FINAL REPORT

JANUARY 1995

FRDC GRANT 92/8

STOCK-STRUCTURE AND RECRUITMENT PROCESSES IN EASTERN KING PRAWNS

Applicants

Dr. David Die
SOUTHERN FISHERIES CENTRE

QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES

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PROJECT TITLE

Stock-structure and recruitment processes in eastern king prawns

PROJECT REFERENCE NUMBER

FRDC 92/8

RESEARCH ORGANISATION

Name:	Fisheries Division
Department:	Queensland Department if Primary Industries
Postal Address:	GPO Box 46, Brisbane, Q 4001
Phone:	07 2393408
Facsimile:	07 2298146

PRINCIPAL INVESTIGATOR

Name:	Dr. David Die[1]
Position:	Fisheries Biologist, QDPI Southern Fisheries Centre
Postal Address:	PO Box 76, Deception Bay, Q 4508
Phone:	07 2031444
Facsimile:	07 2033517

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[1] Present address ; CSIRO Division of Fisheries, PO Box 120, Cleveland Q4163.
Phone (07)286 8219 Fax: (07) 286 2582

SUMMARY

The fishery for eastern king prawns has expanded offshore over the last ten years and fishing effort on the spawning stock has increased accordingly. Circumstantial evidence on recruitment overfishing has lead to the need to increase our understanding of the spawning dynamics of the eastern king prawn stock. This report addresses present research results of a project that attempted to identify natural chemical tracers describing the structure, and the lunar and diurnal cycles affecting the spawning stock of eastern king prawns.

To identify natural tracers samples of juveniles and sub-adult eastern king prawns were obtained from estuaries along the east coast of Australia and the chemical composition of their tissues was analysed with Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). These analyses revealed that prawn tissues from different estuaries can be differentiated on the basis of chemical composition.

Laboratory experiments showed that chemical composition of prawn muscle tissue changes rapidly (in a few weeks) and that the food and seawater composition were two major sources of such change.

Differences in the handling of samples prior to analysis (frozen vs fresh) and injury (marking with streamer tags) also affected the chemical composition of prawn tissue. This makes marking with streamer tags an impractical method for the evaluation of natural tracers.

Effects of lunar phase were detected in the proportion of spawning female prawns and the sex ratio of prawns in trawl catches. The proportion of spawning females follows a 14-day cycle which peaked in the middle of the waxing and waning moon phases. The sex ratio of males to females peaked at around the full moon.

During this project a new method of quantifying ovarian development with Image analysis was developed. The method is more precise and more objective than the traditional visual assessment method.

Given the results of this project and the previous project on eastern king prawns (FIRDC 90/4) we have identified the geographical areas, seasons and stages of the lunar phase which coincide with the highest spawning activity of eastern king prawns. This information can be used by fishery managers to better control the effects of fishing in the spawning stock.

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BACKGROUND

This project is a continuation of the work on spawning dynamics of eastern king prawns initiated by the Queensland Department of Primary Industries and the New South Wales Fisheries Department in 1990 with funds provided by the Fisheries and Industry Research and Development Council (FIRDC Grant 90/4).

The main body of this report presents a brief description of the methodology, the major findings and conclusions derived from the work done as part of FRDC project 92/8. More detailed scientific information derived from this research is included in the appendixes found at the end of the report.

OBJECTIVES

1. Test the use of chemical tracers as natural tags for the study of spawning stock structure in eastern king prawns.

2 Determine if spawning in eastern king prawns is affected by lunar or diurnal cycles.

INTRODUCTION

The eastern king prawn fishery off New South Wales and Queensland has annual landings of between 2,500 and 3,000 metric tonnes and an ex-vessel value between $30 million and $40 million. Most catches come from the area between the Clarence River in NSW and the northern end of the Swains Reefs in Queensland. Eastern king prawn postlarvae settle in estuaries along the coast and develop into sub-adults which contribute to inshore trawl fisheries as they emigrate from those nursery areas. As sub-adults move out of the bays and estuaries into deep water they grow and become the target of an offshore fleet. Mature prawns are found year around in depths over 100 meters, principally in the northern part of the fishery, where the fishery has expanded their operations in recent years.

The fishery is independently managed by each of the states (New South Wales and Queensland) within their coastal waters. In both states trawl licences are used to limit effort in their respective prawn fisheries.

Trawl fishery management objectives in the two states do not always coincide. In Queensland, the eastern king prawn stock is only one of the many prawn stocks managed under the umbrella of the Queensland east coast fishery. Therefore present management of the eastern king prawn stock is based on a mixture of management tools, and is constrained by the management of other prawn stocks.

King prawns are considered to be more resilient to exploitation than tiger prawns because of their behaviour. This perception together with the above mentioned limitations of the present management arrangements have lead to a situation where fishing pressure on the adult spawning stock is not effectively controlled.

The impact of fishing in the spawning stock was not thought to be important when the fishery was mainly confined to estuaries and shallow coastal bars. However over the last ten years fishing activities have expanded to deeper waters and to the northernmost areas of the stocks' distribution. Such increases in fishing effort on the adult stock have generated the fear of recruitment overfishing.

Historically fishery research on eastern king prawns was directed at providing advice for preventing growth overfishing of the stock and describing biological and environmental characteristics of the species (Lucas 1974, Courtney et al 1991, Die et al 1994). Only recently have fishery scientists attempted to study the reproductive biology and the spawning dynamics of the eastern king prawn stock (Courtney et al. in press).

There is mounting evidence that inshore catches of eastern king prawns have declined over the last twenty years as offshore fishing has expanded. Fishers are acutely aware of this decline and some of them attribute these changes to recruitment overfishing.

The lack of long term information on recruitment and spawning stock biomass prevents us to directly assess the presence or absence of a stock-recruitment relationship in eastern king prawns. However, catches from Moreton Bay and its adjacent offshore fishery are known to have declined from about 1,500-2,000 tonnes per year in the 1970s (Young 1975) to about 850 tonnes per year in the late 1980s (Trainor 1991), which gives some credence to industry concerns.

Recent replication by QDPI staff of postlarval prawn studies originally carried out in the early 1970's (Young and Carpenter 1977) indicate that there have been gross changes in prawn species composition in Moreton Bay, and that king prawn abundance has declined (J. Masel, QDPI unpublished data).

There is no quantitative information on the importance of individual estuaries to the catches in offshore waters. Moreton Bay is known to be a significant nursery area for eastern king prawns (Young and Carpenter 1977) but its significance in relation to the entire stock is unknown. Given the present state of conflict between inshore and deep water fleets, and the potential for recruitment and/or growth overfishing in this fishery a major research effort was initiated in the early 1990s to investigate the stock structure of the adult population, the temporal and spatial pattern of reproduction as they relate to recruitment, and ultimately the relationship between parent stock levels and recruitment.

It is very difficult to quantify how much different estuaries contribute to the adult stock and attempts to measure this through tagging experiments have failed because of the difficulty of comparing the probability of recapture of prawns released in different areas. This part of the project looked to identify a more effective method to estimate the origin of prawns based on the assumption that prawns from different estuaries had special chemical characteristics - on the basis of natural tags. This research attempts to identify such natural tags in the chemical composition of prawn tissue.

Once the origin of recruits to the adult stock is defined, there is a need to define the spawning stock. Research on the spawning stock of eastern kings was initiated as part of FIRDC funded project 90/4 (Glaister 1991) that established the seasonal and latitudinal variation in spawning. Results from this work suggested maturation may be also associated with certain lunar phases, particularly the full moon phase of the lunar cycle. Analysis of the SUNFISH logbook data held by the Queensland Fisheries Management Authority indicates that, fishing effort peaks around the full moon in deep water. Therefore the second goal of this project was to determine if there was a relationship between maturation and spawning in eastern king prawns, lunar phase and diurnal cycle .

METHODS

The project was divided into two parts, the first one aimed at developing natural chemical tags and the second at determining lunar and diurnal cycles in spawning.

Natural chemical tags

Juvenile prawns

Juvenile eastern king prawns, *Penaeus plebejus* were sampled from four estuaries (Clarence River, Tweed River, Moreton Bay and Elliott River) from the Australian east coast (Figure 1). The elemental composition (38 chemical elements) of eyes, hepatopancreas, exoskeleton, and abdominal muscle was analysed by ICP-MS (Appendix A).

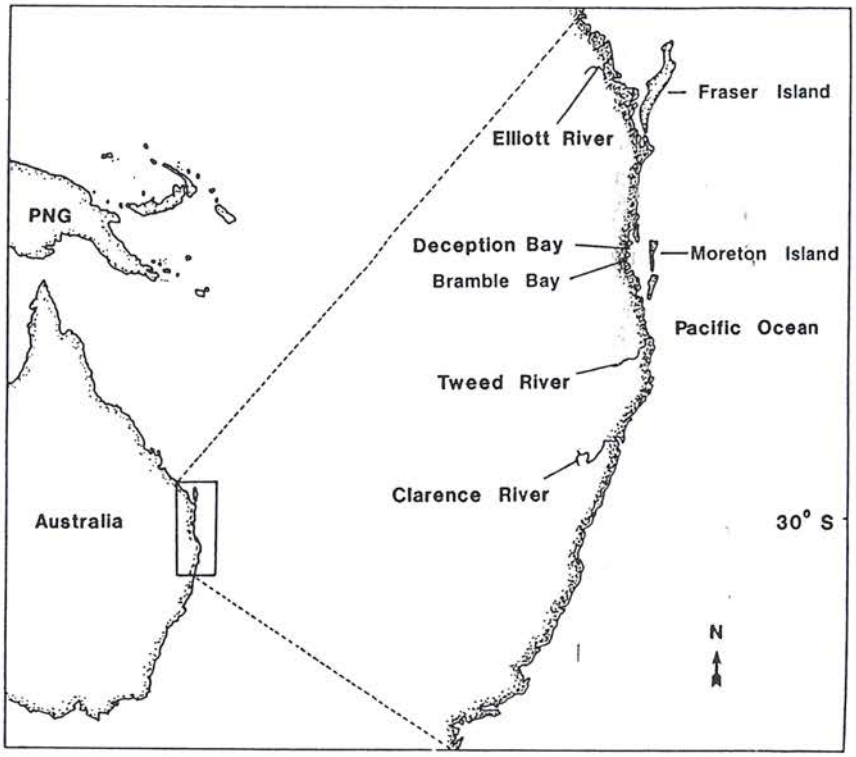

Figure 1: **Locations where juvenile eastern king prawn were sampled (Elliot River, Deception Bay, Tweed River and Clarence River), locations where subadult eastern king prawns were caught (east of Fraser Island, Bramble Bay) and location where tagged subadult eastern king prawns were released (west of Moreton Island).**

Sediment samples were also collected in the same four locations and the elemental composition of the two dominant particle size fractions found on those samples was also analysed with ICP-MS (Courtney et al. 1994, Appendix B).

Sub-adult prawns

A translocation experiment was conducted to test whether adult eastern king prawns could be separated on the basis of their chemical composition. Prawns originating from two different areas were tagged such that the origin of recaptured prawns could be identified from their streamer tags. Tagged prawns were then released at a common site. The performance of natural tags was then tested by comparing the estimated origin of recaptures - estimated with the natural chemical tags - to the known origin of recaptures - identified by the streamer tag.

In January 1994, eastern king sub-adult prawns were translocated from Fraser Island to Moreton Bay (Figure 1). A sample of the surviving sub-adults from Fraser Island were tagged and released in Moreton Bay together with other sub-adult captured in the Bay (Appendix C).

Tagged prawns recaptured by the fishing fleet up to four months after their release where collected and their tail muscle composition was analysed with a ICP-MS.

Samples of sub-adult eastern king prawn from both locations were also kept for ICP-MS analysis of tail muscle. Some of these samples were used to determine the effects of handling (freezing, tagging) in the chemical composition of prawns. The rest of the samples were used to determine differences between the chemical composition of eastern king sub-adult prawns in the two areas.

Uptake of chemicals by prawns

Laboratory experiments were carried out to test whether there were changes in elemental composition of tail muscle of eastern king prawns during periods of up to two months. Experiments were also conducted to determine whether the source of these changes was sediment, food, water or a combination of these three factors (Appendix D).

Lunar and diurnal cycles

Originally this part of the project was planned for 1992/93, but because of confusion pertaining the funding of research vessel costs for the operation of the R.V. Deep Tempest in the first year of the project, the lunar-monthly sampling was delayed until 1993/94. Because of this delay the diurnal cycle surveys had to be cancelled.

Trawl surveys were carried out every 72 hours for two lunar months in May-July 1993 in an area offshore from Moreton Island in South-east Queensland. Ovarian development in prawn sampled in these surveys was determined by microscopic examination of histological sections according to the method of Courtney et al. (in press). Image analysis was also used to develop a new method of quantitative assessment for ovarian development (Appendix E).

Sex ratio, proportion of prawns in early post-moult (soft), catch rates, GSI (gonosomatic index equal to the ratio between gonad weight and total weight) and the proportion of spawning females were estimated throughout two lunar cycles (Appendix F).

RESULTS

Natural chemical tags

Juvenile prawns

Discriminant analysis showed that all four groups of juveniles sampled could be separated with 100% accuracy on the basis of the chemical composition of all tissues examined (Appendix A). Differences in elemental composition of prawn tissue between

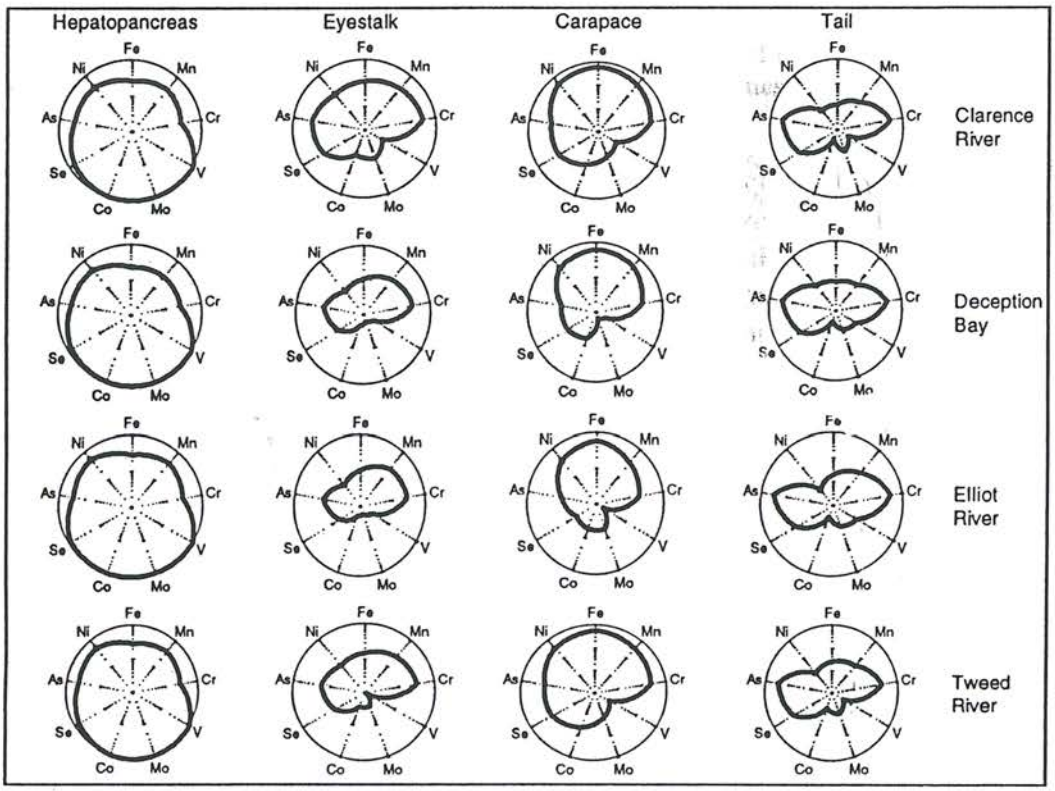

Figure 2: Average concentration of essential elements in tissues of juvenile eastern king prawn from four nursery areas

estuaries are detectable in both essential and non-essential trace elements (Figures 2 and 3). Regression analysis showed that differences in the mean size of juveniles from the four estuaries did not explain the differences in chemical composition of juvenile prawn tissues. Differences could not be attributed to moult stage either, because sampled juvenile prawns from the four estuaries were moulting asynchronously prior to their dissection for ICP-MS analysis.

The elements which contribute to the discrimination between estuaries varied for the different tissues. For eye tissues, the eight elements which had the major contribution to pattern separation were calcium, strontium, sulphur, phosphorous, magnesium, sodium, iron and manganese.

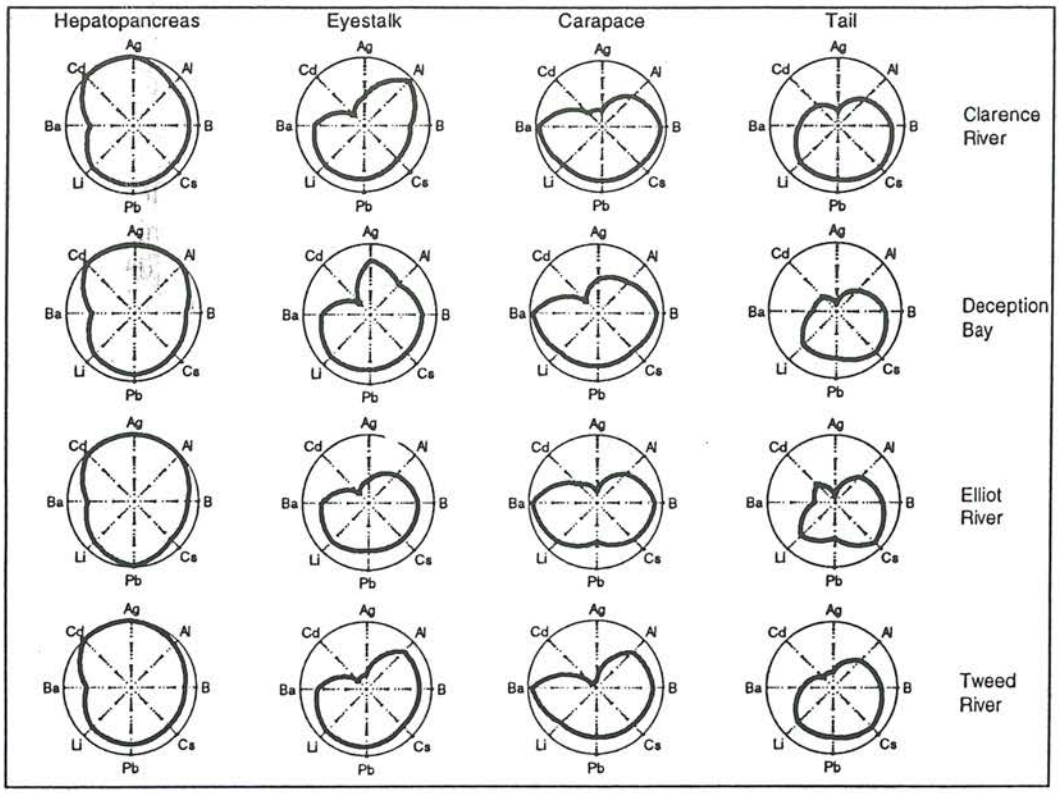

Figure 3: **Average concentration of non-essential elements in tissues of juvenile eastern king prawn from four nursery areas**

The analysis of estuarine sediment composition revealed that there were very large differences[2] in the concentration of several trace elements (Mn, Fe, Co, Cd, Pb, Ba, Ce, Bi, Cs, Ho, Tb, Lu, and Y) within one or both of the sediment fractions.

Sub-adult prawns

The recaptures from the tagging experiment were very disappointing with only four prawns being returned by fishers from the 800 tagged animals that were released (0.5 % rate of recapture). In addition all four recaptured prawns were of the same release group, from Moreton Bay, and were also recaptured within Moreton Bay. The absence of recaptures of prawns translocated from Fraser Island precludes the analysis of the origin of recaptures.

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2 more than 90% of variance in concentration explained by location

There were significant differences in the concentration of several elements (B, K, Mo, Ni, Pb, S, Sb, Se, Sr) within the tail muscle of prawns caught in Moreton Bay and Fraser Island. However prawn handling was also shown to have an effect on elemental composition of tail muscle. Tagging only affected the concentration of Mo, Ni and Sr, however the concentration of several elements (Cr, K, Li, Mo, Na, Ni, P, Sb, Se, Sr and Zn) was different between prawns which were alive up to the dissection and those that had been dead and frozen before the dissection. For some elements (Cs, Mg, Mn, P, and S) it was not possible to separate the effects of tagging and freezing.

Uptake of chemicals by prawns

Laboratory experiments showed that significant changes in the elemental composition of prawn tail muscle could be detected after four weeks. These changes could be attributed to uptake from water (Mn, Co, Pb, Ba), from food (B, Hg) or both (P, Zn, As).

Laboratory experiments showed that substrate was not as important as food or water in determining changes in the elemental composition of prawn tail muscle.

Lunar cycles

Cell diameter, average luminance and percent area of an ovary section within a luminance range were the most useful image analysis variables to discriminate between different ovarian developmental stages. Discriminant analysis successfully separated

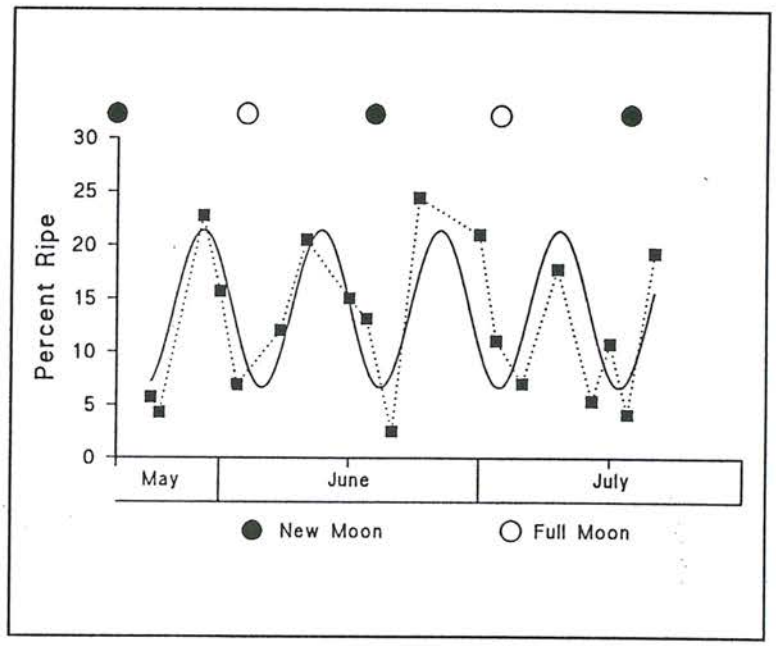

Figure 4: **Percentage of female eastern king prawns ready to spawn in catches made offshore from Moreton Island in the period between 24th May and 23th July 1993 and relationship with the lunar cycle. Continuous line represents a non-linear sinusoidal fit to the data.**

between maturing, mature and resorbing groups in 94% of the samples. The estimated discriminant functions suggest there is a continuum of development which can be characterised by a combination of measurements obtained from histological sections (Appendix E).

Both ovary weight and the proportion of spawning females increased in the middle of the two halves of a lunar cycle - during the waxing and the waning moons (Figure 4). The period for cyclical changes in the proportion spawning was approximately two weeks.

There is also evidence that the ratio of males to females increased very significantly at around the full moon, when catch rates of both males and females peak and are approximately equal (Appendix F). Changes in this ratio are the result of significant changes in the catch rate of male eastern king prawns, because catch rates of females were more or less constant throughout the sampling period (Figure 5).

No evidence was found of a relation between moon cycle and moulting frequency or between moon cycle and the proportion of inseminated females.

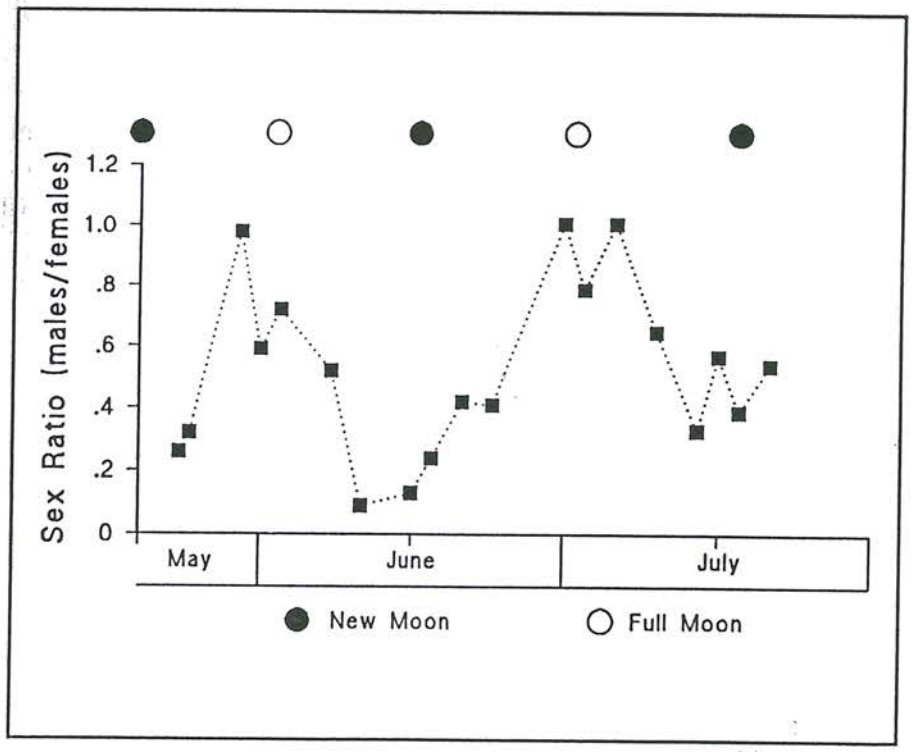

Figure 5: **Lunar variation in sex ratio in eastern king prawns caught offshore from Moreton Island in the period between 24th May and 23rd July**

DISCUSSION

Natural chemical tags

Trace element analysis has been successfully used in Australia for the study of finfish stock discrimination (Edmonds et al. 1989, Edmonds et al. 1991) and insect migration (Fitt, 1986). The present project has successfully proved that there are differences in the concentration of various chemical elements within tissues of eastern king prawns captured at different locations along the east coast of Australia. These difference can be detected at the sub-adult and juvenile stage in shallow waters.

The chemical composition of prawn tissue of specimens captured in a given location may change in time, between years and seasons. Our study did not specifically investigate such changes but we obtained indirect evidence that they occur. This evidence comes from comparing the chemical composition of Elliot River prawns and Moreton Bay prawns captured at different times in the course of this project. The size of these different prawn groups however, was somewhat different, and it is possible that such differences in chemical composition are a result of development.

The overall recapture rate of tagged eastern king prawns was much lower than expected. One explanation for this is that trawl fishing effort during 1994 was unusually low, and therefore the probability of recapture was also low. For this purpose we analysed the SUNFISH logbook data held by the Queensland Fish Management Authority. Trawl fishing effort in the Moreton region (between latitudes 26 30' and 27 30' and longitudes 153 and 154) for the first six months of 1994 was 60 % lower than the effort for the same period for the years 1989, 1990, 1991 and 1993. Only 1992 had a similarly low fishing effort. The tagging experiment conducted herein was designed on the basis of recapture rates estimated from experiments carried out in late 1990 early 1991, when effort was greater than in 1994, something that could not be predicted.

There may be several explanations to the lack of recaptures from tagged Fraser Island prawns. These prawns were released in the western side of Moreton Bay, where water characteristics are similar to waters off Fraser Island. It is possible however, that these prawns did not follow the normal migration route of other Moreton Bay prawns, and were therefore not catchable by the fishing fleet. It is also possible that by an unknown reason they did not survive in the Bay.

Because of our inability to recapture enough tagged prawns we still do not know whether adult eastern king prawns retain the differences in chemical composition that they acquire in the estuaries.

Our laboratory experiments suggest that handling (freezing, storing and tagging) affects the chemical composition of tail muscle. It is therefore difficult to compare the chemical composition of prawns which have suffered different handling. Because

prawns recaptured by fishers may suffer different handling conditions it will be difficult to use tagging as a method to validate natural tags.

The concentrations of many of the chemical elements studied change rapidly in muscle tissue. Of the three possible factors tested (sediment, water and food) we have shown that both water and food are major sources of these changes in concentration. However, our experiments have not explored whether certain elements are more likely to be taken from the food or from the water. To determine that it would be necessary to manipulate experimentally the concentration of individual elements in the diet and in seawater.

Juvenile and sub-adult eastern king prawns move through different kind of environments in their migration from the estuarine nursery areas to the deep-water spawning grounds. In the estuaries, seawater composition is heavily affected by terrestrial inputs and is probably highly variable. In the offshore environment terrestrial inputs have a much smaller effect and seawater composition is more constant.

The range of habitats through which eastern king prawns move from the nursery areas to the spawning grounds is quite diverse. It is therefore likely that the food sources used by eastern king prawns and likewise the chemical composition of muscle tissue change too.

In spite of how dynamic chemical composition of prawn muscle is, it may still be possible to develop natural tags and use them to establish the origin of adult prawns. However validating this method with tagging experiments may be difficult.

As it stands now it is not possible to use natural markers to determine the estuary of origin of adult eastern king prawns.

Lunar cycles

The image analysis method developed in this project is more accurate than the traditional visual staging method for immature ovaries. Future research should focus on determining which other measurements may be appropriate to quantify development of mature, spent and resorbing ovaries.

Quantification of ovarian development with this method was possible in spite of the variability in cell development which occurs within all ovaries. This makes this method more objective than the visual assessment method and more appropriate for studies involving more than one observer. Any species like eastern king prawns - ranging over the coastal waters of two states - is likely to be studied by more than one group of scientists and will benefit from a method that is not observer dependent.

Eastern king prawns spawn over a wide geographical area from Northern New South Wales, to the bottom of the Great Barrier Reef in Queensland. The proportion of spawning females does not significantly change seasonally, but we have shown that it does vary according to the lunar cycle.

Spawning eastern king prawn females are only found in depths over 40 meters. Given that abundance over 40 meters in the Moreton Region peaks in February - March it is likely that the greatest spawning activity takes place during the waxing and waning moons of these two months. Further north, in the Swains Reef, abundance peaks later in the year, April - May. Therefore peak spawning should occur in the corresponding waxing and waning moon phases of those two months.

Not all spawning females contribute equally to recruitment. Spawning far from the main nursery grounds will not generate the same amount of successful postlarval settlement that spawning close to the nursery grounds. Spawning well before or well after those months which seem to be the optimal settlement period (July-August) for postlarvae may not contribute significantly either.

There is no direct evidence of a spawning stock - recruitment relationship in eastern king prawns, but there is circumstantial evidence that decreases in recruitment have coincided with increased fishing pressure directed to the adult stock. If managers of this fishery decide to impose measures to protect or enhance the spawning stock they will have to reduce fishing effort in deep water at the appropriate months, moon phases and areas. Unfortunately such times and areas coincide with the highest catches in the deep water fishery.

To balance the costs of protecting spawners with the benefits of increased recruitment there is a need to continue research on the spawning and recruitment dynamics of eastern king prawns. Some of such research will be done by further analysis of the data collected in this study. However only the continued annual monitoring of the activities of the fleet and its impact on the stocks will allow us to determine the best strategies to manage the spawning stock of eastern king prawns.

CONCLUSIONS

1. Chemical composition of juvenile and subadult eastern king prawns vary between estuaries.

2. Seawater and food type are two important sources of variation in the chemical composition of prawn tail muscle; sediment type is not an important source.

3. Changes in chemical composition of prawn tissue happen rapidly over period of less than 3-4 weeks.

3. Handling (freezing and tagging) affect the chemical composition of prawn tail muscle.

4. Tag-recapture experiments are unlikely to be an appropriate method of validating natural chemical markers.

5. Oscillation in the proportion of females spawning is coupled with the lunar cycle. Highest proportions are found at the waxing and waning moons.

6. Changes in catch rates of male prawns are coupled to the lunar cycle. Highest catches occur during the time of full moon.

7. Image analysis of histological sections is a more precise and objective method of quantifying ovarian development than visual assessment of these sections.

8. Protection of the spawning stock can be achieved by reducing fishing effort in the appropriate time and area. The benefits to the fishery of this protection are not quantifiable now, but continued monitoring of the fishery will ensure these benefits can be estimated in the future.

DISSEMINATION OF RESULTS

Research results from this project have been presented to two scientific meetings:

- 2nd Australian Symposium on Applied ICP-Mass Spectrometry in May 1993 in Brisbane,

- FRDC sponsored meeting on Stock-Recruitment Relationships in Crustacean Fisheries that was held in Bribie Island, Queensland on May 1994

The fishing industry has also been informed of the progress of this project. First news about the research were disseminated in May 1993 with the South eastern Prawn Herald Newsletter, published by the SFC-QDPI, and distributed to commercial fishers and seafood processors. Further results were presented at a meeting of the Queensland Commercial Fishermen's Organisation and the Queensland Fish Management Authority in Mooloolaba, Queensland in November 1993.

The reference section of this report includes papers from this research published to date. These papers and those papers submitted for publication are all included in the appendixes of this report.

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GLOSSARY

Eastern king prawn stock	Is here defined as the eastern king prawn population fished between the Clarence River in New South Wales and the Swains Reefs in Queensland.
Fishing season	Starts in late spring or early summer (September-November), in inshore areas; and in late summer early autumn (January-March), offshore .
Growth overfishing	It occurs when fishing is directed towards prawns that are smaller than the size which produces the highest yield to the fishery.
ICP-MS	Inductively-coupled Plasma Mass Spectroscopy is an analytical process to measure the elemental composition of biological and inorganic samples.
Recruits	Small eastern king prawns caught by the inshore trawl fishery at the beginning of each fishing season.
Recruitment overfishing	It occurs when fishing on the spawning stock leads to reduced recruitment and reduced yield to the fishery.
Stock-recruitment relationship	Describes the relationship between the number of adult eastern king prawns that spawn in a given fishing season and the number of recruits which survive and enter the fishery the following season.

Appendix A: Courtney A.J., D.J. Die and M.J. Holmes. 1994. Discriminating populations of the Eastern King Prawn, *Penaeus plebejus* from different estuaries using ICP-MS Trace Element Analysis. *Atomic Spectroscopy* 15(1): 1-6.

Discriminating Populations of the Eastern King Prawn, *Penaeus plebejus*, From Different Estuaries Using ICP-MS Trace Element Analysis

A. J. Courtney, D. J. Die, and M. J. Holmes
Queensland Department of Primary Industries
Southern Fisheries Centre
P.O. Box 76 Deception Bay
Queensland 4508, Australia

ABSTRACT

ICP-MS is being used to determine if prawns, like some species of fish, possess an "environmental imprint" attained in their juvenile nursery habitats. Such an imprint could act as a naturally occurring tag and provide useful information on nursery grounds, migration patterns, and stock structure. Samples of juvenile eastern king prawns, *Penaeus plebejus*, from four different estuarine nursery areas several hundreds of kilometers apart were distinguished from one another using combinations of the concentration of elements in their body tissues. Four different body tissues were used: eyes, hepatopancreas, abdominal muscle, and exoskeleton.

Canonical-variate (discriminant) analyses showed that each of the four body tissues could be used to correctly classify the samples with high (100%) predictability. The results, although helpful, should be treated with caution as they only provide a static and narrow representation of the prawns' elemental profiles in space and time. Further experiments are in progress to determine if the prawns retain these differences through time in the wild and in the laboratory.

INTRODUCTION

Fisheries biologists and managers rely heavily on the results of tagging studies to estimate growth and mortality, and to provide information on movement and stock structure. The accuracy of these parameters is imperative to the success of any production model used to optimize the resource. Identification, usually through some form of tagging, is also required to assess the success of restocking programs. Tagging, however, can have inadvertent and detrimental effects on the individuals and can bias estimates of the parameters being measured. Penn (1975) and Hill and Wassenberg (1985) have noted some of the effects that tagging has on molting, growth, and activity of marine prawns. Ideally, for any tagging study, what is sought is a completely benign tag that is unique to the individual (or group of individuals), readily identifiable, and retained through time.

Edmonds et al. (1989,1991) concluded that the concentration of trace elements deposited in the sagittal otoliths of certain marine teleosts reflect an environmental history of individual fish. Fish from various geographic regions could be grouped according to the concentration of certain trace elements. These differences were used to support concepts of nonmixing groups and stock structure. Although crustaceans do not possess permanent bony structures, Whyte and Boutillier (1991) found that the concentrations of elements in the carapace of female spot prawns, *Pandalus platyceros*, differed substantially in individuals from separate geographic locations.

Eastern king prawns, *Penaeus plebejus*, are one of the most valuable fisheries species on the east coast of Australia. Tagging studies (Ruello 1975, Potter 1975, Glaister et al. 1987, Montgomery 1990) have revealed that *P. plebejus* undertakes extensive migrations, sometimes exceeding 1000 km, in a northerly direction and from shallow to deep water against the East Australian Current. This migratory behavior results in the prawns crossing state jurisdictional boundaries and complicating management of the fishery. The importance of individual rivers, estuaries, and embayments as nursery grounds and sources of recruitment to offshore stocks is largely unknown. If the prawns were to acquire and retain some type of "environmental imprint," similar to that laid down in the fish otoliths (Edmonds et al. 1989, 1991), then such an imprint could prove to be of value in determining an individual's geographic nursery origin and important areas for recruitment in the fishery.

To be of value to fisheries managers, an environmental imprint must be retained in the prawn through time and be independent of any subsequent change in habitat. Secondly, the imprint must be detectable in adults, or older stages that may have migrated hundreds of kilometers over several months. The body tissues in Crustacea that are most likely to retain an environmental imprint are unknown. It is the objective of this paper to assess inductively coupled plasma-mass spectrometry (ICP-MS) as a means of detecting elemental profiles or combinations of elements, in several prawn tissues that have the potential to be used as environmental imprints.

MATERIALS AND METHODS

Field Sampling

Samples of juvenile *P. plebejus* were obtained at night from four estuarine nursery areas (lower reach of the Elliott River and Deception Bay, Queensland, and the lower reaches of the Tweed and Clarence Rivers in northern New South Wales) on the East Coast of Australia from September 22–October 15, 1992 (Figure 1). About 120 prawns of similar size and ages were sought from each area using a 1-m beam trawl with a 2-mm mesh nylon net attached. The beam trawl was towed along the bottom in approximately 3 m of water using a 5-m aluminum dinghy. In order to minimize stress and mortality on the prawns, the duration of the trawl tows did not exceed five minutes.

A number of steps were adopted to minimize contaminating the prawns and thereby, altering their elemental concentrations. These included sorting the catch on board on a clean plastic surface that had been scrubbed and washed in the surrounding seawater, and handling the prawns with disposable rubber gloves that were also washed in the surrounding seawater. Juvenile *P. plebejus* were quickly identified from other prawn species and placed in plastic 60-liter drums that had been scrubbed, washed, and flushed extensively in seawater. Portable aerators were used to pump air into the seawater in the drums. Air stones were not used to filter and disperse the air as this was considered to be a possible source of contamination. Instead, transparent plastic hosing with numerous tiny puncture holes and sealed at one end were used to aerate the surface of the water.

Laboratory Preparation

The prawns were held in the aerated drums for a minimum of 12 hours after capture and transported live to the laboratory within 48 hours. This allowed the prawns to purge their guts of ingested matter, and it ensured that they were anatomically intact upon arrival. None of the internal body organs, such as the hepatopancreas which ruptures easily, had been damaged or broken down which could have resulted in leaching elements and contaminating other body tissues. Upon arrival at the laboratory, the prawns were individually weighed, placed into plastic storage containers that had been washed in 10% nitric acid, and then killed by chilling in a freezer.

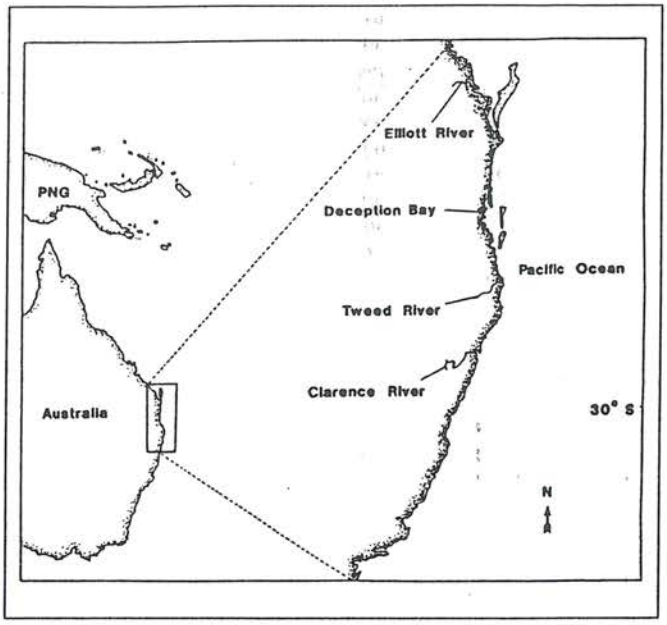

Fig. 1. *The East Coast of Australia showing the positions of the four juvenile eastern king prawn nursery areas sampled during the study.*

Dissections were carried out in a laminar flow cabinet while the prawns were still frozen, using stainless steel scissors, forceps, and a scalpel. A team of two undertook the dissections with each person using a separate set of dissecting instruments. Four body tissues were dissected out to assess their potential to yield a suitable imprint: eyes, hepatopancreas, abdomen with the hindgut removed, and abdominal exoskeleton (shell). For each prawn, the cephalothorax (head) was cut off and given to one person whose task was to remove the eyes (at the base of the compound eye, not including the eye stalk) and cut out the hepatopancreas. The other person received the abdominal half of the prawn and removed carapace and hindgut from the abdominal muscle. Ten samples of each tissue were obtained from each nursery area (10 samples each for abdomen muscle, hepatopancreas, eyes, and abdominal exoskeleton). Each sample contained pooled tissue from 10 or 12 prawns, depending on the number caught in the particular area. Samples were then placed in labeled plastic vials.

To determine if the elemental concentration was independent of prawn size, surplus individual prawns were left intact and analyzed as "whole prawns."

Preparation for ICP-MS

All analyses were carried out on a standard Perkin-Elmer® Sciex ELAN™ 5000 Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). All sample vials and plasticware used for the preparation of standard solutions, dilution, and storage of sample digests were soaked in nitric acid (10%) for a minimum of 48 hours. All items were then rinsed three times in reverse osmosis prepared water, followed by three further rinsings with polished reverse osmosis (ROP) prepared water (18 MΩ). All borosilicate glass volumetric flasks were fitted with PTFE stoppers. These flasks and the PTFE beakers were refluxed with concentrated nitric acid for eight hours, allowed to cool, rinsed three times in RO water, and then soaked and cleaned as per the plasticware. Nitric acid was purified by sub-boiling double distillation of reagent-grade feedstocks in quartz stills.

Mixed multielemental standard solutions were prepared from 1000 ng/L stock solutions. Aluminum standards were prepared separately in TPX (polymethylpentane) volumetric flasks. The adsorption/release equilibriums of glass with aluminum in solution make low level determination of this element in glass highly inaccurate. Sample dissolution was achieved using a nitric acid microwave-assisted digestion. The system used was a Microwave Laboratory Systems MLS 1200, manufactured by MILESTONE, Italy. The frozen samples were placed in a DynaVac Freeze Drying Unit (Model: FD2) and allowed to dry to constant weight. Samples were then ground to a fine powder to achieve a homogeneous final product. Of this material, 100–200 mg was accurately weighed into a TFM™ [Tetrafluormethoxil, a PTFE (Teflon)-based material] insert of the microwave digestion system. Nitric acid (4 mL) was added and the vessels sealed and placed in the microwave oven. The oven program used was as follows:

(a) 250 watts for eight minutes

(b) 400 watts for four minutes

(c) 250 watts for four minutes

It should be noted that 250 watts power with this system is a continuous energy output which results in more even and controlled heating, producing a gradual pressure increase to a maximum of 30 bar. The vessels were then removed from the oven and cooled in an ice-bath for a minimum of one hour. This step is necessary to avoid losses of the sample from aerosols released upon opening of the vessels.

The sample solution was then transferred to a PTFE beaker and made up to 15.0 g with ROP water. Of this solution, 1.5 g was transferred to a polypropylene tube and set aside for mercury determination. A further 6.0 g of the sample solution was placed in a second polypropylene tube and stored as a replacement if required. The remaining 7.5 g of the sample solution in the beaker was taken to near dryness on a ceramic hot plate at 90°C. An additional 2 mL of nitric acid and 0.2 mL of hydrogen peroxide (30% w/v) were added dropwise and again taken to near dryness. This step was included to ensure complete digestion and to remove volatile interfering matrix components. The digestion solution was washed into a 50-mL polypropylene tube using 1% nitric acid, accurately made up to 20.0 g and used for the solution nebulization ICP-MS. The procedure is represented diagrammatically in Figure 2.

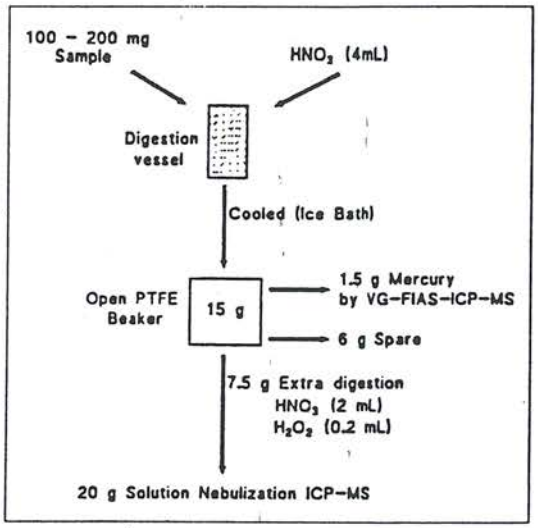

Fig. 2. *Diagrammatic representation of tissue for ICP-MS analyses.*

Instrumental ICP-MS Conditions

The plasma flow, nebulizer flow, and auxiliary flow were set at 15 L/min, 0.92 L/min, and 0.8 L/min, respectively. The RF power was 1000 watts, and the CEM voltage was 0 kV. The sample uptake was set at 1 mL/min.

Statistical Methods

Prawns from the different nursery habitats were treated as separate groups. In order to distinguish between groups, canonical-variate (discriminant) analyses were undertaken, treating the concentration of each element as a discriminating variable. The statistical graphics software package STATGRAPHICS was used to transform and analyze the data.

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RESULTS AND DISCUSSION

We attempted to sample prawns of similar size from each of the four areas to reduce attributing any detectable differences between the areas to differences in prawn size. However, it was not possible to control the exact size or abundance of prawns trawled, and consequently, there was a significant difference (ANOVA $p < 0.01$) in the mean weights of the prawns from each area (Table I).

We then determined whether these differences could influence the overall results by examining possible relationships between elemental concentration and prawn size, using "whole prawns" from several of the sampling areas, ranging in size from approximately 0.3 g to 3.0 g, and several elements such as sodium, aluminum, iron, cadmium, strontium, copper, chromium, caesium, molybdenum, selenium, rhodium, and arsenic. None of the regression analyses were significant ($p > 0.05$). This suggests that for the size range prawns sampled, the concentration of elements was independent of size. Thus, any detectable differences between samples from different nursery areas was unlikely to be due to differences in the size of the prawns.

The canonical variate analyses showed that the prawns could be separated using the concentrations of 15 elements (Table II) in their tissues and that each of the four body tissues was suitable for discriminating (Figure 3a–d). The analyses correctly predicted, for each tissue type, from which nursery area each of the 40 samples was obtained (100% discrimination).

Although the concentration of elements and compounds in various body tissues is known to vary with molt stage (Greenway 1985) and reproductive condition (Galois 1984, Anderson et al. 1985) in Crustacea, it is unlikely that the present results were influenced by such differences in prawn condition. First, the prawns examined were very young, probably no more than four weeks old, and thus all at the same stage of maturation (immature). Second, there was no evidence of synchronized molting, within or between sample areas. After each night of sampling, there were several exuviae (molts) floating on the surface of the water in the 60-L holding drums, indicating that a percentage of any particular population molt every day. Thus, it would be misleading to attribute the observed differences to differences in molt stage between areas.

The results are encouraging because they imply that prawns sampled from different estuarine nursery areas can be distinguished from one another using their elemental compositions. However, the results should also be interpreted with caution, because it is unknown whether these differences are retained in the prawns through time, as they grow, migrate, and undergo reproductive cycles. Furthermore, it is also unknown

TABLE I
Number and Mean Size of Juvenile Eastern King Prawns, *Penaeus plebejus*, Sampled From Four Nursery Habitats From the East Coast of Australia

Estuary/ nursery area	Number of prawns	Mean weight (g)	Standard deviation
Elliott River (Queensland)	120	0.6935	0.2261
Deception Bay (Queensland)	100	1.1136	0.3294
Tweed River (New South Wales)	120	0.7275	0.2482
Clarence River (New South Wales)	120	0.7743	0.2965

TABLE II
Elements Used for Each Tissue Type as Discriminating Variables to Identify Juvenile Eastern King Prawns, *Penaeus plebejus*, from Different Nursery Habitats

Eyes	Hepatopancreas	Abdomen muscle	Exoskeleton
Sodium (Na)	Sodium (Na)	Sodium (Na)	Sodium (Na)
Magnesium (Mg)	Magnesium (Mg)	Magnesium (Mg)	Magnesium (Mg)
Phosphorus (P)	Caesium (Cs)	Phosphorus (P)	Phosphorus (P)
Sulphur (S)	Sulphur (S)	Sulphur (S)	Sulphur (S)
Potassium (K)	Potassium (K)	Potassium (K)	Potassium (K)
Calcium (Ca)	Calcium (Ca)	Calcium (Ca)	Calcium (Ca)
Copper (Cu)	Copper (Cu)	Copper (Cu)	Vanadium (V)
Barium (Ba)	Zinc (Zn)	Barium (Ba)	Cobalt (Co)
Strontium (Sr)	Strontium (Sr)	Strontium (Sr)	Strontium (Sr)
Manganese (Mn)	Boron (B)	Caesium (Cs)	Ytterbium (Y)
Selenium (Se)	Selenium (Se)	Aluminum (Al)	Selenium (Se)
Iron (Fe)	Barium (Ba)	Lithium (Li)	Lithium (Li)
Arsenic (As)	Arsenic (As)	Arsenic (As)	Arsenic (As)
Molybdenum (Mo)	Molybdenum (Mo)	Iron (Fe)	Iron (Fe)
Chromium (Cr)	Cobalt (Co)	Cadmium (Cd)	Nickel (Ni)

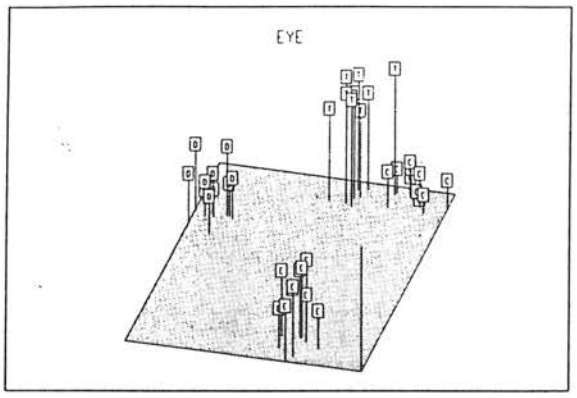

Fig. 3a.

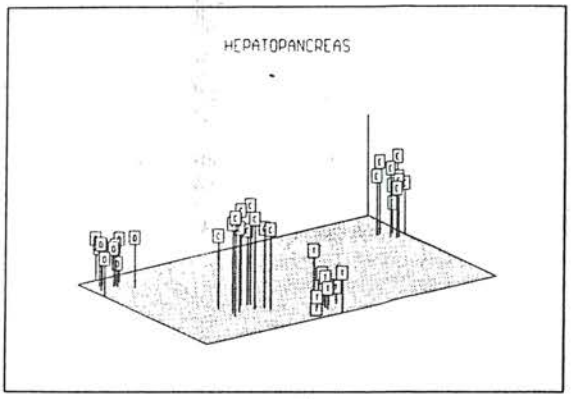

Fig. 3b.

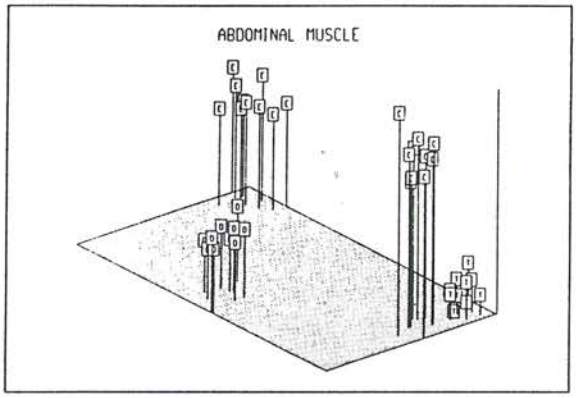

Fig. 3c.

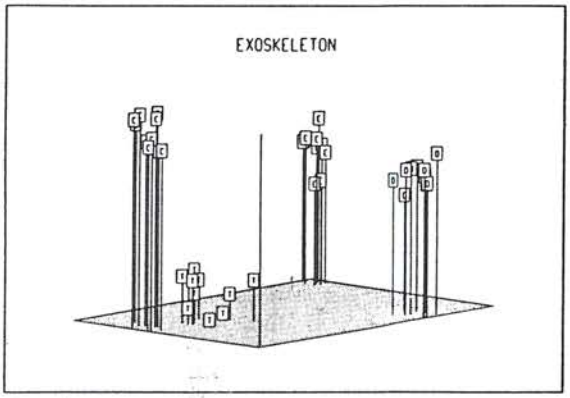

Fig. 3d.

Fig. 3a–d. *Canonical variate analyses based on the concentrations of 15 elements in juvenile eastern king prawns (Penaeus plebejus) from four nursery habitats. Four body tissue types were used (A, eye; B, Hepatopancreas; C, Abdominal Muscle; and D, Exoskeleton).*
The letters refer to the four nursery areas (E = Elliott River, D = Deception Bay, T = Tweed River and C = Clarence River).
The three unlabeled axes represent discriminant functions 1, 2, and 3.

whether the prawns sampled from each particular nursery area can be discriminated from prawns in other sites within the same area, say at different distances upstream. It would also be naive to conclude that the results provide information on the prawns' past environment. The analyses may simply reflect the elemental composition of the prawns at a certain place and at a certain time.

CONCLUSION

The study shows that ICP-MS can be used to measure the concentration of elements in various juvenile prawn body tissues and that combinations of these element concentrations can be used as discriminating variables to distinguish prawns from the different areas they currently inhabit. The value of this methodology for determining a geographic history of the prawns has yet to be demonstrated. Further experiments and analyses of these data are in progress to determine if the prawns retain elemental concentrations or profiles and to determine key elements that are likely to reflect geographic habitat.

ACKNOWLEDGMENT

We would like to thank New South Wales Fisheries and Fisheries Patrol Officers for permission to sample the prawns in the Clarence and Tweed Rivers, located in that state. Mr. Hugh Mawhinney and Mr. Paul Hielscher of the Queensland Department of Primary Industries (QDPI), Animal Research Institute, performed the ICP-MS analyses. Dr. Nigel Preston of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Division of Fisheries Research, provided suggestions about the design of the study. The study was funded by the Fisheries Research and Development Corporation (FRDC).

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Appendix B: Methods of collection and analysis of sediments collected in the four nursery areas where samples of eastern king prawn juveniles were obtained.

Sample collection and preparation

Samples were collected with a hand held sediment grab at the same location where trawl shots where used to collect juvenile prawns. In the laboratory samples where dried and sieved into four fractions:

A: > 2mm
B: 2 - 0.2 mm
C: 0.2 - 0.063 mm
D: < 0.063 mm

Fraction A was considered too coarse for analysis (would require crushing). Fraction D was absent from sediments from the Elliot River, therefore only fractions B and C were analysed with ICP-MS. 3 sub-samples were analysed for each of the four locations given a total of 24 samples (3 samples X 4 locations X 2 fractions).

ICP-MS Analysis

There were 47 elements scanned instead of the 38 elements scanned in the prawn tissue samples. Some elements were scanned in sediment samples and not scanned in prawn tissue samples (Ru, La, Pr, Nd, Sm, Eu, Gd, Dy, Er, Nb, Yb, Hf, Wm, Th, Sb). Some elements were scanned in prawn tissue samples but not in sediments (S, B, Al,Se,Li, Be).

Appendix C: Methods of collection and analysis used to determine differences in the elemental composition of eastern king prawn sub-adults.

In January 1994, live eastern king prawn sub-adults were captured in Moreton Bay and off Fraser Island, with commercial trawl gear towed by the R.V. Deep Tempest . 600 live eastern king prawns were transported on board the R.V. Deep Tempest from Fraser Island to Moreton Bay were 400 survirvors were tagged. Another 400 eastern king prawns captured inside Moreton Bay were tagged.

Prawns were tagged with Hall™ streamer tags impregnated in antibiotic througth their first abdominal segment. All prawns were released the same day within Moreton Bay.

A sample of live and frozen, tagged and untagged, sub-adult eastern king prawns from both Fraser Island and Moreton Bay, were also taken to the laboratory for ICP-MS analysis of their tail muscle.

Fishers participating in the eastern king prawn fishery in the Moreton Bay region were informed of the tagging experiment by mail, and through visits to the major landing places between Wynum, in southern Moreton Bay and Urangan in southern Harvey Bay. Fishers were encouraged to return tagged prawns and lottery tickets were given to those that did.

Tagged prawns recaptured by the fishing fleet up to four months after their release where collected and their dissected tail muscle was analysed with ICP-MS.

Appendix D: Design of experiments used to determine the sources of changes in the elemental composition of eastern king prawns.

Uptake from sediments

Live juvenile eastern king prawns were captured by beam-trawl in the Elliot River in May 1993 and were brought to the Southern Fisheries Centre, where they were held for 2 months in 2 tanks of 4 m diameter.

A sample of 16 of these prawns were used for analysis of the elemental composition of tail muscle and eye at the begining of the experiment. One muscle sample was obtained from the tails of four prawns. Eight pair of eyes produced a single eye sample.

The rest of the prawns were divided in two groups, one group was put in a tank with Deception Bay sediment, the other on a tank with Elliot River sediment.

A month later three prawns were taken out of each tank to produce a single muscle sample and a single eye sample).

The experiment was terminated after 2 months at which point all surviving prawns were used for ICP-MS analysis of eye and tail muscle tissue.

Uptake from seawater and food

Sub-adult eastern king prawns were captured from Moreton Bay in January 1994 and brought to the Southern Fisheries Centre, where they were randomly separated into four groups of 8 prawns each.

Each group of prawns was placed in an 80 liter glass aquarium with undergravel filters. Two of the aquaria were filled with natural seawater from Deception Bay, and the other two aquaria with artificial seawater (Ocean Nature, Aquasonics™).

Two of prawn groups kept in aquaria (one with each type of water) were fed with Penaeus japonicus pellets, and the other two with frozen scallop meat.

After four weeks all surviving prawns were dissected and their tail muscle was analysed with ICP-MS.

Appendix E: Die D.J., J.G. McGilvray, A.J. Courtney (submitted to Canadian Journal of Fisheries and Aquatic Sciences). A quantitative method for staging penaeid prawn ovaries using Image analysis.

A Quantitative method for staging penaeid prawn ovaries using Image Analysis.

D. J. Die[1], J.G. McGilvray, A.J. Courtney and M. G. Cosgrove

Queensland Department of Primary Industries
Southern Fisheries Research Centre
P.O. Box 76, Deception Bay 4508, Queensland, Australia.

[1] Present Address, C.S.I.R.O. Marine Laboratories, P.O. Box 120, Cleveland 4163, Queensland, Australia.

Abstract

A quantitative method of staging reproductive development based on the use of cell measurements and computerised image analysis of histological sections is described. The method has been applied to samples from eastern king prawn, Penaeus plebejus, collected in Queensland, Australia. Luminance measurements of selected tissue structures within the ovary were used to determine three luminance ranges which characterised ovarian development in histological sections. Cell diameter, average luminance and percent area of an ovary section within a luminance range of 200-245 were the most useful variables to discriminate between the different ovarian developmental stages. Discriminant analysis successfully separated maturing, mature and resorbing groups in 94% of the samples, but discrimination of stages within the above groups was as low as 30%. The estimated discriminant functions suggest there is a continuum of development which can be characterised by a combination of measurements obtained from histological sections.

Introduction

Histological development of penaeid prawn ovaries has been described by King (1948), Cummings (1961), Tuma (1966), Yano (1988), Tan-Fermin and Pudadera (1989), and Clark and Pillai (1991). All of these studies distinguish several developmental stages, and describe these on the basis of qualitative assessments of ovarian status. Histological development, however, is a continuous process.

When histological sections are used to determine temporal or spatial spawning patterns in populations, the stage of ovarian development is usually based on the subjective evaluation of the section by an observer who estimates (qualitatively) the dominant cell type in the section. The subjectiveness of qualitative estimation can become a problem when multiple observers are used to conduct the study of gonad development, or when results from two or more studies are to be compared. In such cases, blind test trials should be used to establish how the assignment of developmental stages compares between observers.

In the past, quantitative histological measurement has been difficult, mainly because of the lack of image enhancement technology. The advent of computerised image analysis has facilitated quantitative histological analysis to the point where a large number of precise measurements can be attained cost-effectively. Computer-based image analysis also provides the ability to process images and extract information which, in the past, was impossible to collect with standard optical systems. Heffernan et al. (1989a,b,) and Heffernan and Walker (1989a,b) utilised image analysis to study reproductive cycles in bivalves and found this technique more sensitive to gametogenic development than visual examination .

This paper presents a quantitative method for staging reproductive condition in female penaeid prawns, using computerised image analysis for measuring histological attributes. The method is applied here to samples of ovarian tissue from female eastern king prawns, (Penaeus plebejus), but should be applicable to other penaeid prawns.

Methods

Over a two year period between July 1990 and August 1992 a large number (over 6,000) of female eastern king prawns were sampled from the southeast coast of Queensland (Australia) using a research vessel and commercial trawlers. Ovarian tissue was obtained from each female, sectioned in parafin wax at a thickness of 6μm, and stained with haematoxylin and eosin. The tissue was then examined under an optical microscope and each female qualitatively classified into a stage of development by an observer. Further details of the sampling procedure and preparation of the ovarian tissue can be found in Courtney et al. (in press).

Eighty females in nine different stages of development (Table 1) were identified. Sections from these females were analysed with a black and white image analysis system. The system consisted of an Ikegami ccd video camera, an Olympus™ compound microscope, a Sony™ Trinitron analog video display, a COMPAQ™ Desk Pro 386 personal computer and the Optimas™ software package. All measurements were made under controlled ambient light conditions and constant microscope light intensity and magnification (X200). The system was used to measure cell diameter, luminance and area of tissue within a given range of luminance. Measures of luminance were based upon an arbitrary grey scale, ranging from 0 (black) to 255 (white). The system was calibrated such that white represented the light received by the system in the absence of a histological section on the microscope, and black represented the light received by the system when the microscope light was turned off.

The quantitative staging methodology was developed in three steps. Firstly, luminance ranges associated with different cell structures and different stages in maturation were identified. Secondly, histological measurements were carried out on the 80 females, using results obtained from step one. Thirdly, the histological measurements were used to group individuals into different stages of maturation.

Step 1. Histological structures in the ovary associated with different developmental stages were identified and had their luminance measured. Five females, in each of stages 2,3,4,5 and 6 (Table 1) were used to obtain the luminance values. The structures considered were nucleus, nucleoli, follicle cells, cytoplasm, corticle crypts and the intercellular space within the ovary. Two single-point luminance measures were obtained for each structure (when present) in each female.

Step 2. Five areas were selected randomly from the ovarian tissue section of each female. Each area was displayed on the video screen, which had a 5x4 grid template overlaid on it. The template divided the displayed image into 20 (50mm x 50mm) subareas. Two subareas were then randomly selected within each area, giving a total of 10 subareas per female. For each of these subareas the following measurements were taken; 1) four luminance statistics (average, standard deviation, skewness and kurtosis), 2) the percentage of the subarea within each of three different luminance ranges, and 3) oocyte diameter.

Oocyte diameter for cells containing a nucleus was calculated along an imaginary line passing through the nucleus and joining the two points furthest apart on the cell's membrane. For oocytes without a nucleus, the estimated diameter was calculated from the line joining the two points furthest apart on the cell's membrane. In cases where more than one cell was located fully within the selected area on the video screen, the cell located most central in the grid was measured. When no cell was totally within the selected area the cell occupying the largest space within the selected area was measured.

Step 3. A discriminant function analysis was used to classify the 80 females into the different stages, and determine the gradient of development. The classifications were compared with the results from previous subjective staging by an observer. The subjective staging was used to generate the apriori groups and the discriminant analysis predicted the percentage of females that could be correctly classified into these groups. For each female and for each discriminating variable, the average of the ten subarea measurements was used.

Results

Luminance of tissue structures

Because of the changes that occur in the ovary through development, not all histological structures could be found in all females. Although the luminance of cytoplasm decreased as ovarian maturation progressed, the luminance of other cellular structures did not change between stages of development (Figure 1).

Because their luminance ranges overlapped, cell structures could not be separated on luminance values alone. However the following ranges characterised certain cell structures: low luminance (50-110), mostly follicle cells and nucleoli; medium luminance (125-180), mainly cytoplasm and nuclei; and high luminance (200-245), cortical crypts and inter cellular space (Figure 1).

Discriminant function analysis

Discriminant function analysis indicated that the three most informative histological variables for classifying histological sections into their correct development stages were (1) cell diameter, (2) the percent of area in the high luminance range, and (3) average luminance. The other variables did not significantly increase the number of histological sections that were correctly classified by the discriminant function analysis.

The first discriminant function explained 90.98% of the variation (Table 2). The standardised coefficients (Table 3) indicate that cell diameter was the most important discriminating variable in function 1. Function 2 was related primarily to the percent of area in high luminance, while average luminance was the main discriminating variable in function 3.

The tissue samples representing stages 1,3 and 7, were correctly classified in 100%, 88% and 88% of cases respectively (Table 4). However other stages (particularly stage 8) correctly classified only 33% of the time. For seven of the nine stages, 66% or more of predicted classifications were correct and for six out of the nine stages 75% or more were correct (Table 4).

The analysis also showed that tissue sections could be classified into broader groupings, i.e. maturing (stages 1,2 and 3), mature (stages 4,5 and 6) and spent/resorbing (stages 7,8 and 9) on 93, 100 and 86 % of occasions respectively. Overall only 6% of ovaries were mis-classified with respect to these broad groupings.

The discriminant analysis suggests a continuum associated with ovarian development between stages 1 and 6 (Figure 2). This continuum appears to follow a different plane from that of stages 7 to 9 (Figure 3). Resorbing stages showed most variability in the third discriminant function whilst maturing and ripe stages varied in all three discriminant functions (Figure 3). Stages 7, 8 and 9 are clustered in close proximity to the centroids of stages 1, 2 and 3 (Figure 3) which may account for misclassifications between maturing and spent/resorbing groups.

Discussion and Conclusions

Increase in size of developing oocytes appears to be the main feature allowing separation of maturing stages (Figure 2). Changes in luminance also assist in differentiating between these stages. Large areas of stage 1 ovaries are covered by intercellular space, and this stage therefore has a high percentage of area within the high luminance range. Stage 2 ovaries contain many dark staining (basophilic), pre-vitellogenic cells with a low percentage of area within the high luminance range. An increase in light staining (acidophilic) cytoplasm characterises stage 3 ovaries. These exhibit therefore a higher percentage of area within the high luminance range than stage 2 ovaries.

As maturity approaches, changes in oocyte size become insignificant, cortical crypts lengthen and the nucleus disappears (stage 5) as do the follicle cells (stage 6). The result is that between stages 4 and 6, changes in overall luminance, oocyte size and area within each of the three luminance ranges are small. Therefore whilst stages 4 to 6 were correctly separated from all other stages by the discriminant analysis separation between them was not as successful.

Females with spent or resorbed ovaries are often difficult to distinguish from one another. In both stages, the ovary displays little structural uniformity and any oocytes which may be present appear to be broken down. In spite of the present analysis relying heavily on cell diameter for discrimination, classifications of spent/resorbing stages were as successful as the other two groups (maturing and mature) - stage 8 being the only exception. Stage 8 ovaries share characteristics of stages 1 and 2, as well as stages 7 and 9 (Table 1), and this probably accounts for the high proportion of mis-classifications (66%).

Whilst classification into individual stages was not always successful, the analysis found that three groups, immature (stages 1, 2, and 3), mature (stages 4, 5 and 6) and resorbing (stages 7, 8 and 9) could be easily separated with minimal error. It is specially important for spawning studies that stages within the mature group were always correctly classified as belonging to that group. This is due to the large cell size and high luminance associated with histological sections of mature ovaries.

The misclassification of immature gonads as resorbing gonads and vice versa (Table 4) can be explained by the difficulty of separating the two groups with the visual method (Courtney et al. in press).

We believe that the continuum observed in the discriminant analysis (Figures 2 and 3) can be interpreted as reflecting changes in the characteristics of the gonad:

- an increase in cell size from stage 1 to maturity (stage 4), which can be seen as changes along the axis of discriminant function 1,

- an initial decrease in the amount of light tissue structures (luminance range 200-245), from stage 1 to stage 2 due to the reduction of intercellular space, and a subsequent increase in the amount of light tissue structures as cytoplasm and cortical crypts develop and the nucleus and follicle cells disappear within the ovary.

Because measurements taken with the image analysis system allow us to place an individual prawn within the continuum of ovarian development more precisely, we feel that the method presented herein is more accurate than the traditional visual staging method. This method can quantify, at least for immature ovaries, how developed a gonad is within each of the original visual stages.

The present method however, is limited in its power to quantify differences between stages 4, 5, 6 and between spent/resorbing stages. Future studies should focus on determining which other measurements may be appropriate to separate these stages.

This method will also resolve the problem of standardising staging protocols between observers, because measurements made with the image analysis are not subjective.

From this study it was apparent that some variability in cell development can be observed within the same ovary. This variability has sometimes been cited as a possible source of problems to the qualitative assignment of development stages to a given histological section (Steve Montgomery, NSW Fisheries, Fisheries Research Institute, P.O. Box 21, Cronulla 2230, New South Wales, Australia, Personal communication). In this study we have shown that such variability is not an impediment for the quantitative characterisation of a section as long as several random measurements are taken from each sample tissue.

Acknowledgments

This study was supported by the Australian Fisheries Research and Development Corporation (FRDC grants 90/4 and 92/8). Mike Dredge and Ian Brown provided invaluable comments on an earlier draft of this manuscript. We also thank skippers D. Trama, M. Coglan, K. Walker, P. Seib, S. Wood, W. Sterling and the Mc Ilwain family for assisting in the collection of samples. D. Green and P. Peters carried out most of the laboratory dissections and histological preparations.

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List of Figures

Figure 1 - Luminance range of cell structures in the ovary of female Penaeus plebejus. Five individuals in each stage were used. Two measurements were obtained for each structure in each individual.

Figure 2 - Three dimensional plot of disciminant function values for each ovarian histological section in development stages one to six. Stages one to three and mature animals (4, 5 and 6) show clear group separation whilst groups of stages four to six are hard to differentiate.

Figure 3 - Three dimensional plot of discriminant function values for each ovarian histological section in development stages seven to nine. Included are the group centroids of the first six stages which show immature and mature stages follow a different plane to resorbing stages.

Table 1. Description of nine ovarian developmental stages. Each stage was also considered to be in one of three less descriptive Groups; maturing, mature or spent.

Description of ovarian developmental condition	Stage	Group
Ovaries < 500 μm in diameter. Basophilic oogonia lie scattered in a loose meshwork of connective tissue. Large area of intercellular, vacant space within ovary.	1	
Basophilic oocytes increased in size to 30 - 60 μm. Pre-vitellogenic. Nucleoli appear around the periphery of the nucleus (perinucleolus stage), and are slightly darker staining than the cytoplasm. Basophilic follicle cells (< 5 μm) present, external to, and distributed around the perimeter of the oocyte.	2	Maturing
Oocytes vitellogenic with mean diameter of 210 μm. Massive increase in volume of acidophilic cytoplasm. Basophilic nucleus lies centrally within the cytoplasm with one or two large, dark-staining nucleoli. Basophilic follicle cells (< 5 μm) distributed around the perimeter of oocytes. Little vacant intercellular space. Small groups of perinucleolus stage oocytes.	3	
Oocyte diameter increased to 228 μm. Cortical crypts appear in oocyte cytoplasm, are circular and jelly-like, distributed around periphery of inner cell wall. Nucleus lies centrally within the cytoplasm. Basophilic follicle cells present around the periphery of the oocyte. Basophilic nucleus. Cytoplasm and cortical crypts acidophilic.	4	
Nucleus disappears (germinal vesicle break down, GVBD). Cortical crypts elongate, extending radially inward.	5	Mature
Follicle cells disappear (Ovulation). Elongate corticle crypts. No vacant intercellular space.	6	
Flaccid ovary wall, large intercellular vacant space. Dominant cell type is oogonia. No vitellogenic stages. Spent or resorbed appearance.	7	
Flaccid ovary wall, large intercellular vacant space. Dominant cell type is perinucleous stage. No vitellogenic stages. Spent or resorbed appearance.	8	Spent/ Resorbing
Vitellogenic oocytes with vacuoles present, giving the appearance of breaking down. Lack of uniformity in cell size and shape.	9	

Table 2. Relative percentages of discrimination associated with each discriminant function used to separate development stages from histological sections of eastern king prawn (P. plebejus) ovaries.

Discriminant Function	Relative Percentage
1	90.98
2	6.79
3	2.23

Table 3. Standardised discriminant function coefficients from the discriminant analysis of histological sections of eastern king prawn (P. plebejus) ovaries.

	Discriminant Function 1	Discriminant Function 2	Discriminant Function 3
Cell diameter	0.96475	0.20678	-0.19992
High luminance	-0.69395	1.24397	-1.18155
Average luminance	0.64922	-0.34169	1.70350

Table 4. Classification of histological sections of female eastern king prawn (P. plebejus) ovaries obtained with discriminant analysis. Discriminating variables were cell diameter, percentage area in high luminance range, and average luminance. Rows represent the actual stage and columns the predicted stages. Numbers in bold represent the number of correct classifications for each stage. Shaded cells represent misclassifications of development group (maturing, mature, spent/ resorbing).

	1	2	3	4	5	6	7	8	9	Percent Correct
1	**9**	0	0	0	0	0	0	0	0	100
2	1	**7**	0	0	0	0	0	1	0	77
3	0	0	**8**	0	0	0	0	0	1	88
4	0	0	0	**6**	1	2	0	0	0	66
5	0	0	0	2	**5**	2	0	0	0	55
6	0	0	0	1	1	**6**	0	0	0	75
7	0	0	0	0	0	0	**8**	1	0	88
8	0	2	0	0	0	0	2	**3**	2	33
9	0	0	1	0	0	0	0	1	**7**	77

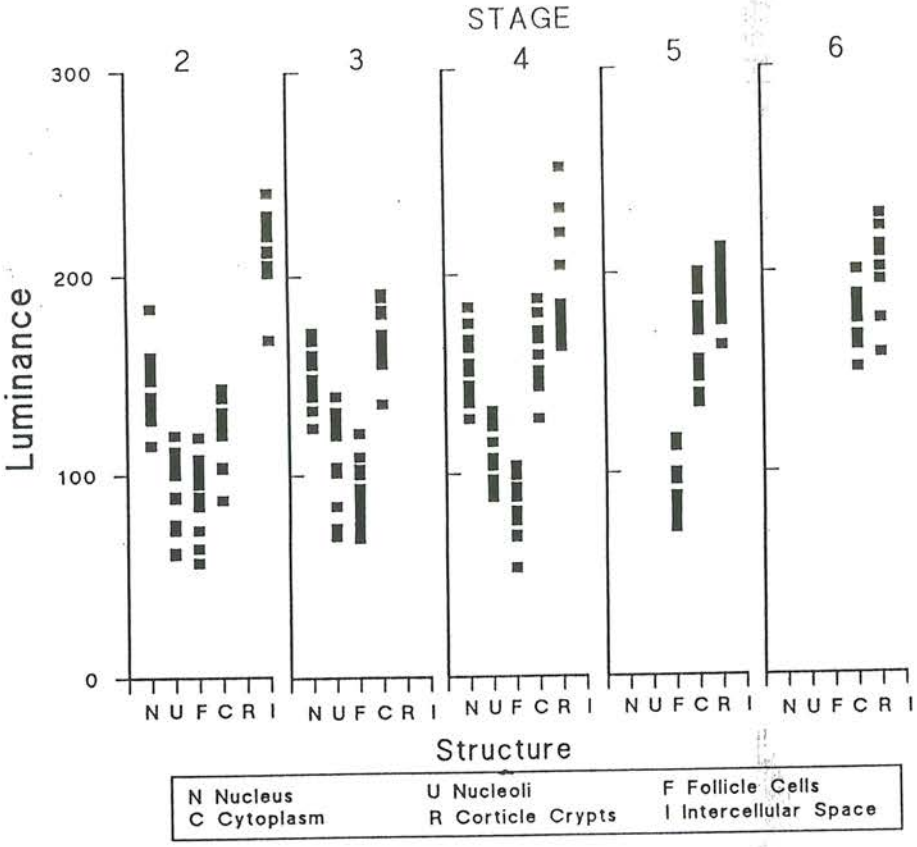

Fig 1

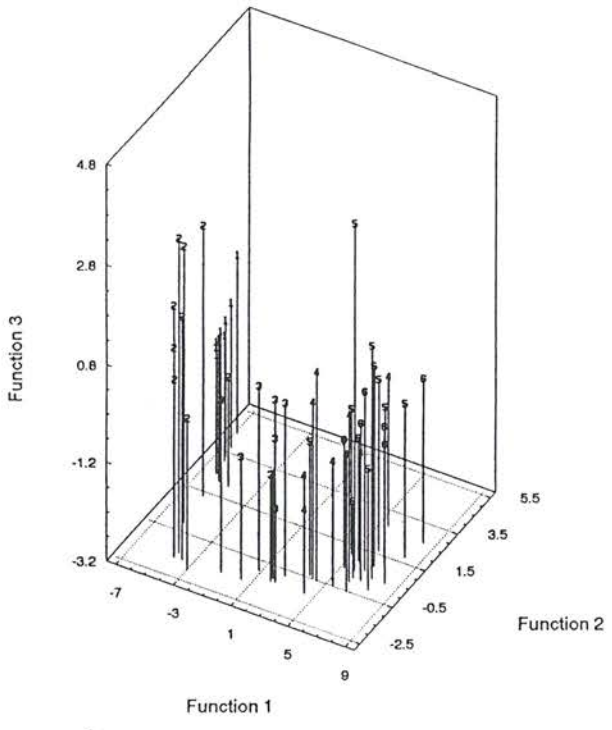

FIGURE 2

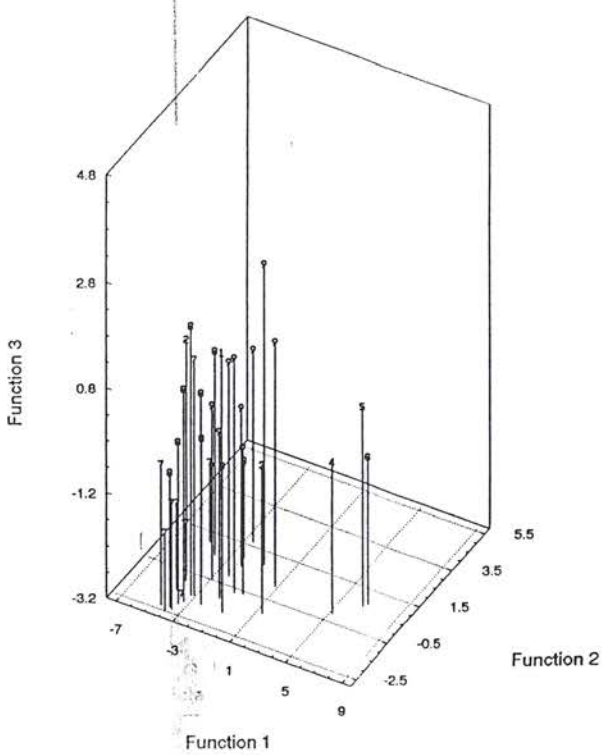

FIGURE 3

Appendix F: Courtney A.J., D.J. Die and J.G. McGilvray. (submitted to Australian Journal of Marine and Frehwater Research). Lunar variation on population stucture and reproduction in *Penaeus plebejus* in coastal waters off southeast Queensland, Australia.

Lunar variation in population structure and reproduction in adult eastern king prawns, *Penaeus plebejus* in coastal waters off southeast Queensland, Australia.

Courtney, A. J., [1]Die, D. J. and McGilvray, J. G.

Queensland Department of Primary Industries
Southern Fisheries Centre
P O Box 76 Deception Bay
Queensland 4508
Australia

[1]CSIRO Marine Laboratories
PO Box 120 Cleveland
Queensland 4136
Australia

Abstract

Introduction

Many coastal marine organisms exhibit physiological and behavioural rhythms which are synchronised with changes in their environment (Neumann 1981). De Coursey (1983) reviewed the literature on crustacean rhythmicity and found examples of tidal, diurnal, semilunar, lunar, and annual periodicities in locomotor activity, moulting and reproduction (courtship and spawning). Physiological and behavioural rhythms may bias estimates of abundance (due to variations in catchability) and other population parameters which are derived from samples. Therefore, whenever populations are sampled for stock assessment, or other purposes, the influence of such rhythms needs to be considered. This can be achieved either by designing the sampling program appropriately, or by standardising the data after it has been obtained. To do this, it is necessary to have some understanding of the physiological and behavioural rhythms of the organisms being studied.

Most of the examples of crustaceans exhibiting rhythmic behaviour in their natural environments are for relatively highly-visible intertidal organisms, such as *Uca* spp. There are fewer examples for subtidal species. For penaeid prawns, catch rates of postlarvae (Barber and Lee 1975, Young and Carpenter 1975) and juveniles (Vance and Staples 1992) display circadian and tidal rhythms. Relatively little information exists on adult prawn rhythmicity apart from laboratory studies of activity patterns (Fuss and Ogren 1966, Moller and Jones 1975, Wassenberg and Hill 1984, Hill and Wassenberg 1985, Natarajan 1989a,b). Natarajan (1989a) noted that while endogenously controlled nocturnal activity has reported for a few species of penaeid prawns, persistent tidal, daily, and semilunar rhythms have generally been poorly demonstrated in migratory, subtidal crustacea.

Eastern king prawns are endemic to coastal waters of eastern Australia. They are the largest,

most oceanic and migratory (Ruello 1975, Potter 1975, Glaister *et al.* 1987, Montgomery 1990) of Australia's endemic penaeid prawns. Eastern king prawns also form the basis of a major, largely mono-specific, otter-board trawl fishery (Glaister *et al.* 1990). Trawling takes place on the continental shelf from shallow coastal estuaries to depths of about 250 m. In the northern range of the fishery (latitude 22° S), trawling takes place up to about 250 km from the coast.

Little quantitative information exists on the physiological or behavioural rhymicity of *P. plebejus*, particularly for adults which occur in relatively deep, offshore waters. Barber and Lee (1975) and Young and Carpenter (1977) found that planktonic postlarvae migrate across coastal bars at night during the first three hours of the flood tide to enter shallow estuarine nurseries. Early observations by Dakin (1938), and some sampling results by Racek (1959) suggested that sub-adults exhibit seasonal trends in catch rate (abundance), display diurnal behavioural patterns (nocturnal activity), and emigrate seaward from coastal lakes and estuaries on a lunar cycle. Racek (1959) also stated that eastern king prawns display a "schooling" behaviour in estuaries shortly before the last quarter of the moon phase, but provided no data to support this.

The aim of this study was to determine if adult eastern king prawns display lunar or semilunar physiological or behavioural rhythms in their natural environment. The general hypotheses being examined were that catch rate, reproductive activity and moulting in adults are independent of lunar phase. The findings are discussed in relation to their significance to the fishery, research sampling and the fishery's stock assessment.

Materials and Methods

Sampling strategy

Three transects were established in a depth of 150 m in an area (27° 02' S, 153° 37' E) approximately 8.7 nautical miles east of Cape Moreton, which is located on Moreton Island, southeast Queensland (Australia) (Figure 1).

Each transect was approximately 4.4 nautical miles long and orientated on a north-south axis. The area is permanently open to commercial prawn trawling. Previous sampling in the vicinity (Courtney *et al.* 1995) and discussions with local fishers indicated that the catches of *P. plebejus* in the area consisted of adult prawns.

Sampling trips were carried out regularly (approximately every 72 hours) using the research trawler "Deep Tempest" for two consecutive lunar cycles from 24th May to 21st July 1993 (59 days, inclusive). On each trip all three transects were sampled once and the order in which they were sampled was randomised. Transects were located using radar and an on-board geographic positioning system (GPS). The duration of each trawl shot was two hours bottom time. The first shot was undertaken early in the evening (from 18:00 to 20:00), the second in the middle of the night (from 22:00 to 24:00) and the third was in the early hours of the morning (from 02:00 to 04:00) before sunrise. The trawl nets consisted of three seven-fathom Florida Flyers with 50 mm (approximately two inch) mesh codends.

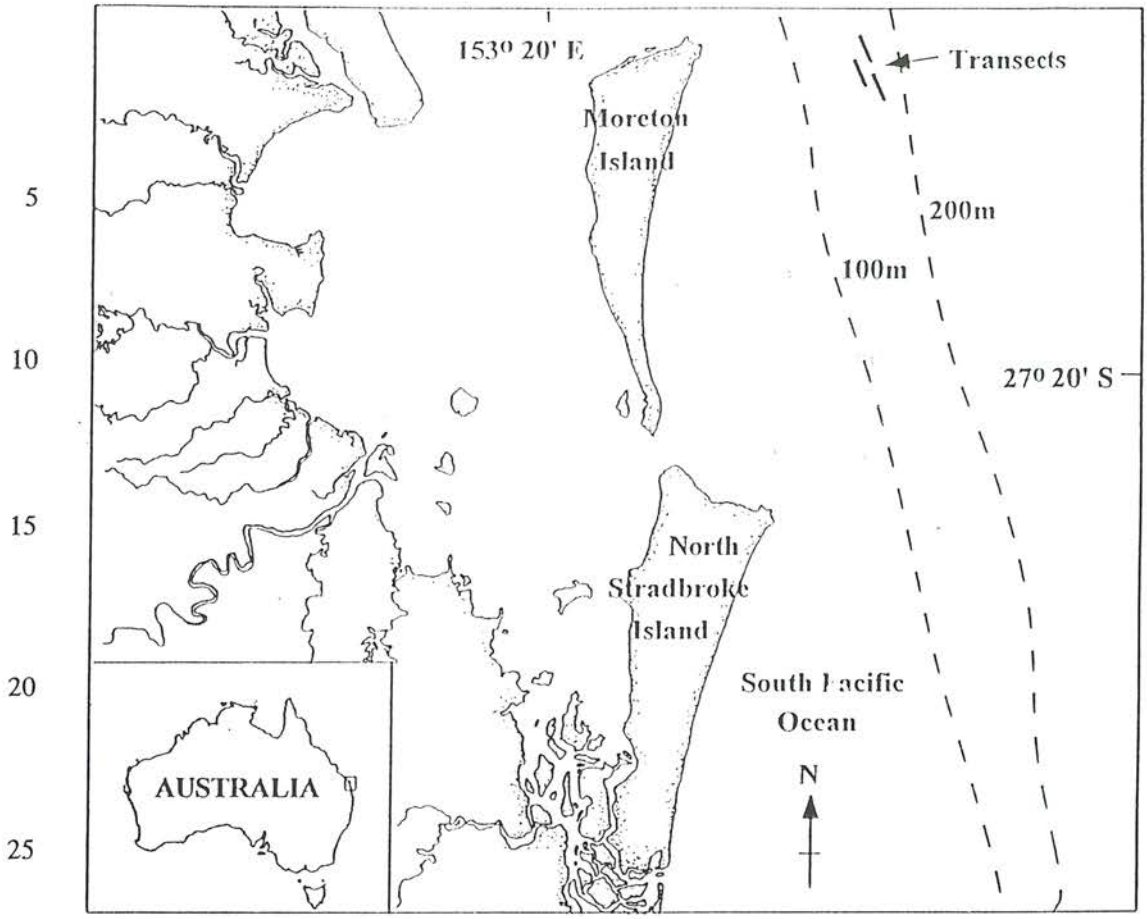

Figure 1. Map of south east Queensland coast showing the positions of the three transects off Moreton Island that were sampled every 72 hours for two complete lunar cycles.

Transects were not trawled in any particular direction (north or south). The only factors influencing the direction of each trawl was the vicinity of the next transect to be sampled, and the strength and direction of the prevailing current. Generally, trawling in the same direction as a strong current was avoided, as this tends to increase the chance of the otter-boards falling over and the nets failing to remain open. Current strength and direction of each shot was not measured, but the direction (north or south) each transect was trawled was recorded.

Processing samples

The catch for each shot was sorted on the vessel's sorting tray immediately after the nets were brought to the surface. For each transect sample, the prawns from all the three nets were pooled, placed into a plastic bag, labelled and frozen on board. Each morning the vessel returned to port with the three transect samples which were then transported to the laboratory. In the laboratory the abundance, size class distribution and mean size of all males and females in each sample was determined. Every prawn had its carapace length (CL) measured to the nearest millimetre and was allocated an approximate moult stage (soft or hard).

Three reproductive characteristics were used to determine change in female reproductive condition; 1) ovary weight, 2) ovary histology and 3) spermatophore insemination. Forty females from each transect sample had their ovaries extracted and weighed to the nearest 0.1 g. In samples with fewer than 40 females, all females had their ovaries dissected out and weighed. Ovarian histological sections were prepared, and the insemination status was determined, for every female. Exceptions were made for samples with large numbers of females. In such cases the number of females was limited to a maximum of 100 per transect sample.

Ovarian tissue used in the histological sections was dissected from the region of the first abdominal somite. The tissue was preserved in 4% (v/v) formaldehyde and later embedded in paraffin, sectioned at 6 μm, and stained with haematoxylin and eosin. A description of histological ovarian development in *P. plebejus* (Courtney *et al.* 1995) was used to stage development in each female. The following post-vitellogenic stages have been shown to occur within a few hours of spawning in another penaeid (Anderson *et al.* 1984) and were therefore assumed to be indicative of imminent spawning:

1) ripe oocytes (also known as the cortical specialisation phase, oocytes have peripheral bodies present). Occurs approximately 90 hours prior to spawning.
2) oocytes in germinal vesicle breakdown (GVBD, characterised by the presence of peripheral bodies in the oocyte but the nucleus is no longer present). This stage is completed approximately 24 hours prior to spawning.
3) ovulation stage (oocytes with peripheral bodies, but no nucleus or follicle cells). Occurs within four hours of spawning.

Females in these stages were pooled and the proportion in each transect sample determined.

Statistical design

Each lunar month was partitioned into four separate phases which were considered as experimental treatments; 1) new moon (± three days), 2) half moon rising to full (± three days), 3) full moon (± three days) and 4) half moon falling to a new moon (± three days). The two lunar months and the three transect sampling times were also considered as treatments. Analysis of variance was used to test whether catch rates, sex ratios, incidence of soft (recently moulted) individuals, incidence of spermatophore insemination and incidence of mature stage females were independent of the above treatments. Prior to ANOVA, percentages and data expressed as a proportion were arcsine transformed if their values were < 30% (0.3) or > 70% (0.7) (Sokal and Rohlf 1981). An earlier study (Courtney *et al.* 1995) found that ovary weight in *P. plebejus* was not independent of carapace length. Therefore, in order to determine if ovary weight was independent of the above treatments, an ANOVA was undertaken using carapace length as a covariate.

Results

Over the 59 day period, 21 sampling trips were planned to be undertaken. Two sampling trips (trips 6 and 13) were abandoned completely due to poor weather. A third trip (trip 8) only had one transect completed before the vessel returned to port, again due to bad weather. A total of 8210 eastern king prawns (both sexes) were captured during the two lunar-month period. Females and males made up 63.4% and 36.6% of the catch respectively. 83.1% of all females had ovaries prepared for histology, 55.1 % of which had ovaries dissected out and weighed to determine gonosomatic indices. The largest female captured had a carapace length of 73.1 mm and the largest male had a carapace length of 52.0 mm.

Sex Ratio and Abundance

Throughout the experiment, females were significantly more abundant than males (Figure 2). At the beginning of the first lunar month females were about three times more prevalent than males in the catch. As the lunar month progressed towards the full moon the sex ratio changed, males became relatively more abundant in catches and as such the ratio changed to approximately 1:1 at around the time of the full moon. As the moon waned, male numbers declined and the ratio returned to being strongly biased by females (around 0.3). These trends in the variation of the sex ratio

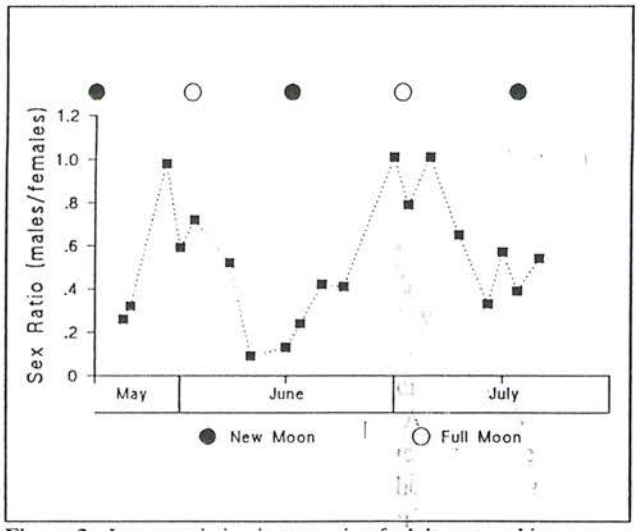

Figure 2. Lunar variation in sex ratio of adult eastern king prawns sampled off Moreton Island in 150m.

for adult eastern king prawns were apparent for both lunar months.

Although the catch rate of both males and females varied, it was the males that showed the most variation. Catch rates of both sexes peaked around the time of the full moon, however, the variability in sex ratio was due mainly to an increase in the relative abundance of males, rather than a decline in female abundance. This trend was consistent for both lunar months (Figure 3).

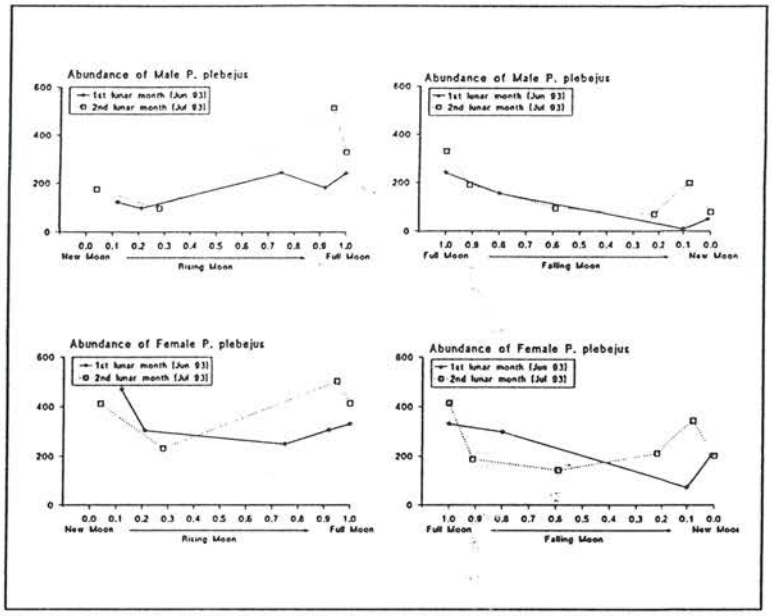

Figure 3. Catch rates of eastern king prawns at different stages of the lunar cycle.

Insemination and Moulting

Insemination rates were high, ranging from 94 to 98% throughout the sampling period (Figure 4). This suggested that, at any one moment, a high proportion of adult females in the population are inseminated.

Although there was no obvious change in the incidence of insemination, mating may still occur at certain lunar phases. The reasons that no obvious change was apparent may be due to:

a) a very high proportion of females

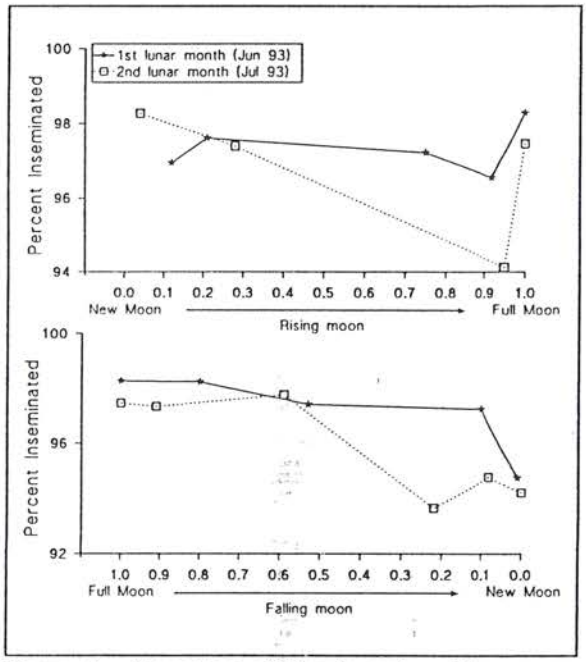

Figure 4. Percentage of female eastern king prawns inseminated at different stages of the lunar cycle.

being inseminated at any one time, and/or

b) females retain the spermatophore until they moult.

There were no obvious trends found in the incidence of recently moulted (soft) prawns (Figure 5). Generally, the incidence of soft cuticle prawns remained at less than 5%. These results suggest that moulting in adult eastern king prawns is independent of lunar phase.

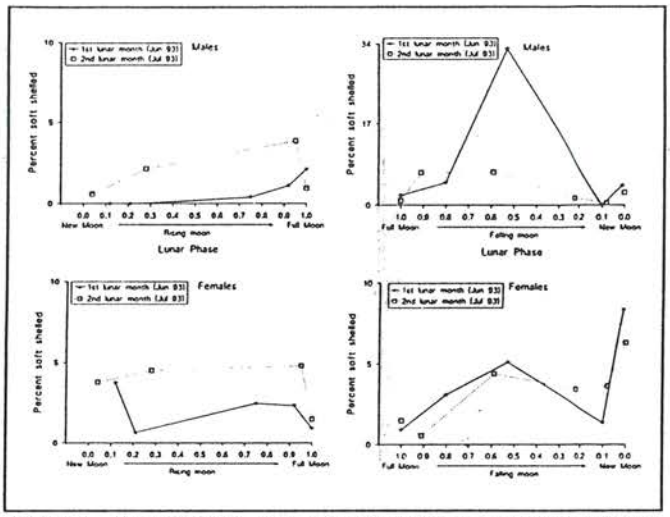

Figure 5. The incidence of *P. plebejus* with soft cuticles (indicating recent moulting) for different lunar phases.

Ovary Weight

ANOVA, using carapace length as the co-variate, indicated that the weight of the ovaries varied significantly between sampling trips ($p < 0.01$).

Figure 6 shows how ovary weight varies through the lunar cycle. The influence of prawn size has been considered by plotting the mean ovary weight for specific size classes of females. The size class groups were a) 40.1 mm to 45 mm, b) 45.1 mm to 50.0 mm, c) 50.1 mm to 55.0 mm and d) larger than 55.0 mm.

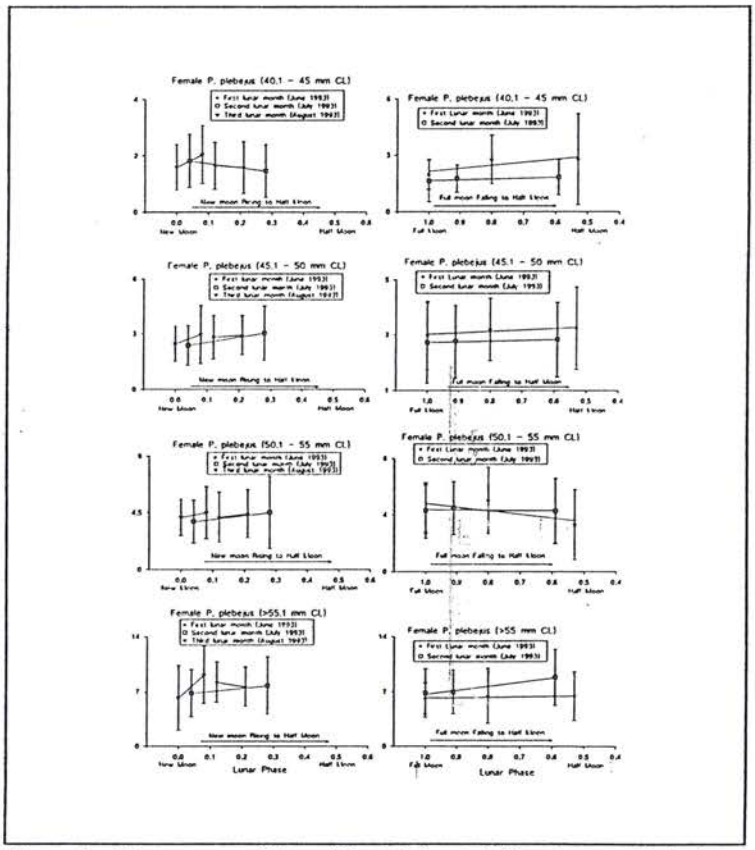

Figure 6. Mean ovary weights of *P. plebejus* at different stages of the lunar cycle. Results are presented for females in four different size classes.

Regressions were fitted in to the data demonstrate the trends in ovary weight with lunar phase. Ovary weights peaked twice for each lunar month the first midway through the waxing moon and the second mid way through the waning moon. There were some exceptions namely the 45.1 to 50 mm group and the >55 mm group which in some instances exhibited downward trends.

An analysis of variance performed on the regressions showed significant differences between ovary weights at different times of the lunar month for the 45.1 to 50mm and 50.1 to 55mm groups.

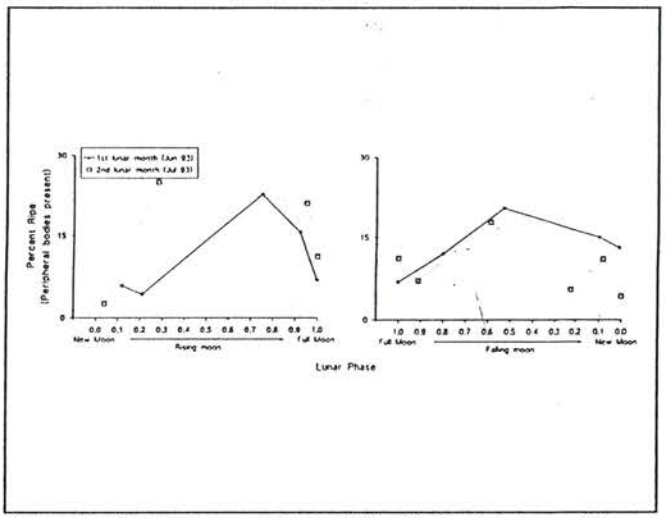

Figure 7. The percentage of "ripe" female *P. plebejus* at different stages of the lunar cycle.

Ovarian histology and spawning

For simplicity, the three histological groups (ripe, GVBD and ovulating) indicative of imminent spawning will be referred to herein as "ripe" females. The percentage ripe throughout the two-lunar-month period never exceeded 30% (Figure 7). However, there was a definite pattern in occurrence which was associated with specific lunar phases.

Two peaks in each lunar month can be discerned; the first about mid way between the new and full moon and the second mid way between the full and new moon. ANOVA confirmed that the percentage of ripe females was significantly ($p < 0.01$) different in the differences stages of the lunar cycle. There was no significant difference between the two lunar months.

When the percentage ripe are plotted on a continuous axis (Figure 8) and a sinusoidal cure imposed over distribution the pattern in the incidence of ripe females is made clearer. the two peaks each lunar month are clearly demonstrated.

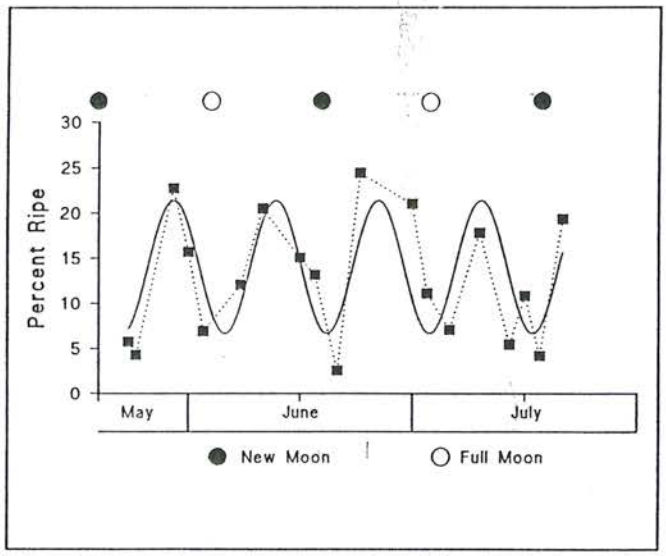

Figure 8. Lunar patterns in the percentage of ripe *P. plebejus*. Dotted line = actual sample percentages. Continuous line = fitted curve.

Discussion

Abundance and sex ratio show only one peak per lunar month. Abundance of females over the two months does not show the variability of the males and as such the peak in sex ratio appears to be attributed to more males being caught than fewer females being caught. We believe the increased catch rate of males at the time of full moon is a result of either reproductive responses or behavioural changes effecting catchability.

The high incidence of insemination 92% - 96% in females at all times of the month suggests that the change in male activity patterns is not due to reproduction. Further, insemination of females must occur post moult while the cuticle is soft. Yet no lunar patterns in the incidence of moulting in females were apparent. The likely scenario of insemination and moulting is when a female moults she is inseminated by a male immediately independent of environmental que's. Thus the increased captures of males are more likely related to factors other than reproduction.

A change in catchability in male *P. plebejus* may explain their increased incidence in trawls at around the full moon. One hypothesis is that males migrate away from the fishing area, making them less catchable, after the full moon whilst females stay on the fishing grounds. Another hypothesis is the change in catchability is related to feeding. Males may feed heavily at the full moon when increased tidal activity disturbs detritus on the sea floor. However a corresponding rise in catches during the new moon spring tides does not occur. Increased light penetration at the full moon may aid in locating food particles. The subsequent energy reserves attained over this time may allow them to survive until the next full moon with minimal activity. Females may need more energy to develop ovaries and as such must feed at a higher rate than the males thus making them susceptible to trawl gear throughout the whole month.

Ovary weight data for all four size groups suggests two reproductive peaks in one lunar month, the first midway through the waxing moon and the second mid way through the waning moon. The peak at the waxing moon appears to be larger than that of the waning moon. The peaks coincide with tides where the difference between the high and low water levels is small.

Histological results reinforces the ovary weight trends. Highest percentages of ripe females coincide with peaks in ovary weight. Histological data could be considered more reliable due to it being size-independent whist ovary weights are still partially size dependent despite attempts to remove the influence of carapace length through the generation of size-class groups.

Spawning has been shown to take place within 90 hours of corticle specialisation (stage4), about 24 hours after germinal vesicle breakdown (stage 5) and approximately four hours after ovulation (stage 6) (Clark and Phillia 1991). The highest percentage of mature animals was stage 4, 10.66 % of total females captured, thus we can assume that the actual spawning event would occur about four days after the peak.

Several authors have alluded to reproductive strategies of marine animals, particularly teleost fishes. Johannes 1978, Pressley 1980 surmise that lunar spawning in small reef fishes maximises flushing of pelagic eggs away from benthic predators. Pressley 1980 continues, moonlight assists a photopositive response in larvae to avoid benthic predators and increase planktonic food supply

in the water column aiding larval survival. Cloudsley - Thompson 1961 believes spawning synchrony within species occurs to ensure high concentrations of reproductive cells to increase the probability of fertilisation and species maintenance. Aggregate spawning also maximises genetic recombination especially if the animals all spawn at the same time. (Helfrick and allen 1957 in Johannes 1978)

Whilst eastern king prawns exhibit different spawning biology to fishes, mechanisms for the survival of pelagic larvae and recruitment as adults should be similar. Environmental conditions must effect larval dispersion and subsequent return to juvenile habitats. Spawning on neap tides may stop larvae from being washed to far away from suitable juvenile habitats. Similarly prevailing westerly winds create lee shores on the outside of the large sand islands endemic to the south east Queensland coast, further assisting in keeping larvae in the area.

The peaks in ovary weight and histology confer that reproduction of eastern king prawns is related to the lunar cycle. Further catchability of males and females appears to be related to lunar cycle whilst insemination of females and moult appear unrelated. The ramifications of this to the fishing industry and managers is unknown however benefits to researchers may be far reaching. Further studies could attempt to pin point the actual spawning event and investigate pelagic egg and larval dispersion and subsequent return to nursery areas. Further research will be aided by this increased knowledge and allow better experimental designs to be devised.

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