FACTORS AFFECTING THE REPRODUCTIVE PERFORMANCE OF CAPTIVE AND WILD BROODSTOCK PRAWNS
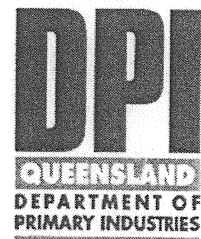
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PRIMARY INDUSTRIES

Project 92/51

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1. NON-TECHNICAL SUMMARY

92/51   FACTORS AFFECTING THE REPRODUCTIVE PERFORMANCE OF CAPTIVE AND WILD BROODSTOCK PRAWNS

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OBJECTIVES:

- To identify the causes of the seasonal variability in reproductive performance of wild-caught and captive broodstock.

- To develop a method for assessing the nutritional condition of broodstock and monitor the seasonal variation in prawns' diet and nutritional condition.

- To assess whether conditioning broodstock prawns in ponds prior to inducing spawning may be used to provide good quality spawners regardless of season or source of supply.

- Develop and evaluate formulations for a cost-effective artificial diet for the pre-spawning conditioning of broodstock prawns.

NON-TECHNICAL SUMMARY

In response to industry concerns about the variable performance and availability of broodstock prawns for hatchery production of postlarvae, this study was undertaken by a team of scientists from CSIRO Division of Fisheries and QDPI Bribie Island Aquaculture Research Centre. The study combined skills in the disciplines of ecology, nutrition and reproductive physiology to provide a strategic approach to understanding some of the factors affecting reproductive performance of broodstock and to be able to improve selection and management of broodstock to enhance reproductive performance

The main findings of the study were:

- Seasonal variation in the reproductive performance of broodstock has been clearly demonstrated. Reproductive performance improves from March to September, then declines by November. The relative effects of season, *per se*, and the age of broodstock were able to be separated. Age of the broodstock is the critical factor.

- Reproductive performance of broodstock improves with the age of the females, up to around 12 months old, thereafter performance declines.

- The nutritional condition of broodstock prawns has a major impact on their reproductive performance.

- Variations in the biochemical composition of tissues and eggs suggest that nutritional condition of broodstock prawns is not a major factor influencing the seasonal variability in reproductive performance.

- No non-invasive technique has been found that adequately assesses the nutritional condition of individual broodstock prawns with ripening ovaries. Assessment of the nutritional condition of males may be useful in determining the nutritional condition of the breeding population as a whole.

- Artificial broodstock diets were developed that enhanced reproductive performance from second and third spawnings but overall the performance was less than that achieved with a mixture of fresh frozen marine invertebrates.

- Results suggest a strong effect of dietary essential highly unsaturated fatty acids on improved reproductive performance. It appears that there is potential for the development of a specific diet for broodstock prawns but further work on this aspect is required.

- Six weeks of pond conditioning of *Penaeus monodon* had little effect on reproductive output of wild-caught broodstock, however holding prawns in the pond for 12 weeks resulted in a halving of reproductive output.

- In this study the reproductive performance of pond-reared *P. monodon* broodstock was poor compared with that of wild-caught spawners.

KEYWORDS:

broodstock, maturation, reproductive performance, nutritional condition

2. BACKGROUND

Prawn aquaculture continues to grow in Australia, with *Penaeus monodon* the predominant species. In the past 5 years farmed prawn production has increased from 100 t to 1600 t ($25M) in 1994/95. Continuing expansion of the industry could see substantial increases in both pond area and spawner demand in the next few years. The reliable supply of quality spawners, and therefore seedstock, remains an impediment to the efficient growth of the industry.

In Australia, as in other countries culturing *P. monodon*, the majority of the spawners are captured from the wild. The distribution of *P. monodon* includes most of Australia's northern waters, but the species is rarely found in commercial numbers and is not targeted by the prawn trawl industry. Currently the aquaculture industry relies on a small number of specialised spawner collectors who trawl the few areas where spawners are available and which have reasonably accessible airport facilities to enable rapid transport to the hatcheries. Two collectors currently supply 80% of the 3000 wild-caught spawners used in the industry each year.

Spawners captured from the wild vary in reproductive performance and availability at different times of year. Australian hatchery demand for spawners is focussed on pulse farm demand in early spring from single crop temperate farms, with a lesser demand at other times of year from farms capable of year-round stocking. The demand for postlarvae coincides with lower spawner availability from the wild and an observed lower reproductive performance of these spawners.

Hatchery operators both in Australia and overseas use wild-caught spawners in preference to pond-reared spawners because of the observed superior quality in terms of eggs and larvae produced. The domestication (closing the reproductive cycle in captivity) of *P. monodon* is possible, but not commercially proven at present. Small scale (confidential) trials by key hatcheries have found low spawning rates and lower larval survival for pond spawners (Macarthur Consulting, 1995). High mortalities of postlarvae in ponds have occurred following periods when wild broodstock have been in short supply and hatcheries have been forced to use the same spawners for extended periods - there appears to be a reduction in the quality of eggs and larvae with successive spawns from the same spawner.

The research approach in this project was to investigate some fundamental causes for seasonal variability in spawner performance, to investigate strategies for pond rearing of broodstock to reduce the need for consistent wild-capture of broodstock, and to develop and evaluate a cost-effective artificial diet to enhance spawner performance.

3. NEED

For most of the year there is an apparent shortfall in prawn supplies on the domestic market, due to the seasonality of wild catches, such that increases in farmed prawn production can be accommodated. However a disproportionate share of the Australian farmed prawn harvest is presently being forced onto the domestic market during the February to June period, when supplies of wild-caught prawns are greatest. Consequently returns for fishers and prawn farmers alike are being affected by lower prices during this period.

The seasonal periodicity in farmed prawn harvests is almost entirely due to seasonal changes in condition and availability of spawners to the prawn farming industry. In 1991 for example the peak demand for postlarvae from hatcheries was during the August-September period. However, because the condition of available wild spawners was poor, hatcheries could not achieve the desired volume of production until November. Because many farmers were forced to stock their ponds late they were marketing their prawns in direct competition with trawl caught prawns during the period of highest catches.

Since 1991 the demand for wild spawners has increased to the point where difficulty in obtaining postlarvae is one of the major problems of the industry. The need for alternative sources of broodstock is acute. The industry would benefit greatly if wild broodstock or on-grown farmed prawns could be successfully held and conditioned in ponds as a source of broodstock during times of low availability from the wild. Further, pond-rearing of broodstock enables selective breeding programs to be undertaken leading to the true domestication of farmed prawns.

4. OBJECTIVES

- To identify the causes of the seasonal variability in reproductive performance of wild-caught and captive broodstock.

- To develop a method for assessing the nutritional condition of broodstock and monitor the seasonal variation in prawns' diet and nutritional condition.

- To assess whether conditioning broodstock prawns in ponds prior to inducing spawning may be used to provide good quality spawners regardless of season or source of supply.

- Develop and evaluate formulations for a cost-effective artificial diet for the pre-spawning conditioning of broodstock prawns.

5. TECHNICAL REPORT - PROJECT IN DETAIL

The project was split into several components: an assessment of seasonal variability in reproductive performance of wild-caught broodstock, an assessment of the nutritional condition of broodstock and the biochemical composition of resultant eggs and larvae, development and testing of broodstock conditioning diets, and an assessment of the spawning performance of pond-reared *P. monodon* broodstock.

5.1 SEASONAL VARIABILITY IN REPRODUCTIVE PERFORMANCE OF WILD-CAUGHT BROODSTOCK.

- The project was successful in identifying the major factors causing variability in the reproductive performance of wild-caught *P. semisulcatus*. There was a seasonal influence and also an influence of the age of the broodstock. The results with *P. monodon* were less clear but the age factor appears to apply to this species as well.

Two species were used to assess seasonal variability in broodstock reproductive performance: *Penaeus semisulcatus* and *P. monodon*. While *P. semisulcatus* is not a commercially farmed species in Australia, we were fortunate to have a detailed knowledge of the seasonal reproductive dynamics and age composition of a *P. semisulcatus* wild population. Therefore, for this species we could select appropriate experimental broodstock to test precisely the effects of inherent temporal reproductive patterns and spawner age on the reproductive performance of captive broodstock. The similarities in the life histories of tropical penaeids make it possible to view this part of the study as a proxy for the general tropical penaeid case. Interpretation of the results of the parallel *P. monodon* study, where such a detailed understanding of the reproductive ecology was not available, is strengthened by this approach.

5.1.1. SEASONAL AND AGE VARIABILITY IN THE REPRODUCTIVE PERFORMANCE OF *P. SEMISULCATUS* BROODSTOCK (CSIRO CLEVELAND)

INTRODUCTION

Hatcheries require quality spawnings from broodstock almost year-round. Knowledge of the natural reproductive patterns in the wild prawn stocks, from which hatchery broodstock are often drawn, may provide a further basis for selection of broodstock to achieve best spawner performance. The reproductive performance of penaeid broodstock in hatcheries has been shown to be affected by a range of variables including eyestalk ablation, nutrition, light intensity and quality, photoperiod, salinity and substrate type (for detailed review see Crocos and Coman 1996). The effects of season and age on the reproductive performance of broodstock have not been well documented.

Penaeid prawns have complex seasonal patterns of reproduction, which vary both within and between species, probably in relation to species, location and variable environmental conditions (Penn 1980, Crocos 1987a, b, Crocos and van der Velde 1995, for review see Dall *et al.* 1990). Typically, tropical penaeids live for about 18

months and have two main spawning periods: when the females reach first maturity at about 6 months, and again at about 12 months old (Garcia 1985, 1988, Crocos 1987a,b, Staples and Rothlisberg 1990, Dall *et al.* 1990). The seasonal variability in spawning patterns of *Penaeus latisulcatus* and *P. esculentus* has been described (Penn 1980, Crocos 1987b). The subsequent recruitment from these spawnings results in a complex size and age composition of the population at any one time, as a consequence of the overlapping generations.

This bimodal seasonal spawning pattern has been described by Crocos and van der Velde (1995) in a *P. semisulcatus* population in Albatross Bay. Seasonal peaks in spawning and egg production occurred in both autumn and spring. The spawners in autumn, mostly 6 month old females, were derived from the previous spring-spawned cohort which recruited to this population during summer. In contrast, the spawners in spring consisted of two age groups; the remainder of the main cohort from the summer recruitment which were now 12 months old, and a 6 month-old cohort which was derived from the autumn spawning.

The approach in this study was to systematically sample females of both cohorts taken from the population throughout the year to test both the effects of seasonality and age in reproductive performance. This enabled us to select experimental broodstock to compare the effects of season and broodstock age on reproductive performance in a hatchery situation.

METHODS

EXPERIMENTAL BROODSTOCK

Penaeus semisulcatus males and females were sampled from a population in Albatross Bay (12° 48' S, 141° 32' E), Gulf of Carpentaria, Australia. This discrete population was the focus of a comprehensive 6 year study of all life history stages (Crocos and van der Velde 1995, Vance *et al.* 1996), hence its recruitment patterns, and its size and age composition in each season were well known.

Experimental broodstock were held in circular fibreglass tanks (3.6 m diameter, 0.9 m water depth) with a 10 cm deep substrate of white sand into which the prawns could bury and thus reduce stress. A sub-sand circulation system prevented the substrate from becoming anaerobic. Filtered seawater (20 μm) flowed through the tanks at an exchange rate of at least 100% per day. Water salinity was 34 ± 2‰ and temperature was maintained at 28 ± 0.5°C. Photoperiod was set at 14 h light and 10 h dark. Tank covers were used to reduce daytime light intensity to 4 to 8 μW cm^{-2} at the substrate surface.

For each trial, two replicate tanks, each with 15 females and 15 males were used to assess reproductive performance. The stocking density was 3 prawns m^{-2}. The prawns were individually marked in each tank by coded clipping of uropods. Prawns were fed daily to excess on the standard CSIRO fresh-frozen marine invertebrate diet for broodstock (FFMI-C), consisting of chopped prawns (*Metapenaeus bennettae*), bivalves (*Plebodonax deltoides*) and squid (*Loligo spp*) (Rothlisberg *et al.* 1991). Prawns were acclimated to the experimental conditions for at least 1 week before the females were unilaterally eyestalk ablated at the beginning of a trial.

REPRODUCTIVE PERFORMANCE TRIALS

A total of 17 reproductive performance trials, each of about 50 d duration, were carried out over the 33 months of the study from March 1992 to December 1994 (Table 1). Two types of trial were carried out: Group A designed to assess reproductive performance of prawns of known but increasing age at different times of year; and Group B designed to assess variability in reproductive performance of prawns of different ages at the same time of year.

TABLE 1

Penaeus semisulcatus. Summary of trials to assess temporal effects on the reproductive performance of *P. semisulcatus* broodstock. (CL = prawn carapace length)

Trial Group	Month	Age at start of trial (months)	Number of trials in each year ('92, '93, '94)	Total number of trials	Total number of females	Mean size of females (mm CL ± SE)
A	March	6	0, 1, 1	2	60	36.5 ± 0.23
	May	8	1, 1, 1	3	74	37.8 ± 0.30
	July	10	0, 1, 0	1	30	40.1 ± 0.28
	September *	12	2 1, 1	4	110	42.6 ± 0.29
	November *	14.	1, 1, 1	3	82	44.6 ± 0.32
B	September	6	0, 1, 1	2	45	35.9 ± 0.90
	November	8	0, 1, 1	2	41	37.7 ± 0.24

*   data from September and November trials in Group A were also
     used for comparison in Group B trials.

For the Group A trials, the strategy was to assess the reproductive performance of the 'spring cohort' (i.e. prawns spawned in spring, recruiting to the population as sub-adults in summer, reaching first maturity in autumn - Crocos and van der Velde 1995) at successive samplings from the time of first maturity in autumn, through winter and into late spring. Hence the first trial began with prawns selected from the 'spring cohort' in March. These prawns were about 6 months old at this time. Based on the known size and age composition of the whole population (Crocos and van der Velde 1995), successive trials using further samplings of progressively larger and older broodstock from this cohort were carried out each 2 months. Thus prawns for the May, July, September and November trials were 8, 10, 12 and 14 months old, respectively. This series of five two-monthly trials was repeated in each of 2 years, 1992 and 1993, with additional trials in 1994.

The Group B trials were designed to separate the effects of season and age. The strategy was to assess the reproductive performance of the 'autumn cohort' (spawned in autumn, reached first maturity in spring) sampled in September when they were 6 months old, and again in November when they were 8 months old.

ASSESSMENT OF REPRODUCTIVE PERFORMANCE

Reproductive performance was assessed over a range of standardised parameters measured at each successive stage of the maturation and spawning process. Rates of survival, maturation (females reaching ovary stage IV), spawning (actual spawns), egg production, nauplii production and protozoeal production were calculated for individual prawns and expressed as the mean number for a treatment per female per 30 prawn-days (1 prawn-day = 1 day's survival by 1 prawn, e.g. a typical value for total female prawn-days per tank would be 15 females x 50 days = 750 prawn-days). This approach provided standard measures of reproductive potential for direct comparisons of performance between treatments, which were unbiased by slight variations in the number of females in a tank or by slight differences in the duration of a trial.

Females were examined nightly for ovarian maturation by shining a light beam through the dorsal exoskeleton. Females at ovary stage IV (ready to spawn) (Crocos and Kerr 1983) were transferred to individual 90 L screened (142 μm) flow-through circular spawning tanks and allowed to spawn; spawned females were returned to the maturation tanks next morning. For each spawning, the eggs in four 250 ml subsamples from the spawning tank were counted to estimate total egg numbers. Eggs were allowed to hatch in the spawning tank and 4 subsamples were taken 2 to 3 h after first hatching to estimate the total number of nauplii. To quantify larval survival and metamorphosis, three subsamples of 300 nauplii from each spawning were reared without food in 3L glass tubes held in a controlled-environment cabinet at 28 ± 0.5°C for another 62 h, by which time they had reached the first protozoeal stage.

For the Group A analyses, the data from the three years of the study were combined into five seasonal categories (March, May, July, September, November). Estimates of variance were based on the individual variability within each treatment (month). Differences in reproductive performance among the five categories were compared by analysis of variance using the SAS (SAS Institute, Inc) software. After examining the data for homogeneity of variance by the F-test, differences among seasonal categories were tested using least significant difference (LSD) pairwise comparisons. For the Group B analyses, a similar procedure was used to test for differences in the reproductive performance of the two age categories in the same season.

RESULTS

GROUP A: REPRODUCTIVE PERFORMANCE OF A SINGLE COHORT THROUGH THE YEAR

BROODSTOCK SURVIVAL

Survival of broodstock was consistently high at each time of year (Fig. 1a). In the March and May trials, survival was more than 95% of the possible prawn-days, and more than 90% in the July, September and November trials; these differences were not significant (P> 0.05).

SPAWNING

Six month old broodstock sampled in March gave the lowest mean spawning rate (0.82 spawns per female per month) (Fig. 1b). In May the spawning rate (1.07) was significantly higher than in March, then declined to an intermediate level in July (0.91). The highest spawning rate was in September (1.47), then declined again in November to the previous level (0.95). Overall, the spawning rate tended to increase from March to a peak in September, then decreased by November.

EGG PRODUCTION

Egg production is a function of both the spawning rate and the number of eggs per spawning. As the size of females increased from 36.5 mm CL in March to 44.6 mm CL in November (Table 1), the mean number of eggs per spawning increased from 38,560 in March to 53,109 in November (Fig. 1c). Even though females became larger through the year to November (Table 1), and the larger females would be expected to produce more eggs per spawning (Crocos 1987a), the actual number of eggs released per spawning did not increase significantly after July (Fig. 1c).

Total egg production followed the same seasonal trend as spawning rate, and increased from a minimum of 32,500 eggs per female per month in March to a peak of 68,300 in September, then declined to 51,200 in November (Fig. 1d). The greatest contribution to the peak egg production in September was made by the higher spawning rate at that time. Despite the slightly higher number of eggs per spawn in November, the associated low spawning rate reduced the total egg production to significantly less than the September value.

EGG HATCHING

The mean hatch rates from July to November (range 52.5% to 61%) were significantly higher than in March (41.3%) (Fig. 1e).

NAUPLII PRODUCTION

The naupliar production rate (NPR), a function of egg production and hatching rate, showed a significant seasonal trend with the lowest value in March (10,700 nauplii per female per month) increasing to a peak in September (36,500), then declining in November (25,400) (Fig. 1f). The lower hatch rate for March depressed the value for the NPR, and further contributed to the trend of lower performance in March which was established largely by the low spawning rate in this month (Fig. 1b).

PROTOZOEAL PRODUCTION

The proportion of nauplii successfully metamorphosing to the protozoeal stage did not differ significantly among months, and varied from 53% in March to 40% in November (Fig. 1g). The protozoeal production rate (PPR) represents the sum of all the various effects at previous stages in the reproductive process. The PPR showed a significant seasonal trend, with lowest value of 5,400 protozoeae per female per month in March, increasing to 10,800 in May, 11,100 in July to a peak of 20,700 in September, then declining to 12,100 in November (Fig. 1h).

FIGURE 1.

Penaeus semisulcatus. Reproductive performance measures (mean and SE) for *P. semisulcatus* at different times of year and age: (a) survival of broodstock females (percent of total prawn-days survived), (b) spawning rate (number of spawns per female per 30 prawn-days), (c) mean number of eggs released per female per spawning, (d) egg production rate (number of eggs per female per 30 prawn-days), (e) hatch (total number of nauplii /total number of eggs, x 100), (f) naupliar production rate (number of nauplii produced per female per 30 prawn-days), (g) metamorphosis (percent nauplii surviving to protozoeae), (h) protozoeal production rate (number of protozoeae produced per female per 30 prawn-days). Within each plot, means with the same superscript are not significantly different (P > 0.05). Numbers of trials and replicates in each year, and numbers of females in each trial are given in Table 1. e.g. Mean values for March represent 2 trials of 2 replicates each, N (females) = 60.
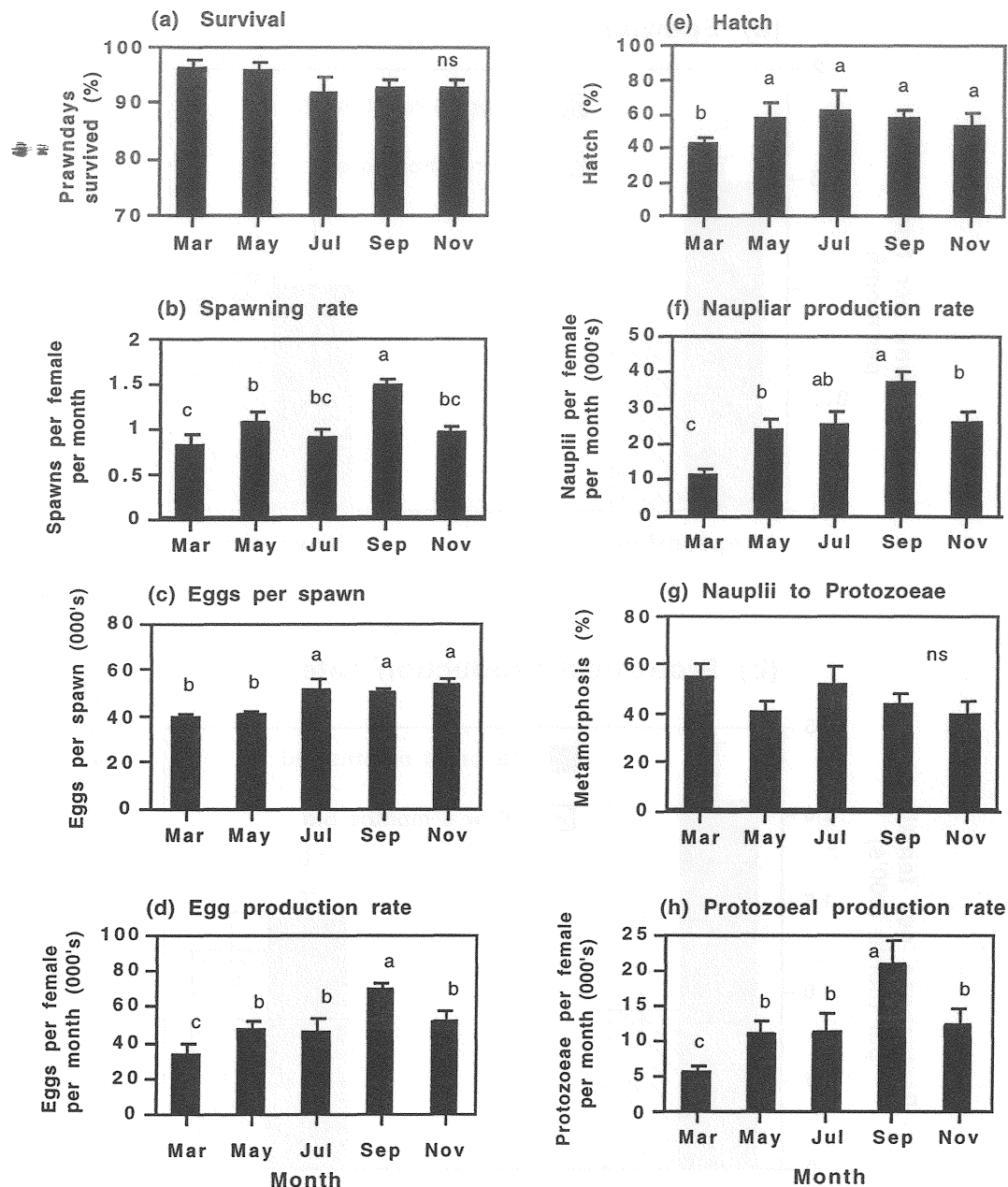
(a) Spawning rate

(b) Protozoeal production rate

Age class
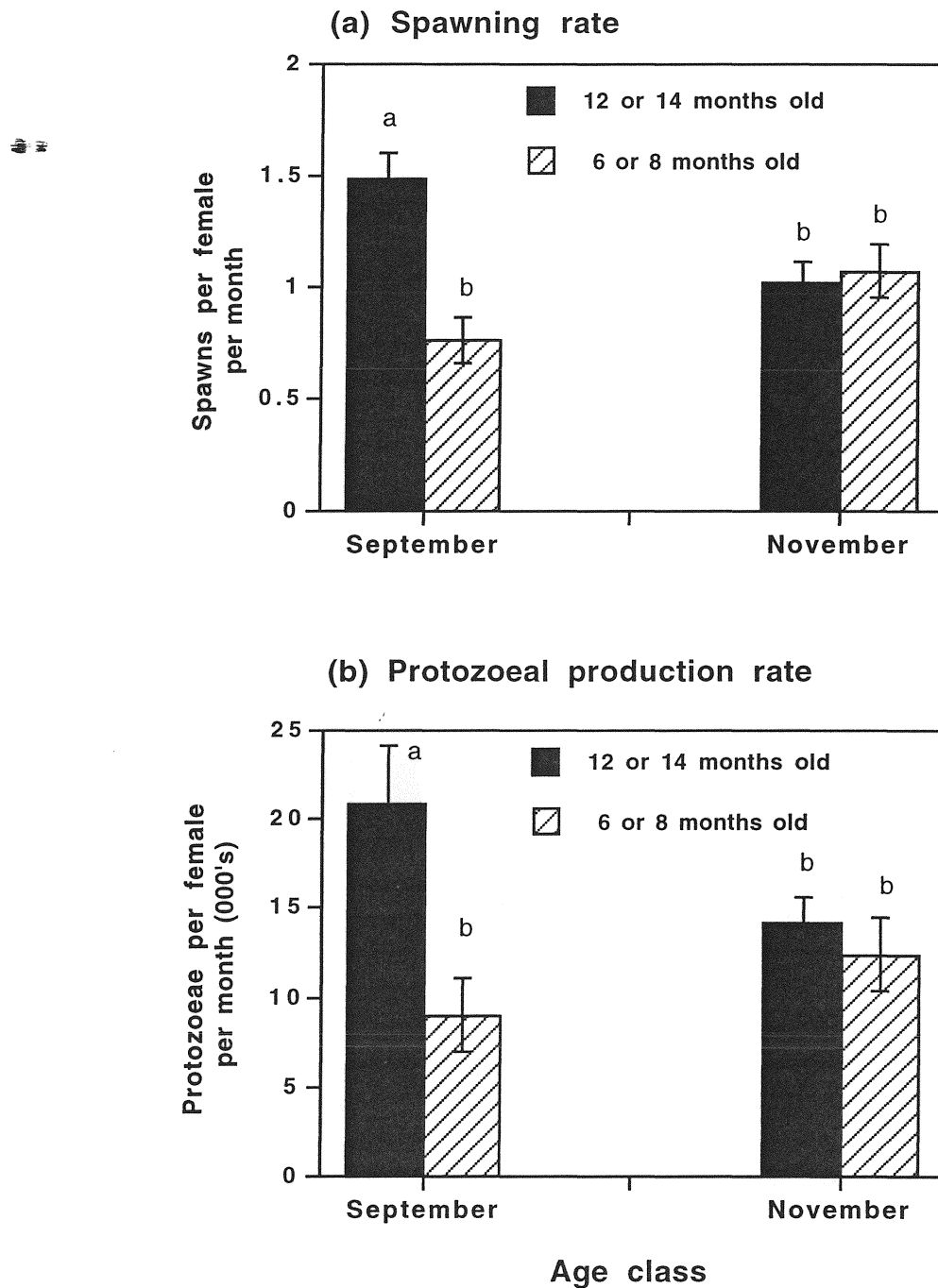
FIGURE 2.

Penaeus semisulcatus. Reproductive performance measures (mean and SE) for *P. semisulcatus* of different ages at the same times of year: Solid histograms represent 'spring cohort' females spawned in September at 12 months old, then another group from the same cohort two months later (14 months old) in November. Hatched histograms represent 'autumn cohort' females at 6 months old in September, then another group from the same cohort at 8 months old in November. (a) spawning rate (number of spawns per female per 30 prawn-days), (b) larval production rate (protozoeae produced per female per 30 prawn-days). Numbers of trials and numbers of females in each trial are given in Table 1. Within each plot, means annotated with the same letter are not significantly different (P > 0.05).

GROUP B: REPRODUCTIVE PERFORMANCE OF DIFFERENT-AGED COHORTS IN THE SAME SEASON

SPAWNING

In September, the spawning rate of 12-month-old females (1.47 spawns per female per month) was significantly higher than that for the 6-month-old females (0.76) (Fig. 2a). Two months later, in November, the spawning rate for the now 14-month-old females (1.01) had declined significantly . In contrast, the spawning performance in November of the now 8-month-old females was higher, but not significantly, than that of the 6-month-old females in September (0.7 to 1.07 spawns per female per month for 6 and 8 month-old respectively). In November, the spawning performance did not differ between the 14 month-old 'spring cohort' females and the 8 month-old 'autumn cohort' females (1.01 and 1.07 spawns per female per month, respectively).

PROTOZOEAL PRODUCTION

Differences in larval production between the 12-month-old and 6-month-old females in September followed the same pattern as for spawning performance. Thus, the PPR was significantly lower for the 6-month-old 'autumn cohort' females in September (9,070 protozoeae per female per month), than for the 12-month-old 'spring cohort' females (20,700) (Fig. 2b). By November, the PPR for the now 14-month-old 'spring cohort' females (14,050) did not differ significantly from that of the now 8-month-old 'autumn cohort' females (12,400).

DISCUSSION:

This study confirms the variability in *P. semisulcatus* reproductive performance at different times of year, and, through an analysis of the effects of the population age structure on broodstock performance, provides the basis to attribute this temporal variability to the effects of either season or broodstock age. The evaluation of reproductive performance at each stage of maturation and spawning makes it possible to assess the relative influence of each factor on overall performance.

SEASONAL CHANGE IN REPRODUCTIVE PERFORMANCE OF THE MAIN COHORT

SPAWNING

The high broodstock survival rates achieved in all months indicate that the prawns were in optimum health and that reproductive performance was unlikely to have been affected by nutritional or physical stresses related to the holding conditions. Rothlisberg *et al.* (1991) demonstrated that broodstock in a treatment with sub-optimal nutrition had both lower survival and lower spawning performance. High and consistent survival therefore provides the basis for controlled trials to assess effects of the specific factors under study.

The broodstock spawning rate increased from March until a peak in September, then declined by November (Fig. 1b). There was some indication of another minor peak in May, but this was not significant. These trends closely parallel the situation described for the wild population by Crocos and van der Velde (1995), where sub-adult prawns recruit to this offshore population over the summer, reach maturity and begin to spawn for the first time in autumn (March-May), at about 6 months old. In this wild population, spawning activity then declines slightly from May to

July before increasing to a major peak in September-October, when most prawns are around 12 months old. After October, the proportion of spawners in this population declines as the majority of the cohort reach 15 to 17 months old by December to February. Also, at this time natural mortality increases as the prawns become senescent (Somers and Kirkwood 1991). For the captive broodstock in the current study, the decline in the spawning rate observed after September suggests that the reproductive fitness of females beyond 12 months old declines. As well as being consistent with the wild stock pattern described above, such a decline is also suggested by Courtney et al. (1995), who reported that the largest females (>55 mm CL) in a wild population of Penaeus plebejus were reproductively senescent and did not contribute to egg production.

EGG AND LARVAL PRODUCTION

The determining factors for egg production are the spawning rate and the number of eggs produced per spawning. Despite the increasing size of the spawners in each subsequent seasonal trial, and the increasing nominal fecundity with size, the number of eggs produced per spawner did not increase after July. As a result the lowest egg production rate was in March. This increased to a maximum in September, largely as a consequence of the increasing spawning rate. Similarly, the lower spawning rate in November determined the lower egg production at this time.

In turn, the naupliar production rate (NPR) is a function of both egg production and the hatch rate; the lower hatch rate in March changed the overall seasonal pattern slightly, resulting in a relatively low NPR for March compared to the egg production pattern. Females spawning in March were just above the size at first maturity; one consequence of this may have been the lower hatch rate of eggs. Otherwise the NPR closely followed the egg production pattern.

Metamorphosis of nauplii to protozoeae was not significantly different between months, so the protozoeal production rate (PPR) was a reflection of the NPR. The PPR is the summation of the effects at each stage of the reproductive process. In this case it is apparent that the temporal pattern established by the spawning rate was only marginally modified by changes in performance at the subsequent stages, notably the effects of eggs per spawn and hatch rate in March. The overall seasonal pattern of reproductive output was therefore lowest in March, higher in May and July, significantly higher in September, followed by a significant decline in November.

AGE EFFECTS

The first part of this study used broodstock of known but increasing age at each time of year. It clearly demonstrates the changing pattern of reproductive performance at different times of year. While this approach provides a robust indication of the expected hatchery performance of broodstock sampled from the main cohort in this population at each time of year, the observed temporal pattern still cannot be attributed to the effects of either season or age. Two hypotheses may explain these performance differences. Firstly, reproductive performance may be higher in September because of favourable factors in the environmental history of the prawns during the preceding few months. For example, seasonal changes in the distribution and abundance of food organisms, or their nutritional quality, may result in better nutritional status for spawners in September. Also, nutrients and

diatom blooms normally start in spring. Such phenomena would likely have effects independent of broodstock age. Secondly, if age were the driving factor, then this would be manifested independently of season.

The possibility of an age effect was tested in the second part of this study. The spawning population during the peak spawning period in September consists of two distinct age classes, 6 and 12 months old (Crocos and van der Velde 1995), which can be separated on size and growth rate data (Somers and Kirkwood 1991). In the September trials, the 12-month-old females outperformed the 6-month-old females in both spawning rate and larval production, which suggested that the age of the broodstock might be a better performance predictor than time of year. However, by November, the performance of the now 14-month-old females had declined, while that of the now 8-month-old females increased. This was consistent with the increased performance demonstrated for 8-month-old females in May versus 6-month-old females in March. Therefore, the trend for the reproductive performance of broodstock to increase from the time of first maturity to about 12 months old was independent of seasonal effects. Hence broodstock age is a more appropriate predictor of performance than season.

Other reports of the effects of season or broodstock age on reproductive performance of penaeid broodstock are scant. Primavera (1978) reported that 5 month old *P. monodon* could mature and spawn after ablation, but produced poor quality larvae. Menasveta *et al.* (1994) evaluated the reproductive success of both large (> 120 g) and small (< 110 g) females for both wild-caught and pond-reared *P. monodon*. They found much higher maturation and spawning rates for the large females, which for the pond-reared group in this study were about 12 months old. Ages for the wild-caught females were unknown, but again the large-sized group performed better than the small-sized group. For pond-reared *P. monodon* broodstock, Niamadio and Kane (1993) reported an optimum age for best performance of at least 12 months (or >90 g). In the wild, Motoh (1981) found that *P. monodon* attained full maturity and spawning at 10 months old. The general case for wild populations of tropical penaeids is for the major spawning to occur at about 12 months of age (Garcia 1985, Crocos 1987a, Crocos and van der Velde 1995, for review see Dall *et al.* 1990).

STRATEGIES FOR HATCHERY BROODSTOCK SELECTION

The seasonal trends in reproductive performance observed in the captive broodstock in this study, which paralleled patterns in the wild population of *P. semisulcatus*, provide a guide to the expected performance of hatchery broodstock selected at each time of year from the major annual cohort of the wild stock. Since these seasonal patterns are common to most tropical penaeid species (Dall *et al.* 1990), it is reasonable to assume that these results may be used as a proxy for the general tropical penaeid case. Thus similar age constraints would be expected to affect the broodstock reproductive performance of other tropical penaeids, and the findings of this study may be used to optimise selection of broodstock for other farmed penaeid species and for *P. monodon* in particular. More specifically, if the ages of the prawns have been determined from known size and age structures in the wild population, it is desirable to select broodstock at around 12 months old for best hatchery performance.

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5.1.2.   TEMPORAL VARIATION IN EGG AND LARVAL PRODUCTIVITY OF *PENAEUS MONODON* BROODSTOCK (QDPI, BIARC)

INTRODUCTION

Despite advances in production techniques in prawn hatcheries, worldwide there is still a general reliance on wild-caught spawners to provide larvae for the culture of the black tiger prawn *Penaeus monodon*. Australian hatcheries used approximately 2,700 wild-caught *P. monodon* spawners in the year 1992-1993 (Lobegeiger 1994). Most of these prawns came from the north Queensland coastal region from Cairns to Townsville. As in other parts of the world, the availability of spawners from this area is variable (Marsden 1992; O'Sullivan 1994). Consequently, prawn hatcheries are sometimes unable to obtain spawners during periods of high demand. As well as this variation in the quantity of spawners available there is anecdotal evidence that the reproductive performance of spawners also varies throughout the year.

Over two years, a series of spawning trials was performed under controlled and replicated conditions to assess the spawning success of broodstock *P. monodon* from one location (Cook Bay) and quantify the temporal variation in the larval productivity.

MATERIALS AND METHODS

Prawns were collected from the wild on seven occasions between September 1992 and July 1994. All spawning trials were conducted at the Bribie Island Aquaculture Research Centre (BIARC). All prawns were captured in the same location (Cook Bay) by the same commercial operator (Cairns Live Prawns), however prawn size varied within each collection and the age of the spawners was not known.

Capture dates were: 26 to 31 August 1992; 29 October to 5 November 1992; 12 to 18 March 1993; 28 April 1993; 28 July 1993; 16 March 1994; 19 to 20 May 1994.

These dates were determined in part by the availability of the prawns since the unpredictable supply of broodstock made sampling at the planned regular intervals impossible.

Prawns were caught by beam trawl, air freighted to Brisbane in polyethylene bags with water and oxygen, and transported by road to BIARC. Upon arrival at the Centre, prawns were weighed and eye-tagged, and females were carapace-marked with nail polish for identification of moulted exuviae. Broodstock were maintained for 42 d in circular fiberglass maturation tanks (4.0m diameter, 0.8m water depth) at an average density of 1.75 prawns m^{-2} and a ratio of one male to two unilaterally eyestalk ablated females. Prawns were fed the standard BIARC fresh diet for broodstock of either squid or mussel, to excess, on alternate days (FFMI-B) at both 10:00 and 17:00 h daily. Seawater (salinity, 33‰ ; pH, 8; dissolved oxygen, 6 mg.L^{-1}) was filtered to 20 µm, heated to 28°C, and exchanged at 200% per day. Light intensity was 5 lux at the water surface, photoperiod was 14L:10D with a 20 min ramp period. Ovarian development was monitored daily using a submerged flashlight to reveal the shadow of the ovary on the dorsal exoskeleton. Mature females were spawned in 0.15 m^3 rectangular drums in 130 L tanks of seawater at 28 °C, filtered to 0.5 µm, and exchanged at 1,000% per day. Numbers of eggs and larvae were estimated by vigorously agitating the water in the spawning drums, then counting four 110 mL subsamples. Larval survival was estimated from known

numbers of larvae at the first nauplius stage (N1) which were left to develop in the spawning drum to the first protozoeal (Z1) stages when they were recounted.

Spawner survival was expressed as a percentage of the possible number of days (maximum 42) survived by each individual. Spawner survival, weight, spawns per prawn, percentage of females that spawned, percentage of females that spawned more than once, fecundity, hatch rate, and larval survival were all compared by analysis of variance (ANOVA).

Protozoeal production values were compared graphically to climatic conditions for the region. Mean monthly air temperature and total monthly rainfall were calculated from data obtained from the Australian Bureau of Meteorology. Values for mean monthly air temperatures were calculated by averaging the mean daily maximum and minimum temperatures for each month. Mean monthly surface water temperatures were calculated from measurements taken at weekly intervals by Cairns Live Prawns, Cairns, Australia.

RESULTS

SPAWNER WEIGHT AND SURVIVAL

The smallest female prawn used in the trials was 57 g in March 1994 and the largest was 246 g in July 1993 (Fig. 3a). The range of prawns that spawned successfully was 61.5 g (March 1993) to 208 g (July 1993). Spawner survival was generally very good, being a minimum of 82% in July 1993 and a maximum of 100% in March 1993 (Fig. 3b).

SPAWNING RATE

The maximum average number of spawns per ablated female was 3.1 ± 0.4 in May 1994, and the minimum was 1.3 ± 0.2 in March 1994 (Fig. 3c). The proportion of prawns that spawned after ablation varied from 91 % (10/11) in October 1992 to 68% (19/28) in March 1993 (Fig. 3d). Of those prawns that spawned successfully, the proportion that spawned again varied from 100% (10/10) in October 1992 and (24/24) in May 1994 to 60% (12/20) in March 1994 (Fig. 3e).

FECUNDITY

The number of eggs produced per spawn varied between trials, and was related to both spawner weight and spawn number (ANCOVA on pooled data, $P < 0.05$); the relation between fecundity and spawner weight was similar between trials (Fig. 3f).

EGG HATCH RATE

The average hatch rate of eggs ranged from 21.1 ± 4. 1 % in August 1992 to 66.5 ± 3.9% in May 1994 (Fig. 3g). Egg hatch rate was related to both spawner weight and spawn number (ANCOVA on pooled data, $p < 0.05$). A test for homogeneity of regressions again found no significant difference between slopes $(P > 0.05)$ between the various trials.

(a) Spawner weight

(b) Spawner survival

(c) Number of spawnings

(d) Spawn rate

(e) Multiple spawns

(f) Eggs per spawn

(g) Hatch

(h) Larval survival

FIGURE 3.

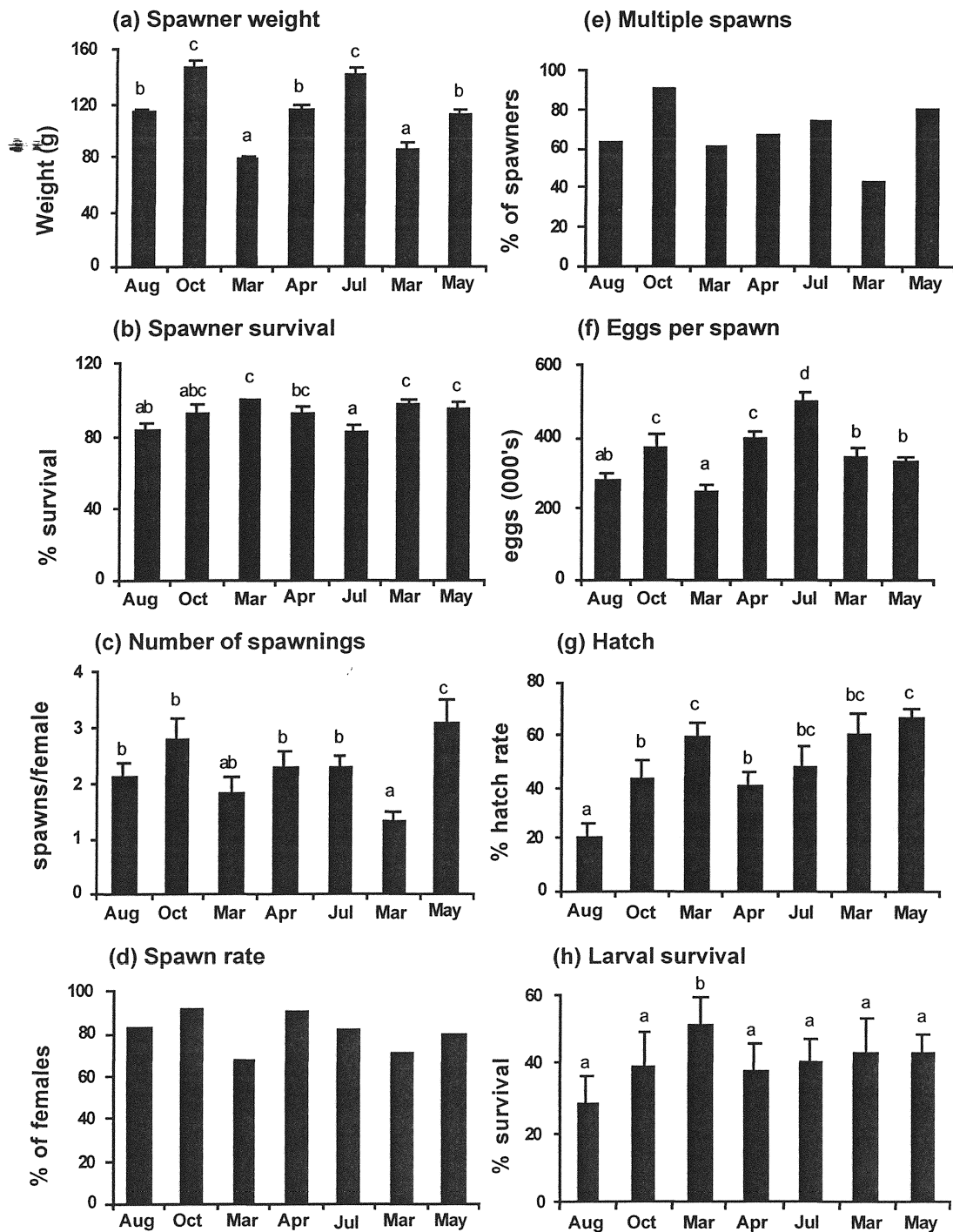*Penaeus monodon*. Seasonal variations in (a) spawner weight, (b) spawner survival,(c) number of spawns per prawn, (d) percentage of females that spawned, (e) percentage of females that spawned more than once, (f) number of eggs per spawn, (g) egg hatch rate, and (h) survival from nauplii to Z1. Mean and standard errors shown, like superscripts denote no significant difference between trials.

LARVAL SURVIVAL

Larval survival varied between trials (Fig. 3h). Survival was unrelated to spawner weight but was related to spawn number (ANCOVA, $P < 0.05$). In all trials larvae produced from a spawner's third spawn or later had a survival rate below 35%. A test for homogeneity of regressions found no significant difference between either slopes or intercepts ($P > 0.05$) between trials.

EGG AND LARVAL PRODUCTION PER PRAWN

The number of eggs and larvae produced per prawn for the seven trials were calculated (Fig. 4). Data were adjusted for spawner weight and spawn number to standardise the data to clarify the effect of season. (An ANCOVA showed the relation between egg output, hatch rate and larval survival, and spawner weight and spawn number). The adjusted total egg production was quite variable though it suggested low numbers of eggs per prawn in spring time (Aug - Oct) and significantly higher levels in autumn and winter (Fig 4a). The adjusted total nauplii production and total zoeal production followed a similar pattern to the total egg production with consistently low values for production in August and the highest production in May (Fig 4b, Fig 4c).

CLIMATIC CONDITIONS

The mean monthly air and surface water temperatures and total monthly rainfall for the Cairns/Cook Bay region varied seasonally (Fig 5). Superimposed on this graph is the average zoeal production per prawn (unadjusted) for the seven trials. The highest rates of zoeal production occurred as water temperature was falling, several months after a peak in monthly rainfall. There was a significant difference in egg hatch rate between April 1993 and May 1994 which may reflect the differences in the monsoonal rainfall between the years.

a. Adjusted total egg production

(Bar chart: x-axis labeled Aug, Oct, Mar, Apr, Jul, Mar, May; y-axis "eggs/prawn (000's)" from 400 to 1000. Bars with superscripts: Aug a (~520), Oct a (~510), Mar bc (~740), Apr bcd (~780), Jul c/d (~860), Mar d (~900), May ab (~650).)

b. Adjusted total Nauplii production

(Bar chart: x-axis labeled Aug, Oct, Mar, Apr, Jul, Mar, May; y-axis "Nauplii/prawn (000's)" from 100 to 500. Bars with superscripts: Aug a (~135), Oct ab (~240), Mar bc (~350), Apr bc (~350), Jul cd (~400), Mar bcd (~390), May d (~490).)

c. Adjusted total Zoea production

(Bar chart: x-axis labeled Aug, Oct, Mar, Apr, Jul, Mar, May; y-axis "Zoea/prawn (000's)" from 0 to 250. Bars with superscripts: Aug a (~57), Oct ab (~92), Mar bc (~183), Apr c (~183), Jul c (~192), Mar bc (~172), May c (~213).)
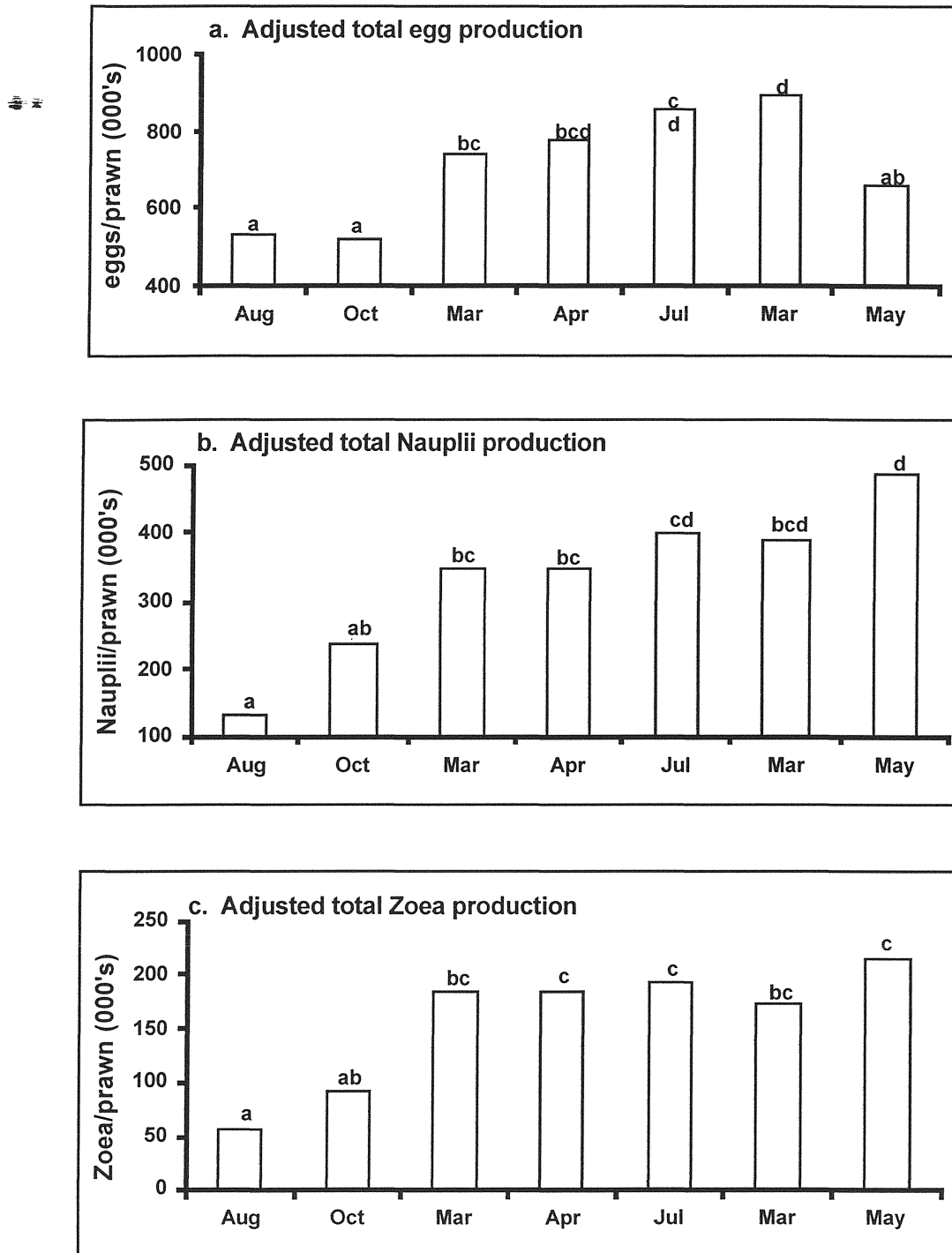
FIGURE 4.

Penaeus monodon. Mean total egg, nauplii and zoea production per prawn for the various trials, adjusted for spawner weight and spawn number. Like superscripts denote no significant difference between trials.
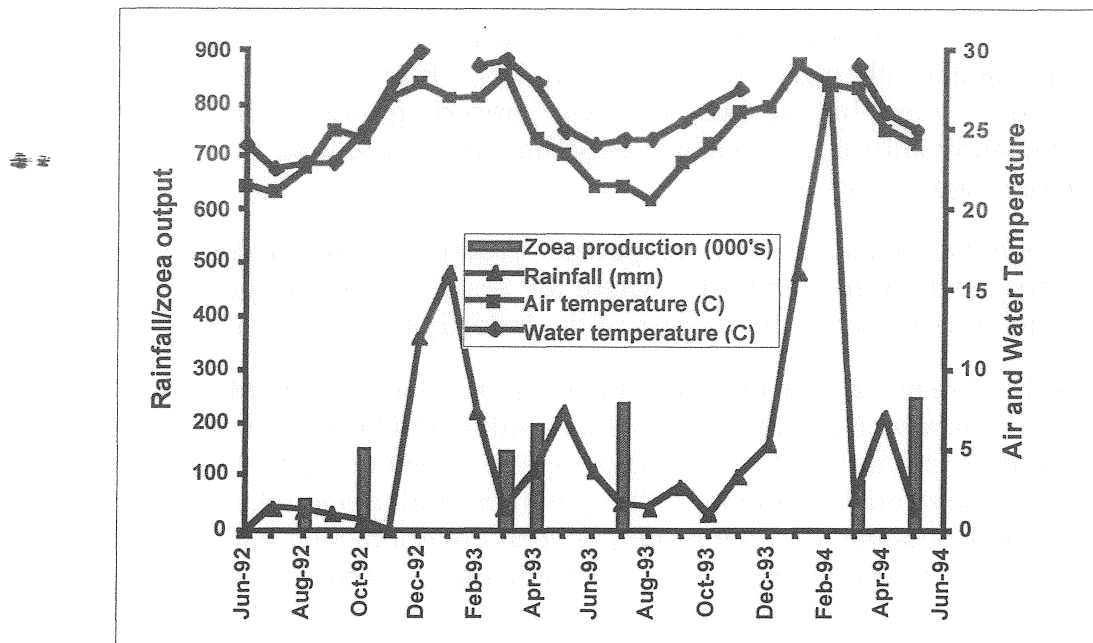
FIGURE 5

Penaeus monodon. Cairns climatic conditions (rainfall, water and air temperatures) vs mean total zoea production per prawn (unadjusted for prawn weight or spawn number) for the various trials.

DISCUSSION

Under standardised spawning and larval rearing conditions, the reproductive performance of *P. monodon* spawners captured from the wild varies throughout the year. Only part of the total variation in egg, nauplii and zoea output per prawn was explained by spawner weight and spawn number. There were still significant differences between trials after correction for prawn weight and spawn number. Between trials, mean fecundity per spawn varied by a factor of up to 2, hatch rate by up to 3.2, larval survival by up to 1.8, and the number of spawns per prawn by up to 2.4. Together these differences resulted in 4.3 times more zoea per prawn in May 1994 than in August 1992.

Causes of this temporal variation in reproductive performance are unknown, but are probably environmentally-induced and/or inherent (age rather than weight-related, see Section 5.1.1). Environmental factors that are likely to affect the reproductive performance of prawns include their nutritional condition, temperature and salinity conditions prior to capture (Harrison 1990).

Some studies have shown that age may be an important factor affecting the spawning performance of wild prawns (Crocos 1994), a factor that has also been established for pond-reared *P. monodon* (Primavera 1978). Age may also have an important influence on the reproductive performance of wild-caught *P. monodon* spawners used by prawn hatcheries; however, little is known about the population dynamics of this species in the wild to enable selection of broodstock based on age.

Climatic conditions were also examined over the sampling period to see if there was a relationship between either temperature or rainfall and reproductive success

(Fig. 5). Interpretation is limited by the low number of replicate trials and the short time span of the study. Water temperature may be a factor affecting larval production in *P. monodon*: survival of larvae from spawners captured in March 1993 was significantly higher compared with those from March 1994, while average temperatures were higher over the two preceding months in 1994. A more extensive study and/or manipulative experiments are required to investigate these leads more thoroughly.

The observed seasonal variation in spawner productivity is a major concern to the Australian prawn farming industry, where the unpredictable supply of postlarvae is seen as an impediment to the success of the industry (O'Sullivan 1994).

This study has shown that the reproductive performance of wild-caught, eyestalk ablated P. *monodon* is highly variable, both within and between trials. Although the cause for most of this variation is unknown, it may be due partly to seasonal effects influencing the overall condition of the spawners, and to age related factors. At this stage it is not possible to differentiate between environmental factors or age of the prawns, hence we cannot predict the reproductive performance of the broodstock based on the current information. Understanding the underlying causes of the seasonal differences in *P. monodon* spawner performance is important if prawn hatcheries are to improve the reliability of supply of larvae obtained from wild-caught prawns.

SUMMARY

- Reproductive performance of *P. monodon* varied throughout the year for spawners caught off Cairns, north Queensland. Some parameters (e.g. spawning frequency and larval survival) contributed more to this variation than others (e.g. fecundity and hatch rates).

- Reproductive performance could vary substantially from month to month and could not be linked to temperature or rainfall.

- Spawner size has an influence on reproductive performance. This may be due to age, but was not demonstrated in the study.

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5.1.3 SUMMARY: SEASONAL AND AGE VARIATION IN REPRODUCTIVE PERFORMANCE OF BROODSTOCK

Both the studies, on *P. monodon* and *P. semisulcatus,* have demonstrated significant variability in reproductive performance of broodstock at different times of the year.

For *P. monodon* the spawning performance was found to be highly variable at different times of the year, and between years, but this variability could not be related to any seasonal pattern or broodstock age effects. However, in the absence of information about the population size and age structure and reproductive patterns of *P. monodon* in the sampled region, it was not possible to select age-standardised broodstock to test these factors critically. The potentially mixed age structure in the experimental trials probably confounded identification of any seasonal or age effects.

For *P. semisulcatus*, the experimental broodstock were able to be selected from a population of known size and age composition, hence the experimental design allowed the effects of season and age to be clearly separated. For *P. semisulcatus* broodstock there was a trend for increasing reproductive performance from a low level in March to a peak in September, followed by a decline by November. It was further shown that this trend was due to the age of the broodstock rather than the particular season. Broodstock performed best at 12 months old. The similarity in the general life cycle biology of *P. monodon* and *P. semisulcatus* suggests that age may also be used as a predictor of reproductive performance in *P. monodon,* even though the actual age of best performance may differ from that of *P. semisulcatus*.

5.2 METHOD OF ASSESSMENT OF NUTRITIONAL CONDITION AND SEASONAL VARIABILITY IN THE NATURAL DIET AND NUTRITIONAL CONDITION OF BROODSTOCK PRAWNS

- Two good indicators of nutritional condition have been established. These are the blood lipid content and the blood refractive index. However, with broodstock prawns with developing ovaries, the sampling of blood is likely to cause unacceptable stress. The abdominal turgidity of prawn, measured by gently squeezing the abdomen, is related to nutritional condition but it is complicated by the subjectivity of the measurement and degree of ovarian development. These indices can contribute to the prediction of reproductive performance but other factors such as age must be taken into consideration.

- No non-invasive technique has been found that adequately assesses the nutritional condition of individual broodstock prawns with ripening ovaries. Assessment of the nutritional condition of males may be useful in determining the nutritional condition of the breeding population as a whole.

- The nutritional condition of broodstock prawns has major impact on their reproductive performance. The levels of the essential highly unsaturated fatty acids appear to be a major factor in influencing a better reproductive performance.

- Variations in the biochemical composition of tissues and eggs suggest that nutritional condition of broodstock prawns was not a major factor influencing the seasonal variability in reproductive performance of the prawns in this study.

5.2.1 METHOD FOR ASSESSING NUTRITIONAL CONDITION (CSIRO, CLEVELAND)

INTRODUCTION

The nutritional condition of a vertebrate can readily be assessed by its external appearance. However, this is not the case for invertebrates. For example, although body weight and volume decrease when brown tiger prawns, *Penaeus esculentus*, are starved, declines in condition are difficult to detect until after 2 or 3 weeks of starvation, because the metabolised tissues are replaced by water (Barclay *et al.* 1983, Dall and Smith 1987). To be useful, an index of nutritional condition of broodstock prawns should show a response to a period of sub-satiation feeding or a very short period of starvation and be measured with a non-invasive technique that would minimise any stress or trauma to the prawn.

There have been no previous reports on a non-invasive technique for the assessment of nutritional condition in crustaceans. Various laboratory-based biochemical indices of nutritional condition have been developed and used but which require the taking of blood samples (which can result in seriously injury to the prawn) or dissection of tissues or organs. As such these techniques are inappropriate to determine the condition of valuable prawns that are required as spawners in a hatchery.

We sought to develop a non-invasive technique to assess nutritional condition of broodstock prawns. We also decided that the technique should not involve the use of expensive scientific instruments which would not be considered appropriate or cost-effective in a prawn hatchery or on a trawler. From previous work on the changes in blood volume with starvation in *Penaeus esculentus* we had noticed a decrease in abdominal turgidity with starvation. The turgidity could be assessed by gently squeezing the abdomen of the prawn between the pads of the fingers and the palm of the hand. Since this method of assessment is very subjective, we needed to develop a more objective way of measuring it.

Another approach is to determine the nutritional condition of males of the species, captured at the same time as the broodstock, and to determine their nutritional condition using a non-lethal biochemical technique. The concentration of protein in the blood decreases in starved prawns and lobsters, due mostly to an increase in blood volume as muscle tissue is metabolised (Dall 1974, Smith and Dall 1982, Depledge and Bjerregard 1989). The moult cycle and probably ovarian development would affect blood protein concentration (Barlow and Ridgway 1969, Smith and Dall 1982). Therefore, if the blood protein concentration is to be used as an index of nutritional condition, the animals assessed must be males (free from the effects of ovarian development) and in the same stage in the moulting cycle. The concentration of protein in the blood is directly proportional to the refractive index of the blood, which is very easily measured using a serum refractometer (Smith and Dall 1982). Measurements of the blood refractive index therefore offer potential as a field method for measuring nutritional condition.

We investigated the changes in lipid content and blood refractive index induced by short periods of starvation in juvenile and adult *Penaeus semisulcatus* to assess their potential as indices of nutritional condition. These findings have been supported by similar work carried out as part of other projects with juvenile *P. esculentus* and postlarval *P. monodon*.

METHODS

ABDOMINAL TURGIDITY EXPERIMENT

A pair of dial calipers was modified to measure the distance a prawn's abdomen would be squeezed laterally by the application of a force of 5 g , 10 g or 15g over a surface area of 0.78 cm^2 . The pressure was applied by the compression of a fine spring and applied evenly to both sides of the first abdominal segment. The width of the abdomen was measured when the contact discs were just touching the sides of the prawn and again when the spring had been compressed 5 mm and 10 mm. The difference in the caliper readings was the distance the sides of the prawn had been pressed inwards.

For the abdominal turgidity study, two 10,000 L tanks were each stocked with 29 male *Penaeus semisulcatus*. The prawns were fed to excess for 2 weeks on the standard CSIRO broodstock diet of fresh frozen prawns, bivalves and squid (FFMI-C). An estimate of the daily food intake was made during this period. For the following 2 weeks one tank was provided with 2/3 of the estimated food intake while the other tank was fed *ad lib* as before. The prawns were then removed from the tank and their moult stage, weight and abdominal turgidity measured.

In a second experiment a 10,000 L tank was stocked with 16 adult male *P. semisulcatus* of 28 to 43 g (32 to 38 mm CL). For 8 weeks during February and March 1993, the prawns were fed to excess on diet FFMI-C until day 0, when feeding ceased. On days 0, 2 and 4, a sample of 5 or 6 prawns in intermoult was taken. The moult stage, weight and carapace length of each prawn was recorded. A sample of about 0.7 µL of blood was taken from the pericardial sinus with a disposable 1 mL syringe with a 0.4 x 13 mm needle. The blood refractive index (RI) was measured immediately, using a clinical serum refractometer (Atago Optical Works Co. Ltd). The prawn was placed on ice before being frozen at -50°C. The remaining blood from the sample was frozen at -50°C. A 3% sodium chloride solution was used to calibrate the refractometer by adjusting the refraction edge to coincide with the 1.334 graduation on the RI scale.

The digestive gland (DG) was dissected from each partially-frozen prawn. The digestive glands and the blood samples were freeze-dried before the lipids were extracted and quantified from a measured volume of blood and 0.3 g samples of the digestive glands. Lipids were extracted with chloroform:methanol (2:1) (Folch *et al.* 1957) and quantified gravimetrically by transferring the lipid extract to a tared HPLC vial insert, evaporating off the solvent under a stream of nitrogen, then drying it further in a vacuum desiccator for 1 h. The mass of lipid was then weighed to the nearest 0.01 mg.

The means and standard errors of blood RI, blood lipid and DG lipid were calculated for each period of starvation. A one-way analysis of variance was used to detect significant differences between the periods of starvation for each parameter, and contrasts were made between all possible pairs of days.

RESULTS

With the modified calipers, we were unable to measure any significant difference between the abdominal turgidity of the prawns fed *ad lib* and the prawns fed about two thirds of a satiation ration. However, our assessment of the turgidity by touch indicated that there was a consistent difference.

The mean blood refractive index (RI) of the starved prawns decreased significantly from 1.3588 to 1.3549 after 4 d (Table 2). However, the mean blood RI on day 2 (1.3577) did not differ significantly from the day 0 value.

The mean blood lipid content also declined significantly from 3.6 mg.mL^{-1} to 1.6 mg.mL^{-1} after 4 d starvation. As with the blood RI, the mean blood lipid content differed significantly between day 0 and day 4, but not between day 0 and day 2 (Table 2).

The mean lipid content of the digestive gland on day 0 (31.5 % of dry weight) did not differ significantly from day 2 (38.5 %) (Table 2). However, after 4 days of starvation, it was about 40 to 50% lower than the day 0 and day 2 values (18.2%).

TABLE 2.

Penaeus semisulcatus. Mean values ($\pm$ standard error) for blood refractive index (RI) and the lipid content of blood (blood lipid) and digestive gland (DG lipid) for *P. semisulcatus* (28 to 43 g wet weight) starved for different periods. Numbers with different superscripts are significantly different ($P < 0.05$); * n=4.

Days Starved	n	Blood RI	Blood lipid $(mg.ml^{-1})$	DG lipid (% dry wt)
0	5	1.3588 $\pm 0.0015^a$	3.6 $\pm 0.2^{*a}$	31.5 $\pm 2.8^a$
2	5	1.3577 $\pm 0.0007^{ab}$	3.1 $\pm 0.4^a$	38.5 $\pm 4.8^a$
4	6	1.3549 $\pm 0.0007^b$	1.6 $\pm 0.1^b$	18.2 $\pm 3.7^b$

DISCUSSION

The apparatus we designed and built to measure abdominal turgidity was not sensitive enough to detect the differences that we could sense through touch. The main failing of the apparatus was the measurement of the abdominal width when there was no pressure being applied to the sides of the prawn. This measurement was inexact whereas the measurement when 5 g or 10 g of pressure were being applied was much more reproducible.

In the second experiment with *P. semisulcatus*, blood RI and blood lipid content declined significantly during the 4 d of starvation. These findings suggest that blood RI and lipid content show potential as indices of nutritional condition for adult prawns. The lipid content of the digestive gland increased during the first 2 d of starvation (although the increase was not significant) and decreased during the next 2 d. This pattern of change indicates that the digestive gland lipid content may not be an ideal indicator of nutritional condition.

Hatchery operators may be trained to assess the nutritional condition of prawns through touch and use that subjective assessment along with other cues that they may believe indicate good health condition to select their broodstock. However, the abdominal turgidity of females will alter irrespective of nutritional condition as the ovaries develop. An alternative approach would be to assess the condition of male prawns that are captured at the same time and place as the females. It would be reasonable to expect that if the males are in a well fed condition, then the females would be in similar condition. The blood refractive index of the male prawns could be used, as the sampling of the blood is unlikely to have an impact on their reproductive performance and will give a sensitive response to their nutritional condition.

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5.2.2   SEASONAL VARIABILITY IN NATURAL DIET AND BIOCHEMICAL COMPOSITION OF TISSUES OF BROODSTOCK PRAWNS (CSIRO, CLEVELAND)

INTRODUCTION

The reproductive performance of broodstock prawns has been shown to be affected by environmental and nutritional factors (Crocos and Coman *in press*, Xu *et al* 1994). The biochemical composition of wild-caught broodstock prawns has been investigated by several researchers (Guary *et al* 1975, Dy-Peñaflorida and Millamena 1990, Millamena and Pascual 1990, Mourente and Rodríguez 1991). However, only recently have researchers related the biochemical composition of the broodstock prawns to their reproductive performance (Cahu *et al.* 1994, Xu *et al.* 1994).

In this project we have studied the seasonal variability of the natural diet of prawns and determined the biochemical composition of the two key tissues involved in nutrient transfer during the reproductive cycle, the digestive gland (sometimes inappropriately referred to as the 'hepatopancreas') and the ovaries. We have looked for correlations between a number of biochemical parameters in the tissues and organs of both *P. semisulcatus* and *P. monodon* with the reproductive performance of prawns caught at the same time and place. This was done in order to determine a biochemical indicator of potential reproductive performance and to gain a better understanding of the relationship between dietary fatty acid levels and reproductive performance. The seasonal changes in reproductive performance are discussed in Section 5.1 of this report.

METHODS
BROODSTOCK PRAWNS

Penaeus semisulcatus broodstock prawns were trawled from Albatross Bay (12° 48'S, 141° 32' E), Gulf of Carpentaria, Australia. These prawns were randomly selected from prawns that were collected for the study into reproductive performance (Section 5.1.1). Prawns were collected seven times at 2-monthly intervals between September 1992 and October 1993. Between 16 and 30 prawns were obtained on each occasion and generally only females were selected but when insufficient numbers were available, males were included in the sample. The prawns were frozen as soon as possible after capture to stop further digestion of food organisms in their foregut and transported to the laboratories for dissection and analysis.

Penaeus monodon broodstock prawns were obtained from Cook Bay, near Cairns, north east Australia. They were captured, frozen and dispatched by a trawler operator, Cairns Live Prawns. These prawns were captured close to the date when broodstock prawns were caught for the reproductive performance study (see Section 5.1.2) but were not part of the same sample.

Frozen prawns were thawed slightly, the moult stage and stage of ovarian development was noted before they were dissected to remove the proventriculus (or foregut), digestive gland, ovary and a sample of abdominal muscle. The complete digestive gland and ovaries were weighed before sub-samples were taken to determine the water and lipid content. The muscle sample was used to determine the water content only.

The proventriculus of each prawn was dissected, weighed and the contents rinsed into a petri dish. The contents were then examined under a microscope to identify the items that the prawn had been feeding on and to assess the number of these items.

Lipids were extracted with chloroform:methanol (2:1) (Folch *et al.* 1957) from the digestive gland and ovaries of eight female prawns from each bi-monthly sample. The total lipid content was determined gravimetrically by transferring the lipid extract to a tared HPLC vial insert, evaporating off the solvent under a stream of nitrogen, then drying it further in a vacuum desiccator for 1 h. The mass of lipid was then weighed to the nearest 0.01 mg. A sub-sample of the total lipid was saponified and esterified in methanol to form the fatty acid methyl esters. The methyl esters were used to determine the fatty acid composition and content of the digestive gland and ovary. Analysis of the neutral lipids and polar lipids was carried out to determine the lipid class composition.

Due to the variation in size of the prawns within and between samples and the ranges of stages in ovarian development, the most meaningful way of analysing the data was to limit the data to prawns in a specific stage of ovarian development. Hence, most of the data analysis reported here has been carried out using only prawns with stage 4 ovaries. In the February 1993 samples there were no *P. semisulcatus* with stage 4 ovaries which limits the dataset to 6 of the 7 bi-monthly samples. Reproductive performance data obtained in the studies into seasonal variability in reproductive performance of wild-caught broodstock (Section 5.1) was used in correlations with the biochemical data. Data analysis was carried out using the procedures, in statistics package SAS, for general linear modelling, correlation and canonical correlation (SAS Institute Inc, Cary, N.C.).

RESULTS

The size of the prawns collected in each of the seven *Penaeus semisulcatus* samples and five *P. monodon* samples varied markedly within samples and between samples. There was a general trend from February through to November of increasing mean weights (Table 3). The mean wet weight of *P. semisulcatus* was 51.1 g and that of *P. monodon* was 119.4 g.

SEASONAL VARIATION IN THE NATURAL DIET

The diets of the two species were broadly similar with various small crustaceans, bivalves, gastropods and polychaetes the most frequently occurring prey items. With *P. semisulcatus* there was a significant variation with time (or season) in the frequency of occurrence of the major prey items which did not occur with *P. monodon* (Table 4, Table 5). In August and October 1993 the main food items occurred more frequently in the proventriculus of *P. semisulcatus* compared with the previous year (September and November 1992) and also in the winter months (April and June 1993) suggesting a lower food availability in those months. With *P. monodon* the peak in frequency of occurrence was in April 1993 but there are no data for the September to November period of that year.

TABLE 3.

Monthly sample mean wet weight of *P. semisulcatus* (P. semi) and *P. monodon* (P. mono) used in the analysis of natural diet and biochemical composition of tissues. Standard error of the means in parentheses.

Species	Sept	Nov	Feb	Apr	Jun/Jul	Aug	Oct
P. semi	40	61	33	52	49	59	62
	(0.7)	(2.4)	(1.3)	(3.8)	(4.0)	(3.6)	(3.2)
P. mono	132	139	79	114	132	-	-
	(2.0)	(13.9)	(9.6)	(9.5)	(5.5)		

TABLE 4.

Penaeus semisulcatus. Seasonal variation in frequency of occurrence of natural food items in the proventriculus of prawns caught at Albatross Bay, Australia. Asterisks indicate significant changes with time (P<0.05).

Organism	Sept 92	Nov 92	Feb 93	Apr 93	Jun 93	Aug 93	Oct 93	Avg	se.
Crustacean*	13	12	11	12	10	21	19	14.0	1.87
Bivalve*	11	12	8	12	11	21	19	13.4	2.08
Gastropod*	12	10	10	7	5	17	15	10.9	1.86
Polychaete*	6	12	5	11	8	11	7	8.6	1.22
Ostracod*	0	8	0	6	2	5	9	4.3	1.62
Ophiuroid*	0	3	2	6	1	4	11	3.9	1.64
Teleost	2	2	2	2	1	1	2	1.7	0.22
Scaphopod*	1	0	6	0	0	0	1	1.1	0.96
Plant	0	2	2	2	0	0	1	1.0	0.44

TABLE 5.

Penaeus monodon. Seasonal variation in frequency of occurrence of natural food items in the proventriculus of prawns caught at Cook Bay, Australia. Asterisks indicate significant changes with time (P<0.05).

Organism	Sept 92	Nov 92	Feb 93	Apr 93	Jun 93	Avg	se.
Crustacean	13	13	18	27	22	18.7	2.59
Bivalve	10	13	15	20	17	15.0	1.70
Gastropod	10	12	12	13	20	13.4	1.72
Polychaete	10	10	10	22	18	14.0	2.53
Ostracod*	2	8	0	7	2	3.7	1.60
Ophiuroid*	5	10	7	18	5	9.0	2.42
Teleost	3	2	7	15	5	6.4	2.31
Scaphopod	0	0	0	0	0	0.0	0.00
Plant	4	7	7	9	6	7.0	1.43

SEASONAL VARIATION IN BIOCHEMICAL COMPOSITION OF BROODSTOCK PRAWNS

Within the samples of *P. semisulcatus* designated as having stage 4 ovaries, there was considerable variation in the level of development of the ovaries as indicated by the gonadosomatic index (GSI) (Table 6). This variability would suggest that the other physiological and biochemical parameters that were measured and which were influenced by the process of ovarian development, would also exhibit considerable variability.

The dry matter content of the abdominal muscle in *P. semisulcatus* showed a significant seasonal trend with the lowest values in June and significantly higher values in September, October and November (Table 6). There was a very strong negative correlation between muscle dry matter and hatch rate ($r=0.841$, $P=0.036$) and the percentage of nauplii that survived and metamorphosed to protozoea ($r=0.901$, $P=0.014$) in *P. semisulcatus* (Table 8). In contrast the muscle dry matter of *P. monodon* was at its highest in April (Table 7) but there was no correlation with the reproductive performance parameters (Table 8). The percentage dry matter of the ovaries in both species was significantly higher in June ($P<0.05$) than in the other months. However, there was no correlation of ovarian dry matter with zoeal production, though there was a weak correlation with metamorphosis of nauplii to zoea ($r=0.798$, $P=0.060$) with *P. semisulcatus*.

TABLE 6.

P. semisulcatus. Bi-monthly means of gonadosomatic index (GSI), muscle dry matter content, ovary dry matter and lipid content. Standard errors in parentheses.

	Sep 92	Nov 92	Apr 93	Jun 93	Aug 93	Oct 93
GSI	0.047	0.057	0.049	0.054	0.066	0.074
	(0.003)	(0.005)	(0.002)	(0.005)	(0.004)	(0.005)
Muscle dry matter (%)	27.0 (0.09)	26.6 (0.12)	26.3 (0.24)	25.5 (0.14)	25.7 (0.23)	26.4 (0.14)
Ovary dry matter (%)	29.5 (0.4)	29.4 (0.6)	29.7 (0.4)	32.5 (0.1)	29.8 (0.5)q	30.2 (0.3)
Ovary lipid (% wet wt)	18.6 (0.6)	15.0 (1.7)	18.8 (0.3)	19.9 (0.6)	17.8 (0.5)	19.7 (0.4)

TABLE 7.

P. monodon. Bi-monthly means of gonadosomatic index (GSI), muscle dry matter content, ovary dry matter and lipid content. Standard errors in parentheses.

	Sep 92	Nov 92	Feb 93	Apr 93	Jul93
GSI	0.082	0.053	0.048	0.076	0.054
	(0.004)	(0.005)	(0.003)	(0.003)	(0.005)
Muscle dry matter (%)	26.4	25.8	25.8	27.1	26.1
	(0.20)	(0.20)	(0.18)	(0.21)	(0.16)
Ovary dry matter (%)	30.9	29.1	30.7	31.6	30.8
	(0.2)	(0.9)	(0.7)	(0.2)	(0.7)
Ovary lipid (% wet wt)	21.7	18.0	20.7	21.9	21.5
	(0.2)	(0.7)	(0.8)	(0.3)	(0.9)

The fatty acid profile of the neutral lipid fraction of the digestive gland lipid of both species generally resembled that of a typical marine oil. The most significant differences in this profile occurred in the proportion of the essential highly unsaturated fatty acids (HUFA) : arachidonic acid (20:4n-6), eicosapentanoic acid (20:5n-3) and docosahexanoic acid (22:6n-3). The neutral lipid of both species had very much higher amounts of 20:4n-6 than is found in fish oil (Fig. 6). The amount of 20:5n-3 and 22:6n-3 in *P. semisulcatus* was similar to that in fish oil but in *P. monodon* these fatty acids were about half of that value. The bi-monthly sampling indicated a slight reduction in the proportions of these three fatty acids during the winter. With *P. semisulcatus* there was a weak negative correlation (r=0.773) between the percentage lipid in the digestive gland (on a wet weight basis) with reproductive performance (Table 8). The percentage lipid in the digestive gland of *P. monodon* was correlated to hatch rate (r=0.890, P=0.043) and naupliar survival (r=0.868, P=0.056). However, the percentage lipid in the ovary was not correlated with the reproductive performance of either species.

Statistical analysis of the digestive gland neutral lipid fatty acids of *P. semisulcatus* showed a number of significant correlations with reproductive performance. The correlations mainly occurred among the HUFAs (Table 8). 20:4n-6 was strongly correlated (r=0.829, P=0.042) with zoeal production while 20:5n-3 and 22:6n-3 were weakly correlated with zoeal production (r=0.785, P=0.064, and r=0.771, P=0.073 respectively). Canonical correlation with spawning performance, naupliar metamorphosis and zoeal production further confirmed the correlation of these HUFA with reproductive performance. There were no useful correlations found between the lipids and fatty acids of the ovaries *P. semisulcatus* and the parameters used to assess reproductive performance.

Analysis of the *P. monodon* data showed a correlation between the essential fatty acid linolenic acid (18:3n-3) of the digestive gland and hatch rate (r=0.944, P=0.016) and also naupliar survival (r=0.994, P=0.001) (Table 8). Again there were no correlations between the fatty acid composition of the ovaries and reproductive performance.
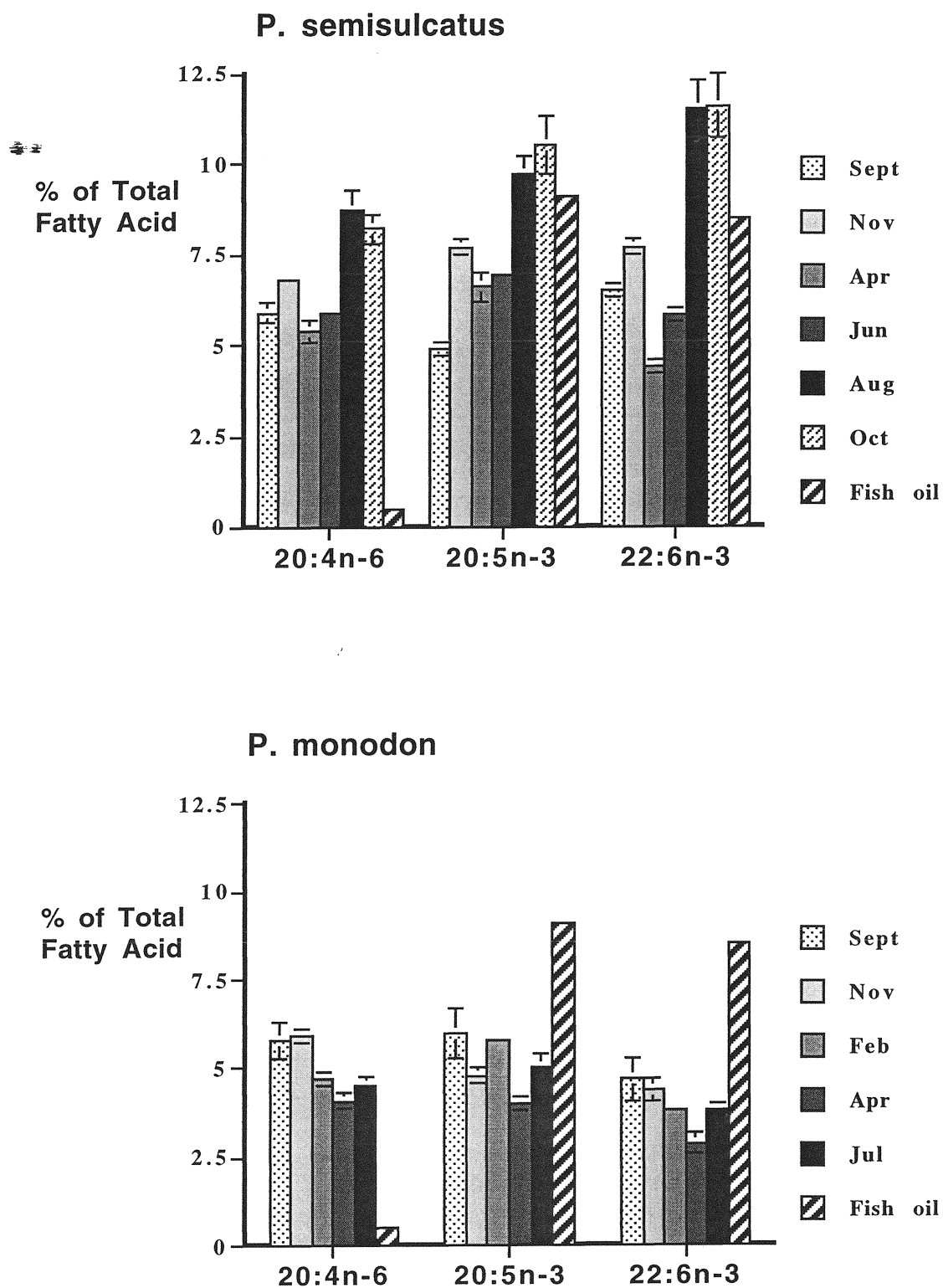
P. semisulcatus

P. monodon

FIGURE 6.

Bi-monthly variation in percentage composition essential HUFAs in the neutral lipid fraction of the digestive gland lipid in *P. semisulcatus* and *P. monodon*. (arachidonic acid 20:4n-6, eicosapentanoic acid 20:5n-3, docosahexanoic acid 22:6n-3). Error bars indicate the standard error of the means.

Table 8.

Correlation coefficient (r) of correlations between reproductive performance parameters and selected physiological and biochemical parameters.(- indicates a negative correlation, * indicates significant correlations)

	Parameter	spawns	hatch rate	naupliar survival	zoeal product'n
P.semisulcatus					
Muscle	Dry matter	0.101	-0.841*	-0.901*	-0.472
Digestive gland	% lipid	-0.772*	-0.024	-0.261	-0.769*
Digestive gland	18:3n-3	-0.156	0.354	0.316	0.326
Digestive gland	20:4n-6	0.568	0.295	0.451	0.829*
Digestive gland	20:5n-3	0.285	0.606	0.651	0.785*
Digestive gland	22:6n-3	0.519	0.253	0.385	0.771*
P. monodon					
Muscle	Dry matter	-0.402	-0.357	-0.407	-
Digestive gland	% lipid	-0.436	0.890*	0.868*	-
Digestive gland	18:3n-3	-0.437	0.944*	0.994*	-
Digestive gland	20:4n-6	0.349	-0.503	-0.417	-
Digestive gland	20:5n-3	-0.605	-0.145	0.049	-
Digestive gland	22:6n-3	0.158	-0.406	-0.321	

DISCUSSION

STANDARDISATION OF ANIMALS

As there was significant variation in the size of the prawns in each bi-monthly sample and in the stage of ovarian development it was necessary to limit analysis of the biochemical data to as close to a 'standard' prawn as possible. Statistical analyses were carried out to include all female prawns in stages 2, 3 and 4; then with stages 3 and 4 and finally with only stage 4 prawns. Results and trends that were clear using only stage 4 prawns were generally also apparent with the stage 3 and 4 grouping. However even with the stage 4 grouping of prawns, the GSI for both species varied from about 0.10 to about 0.02 though the means varied only from 0.045 to 0.082 (Table 6, Table 7). This range in ovarian development makes it meaningless to compare absolute amounts of nutrients in both the ovary and the digestive gland (which is the source of much of the lipid in the developing ovaries). As a consequence the amounts of nutrients are presented either as concentrations or as mass per unit wet weight or dry weight of an organ.

NATURAL DIETS

The natural diets of both species of prawns were very similar. The bulk of their food came from the same four taxonomic groups of animals and these were in much the same order of frequency of occurrence. However, with *P. monodon* there was not a significant variation between months although a minor peak in the frequency of occurrence of the major food items occurred in April (Table 5). With *P. semisulcatus* there was a significant variation, with the major prey items showing a markedly higher frequency of occurrence during August and October 1993 (Table 4). The increase in frequency of occurrence suggests a greater availability of food items and hence suggests the prawns would be in better nutritional condition at this time. The increase in frequency of occurrence appears well correlated to the egg and zoea production observed with the *P. semisulcatus* but there is not a clear relationship with *P. monodon*.

BIOCHEMICAL COMPOSITION OF BROODSTOCK PRAWNS

The dry matter content of abdominal muscle indicates the nutritional condition of non-reproductive prawns. However the situation is much less clear with prawns that are using significant nutrient reserves during maturation. The highest dry matter content of the abdominal muscle of *P. semisulcatus* occurred in September 1992 while the frequency of occurrence of food items was fairly low at this time (Table 4), but dry matter content was close to its lowest point when the frequency of occurrence of food items was at a peak in August 1993. With *P. monodon* the dry matter content was at its highest in April 1993 which coincided with the period of greatest frequency of occurrence of food items (Table 5) and the lowest dry matter content in June which was the period of lowest frequency of occurrence of prey items. Hence there is too much inconsistency with the muscle dry matter content to use it to effectively assess the nutritional condition of prawns that are in the process of ovarian maturation.

The fatty acid profile of the neutral lipid fraction of the digestive gland lipid reflects the composition of the natural diet of prawns. Though the fatty acid profile may not directly indicate the nutritional condition of a prawn, high levels of the HUFAs have been linked to better reproductive performance (Cahu *et al.* 1994, Xu *et al.* 1994). With both species the fatty acid profile resembled that of a typical marine oil but with the major difference being the much higher concentration of 20:4n-6 in the digestive gland lipid. The source of the 20:4n-6 would be the invertebrates in the prawns' diet, particularly the polychaete worms. Though the two species of prawns feed on the same suite of organisms (though the actual prey species would differ), the fatty acid profiles of their digestive gland neutral lipid is significantly different especially in the proportions of 20:4n-6, 20:5n3 and 22:6n-3 (Figure 6). These essential fatty acids have been previously identified as having a major effect on reproduction (Cahu *et al.* 1994, Xu *et al.* 1994). The variation in the concentration of these fatty acids between samples is relatively large but there is a consistent reduction in the concentration of all of them in the winter months. The amount of 20:5n-3 and 22:6n-3 in *P. semisulcatus* was similar to that in fish oil but in *P. monodon* these fatty acids were about half that found in fish oil. As the magnitude of the differences between *P. semisulcatus* and *P. monodon* are even larger than the seasonal variation it would suggest that the lipid composition of their diets is significantly different or that the dietary intake of these fatty acids by *P. monodon* is less than the requirements during ovarian maturation.

Statistical analysis of the fatty acid composition data of the neutral lipid fraction of the digestive gland of *P. semisulcatus* showed a number of significant correlations between the essential HUFAs. 20:4n-6, 20:5n-3 and 22:6n-3 and reproductive performance (Table 8). Regardless of the cause of the low HUFAs levels in *P. monodon* it would appear that their reproductive performance would be enhanced through improved nutrition particularly in the provision of essential fatty acids. The quantitative requirements of broodstock prawns for the essential HUFAs has not been established (Cahu *et al.* 1994, Xu *et al.* 1994) but these comparative results do indicate the approximate concentrations on which further investigation into the requirements would be based.

The aims of this part of the project were to assess the nutritional condition and natural diet composition of broodstock prawns and to determine the relationship between tissue lipid composition and their reproductive performance. A preliminary objective was to develop a method of assessing the nutritional condition of spawners and then apply that technique to spawners collected during the course of the bi-monthly sampling scheme. Because of time constraints the bi-monthly sampling scheme started in August 1992 before the methods of assessing nutritional condition were developed. Samples were frozen immediately on capture to prevent further digestion of food material in the proventriculus and to prevent deterioration of the tissues prior to analysis. Subsequently it was established that the blood refractive index and the blood lipid were good indicators of nutritional condition (Section 5.2.1). The blood could only be collected from live animals so the techniques could not be applied to the samples collected for biochemical analysis. However, the reproductive activity of broodstock prawns has a dramatic effect on their tissue composition which is confounding attempts to assess their nutritional condition. It may be more productive to assess the nutritional condition of males of the species to predict the nutritional condition of the females at that time.

The results of this study have shown that there are significant differences between species in their tissue essential fatty acid composition. The reason for the differences is unclear. These differences could be due to the differences in the fatty acid composition of the diets; alternatively it may be that *P. monodon* is in poorer nutritional condition than the *P. semisulcatus* and that the reproductive activity of *P. monodon* is resulting in a depletion of HUFA reserves. We have not yet established whether the high levels of arachidonic acid, 20:4n-6 seen in the digestive gland, are necessary for consistent high quality spawnings. Though experiments were carried out to address this question (Section 5.3.2.2), the prawns could not utilise the form of 20:4n-6 that was used. Nor is it clear whether the levels of eicosapentanoic acid (20:5n-3) and docosahexanoic acid (22:6n-3) in the *P. monodon* diets are limiting their reproductive performance. Further clarification of these nutrient requirements is necessary in the development of a formulated broodstock diet.

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5.2.3   SEASONAL VARIABILITY IN EGG FATTY ACID PROFILES AND INFLUENCE ON THE HATCH RATE AND SURVIVAL OF PENAEUS MONODON LARVAE (QDPI BIARC)

INTRODUCTION

It has been shown that there is a significant variability in the egg and larval productivity of *Penaeus monodon* caught in one location (Cook Bay, Australia) (Hansford and Marsden, 1995). These seasonal differences may be due to differences in the age of the spawners and/or to environmental factors.

One hypothesis is that differences in the nutrient composition of the eggs is responsible for differences in the hatch rate and larval survival. However, this hypothesis has not been demonstrated conclusively (Cahu *et al.*, 1994). To test the hypothesis, the nutrient composition of *P. monodon* eggs was determined and correlated with hatch rate and larval survival. The aims of the present study were:

- To determine whether there is a seasonal variation in the nutrient composition of eggs, which may correlate with differences in the larval production of prawns,

and, more specifically

- To determine whether one or a combination of fatty acids in prawn eggs were major determinants of larval output, by investigating the correlations between fatty acids and hatch rate and survival of larvae from the first nauplius stage through to the first protozoeal stage, Z1.

MATERIALS AND METHODS

PRAWNS AND EGGS

The eggs were subsampled as part of the study investigating seasonal differences in the reproductive performance of *P. monodon* (Section 5.1.2, Hansford and Marsden 1995). Egg samples were collected during October 1992, December 1992, April 1993, July 1993 and September 1993 from prawns that had been caught within the previous three weeks. For details on the prawn collection and husbandry, and estimation of hatch rate and larval survival see Section 5.1.2.

Only eggs from first spawnings were used in this study. Approximately 2 g of eggs were siphoned from the spawning tub the morning following spawning. A preliminary study showed that there was no change in the fatty acid profile of eggs collected up to 8 h after spawning. Eggs from the samples were counted, to standardise the quantification of nutrients to mg per egg. Biochemical analysis was carried out to determine lipid, fatty acid, cholesterol and phospholipid content of the eggs.

LIPID

Lipid content was determined by Soxhlet extraction with petroleum ether (boiling point 40 to 60°C) for 6 h (AOAC, 1990, method 960.39 for Crude Fat).

FATTY ACID ANALYSIS

Lipids were extracted by the method of Folch *et al.*, (1957) using the modification of Christie (1982). An aliquot of the lipid extract was separated into polar and non-polar fractions using Sep-Pak silica cartridges (Waters Associates, MA, USA). The non-polar fraction was eluted with 15 mL chloroform and the polar fraction with 20 mL of methanol (Christie, 1982). The solvent was removed from each fraction by rotary evaporation and the lipids esterified to fatty acid methyl esters (FAME) by the method of Van Wijngaarden (1967). FAME were separated by capillary gas chromatography using split injection on a 30 m x 0.25 mm i.d. fused silica column coated with 0.25 m of DB-23 (J & W Scientific, Folsom, California). Column temperature was held at 160°C for 10 minutes and then increased at 3°C min^{-1} to 210°C where it was held until all FAME of interest had been eluted. FAME were quantified by comparison with the response of an internal standard (heneicosanoic acid methyl ester). FAME were identified by comparing their retention times with those of authentic standards (Sigma Chemical Company, St. Louis, Missouri).

PHOSPHOLIPIDS

Total phospholipid content was determined by analysis of an aliquot of the lipid extract for phosphorus. The aliquot was digested in a boiling mixture of sulphuric acid/perchloric acid/nitric acid (1:1:3). The phosphorus content was then determined photometrically by the Biggs method using ammonium molybdate to form a blue colour and reading the intensity on a spectrophotometer set at a wavelength of 595 nm.

CHOLESTEROL

Cholesterol was determined by firstly saponifying an aliquot of the lipid extract and then extracting the cholesterol into hexane. The cholesterol was separated by capillary gas chromatography using a 30 m x 0.25 mm i.d. fused silica column, DB5 (J & W Scientific) at a temperature of 280°C. An internal standard, 5α-cholestane, was used for quantification.

STATISTICAL METHODS

Egg fatty acid profiles, total lipids, cholesterol and phospholipids, egg output, hatch rate and naupliar survival to Z1 (first spawns only) were obtained for each of the five seasonal samples. The number of spawns were 15, 5, 11, 14 and 17 for the October 1992, December 1992, April 1993, July 1993 and September 1993 samples respectively. Individual prawns were taken as the independent experimental units. All data were checked for significant differences between seasonal samples using an ANOVA.

Data on individual fatty acids were first summarised and scanned for outliers. Five fatty acids (14:1n-5, 20:3n-6, 22:2n-6, 24:0 and 24:1w9) were found to have constant (zero) values in both the neutral and polar fractions, so were excluded from further consideration. The dependent variates of hatch % and survival % were linearly regressed against each of the fatty acid variables, for mass and percentage basis both the neutral and polar fractions. Multiple regressions were also fitted using the step-forward technique to see if combinations of fatty acids could be used to predict hatch rate or survival.

RESULTS

SEASONAL DIFFERENCES IN NUTRIENT PROFILES AND SPAWNING PERFORMANCE

There was no significant difference in the levels of lipids or cholesterol between seasonal samples (ANOVA) (Fig. 7). The phospholipid levels did differ significantly between seasonal samples ($p < 0.05$). However, phospholipid content appears to be correlated with the lipid content of the eggs.
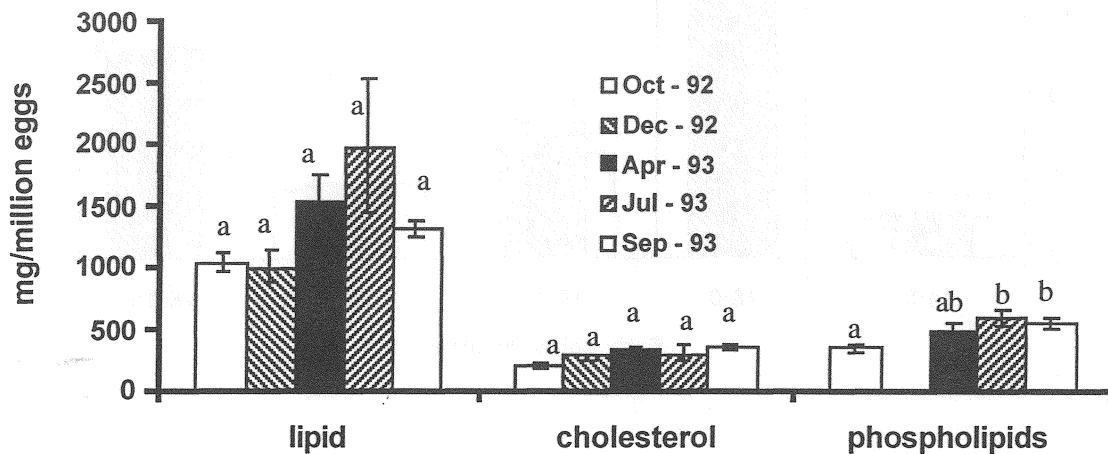

FIGURE 7.

Penaeus monodon. Mean (and standard error) of lipid, cholesterol and phospholipid levels in the eggs collected during each of the five seasonal samples. Annotations with different letters indicate a significant difference between samples.

Though the fatty acid profiles of the neutral lipid fraction and polar lipid fraction of the egg lipids were somewhat different (Fig 8, Fig 9), with each fatty acid there was generally the same pattern of change between seasonal samples. The variability in the proportion of each fatty acid in each fraction was relatively small with the exception of eicosapentanoic acid (22:6n-3). The proportion of this fatty acid was very much higher in the October 1992 eggs than in subsequent ones.

FIGURE 8.

Penaeus monodon. Mean fatty acid levels of the ten most abundant fatty acids in the neutral lipid fraction of the eggs (expressed as a percentage of the neutral lipid fraction), for each of the five seasonal samples. Significant differences in the levels of these fatty acids between seasonal samples are denoted by annotation of the histograms with different letters (ANOVA).
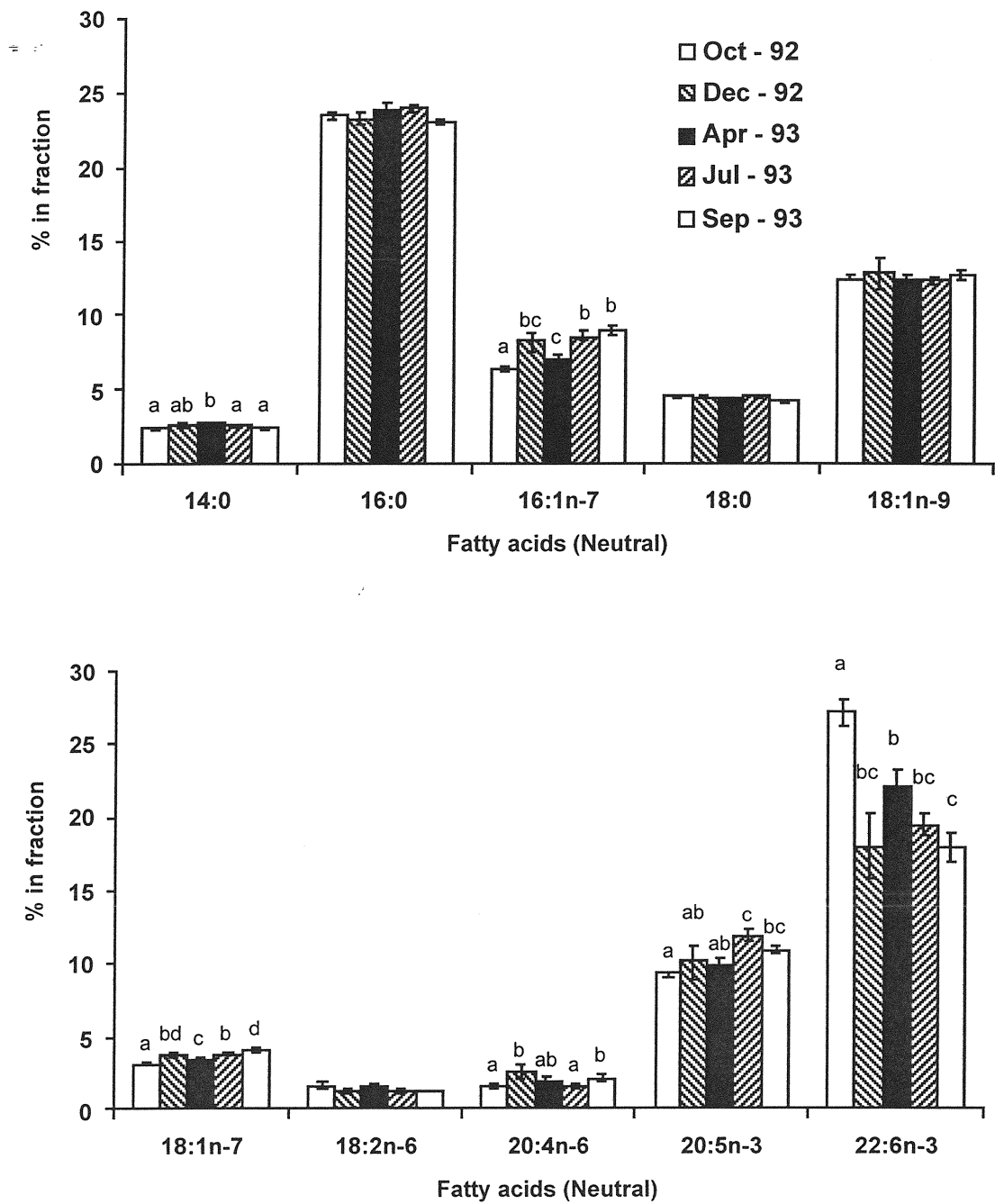
FIGURE 9.

Penaeus monodon. Mean fatty acid levels of the ten most abundant fatty acids in the polar lipid fraction of the eggs (expressed as a percentage of the polar lipid fraction), for each of the five seasonal samples. Significant differences in the levels of these fatty acids between seasonal samples are denoted by annotation of the histograms with different letters (ANOVA).
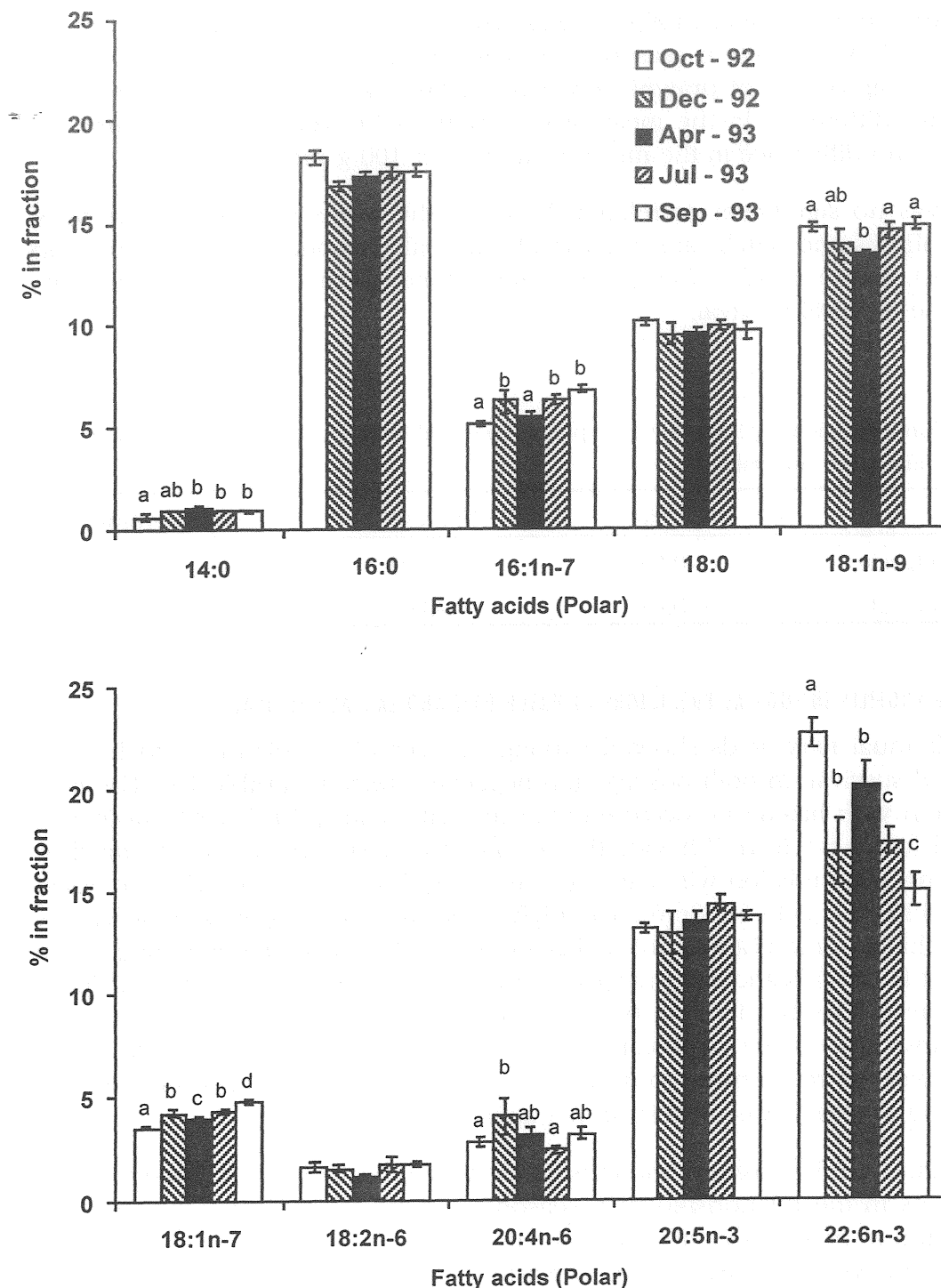
There was a significant difference in the hatch rate of eggs between seasonal samples. The mean hatch rate from the October 1992 samples was lower than that of the April and September 1993 samples. There was no significant difference in the survival of larvae between seasonal samples. There was a significant difference in the mean egg output of prawns between seasonal samples, but this was due to a significant difference in the mean size of prawns between seasonal samples, as there was no difference in the mean fecundity per 100 g prawn.

There was no significant correlation between the levels of lipids, cholesterol or phospholipids and hatch rate or survival when all seasonal samples were pooled (Table 9), indicating that these parameters alone are not major determinants of hatch rate or larval survival.

TABLE 9.

Correlation coefficients (r) of relationship between total lipids, cholesterol, phospholipids, and hatch rate and larval survival.

	Lipid	Cholesterol	Phospholipid
Hatch rate	-0.03	0.00	0.06
Survival	-0.01	-0.06	0.12

FATTY ACID PROFILES IN EGGS AS PREDICTORS OF HATCH RATE AND LARVAL SURVIVAL

The individual fatty acids showed varying degrees of correlation with hatch rate and larval survival, in both positive and negative directions (Table 10). The highest correlation with hatch rate occurred with the fatty acid 18:1n-7 with another non-essential fatty acid 20:1n-7 having the second strongest correlation with hatch rate. The strongest correlation with survival was a negative correlation with the essential fatty acid linoleic acid (18:2n-6). The highest positive correlation with survival was with the highly unsaturated fatty acid 20:4n-3. This fatty acid is not among the most abundant fatty acids and is a precursor for eicosapentanoic acid (20:5n-3). The fatty acids from the neutral fraction (% basis) were subjected to further analysis, along with a few of the more significant fatty acids from the polar fraction (% basis). In no case was the relationship to weight (mg.mg^{-1}total fatty acid) notably better than with %, so the latter was used in further analyses.

The significant relationships, and those close to significant (P<0.10), were tested for differences in the relationship with seasonal samples. In general, the regressions were found to be poolable across seasonal samples, indicating the better predictors of reproductive performance are consistent in their effects. Poolable data are denoted in Table 10 by a cross (for significant correlations only). When data across seasonal samples were not poolable, it is likely that the observed significant correlation was due to chance. Some of the fatty acids (20:3n-3, 20:4n-3, 22:3n-3) were present in very low quantities (less than 1% in fraction). This would reduce the confidence in the correlation as the error about individual values is increased.

Results from the multiple regression analysis showed that there was no improvement in the predictability of percentage hatch rate using combinations of fatty acids rather than one fatty acid alone. On the other hand, percent survival to the first zoeal stage (Z1) could be best predicted using a combination of five fatty acids, these being 18:2n-6 (linoleic acid), 20:4n-3, 22:1n-3, 18:0 and 20:5n-3 (eicosapentanoic acid).

The multiple equation regression which gave the best fit (r = 0.62) was:

% Survival = -2.43(18:2n-6) + 2.19(20:4n-3) - 2.89(22:1n-3) - 3.05(18:0) + 2.09(20:5n-3) + 3.82

The one fatty acid which gave the best predictability of survival was 18:2n-6, linoleic acid (r = 0.32) (Table 10).

TABLE 10.

P. monodon. Correlation coefficients of fatty acid levels in the neutral lipid of eggs of *P. monodon* (expressed as a percentage of the neutral lipid) vs hatch rate (%) and survival to Z1 (%), all seasonal samples pooled. Significant correlations (p<0.10) shown in bold. † denote correlations which were not significantly different across seasonal samples and the data was pooled.

Fatty Acid	Hatch rate (%)	Survival (%)
16:0	-0.048	0.107
16:1n-7	**0.286**[†]	**0.236**[†]
18:0	-0.09	**-0.291**[†]
18:1n-9	0.095	0.032
18:1n-7	**0.388**[†]	0.193
18:2n-6	-0.062	**-0.320**[†]
18:3n-3	0.178	-0.142
18:4n-3	0.019	-0.192
20:0	-0.017	-0.151
20:1n-9	**-0.272**[†]	**-0.228**[†]
20:1n-7	**0.381**[†]	0.064
20:4n-6	0.083	0.121
20:3n-3	**0.297**[†]	0.136
20:4n-3	**0.222**[†]	**0.246**[†]
20:5n-3	**0.286**	0.180
22:0	-0.088	-0.145
22:1n-9	0.01	-0.039
22:1n-7	-0.190	-0.062
22:3n-3	**-0.250**	**-0.257**[†]
22:5n-3	**0.213**	0.052
22:6n-3	**-0.286**[†]	-0.151

A 3-way analysis of variance (polar vs neutral, mg vs % and fatty acid) was conducted on the squares of these data to see which data were the best predictors of hatch rate and survival. Firstly, on average, the fatty acids from the neutral fraction explained about twice the variation that those from the polar fraction did. Secondly, the levels of fatty acids expressed as a percentage of dry matter explained over twice those expressed as mg per million eggs.

DISCUSSION

These results indicate that the total quantities of lipids, cholesterol or phospholipids in eggs are not important determinants of egg performance (hatch rate and larval survival) compared with the fatty acid composition. This finding is consistent with the findings in Section 5.2.2 where no significant correlations were found between ovary lipid content and reproductive performance.

The results relating to egg quality, may have implications for diet formulation. The fatty acid composition of the prawn's diet is reflected very closely in the fatty acid composition of the neutral and phospholipid in the digestive gland, muscle and eggs (Cahu et al. 1994). The variation seen in the proportion of most of the fatty acids in both the neutral lipid and polar lipid fractions between seasonal samples is very small with the exception of 22:6n-3. This result is consistent with results obtained in Section 5.2.2 where the highest levels of HUFA in the digestive gland neutral lipid occurred in September 1992 and these declined slightly with the lowest levels in April 1993. The diets the prawns were given following their capture was constant (FFMI-B), and their natural diet availability and composition appears relatively unchanged throughout the year (Section 5.2.2) so it is possible the larger fluctuations in 22:6n-3 may be associated with the demands for the fatty acid during ovarian development.

The fatty acid that appears to be most strongly positively correlated to hatch rate is 18:1n-7. It is the sixth most abundant fatty acid and can be synthesised de novo. However, it has not previously been identified as having a particularly significant role in animal nutrition and hence this result may not have any nutritional significance.

The concentration of a specific combination of fatty acids was found to predict almost 40% of larval survival. The most significant fatty acid was 18:2n-6, linoleic acid, an essential fatty acid, which was negatively correlated with survival. Also negatively correlated with survival, though without significant differences across seasonal samples, was another essential and highly unsaturated fatty acid, 22:6n-3, docosahexanoic acid which has been widely reported as being a vital nutrient in growth and reproduction (Harrison, 1990). A second essential and highly unsaturated fatty acid, 20:5n-3, eicosapentanoic acid, is positively correlated with naupliar survival. 20:5n-3 is a precursor of 22:6n-3 and has also been found to be important for growth and reproduction. The positive correlation of 20:5n-3 to naupliar survival indicates that it is an important nutrient in the diet of broodstock prawns. This finding is also consistent with the findings in Section 5.2.2. However, the absence of a positive correlation of the other essential highly unsaturated fatty acid 22:6n-3 suggests that the level of this fatty acid in the diet is in excess of requirements. This information will be considered in the formulation of broodstock diets.

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5.3 DEVELOPMENT AND EVALUATION OF BROODSTOCK CONDITIONING DIETS

- A mixture of fresh frozen squid (*Loligo spp.* 37%), chopped prawns (*Metapenaeus bennetae*, 26%) and bivalves (*Plebidonax deltoides* 37%), (FFMIC) was found to be the best hatchery diet for broodstock of both *P. monodon* and *P. semisulcatus* .

- Artificial broodstock diets have been developed which are alginate bound. These diets have not performed as well as the best mixture of fresh frozen marine invertebrates. Artificial diets did result in an increase in survival of nauplii through to the first protozoeal stage whereas the fresh frozen marine invertebrate diet always resulted in a higher number of spawnings. There is potential to develop the artificial broodstock diets further; the definition of the requirements for the essential HUFAs would be of immediate benefit Further research is needed to discover what factor is causing the higher spawning rate of prawns fed the FFMI-C diet.

INTRODUCTION

The specific nutritional requirements of broodstock prawns is very poorly understood, despite considerable interest over the past decade. There have been few successful attempts to determine quantitative requirements of broodstock prawns for specific nutrients (Harrison, 1990; Alava *et al.* 1993; Cahu *et al.* 1994). Researchers have consistently found that a diet consisting of a mixture of fresh frozen marine invertebrates (FFMI) results in a better level of reproductive performance than that achieved with commercially prepared 'maturation' diets or a diet consisting of a single taxa of marine invertebrate (Chamberlain and Lawrence, 1981; Bray *et al.* 1990; Cahu *et al.* 1994). They have also found that the commercial diets were improved when supplemented with a fresh frozen or 'natural' food item (Galgani *et al.* 1989).

Most of the research into specific nutritional requirements has focussed on the lipid component of the diet, particularly the levels of phospholipid, cholesterol and the highly unsaturated fatty acids (HUFA) eicosapentanoic acid (EPA), docosahexanoic acid (DHA) and arachidonic acid (AA) (reviewed by Harrison, 1990; Mourente and Rodriguez, 1991; Alava *et al.* 1993; Cahu *et al.* 1994). Though it has become clear that the dietary intake of these nutrients has a significant effect on reproductive performance, the quantitative requirements for these nutrients has not been determined.

Two confounding aspects of research reported in the literature (reviewed by Harrison, 1990) have been the lack of feed intake data and a diversity of methods used to assess reproductive performance. From the previous research it is not possible to determine if the lower reproductive performance obtained with commercial or single species FFMI diets was due to a low feed intake or basic nutritional deficiencies in the diets. Reproductive performance has often been assessed using only the number of spawnings per prawn and the number of eggs produced. There is a paucity of information on the hatch rate from the spawnings and the percentage of nauplii that metamorphosed into protozoea. A full assessment of reproductive performance should include a range of performance criteria relating to spawnings, egg production, hatch rate and nauplii viability. Analysis of reproductive output under these categories enables a clearer

understanding of how a given treatment is affecting reproduction. Such a procedure was adopted in this study.

To ensure the success of any formulated prawn feed a number of criteria must be met. Foremost of these is that the diet is nutritionally balanced and is consumed in sufficient quantities to meet the specific requirements of the prawn. This study addressed both these aspects of diet development with the initial work focussed on finding a suitable binding method that would ensure diets were palatable, negatively buoyant and stable in water. It was considered that a broodstock diet fed to prawns held in indoor tanks did not require the same handling and storage characteristics considered essential for a growout diet. As relatively small quantities of broodstock diet are used by hatchery operators it was not considered essential to have the diet in a form of dry pellet. With broodstock diets a moist or semi-moist diet is acceptable despite the need to keep it under refrigeration. This storage constraint is offset by their higher level of attractiveness and palatability for prawns.

While some work was being carried out on diet formulations in conjunction with tests on the various binding methods, it was not until the diet had been made with a satisfactory level of water stability that the emphasis shifted to optimising the nutrient profile. Information used in the selection of suitable ingredients was acquired from a number of sources including published research findings on prawn diets (Tacon 1991), diets currently used in commercial hatcheries, natural diets of broodstock in the wild (FRDC 89/50) and the results from the compositional analysis of ovary tissues (FRDC 89/52)

5.3.1 DIET BINDING

INTRODUCTION

Preliminary assessment of existing commercial broodstock diets showed that consumption of the dry pellets was low on a dry weight basis compared to FFMI diets and that the reproductive performance of broodstock fed the commercial diets was low. To maximise the nutritional benefit of the diets and improve attractiveness we sought to avoid binders that require heat to activate the gelatinisation or cross-linking reactions. Based on commercial information (Kelco 1991) and in the scientific literature (Meyers 1980; Heinen 1981; Storebakken 1985; Knauer *et al.* 1993), a range of binders used by the food industry was selected and tested. Sodium alginate was chosen for its ease of use, superior strength and elasticity and also because it was activated by the action of calcium ions rather than a heating process. The calcium, as calcium chloride, could either be added to the diet during preparation (internal setting), or the diet could be extruded into a setting bath containing an aqueous solution of 10% calcium chloride (diffusion setting), (Kelco 1991).

METHODS AND RESULTS

A series of experiments were carried out using diets containing different concentrations of either of two alginate binders (with internal and diffusion setting), and combinations of dry and wet ingredients. Diets were tested for stability, palatability and buoyancy. An alginate inclusion level of 6 to 9% (dry matter) was found to be optimal depending on the ingredients used in the diet. The diffusion setting method was considered preferable to the internal as stability of the diet was more uniform and the diet preparation time was not predetermined as it was when the calcium ions were included in the diet mix.

LEACHING LOSS

The moisture content of the diet ranged from 60 to 80 % depending on the ingredients selected. As moist fresh diets suffer from high leaching rates, tests were also carried out to assess the nutritional stability over time. Replicate samples of a diet with a alginate inclusion level of 9% were placed in water at 28°C and gently agitated. Samples were removed after 1, 2 and 8 h to determine the changes in their biochemical composition caused by leaching. Results showed that leaching rates were lower than previously recorded for FFMI diets. Most of the leaching occurred within the first 2 h with a maximum reduction of 4.5% of protein and 2.3% of lipid (on a dry matter basis). There appeared to be some selective leaching of fatty acids however these were not statistically significant ($P<0.05$).

DIGESTIBILITY

To ensure the inclusion levels of the alginate were not adversely affecting digestibility, ytterbium was included as a marker in a test diet with alginate included at a level of 9%. Results showed that overall digestibility was 80%, and for protein and fatty acids 20:5n3 and 22:6n3 was approximately 95%. These results indicate that the relatively high level of alginate does not have an adverse effect on the digestibility of the diets.

FEED INTAKE

Feed intake by the prawns varied depending on ingredients used in the diet. However, two of the test diets were consumed at a rate of approximately 3 g of dry matter per 100 g wet weight of prawn per day, which was equal to the consumption of the FFMI diets. This suggests that the alginate binding does not have an adverse effect on the palatability of the diets.

5.3.2 DIET FORMULATION

A total of ten experiments were carried out to examine the effect of five different diets on the reproductive performance of *P. semisulcatus* and *P. monodon*. Different approaches were used in formulating the diets once the maximum and minimum levels of protein, lipid and some essential fatty acids were specified. Work then focused on maximising the attractiveness of the diets, and reducing the use of specially prepared ingredients. Though cost of the diet was not a prime consideration, we sought to control the cost through use of commercially available ingredients and by minimising the use of ingredients that required costly preparation procedures such as freeze drying.

5.3.2.1 DIETARY INGREDIENTS AND MACRO NUTRIENTS

MATERIALS AND METHODS

Four sequential experiments were conducted comparing the reproductive performance of prawns fed two formulated broodstock diets, BD1 and BD2, to those fed a FFMI diet of squid/mussel (FFMI-B). The formulated diets employed the same binding method but differed in their biochemical composition. The diets were analysed using standard methods to determine their nutrient composition. Two separate experiments, one in September and one in November, 1993, were carried out to assess the effect of the first version of the formulated diet on reproductive performance. Modifications were then made to the formulation and a further two experiments were carried out, one in April and the other in June, 1994. The experiments were run in duplicate using prawns captured from the wild during different months of the year to test whether the time of capture had any effect on reproductive performance.

For each experiment approximately 60 female and 40 male *P. monodon* were captured from waters off Cairns, Qld, and airfreighted to Brisbane. On arrival at BIARC, females were weighed, eye and carapace tagged. Prawns were then allocated to four tanks such that each tank had a similar size range of animals. Two tanks were fed the diet of squid/mussel (FFMI-B) and two tanks were fed a version of the artificial diet (Tables 11 and 12). After two weeks conditioning the prawns were eyestalk ablated and the reproductive performance monitored over the following 6 weeks.

In the final series of experiments two formulations, BD2 and BD3, that appeared to have considerable potential, were compared against the CSIRO FFMI diet consisting of squid, prawn and mussel (FFMI-C). The mixture used in FFMI-C had been found to result in excellent reproductive performance in *P. semisulcatus* and so was used in this experiment with *P. monodon*. Diet BD3 had a similar nutrient profile as BD2 but was formulated using the ingredients that were commercially available to reduce cost and moisture content. Data collection and tank and spawning conditions are as per Section 5.1.2. of this report.

TABLE 11.

Proximate composition (% dry matter) of experimental diets used with *P. monodon*.

Nutrient	FFMI-B	FFMI-C	BD1	BD2	BD3
Dry Matter	20.2	22.0	26.0	21.5	29.0
Ash	8.2	15.2	12.6	17.0	13.0
Crude Protein	73.5	67.3	52.2	54.6	55.8
Lipid	7.8	8.3	15.6	10.7	10.3
n3/n6	4.5	7.2	5.5	4.5	6.7

TABLE 12.

Ingredient composition of experimental diets used with *P. monodon.*

Ingredient	Weight (g/kg dry diet)		
	BD1	**BD2**	**BD3**
Minced mussel (*P. canaliculatus*)	230.0	220.0	-
Fish roe (*Hoplostethus atlanticus*)	120.0	-	-
Calf liver	-	110.0	-
Artemia enrichment (dry Selco)	-	40.0	-
Squid (*Loligo* so.) meal	410.0	410.0	250.0
Fish meal (Tasmanian)	-	-	300.0
Prawn meal	-	-	150.0
Cod liver oil	24.0	-	7.0
Binder mix	100.0	100.0	60.0
Milled mollusc shell (*C. deltoides*)	20.0	20.0	-
Wheat gluten	-	-	60.0
Starch, Pregelled	-	-	77.6
Lecithin	30.0	30.0	30.0
Vitamin mix (1)	50.0	50.0	21.4
Mineral mix (2)	30.0	30.0	38.0
Cholesterol	11.0	11.0	5.0
Astaxanthin	40.0 ppm	40.0 ppm	70.0 ppm
ß-carotene	40.0 ppm	40.0 ppm	-

1   Vitamin composition (mg 100 g diet^{-1}) p-amino benzoic acid, 15.8; biotin, 0.63; inositol, 632.0; nicotinic acid, 63.2; Ca-pantothenate, 94.8; pyridoxine HCl, 19.0; riboflavin, 12.6; thiamine HCl, 6.32; folic acid, 1.26; cyanocobalamin, 0.13, choline HCl, 948.0; menadione, 6.34; Na ascorbate, 3160.0; calciferol, 1.9; tocopherol, 50.0.

2   Mineral composition (g 100 g diet^{-1}) $K_2H_2PO_4$, 0.70; $Ca_3(PO_4)_2$, 0.95; $MgSo_4.7H_2O$, 1.1; $NaH_2PO_4.2H_2O$, 0.28.

RESULTS

BROODSTOCK DIETS BD1 AND BD2

The initial versions of BD1 resulted in very poor reproductive performance and were reformulated to reduce the total lipid and levels of the fatty acid 18:1n-9, and to increase the level of EPA. These changes were consistent with the nutrient levels in the ovaries of wild-caught prawns and in their natural diet. However it should be noted that in making these changes using non-purified ingredients other changes also occurred in the diet.

From the experiments with the fresh-frozen and the formulated diets it appeared that the diets affected the reproductive performance of prawns in different ways. The diet affected the number of spawnings per female and larval survival with BD2 giving a higher number of spawnings per prawn than the FFMI-B (Fig. 10). The diets tested in each of the four experiments had no effect on spawner survival, fecundity or hatch rate. However, survival of larvae from prawns fed diet BD2 was higher than for the FFMI-B and BD1 diets (Fig. 11). The difference in survival was apparent at the first spawning, and became increasingly apparent with successive spawnings (Fig. 12).
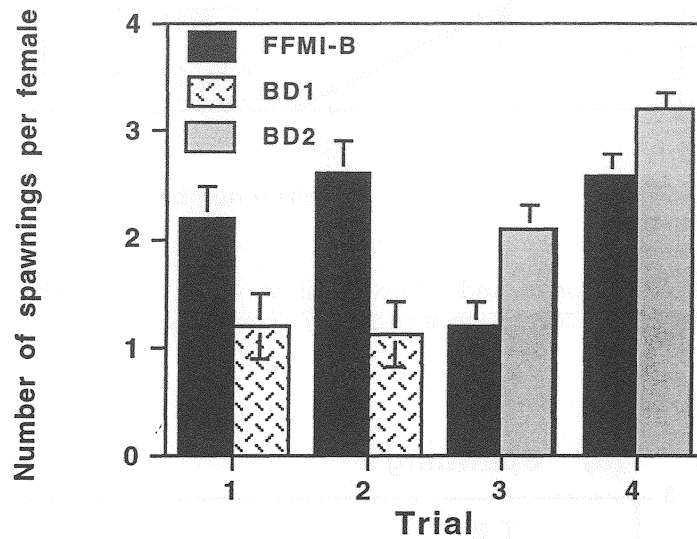

FIGURE 10.

The effect of diet fed prior to and following ablation of *Penaeus monodon* broodstock on number of spawnings produced per female in four successive seasonal samples.
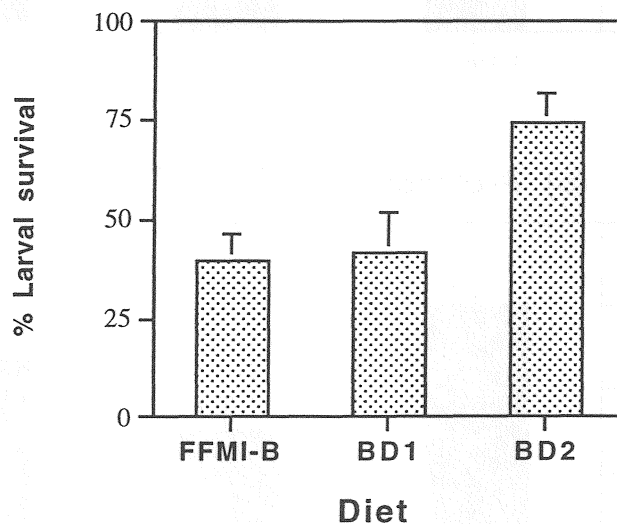

FIGURE 11.

The effect of diet fed prior to and following ablation of broodstock *Penaeus monodon* on percentage larval survival to zoeal stage.
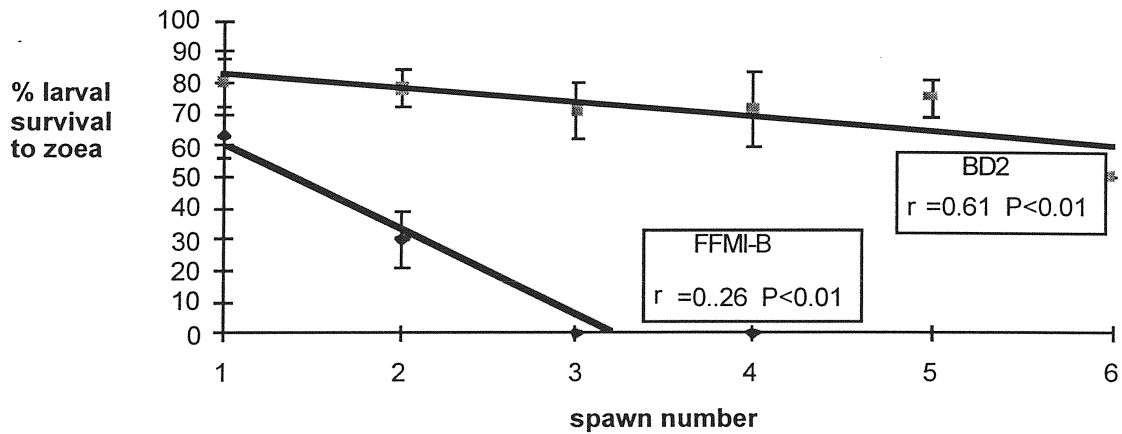
FIGURE 12.

The effect of diet fed prior to and following ablation of broodstock *Penaeus monodon* on percentage larval survival to zoeal stage at successive spawnings.
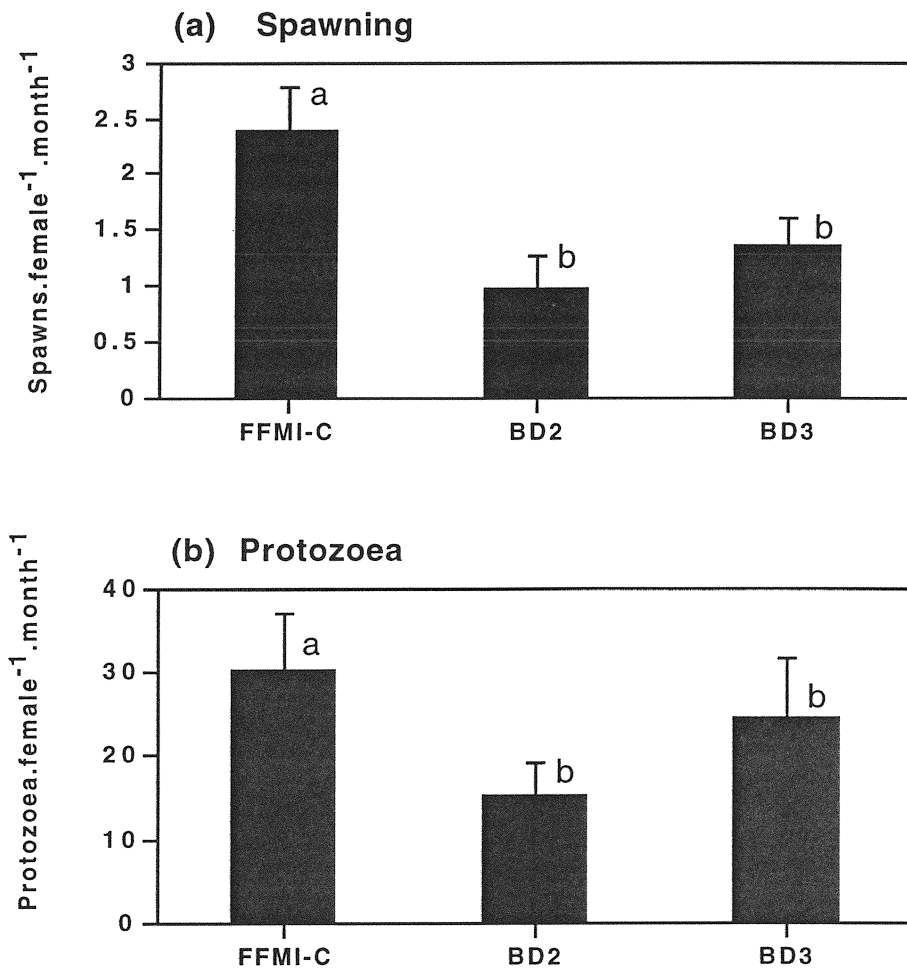

FIGURE 13.

The effect of a fresh frozen marine invertebrate (FFMI-C) diet and two formulated diets (BD2, BD3) on the reproductive performance of *Penaeus monodon*.

BROODSTOCK DIETS BD2 AND BD3 COMPARED WITH FFMI-C

The spawn rate from the prawns fed FFMI-C was higher than that obtained with diets BD2 and BD3 (Fig. 13). This reflects the improvement of FFMI-C over FFMI-B. It .also demonstrates that the diet that provided the best reproductive performance with *P. semisulcatus* resulted in the best reproductive performance with *P. monodon*. The reproductive performance, in terms of spawning frequency and larval survival, of prawns fed the BD3 diet was markedly better than with BD2 but the differences were not statistically significant (Figure 13).

DISCUSSION

From the results of this study it has become evident that some aspects of reproductive performance are more sensitive to nutrition than others. The downward trend in larval survival with successive spawnings which was seen with the FFMI diets did not occur with the formulated diets. This suggests that the nutrient content of the formulated diets was not limiting ovarian development and were enhancing larval survival. With the accelerated rate of spawning due to ablation, it is hypothesised that there is insufficient time to accumulate optimal levels of nutrients in the ovary (Beard and Wickins 1980). It is possible that the higher levels of vitamins and/or carotenoids that are part of the artificial diets have contributed towards the better larval survival in the later spawnings.

The differences in spawning frequency that occurred with diet, suggests an interplay between diet, nutritional condition and the hormones that regulate ovarian development. The major ingredients and the nutrient profiles of the FFMI-C are similar to those in BD3. The BD3 diet has been demonstrated to provide the nutrients required for viable egg production, yet it results in a lower spawning rate. Measurement of feed intake of the broodstock prawns lacked precision but indicated that the dry matter intake of the prawns does not differ significantly when they are fed a FFMI diet or a formulated diet. This suggests that there is some stimulus, possibly a labile micro nutrient, that is missing from the formulated diets.

5.3.2.2 ARACHIDONIC ACID SUPPLEMENTATION (CSIRO, CLEVELAND)

INTRODUCTION

Two experiments were carried out where we evaluated the effect of an elevated level of the highly unsaturated fatty acid, arachidonic acid (20:4n-6), in broodstock diets. During previous research with *P. esculentus* (FRDC 89/50) we found that the arachidonic acid levels in the diets of wild broodstock prawns and the mixture of fresh-frozen marine invertebrates (FFMI-C) was about 0.25% (dry matter, range 0.19% to 0.5%) of the diet. This contrasted with a commercial broodstock diet where the arachidonic acid concentration was about 0.07% (dry matter) and which was not well accepted and resulted in poor reproductive performance. Arachidonic acid is an important precursor of prostaglandins which are associated with reproduction in vertebrates and in arthropods (Stanley-Samuelson and Loher 1986; Brenner and Bernasconi 1989). Its precise requirement in prawn nutrition and reproduction has yet to be investigated.

METHODS

The experiments were run in September 1993 and March 1994 with two age classes of prawns. Groups of 15 male and 15 female *P. semisulcatus*, of reproductive size, were held in six 10,000 L tanks each with sand substrate and an open seawater system. Prawns were fed to excess twice each day and the water was maintained at 27°C . Two tanks served as the controls and were fed FFMI mixture consisting of squid, prawn and mussel (FFMI-C). Two tanks of prawns were fed the base diet, BD4 (Table 13) and two tanks were fed a diet identical to BD4 but which contained an additional 0.3% (dry weight) arachidonic acid (BD5). This level of arachidonic acid was selected as it was very similar to the level in the natural diets of the prawns. The arachidonic acid was converted to the ethyl ester form (Christie 1982) prior to its inclusion in the feed (Lochmann and Gatlin 1993) to minimise leaching loss from the diet and any possible toxic effects of the free arachidonic acid.

RESULTS AND DISCUSSION

The spawn rate of *P. semisulcatus* fed the FFMI-C diet was about double that of the prawns fed the test diets (Fig. 14). In both experiments, the prawns fed the arachidonic acid enhanced diet had a higher spawn rate and level of egg production than those fed the base diet but this was not statistically significant. The hatch rate, number of nauplii produced and the number of zoea produced (Fig. 14), were variable and no benefit from the higher level of arachidonic acid could be detected. However, recent work under the CRC for Aquaculture has demonstrated that prawns do not readily digest the ethyl esters of fatty acids. Since we used the ethyl esters of arachidonic acid in the diets, the prawns would not have gained the benefit of the arachidonic acid supplementation.

Further work on the inclusion of other micronutrients in the broodstock diet was not carried out as the maturation facilities and funds allocated to this work were directed to repeating the collection of broodstock prawns and repeating the feeding experiments with arachidonic acid in March 1994.

TABLE 13.

Ingredient and major nutrients in the base diet (BD4), and exceptional diet (BD5) used in the arachidonic acid experiments. (ingredients as used (g/kg), nutrients as % of dry matter)

Ingredient / Nutrient	Diet BD4	Diet BD5
Wheat Gluten	60	60
Starch	130	130
Tasmanian Fishmeal	230	230
Squid	200	200
Prawn meal	140	140
Methionine	5	5
Vitamin Premix	9	9
Binder	6	6
Humectants	8.8	8.8
Squid oil	17.1	17.1
Lecithin	17.1	17.1
Cholesterol	2.9	2.9
Canola oil	2.9	-
Ethyl arachidonate	-	2.9
Crude Protein	44.5	44.5
Total Lipid	7.8	7.8
Cholesterol	0.7	0.7
Phospholipid	2.3	2.3
n-3 HUFA	1.9	1.9
n-6 HUFA	0.15	0.37
Arachadonic acid	0.03	0.31

Sept 1993

March 1994
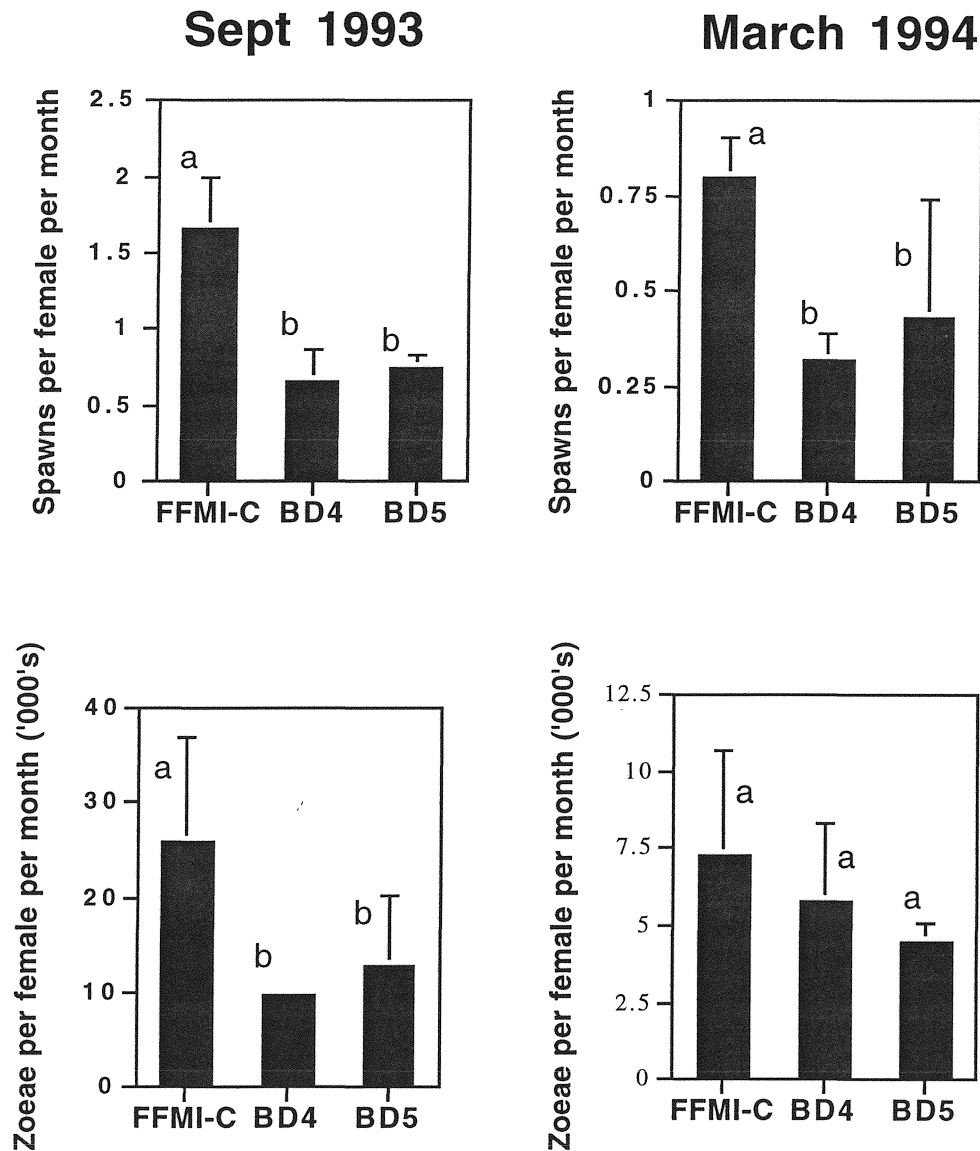
FIGURE 14.

Penaeus semisulcatus. Spawn rate (spawns female^{-1} month^{-1}) and zoeal production (zoeae female^{-1} month^{-1}) of prawns fed one of three diets, a mixture of fresh frozen marine invertebrates (FFMI-C), a base diet (BD4), and the base diet with elevated arachidonic acid content (BD5).

CONCLUSION

This study has demonstrated that the mixture of fresh frozen marine invertebrates, (FFMI-C: 37% pipi, 37% squid and 26% prawn, on a fresh weight basis) as used with *P. semisulcatus* provides the best reproductive performance in *P. monodon*. An alginate binding method has been adopted that enables the preparation of formulated diets with excellent water stability at 4 h. This binding method enables the preparation of diets without the need for heating to activate the binding mechanism. In eliminating the heating step in diet preparation, the attractiveness and palatability of the diets has been retained. The performance of the broodstock diets formulated in this project has been below that achieved with the FFMI-C diet

though they have performed better than the FFMI-B diet which was widely used in commercial hatcheries at the start of the project. Further development of the formulated broodstock diet will require better definition of the essential HUFA requirements and a study to determine how the formulated diets can be improved to result in a spawning rate close to that achieved with the FFMI-C diet.

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5.4 REPRODUCTIVE PERFORMANCE OF POND-REARED *P. MONODON* (QDPI BIARC)

- Using the pond management and hatchery practices employed in this study, pond reared broodstock would not be a viable option for wild-caught spawners. Optimal rearing conditions have not been clearly defined.

BACKGROUND

To culture the black tiger prawn, *Penaeus monodon*, Australian prawn hatchery operators rely on a supply of wild broodstock prawns, most of which are caught in shallow waters off Cairns, Queensland. The disadvantages of using wild broodstock are that catches are low between September and February when demand is high, and reproductive performance varies throughout the year.

This study aimed to assess two methods of alleviating the periodic short supply of wild-caught spawners. The first method is the stockpiling of wild-caught spawners in ponds during times when catches are in excess of hatchery demand. The second method is to use pond-reared broodstock, thereby allowing hatchery production to be independent of wild-caught spawners. Using pond-reared broodstock has the added advantage of enabling genetic selection for stock improvements and offers the potential for pathogen free stocks to be developed.

This study was therefore divided into two parts: broodstock captured at maturity from the wild and stockpiled in ponds, and broodstock reared to maturity in ponds.

5.4.1. ASSESSMENT OF BROODSTOCK CAPTURED AT MATURITY FROM THE WILD AND STOCKPILED IN PONDS

OBJECTIVES

The aims of this part of the study were to assess whether the reproductive performance of wild broodstock *P. monodon* was affected by (i) a 6 or 12 week holding period in a commercial growout pond, and (ii) a 4 week, as opposed to 2 week, pre-ablation acclimation period in the hatchery. These trials were run concurrently with trials aimed at assessing the suitability of an artificial broodstock diet developed as part of this project. The 6 week control and treatment groups were fed the standard BIARC broodstock diet of squid/mussel (FFMI-B). The 12 week control and treatment groups were fed the artificial diet BD2.

METHODS

POND PHASE

Adult *P. monodon* were collected from Cook Bay, Cairns by a commercial operator (Cairns Live Prawns) using a beam trawl.

Two hundred *P. monodon* (133 females, 67 males) caught on the 23 March 1994 were stocked in a 0.15 ha earthen growout pond (Maximum depth 2.0 m) at a commercial prawn farm at Mossman (Sea Ranch). Density at time of stocking was 0.9 prawns m^{-2} as the pond was also stocked with prawns for another experiment. They were fed 4 times a day on a commercial pellet of 50% dry weight protein.

Ponds were managed according to protocols commonly used in the grow-out phase of prawn production.

At the time of stocking, a subsample of broodstock was sent to the Bribie Island Aquaculture Research Centre (BIARC) facilities to have their reproductive performance assessed after 2 weeks acclimation on either the BD2 or FFMI-B diet. After 6 weeks, 28 females and 15 males, harvested using a cast net, together with a sample of broodstock captured at that time from the wild, were shipped to BIARC and spawned. After twelve weeks the pond was drained and the remaining prawns sent to BIARC for assessment. At this time there were no broodstock available from the wild.

CONDITIONING AND HATCHERY PHASE

After arrival at BIARC, prawns were weighed, eye-tagged and allocated a tank according to weight such that each tank was representative of the size range received. Each prawn was also carapace marked with nail polish for identification of moulted exuviae.

Prawns were maintained for 2 or 4 weeks before ablation and fed FFMI-B diet or BD2 *ad libitum* at 10:00 and 17:00 daily. Prawns were held in 10,000 L circular fibreglass tanks (4.0m diameter; 0.8m depth) at an average density of 1.75 prawns m^{-2} and a ratio of two females to one male. Incoming seawater (33‰, pH 8, DO 6 ppm) was filtered to 20 μm, heated to 28°C and exchanged at 200% per day. Light intensity was 5 lux at the water surface. Photo period was 14L:10D, with a 20 minute ramp period.

Ovarian development was determined each afternoon using a submerged flash light to reveal the shadow of the ovary on the dorsal exoskeleton. Females with ripe ovaries (Stage IV) were removed and placed in a 0.15 m^3 spawning drum with filtered seawater (0.5μm) at 28°C with a 1000% daily exchange rate. Egg and larvae numbers were estimated by vigorously agitating the water and counting numbers in 4 x 110 ml aliquots. Larvae were left to develop in the spawning drums and counted at first nauplii stage (Nl) and first protozoeal stage (Zl). Spawner performance parameters recorded were the number of spawns per prawn, the number of eggs per spawn, the egg hatch rate, the survival to Z1, and the days between ablation and first spawn. Differences between groups were assessed using a one-way analysis of variance.

RESULTS

SURVIVAL IN PONDS

The first attempt at assessing the reproductive output of wild-caught spawners held in ponds resulted in 100% mortality in the pond. The major cause of this was thought to be an extended period of heavy rain that resulted in a drop in pond salinity from 33 to 5‰ over a period of less than 1 d. The salinity remained low for 3 d. To test this hypothesis an experiment was carried out comparing the salinity tolerance of 8 g prawns with that of 100 g wild-caught spawners. All prawns were subjected to a drop in salinity from 34 to 5‰ over a 3 h time period. Prawn survival was monitored over the next 6 days. At day four, salinities were brought back up to 34‰. Results of this experiment showed that while the 8 g prawns tolerate both the decline and increase in salinity (100% survival) the 100 g prawns

showed a 35% mortality by the first day increasing to 60% by the third day. No increase in mortality occurred with the increase in salinity. However, the 100 g prawns appeared highly stressed. Prawns in a pond subjected to a similar treatment may be expected to continue to show high mortality even when pond salinities returned to normal.

During the second attempt conditions in the pond showed no severe fluctuations in either temperature or salinity. Total survival for all prawns stocked in March for 12 weeks was 50.4%. Pond conditions for the two groups are summarised as follows:

	Held 6 weeks	**Held 12 weeks**
DO	4.2 - 9.8mgL^{-1}	4.0 - 10.2mgL^{-1}
TEMP	25.0 - 29.0 °C	21.0 - 29.0 °C
SALINITY	34.1 - 35.5‰	34.1 - 36.5‰

REPRODUCTIVE OUTPUT

Diet affected reproductive performance of wild-caught spawners (See Section 5.3). When prawns were fed the BD2 diet (Groups B and D) rather than the FFMI-B diet (Groups A and C), the number of spawns per prawn and larval survival increased (Table 14). However, the use of two diets has limited the comparisons that can be made between wild-caught and the pond held broodstock. As a number of different parameters are used here to measure reproductive performance, it is useful to look at their combined effect on total larval (protozoeal 1) production. The reproductive output of prawns in the various groups (Number of Z1 produced per prawn = # of spawns x # of eggs/spawn x hatch rate x survival to Z1).

Prawns held in the pond for 6 weeks (Group E) showed no change in the number of spawns per prawn or hatch rate when compared to prawns spawned at time of stocking (Group A) (Table 14). Spawner survival and fecundity (despite an increase in prawn weight) were lower, however larval survival was higher. When compared to prawns captured from the wild in June (Group C) the prawns held in the pond for 6 weeks showed less spawns per prawn and lower fecundity and larval survival. The protozoeal production of pond held broodstock was about one third of that of the wild-caught prawns (Group C).

Prawns held in the pond for 12 weeks (Group F) showed an increase in fecundity however hatch rate and larval survival decreased when compared to prawns assessed at the time of stocking (Group B). This resulted in a 50% reduction on the protozoeal production when compared with the wild-caught prawns.

Acclimating prawns for 4 weeks in the hatchery (Group G), as opposed to 2 weeks (Group F), reduced the number of spawns per prawn though on average hatch rate and larval survival increased (Table 14). There was not a marked difference between the numbers of protozoea produced.

Prawns held in the pond for 12 weeks and spawned in July could not be compared to prawns captured from the wild at that time as there was a severe shortage in the supply of wild stock. Prawns are frequently difficult to obtain at that time of the year as demand is high and catches are low.

TABLE 14.
Spawning performance of wild and pond-held broodstock

GROUP	A	B	C	D	E	F	G
ORIGIN	WILD	WILD	WILD	WILD	POND HELD A	POND HELD B	POND HELD C
DIET	FFMI-B	BD2	FFMI-B	BD2	FFMI-B	BD2	BD2
TIME OF TRIAL	MARCH	MARCH	JUNE	JUNE	JUNE	JULY	AUGUST
Spawner weight (g)	82.2 ±4.0a n = 27	91.3 ±4.0a n = 27	111.8 ±3.6b n = 28	111.7 ±3.3b n = 31	103.1 ±3.7b n = 25	122.3 ±3.7c n = 22	127.9 ±3.5c n = 20
Spawner survival (%)	97.4 ±2.6a n = 27	95.7 ±3.0a n = 27	98.2 1 ±.7a n = 29	97.5 ±1.7a n = 31	90.1 ±4.8a n = 25	95.5 ±3.3a n = 22	97.9 ±1.5a n = 20
Spawns per spawner	1.1 ±0.2a n = 27	2.3 ±0.2b n = 27	2.6 ±0.2b n = 29	3.0 ±0.35b n = 31	1.3 ±0.3ac n = 25	2.0 ±0.4bc n = 22	1.5 ±0.4ac n = 20
Eggs/spawn ('000's)	357.8 ±28.8a n = 18	303.0 ±18.6a n = 44	331.0 ±13.9a n = 54	353.4 ±13.9a n = 76	242.8 ±18.5b n = 22	351.3 ±21.7a n = 32	352.8 ±34.3a n = 22
Egg hatch rate (%)	57.5 ±9.0ab n = 18	49.1 ±5.5a n = 43	66.0 ±4.1b n = 54	56.2 ±3.6ab n = 75	54.6 ±8.0ab n = 21	33.1 ±6.0c n = 32	42.3 ±7.5ac n = 22
Survival to Z1 (%)	38.0 ±10.9a n = 13	73.6 ±3.9b n = 38	41.4 ±5.9a n = 51	75.2 ±2.6b n = 69	46.0 ±10.0ac n = 21	56.5 ±6.1ac n = 27	64.3 ±6.2bc n = 20
Days to first spawn (days)	11.1 ±1.5ab n = 17	10.5 ±1.5ab n = 24	8.9 ±0.9a n = 16	8.0 ±0.7a n = 24	9.4 ±1.6ab n = 16	13.3 ±1.9b n = 18	12.2 ±2.9ab n = 13
Protozoeal production (10^3 Z1)	86.0	251.8	235.1	448.1	79.3	131.4	143.9

Within row values with a common superscript letter are not significantly different (P>0.05)
Individual spawner survival values were determined as a percentage of the total length of the trial (approx. 42 days).
A Prawns were held in a pond for 6 weeks before being shipped to the hatchery, then held for 2 weeks before ablation
B Prawns were held in a pond for 3 months before being shipped to the hatchery, then held for 2 weeks before ablation
C Prawns were held in a pond for 3 months before being shipped to the hatchery, then held for 4 weeks before ablation
For all other trials, prawns were held in the hatchery for 1-2 weeks before ablation.

DISCUSSION

From the experiments conducted it would appear that a holding period of 6 weeks had little effect on reproductive output. However the quality of prawns from the wild during March (the time of stocking) was very poor as is evidenced by the higher productivity of prawns captured from the wild in June. This fluctuation in the quality of wild-caught spawners is studied in more detail in Section 5.1. Part of the fluctuation in pond-reared spawners is due to diet. In March the production from prawns fed BD2 was nearly treble the production from prawns fed FFMI-B whereas in June it was about twice the production. This suggests that there was a significant difference in the nutritional condition of the wild broodstock caught in March and June. From the protozoeal production it appears that the prawns captured in March were in poorer condition and that this condition was not improved by the 6 week holding period in the pond.

Holding prawns in the pond for 12 weeks resulted in a halving of reproductive output. The drop in production may be due to unfavourable environmental factors such as temperature, salinity, light regimes and/or density in the ponds. Pond depth is another factor that has been implicated in the determination of spawner quality. It was interesting to note that the lower production rate was due not so much to a

decrease in spawning frequency, which is characteristic of pond reared spawners, but rather a decrease in hatch rate and larval survival.

There are many factors to be considered when planning to stockpile wild-caught spawners in ponds; foremost of these are (i) having access to a pond that will have suitable temperature and salinity regimes over the period concerned, and (ii) there being a surplus in supply of spawners at the appropriate time.

Holding prawns in ponds does reduce the risks of a hatchery being unable to secure spawners from the wild. However, the economic feasibility of holding wild-caught prawns in ponds depends on the initial purchasing price of the prawns, the operating costs of the pond, the survival of prawns in the pond and hatchery phases, and the productive output per spawner.

SUMMARY

- Holding wild-caught prawns in a pond for 12 weeks under the conditions outlined in this study halved the reproductive output.

- Diet fed to the broodstock during the conditioning and hatchery phase had a dramatic effect on reproductive output.

- The nutritional condition of prawns in the wild varies with the time of year and apparently differs from the condition developed in the pond.

- Broodstock diets may need to be developed to specifically meet the needs of prawns held in ponds. These would be more nutrient rich than grow-out diets and could differ from those used during the hatchery phase.

5.4.2. ASSESSMENT OF BROODSTOCK REARED TO MATURITY IN PONDS

INTRODUCTION AND OBJECTIVES

Hatchery operators both overseas and in Australia use wild-caught spawners preferentially to pond-reared broodstock. Trials in the Philippines have shown that pond-reared broodstock had lower spawning frequency and poorer larval survival than the average wild-caught spawner (J. Primavera, SEAFDEC, pers com).

The aim of this study was to (i) assess the reproductive performance of 12 month old pond-reared broodstock, and (ii) to compare the reproductive performance of the 12 month old to that of 14 month old broodstock.

METHODS

Two trials were carried out. Stockings were scheduled so that broodstock would be 12 months of age between August and September when catches from the wild cannot meet the high demand from hatcheries.

TRIAL 1

In January 1993, 200 female and 200 male *P. monodon* juveniles (124 d old) were selected during the harvesting of a commercial growout pond (where they had been grown at a density of 20 m^{-2}) located at Sea Ranch prawn farm, Mossman, Queensland. Following the streamer tagging and weighing of a subsample of 50 females and 50 males, all prawns were transferred to a neighbouring 0.15 ha pond where they were stocked at a density of 0.8 m^{-2} (prawns for other experiments were also stocked in the pond). Ponds were managed in accordance with normal growout practises. Prawns were fed a high protein (50%) commercial pellet.

In September 1993 when the prawns were 12 months old, the pond was drain harvested and the prawns were collected. The 40 females and 30 males that survived the on-growing and the harvesting were kept overnight in tanks, packed and airfreighted to BIARC. Upon arrival, prawns were tagged, weighed and randomly allocated to two tanks (as described in Section 5.4.1). Prawns were acclimated for 2 weeks and then ablated and monitored for 6 weeks. If an individual had not moulted during the 2 week acclimation time, ablation was postponed. Data were collected according to the methods outlined in Section 5.4.1. Prawns were fed an alternating diet of squid and mussel, FFMI-B.

TRIAL 2

In January 1994, 180 female and 100 male juveniles (150 d old) were captured using a cast net from a commercial *P. monodon* pond at the Burdekin Shrimp Farm, Ayr, Queensland. Prawns were weighed and streamer tagged before restocking into a 1 ha commercial pond also containing *Penaeus japonicus* at a density of approximately 18 m^{-2}. Prawns were fed the same commercial prawn pellet fed in Trial 1.

In August 1994, the pond was drain harvested and the 12 month old *P. monodon* collected. All pawns were held in tanks overnight. The following day 35 females and 25 males were randomly selected, packed and airfreighted to BIARC. Upon

arrival at BIARC prawns were handled as per Trial 1 except prawns were fed a diet of squid, mussel, prawns (FFMI-C) and the artificial diet (BD2) on a rotational basis.

RESULTS

The two trials were not designed for direct comparison as the broodstock differed in their origin, their pond rearing conditions were different and the diets used during the hatchery phase differed. Data from wild-caught *P. monodon* spawners captured in July 1993 has also been included in Table 15 for comparative purposes.

The survival to harvest of the pond-reared prawns was 40% in Trial 1 and 60% in Trial 2 (Table 15). There were further mortalities as a result of harvesting and transport to BIARC. The survival of the broodstock in the hatchery tanks was generally consistent with other experiments (See Section 5.1.2) though that of Trial 1 was lower. The reproductive performance of both lots of pond reared broodstock was poor compared to that of the wild-caught spawners captured in late July 1993. This low level of reproductive performance extended from the level of spawning, the fecundity and the hatch rate of the eggs. It is interesting to note that the survival of the nauplii that were produced was not significantly different across treatments.

TABLE 15.
Reproductive performance of pond reared and wild-caught spawners.

	Wild-caught July 1993	Pond reared 1993 (trial 1)	Pond reared 1994 (trial 2)
Age (months)		12	11.3
Survival in pond (%)		40	60
Weight increase (grams/week)		4.0	2.2
Diet (hatchery phase)	FFMI-B	FFMI-B	FFMI-C/BD2
Survival in tanks (%)	82	68	80
Number ablated	33	27	28
Weight (grams)	140	134	87
Ovary developed (%)	90	66	86
Spawns/prawn	2.3	0.07	0.1
Spawned (%)	75	7	11
Fecundity (eggs x 1000)	497 ± 28	110 ± 21	120 ± 32
Hatch rate (%)	47.6 ± 7	30 ± 14	21 ± 10
Protozoeae survival	40.5 ± 7	42 ± 19	50 ± 13

DISCUSSION

Spawning frequency is used as a measure of reproductive performance because there was insufficient data on fecundity, hatch rates and larval survival to give a true indication of all round performance.

The number of spawns per prawn for both groups of pond reared broodstock was poor compared to that of the wild spawners captured during July 1993. This was also found by J. Primavera, SEAFDEC (pers. com.) whose work on *P. monodon* in the Philippines showed a long delay before the first spawnings occurred (average of 23 days post ablation) and between successive spawnings (average of 11 d) when compared to wild-caught spawners. This would suggest an extended post-ablation holding period would increase the number of spawnings per prawn. However, in the current study while in-tank mortality was not particularly high, prawns appeared stressed 3 weeks after ablation and signs of ovary development decreased. Thus it is unlikely that an extended experimental period would have resulted in a higher percentage of prawns spawning.

The reproductive performance of pond-reared *P. monodon* was poor compared to that reported by Makinouchi and Hirata (1995) for similar studies carried out in Malaysia. In their study involving nearly 300 spawners they recorded 51.4 % of prawns spawning. The results from this study were between 7 and 11 %.

The conditions under which the prawns in Trial 1 were reared in the pond and held in the hatchery were similar to the experiments using pond reared (as opposed to wild-caught) males carried out by Makinouchi and Hirata (1995). No mention of stocking rates is made in their study, however both studies used only commercial growout pellets during the on-growing phase in the pond and squid/mussel during the acclimation/hatchery phase. The acclimation time in Makinouchi and Hirata's (1995) study was 3 to 5 days and the experiment went for 35 days. The acclimation time in this study was a minimum of 14 d and the experiment went for 42 days.

Given the similarities between the studies it is interesting to note the large difference in spawning frequency. Age and diet have been shown to influence spawning frequency (see Section 5.1 and 5.2). However, though natural productivity in the ponds probably differed, the diets used in the two studies were similar, as was the age of prawns (approximately 12 months). Therefore, neither age nor diet is likely to be responsible for the differences in spawning frequency found between the two studies.

Trial 2, carried out as part of this study on pond reared broodstock, looked at the feasibility of keeping prawns at a high density throughout the pond phase. This was combined with improvements made to the diet fed during the acclimation and hatchery phase. Weight gain was lower than in Trial 1, however, no other differences in performance were detectable with the amount of data available.

SUMMARY

- Using the current pond management and hatchery practices employed in this study, pond reared broodstock would not be a viable option to wild-caught spawners.

- It is possible that the low levels of reproductive performance were a result of the broodstock being infected with the disease which became chronic in the on-growing phase of Trial 2.

- Many studies have been carried out on pond-reared broodstock, however results are rarely comparable due to differences in experimental procedures. Optimal rearing conditions have not therefore been clearly defined.

- The reproductive performance of broodstock in this study was poor compared with that of broodstock grown and spawned under similar conditions in Malaysia. The diet (fed during the pond and hatchery phases) and age of prawns did not account for the differences, but could be improved.

- While it was not shown by this study, it is hypothesised that geographic and environmental conditions in the ponds (related to seasonal and climatic factors and to pond and disease management) and genetic factors may play important roles in determining the reproductive output of pond reared broodstock.

- More studies assessing the effects of various environmental conditions and enhanced management practices are required if the reproductive performance of pond reared broodstock is to be predictable. For the performance to be improved studies will need to entail hormonal, nutritional and genetic components.

REFERENCES

Makinouchi, S. and Hirata H., 1995. Studies on maturation and reproduction of pond-reared *Penaeus monodon* for developing a closed life-cycle culture system. Israeli Journal of Aquaculture, Bamidgeh. 47: 68-77.

6. SUMMARY AND RECOMMENDATIONS

• Broodstock age is a critical factor to be considered in the selection of broodstock. The critical effect of age on the reproductive performance of *P. semisulcatus* broodstock has been clearly demonstrated in this study. Given the similarity in penaeid prawn life histories, this species can reasonably be regarded as a proxy for the *P. monodon* case; and similar age effects have been alluded to in other studies of *P. monodon*. Therefore, increased reproductive performance could reasonably be expected from broodstock selected at about 12 months old.

• Two good indicators of nutritional condition have been established. These are the blood lipid content and the blood refractive index. However, the sampling of blood is likely to cause unacceptable stress to broodstock prawns with developing ovaries. The abdominal turgidity of prawn, measured by gently squeezing the abdomen, is related to nutritional condition but it is complicated by the subjectivity of the measurement and degree of ovarian development. These indices can contribute to the prediction of reproductive performance but other factors such as age must be taken into consideration.

• Artificial broodstock diets have been developed which are alginate bound. These diets have not performed as well as the best mixture of fresh frozen marine invertebrates which were developed for *P. semisulcatus* and subsequently used with *P. monodon*. Artificial diets did result in an increase in survival of nauplii through to the first protozoeal stage whereas the fresh frozen marine invertebrate diet always resulted in a higher number of spawnings. There is potential to develop the artificial broodstock diets further; the definition of the requirements for the essential HUFAs using the techniques currently in use in the CRC for Aquaculture, would be of immediate benefit. Further research is needed to discover what factor is causing the higher spawning rate of prawns fed the FFMI diets.

• Larvae can be obtained from wild-caught broodstock stockpiled in ponds, however with current practices the hatch rate and quality of larvae produced appears to be reduced. Longer holding periods appear to result in a greater reduction in reproductive performance. From these results the economic feasibility of this method of reducing periodic shortfalls in spawner supply is therefore generally inadvisable, but would depend largely on the initial purchase price of the spawners, the long-term survival in ponds during the holding period, and reproductive outputs.

• Pond-reared broodstock have been used to supplement the supply of wild-caught spawners, however production from pond-reared broodstock in this study was found to be sub-optimal. The major problem was the low spawning rate and resultant low larval production. The factors influencing spawning rates need to be understood before optimal rearing conditions to improve reproductive performance can be established. Based on the findings in Section 5.1, age-based selection is one approach which could provide improved performance. The wider commercial use of pond-reared broodstock is essential for management of introduced disease, development of specific pathogen free (SPF) stocks and selective breeding for other traits.

7. TRANSMISSION OF RESULTS TO THE INDUSTRY

Seminars, meeting presentations, reports:

Crocos, P.J. 1993. Seasonal variability in prawn broodstock reproductive performance, Australian Mariculture Association, Annual Conference, Brisbane, July 1993.

Crocos, P.J. 1995. Factors affecting the reproductive performance of *Penaeus semisulcatus* broodstock. World Aquaculture Society Symposium, San Diego, USA, January 1995.

Crocos, P.J., 1995. Seasonal and age variability in the reproductive performance of *Penaeus semisulcatus* broodstock. Invited oral presentation Larvi 95, Fish and shellfish larviculture symposium, Ghent, Belgium. September 1995

Crocos, P.J. 1996. Assessing reproductive performance of penaeid broodstock. CRC for Aquaculture, Scientific Review Committee. AIMS March 1996.

Crocos, P.J., (in prep). Age is important in the selection of penaeid broodstock. Austasia Aquaculture

8. LIST OF PAPERS ARISING FROM THE PROJECT

Crocos, P.J., and Coman G.J., 1997. Seasonal and age variability in the reproductive performance of *Penaeus semisulcatus* broodstock: optimising broodstock selection. Special publication of papers presented at Larvi 95, Fish and shellfish larviculture symposium, Ghent, Belgium, September 1995, Aquaculture (in press).

Crocos, P.J., and Coman G.J., (in prep). Quality of consecutive spawnings of Penaeus semisulcatus broodstock. Aquaculture

Hansford, S.W. and Marsden, G.E. 1995. Temporal variation in egg and larval productivity of eyestalk ablated spawners of the prawn *Penaeus monodon* from Cook Bay, Australia. Journal of the World Aquaculture Society, 26(4): 396-400.

Moore, L.E., Smith, D.M. and Loneragan, N.R. (in prep) Blood refractive index and whole body lipid content as indicators of nutritional condition for penaeid prawns. Journal of Marine Biology and Ecology

9. BENEFITS

The primary beneficiaries of this research will be hatchery operators and researchers. The secondary beneficiary is the prawn farming industry as these research outcomes lead to improvements in the availability of good quality postlarvae at the times that they are required. The use of 12 month old broodstock will help optimise hatchery effort where there is the availability of broodstock to choose from.

The mixture of fresh frozen marine invertebrates (FFMI-C) for feeding broodstock prawns in the hatchery has been demonstrated to be the best evaluated in this study and provides hatchery operators with an alternative to the diets that they are currently using. The information gathered in this project on the preparation and formulation of broodstock diets and the nutrients that are related to improved reproductive performance will contribute to the future development of a commercially acceptable broodstock diet.

The benefits seen in the original proposal were focussed on improving the availability of spawners for the hatchery sector. The pond conditioning of wild-caught spawners and the on-growing of pond-reared broodstock carried out in this project have not provided a solution to improve the availability of broodstock prawns.

10. INTELLECTUAL PROPERTY AND VALUABLE INFORMATION

- Increased reproductive performance could reasonably be expected from broodstock selected at about 12 months old.

- A mixture of fresh frozen squid *(Loligo spp.)*, chopped prawns *(Metapenaeus bennettae)* and bivalves *(Plebidonax deltoides)* (FFMI-C) was found to be the best hatchery diet for broodstock of both *P. monodon* and *P. semisulcatus* .

- Biochemical analyses of tissues and eggs supports the hypothesis that the arachidonic acid levels of broodstock diets may be a key to improving the reproductive performance through nutrition.

11. FURTHER DEVELOPMENT

- There is potential to develop the artificial broodstock diets further; the definition of the requirements for the essential HUFAs using the techniques currently in use in the CRC for Aquaculture would be of immediate benefit. Further research is needed to discover what factor causes the higher spawning rate of prawns fed the FFMI diets.

- The development of practices that will result in satisfactory reproductive performance of pond-reared broodstock is essential for the continuing viability of farming of *Penaeus monodon*. Successes with *P. japonicus* and *P. esculentus* (CSIRO) suggest that the task is a matter of working out the most appropriate rearing practices.

12. STAFF:

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13. FINAL COST:

	FRDC	CSIRO/QDPI	Other Sources	Total
Salaries and On-costs	$401,818	$512,086	$0	$913,904
Travel	$41,290	$21,280	$0	$62,570
Operating	$274,455	$86,800	$0	$361,255
Capital	$0	$1,200,00	$0	$1,200,00
Total	**$720,563**	**$1,820,166**	**$0**	**$2,537,729**