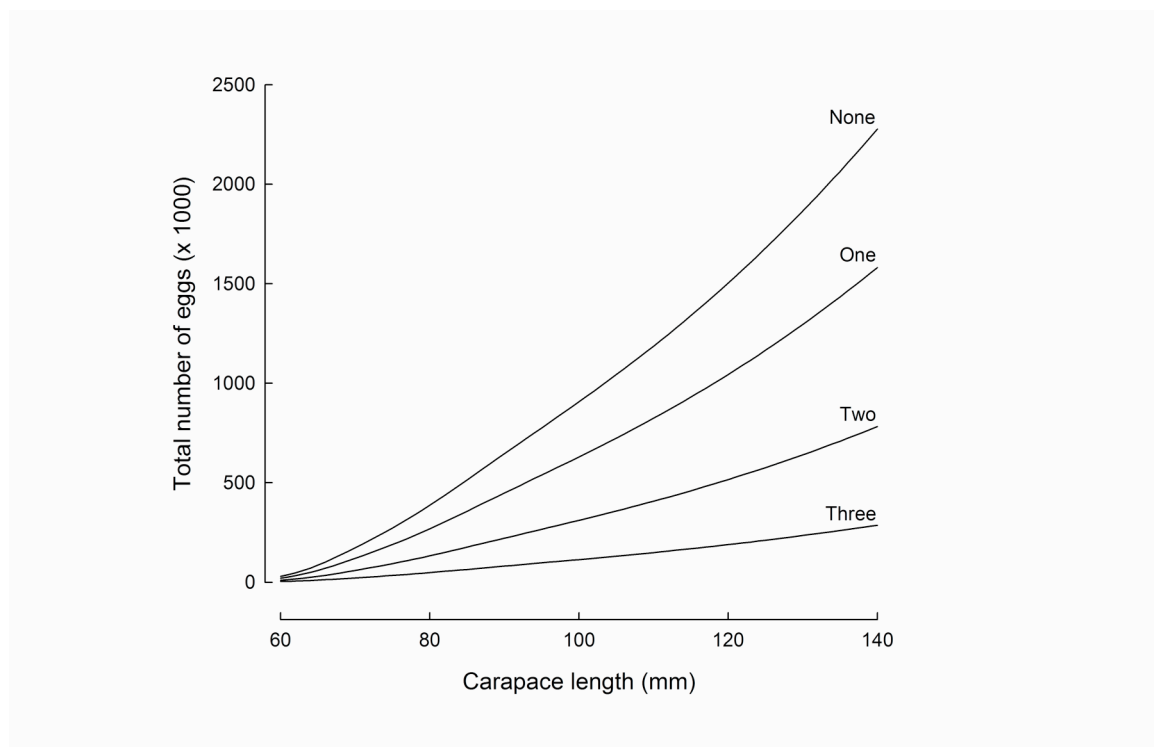
Figure 6. The relationship between total egg production (total fecundity) and carapace length (mm) of female western rock lobster (*Panulirus cygnus*) in one season at the Abrolhos Islands (zone A) with none, one, two, or three damaged appendages.

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.

5.4 Discussion

5.4.1 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.

5.4.2 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.

5.5 Acknowledgements

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

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6.1 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.

6.2 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.

6.3 Methods

6.3.1 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: $Diameter = 2\sqrt{Area/\pi}$.

6.3.2 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.

6.3.3 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 be sure 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.

6.3.4 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.

6.3.5 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.

6.3.6 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.

6.3.7 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).
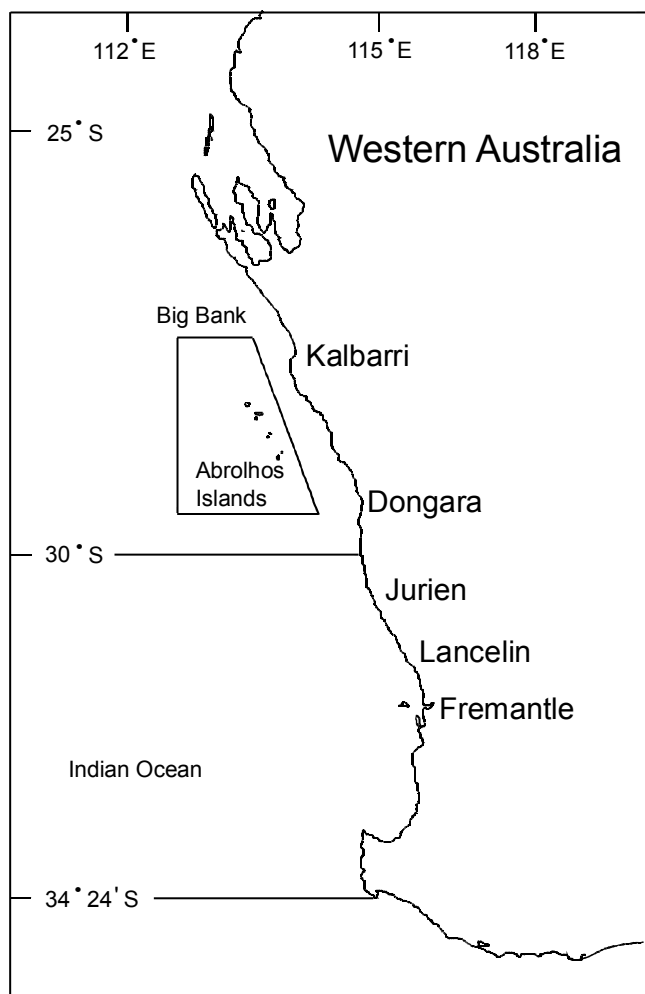
Figure 1. The areas on the Western Australian coast, from where pre-spawning female *P. cygnus* used in this study were collected.

6.4 Results

6.4.1 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., $n = 21$.
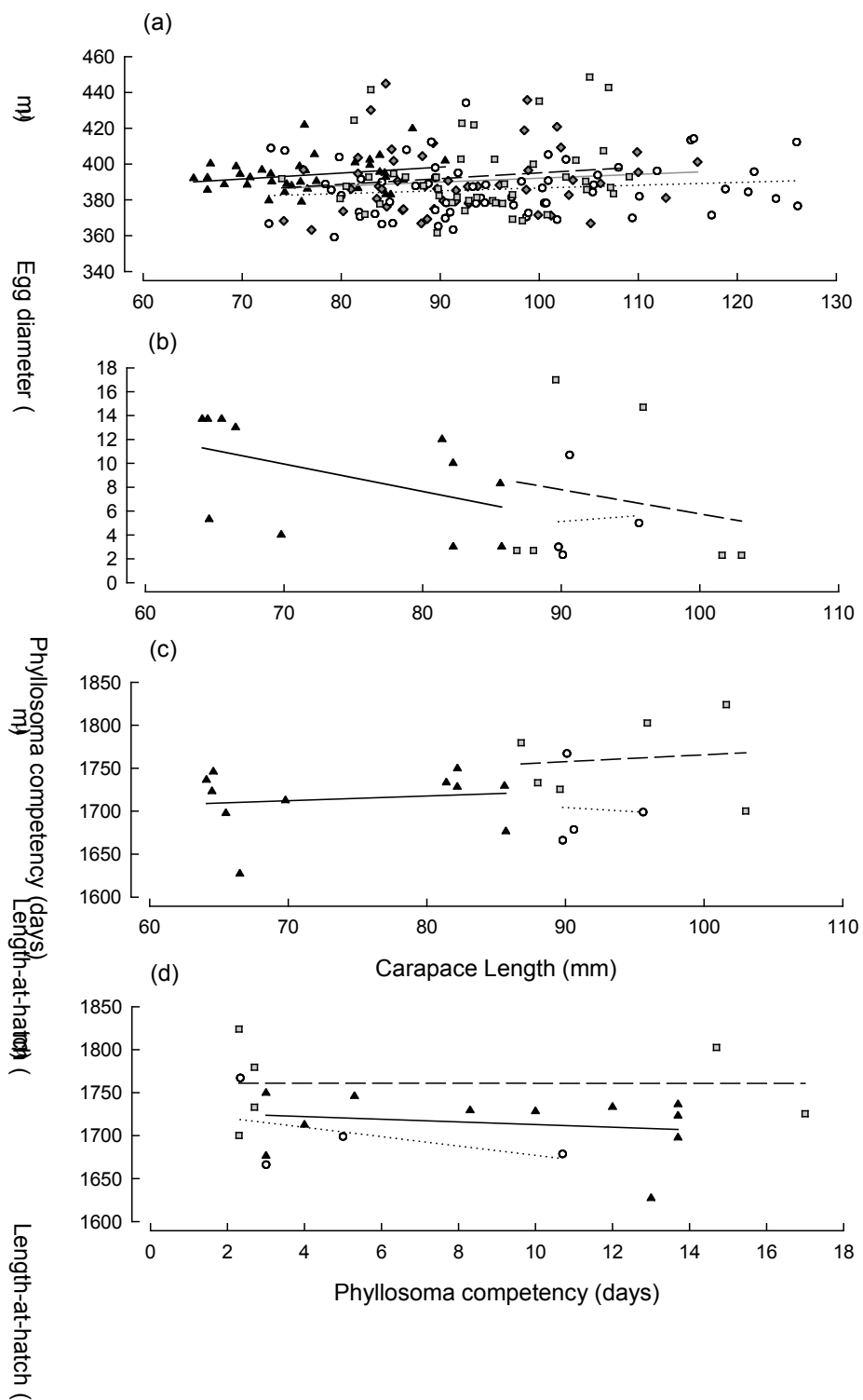
Figure 2. Relationships between the maternal carapace length *and* (a) egg diameter, (b) phyllosoma competency, and (c) phyllosoma length-at-hatch, for different maternal locations of capture. (d) Relationships between phyllosoma competency and phyllosoma length-at-hatch for different maternal locations of capture.

6.4.2 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).