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Final Report

A comparison of techniques for inducing maturation, viable spawning and embryo cryopreservation of Australian penaeid prawn species with potential for aquaculture

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Executive Summary

This study was undertaken by a team of CSIRO Division of Fisheries research scientists combining the disciplines of ecology, nutritional and reproductive physiology, and embryology.

The goals of the study were to:

- induce maturation and spawning using techniques based on cues from the natural environment
- compare maturation-induction techniques and assess their relative suitability for commercial production
- investigate the role of nutrition in maturation and formulate an artificial diet to enhance maturation
- develop techniques for the cryopreservation of prawn embryos and larvae.

The main findings of the study were:

- Rates of maturation, spawning and larval survival can be improved, without eyestalk ablation, by controlling the environment and feeding fresh and formulated diets. However, the overall rates are lower than with ablated prawns.
- The ability to induce spawning in captivity and get viable larvae is related to the age of the spawners.
- The feed formulations (based on the nutritional composition of natural diets) developed by the team achieved higher rates of larval survival than did proprietary feeds.
- Successful cryopreservation of penaeid eggs, embryos and larvae continues to be an elusive goal because of the sensitivity of embryos and larvae to chilling and to changes in solute concentration.

Background and Justification

The developing Australian prawn culture industry is limited by the unpredictable supply of postlarvae. Currently, female spawners are obtained from the wild, which is both costly and unreliable. They are transported to a hatchery and either allowed to spawn naturally or, more usually, induced to spawn by eyestalk ablation.

There is now considerable evidence that ablated prawns held in captivity have a lower fecundity, egg viability and hatch rate than ablated and unablated wild stock (see Primavera, 1985 for review). The inconsistent quality of eggs, particularly with successive spawnings, has been attributed to the abnormally rapid maturation and overstimulation of spawning following ablation. This decline in performance limits the reproductive life of spawners, necessitating frequent and expensive replacement of broodstock.

CSIRO has made considerable progress in closing the life cycle of the two tiger prawn species *Penaeus esculentus* and *P. semisulcatus*. Both have been brought to maturity on a number of occasions and have spawned successfully in the laboratory without eyestalk ablation. These experiments showed that stimulation of maturation and spawning by manipulating the environment is possible without eyestalk ablation (Crocos and Kerr, 1986). However, the process is still poorly understood. In the wild, tropical penaeids typically have two natural spawning periods: an initial small spawning in Autumn of young first-mature adults about 6 months old, then a second larger spawning in Spring at about 12 months old. Both external factors (environment, nutrition) and internal processes need further research; egg production, hatching success and the viability of larvae need to be accurately monitored. Such research should enable the Australian aquaculture industry to become independent of wild breeding stock, especially if a cryopreservation techniques for eggs or early larvae can be successfully developed. This would then allow genetic selection of stocks and more effective disease control of eggs and larvae.

Further, with the real possibility of controversy over the use of the eyestalk ablation technique, and potential conflict with Animal Protection groups, a future ban on this technique cannot be ruled out. If this happens, development and assessment of alternative techniques will be imperative.

In the Indo-West Pacific region the favoured species for aquaculture is *Penaeus monodon*. It has been intensively studied at the Tungkang Marine Laboratory in Taiwan, the Southeast Asian Fisheries Development Center (SEAFDEC) in the Philippines, and the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) in Tahiti, none of which has routinely closed the life cycle. IFREMER uses laboratory-reared prawns, but has to use eyestalk ablation to induce spawning. In Australia, AIMS's genetic research on *P. monodon* depends on closing the life cycle.

Many Australian aquaculturists are concentrating on *P. monodon*. However, this species may not be ideal for all the prawn farming regions of Australia. It requires brackish water for best growth, so is not well suited for many areas; it is not abundant in Australian waters and mature females are sometimes in chronically short supply; and the export market faces severe competition from South East Asian aquaculture farms.

Research in Australia should also include species that are more suited to Australian conditions and have good aquaculture potential. These species include the two

tiger prawns *P. esculentus* and *P. semisulcatus*, the banana prawn *P. merguiensis*, and the kuruma prawn (Japanese king prawn) *P. japonicus*. Both the tiger prawns adapt well to high salinities and are highly regarded on the Japanese market. The brown tiger prawn *P. esculentus* is endemic to Australia and thus has the potential to be a unique export product. The banana prawn is widely adaptable, grows quickly and also commands a good market price. The kuruma prawn is well suited for farming at higher latitudes and there is a well-established export market for this species, which commands the highest price of all the prawns.

Objectives of the Project

(a) To induce gonad maturation and spawning of females using techniques based on cues from the natural environment.

(b) To compare egg production and the viability of larvae from eyestalk-ablated and naturally matured females, and assess which method of handling/treating the females is most suitable for commercial production.

(c) To investigate the role of nutrition in natural gonad maturation from a study of the biochemistry of both the prawn and its natural foods, and to formulate an artificial diet that will enhance maturation.

(d) To develop techniques for the cryopreservation of prawn embryos and larvae.

Technical Report

Maturation And Larval Fitness

Introduction

A regular high quality supply of postlarvae for stocking growout ponds is necessary for the growth of the Australian prawn mariculture industry. The supply of wild-caught spawners is variable; maturation and spawning of prawns in captivity is variable; and the survival of larvae in hatcheries is variable. Current practice is to capture spawners from the wild, which is costly and unreliable. Typically these ripe females are induced to spawn in the hatchery by ablating their eyestalks. This is effective (Primavera, 1985), but has a detrimental effect on fecundity and hatch rate (Emmerson, 1980; Primavera & Posidas, 1981; Browdy & Samocha, 1985). An alternative is to create the environmental conditions in the laboratory that induce spawning at a comparable rate without a decline in fecundity or hatch rate. Other factors that affect reproductive performance, such as diet (Galgani, 1989) and selection of broodstock based on size or age also need to be considered.

This study sought to compare maturation induction techniques and to examine the factors affecting the overall reproductive performance of broodstock with a view to identifying the most benign method of optimising production of healthy larvae.

Methods

1. Reproductive performance trials

The prawns used in the trials were mature-sized adult (<35 mm CL) *P. esculentus* or *P. semisulcatus* collected by a commercial trawler in Moreton Bay or a chartered trawler (FRDC 89/13) in Albatross Bay, Gulf of Carpentaria. The trials were carried out in 3.6 m diameter x 1.2 m deep circular tanks (10,000 litres) provided with a sand substrate to enable the prawns to bury, and supplied with gently aerated flow-through filtered seawater at a turnover rate of 100% per day. Light levels in the tanks were lowered to approximate seabed levels at normal spawning depths. The prawns were stocked at low densities (2.5-5.0 m^{-2}), at a female:male ratio of 1:1. Each prawn was code-marked so its moulting, mating and ovary development could be monitored. The females were inspected daily (without disturbance) to assess ovary development, and ready-to-spawn females were removed to a 100 litre spawning tank. Egg counts were made for all spawnings, and nauplii were counted after hatching. For selected trials, a subsample of eggs was frozen for biochemical analysis, and samples of 3 x 300 nauplii from each spawning were held under standard conditions (28°C, 12:12 h light/dark) for 62 hours to assess survival to the zoeal stage.

2. Factors affecting reproductive performance

The effects of several factors that might influence maturation and spawning of captive broodstock prawns were assessed:

Physical Cues:

A knowledge of temperature and daylength cues from the natural environment that appear to promote maturation and spawning in wild populations, enabled CSIRO to mature and

spawn females without using eyestalk ablation (Crocos & Kerr, 1986). The combination of high temperature (28°C) and long days (14 hours light) produced the best spawning performance from unablated females. One trial (9102, Table 1) assessed the effects on reproductive performance of changing temperature and day length; in all other trials the above conditions were used as a standard, so that the effect of manipulating other factors can be assessed.

Eyestalk ablation:

The reproductive performance of ablated and non-ablated spawners kept in standard physical conditions on standard diets were compared. The performance of ablated prawns provided a standard for the comparative assessment of the suitability for commercial application of non-ablated spawners in terms of quality and quantity of larval output.

Age of spawners:

To assess the effects of age on spawner performance, we compared the reproductive performance of females at 6 and 12 months old. The males and females were from the same recruiting cohort (i.e. of the same age) collected from the wild either at the time of the first natural spawning period (6 months at liberty), or during the second natural spawning period (12 months at liberty).

Diet:

Broodstock diet as a factor affecting reproductive performance was investigated at two levels. Firstly, we formulated a fresh-frozen marine invertebrate diet consisting of chopped frozen prawn (*Metapenaeus bennettae*), bivalves (*Perna canaliculus, Plebodonax deltoides*), squid (*Loligo* spp.) and polychaete worms (*Marphysa sanguinea*): This diet, which is based loosely on the components in the natural diet of wild populations, provides high levels of protein and lipids thought to be essential for maturation. Broodstock prawns in hatcheries are usually fed a similar diet. In addition, there are proprietary artificial dry-pelleted "maturation" diets formulated to provide higher protein and lipid levels than grow-out diets. This study compared reproductive performance on the "Nippai"® artificial maturation diet and on the CSIRO marine invertebrate diet. Secondly, we formulated an artificial diet based on the biochemical composition of the natural diet in the wild, and compared this diet with the CSIRO marine invertebrate diet (see Nutrition section).

3. Assessment of reproductive performance

A standard method of assessing overall reproductive performance was developed to make direct comparisons possible. Maturation and spawning were considered as a series of "stages" so that changes at each stage, from female survival through to larval survival, could be measured, and the effects of the treatment at each stage of the process identified. Accordingly, the measure of reproductive performance of broodstock was: survival rate, percentage of females reproductively active, maturation rate, spawning rate, fecundity, fertilisation rate, hatch rate, biochemical composition of the eggs and production rate of larvae. Larval fitness was measured by the survival rate, production of protozoea, protozoeal weight and biochemical composition.

4. Experimental Treatments

The experiment types and treatments are summarised in Table 1. The 16 reproductive performance experiments on the two species (*P. esculentus* and *P. semisulcatus*), were based on combinations of ablation and non-ablation, and different diets, ages and sources of broodstock. The experiments ranged from 55 to 310 days (average 135 days), with 14 to 200 females (average 64) in each experiment.

Expt. number	Species	Source	Duration (days)	Treatment	No. of females	Matur-ations	Spawn-ings	Larval bioassays	*Biochem. analyses* Eggs	Larvae
8901	PE	MB	310	Abl/N/Diet	200	187	81	36	-	-
8902	PS	GOC	160	Abl/N	40	33	25	12	-	-
9003	PE	MB	164	Abl/N/Diet	120	21	9	2	-	13
9004	PS	GOC	171	Abl/N	120	192	138	37	-	16
9006	PE	TSV/MB	147	Abl/N	42	132	83	63	-	54
9007	PE	GOC	155	Abl/N/Diet	48	169	104	91	-	61
9008	PS	GOC	98	Abl/N	25	64	49	36	-	42
9101	PS	GOC	190	Abl/N/Diet	48	37	23	4	-	-
9102	PE	MB	140	Abl/N/Temp	80	49	19	12	-	-
9103	PS	GOC	119	Abl/N/Hand	25	16	9	3	-	-
9104	PS	GOC	118	Abl/N/Diet	60	89	55	36	2	24
9105	PE	GOC	136	Abl/N/Diet	44	150	92	73	10	48
9201	PE	MB	68	Abl/Diet/Seas	60	56	30	23	-	10
9202	PS	GOC	61	Abl/Seas	26	25	13	7	-	-
9204	PS	GOC	55	Abl/Seas	14	22	14	6	-	-
9205	PE	MB	62	Abl/ZDiet/Seas	60	58	20	9	-	-
Totals:										
16	**2**	**3**	**2154**	**7**	**1012**	**1300**	**764**	**450**	12	**268**

Table 1. Summary of 16 maturation experiments during 1989 to 1992 showing treatment types, number of maturations, spawnings, larval bioassays and samples of eggs and larvae for biochemical analysis completed. Abbreviations: PE, Penaeus esculentus; PS, P. semisulcatus; MB, Moreton Bay; GOC, Gulf of Carpentaria; TSV, off Townsville; Abl, ablated; N, Non-ablated; Temp, elevated and variable temperature treatments (if not listed, experiments run at elevated temperatures); DIET, natural and artificial diet treatments (if not listed prawns fed natural diet); ZDIET, CSIRO maturation diet; HAND, variable prawn handling protocols for parameter measurement); SEAS, treatmen part of a time-series of experiments to assess effects of age and season.

Results

A total of 16 experiments to assess the reproductive performance of females were completed (Table 1). Although analyses of all the experiments is not complete, by way of example the results for experiment 9007 are summarised (see also Rothlisberg et al. 1991, attached). This trial compared the effects of two diets (natural diet and an artificial diet) on the reproductive performance of ablated and non-ablated female *P. esculentus.*

Both ablated and non-ablated females fed the natural diet had a higher survival rate (76.8 and 73.3%) than those fed the artificial diet (49.3 and 31.3%). Their maturation and spawning rates were also generally higher. However, the ablated females whether on natural diet or artificial diet, had a higher maturation rate, spawning rate and fecundity. There was little difference in the hatch rates of ablated and non-ablated females on either diet. The naupliar production rate (NPR) is the cumulative effect of adult survival rate, maturation rate, fecundity, spawning rate and hatch rate. The highest NPR (60,325 nauplii per female per 30 prawn-days) was from ablated females fed the natural diet; non-ablated females fed the same diet had a much lower NPR (20,085); ablated females on the artificial diet had a similar NPR (24,986), but for different reasons. The non-ablated females on a natural diet had a high survival rate and low fecundity, while the reverse applied to females on the artificial diet. The lowest NPR (12,041) was from non-ablated females on the artificial diet due to low adult survival and fecundity, and the lowest spawning rate of all the groups.

Survival of the larvae through the lecithotropic naupliar stages to the first protozoeal stage was higher for larvae spawned by prawns on the artificial diet. However, when coupled to the NPR to produce a protozoeal production rate (PPR), the trend remains the same: the highest number of larvae were produced by ablated adults on the natural diet. Their PPR was four times greater than the non-ablated females on the artificial diet. The artificial diets also produced the heaviest protozoeae.

Other trials (9008, 9101, 9102, 9104, 9105) further demonstrated the effects of ablation on the reproductive performance of both species; early indications are that non-ablated *P. semisulcatus* females are capable of similar reproductive performance to ablated females, but only in the older group of females. For example, trial 9008 showed similar spawning rates and larval production rates for 12 month-old ablated and non-ablated females (1.03, 1.07 and 35,031, 34,726 respectively), but both rates were much higher for ablated 6-month-old females (trial 9101: 0.1, 0.36 and 16,890, 26,630 respectively. In contrast, ablated females *P. esculentus*, always outperformed non-ablated females, even at 12 months old (e.g. the spawning rates of 12-month-old females in trial 9105 were 0.38 (non-ablated) and 0.96 (ablated), while the larval production rates were 16,210 and 23,913 respectively).

Further comparisons of the fresh-frozen invertebrate diet and the proprietary pelleted maturation diet were made in trials 9101, 9104, 9105 and 9205. Again, the invertebrate diet outperformed the pelleted diet (e.g. in trial 9105 the spawning rate on the invertebrate diet was 0.88 and on the pelleted diet 0.65, while larvae production rates were 24,900 and 15,700 respectively. Preliminary analysis of the age trials shows that the reproductive performance of ablated *P. semisulcatus* spawners was higher/better in the older age-group; (e.g. in trial 9204, the monthly spawning rates of 6- and 12-month-old females were 0.61 and 1.32, and the monthly larvae production rates per female were 74,500 and 284,500 respectively.

Table 2. *Penaeus esculentus*. Summary of rates of maturation, spawning, fecundity, and larval survival

	Natural diet		Artificial diet	
Attribute	Non-ablated	Ablated	Non-ablated	Ablated
Reproductive performance				
Female survival (%p-d)[1,2]	76.8	73.3	49.3	31.3
Maturation rate[3]	1.16	1.85	0.86	0.93
Fecundity (eggs/spawn)	52,343	81,082	53,290	70,332
Spawning rate[3]	0.63	1.24	0.42	0.62
Hatching rate (%)	60.9	60.0	53.8	57.3
Nauplier production rate[4]	20,085	60,325	12,041	24,986
Larval fitness				
Larval survival (%)	78.3	68.4	88.4	83.6
Protozoeal production rate[5]	15,724	41,262	10,664	20,888
Protozoeal weight (µg)	1.42	1.46	1.60	1.66

[1] p-d prawn-day
[2] (total p-d/total possible p-d) x 100
[3] number per female per 30 p-d
[4] mean number of nauplii produced per female per 30 p-d survival
[5] egg production rate x larval

Discussion

The results presented here are from preliminary analyses of one of the 16 maturation trials. Ablation increased the reproductive output (NPR) of females on either diet, but the size of the increase depended on diet. The NPR of ablated females on the natural diet was three times higher than that of non-ablated females. Although ablation improved the performance of animals on the artificial diet, NPR values for ablated/artificial diet females were much lower than for ablated/natural diet females, and only reached similar levels to the non-ablated/natural diet females. Larval survival was slightly lower from ablated females in both diets. Total protozoeal production from ablated/natural diet females was over 2.5 times greater than from non-ablated. Similarly, total protozoeal production of ablated females on the artificial diet was around twice that of non-ablated females on the same diet. The lower PPRs of the artificial diet were a result of lower adult survival (30-50%) and lower spawning rates (0.42-0.62), than animals on the natural diets (75% and 1.2-1.8 respectively), even though fecundity and hatch rates were similar. We have inferred larval fitness from larval survival and weight. It appears that the larvae spawned by females on the artificial diet, though lower in number, are fitter.

In the natural environment, females arising from the spring spawning become mature at around 5 to 6 months old in February or March, and begin to spawn at this time. Fewer eggs are spawned than in September and October, when more larger-sized, and therefore more fecund, females are spawning. This study has demonstrated for the first time an inherent difference in the spawning capability of females of different ages. The age of females must therefore be considered when selecting broodstock. Further studies are planned to assess the finer-scale change in reproductive performance with season, and therefore age, as a basis for selecting broodstock with the best spawning potential. This study will also more fully investigate the factors driving a seasonal pattern (age, diet, nutritional condition) to examine the potential for manipulating the inherent pattern.

The overall conclusion from these preliminary trials is that the role of ablation cannot be examined in isolation from the nutrition and age of the prawns. The trials demonstrated both the positive and negative effects of ablation on reproductive output, but more importantly, showed that with good nutrition, non-ablated females can perform at least as well as ablated females on a poorer diet, and that older females perform better than younger ones.

These findings have significant implications for industry's problems with poor spawner performance at some times of year limiting production of postlarvae. To date the industry has not considered age as a factor in the selecting quality broodstock. Consequently, FRDC is supporting further research to investigate more fully the factors underlying seasonal differences in reproductive performance, whether they be age-related physiological factors or nutritional limitations imposed by the environment, and to find ways to overcome the problem of poor spawner performance.

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Nutrition

Introduction

The diet and nutritional condition of wild and captive broodstock prawns prior to and during maturation critically affect the prawns' reproductive performance. There is a need to understand the interactions of nutrition and maturation and to determine the nutritional requirements for successful maturation and production of viable larvae. Despite the improvements in commercially prepared broodstock diets, they have not produced the expected level of reproductive performance (Harrison, 1990; Crocos, unpublished). Hatcheries still have to supplement artificial diets with a mixture of fresh-frozen marine invertebrates. The expense, storage problems and variable quality of fresh feeds, coupled with their contribution to fouling tank water, make the alternative of an effective pelleted maturation diet very attractive.

In this study we investigated the changes in the biochemical composition of the organs and tissues of the tiger prawn *Penaeus esculentus* during maturation to determine the main nutrients required for maturation. We also studied the diets of wild prawns during the main spawning season and determined the biochemical composition of the organisms they ate. With this information, we have formulated, and tested a maturation diet.

Before an animal spawns, all the nutrients the developing embryo and nauplius require for synthesis and energy are deposited in the egg. Lipid is the main energy source and is also used for synthesis and maintaining cell wall function (Harrison, 1990). As the lipid composition is likely to be influenced by the maternal diet and by the method used to induce maturation, we examined the lipid composition of larvae in relation to broodstock treatment and larval viability.

Methods

Tiger prawns, *P. esculentus*, were collected from Moreton Bay during October and November 1989 (the main spawning period) and again in March through to May 1990 when some young adults spawn. The foregut of all the prawns was removed and the contents identified to estimate the numbers of each type of prey organism. The ovaries, digestive glands, abdominal muscles and the remaining tissues of prawns at three stages of ovarian development were dissected for biochemical analysis. The mass of each tissue was measured and subsamples were taken for analysing the lipids and carotenoids and the water content. The total lipid was determined gravimetrically after extraction in chloroform:methanol (Folch *et al.* 1957) and the lipid class and fatty acid composition of the total lipid were then determined (Dall *et al.*, 1991). Total carotenoid was determined spectroscopically from acetone extracts and then analysed by HPLC to quantify the major components.

Benthic organisms were collected from Moreton Bay at the same time and in the same places where the prawns were collected. These samples provided specimens of the benthos to help identify fragments of prey in the guts of the prawns and specimens for biochemical analysis. An Agassiz trawl and a McLeod grab were used to collect the substrate which was progressively washed through screens down to 1.0 mm mesh. The organisms retained by the 1.0 mm screen were collected, identified and counted. Samples of species that were

representative of the main dietary items of the prawns were frozen for later analysis. The average mass, moisture content, ash, crude protein, total lipid, amino acid composition, lipid class and fatty acid composition were determined (Dall *et al.* 1991). These data were combined with the dietary composition data and used to calculate the biochemical profile of the prawns' diet during the spawning season.

A maturation diet was formulated from the nutrient profile of the natural diet. The feed was made up as a semi-moist pellet, with freeze-dried prawn, squid and bivalve as the main protein and lipid sources. It was tested in replicated maturation trials with ablated *P. esculentus*, and the reproductive performance of these prawns was compared with that of a control group fed the diet of fresh-frozen marine invertebrates (referred to as the "invertebrate diet") which is the standard maturation diet at these laboratories.

The lipid composition of the eggs and nauplii produced from some of the maturation experiments has been analysed. For example, in experiment 9007, the maturation and reproductive performance of both ablated and non-ablated *P. esculentus* fed either of the two diets was studied. The lipid composition (total lipid, lipid classes, fatty acids and carotenoids) in the nauplii were related to the rates of hatching and survival of larvae, and to diet.

Results

1. Biochemical composition of *Penaeus esculentus* tissues

The carotenoid content, total lipid, lipid class composition and fatty acid composition had been determined for 7 or 8 replicate samples of ovary, digestive gland, muscle and remaining tissues of the prawn, for each of three stages of ovarian maturation — immature (Stage 2), maturing (Stage 3) and fully mature (Stage 4). Preliminary statistical analysis has been carried out but further work is needed before these data can be presented.

2. Natural diet of *Penaeus esculentus*

In October and November 1989, during the main spawning season of *P. esculentus*, 247 mature prawns were caught from various parts of Moreton Bay. There were roughly equal numbers of males, females with undeveloped ovaries, and females with ripe ovaries. In March, April and May over 1000 tiger prawns were trawled but none had maturing ovaries, which was unexpected; in most years at this time, small numbers of prawns with mature ovaries are caught.

Reproductively active prawns were widely distributed over Moreton Bay in the Spring but were found in significant numbers in three areas: Cribb Island, Bramble Bay and South Tangalooma Road (Moreton Island). The prawns had been feeding on small crustaceans, bivalves, gastropods, ophiuroids and polychaetes but in different proportions at different locations. These proportions appeared to be closely related to the population structure of the benthos and possibly the size of the prawns. There also appeared to be a relationship between the gonadosomatic index and the proportion of gastropods in the diet, but this was not significant due to the variability of the data.

3. Maturation diet

Comparison of the reproductive performance of *P. esculentus* females fed the standard fresh-frozen marine invertebrate diet and the CSIRO-formulated diet indicated a different response to the diet at different stages of maturation and spawning (Fig. 1). The survival rate of females fed the two diet types was similar, but the maturation rates were higher in females fed the invertebrate diet. However more of the females fed the formulated diet actually spawned, so the overall spawning rates for animals on the two diets were similar. Total egg production was slightly higher on the invertebrate diet, but as the hatch rate of eggs was much higher on the formulated diet, these animals had higher overall nauplii production.

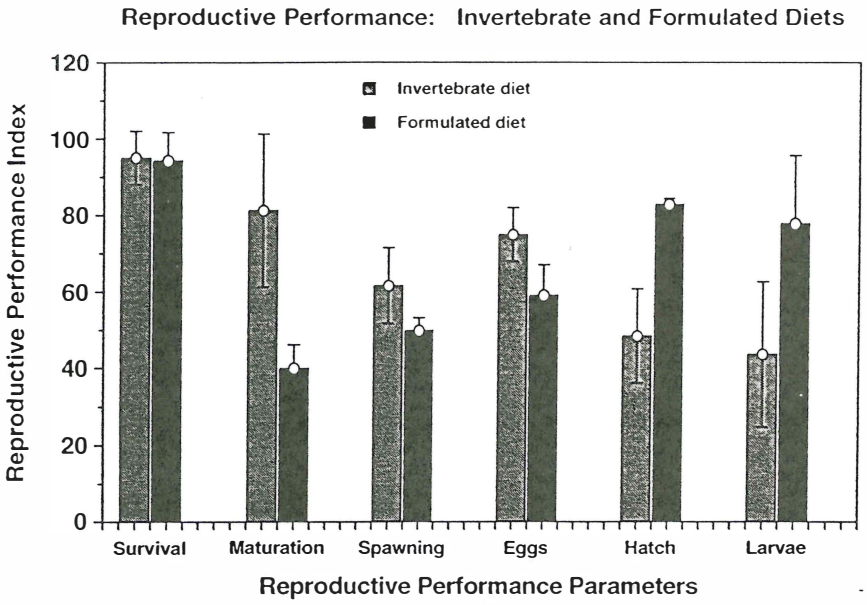

Figure 1. Comparison of aspects of the reproductive performance of *Penaeus esculentus* females fed the standard fresh-frozen marine invertebrate diet and the CSIRO-formulated moist-pellet maturation diet. Reproductive performance parameters: **Survival**, percentage of possible prawn-days survived; **Maturation**, mean number of maturations per female per month; **Spawning**, mean number of spawnings per female per month; **Eggs**, total eggs produced; **Hatch**, percentage of eggs hatching from fertilised spawns; **Larvae**, number of nauplii produced per female per month. Since the units of measurement for these reproductive performance parameters are all different, the values have been standardised to 100 units and expressed as the "Reproductive Performance Index"; hence values should only be compared between the 2 diet treatments in each category. Error bars represent standard errors of means.

4. Nauplia lipid composition

We determined the lipid composition of nauplii hatched and raised in the experiments to compare the effects of diet and ablation on reproductive performance. In all cases there was a positive trend in the relationship between survival of nauplii and their total lipid content, but it was not statistically significant. This trend was most marked in ablated animals fed the invertebrate diet (Figure 2). No correlation was found between naupliar survival and the amount of individual lipid classes or fatty acids in the nauplii.

The fatty acid composition of the nauplii was closely related to that of the diet of the adult females, but the lipid classes were not (Table 2) . Eyestalk ablation of the broodstock did not appear to affect either total lipid or lipid classes, but appears to result in higher C20:1n-9 and C18:n-3 levels and lower levels of C20:4n-6 and C20:5n-3 in their nauplii.

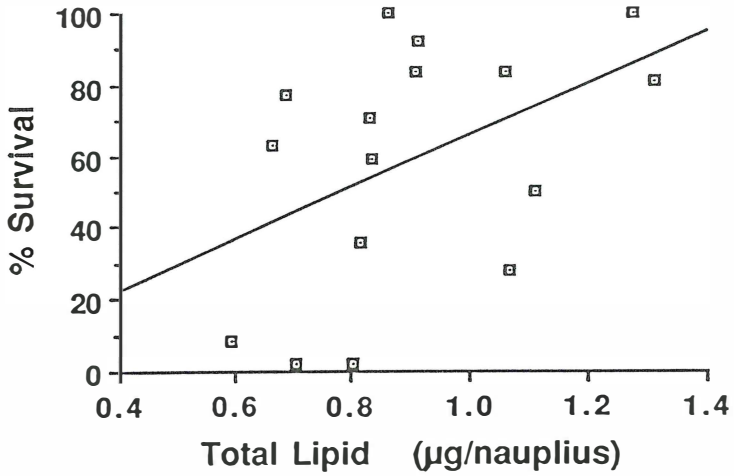

Figure 2. Relationship between the survival rate nauplii after 62 h and their total lipid content from ablated broodstock fed the invertebrate diet. Regression line fits the equation $y = -6.815 + 72.55x$, n=16, R^2=0.205.

Discussion

1. Natural diet of *Penaeus esculentus*

The marked variability in our data precluded us from positively identifying whether the female prawns in the wild preferred a different diet to the males of the same species. The prawns' diet was made up of the same suite of potential food organisms that were present in the area where they were caught, but there were indications that the larger prawns were feeding on a higher proportion of gastropods than were smaller prawns. This is likely to be a reflection of the prawn's ability to ingest the gastropods, rather than a change in preference or dietary need. The apparent relationship between the gonadosomatic index (a measure of the state of ovarian maturation) and the proportion of gastropods in the diet is possibly due to an unrepresentative number of the large prawns being in a late stage of maturity. The outstanding difference between the biochemical profile of the gastropods and that of the other dietary items is its generally higher levels of the essential fatty acid arachidonic acid (C20:4n-6). Arachidonic acid is an important precursor of prostaglandins, which are locally acting hormones with many functions in reproduction (Harrison, 1990).

The lack of prawns in reproductive condition during the autumn prevented a comparison of the seasonal variability of the diets of the broodstock prawns. However, we have found considerable spatial variability in the diets of prawns, and evidence of significant seasonal variability as well. This information has been useful in designing a successful sampling program in Albatross Bay (FRDC 89/13) and the sampling program for a current FRDC-funded project (FRDC 92/51).

2. Maturation diet

We have calculated a nutrient profile of the diet of the broodstock *P. esculentus* from the biochemical composition of its natural prey. This nutrient profile formed the basis of an artificial maturation diet that was tested for its effect on the reproductive performance of *P. esculentus*.

Although this was the first trial of the first version of the CSIRO-formulated diet, its effect on maturation and spawning was apparent. Animals on the formulated diet had a lower overall maturation rate (mean number of maturations per female per month) than those on the invertebrate diet, but more mature animals spawned. Similarly, although animals on the formulated diet produced fewer eggs overall, their hatch rates, and hence overall larval production, were higher. It appears that the formulated diet resulted in better quality maturations and spawnings. The development of a maturation diet that improves larval production will be invaluable to commercial hatcheries.

Table 1. *Penaeus esculentus*. Total lipid and lipid class composition of the broodstock diets and of newly-hatched nauplii of non-ablated and ablated females, and rate of survival of larvae. Lipid class results are expressed as a percentage of the sum of the lipid classes. TG=Triacylglycerol, PE=Phosphatidylethanolamine, PI=Phosphatidylinositol, PC=Phosphatidylcholine

	Invertebrate Diet			Commercial Diet		
	Diet	Nauplii		Diet	Nauplii	
		Non-ablated	Ablated		Non-ablated	Ablated
Mean larval survival (%)		85.3	59.3		78.0	88.0
Number of samples		6	6		4	6
Total lipid ($\mu g.naup^{-1}$)		0.87	0.97		0.99	1.13
Lipid classes						
Sterol ester	2.6	1.3	1.1	4.5	1.2	1.2
Free sterol	3.9	2.0	1.7	2.1	2.0	1.8
TG	29.3	54.8	53.7	45.4	56.2	57.7
PE	7.4	5.8	6.2	2.6	5.6	5.3
PI	25.7	3.7	4.1	22.8	3.2	2.0
PC	31.1	32.4	33.2	22.6	31.8	32.0

Table 2. *Penaeus esculentus.* Fatty acid composition of broodstock diets and newly hatched nauplii. Results are expressed as a percentage of the sum of the fatty acids. PUFA=polyunsaturated fatty acid.

Fatty Acid	Invertebrate Diet			Commercial Diet		
	Diet	Nauplii		Diet	Nauplii	
		Non-ablated	Ablated		Non-ablated	Ablated
Saturates						
C14:0	3.2	2.5	2.4	6.1	2.4	3.1
C15:0	1.2	0.8	0.7	0.5	0.7	0.5
C16:0	20.2	21.0	21.1	23.0	22.4	24.3
C17:0	1.7	1.5	1.2	0.5	1.1	0.7
C18:0	9.8	7.7	7.1	4.3	5.2	4.7
C20:0	0.0	0.6	0.6	0.0	0.5	0.4
C22:0	0.0	0.2	0.1	0.5	0.1	0.0
C24:0	1.0	0.0	0.0	1.4	0.0	0.0
Total	37.1	34.3	33.2	36.3	32.4	33.7
Monoenes						
C14:1n-5	0.0	0.0	0.0	0.0	0.1	0.0
C16:1n-7	6.2	8.0	9.1	6.1	6.3	6.8
C18:1n-7	4.5	3.9	3.9	4.5	4.8	4.9
C18:1n-9	7.4	16.0	16.0	10.9	19.4	19.2
C20:1n-9	1.7	1.1	1.4	2.0	1.8	2.0
C22:1n-9	0.5	0.0	0.0	0.7	0.1	0.1
Total	20.3	29.0	30.4	24.2	32.5	33.0
PUFAS						
C18:2n-6	3.0	1.8	2.1	7.5	6.6	7.8
C18:3n-3	0.7	0.5	0.6	0.7	0.6	0.8
C20:2n-6	1.7	0.9	0.9	0.0	0.7	0.6
C20:3n-3	0.5	0.2	0.2	0.9	0.2	0.1
C20:4n-6	6.7	6.1	4.5	1.8	3.1	1.8
C20:5n-3	13.0	10.0	9.5	13.8	9.1	8.2
C22:3n-3	0.3	0.8	0.5	0.5	0.4	0.0
C22:6n-3	16.7	16.4	18.1	14.3	14.4	14.0
Total	42.6	36.7	36.4	39.5	35.1	33.3

3. Naupliar lipid composition

The lipid content of the nauplii from these experiments was similar to that reported by other workers, about 20% of the dry weight. We will report the lipid content on a per nauplius basis, since this is the most precise way of measuring it and is thus more likely to show up any relationships.

Lipids are used as the prime energy source in nauplii and are also essential for synthesis and the maintenance of cell function. Since nauplii do not feed and all their nutrient requirements must be met from reserves deposited before spawning, the quantity and quality of their lipids are likely to effect their development and survival. In the nauplii, and particularly those from the ablated females fed the invertebrate diet, there was a positive trend in the relationship between numbers of nauplii surviving and their total lipid content, but it was not statistically significant. However, it implies that the lipid content of the nauplii (and by inference, in the maternal diet) was not inadequate. However, the spawnings where only very low numbers of nauplii were produced, were not analysed as there was insufficient material. Had these nauplii been analysed, a stronger relationship

between survival and lipid content would possibly have emerged. Future research in this area (FRDC 92/51) will focus on the analysis of newly spawned eggs so that the spawnings that could not be analysed using the nauplii, will be part of the data set.

The close relationship between fatty acid composition of the nauplii and the maternal diet highlights the importance of providing the broodstock with dietary lipids containing the best possible fatty acid profile for ovarian development and larval survival. Other workers have reported the importance of the lipid in the diets of maturing prawns (Middleditch *et al.* 1979,1980; Millamena 1989; Galgani *et al.* 1989). The importance of the polyunsaturated fatty acid composition of the lipid has become increasingly clear (reviewed by Harrison 1990), but still the required amounts of these fatty acids or their relative proportions in the diets have not been demonstrated. The fatty acid composition of the natural diet of the prawns at the time of greatest reproductive performance, during the spawning season, provides a guide to the fatty acid composition of a maturation diet. The diet we formulated had a nutrient profile similar to that of the natural diet and will be used in future work to investigate the prawns' requirements for individual fatty acids.

Eyestalk ablation of the broodstock did not appear to affect total lipid or lipid classes but their nauplii had higher levels of C20:1n-9 and C18:n-3 and lower levels of C20:4n-6 and C20:5n-3. These differences may be due to ablated prawns having a higher metabolic rate than non-ablated. The two essential fatty acids C20:4n-6 and C20:5n-3 are important in ovarian development (Teshima and Kanazawa, 1983; Teshima *et al.*, 1988; Harrison, 1990); the reduction in their levels may have significant consequences. Further studies are being carried out (FRDC 92/51) to test the benefit of increasing the level of these fatty acids in the diets of ablated broodstock prawns.

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Cryopreservation

Introduction

Cryopreservation of the embryos of penaeid prawns would be of significant value to the prawn-farming industry and to researchers. The ability to stockpile and store embryos for extended periods could avoid the problem of the availability of spawners and the demand for larvae not coinciding. The means to conserve indefinitely such desirable traits as rapid growth and disease resistance would be of considerable benefit to the industry. Because of the poor viability of prawn tissue cultures, cryopreserved embryos could also provide an alternative source of cells for testing responses to pathogens.

Compared to the long-established techniques for the cryopreservation of mammalian embryos (e.g. Whittingham *et al.* 1972) and the more recent ones for insect embryos (e.g. Mazur *et al.* 1992), efforts to cryopreserve the embryos of marine invertebrates have met with little success. Low tolerance of freezing, even in the presence of suitable cryoprotectants, has been found in late-stage embryos of sea urchins (Asahina and Takahashi 1978), mussels (Toledo *et al.* 1989); and early-stage embryos of oysters (Renard 1991). Cryopreservation has been achieved for some aquatic invertebrates, such as rotifers that are, in nature, able to withstand dessication (King *et al.* 1983, Toledo and Kurokura 1990, Toledo *et al.* 1991). However, there are no published accounts of successful cryopreservation of the embryos or early larval stages of marine arthropods. The objective of this study was to determine the principal barriers to the cryopreservation of the embryos or early-stage (nauplii) larvae of penaeid prawns.

Methods

Most of the embryos and larvae used in this study were *Penaeus esculentus*, a subtropical species. The adult prawns were captured with trawled nets from Albatross Bay in the Gulf of Carpentaria, Australia, and air-freighted to the CSIRO Marine Laboratory at Cleveland, Queensland. In the laboratory the prawns completed their ovarian development in 10,000 L maturation tanks (temperature range 25-28°C and salinity range 28-35 ppt). They spawned in individual 90 L tanks maintained at the same temperature and salinity as the maturation tanks. Embryos or larvae at the desired stage of development were collected from the spawning tanks by siphoning into 2 L glass beakers. In addition to *P. esculentus*, some chill and freezing tolerance experiments were done on embryos or nauplii of *P. semisulcatus*, *P. japonicus*, *Metapenaeus endeavouri* and *M. bennettae*.

1. Chill tolerance

The effects of chill tolerance were examined at three stages of development: early embryos (1-2 hours after spawning, at the two-cell stage), late embryos (6-8 hours after spawning, at the multicellular stage with limb development well advanced) and newly hatched nauplii (12-14 hours after spawning). The effects of chilling on the rates of hatching and metamorphosis were determined by exposing embryos or larvae to seawater pre-cooled to 16°, 11°, 6° and 1°C for various intervals. After exposure, they were returned directly to 28°C. The embryos were incubated through to hatching and the nauplii incubated through to moulting to the first protozoeal stage. Tolerance of subzero temperatures was examined by putting embryos and nauplii into seawater pre-chilled to -1°C for 10, 20, 30, 40, or 50 minutes. They were then warmed (80°C/min.) by returning them directly to ambient spawning temperatures.

2. Changes in solute concentration

The tolerance of embryos to hyperosmotic and hyposmotic solutions was determined by measuring the changes in the diameter and viability of one-cell and multi-cell embryos in response to changes in salinity. The diameter of embryos was measured using an ocular microscope and camera lucida.The salinity treatments were 0, 16, 32, 48, 63 and 126 ppt, corresponding to 0, 0.25, 0.75, 1.0 and 2.0 molar solutions. After exposure to the treatment for 20 minutes, a subset of ten embryos was removed from each treatment for measurement, while the remainder were returned to their pre-treatment salinity (30.5 ppt). Twenty minutes later, a further subset of 10 embryos was removed from each treatment and their diameters were measured. The percentage of the remaining embryos, that hatched out was recorded.

3. Cryoprotectants

We tested the tolerance of embryos and nauplii to a variety of low-molecular-weight chemicals (penetrating cryoprotectants), high-molecular-weight chemicals (non-penetrating cryoprotectants), combinations of the chemicals and a vitrification solution. The types and concentrations of cryoprotectants tested are shown in Table 1.

With ultrarapid cooling several orders of magnitude greater than those that just prevent cell shrinkage cells can be cooled so fast that ice does not form. At these cooling rates, liquids solidify by an extraordinary increase in viscosity to attain a vitreous amorphous state (Fahy *et al.*, 1984). This method of cryopreservation requires very high concentrations of cryoprotectants to dehydrate the cells. On the basis of previous success with invertebrate embryos we selected the vitrification solution formulated by Steponkus *et al.* (1990). The tolerance of embryos to this solution was determined for early embryos, late embryos and nauplii.

The protocol for testing the effects of all the cryoprotectants, including the vitrification solution, was to expose the embryos and nauplii to the various concentrations of cryoprotectant for 20 minutes. Embryos and nauplii were then returned to their pre-treatment conditions and their survival rates recorded.

4. The rate of cooling during freezing

The response of embryos and nauplii to variations in freeze/thaw protocols was examined in a programmable, controlled-rate freezing chamber (AGTEC - Embryo Freezer). Two freeze/thaw protocols were used: the relatively rapid freezing protocol described by Asahina and Takahashi (1978) and the relatively slow freezing protocol of Toledo (1989). The temperature and time at which the animals froze was determined with a cryomicroscope. Damage to embryos was recorded on photomicrographs, and post-thaw hatch rates were recorded for each freeze/thaw protocol.

Table 1. Summary of the types and concentrations of cryoprotectants tested. MW = molecular weight

Penetrating compounds	M.W.	CONCENTRATIONS (MOLARITY)					
Methanol	32	0.1	0.5	1.0	2.0	3.0	4.0
Ethylene glycol	62	0.1	0.5	1.0	2.0	3.0	-
Propylene glycol (P.G)	76	0.1	0.5	1.0	2.0	3.0	-
Glycerol	92	0.1	0.5	1.0	2.0	3.0	-
Dimethyl Sulfoxide (D.M.S.O.)	78	0.1	0.5	1.0	2.0	3.0	-
Acetamide	59	0.1	0.5	1.0	2.0	3.0	-
Non-penetrating compounds							
L- Proline1	15	0.1	0.2	-	-	-	-
Glucose	181	0.1	0.2	-	-	-	-
Sucrose	342	0.1	0.2	-	-	-	-
Trehalose	342	0.1	0.2	-	-	-	-
Polyethylene glycol (P.E.G.)	6000	5% by volume					
Polyvinylpyrrolidone (P.V.P)	1000	5% by volume					
Combined compounds							
D.M.S.O. + P.V.P	78 + 1000	0.1 + 5% by volume					
D.M.S.O. + P.E.G.	78 + 6000	0.1 + 5% by volume					
D.M.S.O. + Sucrose	78 + 342	0.1 + 01					
Glycerol + Glucose	92 +181	0.1 + 0.1					
Glycerol + P.V.P.	92 + 1000	0.1 + 5% by volume					
Glycerol + P.E.G.	92 + 6000	0.1 + 5% by volume					
P.G. + P.V.P	76 +1000	0.1 + 5% by volume					
P.G. + P.E.G.	76 + 6000	0.1 + 5% by volume					
P.G. + Sucrose	76 + 342	0.1 + 0.1					
D.M.S.O. + Proline	78 + 115	0.1 + 0.1					
Vitrification compounds							
VSI (Steponkus *et al.* 1990)							

Results

1.Chill tolerance

The chill-tolerance experiments demonstrated that the embryos of tropical penaeid prawns are very sensitive to low water temperatures. For example two-cell stage embryos of *P. esculentus* withstood exposure to 16°C for 2 hours, any longer exposure resulted in very poor hatching success. At all the temperatures lower than 16 °C, hatchings decreased significantly after 30 minutes exposure (Fig. 1). Embryos tolerated exposure to -1°C for 20 minutes, but after 30 minutes hatching success declined to 20% and after 50 minutes to zero(Fig. 2). Later stages of development (8 cells and beyond) had significantly greater tolerance to low temperatures than had earlier stages. However, these changes in chill tolerance did not improve subsequent tolerance to the formation of ice.

Figure 1. The effects of low temperatures on the hatching success of two-cell embryos of *Penaeus esculentus*

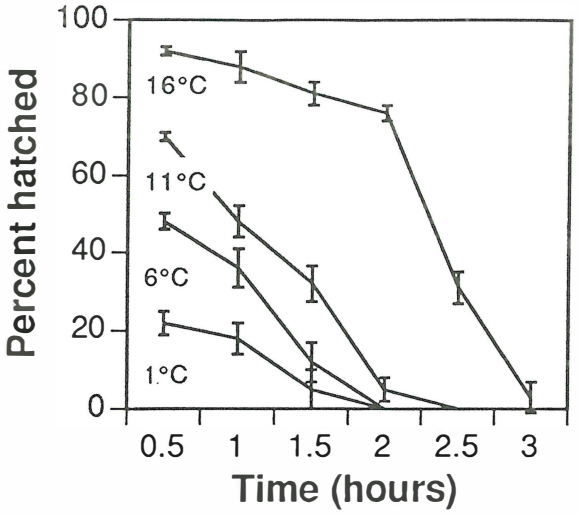
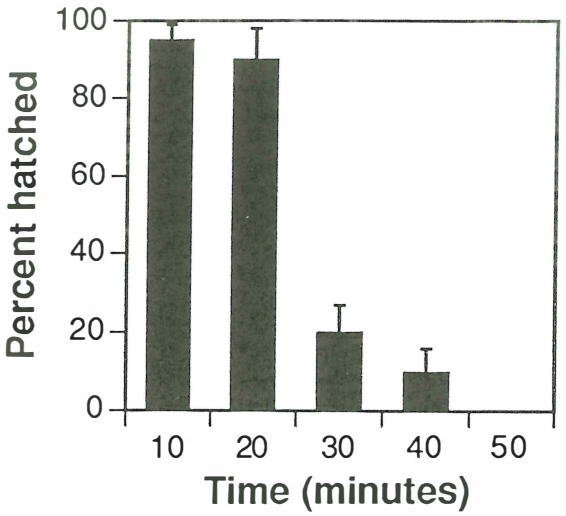


Figure 2. The effects of length of exposure to -1°C on the hatching success of two-cell embryos of *Penaeus esculentus*



2. Changes in solute concentration

Prawn embryos were very sensitive to changes in solute concentration (Table 2). Concentrations above or below 31.6 ppt (0.5 M) produced in rapid changes in the diameter of the hatching envelope, the most pronounced of which was the swelling when exposed to fresh water. On return to ambient salinity, the diameters all returned to close to the same size except for those exposed to 31.6 ppt, which were slightly larger. The only embryos to hatch were those that had been exposed to 31.6 ppt.

Table 2. Changes in the mean diameter (mm ± s.e.) and hatch rates of one-celled embryos of *Penaeus esculentus* in response to changes in salinity. The initial mean diameter of embryos at the ambient salinity (30.5 ppt) was 0.320 (± 0.002) mm.

Salinity	Molarity	Embryo diameter after 20 min exposure	Embryo diameter on return to ambient salinity	% hatched
0	0	0.361 (0.012)	0.317 (0.006)	0
15.8	0.25	0.336 (0.013)	0.316 (0.007)	0
31.6	0.5	0.319 (0.003)	0.320 (0.004)	82
47.4	0.75	0.313 (0.009)	0.316 (0.007)	0
63.2	1.0	0.310 (0.016)	0.316 (0.006)	0
126.4	2.0	0.310 (0.020)	0.316 (0.008)	0

3 Cryoprotectants

The tolerance tests demonstrated that none of the cryoprotectants except acetamide was lethal to embryos at some level of the concentrations tested (Table 3). Three of the penetrating compounds — methanol, propylene glycol and dimethyl sulphoxide — lowered the freezing point of the solution. As a result, the embryos were able to withstand supercooling to temperatures as low as -80°C. However, none of the cryoprotectants or mixtures of cryoprotectants enabled embryos to withstand the formation of ice (Table 3).

Table 3 The responses of embryos (E) and larvae (L) of *Penaeus esculentus* to exposure to various cryoprotectants at different temperatures for 20 minutes. + denotes survival of eggs or nauplii at some level of treatment. 0 denotes no survival of eggs or larvae at any level of treatment. n denotes not tested.

Cryoprotectant	M.W.	Ambient 28°C		Chilled -1°C		S-cooled -80°C		Frozen -198°C	
		E	L	E	L	E	L	E	L
Penetrating compounds									
Methanol	32	+	+	+	+	+	+	0	0
Ethylene glycol	62	+	+	+	+	+	+	0	0
Propylene glycol	76	+	+	+	+	+	+	0	0
Dimethyl sulphoxide (DMSO)	78		+	+	+	+	+	0	0
Glycerol	92	+	+	+	+	n	n	0	0
Acetamide	59	0	0	n	n	n	n	n	n
Non-penetrating compounds									
L- Proline	115	+	+	+	+	0	0	0	0
Galactose	180	+	+	+	+	0	0	0	0
Glucose	181	+	+	+	+	0	0	0	0
Sucrose	342	+	+	+	+	0	0	0	0
Trehalose	342	+	+	+	+	0	0	0	0
Polyethylene glycol (P.E.G.)	6000	+	+	+	+	0	0	0	0
Polyvinylpyrrolidone (P.V.P.)	1000	+	+	+	+	0	0	0	0
Combined compounds									
D.M.S.O. & P.V.P		+	+	+	+	0	0	0	0
D.M.S.O. & P.E.G.		+	+	+	+	0	0	0	0
D.M.S.O. & Sucrose		+	+	+	+	0	0	0	0
Glycerol & Glucose		+	+	+	+	0	0	0	0
Glycerol & P.V.P.		+	+	+	+	0	0	0	0
Glycerol & P.E.G.		+	+	+	+	0	0	0	0
P.G. & P.V.P		+	+	+	+	0	0	0	0
P.G. & P.E.G.		+	+	+	+	0	0	0	0
P.G. & Sucrose		+	+	+	+	0	0	0	0
D.M.S.O. & Proline		+	+	+	+	0	0	0	0
Vitrification compounds									
VSI (Steponkus *et al.*, 1990)		0	0	0	0	0	0	0	0

4. The rate of cooling during freezing

In the absence of cryoprotectants, embryos did not survive any of the freeze-thaw protocols tested. Under certain conditions embryos were able to withstand sub-zero temperatures in the absence of ice (supercooling). This occurred at rapid cooling rates (>80°C/min) with supercooling occurring at temperatures as low as 40°C. The fact that no ice was formed at these low temperatures was established from photomicrographs.

Discussion

This study has shown that there are formidable barriers to the cryopreservation of penaeid prawn embryos or nauplii. None of the species we examined responded well to either the conventional embryo cryopreservation procedures or the new vitrification methods. We have, however, identified some of the barriers that need to be overcome before prawn embryos can be cryopreserved successfully.

The conventional approach to cryopreservation is to cool cells sufficiently slowly to permit intracellular water to be removed by osmosis (Ashwood-Smith 1986). However, this technique was not feasible with *Drosophila*, whose embryos are sensitive to chilling (Mazur *et al* 1992). This also appears to be the case with prawn embryos, which are more sensitive to chilling than *Drosophila*. Similarly the survival of oyster embryos was severely reduced by storage at 0°C for 25 minutes (Renard 1991). The cooled embryos showed a loss of plasma membrane arrangement leading to a massive disruption of the microvilli from the embryonic cells.

One strategy that overcomes chilling injury in some cell types is to cool and warm very rapidly (Fahy *et al.*, 1984). However, this requires high concentrations of permeating cryoprotectants (5 - 9 molar) to induce vitrification and thus avoid the formation of intracellular ice (Fahy *et al.*, 1984). Our study indicates that the sensitivity of prawn embryos to changes in solute concentration is a significant barrier to introducing cryoprotectants at the concentrations required to achieve vitrification. For example, the maximum concentration of ethylene glycol tolerated by prawn embryos was 2.0 M, whereas successful vitrification of *Drosophila* embryos requires a concentration of 8.5 M (Steponkus *et al.*, 1990). Future attempts to develop a vitrification technique for prawn embryos will have to overcome their sensitivity to changes in solute concentration. It also remains to be determined whether the harmful effects are due to electrolytes reaching critical levels (Lovelock 1953), osmotic shrinkage (Meryman 1974) or loss of plasma membrane (Steponkus 1984).

In this study we tested a variety of penetrating and non-penetrating cryoprotectants. Penetrating compounds, which are generally used to protect cells during slow cooling and freezing, act by reducing cell dehydration, maintaining electrolyte balance and increasing the amount of water remaining in the extracellular spaces (Meryman 1971). Non-penetrating cryoprotectants such as sugars and amino acids appear to protect the membrane structure under the stress of freezing and thawing (Rudolph and Crowe, 1985). Prawn embryos tolerated multimolar levels of penetrating cryoprotectants and submolar levels of non-penetrating cryoprotectants. In the presence of these cryoprotectants, they could tolerate short periods of exposure (20 minutes) to very low temperatures, providing no ice was formed either outside or in the cells; cryomicroscopy revealed that ice was lethal to embryos and nauplii. We did not find any combination of cryoprotectant(s) and freeze/thaw protocol that reduced the ice formation to sub-lethal levels.

In summary, we have demonstrated that the sensitivity of prawn embryos and nauplii to chilling, changes in solute concentration and the formation of ice present formidable obstacles to their cryopreservation. Whilst it is encouraging that similar problems have been overcome in *Drosophila*, our study suggests that successful cyropreservation of prawn embryos will be even more challenging.

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Summary and Recommendations

The rates of maturation, spawning and larval survival of unablated *P. esculentus* can be raised to an acceptable level, without eyestalk ablation, by providing a suitable environment and a specifically formulated diet and selecting broodstock by age. Improvements to the formulated diet and precise age-selection is likely to result in the performance of unablated females approaching the level of ablated females. These findings will have a profound effect on broodstock management, and should increase both the quantity and quality of output from captive and wild broodstock.

The ability to induce spawning in captivity and get viable larvae is related to the age of the spawners. This finding should lead to more judicious selection of wild broodstock, reducing the pressure on wild stocks and increasing hatchery productivity and therefore the availability of postlarvae to the industry.

New maturation feed, based on the nutritional composition of natural diets, were developed. They achieved higher rates of larval production than current proprietary feeds. With further refinement these diets should enhance the prospects of raising and keeping broodstock in captivity and inducing controlled spawning. This will further increase the quantity and quality of postlarvae for the industry

Successful cryopreservation of penaeid eggs, embryos and larvae continues to be an elusive goal because of their extreme sensitivity to chilling and to changes in solute concentration. Recent research with insect embryos suggest that the best prospects for achieving successful cryopreservation of prawn embryos lie with developing a vitrification protocol. If successful, this would make it possible to store eggs and embryos, which would make genetic material available for research and breeding programs and overcome the seasonal shortages of eggs that hamper hatchery productivity.

Intellectual Property

The findings of this research project have both scientific and commercial value. Some of the main findings are leading to improved husbandry protocols which allow better selection and management of broodstock and are being adopted by the industry. Based on the early findings in this project, the CSIRO developed maturation diet is being refined in another FRDC-funded project (FRDC92/51). CSIRO and QDPI are testing new versions of the diet on both *Penaeus monodon* and *P. semisulcatus*. This diet is likely to have a large commercial potential throughout Southeast Asia and wherever penaeid broodstock are intensively managed in captivity.

Transmission of Results to Industry

Australian Prawn Farmers Association Workshop Townsville , November 1990

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