

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

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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: (1) ovigerous setae condition (not present, partially developed, and fully developed); (2) the presence (unused or eroded) of a spermatophore mass; (3) the presence and developmental stage of external ova; and (4) 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. 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 modified set of criteria that, if applied to samples collected 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

INTRODUCTION

The fishery for western rock lobster (*Panulirus cygnus* George) is Australia's most valuable single-species fishery: the average harvest of 11 000 t each year is worth c. AU\$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 & Chubb 2001; Caputi et al. 2003). The annual egg production survey is conducted independently of commercial fishing over a 10-day period that spans the last new moon before the start of the commercial fishing season on 15 November. Since collection of all females for dissection in the laboratory is not practical or very sustainable (over 5000 females are sampled each year), the reproductive state of each female is assessed quickly and accurately using external characteristics, thereby allowing them to be returned to the water unharmed.

Studies have generally assumed that female paninurids 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 used by Chubb (1991) was not confirmed histologically by that author and as such, has the potential to miss-assign maturity state.

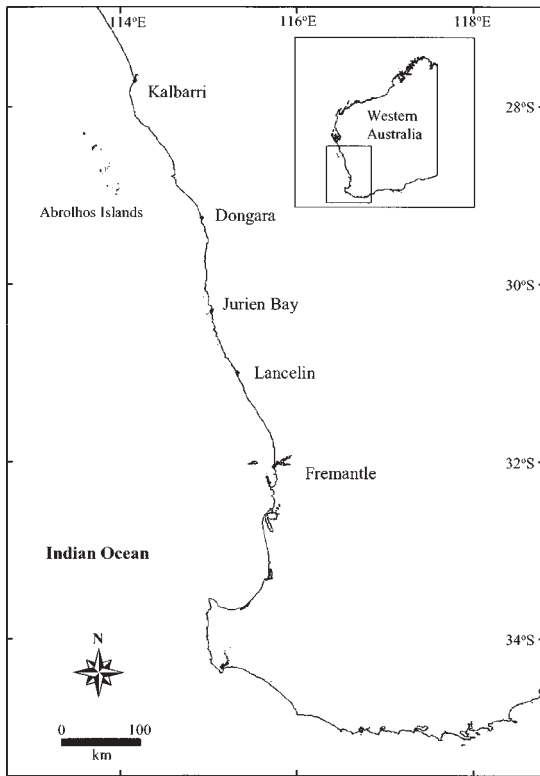


Fig. 1 Map showing the locations of the fishery-independent egg production surveys in October/November 2002.

The present study was initiated 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.

METHODS

Data sources

A total of 8541 female *P. 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 fertilisation 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).

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 & 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 excluded from 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) 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.

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. A further 35 females that proved difficult to assign an ovarian condition on the basis of an external examination were selected to determine histologically their

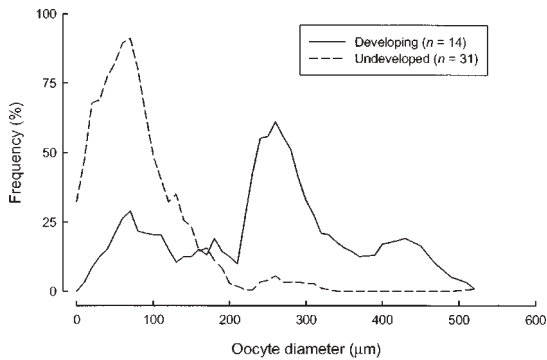


Fig. 2 Oocyte diameters of *Panulirus cygnus* ovaries that were classified as “developing” (yellow/orange) or “undeveloped” (white/translucent) when viewed through the dorso-thoracic musculature.

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 0.025M 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.

RESULTS

External and histological determination of ovarian development

A comparison of the ovaries from 45 female *P. 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 c. 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.

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 2–8 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.

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 double breeders, whereas, with the correct classification of their ovaries now known, they have been reclassified as single breeders.

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.

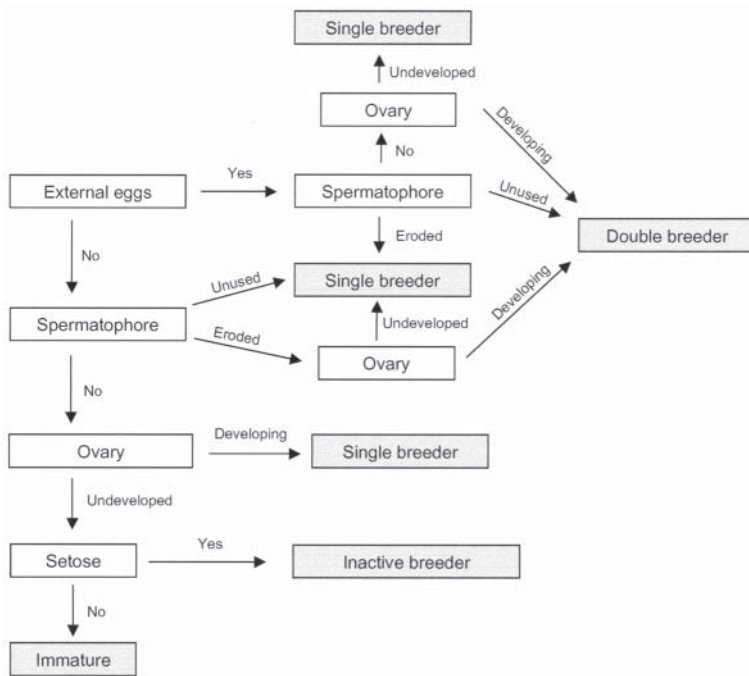


Fig. 3 Flow chart showing the various combinations of external characteristics which, when combined, classify a female *Panulirus cygnus* as immature, inactive breeder, single breeder, or double breeder.

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

During the 2002 survey, 8541 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) whereas 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 instances both sets of criteria classified each lobster as either a single or double

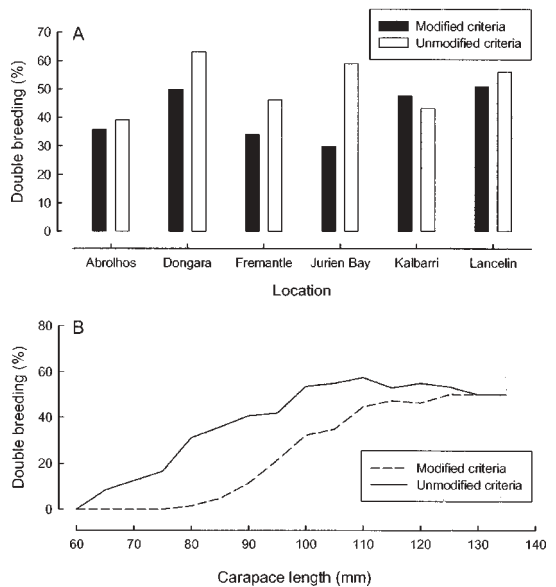


Fig. 4 Comparison between the proportions of *Panulirus cygnus* classified as double breeders at **A**, six locations and **B**, in different carapace length (CL) classes collected at Fremantle, Western Australia, determined from modified criteria (see Fig. 3) and the unmodified criteria (see Chubb et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

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 because of 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).

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 instances 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 before 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 & Bermas 1994; Sharp et al. 1997). Thus, the fact that 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, 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 with 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 & 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.

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