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PRAWN FARMING POND MANAGEMENT

FINAL REPORT TO

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FINAL REPORT

TITLE OF GRANT: **Prawn Farming Pond Management**

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PERSONNEL ENGAGED:

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Senior Research Scientist
(Time spent on Grant - 3%)

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(Time spent on Grant - 100%)

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Other Personnel - D J W Moriarty
B Ag Sc; Ph D
Principal Res. Sci., CSIRO
Dr Moriarty was a co-operating scientist and advised on experimental design and methodology for assessing bacterial productivity in prawn farming ponds)
(Time spent on Grant - 5%)

PERSONNEL TRAINED: Mr S Hopkins, Mrs H Thaggard, Mr A West

Staff received considerable training in analytical methods used in determining water quality and bacterial productivity. In addition training in establishing and running a sophisticated bioassay laboratory, live handling of prawns for experimental purposes and data entry and elementary processing was received.

GRANT REPORT

CONTENTS -

1 OBJECTIVES

Objective A

Using bioassay techniques determine critical concentrations of water quality variables which affect prawn growth and survival.

Objective B

Quantitatively compare the effects of different pond management methods on prawn growth and survival, pond water quality and the population dynamics of bacteria and algae within ponds.

2 RESULTS FOR OBJECTIVE A

2.1 Introduction

For Objective A, a series of experiments was conducted using standard bioassay methodology to determine the critical concentrations of water quality variables which affect prawn growth and survival. The features of the facility which was designed and established for this segment are described below:

* A large volume initial seawater storage unit (capacity - 7000 l) to ensure that irregularities in station seawater supply did not affect water flow in aquaria and to allow settleable solids to be removed from the water supply.

* A filtration system comprising a large capacity commercial sand filter with secondary filtration through a two-stage cartridge filtration unit (5μm and 1μm).

* Seawater heating unit comprising an in-line 6 kw single pass water heater controlled by dual solid state temperature controllers.

* A preliminary dissolved oxygen reduction chamber. This unit was in effect a degassing chamber where the dissolved oxygen level in seawater was greatly reduced through vacuum stripping. By maintaining a vacuum of between -90 & -95 Kpa the dissolved oxygen content of seawater at 27 °C could be reduced to below 2.0mg/l^{-1}.

* Secondary adjustment chambers. Water was supplied to these chambers at a constant rate and was recirculated within each chamber prior to being supplied via gravity feed to individual 70 l experimental containers. Water retention time in each adjustment chamber was approximately 24 minutes. Precisely controlled mixtures of nitrogen and oxygen (or air) were used to adjust dissolved oxygen levels to required concentrations. Using this facility the dissolved oxygen content could be adjusted and maintained at between 0.5 and 20.0 mg/L.

* 18 x 70 l clear perspex aquaria with sealed lids for acute bioassay experiments and 18 x 70 l aquaria with removable lids for chronic bioassay experiments. A flow-meter and valve controlled the water supply to each aquaria, usually at a constant flow of 245 ml min^{-1} via a mixing chamber.

* Toxicant control system comprising 25 l toxicant reservoirs and multi-channel metered peristaltic pumps. Each individual experimental tank had a separate toxicant supply and the desired inputs of toxicants could be added to the seawater supply in the mixing flasks.

* Temperature and photoperiod controlled room was used to house this facility.

The detailed justification, methods, results and implications of the research associated with the bioassay segment are presented in Chapters 2.2 to 2.5. Each Chapter represents a manuscript which has been published, accepted for publication or submitted to an international journal. The titles, authors and publication status of these manuscripts are listed in Chapter 6 of this Grant Report.

2.2 Effect of sediment on growth and acute ammonia toxicity for the school prawn, *Metapenaeus macleayi* (Haswell)

ABSTRACT

Allan, G. L. and Maguire, G.B. Effect of sediment on growth and acute ammonia toxicity for the school prawn, *Metapenaeus macleayi* (Haswell).

Experiments were conducted in 70 l acrylic aquaria to determine; 1) survival and growth of a burrowing penaeid, *Metapenaeus macleayi*, for a number of substrates; 2) the interactive effects of sediment and ammonia on acute ammonia toxicity and emergence from the sediment of *M. macleayi*, and, 3) removal rates of ammonia in marine aquaria with different substrate, filtration and aeration regimes.

Survival rate was high (90-100 %) regardless of substrate type (bare plastic, mud, fine sand or coarse sand) or segregation in mesh cages. Weight gain for prawns in cages was lower ($P<0.01$) and food conversion efficiency poorer ($P<0.01$) than for non-segregated prawns in aquaria with or without sediment. The only other significant difference for weight gain or conversion efficiency among the substrate types was the higher weight gain (2.2 g prawn^{-1}) for fine sand compared with bare aquaria (1.8 g prawns^{-1}) ($P<0.05$). At 1.4 mg unionised ammonia-nitrogen (UAN) l^{-1} in the water column, the presence of sediment significantly reduced mortality of *M. macleayi,* although, at a higher concentration (2.3 mg UAN l^{-1}), all prawns died in 96 h regardless of the presence of sediment. Compared with controls with negligible ammonia concentrations, fewer prawns burrowed when exposed to elevated ammonia concentrations in the water column ($P<0.05$) even though the sediment may have provided a refuge from elevated concentrations. In aquaria with sediment, ammonia concentrations and pH values were always less in water extracted from the sediment (interstitial water) than in the water column.

Ammonia removal in aerated aquaria with sub-sand filters increased in absolute terms but declined as a percentage basis as ammonia input increased from 2 to 20 mg total ammonia -N (TAN) l^{-1} (48.0 % to 16.5 %). At an input of 20 mg TAN l^{-1}, ammonia removal in aquaria with a sub-sand filter was higher than in aquaria with sand and aeration but no filter (16.5 % vs 8.6 %; $P<0.05$). Volatilisation was not a significant pathway for ammonia loss.

The use of sediment in grow-out and bioassay systems for prawns is discussed.

INTRODUCTION

Sediment characteristics can influence the distribution of penaeid prawns in the wild (Williams, 1958; Hughes, 1966; Rulifson, 1981; Somers, 1987) and may be a factor in site selection for prawn farming ponds (Huguenin and Colt, 1989). Soil chemistry and particle characteristics can affect the tendency for the sediment to become chemically reduced, affect pond water turbidity and water quality and influence the availability of fertilisers, eg phosphorus (Boyd, 1982). Food supply

may be affected by sediment type (Williams, 1958; Hughes, 1966) although several species of penaeids have displayed preferences for sediment types irrespective of their food content (Williams, 1958; Ruello, 1973; Aziz and Greenwood, 1982). Ruello (1973) found that, when offered a choice, juvenile *Metapenaeus macleayi*, a burrowing penaeid, preferred fine sand to coarser sediments and attributed this to the ease of burrowing in sediment with a smaller particle size.

Substrate, including absence of sediment, can also affect survival and growth of penaeids. Species which burrow eg *P. japonicus* and *P. kerathurus*, may grow faster when sediment is provided (Liao, 1969; Otazu-Abrill and Ceccaldi, 1981; Klaoudatos, 1980) while infrequent burrowers, eg *P. setiferus*, may grow at similar rates (Sick et al., 1972). However, Chien et al. (1989) concluded that growth rates of *P. monodon*, an infrequent burrower, were significantly higher in aquaria with substrate than without.

Because of its high surface area, a substrate which prawns can burrow into, e.g., sand, can provide an ideal surface for notifying bacteria. Biological filtration is a very common method of ammonia removal (Lucchetti and Gray, 1988) and a number of researchers have investigated removal rates using different types of external filters, filter substrates, ammonia input rates and detention times (Haug and McCarty, 1972; Forster, 1974; Bruce and Gunther, 1981; Manthe and Malone, 1987). Less attention has been given to reduction of ammonia in tanks with sub-sand nitrifying filters, even though this type of filter is all that is needed in most aquarium tanks (Spotte, 1979).

Preliminary studies by the authors indicated that the presence of sediment affected ammonia concentrations, particularly in interstitial water. Numerous studies, mostly based on external biofiltration units, have shown that bacterial populations in sediment, or other biofilter media, remove ammonia (Lucchetts and Gray, 1988). Removal rates depend on ammonia input (Haug and M^{c}Carty, 1972; Foster, 1974; Brune and Gunther, 1981; Manthe and Malone, 1987).

In ponds, sediment suspended by aeration devices can depress photosynthesis and hence ammonia removal by algae (Boyd, 1982). However, the effect of sediment on ammonia concentrations in aquaria is poorly documented although ammonia is very toxic to penaeid prawns (Chin and Chen, 1987; Allan et al., 1990) and can build up to a critically high concentration during intensive prawn culture (Chen et al., 1988). The effects of environmental variables such as temperature and light on burrowing and emergence of penaeids have been investigated (Fuss and Ogren, 1966; Aldrich, 1968; Hughes, 1968; Wickham and Minkler, 1975; Hill, 1985) but no studies were found which described the effects of ammonia on emergence of penaeids.

The aims of this study were to; 1) examine the effects of different substrates on survival and growth of *M. macleayi*; 2) investigate the interactive effects of sediment and ammonia concentration on mortality and emergence of *M. macleayi* and 3) determine the effects of sediment, sub-sand nitrifying filters and aeration on the concentration of ammonia in overlying and interstitial water in marine aquaria

without prawns. *M. macleayi* has often been farmed in New South Wales, NSW, Australia, (Maguire and Allan, in press a), and forms the basis of a large capture fishery in that state (Montgomery, 1988).

MATERIALS AND METHODS

M. macleayi were otter trawled from Port Stephens, NSW (32° 45' S, 152° 04' E). Prawns were acclimatised for at least one week before experiments commenced and were fed freshly shucked and chopped bivalve flesh (pipi, *Plebidonax deltoides*). There were three randomly assigned, 70 l, acrylic aquaria for each treatment. In Experiments 1-2, ten juveniles were placed in each aquarium. As male and female *M. macleayi* can grow at different rates (Maguire and Allan, 1985), five male and five female prawns were used. Seawater from a marine dominated estuary was used (average salinity 34 ‰, range 27-35 ‰). Before use, seawater was filtered through a sand filter and 5 μm and 1 μm cartridge filters. A 12:12 h photoperiod was used and water temperatures were maintained at 25 °C (range 24.5 - 25.8 °C). This was within the optimum temperature range for *M. macleayi* (Maguire and Allan, in press b). Continuously flowing seawater (approximately 250 ml min^{-1} $aquarium^{-1}$, was provided during each experiment, except for Experiment 1, where 90 % of the water in each aquarium was drained and replaced daily with pre-heated water.

For Experiments 2 and 3, concentrated ammonia solutions were prepared in 20 l reservoirs using NH_4Cl, and pH was adjusted to that of incoming seawater (approximately 8.0 pH) using 10 M NaOH. The concentrated ammonia solutions were pumped at 2.5 ml min^{-1} to 500 ml mixing flasks where they were diluted by incoming seawater before entering aquaria. Seawater was vigorously aerated before entering the mixing flasks (Allan et al., 1990).

Experiment 1 - Effect of substrate on growth of M. macleayi

Individually tagged prawns (Allan et al., 1990) with an average initial weight of 2.2 g (range 1.3 - 3.9 g) were provided with four different types of substrate; bare (no sediment), mud, fine sand, and coarse sand. The sediment characteristics (Folk, 1980) are given in Table 1. To assess the importance of interactions (e.g., cannibalism) between prawns, within aquaria without sediment, an additional treatment where prawns were individually segregated in 6 mm plastic mesh cylinders (130 mm long, 50 mm diameter) was provided.

Gentle aeration (0.8 l min^{-1}) was provided, through two air stone diffusers, for each aquarium throughout the 31 d experiment. Prawns were fed fresh, chopped pipi on an *ad libitum* basis, once daily, just before the start of the dark cycle. During the first four days of the experiment, any prawns which died were replaced and these were not included in mortality calculations. Uneaten food and exuviae were recorded and removed daily. An average value for individual prawn weight gain was calculated for each aquarium at the end of the experiment. Food conversion ratio (FRC) (total feed input on a 92 % dry matter basis/wet prawn biomass gain) involved initial biomass estimates corrected for mortality. Feed input data for pipi

was adjusted to 92 % dry weight basis to enable comparison with FCR data recorded for prawns fed pellets in other studies.

Experiment 2 - Effects of sediment on acute toxicity of ammonia to M. macleayi

Prawns (average initial weight 2.3 g; range 1.0 - 3.8 g) were exposed for 96 h to seawater with three ammonia concentrations (<0.05 [controls], 29.5-33.2 or 51.7-55.3 mg total ammonia-nitrogen (TAN) l^{-1}) with or without a layer of sand. No sub-surface filters were used (Table 1). Five males and five females were stocked into each aquarium. The number of dead prawns and those not buried were recorded after 0.5, 1, 2, 4 h and at four hourly intervals thereafter. To reduce disturbance to prawns during observations at night, a shaded 6 V torch was used. 'Emergence' was defined as the average number of prawns not buried at each observation. An average value for all observations during both the dark and light cycle was calculated for each aquarium. Absence of response to touching with a glass rod was the criterion of death, and all dead prawns were removed.

Experiment 3 - Ammonia distribution and loss in aquaria

Six treatments were established. In Treatments 1-3, commercially-available, plastic sub-surface filter plates (555 mm x 265 mm) were placed beneath a layer of sand (Table 1). The sand, for those aquaria with sediment, was obtained from a 0.4 m^3 sub-surface biological filter which had been operating for 12 weeks on a tank stocked at a wet prawn biomass of 0.9 g l^{-1}. The sand was added to the aquaria 25 days before the experiment commenced. The required concentrations of ammonia were added to the aquaria 17 days before the experiment commenced. A single air-lift pump (air flow 0.8 l min^{-1}) circulated water through the filter and aerated each aquarium. No additional aeration was provided and seawater containing 2, 10 or 20 mg TAN l^{-1} was supplied to each aquarium. For treatments 4 - 6 seawater containing 20 mg TAN l^{-1} was supplied to each aquarium and no sub-surface filters were used. For treatment 4, the same quantity of sand (from the same source), as used in Treatments 1 - 3 was placed in each aquarium and aeration (0.8 l min^{-1}) was provided through two air stone diffusers. Treatment 5 had no sand but was aerated as in Treatment 4. Treatment 6 had neither sand nor aeration. This experiment was run for seven days without prawns.

Water quality analyses

Temperature was measured daily and dissolved oxygen (DO) and salinity every 2-3 days in each aquarium during each experiment. For Experiment 1, pH, ammonia and nitrite were measured in each aquarium once, just before the experiment was terminated. For Experiments 2 and 3, pH and ammonia were measured in each aquaria daily and nitrite just before the experiments were terminated. For Experiment 2, ammonia concentrations and pH values in the interstitial water were also measured daily. Samples of interstital water were taken using a 2 ml plastic syringe, with the opening covered by plastic mesh (63 μm), inserted into the sediment to a depth of 5 mm. DO was always above 5.4 mg l^{-1} in

all three experiments.

Salinity was measured using a Yeo-kal (Yeo-Kal Electronics Pty Ltd, Brookvale, NSW) Temperature/Salinity conductivity meter, and DO using a Yeo-Kal DO/Temperature meter. DO was always above 5.4 mg l^{-1} in all experiments. A Metrohm (Metrohm Ltd CH-9100 Herisaw, Switzerland) pH/mV meter with a Metrohm reference and glass electrode assembly was used to measure pH. The Salinity/Temperature meter, the DO/Temperature and the pH meter and electrodes were calibrated as described by Allan et al. (1990). Total ammonia-nitrogen (TAN) was measured using the indophenol blue colourmetric method (Dal Pont et al., 1974). Unionised ammonia-nitrogen (UAN) was calculated from TAN, pH, salinity and temperature (Bower and Bidwell, 1978). Nitrite was measured using colormetric methods (Major et al., 1972) and did not exceed 20 μg NO_2 - N l^{-1}, well below growth reducing levels (Wickins, 1976; Chen et al., 1990).

Statistical analysis

For Experiment 1, two-factor ANOVA was used to investigate the effects of gender, substrate and their interaction on prawn weight gain. As results indicated that neither gender nor the interaction between gender and substrate affected prawn weight gain ($P>0.05$), data for both sexes were combined and re-analysed using single-factor ANOVA. Single-factor ANOVA's were also used to investigate the effects of substrate on FCR and ammonia concentrations.

For Experiment 2, *t*-tests were used to compare mortality in aquaria with different substrates (bare or sand), at particular total ammonia concentrations. Two-factor ANOVA was used to investigate the effects of ammonia input concentration and illumination (dark or light) on emergence of prawns from sediment within aquaria. As there was a significant interaction between illumination and ammonia concentration for prawn emergence ($P<0.01$), single-factor ANOVA was used to investigate treatment effects for each combination of illumination and ammonia concentration (Underwood, 1981).

For Experiment 3, three analyses were carried out; a) two-factor ANOVA's were used to compare effects of ammonia input concentration and site of measurement (water column or interstitial water) on ammonia concentration and pH in Treatments 1-3 which had sub-surface filtration; b) single-factor ANOVA was used to compare the effects of different sediment, filtration and aeration combinations on total ammonia concentration in the water column for Treatments 3-6 which had the same total ammonia input concentration, and, c) *t*-tests (Sokal and Rohlf, 1981) were used to compare the total ammonia concentration in the interstitial water in Treatments 3 and 4 which had sediment and the same total ammonia input concentration.

For all ANOVA's, homogeneity of variance was assessed using Cochran's test (Winer, 1971) and comparisons among means using Tukey's 'honestly significant difference' method (Sokal and Rohlf, 1981). For *t*-tests, homogeneity of variance was confirmed prior to analyses using the F-max test (Sokal and Rohlf, 1981). To

satisfy the assumptions of homogeneity and normality, data for FCR (Experiment 1) and total ammonia concentration (Experiment 3) were transformed (log x) and data for mortality (Experiments 1 and 2) were transformed (arcsine $x^{0.5}$) prior to analysis. Unless otherwise stated, values given in the text are means ± standard error for 3 replicate aquaria.

RESULTS

Experiment 1

Mortality was low (4.7 % overall) and unaffected by segregation or substrate type ($P>0.05$) (Table 2). Prawn weight gain was lowest in the treatment were prawns were segregated in cages ($P<0.01$). Little difference in weight gain between other treatments existed, however, there was a significant difference between bare and fine sand treatments ($P<0.05$) (Table 2). FCR was poorer in aquaria where prawns were held in cages compared with all other treatments ($P<0.01$) (Table 2). The concentration of ammonia in the water column was lowest in the cage treatment and highest in the mud treatment (Table 2).

Experiment 2

Very few prawns died in the low ammonia input treatments (0-6.7 %), while at the highest input concentration (52.8-54.3 mg TAN l^{-1}) all prawns died (Table 3). However, mortality at an input concentration of 31.5-32.6 mg TAN l^{-1} was significantly higher in the bare aquaria (30 %) than in the sand aquaria (6.7 %) ($P<0.05$) (Table 3). Both ammonia input concentration ($P<0.001$) and illumination (dark or light) ($P<0.001$) affected emergence of prawns and the interaction was significant ($P<0.01$). Emergence increased as the ammonia input concentration increased (Table 3). At the lowest ammonia input concentration, only 7.3 % of prawns were emergent at any observation during the light cycle compared with 71.7 % during the dark cycle ($P<0.05$) (Table 3). However, at the two higher ammonia input concentrations, there was no difference in emergence during the light and dark cycles ($P>0.05$) (Table 3).

Experiment 3

For Treatments 1-3 with sub-sand filtration and different total ammonia input concentrations, both total ammonia concentration and pH were lower in interstitial water ($P<0.001$). There was an interaction between ammonia input concentration and site of measurement (water column or interstitial water) for ammonia concentration ($P<0.001$) but not pH ($P>0.05$) (Table 4). The interaction is evident in the ratio of interstitial and water column concentrations for TAN i.e., 0.3, 0.7 and 0.8 for Treatment 1-3 in that order. Ammonia input concentration had no effect on pH ($P>0.05$). As the input concentration of ammonia increased (Treatments 1-3), ammonia removal increased in absolute terms but declined as a percentage basis (Table 4).

For Treatments 3-6 which had similar ammonia input concentrations but different filtration, sediment and aeration regimes, ammonia concentration was lower ($P<0.05$) where sub-sand filtration was used (Treatment 3) and similar ($P>0.05$) for all other treatments (Treatments 4-6). Ammonia removal in Treatment 3 which had sub-surface filtration was higher than in Treatment 4 which had sediment but no filtration ($P<0.05$) and much higher ($P<0.05$) than in Treatments 5 and 6 which had no sediment (Table 4). For the Treatments 3 and 4 which had similar ammonia input concentrations and sediment, the concentration of ammonia in the interstital water was similar ($P>0.05$) (Table 4).

DISCUSSION

Effect of sediment on mortality and growth

An increase in cannibalism in the absence of sediment, as described by Subrahmanyan and Oppenheimer (1969) for *P. duorarum* and *P. aztecus* and Otazu-Abrill and Ceccaldi (1981) for *P. japonicus*, was not evident for *M. macleayi* during present study. Sick et al. (1972) and Chien et al. (1989) also found that absence of sediment did not affect mortality of *P. setiferus* and *P. monodon* respectively. During subsequent short-term (four day) and longer (three week) experiments with both *M. macleayi* and *P. monodon*, mortality of prawns not exposed to high concentrations of ammonia, low concentrations of dissolved oxygen or low pH, was always $\leq$10 % in aquaria without sediment (Allan and Maguire, 1991; Allan and Maguire, in press; Allan et al., 1990). In the present study, prawn weight gain was, however, much slower when prawns were segregated in cages compared with when they were not. Wickins (1984) also found that when *P. monodon* and *P. occidentalis* were segregated individually, growth rates were 45-65 % of those recorded when prawns were not segregated. Wickins (1981) concluded that sensitivity of prawns to a pollutant was affected by growth rate. As mortality was not reduced by the use of cages, and growth and FCR were worse than when prawns were not segregated, experiments on environmental requirements of *M. macleayi* should not be conducted with individually segregated prawns.

For prawns not segregated, differences in average weight gain, in relation to the type of substrate, were relatively minor and were only significant for aquaria with fine sand (2.2 g prawn^{-1}) compared to those aquaria without sediment (1.8 g prawn^{-1}). Despite a clear preference exhibited by juvenile *M. macleayi* for fine sand where approximately 10 % of the sand had a particle size of 0.25-0.50 mm diameter and 87 % was 0.125-0.25 mm diameter (Ruello, 1973), growth of *M. macleayi* during the present study was similar in aquaria with mud, coarse sand or fine sand. Moller and Jones (1975) suggested that reduced activity and energy conservation when *P. semisulcatus* and *P. monodon* were buried may result in increased growth of prawns under artificial conditions. This hypothesis was supported by Lakshmi et al. (1976) who found a parallel relationship between burying response and growth rates for *P. aztecus*. Furness and Aldrich (1979) found growth of *P. aztecus* was positively correlated with increasing pond bottom softness in experimental 0.1 ha ponds. Similarly, Liao (1969) found growth rates

were higher and FCR's lower for *P. japonicus* in aquaria with a sand layer compared to aquaria without any sediment.

Growth in response to the presence or absence of sediment may be related to the burrowing activity of the species being investigated. Sick et al. (1972) found that for *P. setiferus*, an infrequent burrower, growth was not affected by absence of sediment. In the experimental facilities described here, *P. monodon* do not burrow as deeply or as frequently as *M. macleayi*. In experiments with *P. monodon* in 70 l acrylic aquaria no significant differences in weight gain were found for prawns grown with mud, sand or no sediment (Maguire and Allan, unpublished data). Conversely, Chien et al. (1989) found growth of *P. monodon* was reduced when no substrate was present although these results were confounded by differences in water quality between treatments.

The ammonia concentrations measured during Experiment 1 in the present study were all well below growth reducing values (Allan et al., 1990). The lowest concentrations were in the aquaria where prawns were segregated, possibly due to lower ammonia excretion rates for less active, slowly growing prawns receiving lower feed inputs. The highest concentrations were recorded in the aquaria with mud, possibly due to the higher organic content of this substrate compared with others. Decomposition of organic material can result in elevated ammonia concentrations (Boyd, 1982).

Effect of sediment and ammonia on mortality and emergence

Although mortality was not affected by the absence of sediment when ammonia concentrations were low, when ammonia concentrations were elevated (31.5-32.6 mg TAN l^{-1}; Experiment 3), mortality was much higher 30±5.8 % in aquaria without sediment than in aquaria with sand. To prevent disturbance to prawn burrowing activity the concentration of ammonia and pH in the interstitial water were not measured during Experiment 2. However, measurements taken during Experiment 3 (Table 4) indicated that ammonia concentration and pH in the interstitial water, in the zone where prawns bury, were considerably reduced. Thus concentrations of UAN, by far the most toxic form of ammonia (Smart, 1978), in the interstitial water during Experiment 3 were 0.22±0.00 UAN l^{-1} compared with 0.85±0.02 UAN l^{-1} in the water column. Any prawns which were buried in the sediment during Experiment 2 would have been exposed to less toxic conditions than those not buried, although respiratory currents could have drawn in overlying water with higher ammonia concentrations (Ruello, 1973).

Fuss and Ogren (1968), after reviewing burrowing activity of *P. duorarum*, concluded that burrowing served as a protective measure against predation and adverse environmental conditions. Studies of effects of adverse temperatures on emergence support Fuss and Ogren's hypothesis (Aldrich et al., 1968; Hill, 1985). Egusa and Yamamato (1961) found that *P. japonicus* emerged from the sediment when dissolved oxygen concentration fell to 0.5 mg l^{-1} and interpreted this as a mechanism to ensure sediment did not impede the flow of water to the prawns, respiratory siphon when dissolved oxygen concentrations were low. Surprisingly,

in the present study, emergence increased in response to an adverse environmental condition (high ammonia concentration) even though the lower concentrations in the sediment could have provided a refuge. It is possible that *M. macleayi* rarely experience such elevated concentrations of ammonia in the wild, as opposed to low temperatures or dissolved oxygen concentrations, and hence a selective behavioural mechanism for an appropriate burrowing response may not have developed. Alternatively, prawns may have emerged to seek water with a lower ammonia concentration.

Effect of sediment and aeration on ammonia levels

The presence of sediment reduced ammonia concentrations in aquaria and, not surprisingly, this effect was enhanced by arranging the sand layer as a biofilter. The amount of ammonia removed increased in absolute terms but decreased as a percentage basis as input concentrations of ammonia increased in aquaria with sub-surface filters (Table 3). Forster (1974) described a similar response in studies with percolating biological filters. The loading and removal rates for the sub-surface filters used in the present study were calculated using water flow rates (250 ml min^{-1}) and filter volume (6 l). At ammonia input concentrations of 2, 10 and 20 mg TAN l^{-1}, loading rates to each aquarium were 120, 600 and 1200 mg TAN d^{-1} l filter $media^{-1}$ in that order, while removal rates were 60, 102 and 198 mg TAN d^{-1} l filter $media^{-1}$. Although the design of nitrifying filters used for aquaculture varies considerably, these removal rates were less than those reported for percolating filters using polyurethane cubes (99-450 mg TAN d^{-1} filter $media^{-1}$ for input levels of 100-500 mg TAN d^{-1} l filter $media^{-1}$; Brune and Gunther, 1981) or columns of gravel (49-595 mg TAN d^{-1} l filter $media^{-1}$ for input levels of 50-768 mg TAN d^{-1} l filter $media^{-1}$; Haug and McCarty, 1972; Forster, 1974) as the filter media. The difference between ammonia concentrations in the interstital water and the water column, particularly at the lowest ammonia input concentrations, indicates that water flow through the filter was insufficient for adequate mixing. Other limiting factors could be conditioning time for the biofilter, oxygen levels in the sediment layer and clogging of the sand because of small particle sizes (Haug and McCarty, 1972; Forster, 1974; Kaiser and Wheaton, 1983).

Ammonia is volatile and although air-stripping can be used to reduce concentrations it is generally considered impractical for small scale water re-use systems (Lucchetti and Gray, 1988). In this study, light aeration (0.8 l min^{-1}), had no effect on ammonia concentrations.

Application to commercial and experimental prawn culture

The major conclusion from this study is that the choice of substrate material for grow-out systems for *M. macleayi* should not be based on the physical requirements of this species but rather on engineering or sediment chemistry considerations. Similarly, Maguire (1980) found that in pond trials, in which growth and survival rates were quite variable, there was no significant difference in growth or survival rates of *M. macleayi* grown on concrete or fine sand. However, in the present study, the provision of sediment for this burrowing penaeid did reduce the

acute toxic effects of ammonia both through removal of ammonia and lower ammonia concentrations in the interstitial water.

Chen et al. (1988) found survival and average growth rates over 141 days were 44.3 % and 0.07 g prawn day^{-1} respectively for *P. penicillatus* stocked at a density of 286 prawns m^{-2} in earthen ponds where a 10 cm deep layer of sand and gravel was provided. These results were recorded despite average monthly ammonia concentrations reaching 46.1 mg TAN l^{-1}, close to the 96 h LC_{50} for *P. monodon* postlarvae and juveniles and well in excess of growth reducing concentrations for *P. monodon* (Chin and Chen, 1987; Allan et al., 1990). Differences in ammonia tolerance between penaeid species do occur (Allan et al., 1990) and acclimation to high ammonia concentrations could also have been a factor (Ferguson, 1988). However, even though *P. penicillatus* do not usually burrow (Liao and Chien, 1990), the sand and gravel layer may have reduced toxic effects in this high ammonia environment. The sand and gravel layer, together with the high nutrient input, would also have facilitated the development of a microbial community contributing to the decline in pH from 8.1 to 7.3 and hence in the proportion of ammonia in the highly toxic unionised form (Chen et al., 1988).

For *M. macleayi*, absence of sediment does not affect mortality and, to prevent reduction in ammonia and pH in the sediment, bioassays to determine ammonia or pH toxicity should be conducted in aquaria without sediment. It should be noted, however, that the critical values determined without sediment may be conservative estimates for prawns in the wild or under culture conditions where sediment suitable for burrowing is present. Gentle aeration has little effect on ammonia concentrations and is essential during long term bioassays when prawns are fed (Allan et al., 1990). As average growth rates of *M. macleayi* in controls during the static bioassay (Experiment 1) (0.058 g day^{-1}) were slower than those recorded during a continuous-flow bioassay using the same temperature and food and feeding regime (0.071 g day^{-1}; Allan et al., 1990), it is preferable to use continuous-flow bioassays to determine effects of adverse water quality variables on penaeids. For other experiments with penaeid prawns where sediment is provided, the inclusion of sub-surface filters will assist with removal of ammonia. These filters are convenient and inexpensive, although removal rates are not as high as those reported for other types of biological filters.

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REFERENCES

Aldrich, D. V., Wood, C. E. and Baxter, K. N., 1968. An ecological interpretation of low temperature responses in *Penaeus aztecus* and *P. setiferus* postlarvae. Bull. Mar. Sci., 18: 61-71.

Allan, G. L. and Maguire, G. B., 1991. Lethal levels of low dissolved oxygen and effects of short-term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture, 94: 27-37.

Allan, G. L. and Maguire, G. B., in press. Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon*. Aquaculture.

Allan, G. L., Maguire, G. B. and Hopkins, S. J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen levels. Aquaculture, 91: 265-280.

Aziz, K. A. and Greenwood, J. G., 1982. Response of juvenile *Metapenaeus bennettae* Racek and Dall, 1965 (Decapoda, Penaeidae) to sediments of differing particle size. Crustaceana, 43(2): 121-126.

Bower, C. E. and Bidwell, J. P., 1978. Ionization of ammonia in seawater: Effects of temperature, pH and salinity. J. Fish. Res. Board Can., 35: 1012-1016.

Boyd, C. E., 1982. Water Quality Management for Pond Fish Culture. Developments in Aquaculture and Fisheries Science, Vol 9. Elsevier, Amsterdam, 318 pp.

Brune, D. E. and Gunther, D. C., 1981. The design of a new high rate nitrification filter for aquaculture wate reuse. J. World Maricul. Soc., 12(1): 20-31.

Chen, J. C., Liu, P. C., Lin, Y. T. and Lee, C. K., 1988. Super intensive culture of red-tailed shrimp *Penaeus penicillatus*. J. World. Aquaculture Soc., 19(3): 127-131.

Chen, J. C., Liu, P. C. and Lei, S. C., 1990. Toxicities of ammonia and nitrite to *Penaeus monodon* adolescents. Aquaculture, 89: 127-137.

Chein, Y. H., Lai, H. T. and Chang, S. K., 1989. The effects of using steel-making waste slags as substrates on shrimp *Penaeus monodon* reared in aquaria. Asian Fish. Sci., 2: 147-161.

Chin, T. S. and Chen, J. C., 1987. Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*. Aquaculture, 66: 247-253.

Dal Pont, G., Hogan, M. and Newell, B., 1974. Laboratory techniques in marine chemistry. II. Determination of ammonia in seawater and the preservation of samples for nitrate analysis. Aust. CSIRO Div. Fish. Oceanog. Rep., No 55, CSIRO, Sydney, New South Wales, 11 pp.

Egusa, S. and Yamamoto, T., 1961. Studies on the respiration of the 'Kuruma' prawn *Penaeus japonicus* Bate - I. Burrowing behaviour, with special reference to its relation to environmental oxygen concentration. Bull. Jap. Soc. Sci. Fish., 27(1): 22-26.

Ferguson, H., 1988. Water quality diseases. In: Fish Diseases, Refresher Course for Veterinarians. Proc. 106, Post Graduate Committee in Veterinary Science, University of Sydney, Sydney, Australia, pp. 49-54.

Folk, R. L., 1980. Petrology of sedimentary rocks. Hemphill, Austin, TX, 184 pp.

Forster, J. R. M., 1974. Studies on nitrification in marine biological filters. Aquaculture, 4: 387-397.

Furness, G. N. and Aldrich, D. V., 1979. Possible clues to improved pond culture of penaeid shrimp. Proc. World Maricul. Soc., 10: 477-488.

Fuss, C. M. and Ogren, L. H., 1968. Factors affecting activity and burrowing habits of the pink shrimp, *Penaeus duorarum*. Biol. Bull., 130: 170-191.

Haug, R. T. and McCarty, P. L., 1972. Nitrification with submerged filters. J. World Poll. Cont. Fed., 44(1): 2086-2102.

Hill, B. J., 1985. Effect of temperature on duration of emergence, speed of movement, and catchability of the prawn *Penaeus esculentus*. In: P. C. Rothlisberg, D. J. Staples and B. J. Hill, (Editors), Second Aust. Natl. Prawn Sem., 22-26 October 1984. NPS2, Cleveland, Australia, pp. 337-351.

Hughes, D. A., 1966. Factors controlling emergence of pink shrimp (*Penaeus duorarum*) from the substrate. Biol. Bull., 134: 48-59.

Huguenin, J. E. and Coll, J., 1989. Design and Operating Guide for Aquaculture Seawater Systems. Elsevier, Amsterdam, 264 pp.

Kaiser, G. E. and Wheaton, F. W., 1983. Nitrification filters for aquatic culture systems: State of the art. J. World Maricul. Soc., 14: 302-324.

Klaoudatos, S., 1980. Observations on the growth of juveniles of *Penaeus kerathurus* (Forskal) at different stocking densities and varying ecological conditions. FAO, General Fisheries Council for the Mediterranean, Studies and Reviews, Rep., 57: 93-100.

Lakshmi, G. J., Venkataramiah, A. and Gunther, G., 1976. Effects of salinity and photoperiod on the burying behaviour of brown shrimp *Penaeus aztecus* Ives. Aquaculture, 8: 327-336.

Liao, I. C., 1969. Study on the feeding of 'Kuruma' prawn, *Penaeus japonicus* Bate. Coll. Repr. Tungkang Mar. Lab., 1: 19-24.

Liao, I. C. and Chien, Y. H., 1990. Evaluation and comparison of culture practices for *Penaeus japonicus, P. penicillatus* and *P. chinensis* in Taiwan. In: K. L. Main and W. Fulks (Editors), The Culture of Cold Tolerant Shrimp: Proc. Asian-US Workshop on Shrimp Culture, Honolulu, Hawaii, October 2-4, 1989. The Oceanic Institute, Honolulu, Hawaii, pp. 49-63.

Lucchetti, G. L. and Gray, G. A., 1988. Water reuse systems: A review of principal components. Prog. Fish. Cult., 50(1): 1-6.

Maguire, G. B., 1980. Farming of juvenile school prawns (*Metapenaeus macleayi*) in estuarine ponds. Unpublished Ph. D. Thesis, University of New England, Australia, 299 pp.

Maguire, G. B. and Allan, G. L., in press. a. Shrimp culture industry in Australia. In: A. Fast and J. Lester (Editors), Culture of Marine Shrimp: Principles and Practices. Elsevier, Amsterdam.

Maguire, G. B. and Allan, G. L., in press. b. Effects of temperature on growth, food consumption and food conversion for *Penaeus monodon*, *Penaeus plebejus* and *Metapenaeus macleayi*. In: G. L. Allan and W. Dall (Editors), Proc. Aquaculture Nutrition Workshop (ANW), Salamander Bay, April 1991, ANW, Salamander Bay, Australia.

Major, G. A., Dal Pont, J., Klye, J. and Newell, B., 1972. Laboratory techniques in marine chemistry. A manual. Aust. CSIRO Div. Fish. Oceanog. Rep., No 51, CSIRO, Sydney, New South Wales, 55 pp.

Manthe, D. P. and Malone, R. F., 1987. Chemical addition for accelerated biological filter acclimation in closed blue crab shedding systems. Aquacultural Engineering, 6: 227-236.

Moller, T. H. and Jones, D. A., 1975. Locamotory rhythms and burrowing habits of *Penaeus semisulcatus* (de Haan) and *P. monodon* (Fabricius) (Crustacea:Penaeidae). J. Exp. Mar. Biol. Ecol., 18: 61-77.

Montgomery, S. S., 1988. Trends in the New South Wales prawn catch. Aust. Fish., 47(4): 24-30.

Otazu-Abrill, M. and Ceccaldi, H. J., 1981. Contribution a l'etude du comportement de *Penaeus japonicus* (Crustace Decapode) en elevage, vis-a-vis de la lumiere et du sediment. Tethys, 10(2): 149-156.

Ruello, N. V., 1973. Burrowing, feeding, and spatial distribution of the school prawn *Metapenaeus macleayi* (Haswell) in the Hunter River Region, Australia. J. Exp. Mar. Biol. Ecol., 13: 189-206.

Rulifson, R. A., 1981. Substrate preferences of juvenile penaeid shrimps in estuarine habitats. Contrib. Mar. Sci., 24: 35-52.

Sick, L. V., Andrews, J. W. and White, D. B., 1972. Preliminary studies of selected environmental and nutritional requirements for the culture of penaeid shrimp. Fish. Bull., 70(1): 101-109.

Smart, G. R., 1978. Investigations of the toxic mechanisms of ammonia to fish - gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. J. Exp. Biol., 37: 548-556.

Sokal, R. R. and Rohlf, F. J., 1981. Biometry. Freeman, San Francisco, CA, 859 pp.

Somers, I. F., 1987. Sediment type as a factor in the distribution of commercial prawn species in the Western Gulf of Carpentaria, Australia. Aust. J. Mar. Freshwater Res., 38: 133-149.

Spotte, S., 1979. Fish and Invertebrate Culture. Wiley - Interscience. New York, 179 pp.

Subrahmanyam, C. B. and Oppenheimer, C. H., 1969. Food preferences and growth of grooved penaeid shrimp. In: H. W. Youngken Jr (Editor), Food Drugs from the Sea, Proceedings 1969. Mar. Technol. Soc., Washington DC., pp. 65-75.

Underwood, A. J., 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. Ann. Rev., 19: 513-605.

Wickham, D. A. and Minkler, F. C., 1975. Laboratory observations on daily patterns of burrowing and locomotor activity of the pink shrimp *Penaeus duorarum*, brown shrimp *Penaeus aztecus* and white shrimp, *Penaeus setiferus*. Contrib. Mar. Sci., 19: 21-35.

Wickins, J. F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Wickins, J. F., 1981. Water quality requirements for intensive aquaculture: A review. In: K. Tiews (Editor), Aquaculture in Heated Effluents and Recirculation Systems. Heenemann verlagsgellschaft, Berlin, Vol. 1, pp. 17-37.

Wickins, J. F., 1984. The effect of hypercapnic sea water on growth and mineralisation in penaeid prawns. Aquaculture, 41: 37-48.

Williams, A. B., 1958. Substrates as a factor in shrimp distribution. Limnol. Oceanogr., 3(3): 283-290.

Winer, B. J., 1971. Statistical Principles in Experimental Design. 2nd Edition. McGraw-Hill, New York, 907 pp.

Wyban, J. A. and Sweeney, J. N., 1989. Intensive shrimp growout trials in a round pond. Aquaculture, 76: 215-225.

TABLE 1

Grain size characteristics (Folk, 1980) and organic content of sediment used in marine aquaria (% of dry matter). Each aquarium had a 40 mm deep layer of sediment (6.5 kg dry weight)

Sediment description	Organic content (%)	Particle size (mm)				<0.063 mm component	
		1.0-2.0 (%)	0.25-1.0 (%)	0.063-0.25 (%)	<0.063 (%)	Clay (%)	Silt (%)
Mud[1]	6.6	0	0.5	0.7	98.8	33.8	65.0
Fine sand[1]	0.6	0	91.9	6.4	1.7	0.6	1.1
Coarse sand[1]	0.2	1.9	97.6	0.1	0.4	0.4	0
Sand[2]	0.3	0.9	98.0	0.2	0.9	0.9	0

[1] Experiment 1

[2] Experiments 2 and 3

TABLE 2

Mortality, weight gain and food conversion ratio over 31 days for *Metapenaeus macleayi* and ammonia concentration in the water column, in marine aquaria with different substrates during Experiment 1[2]

Treatment	Mortality[3] (%)	Average weight gain (g prawn^{-1})	Food Conversion Ratio[4] (FCR)	Ammonia (mg TAN l^{-1})
Cage[2]	3.3±3.3^{a}	0.6±0.1^{a}	4.4±0.5^{a}	0.3±0.1^{1a}
Bare	10.0±0.0^{a}	1.8±0.1^{b}	1.7±0.1^{b}	1.0±0.1^{bc}
Mud	6.7±3.3^{a}	2.0±0.1^{bc}	1.6±0.1^{b}	1.4±0.03^{c}
Coarse sand	0^{a}	2.0±0.1^{bc}	1.5±0.03^{b}	0.6±0.2^{ab}
Fine sand	3.3±3.3^{a}	2.2±0^{c}	1.3±0.1^{b}	0.9±0.1^{bc}

1 Values are means ± standard error for 3 replicate aquaria. Within each column, means sharing a letter in the superscript are not significantly different ($P>0.05$)

2 Individual prawns were held separately in 6 mm mesh cylinders (130 mm long)

3 Data were transformed (arcsine $[0.01x]^{0.5}$) prior to statistical analyses

4 Data were transformed (log x) prior to statistical analyses

TABLE 3

Mortality and emergence of *Metapenaeus macleayi* from the sediment layer in aquaria with different ammonia input concentrations and sediment (Experiment 2)

Ammonia input[1]		Sediment	Mortality after 96 h[1, 2, 3]	Emergence[1, 2, 4]	
(mg TAN[5] l^{-1})	(mg UAN[6] l^{-1})	(kg)	(%)	Light (%)	Dark (%)
<0.05	<0.002	6.5	0	7.3 ± 1.7^{a}	71.7 ± 6.7^{bc}
<0.05	<0.002	0	6.7±3.3	-	-
31.5±0.5	1.4±0.05	6.5	6.7±6.7	48.4 ± 8.9^{b}	67.4 ± 10.3^{b}
32.6±0.2	1.4±0.02	0	30.0±5.8	-	-
52.8±0.4	2.3±0.05	6.5	100±0	79.2 ± 1.4^{bc}	94.2 ± 2.5^{c}
54.3±0.8	2.3±0.03	0	100±0	-	-

1 Values are means ± standard error for replicate aquaria
2 Data transferred (arcsine $x^{0.5}$) prior to statistical analysis
3 Means for any combination of light or dark and ammonia input, sharing a similar letter in the superscript are not significantly different ($P>0.05$)
4 TAN = total ammonia-nitrogen
5 UAN = unionised ammonia-nitrogen

TABLE 4

Ammonia concentrations and pH values in the water column and interstitial water, and removal of ammonia from the water column, in marine aquaria with different ammonia input concentrations, sub-sand filtration, sediment and aeration regimes (Experiment 3).

Treatment	Ammonia input	Sediment	Aeration	Sub-surface filter	Water column[1, 2, 3]			Interstitial water[1, 2, 4]	
	(mg TAN l^{-1})	(kg dry wt)	(l min^{-1})		pH	ammonia[6] (mg TAN l^{-1})	ammonia removal (% TAN)	pH	ammonia[6] (mg TAN l^{-1})
1	2.0	6.5	0.8	Yes	7.9±0.03	1.0±0.1	48.0±6.4	7.6±0.03	0.3±0.1
2	10.0	6.5	0.8	Yes	7.9±0.1	8.3±0.8	16.8±8.1	7.5±0.1	5.9±1.0
3	20.0	6.5	0.8	Yes	7.9±0.03	16.7±0.2[a]	16.5±1.0[a]	7.5±0.03	14.1±0.5
4	20.0	6.5	0.8	No	8.0±0.03	18.3±0.3[b]	8.6±1.3[b]	7.6±0.03	12.5±0.2
5	20.0	0	0.8	No	8.1±0.03	19.4±0.4[b]	3.0±1.8[b]		
6	20.0	0	0	No	8.0±0.03	19.6±0.9[a]	2.1±2.0[b]		

1 Values are means ± standard error for 3 replicate aquaria

2 For Treatments 1-3, ammonia input level, site of measurement (water column or interstitial water) and their interaction all affected ammonia concentration ($P<0.001$). Site of measurement affected pH level ($P<0.001$) but effects of ammonia input level and the interaction between ammonia input and site of measurement were not significant ($P>0.005$)

3 For Treatments 3-6, means with a common letter in the superscript are not significantly different ($P>0.05$)

4 For Treatments 3 and 4, mean levels of ammonia in interstitial water were not different ($P>0.05$)

5 TAN: total ammonia-nitrogen

6 Data transformed (log x) prior to statistical analysis

2.3 Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen concentrations

ABSTRACT

Allan, G. L., Maguire, G. B. and Hopkins, S. J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen concentrations. Aquaculture, 91: 265-280.

Acute toxicity of ammonia was estimated as 96 h LC_{50} values. For juvenile, *Metapenaeus macleayi*, and, *Penaeus monodon*, these were 1.39 and 1.69 mg unionised ammonia-nitrogen (UAN) l^{-1} (26.3 and 37.4 mg total ammonia-nitrogen (TAN) l^{-1}) respectively. Reduced dissolved oxygen (DO) concentrations significantly ($P<0.05$) increased the acute toxicity of ammonia to *P. monodon*. 90.0 % of prawns held for 96 h at a DO concentration of 2.3 mg l^{-1} and an ammonia concentration of 1.60 mg UAN l^{-1} (33.5 mg TAN l^{-1}) died, whereas only 33.3 % died at a DO concentration of 5.7 mg l^{-1} and a similar ammonia concentration of 1.63 mg UAN l^{-1} (33.9 mg TAN 1^{-1}). The "maximum acceptable" concentration of ammonia was defined as that which reduced growth by 5 % over three weeks. For *M. macleayi* and *P. monodon* these concentrations were 0.35 and 0.21 mg UAN l^{-1} (7.7 and 4.1 mg TAN l^{-1}) respectively.

INTRODUCTION

Ammonia is very toxic to aquatic animals and can cause impairment of cerebral energy metabolism and damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and molluscs (Smart, 1978; Colt and Armstrong, 1981). It can limit production in intensive fish and crustacean aquaculture (Delistraty et al., 1977; Colt and Armstrong, 1981) and has killed fish in the wild (Tarazona et al., 1987).

In solution total ammonia comprises un-ionised ammonia (NH_3) and ionised ammonia (NH_4^+) in equilibrium. The proportions of each depend mainly on pH, but also on temperature, salinity and pressure (Whitfield, 1974). NH_3 is by far the more toxic (Smart, 1978); however, NH_4^+ may also become toxic, especially at low pH levels when the proportion of ammonia as NH_4^+ is very high (Shaw, 1960; Armstrong et al., 1978).

Ammonia is the principal nitrogenous product excreted by crustaceans (Claybrook, 1983) and may also accumulate in culture systems following microbial decomposition of organic material (Stanier et al., 1976) and with some fertilisation practices (Boyd, 1982). As low dissolved oxygen (DO) concentrations can occur following organic decomposition, an interaction between ammonia and DO could be important for aquaculturists.

Low dissolved oxygen concentrations increase the toxicity of ammonia to fish (Alabaster et al., 1979; Thurston et al., 1981) and Lloyd (1961) attributed this to an increase in the uptake of ammonia as gill ventilation rates increased to prevent

hypoxia. When the present study was undertaken, no reports describing the effects of low dissolved oxygen concentrations on ammonia toxicity for crustaceans were found. However, recent work has shown that low DO increased the toxicity of ammonia to *P. semisulcatus* (Wajsbrot et al., 1990).

Previous studies on the toxicity of ammonia to crustacean larvae have involved lobsters *Homarus americanus* (Delistraty et al., 1977), freshwater prawns *Macrobrachium rosenbergii* (Armstrong et al., 1978) and penaeids; *Penaeus indicus* (Jayasankar and Muthu, 1983) and *P. monodon*, (Chin and Chen, 1987). One study used juvenile penaeids (< 2g) but the results for several species were pooled (Wickins, 1976). Recent studies have used juvenile *P. semisulcatus* (0.3-2.4 g) (Wajsbrot et al., 1990), juvenile *P. monodon* (3.9-6.2 g) (Chen et al., 1990) and juvenile *P. japonicus* (0.025 g) (Kou and Chen, 1991). All used static bioassay methods to estimate critical concentrations of ammonia. This approach is limited, as animals are stressed when test solutions are replaced, and metabolic wastes can increase the concentrations of ammonia between water exchanges, especially when larger animals are used and in chronic bioassays when animals are fed.

Most studies of ammonia toxicity involve a single species. Comparisons between species are made using results from different bioassay systems; however, comparisons are more valid if only one bioassay system is used. In the present study continuous flow bioassays were used to determine the acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *P. monodon* both commonly farmed in Australia (Maguire and Allan, 1985; Maguire et al., 1988). The effects of low dissolved oxygen on acute toxicity of ammonia to *P. monodon* were also investigated. Results from this study may assist with the management of prawn culture systems especially intensive nursery and grow-out units which can experience high ammonia levels (Chen et al., 1988).

MATERIALS AND METHODS

Experimental animals

P. monodon came from prawn farming ponds (Goodwood Island, NSW) and *Metapenaeus macleayi* were otter trawled from Port Stephens, NSW. They were acclimatised in the experimental aquaria for at least a week before experiments commenced. During both acclimation and chronic bioassays, prawns were fed freshly shucked and chopped bivalve flesh (pipi, *Plebidonax deltoides*).

Bioassay system

Continuous flow bioassays were conducted in 18 acrylic aquaria (70 l) with 10 prawns in each. All experiments had six treatments with three randomonly assigned replicate aquaria per treatment. Except for the low dissolved oxygen experiment (Experiment 3) treatments included one control, with no added ammonia, and five with different ammonia concentrations. Concentrated ammonia solutions were prepared in 20 l reservoirs and pumped at 2.5 ml min^{-1} to 500 ml mixing flasks, diluted by flowing seawater and then supplied to the aquaria.

Seawater was filtered through a commercial sand filter and 5 μm and 1 μm cartridge filters before entering the mixing flasks at a controlled flow rate of 245 ml min^{-1}. The combined flow (ammonia solution plus seawater) of 247.5 ml min^{-1} replaced approximately 90 % of each 70 l aquarium in 10 h as recommended by Sprague (1969). Except for Experiment 3, seawater was vigorously aerated before reaching the mixing flasks. Ammonium chloride (NH_4Cl) was used to prepare ammonia solutions, and pH was adjusted in the 20 l reservoirs to that of incoming seawater by the addition of 10 M NaOH.

No sediment was provided for the prawns because it reduced the concentration of ammonia, presumably due to nitrification. Control of pH was also harder when sediment was provided. The absence of sediment did not increase cannibalism among starved *M. macleayi* over 96 h or significantly affect growth ($P > 0.05$) in 3-4 weeks when *M. macleayi* or *P. monodon* were fed *ad libitum* with pipi flesh (Allan and Maguire, unpublished data, 1989). Seawater was maintained at constant temperatures of 25±1 °C and 27±2 °C for bioassays with *M. macleayi* and *P. monodon* respectively. These temperatures were within the optimum range for growth of each species (Maguire and Allan, unpublished data, 1989). A 12:12 hour photoperiod of light:dark was maintained. Except for Experiment 3, dissolved oxygen levels were maintained above 5.5 mg l^{-1} in all experiments either by vigorously aerating incoming water (Experiments 1 and 2) or by using two airstone diffusers in each aquarium (Experiments 4 and 5).

The bioassay seawater was essentially oceanic (salinity 30.4-36.3 ‰) with slightly elevated levels of suspended solids as described by Scribner (1986). Total nitrite-N plus nitrate-N levels in acute bioassays (1 - 3) remained below 30 μg l^{-1}, but in chronic bioassays (4 - 5) levels reached 111 μg l^{-1}, still well below those which reduced growth of juvenile penaeids (Wickins, 1976).

Water quality analyses

The ammonia concentration, pH and temperature in each aquarium were measured daily. The indophenol blue colourmetric method (Dal Pont et al., 1973) was used to measure ammonia. Un-ionised ammonia-nitrogen (UAN) concentrations were calculated daily from measurements of total ammonia-nitrogen (TAN), pH, temperature and salinity (Bower and Bidwell, 1978). Orion and Metrohm pH/mV meters, with Ross combination and Metrohm reference and glass electrodes respectively, were used to measure pH. The electrodes were calibrated with phosphate and borate buffers (American Public Health Association [A.P.H.A.], 1971) and both systems agreed (± 0.01 pH units). Nitrite and nitrate were measured occasionally using colourmetric methods (Major et al., 1972). Salinity was measured regularly using a Yeo-Kal temperature/salinity conductivity meter (Yeo-Kal Electronics, Brookvale, Sydney, N.S.W. 2100 Australia) calibrated with sub-standard seawater and a standard thermometer. Dissolved oxygen was measured with a Yeo-Kal dissolved oxygen/temperature meter calibrated before each set of measurements with "air-saturated" seawater and periodically using Winkler's titration (A.P.H.A., 1971).

Experiments 1 and 2 - Acute bioassays

To assess the acute toxicity of ammonia to *M. macleayi* and *P. monodon* six experimental concentrations ranging from 0.003 - 3.54 mg UAN l^{-1} (0.06 - 66.4 mg TAN l^{-1}) and 0.002 - 2.27 mg NH_3-N UAN l^{-1} (0.04 - 51.0 mg TAN l^{-1}) respectively were established. Just before the start of each acute bioassay, ammonia concentrations and pH in each aquarium were quickly adjusted with ammonium chloride and sodium hydroxide. The initial mean weights for *M. macleayi* and *P. monodon* were 2.0 g (range 0.9 - 4.8 g) and 2.2 g (range 0.8 - 4.5 g) respectively. Prawns were not sexed for the acute bioassays, no aeration was provided and prawns were not fed. Absence of response to touching with a glass rod was the criterion of death and all dead prawns were removed. Following A.P.H.A. (1971) and Sprague (1969), the acute bioassays were run for 96 h. Longer periods were not considered appropriate due to stress and cannibalism. Mortality checks were made after 0.5, 1, 2 and 4 h and at four hourly intervals thereafter.

Experiment 3 - Effect of low dissolved oxygen on acute ammonia toxicity

In this experiment only unsexed *P. monodon* were used and, except for oxygen control, similar methods to those described for Experiments 1 and 2 were used. Average initial prawn weight was 4.2 g (range 2.0 - 8.3). The dissolved oxygen concentration was reduced for five of the six treatments to approximately 2.2 mg l^{-1} using a vacuum, gas-stripping apparatus similar to that described by Mount (1961, 1964).

One of these five treatments was a control (no added ammonia) and in the other four ammonia was added to give 0.53, 0.95, 1.30 and 1.63 mg UAN l^{-1} (12.6, 19.9, 27.0 and 33.9 mg TAN l^{-1}). For the sixth treatment vigorously aerated seawater was used and ammonia was added to give 1.60 mg UAN l^{-1}.

Experiments 4 and 5 - Chronic bioassays

To assess the effects of ammonia on weight gain, survival and food conversion efficiency for school and *P. monodon*, six experimental levels ranging from 0.01 - 1.20 mg UAN l^{-1} (0.2 - 24.2 mg TAN l^{-1} N l^{-1}) and 0.02 - 1.60 mg UAN l^{-1} (0.3 - 32.9 mg TAN l^{-1}) respectively were established. For Experiment 4 *M. macleayi* averaged 2.5 g (range 0.9 - 5.6 g) and for Experiment 5 *P. monodon* averaged 3.7 g (range 1.6 - 6.9 g). Prawns were individually tagged before stocking by injecting a marker of petroleum jelly impregnated with a fluorescent dye, "saturn yellow", into the musculature of one of the abdominal segments on either side (Klima, 1965). Prawns were fed *ad libitum*, and gentle aeration was provided throughout. As juvenile male and female *M. macleayi* grow at different rates, (Maguire and Allan, 1985), equal numbers of males and females were stocked into each aquarium for Experiment 4, but as *P. monodon* grow at similar rates when <13 g (Liao, 1977), prawn sex was not recorded for Experiment 5. At the start of each chronic bioassay, ammonia concentrations were allowed to rise gradually, reaching required concentrations within 48 h.

After 3 weeks exposure, the average individual prawn weight gain was determined for each aquarium. Weight gain values were based on individual measurements of initial and final weights for prawns that survived or that died during the last three days of an experiment. The minimum numbers of prawns, in any aquarium, used for average individual weight gain determinations were $n \geq 6$ for *M. macleayi* and $n \geq 7$ for *P. monodon*.

Statistical analyses

For acute bioassays (Experiments 1 and 2) mortality data represent deaths over 96 h unless stated. The dose response at each concentration was determined with probit analysis (Busvine, 1957), using pooled data for replicate aquaria. Acute toxicity was expressed as 96 h LC_{50}, that concentration which killed 50 % of the prawns in 96 h. To enable comparisons with other studies, 48 h LC_{50} values were also calculated.

For all other experiments (3 - 5), treatment effects were identified using analysis of variance (ANOVA), and multiple comparisons among means were made using Tukey's honestly significant difference techniques (Sokal and Rohlf, 1981). Homogeneity of variance was confirmed using Cochran's test (Winer, 1971). Throughout the paper mean ± S.E. values are given, except where 95 % confidence limits are indicated.

For the chronic bioassay with *M. macleayi* (Experiment 4) two-factor ANOVA was used to investigate the effects of ammonia concentration and sex on growth. For all other analyses, including the effect of ammonia concentration on *P. monodon* growth (Experiment 5), single factor ANOVA's were used. Survival and food conversion efficiency for each aquarium were also calculated. The food conversion ratio (FCR) was estimated by dividing the total amount of food eaten by the prawns in each aquarium by the total prawn biomass increase in each aquarium (g wet weight). Weight of bivalve fed was adjusted to 92 % dry weight for comparison with values recorded in trials with pelleted artificial diets (Maguire et al., 1988). In aquaria where mortality occurred, the initial weights of the prawns that died were not considered in FCR calculations. When survival was less than 80 % within an aquarium, the data for that treatment were not included in statistical analysis of FCR. To meet the assumptions of normality and homogeneity of variance, FCR data were transformed ($\log x$) prior to analysis.

The EC_5 value, that concentration at which prawn growth was reduced by 5 %, was considered the "maximum acceptable" concentration of ammonia. This was estimated from two intersecting linear regressions (Sedgwick, 1979; Maguire and Hume, 1982). The first regression line was based on the control and those treatments where growth was not obviously affected by ammonia concentration. The second was based on all remaining treatments plus the results from the treatment with the highest ammonia concentration from the first regression analysis. The point of intersection estimates the maximum concentration of ammonia that did not reduce prawn growth. This point and the second regression equation were used to predict the concentration of ammonia expected to result in a 5 % reduction

in growth (Figs. 1 and 2).

RESULTS

Experiments 1 and 2 - Acute bioassays

Mortality of *M. macleayi* over 96 h increased from 33.3 % to 100 % as ammonia increased from 1.33 to 2.64 mg UAN l^{-1} (Table 1). Because most prawns died in four of the six treatments (90.0 - 100 %), lower concentrations were used in the acute bioassay with *P. monodon* (Table 2). With *P. monodon* few mortalities (0 - 3.3 %) were recorded up to 1.42 mg UAN l^{-1} with mortality increasing to 96.7 % as the concentration increased to 2.27 mg UAN l^{-1} (Table 2). Lethal concentrations (96 h LC_{50} values) were higher for *P. monodon* (1.69 mg UAN l^{-1}, 95 % confidence limits; 1.69, 1.84 mg UAN l^{-1}) than for *M. macleayi* (1.39 mg UAN l^{-1} 95 % confidence limits; 1.22, 1.57 mg UAN l^{-1}).

Experiment 3 - Effect of low DO on acute ammonia toxicity

At a low DO concentration (2.2 mg l), mortality of *P. monodon* was low (0 - 10.0 %) and not significantly affected ($P>0.05$) by ammonia concentration in the range 0.004 - 1.30 mg UAN l^{-1} (Treatments 1 - 4; Table 3). However, at 1.63 mg UAN l^{-1}, 90.0 % died at the low DO concentration. At a similar ammonia concentration (1.60 mg UAN l^{-1}) prawns held in well-oxygenated water (5.7 mg l^{-1}) had significantly ($P<0.05$) lower mortality (33.3 %).

Experiment 4 - Chronic bioassay with M. macleayi

Female *M. macleayi*, in the presence of males, grew faster ($P<0.05$) than males in the presence of females in all treatments. As there was no sex-ammonia interaction ($P>0.05$) the data for both sexes were combined to compare means (Table 4) and to estimate the EC_5 (Figure 1). Growth relative to the control (0.01 mg UAN l^{-1}) was not significantly ($P>0.05$) reduced from 0.10 - 0.64 mg UAN l^{-1}, however, at 0.89 and 1.20 mg significant reductions occurred ($P<0.05$) (Table 4). The EC_5 value was 0.35 mg UAN l^{-1} (7.6 mg TAN l^{-1}). Survival was high (90 - 100 %) at 0.01 - 0.64 UAN l^{-1} but was significantly reduced at the highest concentration (1.20 mg UAN l^{-1}) ($P<0.05$). FCR was unaffected at ammonia concentrations where survival exceeded 80 % in each replicate ($P>0.05$) (Table 4).

Experiment 5 - Chronic bioassay with P. monodon

Growth relative to the control (0.02 mg UAN l^{-1}) was not significantly reduced ($P>0.05$) from 0.10 - 0.45 mg UAN l^{-1}, however, at 0.78 and 1.08 mg significant reductions occurred ($P<0.05$) (Table 5). The EC_5 value was 0.21 mg UAN l^{-1} (4.1 mg TAN l^{-1}). Ammonia concentration also significantly reduced survival ($P<0.05$) at 1.08 and 1.60 mg UAN l^{-1} and FCR ($P<0.05$) at 0.78 mg UAN l^{-1}.

DISCUSSION

During all experiments, survival among controls exposed to negligible ammonia concentrations was > 90 %, and relatively stable ammonia, pH, temperature and DO concentrations were maintained in all aquaria. Although stocking density in the aquaria was equivalent to approximately 45 prawns m^{-2}, growth of *M. macleayi* in the controls was 0.07 g day^{-1} during Experiment 4. This compared well with that recorded during four 8 - 12 week farming trials (0.06 - 0.08 g day^{-1}) with this species at between 17.4 and 27.0 prawns m^{-2} in a 1 ha pond (Maguire and Allan, 1985). Similarly, growth of *P. monodon* (0.18 g day^{-1}) in the controls in Experiment 5 was comparable with that measured by the authors in model ponds (0.17 ± 0.01 g day^{-1}) at 25 prawn m^{-2} and a similar average water temperature (26.9 °C). However, faster growth rates for this species (0.3 g day^{-1}) have been recorded (Liao, 1977). As survival and growth rates were comparable with larger scale facilities, the continuous flow bioassay system used here was considered appropriate for assessing both the acute and the chronic toxicities of ammonia.

The acute toxicity results obtained are compared with other published values for crustaceans in Table 6. As different levels of water quality variables which affect the NH_3/NH_4^+ equilibrium were used, e.g., pH, comparisons are made between concentrations of un-ionised ammonia (NH_3-N), the more toxic form of ammonia. Previous studies with penaeid larvae have shown that tolerance to acutely toxic concentrations of ammonia increases with age (Jayasankar and Muthu, 1983; Chin and Chen, 1987). For example the 24 h LC_{50} for *P. monodon* increased from 0.54 to 4.70 mg UAN l^{-1} as the animals progressed from nauplii to postlarvae (Chin and Chen, 1987). Here the 96 h LC_{50} value for juvenile *P. monodon* (1.69 mg UAN l^{-1}) was higher than for postlarvae (1.04 mg UAN l^{-1}) (Chin and Chen, 1987) although the 48 h LC_{50} values were more similar (2.33 and 2.50 mg UAN l^{-1} respectively).

Wickins (1976) used small (0.5 - 1.5 g) juvenile penaeids of seven mixed species and found a lower 48 h LC_{50} value (1.29 mg UAN l^{-1}) than those for *M. macleayi* and *P. monodon* determined here. Differences in size (age) and species may have accounted for differences in susceptibility. Wajsbrot et al. (1990) reported a 96 h LC_{50} of 1.43 mg UAN l^{-1} for *P. semisulcatus*, very similar to the concentration determined here for M. macleayi. The 96 h LC_{50} value of 3.0 mg UAN l^{-1} for *P. japonicus* juvenile determined by Kou and Chen (1991) was the highest equivalent value recorded for an aquatic crustacean (Table 6). The 96 h LC_{50} values for *P. monodon* determined here are the next highest equivalent values presented in Table 6 indicating that, compared with other penaeids, this species is also tolerant of acutely toxic concentrations of ammonia.

Recently, Chen et al. (1990) reported a 96 h LC_{50} for *P. monodon* juveniles (3.9-6.2 g) of 0.96 mg UAN^{-1}, considerably lower than the value estimated here for the same species. Chen et al. (1990) used static bioassay methods, the prawns were fed, and only 10 l of test solution was provided in each aquaria containing 10 prawns. The prawns may have been stressed and more susceptible to ammonia poisoning than the ones used in the present study.

In a chronic bioassay for low DO, Seidman and Lawrence (1985) found that while growth of *P. monodon* was depressed at a very low DO concentration (1.2 mg l^{-1}), neither survival nor growth were affected at a concentration of 2.2 mg l^{-1} or above. In the present study, a DO concentration of 2.2 mg l^{-1} together with ammonia concentrations ranging from 0.004 - 1.30 mg UAN l^{-1} (Table 3) had no significant effect on survival of *P. monodon*. However, at higher ammonia concentrations (1.60 - 1.63 mg UAN l^{-1}) mortality was much greater (90 %) at a DO concentration at 2.3 mg l^{-1} than at a DO concentration of 5.7 mg l^{-1} (33.3 %). This demonstrates that low DO increases acute ammonia toxicity. In experiment 3 the 96 h LC_{50} at low DO concentrations (2.2 - 2.3 mg l^{-1}) lay in the range 1.30 - 1.63 mg UAN l^{-1}, compared with the 96 h LC_{50}, estimated in well oxygenated water (Experiment 2), of 1.69 mg UAN l^{-1} (95 % confidence limits: 1.56, 1.84 mg UAN l^{-1}). This indicates that the low DO caused a reduction in the acutely lethal concentrations of ammonia to *P. monodon* of up to 0.5 mg UAN l^{-1}. For this comparison to be valid the two data sets (Experiments 2 and 3) must be comparable. To confirm this assumption, the mortality data (33.3 %) for Treatment 6 (high DO; 1.60 mg UAN l^{-1}) in Experiment 3 (Table 3) was used in the probit regression equation generated from the acute bioassay in oxygenated water (Experiment 2). The ammonia concentration which was predicted to give this mortality after 96 h was 1.61 mg UAN l^{-1} (95 % confidence limits 1.47, 1.76 mg UAN l^{-1}). This compares very well with the actual ammonia concentration used for Treatment 6 in Experiment 3 (1.60 mg UAN l^{-1}) (Table 3). Recently, Wajsbrot et al. (1990) also found ammonia toxicity to *P. semisulcatus* increased when DO was reduced to concentrations below 55 % saturation. At 27 % saturation, the ammonia toxicity (96 h LC_{50}) was doubled (Wajsbrot et al., 1990).

Toxicity studies of water quality variables to aquaculture species usually indicate "safe" concentrations for routine farm management. Here EC_5 values, calculated from 3 week experiments, were used to indicate the maximum acceptable concentrations. For *M. macleayi* and *P. monodon* these were 0.35 and 0.21 mg UAN l^{-1}, respectively. In the only other published study on the effects of ammonia on penaeid growth, the average of the EC_1 and EC_2 values (those concentrations which reduced growth by 1 and 2 %) was used as an estimate of the "maximum acceptable" concentration (Wickins, 1976). Six mixed species were used and the concentration reported was 0.10 mg UAN l^{-1} (Wickins, 1976). If the same technique is applied here the "maximum acceptable" concentration for *M. macleayi* and *P. monodon* would be 0.30 and 0.17 mg UAN l^{-1} respectively, higher than Wickins' value and indicating that *M. macleayi* and *P. monodon* are also tolerant of sub-lethal concentrations of ammonia.

Several other authors have reported 'safe' concentrations which have been based on the multiplication of experimentally determined LC_{50} values by an 'application factor' (Delistraty 1977, Jayasankar & Muthu, 1983; Chin & Chen, 1987). At best this technique can only be described as providing a tentative estimate as it relies upon an arbitrarily determined factor, usually 0.1 (Sprague, 1971). If an application factor of 0.1 and the 96 h LC_{50} determined for *P. monodon* during the present study are used, the estimated 'safe' concentration (0.17 mg UAN l^{-1}) is in close agreement to the EC_5 determined experimentally (0.21 mg UAN l^{-1}: 95 %

confidence limits = 0.13-0.25 mg UAN l^{-1}). For *M. macleayi*, however, the 'safe' concentration estimated using the application factor (0.14 mg UAN l^{-1}) is considerably lower than that determined experimentally (0.35 mg UAN l^{-1}: 95 % confidence limits = 0.25-0.56 mg UAN l^{-1}).

The higher EC_5 value estimated for *M. macleayi* compared with *P. monodon* was anomalous, considering that the 96 h LC_{50} for *M. macleayi* was lower (1.39 mg UAN l^{-1}) than that for *P. monodon* (1.69 mg UAN l^{-1}). The two species may differ in their relative susceptibility to acute and chronic ammonia poisoning. However, it should be noted that during the chronic bioassay with *P. monodon*, prolonged dry weather resulted in elevated salinities (36.0 ‰) which were above the optimum for osmoregulation (Cawthorne et al., 1983) and this may have affected their susceptibility to chronic ammonia poisoning. Increased salinities have been shown to reduce ammonia excretion rates (Spaargaren et al., 1982; Claybrook, 1983). In contrast salinities during the chronic bioassay with *M. macleayi* were lower (31.5 ‰) and close to their optimum for osmoregulation (Maguire and Allan, unpublished data, 1989).

The concentration of ammonia in prawn ponds or tanks should not be allowed to rise above "maximum acceptable" concentrations (EC_5) and should certainly not be allowed to approach lethal concentrations (96 h LC_{50}). Total ammonia concentrations in excess of the LC_{50} values determined here have been recorded during intensive culture of *Penaeus penicillatus* (Chen et al., 1988), however, species tolerances do differ. Temperature, salinity, dissolved oxygen and pH should also be monitored as unfavourable levels may synergistically affect ammonia toxicity.

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REFERENCES

Alabaster, J. S., Shurben, D. G. and Knowles, G., 1979. The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, *Salmo salar* L. J. Fish Biol., 15: 705-712.

American Public Health Association (A.P.H.A.), 1971. Standard Methods, 13th Edition. A.P.H.A., Washington D.C., 874 pp.

Armstrong, D. A., Chippendale, D., Knight, A. W. and Colt, J. E., 1978. Interaction of ionized and un-ionized ammonia on short-term survival and growth of prawn larvae, *Macrobrachium rosenbergii*. Biol. Bull., 154: 15-31.

Boyd, C. E., 1982. Water Quality Management for Pond Fish Culture. Elsevier, Amsterdam, 318 pp.

Bower, C. E. and Bidwell, J. P., 1978. Ionization of ammonia in seawater: Effects of temperature, pH and salinity. J. Fish. Res. Board Can., 35: 1012-1016.

Busvine, J. R., 1957. A Critical Review of the Techniques for Testing Insecticides. Commonwealth Institute of Entomology, London, 208 pp.

Cawthorne, D. F., Beard, T., Davenport, J. and Wickins, J. F., 1983. Responses of juvenile *Penaeus monodon* Fabricius to natural and artificial sea waters of low salinity. Aquaculture, 32: 165-174.

Chen, J. C., Liu, P. C. and Lin, Y. T., 1988. Super intensive culture of red-tailed shrimp *Penaeus penicillatus*. J. World Aquaculture Soc., 19(3): 127-131.

Chen, J. C., Liu, P. C. and Lei, S. C., 1990. Toxicities of ammonia and nitrite to *Penaeus monodon* adolescents. Aquaculture, 89: 127-137.

Chin, T. S. and Chen, J. C., 1987. Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*. Aquaculture, 66: 247-253.

Claybrook, D. L., 1983. Nitrogen metabolism. In: L. H. Mantel (Editor), The Biology of Crustacea. Volume 5 - Internal Anatomy and Physiological Regulation. Academic Press, New York/London/Paris, pp. 163-213.

Colt, J. E. and Armstrong, D. A., 1981. Nitrogen toxicity to crustaceans, fish and molluscs. In: L. J. Allen and E. C. Kinney (Editors), Proceedings of the Bio-Engineering Symposium for Fish Culture. Fish Culture Section of the American Fisheries Society (FCS publ. 1), pp. 34-47.

Dal Pont, G., Hogan, M. and Newell, B., 1973. Laboratory techniques in marine chemistry. II. Determination of ammonia in seawater and the preservation of samples for nitrate analysis. Aust. C.S.I.R.O. Div. Fish. Oceanogr. Rep., No. 55, CSRIO, Sydney, New South Wales, 11 pp.

Delistraty, D. A., Carlberg, J. M., Van Olst, J. C. and Ford, R. F., 1977. Ammonia toxicity in cultured larvae of the American lobster (*Homarus americanus*). Proc. Ann. Meet. World Mariculture Soc., 8: 647-672.

Jayasankar, P. and Muthu, M. S., 1983. Toxicity of ammonia to the larvae of *Penaeus indicus* H. Milne Edwards. Indian J. Fish., 30(1): 1-12.

Klima, E. F., 1965. Evaluation of biological stains, ink and fluorescent pigments as marks for shrimp. US Fish. Wildl. Serv. Spec. Sci. Rep., No. 511, 8 pp.

Kou, Y. Z. and Chen, J. C., 1991. Acute toxicity of ammonia to *Penaeus japonicus* Bate juveniles. Aquaculture Fish. Manag., 22: 259-263.

Liao, I. C., 1977. A culture study on grass prawn, *Penaeus monodon*, in Taiwan - the patterns, the problems and the prospects. J. Fish. Soc. Taiwan, 5(2): 11-29.

Lloyd, R., 1961. Effect of dissolved oxygen concentrations on the toxicity of several poisons to rainbow trout (*Salmo gairdnerii* Richardson). J. Exp. Biol., 38: 447-455.

Maguire, G. B. and Hume, I. D., 1982. A study of the nutritional requirements of *M. macleayi Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture, 29: 261-278.

Maguire, G. B. and Allan, G., 1985. Development of methods for growing juvenile *M. macleayi*, *Metapenaeus macleayi*, in estuarine ponds. In: P. C. Rothlisberg, B. J. Hill and D. J. Staples (Editors), Second Aust. Nat. Prawn Sem., NPS2, 22-26 October 1984, Cleveland. NPS2 Cleveland, Queensland, pp. 337-351.

Maguire, G. B., Allan, G. L., Baigent, R. and Frances, J., 1988. Evaluation of the suitability of some Australian and Taiwanese diets fed to *P. monodon* (*Penaeus monodon*) in ponds. In: L. H. Evans and D. O'Sullivan (Editors), Proc. First Aust. Shellfish Aquaculture Conf., Perth, Australia, 23-27 October 1988. Curtin University of Technology, Perth, pp. 89-104.

Major, G. A., Dal Pont, J., Klye, J. and Newell, B., 1972. Laboratory techniques in marine chemistry. A manual. Aust. CSIRO Div. Fish. Oceanogr. Rep., No. 51, CSIRO, Sydney, New South Wales, 55 pp.

Mount, D. I., 1961. Development of a system for controlling dissolved-oxygen content of water. Trans. Am. Fish. Soc., 90(3): 323-327.

Mount, D. I., 1964. Additional information on a system for controlling the dissolved oxygen content of water. Trans. Am. Fish. Soc., 93: 100-103.

Scribner, E. A., 1986. Report on seawater supply, Brackish Water Fish Culture Research Station. Div. Fish., N.S.W. Dept. Agric. Int. Rep., (unpubl.), 6 pp.

Sedgwick, R. W., 1979. Influence of dietary protein and energy on growth, food consumption, and food conversion efficiency in *Penaeus merguiensis* De Man. Aquaculture, 16: 7-30.

Seidman, E. R. and Lawrence, A. L., 1985. Growth, feed digestibility, and proximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. J. World Mariculture Soc., 16: 333-346.

Shaw, J., 1960. The absorption of sodium ions by the crayfish *Astacus pallipes* Lereboullet. III. The effect of other cations in the external solution. J. Exp. Biol., 37: 548-556.

Smart, G. R., 1978. Investigations of the toxic mechanisms of ammonia to fish - gas exchange in rainbow trout (*Salmo gairdneri*) exposed to acutely lethal concentrations. J. Fish Biol., 12: 93-104.

Sokal, R. R. and Rohlf, F. J., 1981. Biometry. W. H. Freeman and Company, San Francisco, 859 pp.

Spaargaren, D. H., Richard, P. and Ceccaldi, H. J., 1982. Excretion of nitrogenous products by *Penaeus japonicus* Bate in relation to environmental osmotic conditions. Comp. Biochem. Physiol., 72A(4): 673-678.

Sprague, J. B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res., 3: 793-821.

Stanier, R. Y., Aderlberg, E. A. and Ingraham, J. L., 1976. General Microbiology. MacMillan Press Ltd, London, 871 pp.

Tarazona, J. V., Munoz, M. J., Ortiz, J. A., Nunez, M. O. and Camargo, J. A., 1987. Fish mortality due to acute ammonia exposure. Aquaculture and Fish. Manag., 18: 167-172.

Thurston, R. V., Phillips, G. R. and Russo, R. C., 1981. Increased toxicity of ammonia to rainbow trout (*Salmo gairdneri*) resulting from reduced concentrations of dissolved oxygen. Can. J. Fish. Aquat. Sci., 38: 983-988.

Wajsbrot, N., Gasith, A., Krom, M. O. and Samocha, T. M., 1990. Effects of dissolved oxygen and the moult stages on the acute toxicity of ammonia to juvenile green tiger prawn *Penaeus semisulcatus*. Environ. Toxicol. Chem., 9: 497-504.

Whitfield, M., 1974. The hydrolysis of ammonium ions in sea water - a theoretical study. J. Mar. Biol. Ass. U.K., 54: 565-580.

Wickins, J. F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Winer, B. J., 1971. Statistical Principles in Experimental Design, 2nd Edition. McGraw-Hill, New York, NY, 907 pp.

TABLE 1

Mortality of *Metapenaeus macleayi* exposed to a range of ammonia concentrations (Experiment 1)[1].

Ammonia (mg N l^{-1})[2,3]		Mortality (%)	
Total	Un-ionised	48 h	96 h
0.06±0.01	0.003±0.0003	3.3±3.3	10.0±10.0
25.0±0.3	1.33±0.02	16.7±12.0	33.3±14.5
31.0±0.7	1.60±0.06	43.3±16.7	90.0±5.8
41.2±1.2	2.19±0.06	86.7±6.6	96.7±3.3
49.6±2.4	2.64±0.13	100	100.0
66.4±1.7	3.54±0.09	100	100.0

1 Values are means±S.E. (n=3) for each treatment.

2 Mean of the average values (based on daily measurements) for each replicate aquarium.

3 Average water temperature, pH and salinity values for all treatments were 25.1 °C, 8.0 ± 0.1 and 34.5 ‰ in that order.

TABLE 2

Mortality of *Penaeus monodon* exposed to a range of ammonia concentrations (Experiment 2)[1].

Ammonia (mg N l^{-1})[2,3]		Mortality (%)	
Total	Un-ionised	48 h	96 h
0.04±0.003	0.002±0.0003	0	0
10.5±0.3	0.48±0.01	0	0
21.2±0.7	0.96±0.02	0	0
31.0±0.4	1.42±0.05	0	3.3±3.3
41.1±1.1	1.85±0.03	10.0±5.8	86.7±3.3
51.0±2.4	2.27±0.05	43.3±8.8	96.7±3.3

1 Values are means±S.E. (n=3) for each treatment.

2 Mean of the average values (based on daily measurements) for each replicate aquarium.

3 Average water temperature, pH and salinity values for all treatments were 26.0 °C, 0.1 ± 8.0 and 34.0 ‰ in that order.

TABLE 3

Mortality of *Penaeus monodon* at different ammonia and dissolved oxygen concentrations (Experiment 3)[1].

Treat-ment	Ammonia (mg N l^{-1})[2,3]		Dissolved[2] oxygen (mg l^{-1})	Mortality[3,4] (%)
	Total	Un-ionised		96 h
Not aerated				
1	0.1±0.01	0.004±0.0004	2.2±0.09	6.7±6.7^{ab}
2	12.6±0.2	0.53±0.04	2.2±0.09	0^{a}
3	19.9±0.3	0.95±0.01	2.2±0.03	3.3±3.3^{ab}
4	27.0±0.4	1.30±0.02	2.2±0.06	10.0±10.0^{ab}
5	33.9±0.5	1.63±0.03	2.3±0.03	90.0±5.8^{c}
Aerated				
6	33.5±0.5	1.60±0.03	5.7±0.01	33.3±5.8^{b}

1 Values are means±S.E. (n=3) for each treatment.

2 Mean of the average values (based on daily measurements) for each replicate aquarium.

3 Average water temperature, pH and salinity values for all treatments were 26.0 ± 0.2 °C, 8.0 ± 0.1 and 31.0 ‰ in that order.

4 Means, within a column, sharing a common superscript are not significantly different ($P>0.05$). Data were transformed (arcsine [$x^{0.5}$]) prior to statistical analysis.

TABLE 4

Effects of ammonia on weight gain, survival and food conversion ratios (FCR) for *Metapenaeus macleayi* over 21 day (Experiment 4)[1].

Ammonia (mg N l^{-1})[2,3]		Wt. gain[4] (g $prawn^{-1}$)	Survival[4] (%)	FCR[4,5]
Total	Un-ionised			
0.3±0.01	0.01±0.0	1.5±0.1[a]	100[a]	2.4±0.26[a]
2.2±0.04	0.10±0.003	1.6±0.2[a]	100[a]	2.2±0.57[a]
6.2±0.1	0.27±0.003	1.6±0.2[a]	100[a]	2.1±0.20[a]
13.6±0.1	0.64±0.09	1.3±0.2[a]	90.0±0.6[a]	2.8±0.72[a]
18.5±0.2	0.89±0.01	0.6±0.2[b]	73.3±1.2[ab]	*
24.2±0.6	1.20±0.04	0.5±0.1[b]	50.0±1.2[b]	*

1 Values are means±S.E. (n=3) for each treatment.

2 Mean of the average values (based on daily measurements) for each replicate aquarium.

3 Average water temperature, pH and salinity values for all treatments were 25.0 ± 0.2 °C, 8.0 ± 0.1 and 31.5 ‰ in that order.

4 Means, within a column, sharing a common superscript are not significantly different (P>0.05).

5 Data were transformed (log x) prior to statistical analysis.

* Excessive mortality (>80 %) for reliable FCR estimation.

TABLE 5

Effects of ammonia on weight gain, survival and food conversion ratios for *Penaeus monodon* over 21 day (Experiment 5)[1].

Ammonia (mg N l^{-1})[2,3]		Wt.gain[4] (g $prawn^{-1}$)	Survival[4] (%)	FCR[4,5]
Total	Un-ionised			
0.30±0.03	0.02±0.006	3.8±0.26[a]	90.0±1.00[a]	1.6±0.17[a]
2.1±0.04	0.10±0.01	3.8±0.27[a]	100[a]	1.4±0.06[a]
9.8±0.3	0.45±0.02	2.9±0.18[a]	96.7±0.33[a]	1.7±0.07[a]
17.1±0.2	0.78±0.01	1.4±0.20[b]	100[a]	2.6±0.29[b]
24.9±0.1	1.08±0.02	0.4±0.06[c]	46.7±0.88[b]	*
32.9±1.0	1.60±0.07	-	0[c]	*

1 Values are means±S.E. (n=3) for each treatment.

2 Mean of the average values (based on daily measurements) for each replicate aquarium.

3 Average water temperature, pH and salinity values for all treatments were 26.9 ± 0.1 °C, 8.0 ± 0.1 and 36.0 ‰ in that order.

4 Means, within a column, sharing a common superscript are not significantly different ($P>0.05$).

5 Data were transformed (log x) prior to statistical analysis.

* Excessive mortality (>80 %) for reliable FCR estimation.

TABLE 6

Comparison of acute ammonia toxicity to aquatic crustaceans.

Species	Stage	LC_{50} (hrs)	Un-ionised ammonia (mg NH_3-N l^{-1})
Homarus americanus [1]	4th stage larvae	Incipient*	1.40
Macrobrachium rosenbergii [2]	larvae 3-8d post-hatch	144	0.26[a] 0.80[b] 1.35[c]
Penaeid prawns [3] (Data for seven species pooled)	juveniles (0.5 - 1.5g)	48	1.29
Penaeus indicus [4]	nauplii	24	0.29
	protozoeae	24	0.95
	mysis	24	3.17
	protozoeae	48	1.18
	nauplii-postlarvae	Incipient*	0.93
P. monodon [5]	nauplii	24	0.54
	protozoeae	24	0.76
	mysis	24	2.17
	mysis	48	1.30
	postlarvae	24	4.70
	postlarvae	48	2.50
	postlarvae	96	1.04
P. monodon [6]	juveniles (3.9 - 6.2 g)	96	0.96
		144	0.77
P. monodon [7]	juveniles (0.8-4.5 g)	48	2.33
		96	1.69
P. semisulcatus [8]	juveniles (0.4 - 2.4 g)	96	1.43
P. japonicus [9]	juveniles (0.025 g)	96	3.0
Metapenaeus macleayi [7]	juveniles (0.9-4.8 g)	48	1.66
		96	1.39

1 Delistraty et al. (1977);
2 Armstrong et al. (1978) [a] pH = 6.83, [b] = 7.60, [c] pH= 8.34;
3 Wickins (1976);
4 Jayasankar and Muthu (1983);
5 Chin and Chen (1987);
6 Chen et al. (1990);
7 Present study;
8 Wajsbrot et al. (1990);
9 Kou and Chen (1991).

* Incipient LC_{50} - lethal concentration for 50% of individuals on long exposure.

Figure 1 Effects of ammonia on growth of *Metapenaeus macleayi* over 21 day (Experiment 4).

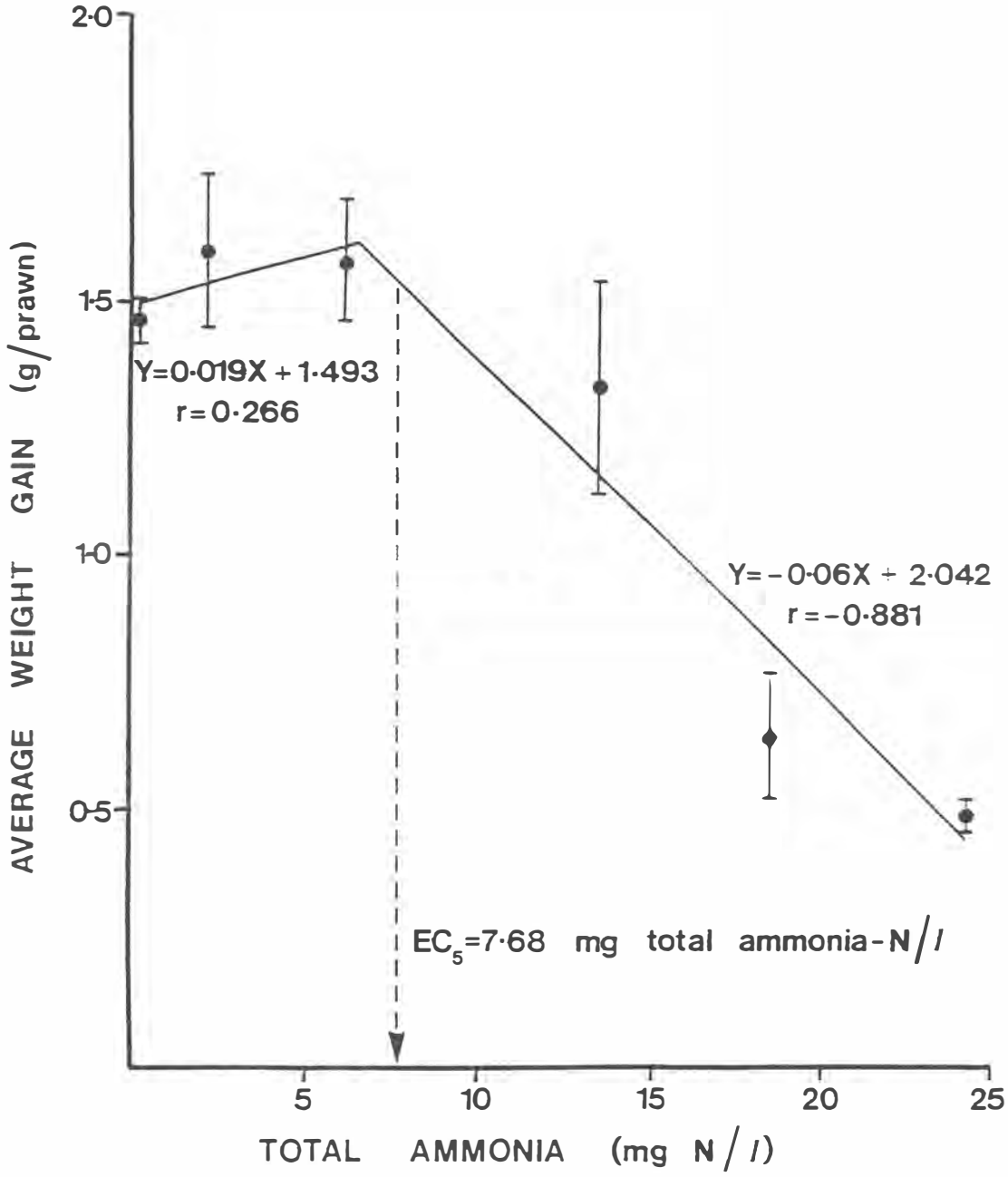
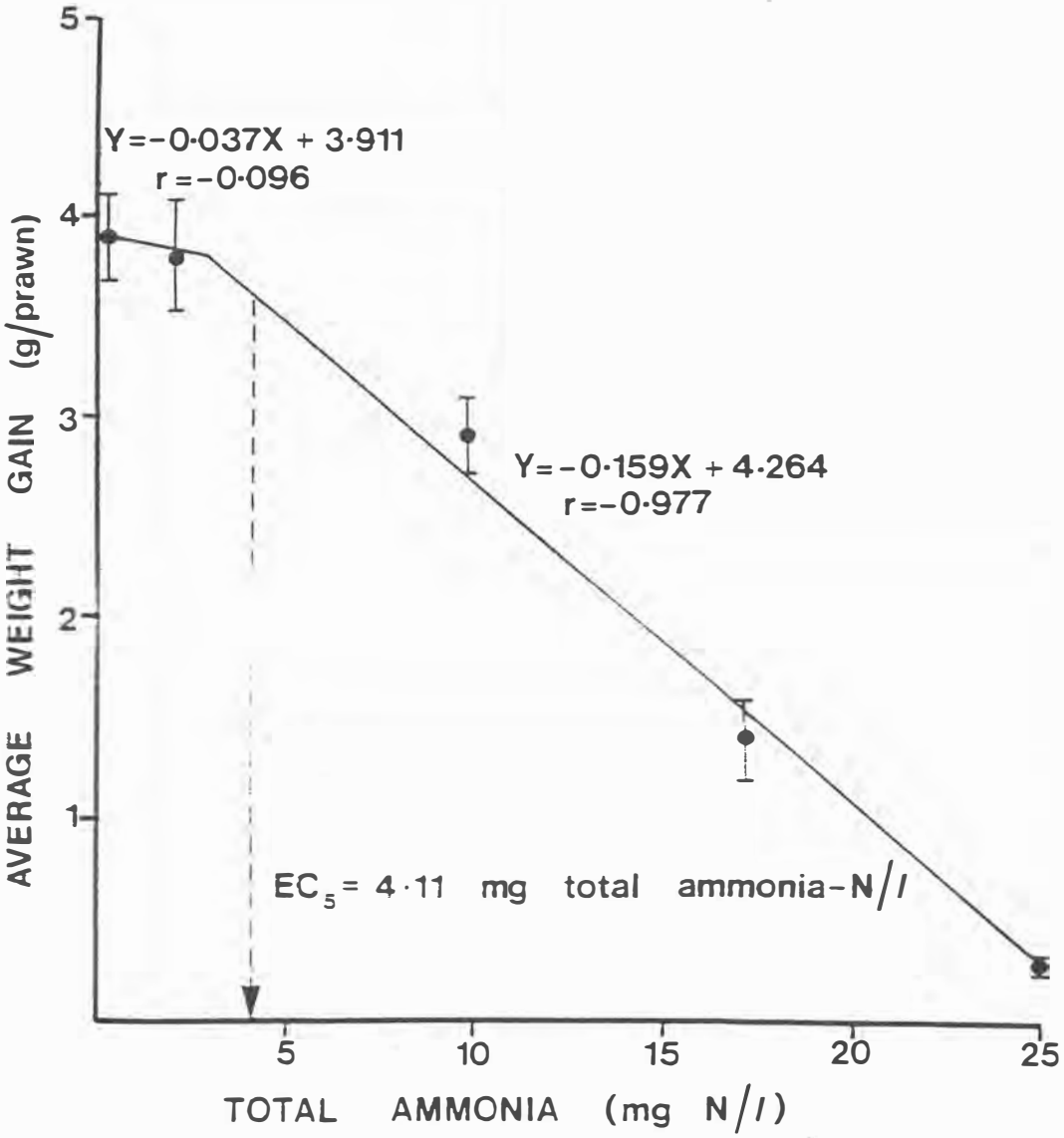

2·0
1·5
1·0
0·5
AVERAGE WEIGHT GAIN (g/prawn)
Y=0·019X + 1·493
r=0·266
Y=-0·06X + 2·042
r=-0·881
EC5=7·68 mg total ammonia-N/l
5
10
15
20
25
TOTAL AMMONIA (mg N/l)

Figure 2 Effects of ammonia on growth of *Penaeus monodon* over 21 day (Experiment 5).


Y=-0·037X + 3·911
r=-0·096
Y=-0·159X + 4·264
r=-0·977
EC5 = 4·11 mg total ammonia-N/l
AVERAGE WEIGHT GAIN (g/prawn)
TOTAL AMMONIA (mg N/l)
1
2
3
4
5
5
10
15
20
25

2.4 Lethal concentrations of low dissolved oxygen and effects of short term oxygen stress on subsequent growth of juvenile *Penaeus monodon*

ABSTRACT

Allan, G. L. and Maguire, G. B., 1991. Lethal concentrations of low dissolved oxygen and effects of short term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture, 94: 27-37.

The lethal concentration (96 h LC_{50}) of dissolved oxygen (DO) for juvenile *Penaeus monodon* was estimated to be 0.9 mg O_2 l^{-1} (95% confidence limits: 0.8, 1.0 mg O_2 l^{-1}). Other replicate groups of *P. monodon* were subjected to short-term, severe DO stress and then grown under conditions approaching DO saturation. The aim was to simulate the type of event which can occur in ponds when emergency aeration successfully raises DO after an oxygen crisis. Neither the duration (4, 8 or 12 h), nor the concentration (0.5 - 0.6 or 1.0 - 1.1 mg O_2 l^{-1}) of the DO stress significantly ($P>0.05$) reduced subsequent growth or food conversion efficiency at favourable DO concentrations over 21 days. Prawn pond managers should not need to reduce feeding levels or consider premature harvest after a single, short-term DO crisis when mass mortality is avoided. This is provided that other water quality variables do not reach critical levels during the crisis.

INTRODUCTION

Low dissolved oxygen (DO) concentrations are the major limiting water quality variable in intensive aquaculture (Boyd and Watten, 1989). Critically low DO concentrations occur in ponds, particularly following die-off and subsequent decomposition of algal blooms, (Chang and Ouyang, 1988), and can cause stress or even mortality of prawns in ponds (Shigueno, 1975; Wickins, 1976a; Madenjian et al., 1987). Emergency aeration can often prevent mortality (Boyd and Watten, 1989), however, chronically low DO concentrations can reduce growth, feeding and moulting frequency (Seidman and Lawrence, 1985; Clark, 1986; Chang and Ouyang, 1988). Lethal concentrations in the range 0.2 - 1.0 mg O_2 l^{-1} have been reported for a number of species of penaeids including *Penaeus japonicus* (Egusa, 1961), *P. japonicus* and *P. kerathurus* (Tournier, 1972), *P. schmitti* (MacKay, 1974) and *P. monodon* (Liao and Huang, 1975). In all these studies lethal concentrations were estimated following progressive reduction of DO until mortality occurred. No studies were found which determined lethal DO concentrations for penaeids using toxicological methodology, although Morrissy et al. (1984) estimated a 24 h LC_{50} for the freshwater crayfish *Cherax tenuimanus* as 7.25% of oxygen saturation, or approximately 0.7 mg O_2 l^{-1} at 20°C.

The effects of sub-lethal concentrations of DO have been documented by several authors. The incipient limiting concentration (that concentration which separates respiratory independence from respiratory dependence) was estimated as being close to the air saturation value for *P. japonicus* (Egusa, 1961) and between 4.0 and 4.3mg l^{-1} for *P. monodon* (Liao and Murai, 1986). However, Seidman and

Lawrence (1985) found that growth of *P. vannamei* or *P. monodon* was not affected at constant average DO concentrations of 1.9 - 4.0 mg O_2 l^{-1} but was significantly reduced at 1.2 mg O_2 l^{-1}. Aquacop et al. (1988) reported that growth, survival and moulting frequency were significantly depressed when *P. vannamei* and *P. stylirostris* were maintained at a diurnally fluctuating DO regime (6:18 h; 1.5 mg O_2 l^{-1} and saturation). Clark (1986) found that mortality occurred and moulting was inhibited when *P. semisulcatus* were kept at a DO concentration of 2 mg O_2 l^{-1} for 17 days. Mortality ceased and moulting frequency returned to normal when DO was elevated to 5 mg O_2 l^{-1}.

No reports were found which assessed the effects of short-term, severe, low DO concentrations on subsequent growth and feeding at favourable DO concentrations for prawns or for any aquatic organism. Such a DO regime might occur when emergency aeration successfully raises and maintains DO concentrations after an oxygen crisis (Boyd and Watten, 1989). An understanding of these longer-term effects has important ramifications for pond management, particularly for planning feeding and harvesting strategies.

The objectives of this study were firstly to determine the lethal concentration of low DO for juvenile *P. monodon* using toxicological methodology and, secondly, to investigate the effects of a short-term, severe low DO stress on subsequent growth and food conversion efficiency.

METHODS

Juvenile prawns were purchased from prawn farms (Goodwood Island, NSW) and were acclimatised in the experimental aquaria for at least a week before experiments commenced. Prawns were fed commercially available, Taiwanese, prawn grower pellets (46.7 % crude protein [N x 6.25], dry basis).

DO concentrations in seawater were adjusted using nitrogen gas and an apparatus similar to that described by Seidman and Lawrence (1985). Six 1.8 m long by 300 mm diameter PVC columns were used as "degassing chambers" and were supplied with a constant flow of seawater which had been filtered through a sand filter and 5 µm and 1 µm cartridge filters. Nitrogen gas was supplied through air-stone diffusers at different metered rates to each degassing chamber. Seawater, with reduced DO concentrations, was gravity fed from each chamber to three randomly assigned 70 l acrylic aquaria, each containing ten prawns. To prevent prawns coming in contact with the air-water interface, and to reduce the opportunity for partial re-oxygenation of seawater, a layer of "bubble" packaging plastic was placed on top of the water in each aquarium. Care was taken not to trap any air beneath the flat surface of the plastic. These covers were used throughout the first experiment and during the period of oxygen stress in the second experiment.

No sediment was provided for the prawns and water temperatures were maintained at 27.1 $\pm$ 1.1°C, within the optimum range for this species (G. Maguire and G. Allan, unpublished data, 1988). Salinity ranged from 32 - 35 ‰ and a 12:12 h

photoperiod was maintained.

Water quality analyses

Throughout the first experiment, DO concentrations were measured in each aquarium every 4 h and in the second experiment every 2 h during the period of oxygen stress and at regular intervals thereafter. A Yeo-Kal polargraphic DO/temperature meter (Yeo-Kal Electronics, Brookvale, NSW, 2100 Australia) was used and was calibrated before each set of measurements using "air-saturated" seawater and daily using the Winkler method (APHA, 1971). Temperature was measured daily and salinity occasionally using a Yeo-Kal temperature/salinity conductivity meter. Regular measurements of pH were made, using an Orion pH/MV meter (Orion Research Inc. Cambridge, MA., 02139 USA) with a Ross combination electrode, and ammonia and nitrite were measured using the methods described by Dal Pont et al. (1973) and Major et al. (1972) respectively.

The seawater used in both experiments was essentially oceanic as described by Allan et al. (1990). Nitrite and ammonia concentrations did not exceed 20 μg NO_2-N l^{-1} and 405 μg total ammonia-N l^{-1} respectively in either experiment. These concentrations were below those which reduced growth of penaeids (Wickins, 1976b; Allan et al., 1990). Salinity and pH ranges were 30 - 34 ‰ and 7.9 - 8.1 respectively.

Experiment 1

For the acute bioassay, six treatments were established; one control in which seawater was vigorously aerated to saturation before being supplied to experimental aquaria and five others with nearly constant average DO concentrations of 2.1, 1.6, 1.0, 0.6 and 0.3 mg O_2 l^{-1} (Table 1). The flow-rate of seawater from the degassing chambers to each aquarium was 269 ml min^{-1} which replaced more than 90% of seawater in less than 10 h as recommended by Sprague (1969).

DO reached desired concentrations within 8 h after nitrogen gas was first introduced into degassing columns. For the purpose of calculating LC_{50}'s the bioassay was considered to have commenced 8 h after nitrogen gas was first introduced. The average individual initial wet weight of prawns was 4.2 g (range 2.0 - 8.3 g) and animals which had recently moulted were not used. Prawns were not sexed, as gender did not significantly affect lethal DO concentrations for *P. aztecus* (Kramer, 1975). No aeration was provided and prawns were not fed. Absence of response to touching with a glass rod was the criterion of death, and all dead prawns were removed. Following APHA (1971) and Sprague (1969) the bioassay was run for 96 h. Longer periods were not considered appropriate due to stress and cannibalism. The numbers of moribund prawns and exuviae were recorded after 0.5, 1, 2 and 4h and at four hourly intervals thereafter.

Experiment 2

This experiment assessed the effects of short-term DO stress on subsequent growth. Prawns (average individual initial wet weight 2.6 g, range 1.7 - 3.7 g) were individually weighed and tagged by injecting a marker of petroleum jelly impregnated with fluorescent dye, "saturn yellow", into the musculature on one side of an abdominal segment (Klima, 1965). Prawns were not sexed as juvenile male and female *P. monodon* grow at similar rates in this size range (Motoh, 1981). For the first treatment (control) seawater was vigorously aerated before being supplied to experimental aquaria and DO concentrations were maintained $\geq$ 6.0 mg O_2 l^{-1}. The six other treatments were combinations (2 x 3 factorial) of two ranges of low DO (0.5 - 0.6 or 1.0 - 1.1 mg O_2 l^{-1}) and three time periods (4, 8 or 12 h).

The flow-rate to each aquarium was 245 ml min^{-1}. The stress period was considered to have commenced 10 h after nitrogen gas was first introduced into the degassing columns as the desired concentrations of DO were all achieved within this time. After prawns had been stressed for the appropriate period, nitrogen gas was turned off, degassing columns were vigorously aerated and plastic covers on the water surface in each aquarium were removed. DO concentrations in all aquaria had recovered to above 4.0 mg O_2 l^{-1} within 4 h. Two hours later each aquarium was gently aerated using two air-stone diffusers and DO concentrations were maintained > 6.0 mg O_2 l^{-1} for the remainder of the experiment. Prawns were first fed 12 h after the end of the longest DO stress (12 h) and thereafter twice daily on an *ad libitum* basis. Following the DO stress, this bioassay was run for 21 days then prawns were individually weighed and an average individual prawn weight gain for each aquarium calculated. Food conversion ratio (FCR) was calculated by dividing the total weight of pellets eaten by the prawns (g dry weight) by the total prawn biomass increase (g wet weight) for each aquarium. In aquaria where mortality occurred, the initial weights of the prawns which died were not considered in growth or FCR calculations.

Statistical analyses

LC_{50} values were calculated using probit analysis (Busvine, 1957) and a computer package developed by Mr. A. Woods (University of NSW, Sydney, NSW, Australia). Homogeneity of variance was assessed using Cochran's test (Winer, 1971), treatment effects using single-factor ANOVA, and differences between means using Tukey's "honestly significant differences" technique (Sokal and Rohlf, 1981). A likelihood ratio test was used to examine differences in survival at all combinations of DO and time (Sokal and Rohlf, 1981).

RESULTS

In both experiments the initial behavioural response of the prawns to reduced DO concentrations included increased activity and attempts to surface. However, as DO declined further this activity ceased and by the time desired DO concentrations (2.1, 1.6, 1.0, 0.6 and 0.3 mg O_2 l^{-1}) were reached prawns were very still and there was no apparent interaction between individuals. Similar behavioural responses to

a gradual reduction in DO were reported for *Crangon vulgaris* (Huddart and Arthur, 1971).

Experiment 1

No prawns died in the control aquaria and only one died in each of the treatments where DO was maintained at 2.1 and 1.6 mg O_2 l^{-1} for 96 h. At lower DO concentrations (1.0 and 0.6 mg O_2 l^{-1}) higher mortality rates were recorded (26.7 and 96.7 % after 96 h respectively) (Table 1). At 0.3 mg O_2 l^{-1} all prawns died within 12 h. The LC_{50} values ranged from 0.5 mg O_2 l^{-1} after 16 h to 0.9 mg O_2 l^{-1} after 96 h (Fig. 1). Only 10 prawns moulted during the experiment, mostly in the treatments with higher DO concentrations (Table 1). However, at 1.0 mg O_2 l^{-1} both prawns which moulted died within 4 h and at 1.6 and 2.1 mg O_2 l^{-1} the only prawns which died had moulted in the past 4 h.

Experiment 2

No prawns died in the control aquaria, and survival rates among the treatments at the end of the growth trial ranged from a mean of 76.7 % in the treatment exposed to 0.5 mg O_2 l^{-1} for 12 h to 100 % in the treatment exposed to 0.6 mg O_2 l^{-1} for 4 h (Table 2). However, the effects of DO stress on survival were not significant ($\chi^2=2.3$, d.f.=5, $P>0.05$). Neither was the interaction between time and DO concentration ($\chi^2=0.47$, d.f.=2, $P>0.05$). The severe DO stress had no significant effect ($P>0.05$) on prawn weight gain or on FCR when favourable conditions were maintained for 21 days (Table 2).

DISCUSSION

Even though different methodology has been used, the results of Experiment 1 are in agreement with other studies which reported lethal DO concentrations for a number of penaeids of between 0.5 and 1.0 mg O_2 l^{-1} (Egusa, 1961; Tournier, 1972; MacKay, 1974; Kramer, 1975). However, for *P. monodon*, Liao and Huang (1975) estimated lethal concentrations of between 0.2 and 0.3 mg O_2 l^{-1} at a salinity of 30 ‰, considerably lower than the 96 h LC_{50} of 0.9 mg O_2 l^{-1} estimated here. Indeed all prawns exposed to a DO of 0.3 mg O_2 l^{-1} in the present study were dead within 12 h. The prawns used by Liao and Huang (1975) were smaller (maximum 1.5 g) and may have been more tolerant. Oxygen consumption in crustaceans is size dependent (Bridges and Brand, 1980; Dall, 1986) and Kramer (1975) reported significantly lower lethal DO concentrations for *P. aztecus* juveniles (1.37 g) compared with sub-adult prawns (6.12 g) at 2.5 and 36 ‰ and an oxygen reduction rate of 2.6 mg O_2 l^{-1} h^{-1}. Prawns may also have had more opportunity to acclimate to reduced DO concentrations in the Liao and Huang (1975) study. Shepard (1955) found resistance times for fish at given lethal concentrations increased by as much as five times following acclimation.

The 24 h LC_{50} estimated here (0.6 mg O_2 l^{-1}, 95% confidence limits 0.5, 0.7 mg O_2 l^{-1}[Fig. 1]) was also similar to the 24 h LC_{50} estimated by Morrissy et al. (1984) for the freshwater crayfish *Cherax tenuimanus* (0.7 mg O_2 l^{-1}).

Although few prawns moulted during Experiment 1, prawns which did moult appeared to be more susceptible to reduced DO. Dall (1986) found that oxygen consumption of *P. esculentus* increased by 55% three days prior to ecdysis, returning to normal one day after. A similar response during moulting was reported for the lobster *Homarus americanus* (Penkoff and Thurberg, 1982). No reports of increased toxicity of DO to penaeids during ecdysis were found, although, the close association between increased mortality and ecdysis and another water quality variable, pH, has been recorded for other crustaceans (Zimmer and Storr, 1983; Zimmer, 1987).

Ideally, acute toxicity tests should continue for extended periods to enable the concentration at which acute toxicity stops increasing with time, i.e., the incipient lethal concentration or the lethal threshold concentration (Sprague, 1969), to be determined. The present bioassay was only run for 96 h because of previously observed problems with cannibalism and mortality, exacerbated by starvation, when animals were held for longer than 96 h (Allan and Maguire, unpublished data, 1988). However, the apparent plateau of LC_{50} values after 72 h indicates that a threshold concentration was reached within the period of this study.

The concentrations of DO used in Experiment 2 for the short-term severe stress (0.5 - 0.6 and 1.0 - 1.1 mg O_2 l^{-1}) were chosen to be sufficiently low to cause mass mortality if maintained for extended periods. Concentrations below and just above the 96 h LC_{50} were used. In a pond the duration of any DO crisis will depend upon the time taken to detect and effectively respond to the problem, eg, through emergency aeration and water exchange. Short-term (2-6 h) declines in DO have been recorded in experimental fish ponds (Rapport et al., 1976) and in model prawn farming ponds (Allan et al., unpublished data, 1990). Boyd and Tucker (1979) found that a paddle wheel aerator was capable of elevating the DO concentration from 0.05 to 4.90 mg O_2 l^{-1} within 4 h at some stations in a 0.57 ha pond. Boyd and Watten (1989) provided standard aeration efficiency rates for "Taiwanese style" paddle wheel aerators (the type most commonly used in Australia) and procedures for calculating DO increases. Using these, it was estimated that four (0.75 kW) aerators in a 1 ha pond (25 °C, 25 ‰) could raise DO concentrations from 0.5 mg O_2 l^{-1} to 3.0 mg O_2 l^{-1} in about 9 h. Thus the time periods for DO stress in the present study were similar to those that may occur in commercial ponds.

The results of this study indicate that *P. monodon* are very resilient in response to a short-term DO stress. However, in ponds other water quality variables may change during a DO crisis. Notably, an increase in ammonia concentration and a decrease in pH, often accompany rapid DO declines (Boyd, 1982). Allan et al. (1990) found that a low DO (2.1 mg 0_2 l^{-1}) significantly increased the toxicity of ammonia to *P. monodon* and Wajsbrot et al. (1990) found that at 27 % dissolved oxygen saturation, the 96 h LC_{50} of ammonia for *P. semisulcatus* was doubled. The effect of any increase in ammonia may however accompany a decline in DO be mitigated by the accompanying decrease in pH which reduces the proportion of ammonia in the highly toxic un-ionised form. At the same total ammonia concentration (250 mg ammonia-N l^{-1}), Chen and Chin, (1989) found that the LT_{50}

of ammonia to *P. monodon* postlarvae increased from 25 min to 146 min when pH was reduced from 8.3 to 7.6.

Ideally, conclusions reached from aquarium studies should be validated against observations from pond studies. In another study, in 10 m^2 model ponds, one group of *P. monodon* grew at similar rates as unstressed groups despite being exposed to a single, low DO stress (morning DO of 0.9 mg O_2 l^{-1}) for ≤12 h. A rapid improvement in DO was then effected through aeration and water exchange. This indicated that the results from Experiment 2 could be extrapolated to ponds and should assist with the planning of pond management strategies.

Providing other water quality variables do not reach critical concentrations, premature harvesting, following a DO crisis where mass mortality has been avoided and DO concentrations have been quickly returned to normal, should not be necessary. Feeding should also be maintained at pre-crisis rates. However, this does not mean that morning DO concentrations can be allowed to regularly fall to stressful concentrations within any one pond. The results summarised by Aquacop et al. (1988) indicate that such a situation would result in depressed growth of penaeids.

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REFERENCES

Allan, G. L., Maguire, G. B. and Hopkins, S. J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen levels. Aquaculture.

American Public Health Association (APHA), 1971. Standard Methods, 13th Edition. APHA., Washington DC., 874 pp.

Aquacop, Bedier, E. and Soyez, C., 1988. Effects of dissolved oxygen concentration on survival and growth of *Penaeus vannamei* and *Penaeus stylirostris*. J. World Aquaculture Soc., (Aquaculture communiques), 19(1): 13A.

Boyd, C. E., 1982. Water Quality Management for Pond Fish Culture. Developments in Aquaculture and Fisheries Science, Volume 9. Elsevier, Amsterdam, 318pp.

Boyd, C. E. and Tucker, C. S., 1979. Emergency aeration of fish ponds. Trans. Am. Fish. Soc., 108: 299-306.

Boyd, C. E. and Watten, B. J., 1989. Aeration systems in aquaculture. Rev. in Aq. Sci., 1(3): 425-472.

Bridges, C. R. and Brand, A. R., 1980. Oxygen consumption and oxygen-independence in marine crustaceans. Mar. Ecol. Prog. Ser., 2: 133-141.

Busvine, J. R., 1957. A Critical Review of the Techniques for Testing Insecticides. Commonwealth Institute of Entomology, London, 208 pp.

Chang, W. Y. B. and Ouyang, H., 1988. Dynamics of dissolved oxygen and vertical circulation in fish ponds. Aquaculture, 74: 263-276.

Chen, J. C. and Chin, T. S., 1989. Effect of ammonia at different pH levels on *Penaeus monodon* postlarvae. Asian Fish. Sci., 2: 233-238.

Clark, J. V., 1986. Inhibition of moulting in *Penaeus semisulcatus* (De Haan) by long-term hypoxia. Aquaculture, 52: 253-254.

Dall, W., 1986. Estimation of routine metabolic rate in a penaeid prawn, *Penaeus esculentus* Haswell. J. Exp. Mar. Biol. Ecol., 96: 57-74.

Dal Pont, G., Hogan, M. and Newell, B., 1974. Laboratory techniques in marine chemistry. II. Determination of ammonia in seawater and the preservation of samples for nitrate analysis. Aust. CSIRO Div. Fish. Oceanogr. Rep., No.55, CSIRO, Sydney, New South Wales, 11 pp.

Egusa, S., 1961. Studies on the respiration of the "Kuruma" prawn, *Penaeus japonicus* Bate. II. Preliminary experiments on its oxygen consumption. Bull. Jpn. Soc. Sci. Fish., 27(7): 650-659.

Huddart, R. and Arthur, D. R., 1971. Shrimps in relation to oxygen depletion and its ecological significance in a polluted estuary. Environ. Pollut., 2: 13-35.

Klima, E. F., 1965. Evaluation of biological stains, ink and fluorescent pigments as marks for shrimp. US Fish. Wildl. Serv. Spec. Sci. Rep., No. 511, 8 pp.

Kramer, G. L., 1975. Studies on the lethal dissolved oxygen levels for young brown shrimp, *Penaeus aztecus* Ives. Proc. Ann. Meet. World Maricul. Soc., 6: 157-167.

Liao, I. C. and Huang, H. J., 1975. Studies on the respiration of economic prawns in Taiwan. I. Oxygen consumption and lethal dissolved oxygen of egg up to young prawn of *Penaeus monodon* Fabricius. J. Fish. Soc. Taiwan, 4(1): 33-50.

Liao, I. C. and Murai, T., 1986. Effects of dissolved oxygen, temperature and salinity on the oxygen consumption of the grass shrimp, *Penaeus monodon*. In: J. L. Maclean, L. B. Dizon and L. V. Hosillos (Editors), The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp. 641-646.

MacKay, R. D., 1974. A note on minimal levels of oxygen required to maintain life in *Penaeus schmitti*. Proc. Ann. Meet. World Maricul. Soc., 5: 451-452.

Madenjian, C. M., Rogers, G. L. and Fast. A. W., 1987. Predicting night time dissolved oxygen loss in prawn ponds of Hawaii: Part I. Evaluation of traditional methods. Aquacultural Engineering, 6: 191-208.

Major, G. A., Dal Pont, G., Klye, J. and Newell, B., 1972. Laboratory techniques in marine chemistry. A manual. Aust. CSIRO Div. Fish. Oceanogr. Rep., No. 51, CSIRO, Sydney, New South Wales, 55 pp.

Morrissy, N. M., Caputi, N. and House, R. R., 1984. Tolerance of marron (*Cherax tenuimanus*) to hypoxia in relation to aquaculture. Aquaculture, 41: 61-74.

Motoh, H., 1981. Studies on the fisheries biology of the giant tiger prawn, *Penaeus monodon* in the Philippines. Southeast Asian Fisheries Development Centre Tech. Rep. No 7, 128 pp.

Penkoff, S. J and Thurberg, F. P., 1982. Changes in oxygen consumption of the American lobster, *Homarus americanus*, during the molt cycle. Comp. Biochem. Physiol., 72A(4): 621-622.

Rapport, A., Sarig, S. and Marek, M., 1976. Results of tests of various aeration systems on the growth regime in the Genosar experimental ponds and growth of fish there in 1975. Bamidgeh, 28(3): 35-49.

Seidman, E. R. and Lawrence, A. L., 1985. Growth, feed digestibility, and proximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. J. World Maricul. Soc., 16: 333-346.

Shepard, M. P., 1955. Resistance and tolerance of young speckled trout (*Salvelinus fontinalis*) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Bd. Can., 12(3): 387-446.

Shigueno, K., 1975. Shrimp Culture in Japan. Association for International Technical Promotion, Tokyo, 153 pp.

Sokal, R. R. and Rohlf, F. J., 1981. Biometry. W. H. Freeman and Company, San Francisco, 859 pp.

Sprague, J. B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res., 3: 793-821.

Tournier, H., 1972. Conditions d'acclimatation des crevettes *Penaeus kerathurus* et *P. japonicus* dans les eaux du littoral L. Languedocien - Science et Peche, Bull. Inst. Peches maritimes, No. 213, 13 pp.

Wajsbrot, N., Gasith, A., Krom, M. O. and Samocha, T. M., 1990. Effect of dissolved oxygen and the moult stage on the acute toxicity of ammonia to juvenile green tiger prawn *Penaeus semisulcatus*. Environ. Toxicol. Chem., 9: 497-504.

Wickins, J. F., 1976a. Prawn biology and culture. Oceanogr. Mar. Biol. Ann. Rev., 14: 435-507.

Wickins, J. F., 1976b. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Winer, B. J., 1971. Statistical Principles in Experimental Design. 2nd Edition. McGraw-Hill, New York, NY, 827 pp.

Zimmer, D. J., 1987. Effects of low pH acclimation on cladocerans: Clues to the interaction of physiology and ecology of acid lake zooplankton. In: H. Witters and O. Vanderborght (Editors), Ecophysiology of acid stress in aquatic organisms. Annls Soc. r. zool. Belg., 117(1): 139-149.

Zimmer, D. J. and Storr, J. F., 1984. The effects of environmental pH on life history parameters of *Daphnia parvula* (Crustacea; Cladocera). In: J. S. Jacobson and L. S. Raymond (Editors), Proc. Second New York State Symp. on Atmospheric Deposition, October 26-27, 1983, Albany, NY, New York State College of Agriculture and Life Sciences, NY, pp. 209-216.

TABLE 1

Dissolved oxygen levels, mortality over 48 and 96 h and moulting frequency of *Penaeus monodon*[1].

DO (mg l^{-1})		Mortality (%)				Exuviae[2]
		48h		96h		
mean	range	mean	SE	mean	SE	(Total No.)
5.6	5.2 - 5.80	0	0	0	0	3
2.1	1.9 - 2.3	0	0	3.3	3.3	3
1.6	1.2 - 1.7	0	0	3.3	3.3	2
1.0	0.9 - 1.2	16.7	8.8	26.7	8.8	2
0.6	0.5 - 0.7	66.7	6.7	96.7	3.3	0
0.3	0.3 - 0.3	100	0	100	0	0

1 Mean and standard error (SE) based on n =3 replicate aquaria.

2 Total numbers per treatment after 96 h.

TABLE 2

Periods of exposure at low dissolved oxygen (DO) levels and survival, growth and food conversion efficiency for *Penaeus monodon* held in well oxygenated conditions for 21 days after low DO stress[1].

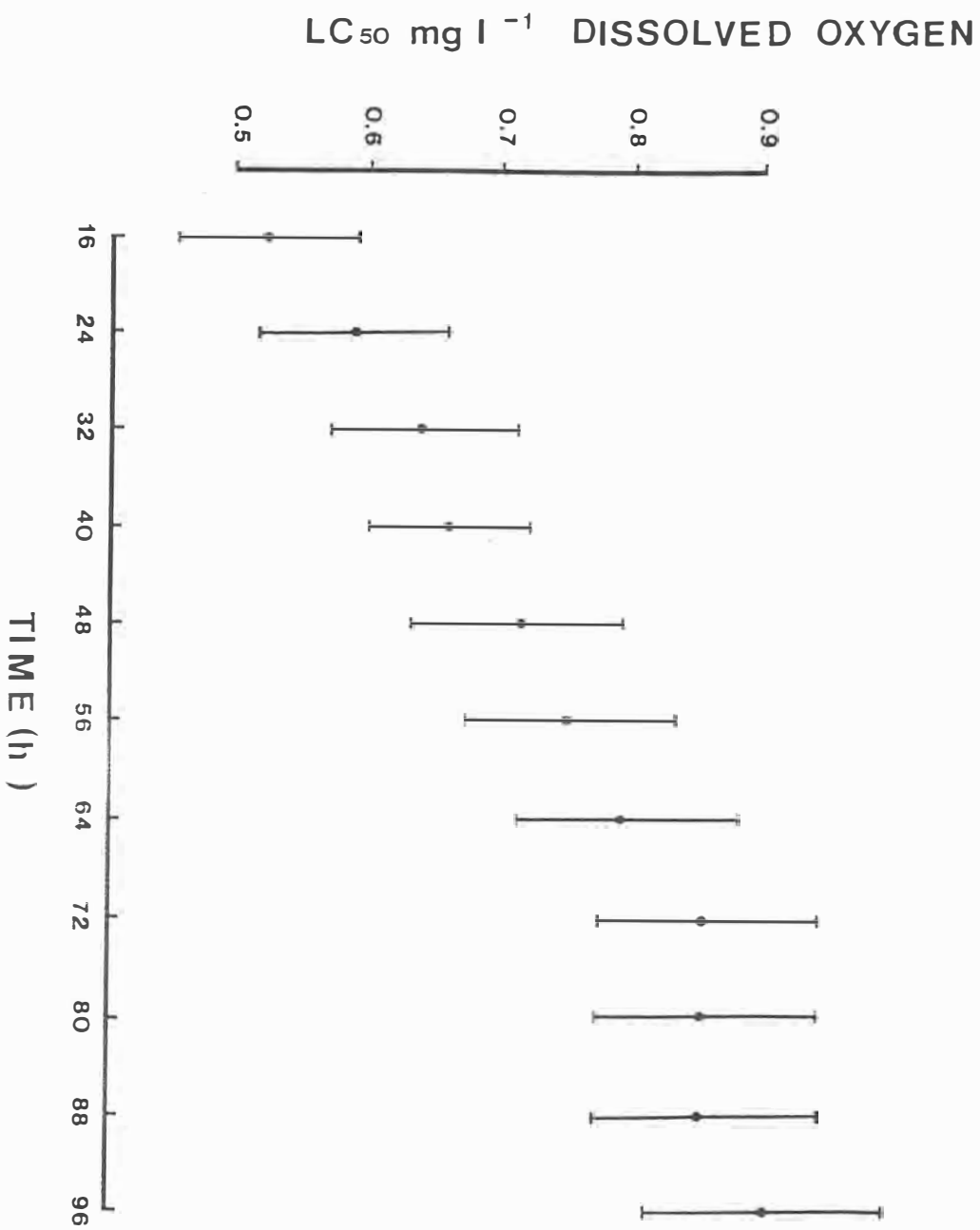
DO (mg l^{-1})		Period of Stress (h)	Survival[2] (%)		Wt Gain[3] (g $prawn^{-1}$)		FCR[3]	
mean	range		mean	SE	mean	SE	mean	SE
6.1	6.0-6.3	0	100	0	3.3	0.1	1.5	0.02
1.0	0.8-1.4	4	96.7	3.3	2.8	0.2	1.5	0.1
1.1	0.9-1.3	8	96.7	3.3	3.0	0.2	1.6	0.1
1.1	0.9-1.3	12	90.0	5.8	2.9	0.2	1.4	0.2
0.6	0.5-0.8	4	100	0	2.8	0.2	1.6	0.1
0.6	0.5-0.8	8	90	5.8	3.3	0.3	1.5	0.1
0.5	0.5-0.7	12	76.7	6.7	3.5	0.1	1.3	0.1

1 Mean and standard error (SE) based on n=3 replicate aquaria.

2 The effects of DO stress on survival were not significant (χ^2=2.3, d.f.=5, P>0.05).

3 ANOVA revealed no significant treatment effect for this variable (P>0.05).

Figure 1 LC_{50} (dot) and 95% confidence limits (bar) at 8 hourly intervals from 16-96 h for *Penaeus monodon* exposed to low dissolved oxygen concentration.


LC50 mg l -1 DISSOLVED OXYGEN
0.5
0.6
0.7
0.8
0.9
TIME (h)
16
24
32
40
48
56
64
72
80
88
96

2.5 Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon*

ABSTRACT

Allan, G. L. and Maguire, G. B. Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon*.

Critical levels of low pH for juvenile *Penaeus monodon* (4.2-5.5g average weight), were determined using static bioassays with seawater acidified using hydrochloric acid (HCl). The lethal pH (96 h LC_{50}) was 3.7 (95 % confidence limits; 3.4 and 4.1) at a salinity of 32 ‰. The "minimum acceptable" pH, defined as that pH which reduced growth by 5 % over 23 days, was estimated as 5.9 at a salinity of 30 ‰. In comparison to a pH of 7.8, long-term (23 days) exposure to low pH (4.9) at 30 ‰ salinity also significantly decreased dry matter content of the prawns ($P<0.001$) and increased moulting frequency ($P<0.05$). For prawns held at different pH (5.5 or 7.8) and salinity (15 or 30 ‰) combinations, growth was reduced by low pH ($P<0.001$) but was unaffected by salinity ($P>0.05$) while the pH x salinity interaction was significant ($P<0.05$). In a separate factorial experiment with prawns held at different pH (5.6 or 7.8) and salinity (15 or 30 ‰) combinations, haemolymph osmoregulation was worse at high salinity ($P<0.001$) and low pH ($P<0.01$), compared with low salinity or high pH and there was no significant interaction ($P>0.05$). The estimation of lethal and "minimum acceptable" low pH values should assist prawn farmers with the management of acid ponds.

INTRODUCTION

No comprehensive studies of acute or chronic toxicity of acidified seawater to penaeids have been published and, in general, most studies of that type have been for freshwater rather than marine animals. A reduction in pH ensues when respiration exceeds photosynthesis, thereby affecting the $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$ equilibrium (Boyd, 1982). Reduced pH also occurs in recirculation systems following nitrification (Wickins, 1983). More severe reductions can occur when water interacts with sediments which have a high cation exchange capacity (Boyd, 1982), or when acid-sulphate sediments which contain pyrite are exposed to air and water and the pyrite is oxidised to form sulphuric acid (Webber and Webber, 1978; Boyd, 1982; Simpson et al., 1983; Gaviria et al., 1986; Lin, 1986). Direct release of acidic effluent and seepage from acid mine spoils can also lower pH (Boyd, 1982; Ferguson, 1988), and in the northern hemisphere acid precipitation has led to reduced pH in freshwater environments, including aquacultural systems (Haines, 1981; Hobe et al., 1983, 1984b; Anon., 1987).

Low pH can cause damage to gill tissues of fish (Ferguson, 1988) and can also influence the impact of potential toxins, e.g. ammonia (Alabaster and Lloyd, 1980, Colt and Armstrong, 1981) and heavy metals (Boyd, 1989). In acid waters, crustaceans and fish may experience impaired ionic regulation (Morgan and McMahon, 1982; Havas and Hutchinson, 1983; Hobe et al., 1983, 1984b).

Crustaceans can, however, regulate internal pH to some extent through Cl^-/HCO_3^- and Na^+/H^+ exchanges via the gills (Henry et al., 1981; Wickins, 1984a). The dissolution of exoskeletal calcium carbonate (Defur et al., 1980; Morgan and McMahon, 1982; Wickins, 1984b) and the release of ammonia and amino acids (Brehm and Maijering, 1986) can also buffer pH changes to some extent.

Tsai (1990) considered pH values of below 4.8 or above 10.6 lethal to penaeids with an optimum range, outside of which growth and food conversion efficiency were reduced, of 6.6 to 8.5. Apud et al. (1985) observed that a pH of 5 or below in ponds caused mortality of penaeids while lethal values of low pH (96 h LC_{50}'s) for the crayfish *Procambarus clarki* and *Orconectes rusticus* in acidified water were 2.5 to 2.8 (Morgan and McMahon, 1982). Growth and moulting frequency of *Penaeus occidentalis* and *P. monodon* were reduced and carapace dry weight increased when prawns were exposed for 36 to 56 days to seawater where pH was reduced from 7.9 to 6.4 by the addition of carbon dioxide (Wickins, 1984a). Sub-lethal low pH can also affect maturation and reproduction in crustaceans (Walton et al., 1982; Zimmer, 1987; Zimmer and Storr, 1984).

In aquaculture ponds built in acid-sulphate soils, pH reductions can be exacerbated during periods of heavy rain, as acid soils in pond dykes erode into ponds (Webber and Webber, 1978; Lin, 1986; Boyd, 1989). In such cases decreased salinity may accompany decreased pH. No studies were found which assessed interactive effects of pH and salinity for penaeids although such an interaction might be expected as the acid-base balance of crustaceans is directly affected by salinity (Truchot, 1983).

The aims of this study were to estimate lethal and "minimum acceptable" pH values, using acidified seawater, for *P. monodon* which dominates cultured prawn production both worldwide (Rosenberry, 1991) and in Australia (Maguire and Allan, in press). Interactive effects of reduced salinity and low pH on weight gain, moulting frequency, dry matter content and haemolymph osmotic pressure for this species were also investigated.

METHODS

Experimental animals

Juvenile *Penaeus monodon* were purchased from prawn farms in the Clarence River region, (New South Wales, Australia), and were acclimatised in experimental aquaria for at least a week before experiments commenced. Prawns were fed commercially available Taiwanese prawn pellets (46.7 % crude protein [Nx6.25], dry weight basis). Prawns were not sexed as male and female *P. monodon* grow at similar rates in the size range used (Motoh, 1981). Prawns which had moulted in the two days before stocking were not used.

Experimental systems

Three replicate, 70 l acrylic aquaria, with ten prawns in each, were used for each treatment in each experiment. The seawater used in all experiments was essentially oceanic as described by Allan et al. (1990). No sediment was provided and an average water temperature of 26.0 °C (range 24.9-27.1 °C) and a 12:12 h photoperiod were maintained. Static bioassays were used and each day 95 % of water in each aquarium was drained then replaced. The pH levels in the replacement water were adjusted to those required using 10 M HCl. For Experiments 2 and 3, salinity was adjusted in these reservoirs using rainwater. In each aquarium the low aeration rate (approximately 100 ml min^{-1} through air-stone diffusers) was sufficient to maintain dissolved oxygen above 5.0 mg l^{-1}. Deviations from desired pH levels in experimental aquaria, due in part to aeration, were compensated for by continuous additions of dilute acid from peristaltic pumps (approximately 1.0 ml min^{-1}).

Water quality analyses

The pH levels were measured in each aquarium every four hours during Experiment 1, and two to three times daily during Experiments 2 and 3. A Metrohm, model 605, (Metrohm Ltd. Ch 9100 Herisaw, Switzerland) pH/mV meter, with a Metrohm glass and reference electrode assembly, was used to measure pH. Electrodes were calibrated with phosphate and phthalate buffers (Chemical Rubber Company [CRC], 1971). Although these are low ionic strength buffers, Whitfield et al. (1985) found that for a single electrode pair, pH measurements made with these buffers were as precise as those made using high ionic strength buffers. Salinity and temperature were measured daily using a Yeo-Kal (Yeo-Kal Electronics, Brookvale, NSW, 2100, Australia) Temperature/Salinity conductivity meter calibrated with sub-standard seawater and a standard thermometer. Dissolved oxygen (DO) was measured with a Yeo-Kal DO/Temperature meter calibrated daily with "air-saturated" seawater and periodically using the Winkler titration (American Public Health Association [APHA], 1971). The concentrations of ammonia and nitrite were measured at the end of each experiment using methods described by Dal Pont et al. (1973) and Major et al. (1972) respectively. Alkalinity, expressed as equivalent bicarbonate concentration (mg HCO_3 l^{-1}) was measured using the methods described by APHA (1971).

Experiment 1 - Acute bioassay

Seven treatments with different levels of pH (3.0-7.8) were established to assess the lethal effects of acidified seawater (32 ‰) on *P. monodon*. Just before the start of the acute bioassay, pH was adjusted in each aquarium to the level required with 1 M HCl. The initial mean weight for this experiment was 4.2 g $prawn^{-1}$ (range 2.2 to 6.8 g). Prawns were not fed during the experiment. Following APHA (1971) and Sprague (1969) the acute bioassay was run for 96 h. Longer periods were not considered appropriate due to stress and cannibalism. The number of dead prawns and exuviae were recorded after 0.5, 1, 2 and 4 h and at four hourly intervals thereafter. The absence of response to touching with a glass rod was

used as the criterion of death and all dead prawns and exuviae were removed. Throughout the 96 h period, pH values were within 0.2 pH units of the desired levels.

Experiment 2 - Chronic bioassay

Prawns (average initial weight 5.5 g prawn^{-1}, range 3.6-7.8 g) were individually weighed and tagged by injecting a marker of petroleum jelly impregnated with fluorescent dye, "saturn yellow", into the musculature of one side of an abdominal segment (Klima, 1965). Eight treatments were established; six at a salinity of 30 ‰ with average pH values of 7.8, 7.3, 6.7, 6.1, 5.5 and 4.9 and two others at a salinity of 15 ‰ with average pH values of 7.8 and 5.5. Experimental levels of pH and salinity were reduced gradually over three days. The experiment was then run for 23 days. With the exception of one day, pH values were within 0.4 pH units of desired levels. An average individual prawn weight gain for each aquarium was determined at the end of the experiment. Prawns which died were replaced to maintain constant prawn densities and to prevent density related differences in water quality (particularly ammonia) between aquaria. Prawns which were replaced within the final 14 days of the experiment (11 prawns in total) were not used in weight gain estimations. Prawns were fed twice daily (1/3 of daily ration in the morning, 2/3 in the afternoons) on an *ad libitum* basis. Uneaten food and any exuviae were recorded and removed daily.

Experiment 3 - Interactive effects of pH and salinity on haemolymph osmotic pressure

The initial mean weight in this experiment was 5.5 g prawn^{-1} (range 3.6-8.4 g). In this factorial experiment there were four combinations of two salinity (30 and 15 ‰) and two pH levels (7.8 and 5.6). For the lower levels, salinity and pH were reduced gradually over three days and then all levels were maintained for a further three days. During the 24 h before osmotic pressure measurements were taken, pH levels were stable (within 0.2 pH units of required levels) in all aquaria. Dall and Smith (1981) found that constant haemolymph osmotic pressure was attained in *P. plebejus* at extreme salinities in <24 h. Prawns which died were not replaced, and, at the end of the experiment the osmotic pressure of the seawater in each aquarium and the haemolymph of all surviving prawns was measured with a Wescor Model 5100B vapor pressure osmometer (Wescor Inc., 459 South Main Street, Logan, Utah 84321, USA). Whole blood samples from the prawns were withdrawn from the pericardium using chilled, disposable 1 ml plastic syringes and 30G needles, and analysed immediately. An average value based on $n \geq 7$ prawns was calculated for each aquarium.

Statistical analyses

LC_{50} values were calculated using probit analysis (Busvine, 1957) and a computer package developed by Mr A Woods (University of New South Wales, Australia). Single factor ANOVA was used to compare the effect of pH on ammonia levels for Experiments 1 and 2. Because several treatments yielded zero variance in

Experiment 2, a single sample chi-squared test was used to examine differences in survival. In Experiment 2 the mean individual prawn weight gain for each aquarium was calculated and differences in weight gain, number of exuviae and dry weight between all treatments were assessed using single factor ANOVA. Comparisons between means were made using Tukey's "honestly significant differences" procedure (Winer, 1971). Data from treatments 1 and 7 (pH 7.8; salinity 30 and 15 ‰) and 5 and 8 (pH 5.6, salinity 30 and 15 ‰) were also used in a two factor ANOVA to assess the interactive effects of pH and salinity.

For Experiment 3 mean values for water and haemolymph osmotic pressure, and the difference between the two (D_{OP}), were calculated for each aquarium and the data were analysed using two factor ANOVAs to assess the interactive effects of pH and salinity. For all analyses of variance, homogeneity was assessed using Cochran's test (Winer, 1971).

The EC_5 value, that pH at which prawn growth was reduced by 5 %, was considered the "minimum acceptable" pH. This was estimated for a salinity of 30 ‰ using two intersecting regression lines (Allan et al., 1990) based on the data from Experiment 2 (Fig. 1). Linear regression was used to investigate the relationship between pH and the number of prawns which moulted in aquaria with seawater at 30 ‰ salinity (Experiment 2).

RESULTS

Water quality

The maximum ammonia concentration reached in an aquarium during any static bioassay in the present study (1.04 mg total ammonia-N l^{-1}) was considerably higher than that recorded in controls during continuous flow bioassays with the same species (Allan et al., 1990). However, this concentration was still below the maximum acceptable concentration for penaeids (Wickins, 1976; Allan et al., 1990). There was a tendency for ammonia concentrations to be higher in treatments with the lowest pH during both Experiment 1 and 2, however, differences between treatments were not significant ($P>0.05$). Nitrite concentrations did not exceed 20 μg NO_2-N l^{-1}, well below concentrations which reduce growth of penaeids (Wickins, 1976).

Average alkalinity for Experiment 2 ranged from 0 mg HCO_3^- l^{-1} for the treatment with a pH of 4.9 to 117.6 mg HCO_3^- l^{-1} for the treatment with a pH of 7.8 (30 ‰). At a similar pH, alkalinity was reduced at lower salinities (Table 2).

Experiment 1

Few prawns died at pH 7.8-4.1, although all prawns died within eight hours at pH 3.0 (Table 1). The 96 h LC_{50} value was 3.7 (95 % confidence limits 3.4, 4.1). There was little difference between mortality after 48 h and 96 h indicating that a lethal toxicity threshold (Sprague, 1969) was reached. No prawns held at pH 7.8, 7.0, 6.1, 5.1 or 3.0 moulted. Three prawns moulted at pH 4.1, one of which died

during, or soon after, moulting. At pH 3.8, seven prawns moulted and all of these died within four hours of moulting.

Experiment 2

The effects of treatment on survival were not significant ($\chi^2=1.1$, d.f.=7, $P>0.05$) (Table 2). In comparison to results for pH 7.8 and 30 ‰, growth was depressed at pH ≤ 5.5 at both 15 and 30 ‰ (Table 2) and, although salinity did not affect growth ($P>0.05$), there was a significant pH x salinity interaction ($P<0.05$) (Table 3). The interaction occurred because, at pH 7.8, average weight gain was higher at 30 ‰ than at 15 ‰ salinity, while at pH 5.6, growth was similar at the two salinity concentrations (Fig. 1). The number of exuviae was highest at pH 4.9 (30 ‰) ($P<0.01$) (Table 2) and was inversely related to salinity ($P<0.001$), while the interaction between pH and salinity was not significant ($P>0.05$) (Table 3). The number of moults (Table 2) was related to pH (number of moults = 18.15 - 2.15 pH; $r = 0.656$; $P<0.01$, df = 16). The dry matter content was depressed at pH 4.9 (30 ‰) (Table 2) but unaffected by salinity or the interaction ($P>0.05$) (Table 3).

For *P. monodon*, at a salinity of 30 ‰, the EC_5 value estimated for reduced pH was 5.9 (Fig. 1).

Experiment 3

The effects of salinity on water osmotic pressure were significant ($P<0.001$), but pH effects were not significant ($P>0.05$), and there was no pH x salinity interaction (Tables 4 and 5). The effects of salinity on haemolymph osmotic pressure were also significant ($P<0.001$). However, at both salinities (15 and 30 ‰) haemolymph osmotic pressure was closer to ambient osmotic pressure at reduced pH (5.6) (Table 4) and consequently pH effects were not significant ($P>0.05$), although, there was a significant ($P<0.05$) pH x salinity interaction (Table 5). Both salinity ($P<0.001$) and pH ($P<0.01$) significantly reduced D_{OP}, an indicator of osmoregulatory performance, and there was no interaction ($P>0.05$) (Table 5).

DISCUSSION

Mortality (Apud et al., 1985), and poor growth (Webber and Webber, 1978), of penaeids occur when acid sulphate soils acidify pond waters. However, no studies using standard toxicological methodology (Sprague, 1969) were found which described lethal or "minimum acceptable" values of pH for penaeids.

Continuous-flow bioassays are preferable to static bioassays as animals are not subject to the stresses associated with the replacing test solutions (Allan et al., 1990). However, during preliminary trials, pH could not be maintained with any precision in the aquaria available when continuous-flow bioassays were used. Consequently, static bioassays had to be used. In Experiment 2, growth of prawns at pH 7.8 and 30 ‰ salinity was equivalent to 0.10 g day^{-1}. This was slower than average growth rates of 0.18 g day^{-1} or 0.16 g day^{-1} recorded for control groups of similar size *P. monodon* during continuous-flow bioassays with ammonia or

dissolved oxygen over 21 days (Allan et al., 1990; Allan and Maguire, 1991).

The growth results (Experiment 2; Table 2), however, did indicate that *P. monodon* were also tolerant of sub-lethal exposure to acidified seawater over 23 days and allowed the estimation of a "minimum acceptable" pH (5.9). Growth was not significantly reduced ($P>0.05$) at pH 6.1 or above, although, significant reductions were recorded at pH 5.5 and 4.9. Similarly, Wickins (1984b) found that growth of *P. monodon* was unaffected in acidified seawater with a pH of 6.7. Conversely, Wickins (1984a) found that growth of *P. monodon* and *P. occidentalis* was reduced when pH was lowered to 6.4 using seawater enriched with carbon dioxide. However, in this last case, growth could have been reduced by direct toxicity of CO_2 rather than simply by a reduction in pH.

At the lowest pH in Experiment 2 (4.9), the number of exuviae was significantly higher ($P<0.05$), compared with the number recorded at pH 7.8, 7.3, 6.1 and 5.5, even though growth was significantly reduced ($P<0.05$) (Table 2). Brown et al. (1991) also found that an increase in moulting frequency for *Macrobrachium rosenbergii* could accompany reduced growth. Other studies have shown that under adverse conditions prawns may continue to moult but not grow (Reeve, 1969) and Joshi et al. (1987) found that exposure to sublethal levels of two insecticides increased moulting frequency in *P. monodon*. Chang (1989) suggested that environmental variables may regulate the endocrines involved in crustacean moulting by affecting the synthesis and/or secretion of moult inhibiting hormones.

During this study, prawns were not individually segregated and we could not indentify which prawns moulted, nor measure the growth increment between moults. Brown et al. (1991) found that the growth increment between moults was greater for *M. rosenbergii* (initial weight 0.1 - 0.35 g) at low water hardness concentrations (<53 mg Ca CO_3 l^{-1}) although the converse has been found for larger individuals (initial weight 3.8 - 32.6 g) of the same species (Cripps and Nakumura, 1979).

Prawns exposed to the lowest pH (4.9) also had lower ($P<0.001$) dry matter contents (Table 2). Although smaller decreases in dry matter content have also been recorded for penaeids which grew slowly under other unfavourable conditions (low feed rate or temperature) (Maguire, unpublished data, 1990), the decrease recorded during the present study may have been due to exoskeletal dissolution in the acid conditions, as has been recorded for other crustaceans (Defur et al., 1980; Morgan and McMahon, 1982). Although no measurements of carapace weight were made during the present study, the exoskeletons of prawns exposed to the lowest pH (4.9) were noticeably softer than for prawns exposed to the highest pH (7.8). Wickins (1984b) found that carapace weight of *P. monodon* was reduced following exposure to low pH (6.7) and low inorganic carbon concentration. When pH was reduced to 6.4, by adding carbon dioxide (thus maintaining inorganic carbon concentrations), carapace weight gain in *P. monodon* occurred, possibly as a result of increased uptake of bicarbonate (Wickins, 1984a).

The effects of pH and salinity, and their interaction, on haemolymph osmotic concentration were investigated in Experiment 3 to determine if differences in osmoregulatory ability in acid waters at different salinities may have contributed to the interaction of salinity and pH on growth, observed in Experiment 2 (Table 3). Reduced osmolarity, or a loss of haemolymph ions (in particular Na^+), has been recorded following exposure to acid for several species of freshwater fish and crustaceans (Morgan and McMahon, 1982; McMahon and Morgan, 1983; Hobe et al., 1983; 1984a, 1984b). *P. monodon* is an efficient osmoregulator in the range 15-30 ‰ and has an isosmotic concentration of between 23-25 ‰ (Cawthorne et al., 1983); this is close to mid way between the two salinities tested in the present study. At both salinities, haemolymph osmotic concentrations were closer to ambient osmotic concentrations when prawns were exposed to acidified conditions (pH 5.6) compared with those for prawns in normal seawater (pH 7.8). When an indicator of osmoregulatory performance (D_{OP}), was analysed, both pH and salinity effects were significant ($P<0.01$), and there was no interaction ($P>0.05$) (Table 3). This suggests an impairment of osmoregulatory function at low pH (5.6) and is consistent with the conclusion made by McMahon and Morgan (1983) that the loss in total osmolarity in *Procambarus clarkii* and *Orconectes rusticus*, following exposure to acidified water (pH 4.0), resulted from an inhibition of ion uptake as well as an increase in passive ion efflux. However, although the apparent impairment of osmoregulatory ability in acidified conditions (pH 5.5) was slightly greater for the higher salinity (30 ‰), the pH x salinity interaction was not significant ($P>0.05$) (Table 5). Thus the differences in osmoregulatory ability did not necessarily explain the significant pH x salinity interaction on growth in Experiment 2. The interactive effect of pH and salinity on ionic regulation may warrant further study.

Inorganic carbon levels are reduced by acidification as indicated by the reduction in alkalinity to 0 mg $HCO_3^{-1}l^{-1}$ in the treatment with the lowest pH (4.9). The current study was not aimed at investigating the direct effects of reduced alkalinity of *P. monodon* but this may warrant further study. It may have indirect effects through inhibiting algal blooms in farming ponds (Boyd, 1982).

The absence of a significant difference in growth between salinities of 15 and 30 ‰ was surprising as prawn farmers in Australia and overseas place great emphasis on maintaining low salinity levels in *P. monodon* ponds (New and Rabanal, 1985; Chien and Liao, 1987). However, data recently presented by Shiau et al. (1991) for juvenile *P. monodon* fed experimental diets with six dietary protein contents at 16 ‰ and 32 ‰, indicate that average growth results (data for different protein levels combined) for the two salinity levels were similar (289.7 % and 337.6 % increases in average weight respectively). Thus low salinity does not appear necessary in terms of the physiological requirements of *P. monodon* although low salinity could influence other biotic components of pond ecosystems. It is noteworthy that low salinity (15 ‰) increased moulting frequency as a variety of environmental stresses, eg rapid reduction in salinity, increased temperature through partial pond drainage and additions of copper, have been used by prawn farmers in an attempt to stimulate moulting and growth (Robertson, 1988). Clearly, an increase in moulting frequency does not necessarily improve growth.

A major aim of this study was to provide data to assist with the management of acidic prawn farming ponds. However, results from aquaria are not necessarily directly applicable to pond situations (Maguire and Allan, 1985). Apud et al (1985) observed mortality among penaeids at pH ≤ 5 and in the present study mortality occurred at pH ≤ 5.1 although the 96h LC_{50} was 3.7. Within ponds, toxic effects of low pH can involve interaction with other water quality variables e.g., low pH reduces ammonia toxicity (Whitfield, 1974) and mobilises heavy metals, including iron and aluminium which can reduce yields in aquaculture ponds (Simpson et al., 1983).

Low pH can also reduce natural pond productivity presumably by reducing the availability of nutrients (Alabaster and Lloyd, 1980) including phosphorus (Boyd, 1982) and carbon sources for photosynthesis e.g., bicarbonate and hence alkalinity. Liming usually involves applications of calcium carbonate which assists with overcoming these problems and Simpson et al. (1983) specifically recommended regular monitoring of alkalinity in acid ponds. Clearly the interrelationships between pH and pond water quality variables are complex. Nevertheless, if the pH in ponds falls below 5.9 the likelihood of a decline in prawn growth warrants remedial action such as water exchange or the addition of lime. If pH falls below about 4.0, immediate action such as transferring prawns to another pond or premature harvest is recommended. If the pH is reduced, problems may be encountered with soft prawn exoskeletons and this could affect marketability.

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REFERENCES

Alabaster, J. S. and Lloyd, R., 1980. Water Quality Criteria for Freshwater Fish. Butterworths, London. 297 pp.

Allan, G. L. and Maguire, G. B., 1991. Lethal levels of low dissolved oxygen and effects of short term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture, 94: 27-37.

Allan, G. L., Maguire, G. B. and Hopkins, S. J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen levels. Aquaculture, 91: 265-280.

American Public Health Association (APHA), 1971. Standard Methods, 13th Edition. APHA, Washington DC., 874 pp.

Anonymous, 1987. Bioakva hatchery solves problem of acid water. Fish Farming International, August, pp. 32.

Apud, F. D., Primavera, J. H. and Torres, P. L. (Jr), 1985. Farming of Prawns and Shrimps. Aquaculture Extension Manual No. 5 (3rd Edition), South East Asian Fish. Dev. Cent., Tigbauan, Iloilo, Philippines, 66 pp.

Boyd, C. E., 1982. Water Quality Management for Warm Water Fish Culture. Developments in Aquaculture and Fisheries Science, Vol. 9. Elsevier, Amsterdam, 318 pp.

Boyd, C. E., 1989. Water Quality Management and Aeration in Shrimp Farming. American Soybean Assoc. and US Wheat Associates, Singapore, 70pp.

Brehm, J. and Meijering, M. P. D., 1986. On the sensitivity of low pH of some selected freshwater crustaceans (*Daphnia* and *Gammarus*, Crustacea). Can. Transl. Fish. Aquat. Sci., 15 pp.

Brown, J. H., Wickins, J. F. and MacLean, M. H., 1991. The effect of water hardness on growth and carapace mineralization of juvenile freshwater prawns, *Macrobrachium rosenbergii* de Man. Aquaculture, 95: 329-345.

Busvine, J. R., 1957. A Critical Review of the Techniques for Testing Insecticides. Commonwealth Institute of Entomology, London, 208 pp.

Cawthorne, D. F., Beard, T., Davenport, J. and Wickins, J. F., 1983. Responses of juvenile *Penaeus monodon* Fabricius to natural and artificial sea waters of low salinity. Aquaculture, 32: 165-174.

Chang, E. S., 1989. Endocrine regulation of moulting in crustacea. Rev. Aquat. Sci., 1(1): 133-157.

Chien, Y. H. and Liao, I. C., 1987. Bioeconomic considerations for prawn farming. In: P. Sloane (Editor), Proc. SCP Prawn Farming for Profit Seminars, Sydney/Brisbane/Cairns, Australia, February 1987, pp. 41-49.

Colt, J. E. and Armstrong, D. A., 1981. Nitrogen toxicity to crustaceans, fish and molluscs. In: L. J. Allen and E. C. Kinney (Editors), Proceedings of the Bio-Engineering Symposium for Fish Culture. Fish Culture Section of the American Fisheries Society (FCS publ. 1), pp. 34-47.

Cripps, M. C. and Nakamura, R. M., 1979. Inhibition of growth of *Macrobrachium rosenbergii* by calcium carbonate water hardness. Proc. World Maricul. Soc., 10: 575-580.

Dal Pont, G., Hogan, M. and Newell, B., 1974. Laboratory techniques in marine chemistry. II. Determination of ammonia in seawater and the preservation of samples for nitrate analysis. Aust. CSIRO Div. Fish. Oceanogr. Rep., No. 55, Sydney, Australia, 11 pp.

Dall, W. and Smith, D. M., 1981. Ionic regulation of four species of penaeid prawn. J. Exp. Mar. Biol. Ecol., 55: 219-232.

Defur, P. L., Wilkes, P. R. H. and McMahon, B. R., 1980. Non-equilibrium acid-base status in *C. productus:* Role of exoskeletal carbonate buffers. Respir. Physiol., 42: 247-261.

Ferguson, H., 1988. Water quality diseases. In: Fish Diseases, Refresher Course for Veterinarians. Proceedings 106, Post Gratuate Committee in Veterinary Science, University of Sydney, Australia, pp. 49-54.

Gaviria, J. I., Schmittou, H. R. and Grover, J. H., 1986. Acid sulphate soils: Identification, formation and implications for aquaculture. J. Aqua. Trop., 1: 99-109.

Haines, T. A., 1981. Acidic precipitation and its consequences for aquatic ecosystems: A review. Trans. Am. Fish. Soc., 110(6): 669-707.

Havas, M. and Hutchinson, T. C., 1983. Effect of low pH on the chemical composition of aquatic invertebrates from tundra ponds at the Smoking Hills, N.W.T., Canada. Can. J. Zool., 61(1): 241-249.

Hobe, H., Laurent, P. and McMahon, B. R., 1984a. Whole body calcium flux rates in freshwater teleosts as a function of ambient calcium and pH levels: a comparison between the euryhaline trout, *Salmo gairdneri* and stenohaline bull-head, *Ictalurus nebulosus*. J. Exp. Biol., 113: 237-252.

Hobe, H., Wood, C. M. and McMahon, B. R., 1984b. Mechanisms of acid-base and ionoregulation in white suckers (*Catostomus commersoni*) in natural soft water. J. Comp. Physiol. B., 154: 35-46.

Hobe, H., Wilkes, P. R. H., Walker, R. L., Wood, C. M. and McMahon, B. R., 1983. Acid-base balance, ionic status, and renal function in resting and acid-exposed white suckers (*Catostomus commersoni*). Can. J. Zool., 61: 2660-2668.

Joshi, H. C., Mukhopadhyay, M. K. and Nath, D., 1987. Toxicity of quinalphos and endosulphan to different life stages of tiger prawn *Penaeus monodon*. Symposium on the Impact of Current Land Use Pattern and Water Resources Development on Riverine Fisheries. Cent. Inland Capture Fish. Res. Inst., Barrackpore, India, April 25-27, 1987, Abstracts, pp. 21.

Klima, E. F., 1965. Evaluation of biological stains, inks and fluorescent pigments as marks for shrimp. US Fish Wildl. Serv. Spec. Sci. Rep., No. 511, 8 pp.

Lin, C. K., 1986. Acidification and reclamation of acid sulfate soil fishponds in Thailand. In: J. L. Maclean, L. B. Dizon and L. V. Hosillos, (Editors), The First Asian Fisheries Forum. Asian Fish. Soc., Manilla, Philippines, pp. 71-74.

Maguire, G. B. and Allan, G. L., in press. Shrimp culture industry in Australia. In: A. Fast and J. Lester (Editors), Culture of Marine Shrimp: Principles and Practices. Elsevier, Amsterdam, pp. 763-776.

Major, G. A., Dal Pont, G., Klye, J. and Newell, B., 1972. Laboratory techniques in marine chemistry. A manual. Rep. CSIRO Div. Fish. Oceanogr., Sydney, Australia, No. 51, 55 pp.

McMahon, B. R. and Morgan, D. O., 1983. Acid toxicity and physiological responses to sub-lethal acid exposure in crayfish. In: C. R. Goldman (Editor), Freshwater Crayfish, 5. Papers From the Fifth International Symposium on Freshwater Crayfish, Davis, CA, pp. 71-85.

Morgan, D. O. and McMahon, B. R., 1982. Acid tolerance and effects of sublethal acid exposure on iono-regulation and acid-base status in two crayfish *Procambarus clarki* and *Orconectes rusticus*. J. Exp. Biol., 97: 241-252.

Motoh, H., 1981. Studies on the fisheries biology of the giant tiger prawn, *Penaeus monodon* in the Philippines. SEAFDEC Tech. Rep., No. 7, 128 pp.

New, M. B. and Rabanal, H. R., 1985. A review of the status of penaeid aquaculture in South East Asia. In: P. C. Rothlisberg, B. J. Hill and D. J. Staples (Editors), Second Aust. Nat. Prawn Sem., NPS2, Cleveland, Qld, pp. 307-326.

Reeve, M. R., 1969. The laboratory culture of the prawn *Palaemon serratus*. Fishery Invest. Lond. Ser. 2, 26(1): 1-38.

Robertson, C. H., 1988. Prawn farming in Queensland - Problems and progress so far. In: L. H. Evans and D. O'Sullivan (Editors), Proc. First Aust. Shellfish Aquaculture Conf. Curtin Univ. of Technology, Perth, Australia, pp. 17-27.

Rosenberry, R., 1991. World Shrimp Farming 1990. Aquaculture Digest, San Diego, CA, 31pp.

Shiau, S. Y., Kwok, C. C. and Chou, B. S., 1991. Optimum dietary protein level of *Penaeus monodon* reared in seawater and brackishwater. Nippon Suisan Gakkaishi, 57(4): 711-716.

Simpson, H. J., Ducklow, H. W., Deck, B. and Cook, H. L., 1983. Brackish-water aquaculture in pyrite-bearing tropical soils. Aquaculture, 34: 333-350.

Sprague, J. B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Wat. Res., 3: 793-821.

Truchot, J. P., 1983. Regulation of acid-base balance. In: L. H. Mantel, (Editor), The Biology of Crustacea. Vol. 5: Internal Anatomy and Physiological Regulation. Academic Press, New York, NY, pp. 431-457.

Tsai, C. K., 1990. Water quality management. In: D. M. Akiyama (Editor), Proc. Southeast Asia Shrimp Farm Management Workshop, Philippines, Indonesia, Thailand. July 26 - August 11, 1989. American Soybean Assoc., Singapore, pp. 56-63.

Walton, W. E., Compton, S. M., Allan, J. D. and Daniels, R. E., 1982. The effect of acid stress on survivorship and reproduction of *Daphnia pulex* (Crustacea:Cladocera). Can. J. Zool., 60(4): 573-579.

Webber, R. J. and Webber, H. H., 1978. Management of acidity in mangrove sited aquaculture. Rev. Biol. Trop., 26 (Supl.1): 45-51.

Whitfield, M., 1974. The hydrolysis of ammonium ions in sea water - a theoretical study. J. mar. biol. Ass. UK., 54: 565-580.

Whitfield, M., Butler, R. A. and Covington, A. K., 1985. The determination of pH in estuarine waters. I. Definition of pH scales and the selection of buffers. Oceanol. Acta, 8(4): 423-432.

Wickins, J. F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Wickins, J. F., 1983. Studies on marine biological filters. Wat. Res., 17(12): 1769-1780.

Wickins, J. F., 1984a. The effect of hypercapnic sea water on growth and mineralization in penaeid prawns. Aquaculture, 41: 37-48.

Wickins, J. F., 1984b. The effect of reduced pH on carapace calcium, strontium and magnesium levels in rapidly growing prawns (*Penaeus monodon* Fabricius). Aquaculture, 41: 49-60.

Winer, B. J., 1971. Statistical Principles in Experimental Design. 2nd Edition. McGraw-Hill, New York, NY, 907 pp.

Zimmer, K. J., 1987. Effects of low pH acclimation on cladocerans: Clues to the interaction of physiology and ecology of acid lake zooplankton. In: H. Witters and O. Vanderborght (Editors), Ecophysiology of acid stress in aquatic organisms. Annls. Soc. r. zool. Belg., 117 (Suppl. 1): 139-149.

Zimmer, D. J. and Storr, J. F., 1984. The effects of environmental pH on life history parameters of *Daphnia parvula* (Crustacea:Cladocera). Proc. Second New York State Symp. on Atmospheric Deposition, Albany, New York, NY, 1983, pp. 209-216.

TABLE 1

Mortality of *Penaeus monodon* after exposure to acidified seawater (32 ‰) for 48 and 96 h (Experiment 1)[1].

pH		Mortality (%)[2]	
mean	range	48 h	96 h
7.8	7.6-8.0	0	3.3±3.3
7.0	6.9-7.1	0	0
6.1	6.0-6.2	0	0
5.1	5.0-5.3	10.0±5.8	10.0±5.8
4.1	4.0-4.2	13.3±6.7	20.0±0
3.8	3.6-3.9	30.0±11.5	36.7±6.7
3.0	3.0-3.1	100	100

[1] The mean temperature value for all treatments was 26.2±0.4 °C. Total ammonia and nitrite levels did not exceed 400 µg NH_3-N l^{-1} and 20 µg NO_2-N l^{-1} respectively.

[2] Values are means ± standard error (n=3 replicate aquaria).

TABLE 2

Performance of *Penaeus monodon* following chronic (23 d) exposure to combinations of pH and salinity (Experiment 2).

Treatment	pH[1] mean	pH[1] range	Alkalinity[2,3] (mg l^{-1})	Survival[2,4] (%)	Weight gain[2,5] (g $prawn^{-1}$)	Exuviae[2,5] (No. $aquarium^{-1}$)	Dry matter content[2,5] (%)
30 ‰							
1	7.8	7.6-7.9	117.6±0.7	100 ± 0	2.2 ± 0.1^{a}	2.7 ± 0.9^{ab}	25.2 ± 0.5^{ab}
2	7.3	6.9-7.6	102.0±1.3	100 ± 0	1.9 ± 0.1^{a}	2.0 ± 1.2^{a}	25.5 ± 0.8^{ab}
3	6.7	6.5-7.2	83.6±0.7	100 ± 0	1.8 ± 0.1^{ab}	4.3 ± 0.9^{abc}	27.0 ± 0.5^{a}
4	6.1	5.8-6.8	49.3±1.3	100 ± 0	1.8 ± 0.1^{ab}	3.7 ± 0.7^{ab}	25.8 ± 0.2^{ab}
5	5.5	4.7-6.7	9.2±0.7	96.7 ±3.3	1.2 ± 0.1^{c}	3.7 ± 2.2^{ab}	24.4 ± 0.2^{bc}
6	4.9	4.4-6.3	0	86.7 ±8.8	0.5 ± 0.2^{d}	10.3 ± 1.5^{c}	22.7 ± 0.2^{c}
15 ‰							
7	7.8	7.2-7.8	57.0±1.0	100 ± 0	1.7 ± 0.1^{abc}	8.0 ± 1.5^{abc}	25.1 ± 0.5^{ab}
8	5.5	4.8-6.7	3.1±0.4	86.7±6.7	1.3 ± 0.1^{bc}	8.7 ± 0.3^{bc}	24.7 ± 0.6^{bc}

1 In each aquarium the extreme pH values were recorded once during the experiment. Apart from this single occassion, pH values were within 0.4 pH units of desired levels.

2 Values are means ± standard error (n=3 replicate aquaria).

3 Alkalinity expressed as mg l^{-1} HCO_3^{-1} based on acid titration (APHA, 1971).

4 The effects of pH on survival were not significant ($\chi^2=2.6$, d.f.=7, $p>0.05$).

5 Within a column means sharing the same letter in the superscript were not significantly different ($P>0.05$).

TABLE 3

Summary of Analysis of Variance for effects of combinations of two pH (7.8 and 5.5) and salinity (15 and 30 ‰) levels on *Penaeus monodon* (Treatments 1, 5, 7 and 8; Experiment 2).

Performance index	pH	Salinity (‰)	pH x salinity interaction
Weight gain	$P<0.001$	ns[1]	$P<0.05$
Moulting frequency	ns	$P<0.01$	ns
Dry matter content	ns	ns	ns

[1] ns = not significant ($P>0.05$)

TABLE 4

Osmotic pressure readings for water and *Penaeus monodon* haemolymph following short term (3 d) exposure to combinations of two salinity and pH levels (Experiment 3).

Salinity	pH		Osmotic Pressure (mOsm l^{-1})[1]		
(%)	mean	range	Water	Haemolymph	D_{OP}[2]
15	7.8	7.7-7.9	429±9	652±8	223±8
15	5.6	5.4-5.7	428±3	635±7	207±8
30	7.8	7.7-7.9	860±4	708±5	152±2
30	5.6	5.5-5.7	861±2	732±5	129±2

1 Values are means ± S.E. (n=3 replicate aquaria).

2 D_{OP} = Difference between haemolymph osmotic pressure and the osmotic pressure of the water. This is an indicator of osmoregulatory performance.

TABLE 5

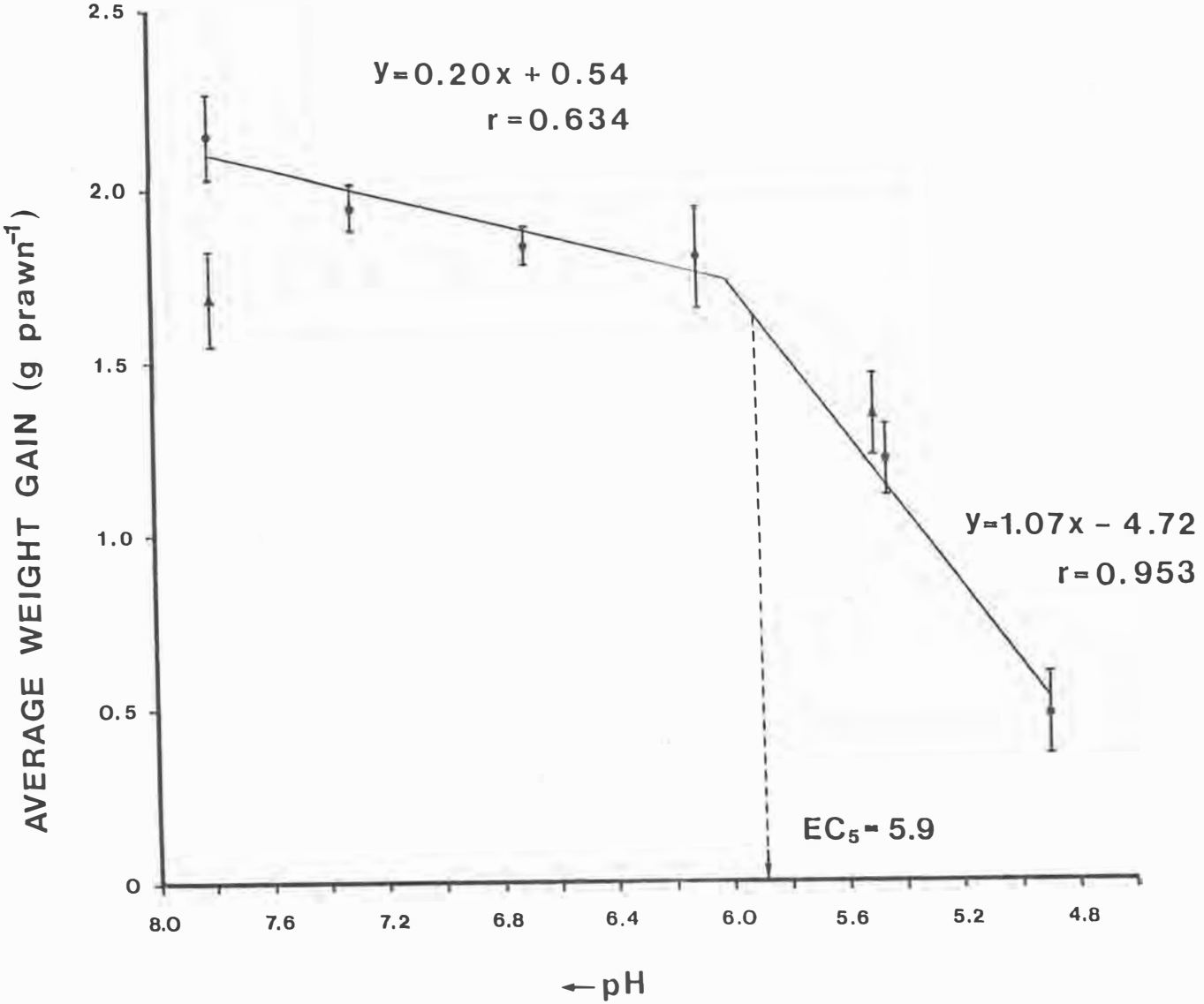
Summary of Analysis of Variance for effects of short term (3 day) exposure to combinations of two pH (7.8 and 5.5) and salinity (15 and 30 ‰) levels on *Penaeus monodon* haemolymph and water osmotic pressures and D_{OP}[1] (Experiment 3).

Osmotic pressure (mOsm l^{-1})	pH	Salinity (‰)	pH x salinity interaction
Haemolymph	ns[2]	$P<0.001$	$P<0.05$
Water	ns	$P<0.001$	ns
D_{OP}	$P<0.01$	$P<0.001$	ns

[1] D_{OP} = Difference between haemolymph osmotic pressure and the osmotic pressure of the water (either 15 or 30‰). This is an indicator of osmoregulatory performance.

[2] ns = not significant ($P>0.05$)

Figure 1 Effects of low pH on growth of *Penaeus monodon* over 23 d. ● = 30 ‰ salinity ▲ = 15 ‰ salinity. Vertical bars indicate standard error of the mean.


y=0.20x + 0.54
r=0.634
y=1.07x - 4.72
r=0.953
EC5 = 5.9
AVERAGE WEIGHT GAIN (g prawn-1)
2.5
2.0
1.5
1.0
0.5
0
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
← pH

3 RESULTS FOR OBJECTIVE B

3.1 Introduction

Objective B was to quantitatively compare the effects of different pond management methods on prawn growth and survival, pond water quality and the population dynamics of bacteria and algae within ponds. The overall aim of the program was to conduct research aimed at improving the efficiency of both pond water quality management and the utilisation of supplementary artificial diets in prawn farming ponds. The results of the first segment (Bioassay Experiments) were used to assess the effect of pond management decisions on water quality and to relate these effects to prawn growth and survival.

This series of experiments was conducted in sixteen 3.5 m diameter x 1.2 m high fibreglass tanks supplied by NSW Fisheries. These units were used to simulate small prawn farming ponds. The water supply system comprised a 6" axial flow water pump with dual delivery lines and float valves on each tank. Drainage facilities and an aeration system were also provided. Sediment was added to the tanks, they were filled with seawater and managed in a similar manner to commercial prawn farming ponds.

The detailed justification, methods, results and implications of the research associated with this segment are presented in Chapters 3.2 - 3.4. Each chapter represents a manuscript which has been submitted or prepared for submission to an international journal. The titles, authors and publication status of these manuscripts are listed in Chapter 6 of this Grant Report.

3.2 The use of model ponds to evaluate phytoplankton blooms and benthic algae mats for *Penaeus monodon* culture

ABSTRACT

Fibreglass pools (3.5 m diameter, 1.2 m high) were evaluated as model ponds for use as experimental units to assess the effects of pond management strategies on production of *Penaeus monodon*, and pond water quality. During an eight week trial, the effects of two fertilisation strategies designed to promote either phytoplankton blooms or benthic algae mats, on prawn production and water quality, were compared. Eight replicate pools were used for each treatment. As production results were similar to those reported from commercial pond farming trials in Australia and Taiwan, the pools were considered appropriate experimental units for assessing pond management strategies. Large blooms of filamentous algae developed in four of the eight pools with benthic algae. In these, individual prawn growth (weight gain), biomass gain, food conversion efficiency and drain harvest efficiency were all lower ($P<0.05$) than those achieved in pools with phytoplankton. Abundance of filamentous algae was negatively correlated ($P<0.05$) with prawn weight gain ($r = -0.80$) and drain harvest efficiency ($r = -0.76$). For all pools, drain harvest efficiency was positively correlated with prawn size at harvest ($r=0.6$; $P<0.01$). Differences in water quality arising from alternative fertilisation strategies occurred but did not explain differences in prawn production indices. The results of the experiment indicate that ponds used for monoculture of *Penaeus monodon* should be fertilised so as to stimulate and maintain phytoplankton blooms and to discourage blooms of filamentous algae.

INTRODUCTION

A large array of experimental rearing systems have been used to investigate variables relevant to prawn farming pond management. These have included earthen ponds (Maguire & Allan 1985; Wyban, Pruder, Leber & Burzell, 1989; Reymond & Legardere 1990), netting enclosures within ponds (Primavera, Apud & Usigan 1976; Maguire & Leedow 1983) and a large variety of different size tanks and other containers (Sohier & Bianchi 1983; Sandifer, Hopkins & Stokes 1987; Wyban & Sweeney 1989; Seidman & Lawrence 1985). While very few studies have attempted to assess the applicability of the experimental facilities used, Chen, Liu & Lin (1989) did compare production results from intensively stocked experimental ponds (0.14 ha) with those obtained from commercial ponds (0.25 - 1.50 ha) and found that growth was similar but production was higher in experimental ponds. Maguire & Allan (1985) presented research results from a number of experimental systems and concluded that while each system had specific contributions to make, information from pond trials should be the most relevant to commercial farming operations. Shell (1966, 1983) also concluded that trials in larger, earthen ponds were most applicable to commercial channel catfish *Ictalurus punctatus*, farming operations.

Pond trials nevertheless suffer a number of major disadvantages for pond management research. It can be very difficult to minimise intrinsic variation between ponds (Wee 1989) and the complex interaction of a number of physico-chemical variables (eg water quality variables) can confound interpretation of treatment effects. Operating sufficient numbers of large ponds concurrently as replicates is also logistically difficult and additional extraneous variation imposed by sequential trials can also make separation of treatment effects more difficult. The use of above-ground pools or large tanks as model ponds can alleviate many of these problems although the effects of their smaller size, eg large wall surface to volume ratio and smaller water surface area, need to be considered. One way of assessing the applicability of experimental systems is to compare production results with those obtained during commercial farming operations where similar methods (eg stocking density) and conditions (eg water temperatures) apply.

Increases in yield following fertilisation have been well documented for aquaculture systems (Boyd 1982; Fair & Fortner 1981; Geiger 1983; Perschbacher & Strawn 1984), including penaeid prawn farming ponds (Furness & Aldrich 1979; Rubright, Harrell, Holcomb & Parker 1981; Lee & Schlesser 1984; Garson, Pretto & Rouse 1986; Wyban, Lee, Sato, Sweeney & Richards 1987; Lanari, Balliestrazzi & Tibaldi 1989; Subosa & Bautista 1991). The usual response to pond fertilisation is the stimulation of an algal bloom (Boyd 1982). This increase in primary productivity following fertilisation can stimulate the production of natural food organisms and increase shrimp production (Furness & Aldrich 1979; Rubright *et al.* 1981; Lee & Shlesser 1984; Lanari *et al.* 1989). Algae also assimilate ammonia and other potentially toxic, nitrogenous, metabolic waste products (Laws & Malecha 1981; Millis 1981; Maestrini, Robert & Truquet 1982). In addition, actively photosynthesising algae can help maintain dissolved oxygen concentrations (Laws & Malecha 1981; Boyd 1982). Careful management is, however, required to avoid excessive blooms, depletion of nutrients, and subsequent rapid die-off and decomposition of algae, leading in turn to dissolved oxygen problems (Boyd 1982).

Penaeus monodon, the most cultured penaeid prawn both worldwide (Rosenberry 1991) and in Australia (Maguire & Allan in press), is traditionally cultured in Asia using extensive techniques, often within pond facilities originally constructed for milkfish (*Chanos chanos*) culture (Wickins 1976; New & Rabanal 1985). For *C. chanos* culture, ponds are fertilised to encourage the growth of a benthic algal mat (Chen 1972; Djajadiredja & Poernomo 1972). This benthic algal community, called "lab lab" in the Philippines (Apud 1985) and "kelekap" in Indonesia (Djajadiredja & Poernomo 1972), is an association of many plants and animals but is usually dominated by diatoms and blue-green algae (Apud 1985; New & Rabanal 1985). This method of fertilisation is still practised in some regions, especially where *P. monodon* is cultured using extensive techniques (Chakraborti, Haldar, Das, Mandal & Bhowmik 1986).

An alternative, and more widely used method of fertilisation is the stimulation and maintenance of a phytoplankton bloom (Furness & Aldrich 1979; Apud, Primavera & Torres 1985; Clifford 1985). This method is generally used for *P. monodon* culture in Taiwan (New & Rabanal 1985), by most farmers in Australia (Maguire &

Allan in press), and is recommended for *P. vannamei* culture in the western hemisphere (Clifford 1985). Phytoplankton blooms have the advantage of reducing light penetration and thus helping to prevent colonisation of the pond bottom by attached macrophytes (Laws & Malecha 1981; Boyd 1982).

The aims of this study were; 1) to compare the prawn production results obtained in fibreglass pools (used as model ponds) with those obtained during commercial farming trials with *P. monodon*, and, 2) to compare the effects of fertilisation strategies, designed to promote either benthic algae mats or phytoplankton blooms, on prawn production and water quality in model ponds.

MATERIALS AND METHODS

Experimental facilities and procedures

This study was carried out at the Brackish Water Fish Culture Research Station at Port Stephens, New South Wales, Australia (32° 45'S, 152° 04'E) in 3.5 m diameter, 1.2m high fibreglass pools. A 100 mm layer of sediment with 0.6 % organic matter (dry weight) was placed in each pool. Particle size analysis (Folk 1980) of this sediment indicated 92.0 % of the sediment had a grain size diameter of 0.25 - 1.0 mm, 6.4 %, was 0.063 - 0.25 mm and 1.6 % was <0.063 mm. Two months prior to stocking, all pools were filled with seawater and fertilised with the equivalent of 100 kg ha^{-1} of ammonium nitrate ('Nitram', Greenleaf Fertilisers Pty Ltd, Heron Road, Kooragang Island, NSW, 2304, Australia; *N:P:K*=24:0:0) and di-ammonium phosphate (DAP, Greenleaf Fertiliser Pty Ltd; *N:P:K*=20:20:0) to stimulate a phytoplankton bloom and enhance natural pond productivity. One month prior to stocking, all pools were completely drained and allowed to dry out for several days, thereby simulating commercial pond management practices (Apud *et al.* 1985).

Two algal management treatments were then established with eight replicate pools per treatment. Phytoplankton blooms were stimulated by the addition of Nitram or 'Aquasol' (Trade mark of Hortico Pty Ltd, Raymond Road, Laverton, Victoria, 3026, Australia; *N:P:K*=23:4:18 with trace amounts of Zn, Cu, Mo, Mn Fe and B) and DAP, at the rate of 100 kg ha^{-1} for each fertiliser, to the pools after they had been filled to a depth of 1 m. To maintain the phytoplankton bloom throughout the experiment, smaller additions (between 5-25 kg ha^{-1} for each fertiliser) were added periodically when measurements of dissolved oxygen (DO) and pH levels, and/or the concentrations of plant pigments, indicated a decline in algal density.

Benthic algal mats were stimulated in the remaining eight pools by the addition of the equivalent of 2 t ha^{-1} of cow manure (2.0 % *N*; 0.25 % *P*; 7.6 % moisture) and approximately 70 kg ha^{-1} of DAP. Fertilisers were added when the pools were dry and then, as recommend by Djajadredja & Poernomo (1972), water was added to a depth of only 10 cm to allow maximum penetration of sunlight to the pool bottom. A vigorous benthic algal mat was established in about two weeks. Pools were gradually filled over the ensuing 10 days to a depth of 1 m. Total initial inputs of *N* and *P* (whether added as inorganic fertiliser only or organic

plus inorganic) were similar for both treatments. However, as additions of fertiliser to the benthic algae pools during the experiment when the pools were full might have stimulated phytoplankton and reduced the benthic algae through shading, maintenance additions of fertiliser to these pools were kept to a minimum. Consequently the total inputs of *N* and *P* were much greater for pools managed with phytoplankton blooms (see Results).

Juvenile prawns (mean weight 2.2 g, range 1.2-3.4 g) were obtained from a commercial prawn farm in north Queensland, Australia. Batches of 25 prawns were blotted dry with a sponge, weighed and stocked sequentially into pools. All pools were stocked with 150 prawns (15 prawns m^{-2}). During the first four days all pools were half drained daily to allow removal and replacement of dead prawns with live prawns of a similar size. The experiment was run for 56 days.

Each pool was aerated (2.0 l min^{-1} $pool^{-1}$) with two airstone diffusers for an average of 5.6 h day^{-1} (1 h between 1500 and 1600 h to simulate mixing and aeration caused by prevailing afternoon winds and 4.6 h between 0400 and 0900 h). Water exchange was provided to each pool as needed using DO and pH levels as a guide.

Every two weeks a sample of prawns (n>20 prawns) was taken from each pool, weighed and returned to the pools. Prawns were fed a commercial diet imported from Taiwan (46.7 % protein [*N*x6.25, dry basis], 9.3 % moisture). Prawns were fed twice daily; 33.3 % of the ration in the morning and 66.7 % in the evening. Food consumption was monitored twice daily using plastic mesh trays (0.6 m^2) supplied with a proportional amount of food. Prawns were initially fed 4 % wet prawn biomass ([initial biomass + final biomass]/2) day^{-1} and this was adjusted according to food consumption and prawn growth rates. Average consumption (feed input) rates are given in Table 1.

One of the major differences between the model ponds (pools) used here and ponds used for commercial culture of *P. monodon* was the much greater wall to volume ratio of the pools. Growth of attached macrophytes on the walls could make a significant contribution to primary production in the pools while this is less likely in larger commercial ponds. The wall of each pool was therefore cleaned several times during the experiment to prevent excessive growth of attached macrophytes.

At the end of the experiment the pools were drained and the prawns harvested. Male and female prawns were separated and the number and total weight of each gender recorded. As a measure of drain harvest efficiency, the number of prawns which passed through a 100 mm drain at the sediment surface were counted and expressed as a percentage of the total number of surviving prawns.

The total quantity of food added to each pool was used as an estimate of food consumption. The conversion efficiency (S) of supplementary feed, where natural food is also present, was estimated using the following formula (Parker, 1987): S = weight of feed (92 % dry basis) added to pond/wet prawn biomass gain. This

inverse index overestimates the energetic efficiency of food conversion because the contribution made by natural food organisms is not considered (Parker, 1987). It is nevertheless useful for comparing inputs and outputs. The abundance of filamentous algae in the pools was measured in the week before harvest by estimating the total volume of the pool occupied by suspended filamentous algae.

Water quality analysis

Temperature, DO and pH were measured twice daily (morning 0700-0900 and afternoon 1600-1800) using a Yeokal (Yeokal Electronics, Brookvale, NSW, 2100) model 603 DO/T meter and an Orion (Orion Research Inc., Main Street, Boston, MA 021129, USA) pH/mV meter with a Ross (Orion Research Inc) combination glass electrode. Salinity was measured regularly using a Yeokal temperature/salinity conductivity meter. All meters were regularly calibrated as described by Allan, Maguire and Hopkins (1990). Nutrients were measured every seven days using the methods described by Dal Pont, Hogan & Newell (1974), for ammonia and the methods described by Major, Dal Pont, Kyle and Newell (1972) for nitrite plus nitrate and reactive phosphorus. Concentrations of the plant pigments chlorophyll a, b and c and pheophytin were measured during week 1, 2, 5 and 10 using the spectrophotometric methods described by Major *et al.* (1972). As all major groups of phytoplankton contain the pigment chlorophyll a (Jeffrey & Vesk 1981) the concentrations of this pigment was measured to provide an indication of the abundance of live phytoplankton in each pond at the time of each sampling. The concentrations of chlorophyll b and c were measured to indicate relative abundance of various divisions of phytoplankton which contain either or both of these pigments (Jeffrey 1981). Pheophytin, a degradation product of chlorophyll a (Jeffrey 1981), was measured to indicate abundance of senescent algal cells.

Statistical analysis

Two-factor ANOVA was used to investigate whether treatment, gender or the interaction between treatment and gender affected harvest weight. Differences in prawn performance indices, pond management variables (water exchange rate and input of *N* and *P*) and water quality were assessed using *t*-tests (Sokal & Rohlf 1981). In four of the pools where benthic algal blooms were stimulated, blooms of the filamentous algae *Enteromorpha* sp. developed. Single-factor ANOVA was used to determine whether the dominant bloom in the pools at the time of harvest (Phytoplankton, [P], benthic algae without filamentous algal bloom [B] or benthic algae with filamentous algal bloom [BF]) affected prawn performance indices or water quality variables. Sample sizes were n=8 for P and n=4 for B and for BF pools. Where significant differences were found, means were compared using Tukey's *w* (Sokal & Rohlf 1981).

Homogeneity of variance was comfirmed prior to *t*-test using the F-test (Sokal & Rohlf 1981) and prior to ANOVA using Cochran's test (Winer 1971). To satisfy the assumptions of normality and/or homogeneity of variance the following transformations were made prior to statistical analysis: Survival, S and drain harvest efficiency - (arcsine $x^{0.5}$); ammonia, nitrite plus nitrate, chlorophy a, b, c and

pheophytin - (log x).

To investigate whether the abundance of filamentous algae was correlated with prawn performance indices, the abundance of filamentous algae in benthic algae pools (B+BF) was estimated in the week prior to harvest and used in a correlation analysis (Sokal & Rohlf 1981) with data for survival, prawn weight gain, final biomass, biomass gain, food consumption, S and drain harvest efficiency. Using the data from all pools, prawn size at harvest was also used in a correlation with drain harvest efficiency.

RESULTS

Survival rates in all pools were ≥88.7 % and were unaffected ($P>0.05$) by the algal management treatments (phytoplankton or benthic algae) or by the dominant algal bloom at harvest P, B or BF (Table 1). Two-factor ANOVA showed that treatment effects (P, B plus BF) on harvest weight were not significant ($P>0.05$) although gender was significant ($P<0.05$). Female prawns were larger than males in both treatments (data for both treatments; average final weights were females 14.2 g, males 13.5 g). As there was no interaction between treatment and gender ($P>0.05$), data for females and males were combined to assess effects of treatment on prawn weight gain.

Prawns grew rapidly (Fig. 1) and gained between 9.6 and 13.1 g over 56 days, based on average gain within each pool. Differences in growth between algal management treatments were not significant ($P>0.05$), however, when the effects of the three dominant blooms at harvest were assessed, prawns in BF pools had significantly ($P<0.05$) lower average weight gain (Table 1). Similarly, final biomass and biomass gain results were not affected by treatment ($P>0.05$) but were significantly reduced ($P<0.05$) in BF pools (Table 1). Food consumption was unaffected ($P>0.05$) by treatment or dominant bloom at harvest (Table 1) but S ($P>0.05$) and drain harvest efficiency ($P<0.01$) were both worse in BF pools compared with P pools (Table 1).

Water exchange rates (mean±SE) were similar ($P>0.05$) in both treatments (7.4±0.2 % d^{-1} and 7.3±0.4 % d^{-1} for P and B+BF respectively). Total inputs of both *N* and *P* were higher ($P<0.001$) for the P pools (27.1±0.3 g *N* m^{-2} and 7.8±0.3 g *P* m^{-2} respectively) than for the B+BF pools (13.8±0.2 g *N* m^{-2} and 3.9±0.1 g *P* m^{-2} respectively). Mean minimum and maximum temperatures and salinity were 23.0 °C (range 19.2 - 25.9 °C), 25.6 °C (range 21.8 - 29.3 °C) and 31.2 ‰ (range 28.5 - 33.3 ‰) respectively and were similar for all pools. Morning DO concentrations and morning and afternoon pH values were unaffected by treatment or dominant algal bloom at harvest ($P>0.05$) (Table 2) although, afternoon DO, while unaffected ($P>0.05$) by treatment, was affected ($P<0.05$) by the dominant bloom at harvest (Table 2). Ammonia, nitrite plus nitrate and reactive phosphorus concentrations were all higher in P pools than the B or BF pools ($P<0.05$) (Table 2). Chlorophyll a concentrations were similar in P and B pools ($P>0.05$) and higher than in BF pools ($P<0.05$) (Table 2). Chlorophyll b and pheophytin concentrations were highest in P pools and chlorophyll c

concentrations were similar in P, B or BF pools (Table 2).

Filamentous algae occupied an estimated 20, 60, 70 and 80 % of the pond volume in BF ponds. The abundance of filamentous algae correlated significantly ($P<0.05$) with prawn weight gain ($r = -0.80$) and harvest efficiency ($r = -0.76$) but not with final biomass, biomass gain, food consumption or S ($P>0.05$). Using data for all pools, prawn size at harvest was significantly correlated with drain harvest efficiency ($r=0.64$; $P<0.01$).

DISCUSSION

Experimental systems

Survival rates in the present study were higher than for commercial pond trials with *P. monodon* in New South Wales (NSW), Australia (mean 63.9 %) (Allan 1989) or commercial pond trials with *P. monodon* in Taiwan (mean 59.4 %, range 30.5 - 81.9 %) (Chen *et al.* 1989). This is to be expected because, juvenile prawns (mean weight 2.2 g prawn^{-1}) were used in the present study rather than postlarvae. The present study was also of short duration compared with the usual commercial grow-out period, and faster growth rates and better food conversion efficiencies have been associated with an increased contribution of natural food during the early stages of prawn culture in ponds (Rubright et al. 1981). In addition, because of the relatively small size of the pools, there was a greater capacity for aeration and water exchange than is usually provided for commercial ponds. Nevertheless, the average growth rates recorded during this study (0.21 g day^{-1}) were similar to those recorded for *P. monodon* (0.17-0.25 g day^{-1}) by commercial farmers in NSW, Taiwan, Philippines and India for densities at harvest ranging from 3-18.1 prawn m^{-2} (Apud *et al.* 1985; Chakraborti *et al.* 1986; Allan 1989; Chen *et al.* 1989). Average values for S during this experiment (1.1:1) and for favourable treatments in other pool experiments (1.6 - 2.1; Allan and Maguire, unpublished data) were also similar to those reported for NSW and Taiwan (1.4:1 - 2.2:1) (Allan 1989; Chen *et al.* 1989). Although faster growth rates have been recorded for *P. monodon* (eg. 0.32-39 g prawn^{-1} day^{-1}; Liao 1977; Sundararajan, Victor Chandra Bose and Verkatesan 1979), these have usually been for culture under higher water temperatures and lower stocking densities than those used in the present study.

Shell (1966, 1983) found that yield (production per ha) for channel catfish, *Ictalurus punctatus*, was many times greater using earthen ponds (0.1 ha) than plastic-lined pools (0.00059 ha) or concrete tanks (0.002) even though stocking density, feeding and fertilisation practices were the same. In earthen ponds, Chen *et al.* (1989) found that *P. monodon* yield was inversely related to pond size although, as stocking density was higher in his smaller ponds, this result was not an unconfounded pond size/yield relationship. The results for survival, growth and S obtained during the present study are similar to those reported for commercially farmed *P. monodon* in NSW and Taiwan under similar water temperature regimes and stocking densities. On this basis, the pools described here are considered appropriate experimental units for pond management research with *P. monodon.*

Where an estimate of the variability in an experimental system is known, the number of replicates needed to detect a difference between treatments with a given degree of accuracy can be predicted (Roberts 1983). Using the results for weight gain from P and B pools (mean 11.95 g prawn^{-1}, standard deviation 0.76 g prawn^{-1}, n=12 pools), four replicate pools would have been sufficient to detect a 15 % difference in weight gain at P=0.001.

Fertilisation strategies

During this study there were no differences in prawn survival or growth in phytoplankton pools or benthic algae pools without filamentous algae. Apud (1985), however, recommended against the use of benthic algae mats on two grounds. Firstly, conditions most suitable for the growth of benthic algae mats, ie. shallow depth (20-25 cm), high salinity (>28 %), are not ideal for *P. monodon* and, secondly, deterioration of pond bottoms, production of sulphides and other toxic gases and reduced DO were common in ponds with excessive growth of benthic algae mats. During the present study, excessive growth of filamentous algae was likely in ponds with benthic algal mats.

Growth of filamentous algae in monoculture *P. monodon* ponds should be avoided for three reasons. Firstly, the growth rate and yield of prawns in ponds with extensive blooms of *Enteromorpha* sp. was lower (P<0.05) than in either phytoplankton ponds or ponds with benthic algal blooms and no filamentous algae (Table 1). This may have been related to decreased accessibility of pellets, some of which became entangled in the filamentous algae. This problem is likely to be worse in commercial ponds where mechanised food distribution systems, e.g., blowers, are used rather than hand feeding as in this study. Secondly, excessive growth of filamentous algae, such as *Chaetomorpha* sp, can cause problems by entangling postlarvae (Apud 1985). Thirdly, and possibly most importantly, filamentous algae blooms seriously impair effective pond harvest. For example, in the pool with the greatest abundance (80 %) of filamentous algae only 20 % of the prawns were recoved from effluent water when the pond was drained. Commercial prawn farmers in Australia and Taiwan prawn usually harvest prawns by either drain harvesting or using nets (Chiang & Liao 1985; Maguire & Allan 1991). Both methods are impeded by filamentous algae. In the present study, drain harvest efficiency was positively correlated with prawn size, and the prawns in the pools with filamentous algae (BF pools) were smaller. It should be noted, however, that this relationship applied for smaller prawns than are often harvested for commercial *P. monodon* ponds (ie., >25 g).

The maximum levels of ammonia and nitrite plus nitrate in all pools remained below growth reducing inhibiting concentrations for *P. monodon* reported by Wickins (1976) and Allan *et al.* (1990). Similarly, DO concentrations (highest in BF pools [P<0.05] with extensive blooms of *Enteromorpha* sp) and pH values were well above growth inhibiting concentrations for *P. monodon* (Seidman & Lawrence 1985; Allan & Maguire 1991; Allan & Maguire in press) and did not account for the reduced weight gain of prawns in BF pools compared with those in P or B pools.

Despite similar inputs of *N* and *P* to B and BF pools, measured concentrations of ammonia and reactive phosphorous in the B and BF pools were significantly different ($P<0.05$: Table 2). The uptake kinetics of *N* and *P* vary for different algal species (Kautsky 1982) and may explain these differences. For example blue green algae are usually a major component of benthic algae mats in prawn farming ponds (New & Rabanal 1985) and were more prevalent in B than BF pools. Blue green algae are able to assimilate dissolved atmospheric nitrogen and reproduce and grow in waters with low levels of dissolved nitrogen (Sevrin-Reyssac & Pletikosic 1990). Compared with P pools, the BF pools were characterised by low levels of chlorophyll a and b and phaeophytin indicating an inverse relationship between occurence of filamentous algae and phytoplankton. Liao (1977) noted problems with filamentous algae in *P. monodon* farming ponds and he concluded that *Enteromorpha* sp did not grow when water transparency was low.

CONCLUSIONS

The results from the present study showed survival, growth and food conversion efficiency for prawns reared in experimental, above-ground pools are similar, to those reported from commercial pond farming trials in NSW (Allan 1989) and Taiwan (Chen *et al.* 1989). Consequently, although care should be taken when directly extrapolating results from studies using model ponds to commercial size ponds, the overall conclusions reached should be relevant. Results from this study indicate that ponds used for the monoculture of *P. monodon*, managed using similar methods to those described here, should be fertilised to stimulate and maintain phytoplankton blooms to avoid growth of filamentous algae. Addition of fertilisers should be made when the intensity of phytoplankton blooms, and hence water turbidity, decreases.

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REFERENCES

Allan, G. L., 1989. Results from commercial and experimental prawn farming ponds in New South Wales during 1988/89. In: Proceedings of the Australian Mariculture Association 4th Annual Conference, Brisbane, Queensland July 1989, (ed. by J. Paynter and N. Preston), pp. 51-57. Australian Mariculture Association, Brisbane, Queensland.

Allan, G. L., Maguire, G. B. & Hopkins, S. J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen levels. Aquaculture, 91: 265-280.

Allan, G. L. & Maguire, G. B., 1991. Lethal levels of low dissolved oxygen and effects of short-term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture, 9: 27-37.

Allan, G. L. & Maguire, G. B., in press. Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon*. Aquaculture.

Apud, F. D., 1985. Extensive and semi-intensive culture of prawn and shrimp in the Philippines. In: Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps (ed. by Y. Taki, J. H. Primavera and J. A. Llobrera), pp. 105-113. Southeast Asian Fisheries Development Centre, Iloilo City, Philippines.

Apud, F. D., Primavera, J. H. & Torres, P. L. (Jr), 1985. Farming of Prawns and Shrimps. Aquaculture Extension Manual No. 5 (3rd Edition), South East Asian Fisheries Development Centre, Tigbauan, Iloilo, Philippines.

Boyd, C. E., 1982. Water Quality Management for Pond Fish Culture. Developments in Aquaculture and Fisheries Science, 9, Elsevier, Amsterdam.

Chakraborti, R. K, Halder, D. D., Das, N. K., Mandal, S. K. & Bhowmik, M. L., 1986. Growth of *Penaeus monodon* Fabricius under different environmental conditions. Aquaculture, 51: 189-194.

Chen, J. C., Liu, P. C. and Lin, Y. T., 1989. Culture of *Penaeus monodon* in an intensified system in Taiwan. Aquaculture, 77: 319-328.

Chen, T. P., 1972. Fertilization and feeding in coastal fish farms in Taiwan. In: Coastal Aquaculture in the Indo-Pacific Region (ed. by J. V. R. Pillay), Fishing News (Books) Ltd, London, pp. 68-73.

Chiang, P. & Liao, I. C., 1985. The practice of grass prawn (*Penaeus monodon*) culture in Taiwan from 1968 to 1984. Journal of the World Mariculture Society, 16: 297-315.

Clifford, H. C., 1985. Semi-intensive shrimp farming. In: Texas Shrimp Farming Manual - An Update on Current Technology (ed. by G. W. Chamberlain, M. G. Haby & R. J. Miget), Texas A & M University System, Research and Extension Centre, Corpus Christi, Texas. Chapter 4, pp. 15-43.

Djajadiredja, R. & Poernomo, A., 1972. Requirements for successful fertilization to increase milkfish production. In: Coastal Aquaculture in the Indo-Pacific Region (ed. by J. V. R. Pillay), Fishing News (Books) Ltd, London, pp. 398-409.

Dal Pont, G., Hogan, M. & Newell, B., 1974. Laboratory techniques in marine chemistry. II. Determination of ammonia in seawater and the preservation of samples for nitrate analysis. CSIRO Division of Fisheries and Oceanography, Sydney, New South Wales, Report No. 55, 11pp.

Fair, P. H. & Fortner, A. R., 1981. The role of formula feeds and natural productivity in culture of the prawn, *Macrobrachium rosenbergii*. Aquaculture, 24: 233-243.

Folk, R. L., 1980. Petrology of Sedimentary Rocks. Hemphill, Austin, Texas.

Furness, G. N. & Aldrich, D. V., 1979. Possible clues to improved pond culture of penaeid shrimp. Proceedings of the World Mariculture Society, 10: 477-488.

Garson, G. I., Pretto, R. M. & Rouse, D. B., 1986. Effects of manures and pelleted feeds on survival, growth, and yield of *Penaeus stylirostris* and *Penaeus vannamei* in Panama. Aquaculture, 59: 45-52.

Geiger, J. G., 1983. A review of pond zooplankton production and fertilisation for the culture of larval and fingerling striped bass. Aquaculture, 35: 353-369.

Jeffrey, S. W., 1981. Phytoplankton ecology - with particular reference to the Australasian region. In: Marine Botany: An Australasian Perspective (ed. by M. N. Clayton and R. J. King), pp. 241-291. Longman Cheshire, Melbourne.

Jeffrey, S. W. & Vesk, M., 1981. The phytoplankton - systematics, morphology and ultrastructure. In: Marine Botany: An Australasian Perspective (ed. by M. N. Clayton & R. J. King), pp. 138-179. Longman Cheshire, Melbourne.

Kautsky, L., 1982. Primary production and uptake kinetics of ammonium and phosphate by *Enteromorpha compressa* in an ammonium sulfate industry outlet area. Aquatic Botany, 12: 23-40.

Lanari, D., Ballestrazzi, R. & Tibaldi, E., 1989. Effects of fertilisation and stocking rate on the performance of *Penaeus japonicus* (Bate) in pond culture. Aquaculture, 83: 269-279.

Laws, E. A. & Malecha, S. R., 1981. Application of a nutrient-saturated growth model to phytoplankton management in freshwater prawn (*Macrobrachium rosenbergii*) ponds in Hawaii. Aquaculture, 24: 91-101.

Lee, C. S. & Shlesser, R. A., 1984. Production of *Penaeus vannamei* in cattle manure - enriched ecosystems in Hawaii. Journal of the World Mariculture Society, 15: 52-60.

Liao, I. C., 1977. A culture study on grass prawn, *Penaeus monodon* in Taiwan - the patterns, the problems and the prospects. Journal of the Fisheries Society of Taiwan, 5(2): 11-29.

Maestrini, S. Y., Robert, J. M. & Truquet, I., 1982. Simultaneous uptake of ammonia and nitrate by oyster-pond algae. Marine Biology Letters, 3: 143-153.

Maguire, G. B. & Leedow, M. T., 1983. A study of the optimum stocking density and feed rate for school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture, 30: 285-297.

Maguire, G. B. & Allan, G. L., 1985. Development of methods for growing juvenile school prawns, *Metapenaeus macleayi*, in estuarine ponds. In: Proceedings of the Second Australian National Prawn Seminar, Kooralbyn, Queensland, October, 1984, (ed by P. C. Rothlisberg, B. J. Hill & D. J. Staples), pp. 337-351. NPS2, Cleveland, Queensland.

Maguire, G. B. & Allan, G. L., 1991. Shrimp Culture industry in Australia. In: Culture of Marine Shrimp: Principles and Practices (ed. by A. Fast & J. Lester), Elsevier, pp. 763-776.

Major, G. A., Dal Pont, G., Klye, J. & Newell, B., 1972. Laboratory techniques in marine chemistry. A manual. CSIRO Division of Fisheries and Oceanography, Sydney, New South Wales, Report No. 51, 55 pp.

Millis, N. F., 1981. Marine microbiology. In: Marine Botany: An Australasian Perspective (ed. by M. N. Clayton & R. J. King), pp. 35-60. Longman Chesire, Melbourne.

New M. B. & Rabanal, H. R., 1985. A review of the status of penaeid aquaculture in South East Asia. In: Second Australia National Prawn Seminar (ed. by P. C. Rothlisberg, B. J. Hill & D. J. Staples), pp. 307-326. NPS2, Cleveland, Queensland.

Parker, N. C., 1987. Food conversion indices: Controversy or convention. Progressive Fish Culturist, 49(3), 161-166.

Perschbacher, P. W. & Strawn, K., 1984. Depth distribution and composition of macroinvertebrate communities in enriched, low-salinity fish culture ponds. Journal of the World Mariculture Society, 15: 341-354.

Primavera, J. H., Apud, F. & Usigan, S., 1976. Effect of different stocking densities on survival and growth of sugpo (*Penaeus monodon* Fabricius) in a milkfish - rearing ponds. The Philippine Journal of Science, 105(3): 193-203.

Reymond, H. & Legardere, J. P., 1990. Feeding rhythms and food of *Penaeus japonicus* Bate (crustacean, Penaeidae) in salt marsh ponds: Role of halophilic entomofauna. Aquaculture, 84: 125-143.

Roberts, P., 1983. The number of replicates and other considerations in the design of field trials. In: Recent Advances in Animal Nutrition - 1983 (ed. by W. Haresign), pp. 3-11. Butterworths, London.

Rosenberry, R., 1991. World Shrimp Farming 1990. Aquaculture Digest, San Diego, California.

Rubright, J. S., Harrell, J. L., Holcomb, H. W. & Parker, J. C., 1981. Responses of planktonic and benthic communities to fertilizer and feed applications in shrimp mariculture ponds. Journal of the World Mariculture Society, 12: 281-299.

Sandifer, P. A., Hopkins, J. S. & Stokes, A. D., 1987. Intensive culture potential of *Penaeus vannamei*. Journal of the World Aquaculture Society, 18(2): 94-100.

Seidman, E. R. & Lawrence, A. L., 1985. Growth, feed digestibility and proximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. Journal of the World Mariculture Society, 16: 333-346.

Sevrin-Reyssac, J. & Pletikosic, M., 1990. Cyanobacteria in fish ponds. Aquaculture, 88: 1-20.

Shell, E. W., 1966. Comparative evaluation of plastic and concrete ponds and earthern ponds in fish-cultured research. Progressive Fish Culturist, 28(4): 201-205.

Shell, E. W., 1983. Fish Farming Research. Alabama Agricultural Experiment Station, Auburn University, Alabama.

Sohier, L. & Bianchi, M., 1983. Regulation per des bacteries nitritiantes des taux d'ammoniaque et de nitrite dans bassins d'aquaculture marine. Oceanis, 9(3): 265-273.

Sokal, R. R. & Rohlf, F. J., 1981. Biometry. Freeman & Company, San Francisco, CA.

Sundararajan, D., VictorChandra Bose, S. & Venkatesan, V., 1979. Monoculture of tiger prawn, *Penaeus monodon* Fabricius, in a brackish water pond at Madras, India. Aquaculture, 16: 73-75.

Subosa, P. F. & Bautista, M. N., 1991. Yield of *Penaeus monodon* Fabricius in brackish water ponds given different fertiliser combinations. Aquaculture, 94: 39-48.

Wee, K. L., 1989. Pond experiment methodology. In: Proceedings of the Symposium on Tropical Aquaculture. Darwin, Northern Territory, August, 1989, pp. 92-103. Australian Institute of Biology, Darwin, Northern Territory.

Wickins, J. F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Winer, B. J., 1971. Statistical Principles in Experimental Design. 2nd Edition. McGraw-Hill, New York.

Wyban, J. A., Lee, C. S., Sato, V. T., Sweeney, J. N. & Richards, W. K. Jr., 1987. Effect of stocking density on shrimp growth rates in manure-fertilized ponds. Aquaculture, 61, 23-32.

Wyban, J. A. & Sweeney, J. N., 1989. Intensive shrimp growout trials in a round pond. Aquaculture, 76: 215-225.

Wyban, J. A., Pruder, G. D., Leber, K. M. and Burzell, L., 1989. Paddlewheel effects on shrimp growth, production and crop value in commercial earthen ponds. Journal of the World Aquaculture Society, 20(1): 18-23.

TABLE 1

Performance data for *Penaeus monodon* reared in model prawn farming ponds (pools) with different dominant algal blooms[1].

Indice	Dominant algal bloom		
	Phyto-plankton (P)	Benthic algae (B)	Filamentous algae[2] (BF)
Survival (%)[3]	94.0±0.9 [a]	92.2±1.7 [a]	91.9±1.2 [a]
Weight gain (g prawn^{-1})	11.9±0.3 [a]	12.1±0.3 [a]	10.4±0.3 [b]
Final biomass (g m^{-2})	199.5±4.0 [a]	197.2±6.4 [ab]	177.0±5.2 [b]
Biomass gain (g m^{-2})	165.9±4.0 [a]	163.5±6.2 [ab]	143.5±5.2 [b]
Food consumption (% biomass d^{-1})	2.5±0.1 [a]	2.6±0.1 [a]	2.7±0.1 [a]
S[3,4]	0.97±0.03 [a]	1.01±0.04 [ab]	1.13±0.04 [b]
Drain harvest efficiency (%)[3,5]	85.0±3.3 [a]	74.3±6.5 [ab]	50.3±10.4 [b]

1 Data are means ± SE (n=8 replicate pools for P and n=4 replicate pools each for B and BF). Within a row, means sharing a letter in the superscript are not significantly different (P>0.05)

2 Although benthic algal blooms were stimulated initially, extensive blooms of the filamentous alga *Enteromorpha* sp developed

3 Data transformed arcsine ($x^{0.5}$) prior to statistical analysis

4 S = total weight of feed added to pools/prawns^{-1} biomass gain (Parker 1987)

5 Drain harvest efficiency = number of prawns (expressed as a percentage of total recovered) that were drain harvested through a 100mm diameter outlet

TABLE 2

Water quality in model farming ponds (pools) with different dominant algal blooms[1].

Indice	Dominant algal bloom		
	Phyto-plankton (P)	Benthic algae (B)	Filamentous algae[2] (BF)
Morning DO (mg l^{-1})	6.3±0.1 [a]	6.0±0.04 [a]	6.7±0.5 [a]
Afternoon DO (mg l^{-1})	8.3±0.2 [ab]	7.6±0.2 [a]	9.1±0.6 [b]
Morning pH	8.1±0.1 [a]	8.0±0.04 [a]	8.5±0.2 [a]
Afternoon pH	8.3±0.1 [a]	8.2±0.04 [a]	8.6±0.2 [a]
Ammonia (μg TAN l^{-1})[3,4]	384.3±48.4[a]	42.7±16.0 [b]	11.0±2.0 [b]
Nitrite plus nitrate (μg N l^{-1})[4]	298.5±43.6[a]	4.9±0.3 [b]	3.3±0.8 [b]
Reactive phosphorous (μg P l^{-1})	506.9±26.3[a]	110.4±24.8[b]	238.4±37.6 [b]
Chlorophyll a (μg l^{-1})[4]	16.0±1.9 [a]	12.3±0.9 [a]	4.7±0.8 [b]
Chlorophyll b (μg l^{-1})[4]	4.0±0.4 [a]	1.6±0.3 [b]	0.9±0.2 [b]
Chlorophyll c (μg l^{-1})[4]	4.4±0.7 [a]	4.8±0.4 [a]	2.0±0.4 [a]
Pheophytin (μg l^{-1})[4]	14.4±1.7 [a]	10.8±0.9 [b]	4.3±0.7 [b]

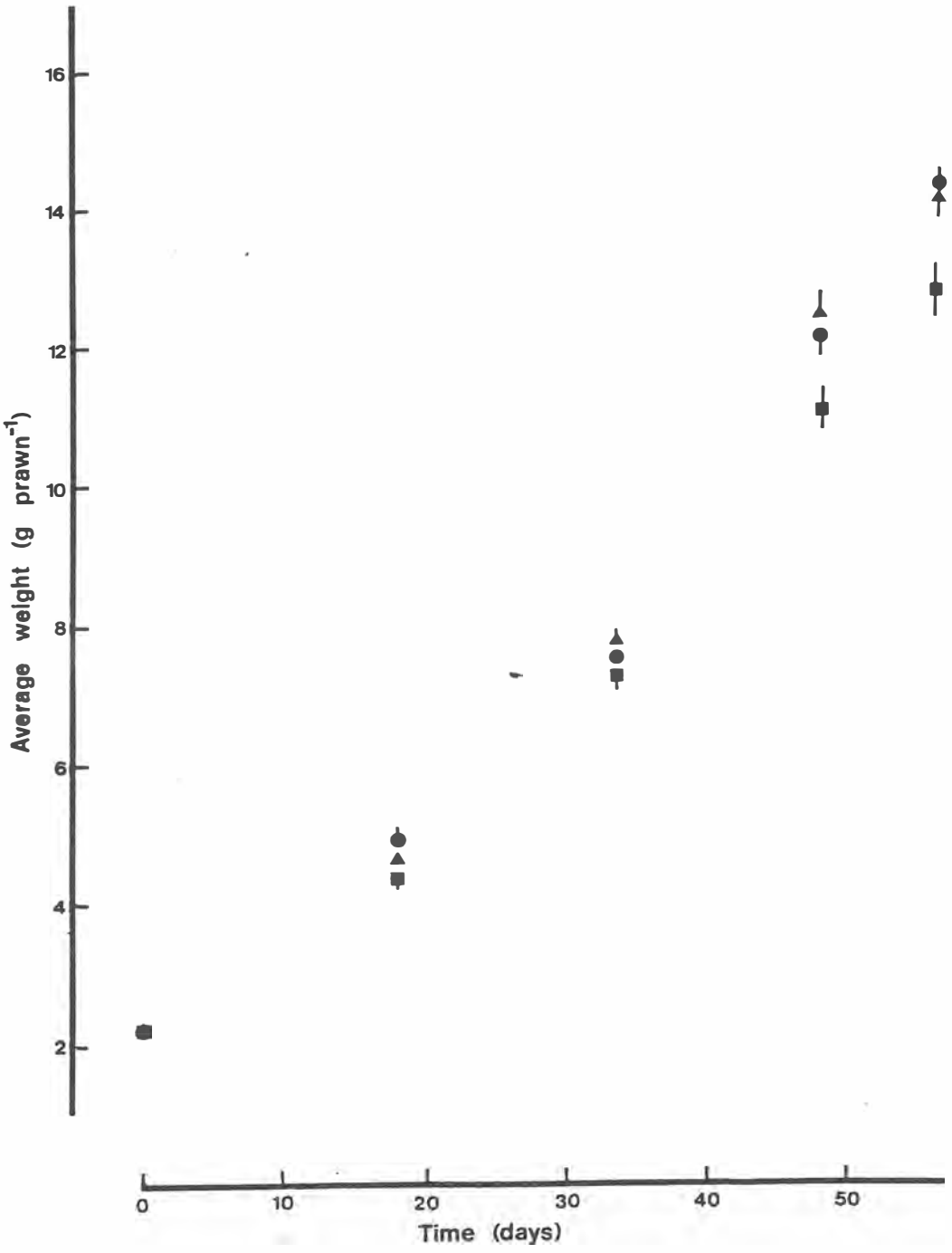
1 Data are means ± SE (n=8 replicate pools for P and n=4 replicate pools each for B and BF). Within a row, means sharing a letter in the superscript are not significantly different (P>0.05)

2 Although benthic algal blooms were stimulated initially, extensive blooms of the filamentous alga *Enteromorpha* sp developed

3 TAN = total ammonia nitrogen

4 Data transformed (log x) prior to analysis

Figure 1 Growth of *Penaeus monodon* in model prawn farming ponds over 56 days with different algal management strategies. o average for all treatments; ▲ phytoplankton pools (P); ● benthic algal pools (B); ■ benthic algal pools where extensive blooms of the filamentous alga *Enteromorpha* sp also developed (BF). Symbols represent the mean of n=8 pools for P and n=4 pools for B and for BF. Bars represent standard error of the mean.


Average weight (g prawn^{-1})
16
14
12
10
8
6
4
2
0
10
20
30
40
50
Time (days)

3.3 Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds

ABSTRACT

Allan, G. L. and Maguire, G. B. Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds.

Sixteen 3.5m diameter, 1.2m high fibreglass pools with sediment were used as model prawn farming ponds to investigate the effects of four stocking densities (5, 15, 25, and 40 prawns m^{-2}) on prawn survival, growth and biomass gain, water quality, natural food resources, prawn feeding behaviour and economic return. Prawn survival was high (>88%) and unaffected ($P>0.05$) by density and there was a significant ($P<0.05$) decreasing exponential relationship between weight gain and density ($y=9.177e^{-0.0103x}1$; $r=-0.88$) and a significant ($P<0.001$) increasing linear relationship between final total biomass (g m^{-2}) and density ($y=21.044+8.348x$; $r=0.99$). Biomass gain, apparent food consumption (feed input) and food conversion ratio increased ($P<0.001$) as density increased. A simple economic analysis indicated that returns were affected by density ($P<0.01$), but, differences among more profitable treatments (15, 25 and 40 prawns m^{-2}) were not significant ($P>0.05$). Dissolved oxygen and concentration of algal pigments were affected by density ($P<0.001$), although, density-related effects on water quality did not explain reductions in prawn growth with increased density. The population density of macrobenthos, but not meiobenthos, within the sediment layer in each pool declined with increasing stocking density, however, the decline in growth of prawns at higher densities may have been due to decreased grazing activity as indicated by the number of stomachs containing neither natural nor supplementary feed.

INTRODUCTION

Penaeus monodon is the most widely cultivated prawn both worldwide (Rosenberry, 1991) and in Australia (Maguire and Allan, in press). Although a number of studies have investigated the effects of stocking density for this species, they have usually either been for nursery culture (Ravichandran et al., 1982; Tabbu, 1985), extensive culture in combination with milkfish (Primavera et al., 1976; Ranoemihardjo et al., 1980; Eldani and Primavera, 1981) or, have only considered relatively low maximum densities eg 20 prawns m^{-2} or less (Apud et al., 1981; Verghese et al., 1982; Tiro et al., 1986).

Increasing the stocking density of fish or crustaceans in ponds usually exacerbates problems with water quality and sediment deterioration (Avnimelech et al., 1981; Emmerson and Andrews, 1981; Chien and Lai, 1988; Hopkins et al., 1988; Wyban and Sweeney, 1989). However, available literature does not discuss the effects of stocking density on water quality, natural food resources or feeding behaviour in *P. monodon* farming ponds. Increasing density also increases the susceptibility of prawns to disease (Hanson and Goodwin, 1977; Baticados et al., 1986; Doubrousky et al., 1988). In addition, increasing the density raises pressure on

natural food resources (Hopkins et al., 1988) and, as food conversion efficiency is often reduced (Sandifer et al., 1987), the total feed costs rise (New, 1987). Conversely, optimum returns on capital and labour depend upon using the highest possible stocking densities which are consistent with good survival and growth (Hanson and Goodwin, 1977). Hardman et al. (1991) found that the profitability of prawn farming in Australia was extremely sensitive to market price, yield and farm size and stressed the importance of stocking density on yield.

Trials in replicated model prawn farming ponds provide consistent estimates of major inputs (Maguire and Allan, 1985) yet no studies have assessed the impact of stocking density on estimated returns for *P. monodon* farming using these systems. The aims of this study were to investigate the effects of stocking density in model ponds on (1) survival, growth and production of prawns, (2) relative economic return, (3) pond water quality, and (4) natural food resources (macrofauna and meiofauna) and feeding behaviour.

MATERIALS AND METHODS

Experimental facilities and procedures

This study was carried out at the Brackish Water Fish Culture Research Station at Port Stephens, New South Wales (NSW), Australia (32° 45'S, 152° 04'E). Sixteen 3.5 m diameter, 1.2 m high fibreglass pools were used as model prawn farming ponds. A 100 mm deep layer of sediment with 0.6 % organic matter (dry weight) was placed in each pool. Particle size analysis (Folk, 1980) indicated that 92 % of this sediment had a grain size of 0.25 - 1.0 mm diameter, 6.4 % was 0.063 - 0.25 mm diameter and 1.6 % was <0.063 mm diameter. To help stimulate an algal bloom and the production of natural food organisms, pools were fertilised two weeks prior to stocking at a rate of 86 kg ha^{-1} Aquasol (Trade mark of Hortico Pty Ltd, Raymond Road, Laverton North, Victoria, 3026, Australia; N:P:K=23:4:18 with trace ammounts of Zn, Cu, Mo, Mn, Fe and B) and 4 kg ha^{-1} Di-ammonium phosphate (DAP; Greenleaf Fertilisers Pty Ltd, Heron Road, Kooragang Island, NSW, 2304; N:P:K=20:20:0). Each pool was managed on an individual basis and further additions of fertiliser were made when measurements of dissolved oxygen (DO), pH and/or the concentration of algal pigments indicated a reduction in algal density. Similarly, water exchange was used to dilute excessive blooms or provide well oxygenated water when DO concentrations were low (e.g. morning concentrations <5.0 mg l^{-1}) (Fig. 1A).

Juvenile prawns (mean weight 3.5 g, range 2.0 - 5.6 g) were obtained from a commercial prawn farm. Groups of 25 prawns were blotted dry, weighed and stocked into pools until the required densities were reached. Four treatments, each with four randomly allocated replicate pools, were established with stocking densities of 5, 15, 25 and 40 prawns m^{-2}. On each of the first four days all pools were drained to 50 % volume to allow the estimation of post-stocking mortality and the dead prawns (3 in total) were removed and replaced with live prawns of a similar size. The experiment ran for 59 days from February - April 1988 and was terminated because of declining water temperatures.

An air compressor supplying two airstone diffusers per pool (average flow rate = 1.0 l min^{-1} for each diffuser) was used for aeration and, following reduced DO concentrations in pools stocked at 40 prawns m^{-2} (morning concentrations <5.0 mg l^{-1}) during Week 1, longer daily periods of aeration were provided. Average rates of aeration for treatments with 5, 15, and 25 prawn m^{-2} were 7.3 h day^{-1} and 12.0 h day^{-1} for pools with 40 prawns m^{-2}. With the exception of a one hour period of aeration between 1500 h and 1600 h to all pools to simulate prevailing afternoon winds, all aeration was provided between 2200 h and 0900 h.

Every two weeks a sample of prawns (n>10 % of total population) was taken from each pool and an average individual weight determined. These prawns were returned to the pools. Prawns were fed a commercially available diet imported from Taiwan (46.7 % protein [Nx6.25, dry basis]; 9.3 % moisture), initially at a rate of 4 % biomass day^{-1}, twice daily; 33.3 % of the ration in the morning and 66.7 % in the evening. The average total inputs of nitrogen and phosphorus, from both feed and fertilizer, for each treatment, are given in Fig. 1B. Food consumption was monitored visually twice daily using one (0.6 m^2) plastic mesh tray per pool supplied with an amount of feed proportional to its size and, together with biomass estimates based on bi-weekly prawn size sampling, used to adjust feed rates.

At the termination of the experiment, the ponds were drained, the prawns collected, and survival, average individual harvest weight for each gender, average individual weight gain and total biomass gain were calculated for each pool.

The total quantity of food added to each pond was used as an estimate of food consumption. The conversion ratio (S) for supplementary feed, (in the presence of natural food), was estimated using a formula similar to that described by Parker (1987):

$$S = \frac{\text{weight of feed added to pond}}{\text{prawn biomass gain}}$$

This inverse index, which is equivalent to apparent food conversion ratio (AFCR) (New, 1987), overestimates the energetic efficiency of food conversion, because the contribution made by natural food organisms is not considered (Parker, 1987) but it is useful for comparing inputs and outputs. The average input of feed for each treatment is given in Fig 1C.

The relative economic performance for each treatment was estimated using a economic model which included costs for stock, feed, and electricity for pumping and aeration, and value of prawns produced. Electricity costs for pumping and aeration were included to take into account increased costs of maintaining water quality associated with high density culture. However, the model did not include costs associated with capital and labour which can also increase with density (Hardman et al., 1991). The estimate for the cost of stock (A$0.35 $prawn^{-1}$) was relatively high to take into account the large size (3.5 g average initial weight) of

prawns used. The estimate for feed cost (A$1420 t^{-1}) was based on prices for Taiwanese prawn pellets in Australia in 1990 (Maguire, 1990). Electricity costs were calculated based on rates (A$0.07 $kW^{-1}h^{-1}$) and performance estimates for 50 kW electric water pumps and 1 kW paddle wheel aerators in use at a prawn farm in northern NSW (28 ha of ponds). As the market value of prawns depends on size, the estimate for value of prawns from each pool was adjusted according to average individual prawn weight at harvest. A base price of A$8 kg^{-1} was adjusted by A$0.25 for every 1 g above or below an average individual final weight of 10 g $prawn^{-1}$. This simple size/price model was considered accurate for prawns in the 9.5 - 13.5 g size range but may have overestimated the value of larger prawns, eg >18 g.

Water quality analyses

Temperature, DO and pH were measured twice daily (morning 0700-0900 and afternoon 1600-1800) using a Yeokal (Yeokal Electronics, Brookvale, NSW, 2100) model 603 DO/T meter and an Orion (Orion Research Inc., Main Street, Boston, MA 02129, USA) pH/mV meter with a Ross (Orion Research Inc.) combination glass electrode. Salinity was measured regularly using a Yeokal temperature/salinity conductivity meter. All meters were regularly calibrated as described by Allan et al. (1990). Concentrations of the pigments chlorophyll a, b and c and pheophytin were measured every three or four days using the spectrophotometric methods described by Major et al. (1972). As all major groups of phytoplankton contain the pigment chlorophyll a (Jeffrey and Vesk, 1981), the concentration of this pigment was measured to provide an indication of the abundance of live phytoplankton in each pool at the time of each sampling. The concentration of chlorophyll b and c were measured to indicate relative abundance of various divisions of phytoplankton which contain either or both of these pigments (Jeffrey, 1981). Pheophytin, a degredation product of chlorophyll a (Jeffrey, 1981), was measured to indicate algal senescence. Nutrients were measured during Week 1, Week 5 and Week 8 using the methods described by Dal Pont et al. (1974) for ammonia and by Major et al. (1972) for nitrite plus nitrate and reactive phosphorus.

Macrobenthos and meiobenthos

For macrobenthos estimates, two cores (92 mm diameter; 100 mm deep) were taken from each pool and combined for each sample. One sample was taken from each pool at the end of the experiment. Each sample was sieved through a 500 μm screen to separate macrofauna and detritus from sediment and the macrobenthos were identified and counted after preservation in 10% buffered formalin. For meiobenthos estimates, two cores (26 mm diameter; 20 mm deep) were taken from each pool and combined for each sample. Each sample was preserved in a 10 % buffered formalin solution and stained with rose bengal. Three samples were taken from each pool at the end of the experiment. Prior to analysis each 70 ml sample container was filled with distilled water, shaken vigorously and then the supernatant (including the water and organics) quickly decanted. This procedure was repeated at least five times. Microscopic examination revealed very few or no animals left in the remaining sediment after this treatment. The organic material

was washed through two seives, a 500 μm screen to separate macrobenthos and large detritus and a 63 μm screen to retain meiobenthos.

Prawn stomach contents

After harvest, 15 prawns from each pool were preserved in 10 % buffered formalin. The stomachs were dissected and the contents observed under a binocular microscope. The occurrence method (Hyslop, 1980) was used to quantify the effect of stocking density on feeding behaviour. For each treatment the number of stomachs containing one or more individuals of each food category was expressed as a percentage of the total number of stomachs examined. The number of empty stomachs for each replicate pool was also recorded.

Statistical analysis

Two-factor ANOVA was used to investigate whether treatment, gender or the interaction between treatment and gender affected average individual prawn weight gain. Differences in other prawn performance indices and water quality variables among treatments were assessed using single-factor ANOVA. Where differences were significant ($P<0.05$) comparisons between means were made using Tukey's *w* (Winer, 1971). Homogeneity of variance was assessed using Cochran's test (Winer, 1971). To satisfy the assumptions of normality and/or homogeneity of variance, the following transformations were performed prior to statistical analysis: Survival and S - (arcsine $[x^{0.5}]$); final biomass, biomass gain, ammonia, nitrite plus nitrate, reactive phosphorus, chlorophyll - a, b and c and pheophytin concentrations - (log x).

Regression analysis was used to model the effects of stocking density on weight gain and final biomass. A one-sample Chi-square test was used to determine whether the total number of empty stomachs for each treatment differed from the mean number of empty stomachs for all treatments.

RESULTS

Prawn performance indicators

Treatment ($P<0.001$) and gender ($P<0.05$) both affected average individual prawn weight gain and females grew faster than males in all treatments (average for all treatments: females 11.2 g, males 10.7 g). As the interaction between treatment and gender was not significant ($P>0.05$) and, as there was no effect ($P>0.05$) of density on sex ratio, data for both males and females were combined to assess effects of treatment on prawn weight gain.

Survival rates were high (>88 %) and unaffected ($P>0.05$) by stocking density. Average individual prawn weight gain was reduced ($P<0.001$) as stocking density increased and there was a significant ($P<0.05$) decreasing exponential relationship ($y=9.177e^{-0.0103x}$ $r=-0.88$) between growth (weight gain) and density (Fig. 2A). Final biomass increased linearly ($y=21.044 + 8.348x$; $r=0.99$) as density increased

($P<0.001$) (Fig. 2B). Similarly, biomass gain (Fig. 2B), apparent food consumption (as indicated by feed input, Fig. 1C) and S (Fig. 2C) all increased ($P<0.001$) as density increased. Economic return was also affected ($P<0.01$) by density with the highest, and similar ($P>0.05$), returns recorded at densities of 15, 25 and 40 prawns m^{-2} and the lowest return ($P<0.05$) at 5 prawns m^{-2} (Fig. 2D). Density had no effect ($P>0.05$) on size range for either gender. The average size ranges for males and females were 6.0 g (range 4.5 - 8.0 g) and 6.4 g (range 4.5 - 8.4 g) respectively.

Water quality

For all pools, average salinity was 30.4 ‰ (range 26.0 - 35.0 ‰) and morning and afternoon temperatures were 21.8 °C (range 16.9 - 26.2 °C) and 24.1 °C (range 17.9-29.1 °C) in that order. For all treatments, morning DO concentrations were always above 4.2 mg l^{-1}, however, those stocked at 40 prawns m^{-2} had significantly lower ($P<0.05$) morning DO than all other treatments (Fig. 3A). Afternoon DO concentrations were lower ($P<0.05$) in the pools stocked at the lowest density (Fig. 3A). Morning and afternoon pH values ranged from 7.3 - 9.0 and 8.1 - 9.3 respectively, and were unaffected ($P>0.05$) by density. Although average ammonia concentrations increased markedly as density increased, differences between treatments were not significant ($P>0.05$) (mean ± standard error 12.9 ± 3.1, 46.0 ± 38.6, 37.3 ± 33.8 and 147.4 ± 96.6 μg total ammonia - nitrogen [TAN] l^{-1}, for 5, 15, 25 and 40 prawns m^{-2} respectively). Similarly, nitrite plus nitrate (range for all pools 0 - 67 μg NO_2 + NO_3 - N l^{-1}) and reactive phosphorus (range for all pools 0 - 103 μg PO_4 - P l^{-1}) were unaffected ($P>0.05$) by density. Phytoplankton blooms, as indicated by the concentration of chlorophyll a, were most intense in the most densely stocked pools which received the highest inputs of nutrients (Fig. 3B). Similarly, chlorophyll c (14.6 ± 2.3, 34.2 ± 5.2, 40.8 ± 8.7 and 59.8 ± 4.9 μg l^{-1} for 5, 15, 25 and 40 prawns m^{-2} in that order) and pheophytin (Fig. 3C) increased ($P<0.05$) with density. However, chlorophyll b (range for all pools 0 - 67 μg l^{-1}) was unaffected ($P>0.05$) by density.

Weekly microscopic examination of the species composition of the algal blooms indicated that the blooms in all pools were dominated by the Divisions Chrysophyta, Dinophyta and Bacillariophyta. Chlorophyts were relatively scarce, a finding consistent with the low concentration of chlorophyll b in all pools.

Macrobenthos and meiobenthos

The major categories of macrobenthos and meiobenthos found in the pool sediment and prawn stomachs were classified to class level (Table 1). Polychaetes dominated the macrobenthos in pools stocked at the lowest density and average numbers of polychaetes for this treatment were much greater than for any other treatment (Fig. 4A). With the exception of algae, which was present in similar amounts in all treatments, all major categories of macrobenthos were more abundant in treatments with 5 or 15 prawns m^{-2} (Fig. 4A). Numerically, the most abundant categories of meiobenthos; diatoms, others (including forams, ostracods, eggs, amphipods, cirripeds and hydrozoans) and ciliates, were most plentiful in the

sediment of pools stocked at 25 prawns m^{-2}, while nematodes were most abundant in pools stocked at 40 prawns m^{-2}. Copepods were found in similar numbers in all treatments (Fig. 4B).

Prawn stomach contents

The total number of prawns with empty stomachs for treatments stocked with 5, 15, 25 and 40 prawns m^{-2} were 7, 4, 7 and 18 in that order. The treatment effect (based on a null hypothesis of 1:1:1:1) was significant (x^2=12.7, df=3, P<0.01). Similarly, considerable variation was recorded in stomach contents among pools both within a treatment level and between treatment levels. However, for all treatment levels supplementary food was found in more stomachs than any other food category. The number of stomachs which contained the major food categories, such as supplementary feed, copepods, others and algae was also greatest for the pools stocked at the lowest density, and, tended to decrease as density increased (Fig. 4C).

DISCUSSION

An increase in stocking density from 5 to 40 prawns m^{-2} had no effect (P>0.05) on survival of *P. monodon*. Similarly, Sandifer et al. (1987) (average initial weight 1.3g and densities of 10-40 prawns m^{-2}) and Wyban et al. (1987) (average initial weight 2.7g and densities of 5-20 prawns m^{-2}) found density had no effect (P>0.05) on survival for *P. vannamei*. However, these results were in contrast to those reported by Apud et al. (1981) who found that increasing density of *P. monodon* (initial weight 0.5 g) from 2.5 to 20 prawns m^{-2} resulted in slight but significant (P<0.05) reductions in survival. During the present study, maximum growth rates for *P. monodon* (0.16 g day^{-1}) were relatively slow (Liao, 1977; Chen et al., 1989) probably because water temperatures were below the optimum for this species (27-33°C [Maguire and Allan, unpublished data]). However, growth rates were not dissimilar to average results (0.17 g day^{-1}) for commercial ponds stocked with *P. monodon* in NSW (Allan, 1989).

As with results from other penaeid studies (Apud et al., 1981; Maguire and Leedow, 1983; Sandifer et al., 1987; Wyban et al., 1987) growth declined and yield increased as density increased. The exponential decay model used here is consistent with that adopted by Maguire and Leedow (1983), for *Metapenaeus macleayi* and Edwards (1977) for *P. vannamei*. Increased prevalence of disease has been associated with increased stocking density (Hanson and Goodwin 1977; Baticados et al., 1986; Doubrousky et al., 1988) but there was no evidence of any disease during the present study. Minimum DO was always well above lethal (Allan and Maguire, 1991) or growth limiting (Seidman and Lawrence, 1985) concentrations for *P. monodon* but was lower (morning concentrations) at higher densities (P<0.05) (Fig. 3A). Similarly, build-up of metabolic waste products during this experiment is unlikely to have affected growth. There was a trend towards average ammonia concentration increasing with density, however, it was not significant (P>0.05) and the maximum ammonia concentrations recorded were well below those reported to have reduced growth or survival of *P. monodon* (Allan et

al., 1990). Nitrite plus nitrate also remained well below growth inhibiting concentrations (Wickins, 1976). The increase in the input of nutrients with density (Fig. 1B), largely as a result of the increase in supplementary food added, did not lead to significant increases in measured nutrient concentrations in the water column, probably due to rapid uptake of nutrients by phytoplankton. The intensity of the blooms, as indicated by the concentration of the pigments chlorophyll a and pheophytin, increased ($P<0.05$) with density (Figs. 3B & 3C). There is no evidence that high concentrations of plant pigments are associated with growth depression. In fact, Allan and Maguire (unpublished data) found that, while low water exchange rates increased plant pigment concentrations, growth of *Metapenaeus macleayi* was unaffected ($P>0.05$).

The importance of natural food items in the diet of penaeid prawns has been stressed (Maguire and Bell, 1981; Lilyestrom and Romaire, 1987; Reymond and Legardere, 1990) and Anderson et al. (1987) estimated that between 53 and 77% of *P. vannamei* growth, in cages stocked at 20 prawns m^{-2} within nursery ponds, was due to grazing on pond biota. A reduction in the availability of natural food at high stocking densities as a result of increased grazing pressure has been suggested as contributing to growth reductions (Hanson and Goodwin, 1977; Maguire and Leedow, 1983) and Ordner and Lawrence (1987) found that populations of polychaetes in penaeid prawn farming ponds declined as prawn density and grazing pressure increased. The abundance of macrofauna in the present study was clearly affected by density (Fig. 4A). As in other studies in penaeid prawn farming ponds (Rubright, 1978; Maguire et al., 1984; Ordner and Lawrence, 1987), the macrofauna in ponds stocked at low or moderate densities (5-25 prawns m^{-2}) was dominated by polychaetes. In the present study polychaete numbers declined (1241, 263, 132 and 0 m^{-2}) as density increased (5, 15, 25 and 40 prawns m^{-2} in that order). For all other major categories of macrobenthos, except for insect remains, a similar trend of decreasing abundance with increasing stocking density was evident (Fig. 4A).

With the exception of copepods, present in similar numbers in pools at all densities, numbers of the major groups of meiobenthos were highest at densities of either 25 or 40 prawns m^{-2} (Fig. 4B). A reduction in the availability of natural food organisms (macrobenthos and/or meiobenthos) may also occur following adverse environmental conditions which can develop in high density systems. Josefson and Widbom (1988) found that during hypoxic conditions in a natural environment the macrofaunal component of the benthic community disappeared, although, the meiofaunal component was unaffected. Although low DO concentrations were not recorded in the water column during this study, sediment deterioration was clearly related to density and low DO concentrations may have occurred in interstitial waters, especially in pools stocked at high densities. A reduction in the growth of *Macrobrachium rosenbergii,* as density increased to 31 prawns m^{-2}, was attributed to the deterioration in surface sediments and an increase in nitrogenous wastes (Chien and Lai,1988).

The importance of polychaetes in the diet of prawns in ponds has also been stressed (Rubright, 1978; Maguire and Bell, 1981; Rubright et al., 1981; Maguire et al., 1984; Nailon, 1985). However, one of the major problems with stomach content analysis in that only recently ingested material is easily identifiable and often large masses of unidentified organic material are recorded (Schroeder, 1983; Lilyestrom and Romaire, 1987). The number of stomachs containing food decreased as density increased (Fig. 4C). A decrease in the availability of natural food items with increased density, as a result of increased grazing pressure, or changes in the benthic community structure, may explain the reduced presence of natural food organisms in prawn stomachs but not the reduction in supplementary food or the increase in the number of empty stomachs. These results suggest that feeding activity was reduced as density increased, independent of the availability of food. Feed input, adjusted on the basis of feed tray results, actually increased with density (Fig. 1C) casting doubt on the reliability of feeding trays as a method for estimating food consumption. In a subsequent experiment in the same pools with *P. monodon*, feeding trays did not indicate overfeeding (they were usually empty), despite S values of as high as 5.1 being recorded. Prawns appeared to feed preferentially from the trays, possibly using these as a refuge from reduced pond sediment (Allan, Moriarty and Maguire, unpublished data).

In fish farming ponds receiving supplementary feed, reduced grazing by fish on natural pond biota over time, even where large amounts of natural food were present, was associated with deteriorating sediments and the development of anaerobic bottom conditions, (Avnimelech et al., 1981). The possibility of reduced grazing activity in pools stocked at higher densities represents an alternative to the hypothesis that growth declined at higher densities because of competition for natural food.

During this study, maximum economic return was recorded in pools stocked at 15-40 prawns m^{-2} (Fig. 2D). Chiang et al. (1986) analysed the economics of *P. monodon* culture in Taiwan and found that maximum return on investment was predicted at 30-40 prawns m^{-2}. However, it should be noted that higher land costs in Taiwan compared to Australia (Chiang et al, 1986; Hardman et al., 1991) would favour a high stocking density strategy. Maguire and Leedow (1983) found that for *Metapenaeus macleayi*, economic return increased with density up to 18.2 prawns m^{-2} followed by a slight but non-significant ($P>0.05$) reduction at 21.2 prawns m^{-2}. Conversely, Pardy et al. (1983) found that economic returns above selected costs (RASC) were greatest at lower densities. Maximum RASC was obtained at approximately 9.9 prawns m^{-2} for *P. stylirostris* and 13.6 prawns m^{-2} for *P. vannamei* (Pardy et al., 1983). It should be noted that although *P. monodon* are sometimes marketed in Australia at a similar size to those harvested during this study, the preferred size is considerably larger e.g., >25 g. Had water temperatures been higher, allowing the experiment to continue, it is likely that economic return at the higher densities would have deteriorated due to lower growth rates. In addition, as maintaining water quality is more difficult in ponds stocked at higher densities (Sandifer et al., 1987; Hopkins et al., 1988), and disease risk is likely to increase (Hanson and Goodwin, 1977), recommended stocking densities for *P. monodon*, grown under similar conditions, to those described here, would be 15-25 prawns

m^{-2}. The choice of optimum stocking density will however also depend upon the intended market size of prawns and on the number of crops per year which the prawn farmer intends to grow (Treadwell et al., 1991).

In eastern Australia, commercial prawn farmers are subject to regulations governing nutrient and algal levels in discharge (effluent) water (Allan and Maguire, 1987; Mitchell, 1987). The effect of increasing stocking density on effluent can be estimated by multiplying the total quantity of water released during water exchange by the average concentrations of nutrients (total ammonia - N, $[NO_2 + NO_3]$ - N and total P) and chlorophyll a. As density increased from 5 to 40 prawns m^{-2}, total nitrogen and algal output levels increased by approximately 795 % and 640 % respectively, while total phosphorus increased by approximately 45 %. As, with the same increase in density, final biomass increased by 520 %, the total nitrogen and chlorophyll a levels in the effluent, per kg of prawns produced, increased with density while phosphorus concentration decreased. However, a proportion of this phosphorus would have remained in algal cells or been absorbed onto pool sediments (Boyd, 1982). The use of high stocking densities in *P. monodon* ponds involves the input of large amounts of nutrients, often in a situation where salinity levels are being deliberately or inadvertantly reduced. These conditions can be conducive to the development of toxic algal blooms (Shumway, 1990) which are of concern to prawn farmers and regulatory authorities. This issue also highlights the economic risks associated with increasing stocking densities.

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REFERENCES

Allan, G. L., 1989. NSW prawn production up. Austasia Aquaculture Mag., 4(5): 23-25.

Allan, G. L. and Maguire, G. B., 1987. Government requirements for the establishment of marine prawn farms in New South Wales. In: P. Sloane (Editor), Proc. SCP Prawn Farming Sem. SCP Fisheries Consultants Australia Pty Ltd, Sydney, pp. 50-61.

Allan, G. L. and Maguire, G. B., 1991. Lethal levels of low dissolved oxygen and effects of short term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture, 94: 27-37.

Allan, G. L., Maguire, G. B. and Hopkins, S. J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen levels. Aquaculture, 91: 265-280.

Anderson, R. K., Parker, P. L. and Lawrence, A., 1987. A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond growout system. J. World Aquaculture Soc., 18(3): 148-155.

Apud, F. D., Gonzales, K. and Deatras, N., 1981. Survival, growth and production of *Penaeus monodon* Fabricius at different stocking densities in earthen ponds with flow-through system and supplemental feeding. Fish. Res. J. Philipp., 6(2): 1-9.

Avnimelech, Y. and Lacher, M., Raveh, A. and Zur, O., 1981. A method for the evaluation of conditions in a fish pond sediment. Aquaculture, 23: 361-365.

Baticados, M. C. L., Coloso, R. M. and Duremdez, R. C., 1986. Studies on the chronic soft-shell syndrome in the tiger prawn *Penaeus monodon* Fabricius from brackishwater ponds. Aquaculture, 56: 271-285.

Boyd, C. E., 1982. Water Quality Management for Pond Fish Culture. Elsevier, Amsterdam, 318 pp.

Chen, J. C., Liu, P. C. and Lin, Y. T., 1989. Culture of *Penaeus monodon* in an intensified system in Taiwan. Aquaculture, 77: 319-328.

Chiang, P., Wang, T., Kuo, C. M. and Liao, I. C., 1986. The prospects of the grass shrimp culture industry in Taiwan. In: J. L. Maclean, L. B. Dizon and L. V. Hosillos (Editors), The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp. 25-29.

Chien, Y. H. and Lai, H. T., 1988. The effects of aged sediment and stocking densities on freshwater prawn *Macrobrachium rosenbergii* culture. J. World Aquaculture Soc. (Aquaculture Communiques), 19(1): 22A-23A.

Dal Pont, G., Hogan, M. and Newell, B., 1974. Laboratory techniques in marine chemistry. II. Determination of ammonia in seawater and the preservation of samples for nitrate analysis. CSIRO Div. Fish. Oceanogr. Rep., No. 55, CSIRO, Sydney, New South Wales, 11 pp.

Doubrovsky, A., Paynter, J. L., Sambhi, S. K., Atherton, J. G. and Lester, R. J. G., 1988. Observations on the ultrastructure of baculovirus in Australian *Penaeus monodon* and *Penaeus merguiensis*. Aust. J. Mar. Freshwater Res., 39: 743-749.

Edwards, R. R. C., 1977. Field experiments on growth and mortality of *Penaeus vannamei* in a Mexican coastal-lagoon complex. Estuar. coastal mar. Sci., 5: 107-120.

Eldani, A. and Primavera, J. H., 1981. Effect of different stocking combinations on growth, production and survival of milkfish (*Chanos chanos* Forskal) and prawn (*Penaeus monodon* Fabricius) in polyculture in brackishwater ponds. Aquaculture, 23: 59-72.

Emmerson, W. D. and Andrews, B., 1981. The effect of stocking density on growth, development and survival of *Penaeus indicus* Milne Edwards larvae. Aquaculture, 23: 45-57.

Folk, R. L., 1980. Petrology of Sedimentary Rocks. Hemiphill, Austin, TX, 184 pp.

Hanson, J. E. and Goodwin, H. L., 1977. Shrimp and Prawn Farming in the Western Hemisphere. Dowden, Hutchinson and Ross Inc., Stroudsburg, PA, 439 pp.

Hardman, J. R. P., Treadwell, R. and Maguire, G. B., 1991. Economics of prawn farming in Australia. In: Proc. 1990 International Crustacean Conference, Brisbane, 2-6 July 1990, Mem. Qld. Museum, 31: 421-434.

Hopkins, J. S., Baird, M. L., Grados, O. G., Maier, P. P., Sandifer, P. A. and Stokes, A. D., 1988. Impacts of intensive shrimp production on the culture pond ecosystem. J. World Aquaculture Soc. (Aquaculture Communiques), 19(1): 37A.

Hyslop, E. J., 1980. Stomach contents analysis - a review of methods and their application. J. Fish Biol., 17: 411-429.

Jeffrey, S. W., 1981. Phytoplankton ecology - with particular reference to the Australasian region. In: M. N. Clayton and R. J. King (Editors), Marine Botany: An Australasian Perspective. Longman Cheshire, Melbourne, Victoria, pp. 241-291.

Jeffrey, S. W. and Vesk, M., 1981. The phytoplankton - systematics, morphology and ultrastructure. In: M. N. Clayton and R. J. King (Editors), Marine Botany: An Australasian Perspective. Longman Cheshire, Melbourne, Victoria, pp. 138-179.

Josefson, A. B. and Widbom, B., 1988. Differential response of benthic macrofauna and meiofauna to hypoxia in the Gullmar fjord basin. Mar. Biol., 100: 31-40.

Liao, I. C., 1977. A culture study on grass prawn, *Penaeus monodon*, in Taiwan - the patterns, the problems and the prospects. J. Fish. Soc. Taiwan, 5(2): 11-29.

Lilyestrom, C. G. and Romaire, R. P., 1987. Diet and food assimilation by channel catfish and Malaysian prawns in polyculture as determined by stomach content analysis and stable carbon isotope ratios. J. World Aquaculture Soc., 18(4): 278-288.

Maguire, G. B., 1990. A comparison of commercial diets for leader prawns (*Penaeus monodon*). Austasia Aquaculture Mag, 5(2): 34.

Maguire, G. B. and Allan, G. L., 1985. Development of methods for growing juvenile school prawns, *Metapenaeus macleayi*, in estuarine ponds. In: P. C. Rothlisberg, B. J. Hill and D. J. Staples (Editors), Second Australian National Prawn Seminar, NPS2, Cleveland, Queensland, pp. 337-351.

Maguire, G. B. and Allan, G. L. (1991). Shrimp Culture industry in Australia. In: A. Fast and J. Lester (Editors), Culture of Marine Shrimp: Principles and Practices. Elsevier, Amsterdam, pp. 763-776.

Maguire, G. B. and Bell, J. D., 1981. The effects of fish on growth and survival of school prawns *Metepenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture, 24: 267-283.

Maguire, G. B. and Leedow, M. I., 1983. A study of the optimum stocking density and feed rate for school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture, 30: 285-297.

Maguire, G. B., Gibbs, P. J. and Collett, L. C., 1984. The macrobenthic fauna of brackish water prawn farming ponds at Port Stephens, New South Wales. Aust. Zool., 21(5): 445-458.

Major, G. A., Dal Pont, G., Klye, J. and Newell, B., 1972. Laboratory techniques in marine chemistry. A manual. CSIRO Div. Fish. Oceanogr. Rep., No. 51, Sydney, New South Wales, 55 pp.

Mitchell, W. D., 1987. Legislative requirements for prawn farming - the Queensland situation. In: P. Sloane (Editor), Proc. SCP Prawn Farming Sem. SCP Fisheries Consultants Australia Pty Ltd, Sydney, New South Wales, pp. 62-66.

Nailon, R. W., 1985. Extensive grow-out system for shrimp. In: G. W. Chamberlain, M. G. Haby and R. J. Miget (Editors), Texas Shrimp Farming Manual: An Update on Current Technology. Texas A & M University Systems Research and Extention Centre, Corpus Christi, TX, pp. IV43-IV48 Ch.

New, M. B., 1987. Feed and Feeding of Fish and Shrimp - A Manual on the Preparation and Presentation of Compound Feeds for Shrimp and Fish in Aquaculture. Report ADCP/REP/87/26. United Nations Development Program, Food and Agriculture Organization of the United Nations, Rome, Italy, 275 pp.

Ordner, M. T. and Lawrence, A. L., 1987. Importance of polychaetes to penaeid pond culture. J. World Aquaculture Soc. (Aquaculture Communiques), 18(1): 36A-37A.

Pardy, C. R., Griffin, W. L., Johns, M. A. and Lawrence, A. L., 1983. A preliminary economic analysis of stocking strategies for penaeid shrimp culture. J. World Maricul. Soc., 14: 49-63.

Parker, N. C., 1987. Feed conversion indices: Controversy or convention. Prog. Fish Cult., 49(3): 161-166.

Primavera, J. H., Apud, F. and Usigan, C., 1976. Effect of different stocking densities on survival and growth of sugpo (*Penaeus monodon* Fabricius) in a milkfish-rearing pond. Phil. J. Sci., 105(3): 193-203.

Ranoemihardjo, B. S., Sudarno, and Kusnendar, K, E., 1980. The effect of stocking density on the rate of the growth of tiger shrimp (*Penaeus monodon* Fabricius) and milkfish (*Chanos chanos* Forskal). Bull. Brackishwater Aqua. Dev. Cent. Philippines, 6(1&2): 454-461.

Ravichandran, P., Verghese, P. U. and Rajyalakshmi, T., 1982. Effect of stocking density and size on *Penaeus monodon* (Fabricius) postlarvae in nursery rearing experiments. In: Proc. Symp. Coastal Aquaculture, Cochin, India, 12-18 January 1980, Part I: Prawn Culture, pp. 412-413.

Reymond, H. and Lagardere, J. P., 1990. Feeding rhythms and food of *Penaeus japonicus* Bate (Crustacea, Penaeidae) in salt marsh ponds: role of halophilic entomofauna. Aquaculture, 84: 125-143.

Rosenberry, R., 1991. World Shrimp Farming 1990. Aquaculture Digest, San Diego, CA, 31pp.

Rubright, J. S., 1978. An investigation into the role of meiofauna in the food chain of a shrimp mariculture pond system. Unpublished MSC Thesis, Texas A&M University, TX, 56pp.

Rubright, J. S., Harrell, J. L., Holcomb, H. W. and Parker, J. C., 1981. Responses of planktonic and benthic communities to fertilizer and feed applications in shrimp mariculture ponds. J. World Maricul. Soc., 12(1): 281-299.

Sandifer, P. A., Hopkins, J. S. and Stokes, A. D., 1987. Intensive culture potential of *Penaeus vannamei*. J. World Aquaculture Soc., 18(2): 94-100.

Schroeder, G. L., 1983. Stable isotope ratios as naturally occurring tracers in the aquaculture food web. Aquaculture, 30: 203-210.

Seidman, E. R. and Lawrence, A. L., 1985. Growth, feed digestibility, and proximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. J. World Maricul. Soc., 16: 333-346.

Shumway, S. E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. J. World Aquaculture Soc., 21(2): 65-104.

Tabbu, N. S., 1985. Growth and survival of *Penaeus monodon* postlarvae with different feeding regimes and stocking densities in earthen brackishwater nursery ponds. In: Y. Taki, J. H. Primavera and J. A. Llobrera (Editors), Proc. First International Conf. on the Farming of Penaeid Prawns/Shrimp. Iloilo City, Philippines, 4-7 December 1984, pp. 180.

Tiro, L. B., Pudadera, B. J., Potestas, I. O. and Kungvankij, P., 1986. Intensification techniques in the extensive culture of *Penaeus monodon*. In: J. L. Maclean, L. B. Dizon and L. V. Hosillos (Editors), The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp. 93-95.

Treadwell, R., McKelvie, L. and Maguire, G. B., 1991. Profitability of selected aquacultural species. ABARE Discussion Paper 91-11, Aust. Govt. Publishing Service, ACT, Australia, 85 pp.

Verghese, P. U., Ravichandran, P. and Pillai, S. M., 1982. Growth and production of *Penaeus monodon* Fabricius in short-term field rearing experiments. In: Proc. Symp. Coastal Aquaculture, Cochin 12-18 January 1980. Part I: Prawn Culture, pp. 419.

Wickins, J. F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Winer, B. J., 1971. Statistical Principles in Experimental Design. 2nd Edition. McGraw-Hill, New York, NY, 907 pp.

Wyban, J. A. and Sweeney, J. N., 1989. Intensive shrimp growout trials in a round pond. Aquaculture, 76: 215-225.

Wyban, J. A., Lee, C. S., Sato, V. T., Sweeney, J. N. and Richards, W. K. Jr., 1987. Effect of stocking density on shrimp growth rates in manure-fertilized ponds. Aquaculture, 61: 23-32.

TABLE 1

Major categories of benthos and their relative abundance in macrobenthos, meiobenthos and prawn stomach samples

Benthos[1]	Relative abundance[2]		
	Macro-benthos	Meio-benthos	Prawn stomachs
Phylum Chlorophyta	N	A	R
Phylum Chryosphyta			
Class Bacillariophyceae	N	A	R
Superphylum Protozoa			
Phylum Sarcodina			
Class Rhizopoda	R	A	A
Phylium Ciliophora			
Class Ciliatea	N	A	N
Phylum Cnideria			
Class Hydrozoa	N	R	R
Phylum Nematoda			
Class Adenophora	N	A	N
Phylum Mollusca			
Class Gastropoda	N	R	R
Class Bivalvia	A	C	R
Phylum Annelida			
Class Polychaeta	A	C	R
Phylum Arthropoda			
Subphylum Crustacea			
Class Ostracoda	N	A	R
Class Copepoda	N	A	A
Class Cirripedia	N	R	N
Class Amphipoda	N	R	N
Class Decapoda[3]	N	N	C
Class Insecta	C	N	C
Phylum Bryozoa			
Class Gymnolaemata	R	N	N

1 Organisms were usually only classified to class

2 N = none found, R = rare, C = common, A = abundant

3 Moults from prawns were most common, although some prawn tissue was found in stomachs

Figure 1 Water exchange rates and total input of nutrients for model prawn farming ponds stocked with *Penaeus monodon* at different densities: A) water exchange rate, B) total input of nutrients; ● = total nitrogen (g N m^{-2}), ▲ = total phosphorus (g P m^{-2}), C) Feed input. Symbols are means and vertical bars are standard errors of the means for n = 4 replicate ponds. Means sharing a similar letter are not significantly different ($P>0.05$).

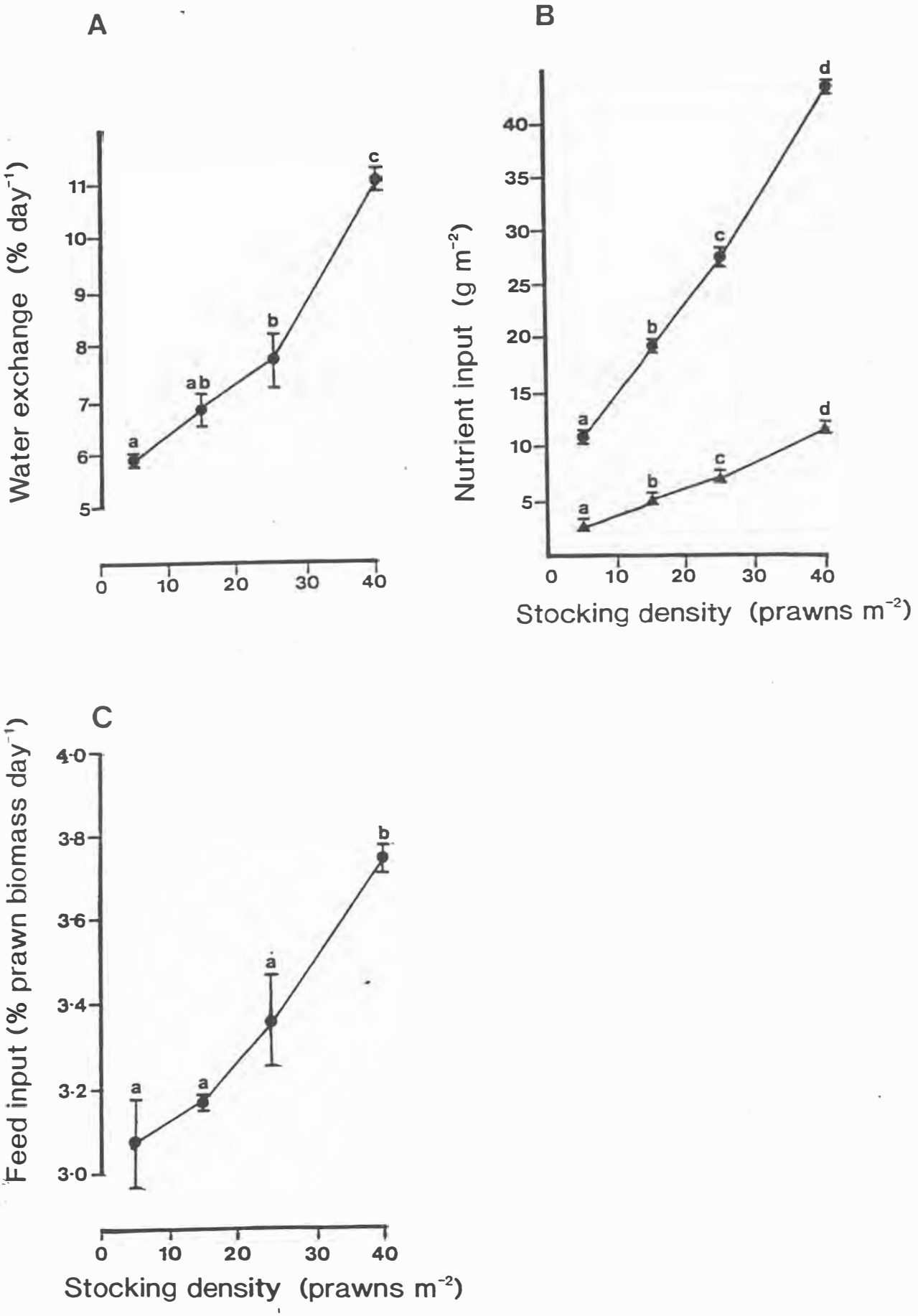

A
Water exchange (% day⁻¹)
11
10
9
8
7
6
5
a
ab
b
c
0
10
20
30
40
B
Nutrient input (g m⁻²)
40
35
30
25
20
15
10
5
a
b
c
d
a
b
c
d
0
10
20
30
40
Stocking density (prawns m⁻²)
C
Feed input (% prawn biomass day⁻¹)
4·0
3·8
3·6
3·4
3·2
3·0
a
a
a
b
0
10
20
30
40
Stocking density (prawns m⁻²)

Figure 2 Production results for model prawn farming ponds stocked with *Penaeus monodon* at different densities: A) average individual weight gain, B) biomass; ● = final biomass, ▲ = biomass gain, C) supplementary food conversion ratio (S), and d) economic return. Symbols are means and vertical bars are standard errors of the means for n = 4 replicate ponds. Means sharing a similar letter are not significantly different ($P>0.05$).

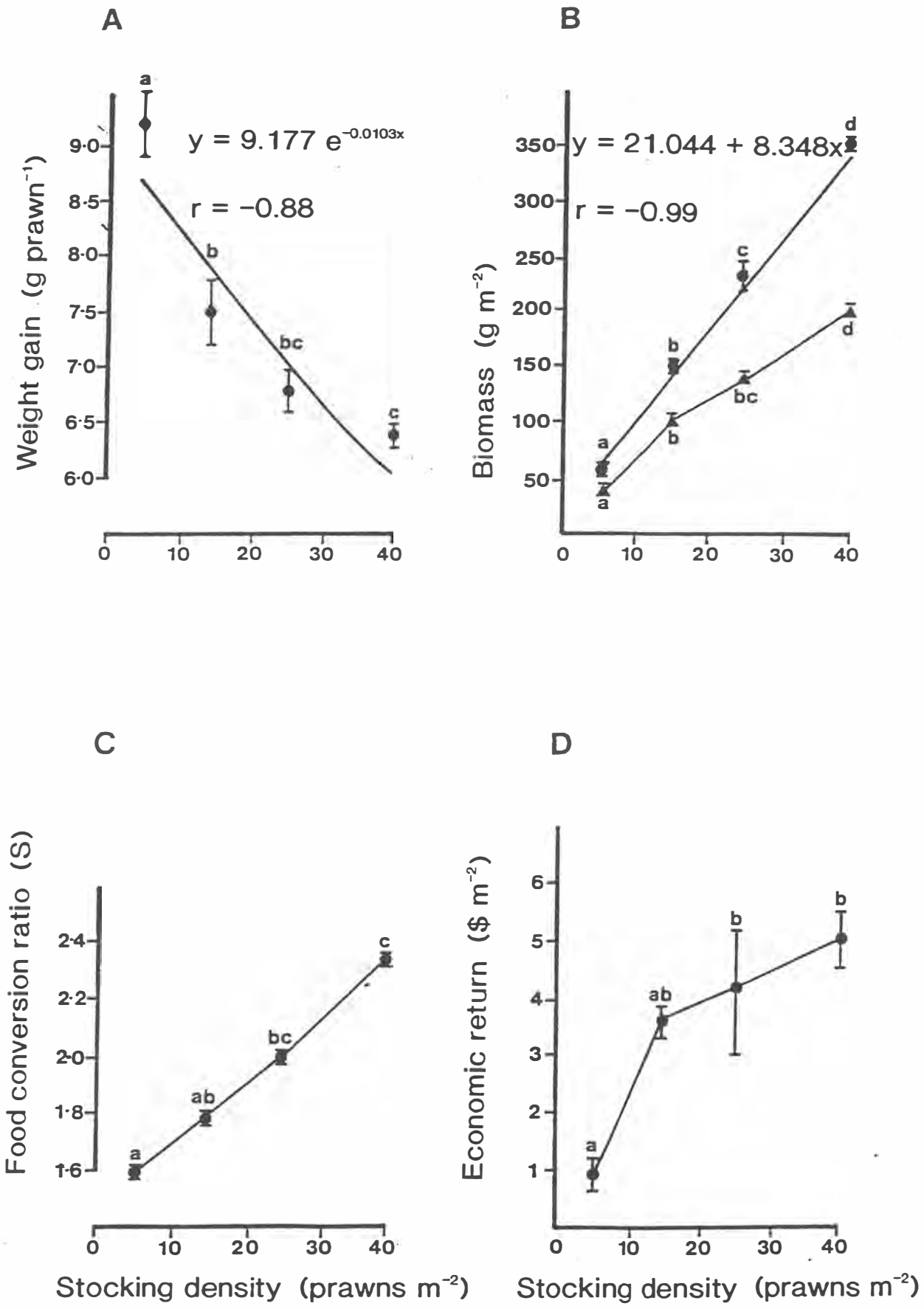

A
B
C
D
y = 9.177 e^-0.0103x
r = −0.88
y = 21.044 + 8.348x
r = −0.99
Weight gain (g prawn⁻¹)
Biomass (g m⁻²)
Food conversion ratio (S)
Economic return ($ m⁻²)
Stocking density (prawns m⁻²)
Stocking density (prawns m⁻²)

Figure 3 Water quality in model prawn farming ponds stocked with *Penaeus monodon* at different densities: A) dissolved oxygen; ● = afternoon levels, ▲ = morning levels, B) chlorophyll a concentration, and C) pheophytin concentration. Symbols are means and vertical bars are standard errors of the means for n = 4 replicate ponds. Means sharing a similar letter are not significantly different (P>0.05).

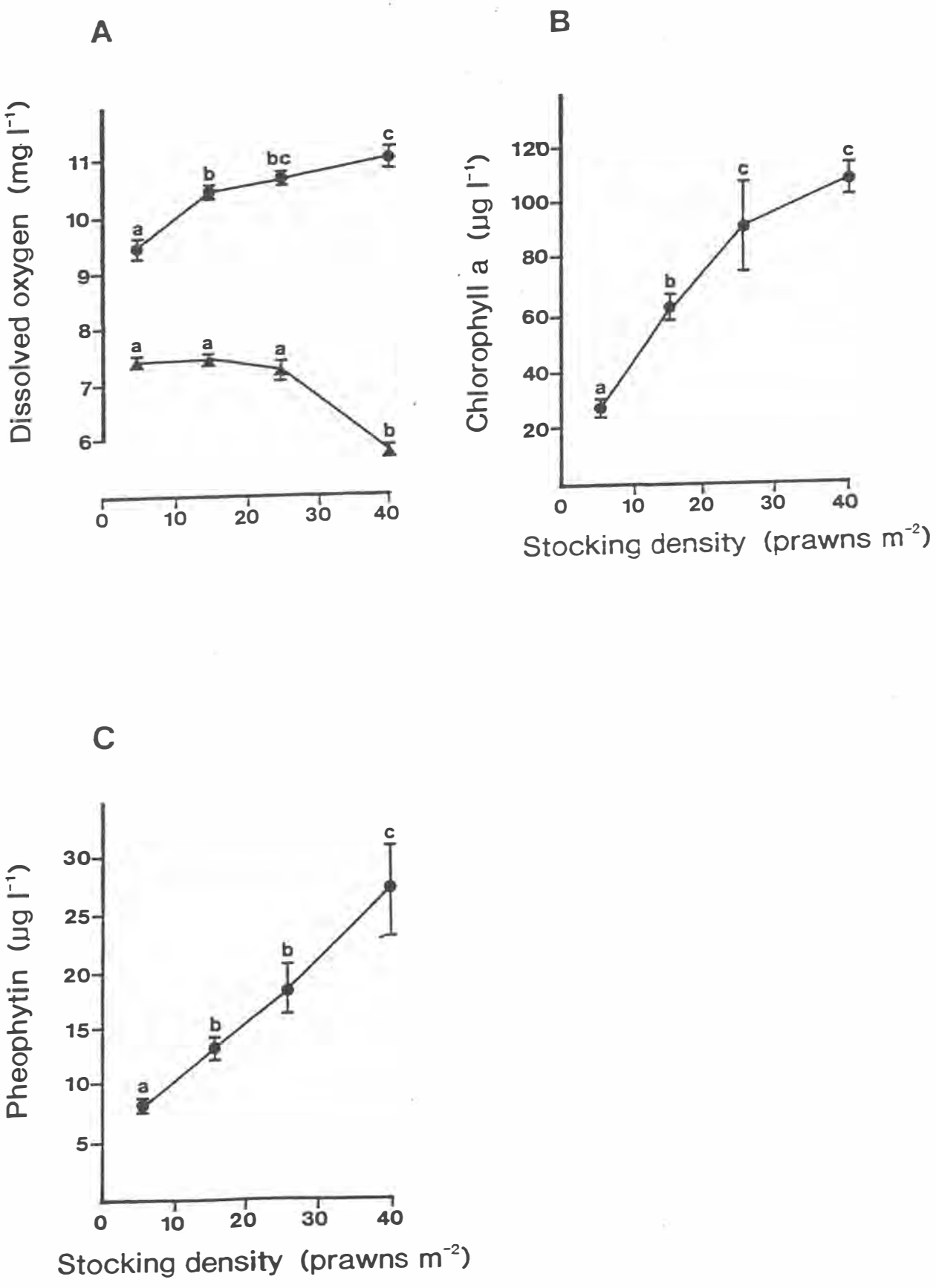
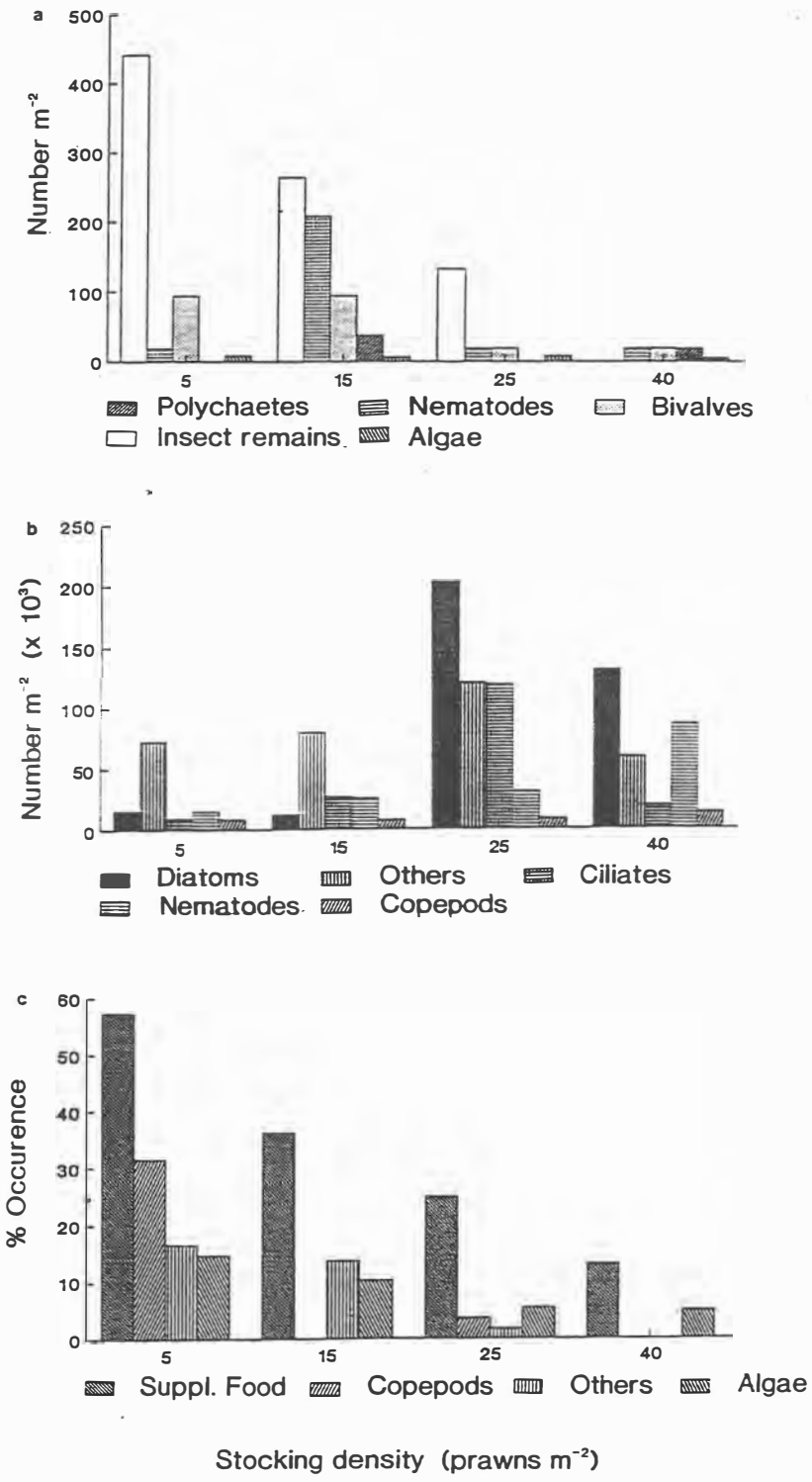

A
Dissolved oxygen (mg l⁻¹)
11
10
9
8
7
6
a
b
bc
c
a
a
a
b
0
10
20
30
40
B
Chlorophyll a (µg l⁻¹)
120
100
80
60
40
20
a
b
c
c
0
10
20
30
40
Stocking density (prawns m⁻²)
C
Pheophytin (µg l⁻¹)
30
25
20
15
10
5
a
b
b
c
0
10
20
30
40
Stocking density (prawns m⁻²)

Figure 4 Macrobenthos, meiobenthos and prawn stomach contents in model prawn farming ponds stocked with *Penaeus monodon* at different densities: A) macrobenthos, B) meiobenthos, and C) prawn stomach contents.


a
Number m⁻²
500
400
300
200
100
0
5
15
25
40
Polychaetes
Nematodes
Bivalves
Insect remains
Algae
b
Number m⁻² (x 10³)
250
200
150
100
50
0
5
15
25
40
Diatoms
Others
Ciliates
Nematodes
Copepods
c
% Occurence
60
50
40
30
20
10
0
5
15
25
40
Suppl. Food
Copepods
Others
Algae
Stocking density (prawns m⁻²)

3.4 Effects of pond preparation and feeding rate on production of *Penaeus monodon* Fabricus, water quality, bacteria and benthos in model farming ponds

ABSTRACT

Allan, G. L., Moriarty, D. J. W. and Maguire, G. B. Effects of pond preparation and feeding rate on production of *Penaeus monodon* Fabricius, water quality, bacteria and benthos in model farming ponds.

Sixteen 3.5 m diameter, 1.2 m high, fibreglass pools with sediment were used as model farming ponds to investigate the interactive effects of pond preparation and feeding rate on prawn production indices, apparent, water quality, bacterial dynamics, abundance of benthos and prawn feeding behaviour. Ponds were either fertilising one month (prepared) or two days (unprepared) prior to stocking and average feed inputs similar to those recommended for commercial farms (5.0 % wet prawn biomass day^{-1}; high rate) or half this rate (2.5-2.6 % day^{-1}; low rate) were used. Juvenile *Penaeus monodon* (2.0-7.5 g), stocked at 15 prawns m^{-2}, were cultured for 71 days. With the exception of one prepared, high feeding rate pond where mass mortality (>80 %) of prawns occurred following an interruption to aeration, prawn survival was high (>86%) and unaffected by preparation, feeding rate or their interaction ($P>0.05$). Preparation resulted in major (approximately 20 %) increases in growth, biomass gain and S ($P<0.01$). Growth was higher with the higher feeding rate (4 %) but biomass gain was not affected ($P>0.05$) and, as S was much worse ($P<0.001$), the use of the lower feeding rates used here offers considerable scope to reduce feeding costs especially during cooler periods. There was no interaction ($P<0.05$) in relation to growth between preparation and feeding rate.

The interactive effects of preparation and feeding rate on pond water quality, bacterial dynamics, abundance of benthos and the effects of these variables on production of prawns are discussed.

INTRODUCTION

Almost all prawn farming occurs in earthen ponds and these can contain biota of nutritional value to prawns (Maguire and Bell, 1981; Anderson et al., 1987; Gonzales, 1988; Reymond and Lagardere, 1990; Rosenberry, 1991). Maguire and Bell (1981) found natural food items were a significant component of the stomach contents of school prawns (*Metapenaeus macleayi*) in earthern ponds while Reymond and Lagardere (1990) found that in ponds with about 10 prawns m^{-2}, naturally occurring prey were the largest food source for *Penaeus japonicus*, despite daily inputs of pellet feed. Gonzales (1988) estimated that natural food contributed between 51 and 89 % of the total food intake for juvenile (0.02 g $prawn^{-1}$) *P. monodon*.

Using the ratio of stable carbon isotopes as a naturally occurring tracer to follow the metabolic pathways of food consumed, Anderson et al. (1987) estimated that for juvenile *P. vannamei* (initial weight 1.5 g prawn^{-1}) stocked at 20 prawns m^{-2}, between 53 and 77 % of prawn growth over 28 days was due to grazing on pond biota. Conversely, Schroeder (1983) measured the carbon isotope ratio of penaeid prawns (harvest weight approximately 15 g prawn^{-1}), supplementary feed ingredients and pond biota in Isreali ponds (unspecified stocking density) and found that only selected components of the prawn pellets (corn and sorghum) were metabolised to any extent. It is possible, however, that a food chain via bacteria and/or meiofauna or small macrofauna developed between the feed pellets and prawns in these ponds.

The fertilisation of ponds to stimulate the production of natural food is used extensively in pond based aquaculture, partly as a method of sparing requirements for expensive supplementary diets (New, 1987). Most of the research investigating the effects of fertilisation in penaeid prawn farming ponds has been conducted using low stocking densities (<10 prawn m^{-2}) and increased yields usually follow fertilisation (Rubright et al., 1981; Garson et al., 1986; Lanari et al., 1989). In one case fertilisation did not increase growth (Lee and Schlesser, 1984). Although recommendations exist for application rates for various fertilisers used in penaeid prawn farming ponds (Apud et al., 1985; Clifford, 1985), the importance of fertilising ponds well in advance of stocking to allow the communities of natural food organisms time to develop has not been investigated.

In several reviews of trophic pathways. The importance of bacteria and meiobenthos in aquaculture ponds or other aquatic ecosystems has been stressed as has the role of bacteria in decomposition and nutrient cycling and as a trophic link with meiobenthos and then prawns (Moriarty, 1986a; Anderson, 1987; Coleman and Edwards, 1987; Fry, 1987). In ponds fertilised with chicken manure and used for the culture of *P. monodon* and *P. merguiensis* in Malayasia, Moriarty et al. (1987) and Moriarty (1986a) found that the feed pellets were primarily the base for a microbial food chain rather than being used directly as a food for the prawns. Polychaetes and other benthic fauna are also important (Rubright, 1978; Rubright et al., 1981; Nailon, 1985; Moriarty, 1986a; Moriarty et al., 1987; Ordner and Lawrence, 1987). The dynamics of bacteria and meiobenthos in ponds with differing types of pond preparation and feeding (feed input) rates have not been studied.

Optimum feeding rates for maximum growth of *P. monodon* and *P. merguiensis* (0.1-1.8 g prawns^{-1}) in aquaria have been estimated as between (11-12 % prawn biomass per day as dry pellets Lee, 1971; Sedgwick, 1979). In pens within an earthen pond where prawns had access to natural food items, Maguire and Leedow (1982) found the optimum feeding rate for maximum growth of *Metapenaeus macleayi* (3-10 g prawn^{-1}) was 5 % of prawn biomass per day as dry pellets.

There are a number of recommended feeding rates for commercial culture of *P. monodon* (Table 1), however, no studies were found which assessed optimum feeding rates for this species in earthen ponds (where prawns have access to natural food items) using nutritionally adequate diets. Akiyama and Chwang (1990) recommended reduced rates of feeding where lower stocking densities were used, because of the contribution of natural food, but the interaction between feeding rate and pond preparation has not been investigated even though increased inputs of supplementary food might be necessary when ponds have not been adequately prepared.

The objectives of this study were firstly to assess whether preparing ponds, by fertilisation prior to stocking, enhanced production of natural food organisms and prawn growth, and, secondly to investigate the interactive effects of feeding rate and pond preparation on prawn growth and survival, pond water quality and natural food resources.

MATERIALS AND METHODS

Experimental facilities and procedures

This study was carried out at the Brackish Water Fish Culture Research Station at Port Stephens, New South Wales (NSW), Australia (32° 45'S, 152° 04'E). Sixteen 3.5 m diameter, 1.2 m high, fibreglass pools were used as model prawn farming ponds. A 100 mm deep layer of sediment containing 0.6 % organic matter was placed in each. The grain size characteristics of the sediment Folk (1980), were: 0.25 - 1.0 mm diameter - 92.0 %, 0.063 - 0.25 mm - 6.4 %, <0.063 mm - 1.6 %. Juvenile prawns (mean 4.5 g, range 2.0 - 7.5 g) were obtained from a commercial prawn farm on Micalo Island, NSW. Groups of 25 prawns were blotted dry, with a sponge, weighed and stocked into pools at a density of 15 prawns m^{-2}. During the first four days all pools were drained daily from 1.0m depth to 50 % volume to estimate post-stocking mortality and all dead prawns (ten in total) were replaced with live prawns of a similar size. The experiment was conducted for 71 days from 20/1 - 31/3/1989 and was terminated when water temperatures declined.

An air compressor supplying two air-stone diffusers per pool (average flow rate 1.0 l min^{-1} for each diffuser) was used for aeration. All pools were aerated for an average of 10 h day^{-1}; 9 h between 2300 and 0800 h and 1 h between 1500 and 1600 h. The latter period of aeration was provided to simulate the wind mixing of larger commercial prawn farming ponds in northern NSW which typically occurs in summer months as a result of prevailing north-easterly winds. Water exchange was provided to each pond when needed using dissolved oxygen (DO) and pH levels as a guide.

Four treatment combinations, in a two x two factorial design, were established with four randomly allocated, replicate pools per treatment combination. The two factors were preparation (pools were 'prepared' [P] or 'unprepared' [U]) and feeding rate (high [H] or low [L]). Four months prior to stocking, all pools were fertilised with the equivalent of 99 kg ha^{-1} Aquasol (Trade mark of Hortico Pty Ltd,

Raymond Road, Laverton North, Victoria, 3026, Australia; N:P:K=23:4:18 with trace amounts of Zn, Cu, Mb, Mn Fe and B) and 22 kg ha^{-1} Di-Ammonium Phosphate (DAP; Greenleaf Fertilisers Pty Ltd, Heron Road, Kooragang Island, NSW, 2304, Australia; N:P:K=20:20:0). This stimulated a phytoplankton bloom which was maintained with regular, smaller additions of fertilisers and water exchange for two months. After this time, all pools were drained and allowed to dry out (to simulate typical commercial practice between crops). One month prior to stocking, the eight 'prepared' pools were refilled, fertilised (as above) and water was exchanged regularly to stimulate and maintain a phytoplankton bloom and to allow colonization of the pool by benthic fauna. Two days prior to stocking the remaining eight 'unprepared' pools were filled and fertilised (as above). After stocking, all pools were managed in a similar manner to maintain phytoplankton blooms. Two feeding rates were used. The 'high' rate was similar to that advocated in a number of practical prawn farming manuals (Anon, 1984; Apud et al., 1985; Chiang and Liao, 1985; Clifford, 1985; Pascual, 1985) or feed manufacturing companies, while the 'low' rate was 50 % of the high rate (Table 1) and was similar to that used by the authors in previous trials with *P. monodon* in model ponds (Allan and Maguire, 1988; Allan and Maguire, in press).

Every two weeks a sample of prawns (n>30) was taken from each pool and an average individual weight determined. These prawns were returned to the pools alive. Prawns were fed a commercially available diet imported from Taiwan (46.7 % protein [Nx6.25, dry basis], 9.3 % moisture). Prawns were fed twice daily; 33.3 % of the ration in the morning and 66.7 % in the evening. Apparent food consumption was monitored twice daily in each pool using plastic mesh trays (0.6 m^2) supplied with a proportional amount of food on a surface area basis. The total quantity of food added to each pool was used as an estimate of apparent food consumption.

At the end of the experiment the prawns were harvested and male and female prawns were separated and the number and weight in bulk of each sex recorded. Survival, average individual weight gain, total biomass gain and sex ratio were determined for each pool. The conversion ratio (S) for supplementary feed (in the presence of natural food) was estimated using the following formula (Parker, 1987): S = weight of feed (92 % dry matter) added to pool / increase in wet prawn biomass. This inverse index, which is equivalent to the apparent food conversion ratio (AFCR) (New, 1987), overestimates the energetic efficiency of food conversion, because the contribution made by natural food organisms is not considered, but it is useful for comparing inputs and outputs. At the end of the experiment, S was estimated for each two-week growth period. For these estimations, prawn biomass increase was calculated by multiplying weight gain values by an estimate of the number of live prawns in each pool. Prawn mortality was assumed to be constant over time for the purpose of this estimation.

Water quality analysis

Temperature, DO and pH were measured twice daily (morning 0700-0900 and afternoon 1600-1800) using a Yeokal (Yeokal Electronics, Brookvale, NSW, 2100,

Australia) model 603 DO/T meter and an Orion (Orion Research Inc., Main Street, Boston, MA 02129, USA) pH/mV meter with a Ross (Orion Research Inc.) combination glass electrode. Salinity was measured regularly using a Yeokal temperature/salinity conductivity meter. All meters were calibrated regularly as described by Allan et al. (1990). Concentrations of the plant pigments chlorophyll a, b and c and pheophytin were measured during weeks 1, 2, 5 and 10 using the spectrophotometric methods described by Major et al. (1972). As all major groups of phytoplankton contain the pigment chlorophyll a (Jeffrey and Vesk, 1981) the concentration of this pigment was measured to provide an indication of the abundance of live phytoplankton in each pool at the time of each sampling. The concentrations of chlorophyll b and c were measured to indicate relative abundance of various divisions of phytoplankton which contain either or both of these pigments (Jeffrey, 1981). Pheophytin is a degradation product of chlorophyll a (Jeffrey, 1981) and was measured to indicate algal senescence.

Nutrients were measured during weeks 1, 5 and 10 using the methods described by Dal Pont et al. (1974) for ammonia, and the methods described by Major et al. (1972) for nitrate, nitrite and reactive phosphorus.

Bacterial Dynamics

During weeks 1, 5 and 10 direct counts of bacteria and experiments to estimate bacterial growth rates were carried out on samples from the water column and sediment in each pool. For bacterial estimations in the water column in each pool, five 70 ml samples were collected at different, evenly spaced positions about 200 mm in from the pool side at a depth of approximately 500 mm. These were combined, mixed, and a series of 5 ml subsamples taken using a pipette. For bacterial estimations in the sediment, ten cores (26 mm diameter, 8 mm deep; 2.25 ml) were taken from each pool. This sediment was combined, mixed and five 0.25 g sub-samples were taken using a corer (9 mm diameter, 2 mm deep; 0.13 ml). Two sub-samples each from the water column and sediment were preserved by the addition of formaldehyde (final concentration of 2 %), stored in the refrigeration and, to estimate the number of bacteria at the time of sampling (Nt), the bacteria were counted after staining with acridine orange using the modified epi-fluorescence technique (Daley and Hobbie, 1975). Estimates of the volume (v) of bacteria were made by measuring the length (l) and width (w) of representative bacteria from the water column and sediment using a calibrated ocular micrometer and the equation: $v = (w^2 \pi/4)(l-w) + \pi w^3/6$ (Fry, 1988). It should be noted that this method may lead to overestimation (Fry, 1988). Samples of $n>350$ bacteria from both the water column and sediment from each sampling period were measured. As differences between the size of bacteria from the different treatments and different sampling times were not significant ($P>0.05$), average volumes for both the water column (0.16 μm^3) and the sediment (0.19 μm^3) were used for productivity calculations.

Estimates of bacterial growth rates were made by measuring the incorporation of tritiated thymidine into DNA as in Moriarty (1986b, 1990) and Moriarty and Pollard (1981). Two blank and three replicate subsamples were used to calculate average

values for both the water column and sediment in each pool at each sampling time. Tritiated thymidine (25 μl; S.A. 25 ci/mmol) was added to each subsample and incubated for 30 min. Uptake of tritiated thymidine was terminated in the water column subsamples by the addition of 2 ml buffered 37 % formaldehyde solution (containing 0.1 g thymidine per 100 ml) and, in the sediment subsamples, by the addition of 8 ml 80 % ethanol (containing 0.1 g thymidine per 100 ml). For the blanks, formaldehyde (water column samples) or ethanol (sediment) was added prior to the addition of tritiated thymidine.

The total number of cells dividing per hour (Nh^{-1}) was estimated using the formula described by Moriarty (1990): Nh^{-1} = dpm x 1.62 x 10^4 / S.A. x t where dpm = disintegrations per minute; S.A. = specific activity of the isotope; and t = incubation time in minutes.

The specific growth rates of bacteria (μ) in the water column and the sediment were estimated as: $\mu = Nh^{-1} / Nt$. Productivity of bacteria, in terms of carbon, was estimated as: $gC = Nh^{-1}$ x V x specific gravity x carbon content where V = volume of bacteria (estimated by multiplying Nt by average biomass of bacteria [v] from water column or sediment); specific gravity = 1.1 (Fry, 1988); and carbon content = 22 % wet weight (Bratbak and Dundas, 1984).

Meiobenthos and macrobenthos

The abundance of major groups of meiobenthos was estimated for each pond from samples taken during weeks 1, 5 and 10. Two cores (26 mm diameter, 20 mm deep) were taken and combined for each sample. Each sample was preserved in a 10 % buffered formalin/seawater solution and stained with rose bengal. Three replicate samples were taken from each pool at each sampling time. Prior to analysis, 70 ml distilled water was added to each sample, the combination was shaken vigorously and then the supernatant (including the water and organics) quickly decanted. This procedure was repeated at least five times. Microscopic examination revealed very few or no animals left in the remaining sediment after this treatment. The organic material was washed through two seives, a 500 μm screen to separate macrobenthos and large detritus and a 63 μm to retain meiobenthos.

Two cores (92 mm diameter, 100 mm deep) were taken for macrobenthos estimates and combined for each sample. Each sample was preserved with 10 % buffered formalin solution. One sample was taken from each pool at the end of the experiment. Sampling of macrobenthos was not carried out during the experiment to avoid the major disturbance to prawns caused by taking the large cores for macrobenthos estimation. Each sample was sieved through a 500 μm mesh screen to retain macrobenthos and detritus.

Prawn stomach contents

After harvest the heads of 15 prawns from each pond were preserved in a 10 % buffered formalin solution. The stomachs were dissected and the contents

observed under a binocular microscope. The occurrence method (Hyslop, 1980) was used to quantify the effects of treatment on feeding behaviour. For each treatment the number of stomachs containing one or more individuals of each food category was expressed as a percentage of the total number of stomachs examined. The number of empty stomachs for each treatment was also recorded.

Statistical analysis

The effects of pond preparation, feeding rate and their interaction on prawn performance indices, (except S) water quality variables, bacterial dynamics and the numbers of each major group of meiobenthos were assessed using two-factor ANOVA. To assess the interactive effects of pond preparation, feeding rate and time on S and the number, growth rate and productivity of bacteria, and the abundance of meiofauna, these data were also analysed using three-factor ANOVA. Homogeneity of variance was assessed using Cochran's test (Winer, 1971) and to satisfy the assumptions of normality and/or homogeneity of variance the following transformations were performed prior to statistical analysis: Survival number of empty stomachs (as a percentage of the total number of stomachs examined) and % occurrence of all major food categories in the prawn stomachs (arcsine $[(0.01\ x]^{0.5}]$); food conversion ratio (S), afternoon DO, afternoon pH, ammonia, nitrite, nitrate, phosphorus, chlorophyll b concentration, specific growth rate of bacteria and productivity of bacteria - (log x); number of bacteria - $(x^{0.5})$; and abundance of all major categories of meiobenthos - $([x+1]^{0.5})$. Unless otherwise indicated values in the text are means ± standard errors.

RESULTS

Prawn performance indicators

Prawn growth (weight gain) was highest in the prepared pools where the highest feeding rate was used (Table 2) and both preparation ($P<0.001$) and feeding rate ($P<0.05$) affected weight gain, although there was no interaction ($P>0.05$) (Table 2). Although average prawn weight at each bi-weekly sampling interval was higher in prepared pools ($P<0.001$), feeding rate did not affect average prawn weight ($P>0.05$) until the week when prawns were harvested. By the end of the experiment average weight gain of prawns in the prepared pools was approximately 20 % greater than in the unprepared pools but the difference between high and low feeding rate treatments was only 4 %. The average weight gain between sampling periods for prawns in all treatments indicated that the difference in weight gain between prepared and unprepared pools was only pronounced during the first two bi-weekly sampling periods (Fig. 1).

Final biomass and biomass gain were significantly ($P<0.001$) higher in the prepared pools (Table 2) but were unaffected by feeding rate or the interaction between preparation and feeding rate ($P>0.05$). Apparent food consumption was significantly affected by feeding rate ($P<0.001$) but not by preparation or their interaction ($P>0.05$) (Table 2). S was worse (higher) for unprepared pools and for pools where the higher feeding rate was used ($P<0.001$). For all treatment

combinations S deteriorated over time ($P<0.001$) (Fig. 2). As the difference between S during the early stages of the experiment (Week 2) and at the end of the experiment was greater from prepared pools compared with unprepared pools, the interaction between preparation and time was significant ($P<0.001$) (Fig. 2). Interactions between preparation and feeding rate, feeding rate and time and preparation, feeding rate and time were all non-significant ($P>0.05$).

During the experiment two interruptions to the air supply to all pools occurred. During the first interruption in Week 1, air supply ceased for between 4-8 h on a hot, still night and, in one pool (Pool 2), DO declined to 0.1 mg l^{-1}. More than 80 % of prawns died and results from this replicate (from the prepared, high feeding rate treatment combination) were not included in any statistical analyses. Concentrations of DO in all other pools were >2.0 mg l^{-1} and no evidence of mortality was found. The DO concentration on the morning following the interruption was not affected by preparation, feeding rate or their interaction ($P>0.05$). During the second 4-8 h electricity interruption in the week prior to harvest, DO concentrations in a number of pools declined (minimum 0.6 mg DO l^{-1}), although no prawns died. The lowest concentrations were recorded in the high feeding rate pools ($P<0.05$). Previous research had shown that short periods (4-12 h) of sub-lethal DO stress (0.8 or 1.0 mg l^{-1}) did not affect subsequent growth of *P. monodon* juveniles were DO concentrations were returned to normal (Allan and Maguire, 1991).

Water quality analysis

Average salinity and morning and afternoon temperatures for all pools were 30.5 ‰ (range 28.7-34.5 ‰), 22.9 °C (range 17.2-26.5 °C) and 25.3 °C (range 18.6-28.7 °C) respectively. After an initial rise during Weeks 2 to 4, temperature tended to decline towards the end of the experiment (Fig. 10.1b). The concentration of dissolved oxygen was lower in the morning in pools receiving the higher feeding rate, regardless of whether pools were prepared or unprepared ($P<0.05$; Table 3). Concentrations of dissolved oxygen in the aftern were higher in the unprepared pools receiving the higher feeding rate, compared with those receiving the lower feeding rate, while the converse was true for the prepared pools (Table 3). Average pH values differed by only 0.3 pH units but pH was lower in the pools with the higher feeding rate in the morning and afternoon ($P<0.001$). For both prepared and unprepared pools, ammonia concentrations were much higher in the pools receiving the higher feeding rate than those receiving the lower feeding rate ($P<0.001$; Table 3). Concentrations of nitrate, nitrate and phosphorus were not significantly affected by preparation, feeding rate or their interaction ($P>0.05$; Table 3). The abundance of phytoplankton, as indicated by chlorophyll a concentration, was variable but was greater for pools receiving the lower feeding rate than the higher feeding rate than the higher feeding rate ($P<0.05$) and for both feeding rates, was greater in prepared pools ($P<0.01$; Table 3). There was a higher concentration of chlorophyll c (a pigment indicative of diatoms) in prepared pools ($P<0.05$) while chlorophyll b and pheophytin were not significantly affected by preparation, feeding rate or their interaction ($P>0.05$; Table 3).

Pond management variables

Total input of nutrients, nitrogen and phosphorus (feed and fertiliser combined) were highest in the prepared pools with the higher feeding rate ($P<0.001$) (Table 3). The requirement for water exchange (excluding partial drainage for a mortality count soon after stocking) was highest in prepared pools ($P<0.01$) but was not unaffected by feeding rate (Table 2).

Bacterial dynamics

Total bacterial carbon contents for the water column and sediment, calculated using average bacteria cell volumes, carbon content and specific gravity (see methods section) were 39 and 46 fg C $cell^{-1}$ respectively. The total numbers of bacteria in the water column and sediment for all pools for all treatments for all sampling periods ($n=45$) were $4.11 \times 10^6 \pm 0.19 \times 10^6$ ml^{-1} (range 2.27×10^6 - 8.74×10^6 ml^{-1}) and $8.39 \times 10^8 \pm 0.35 \times 10^8$ cm^{-3} (range 4.03×10^8 - 123.6×10^8) respectively. Average values for productivity were 0.03 ± 0.002 μg ml^{-1} day^{-1} (ranges 0.005-0.08 μg ml^{-1} day^{-1}) and 2.1 ± 0.2 μg C cm^{-3} day^{-1} (range 0.6-4.4 μg C cm^{-3} day^{-1}) for water column and sediment respectively.

When data for all sampling times were analysed pond preparation only significantly affected the total number of bacteria in the water column ($P<0.05$) while feeding rate did not affect any of the bacterial indicators ($P>0.05$) (Table 7). Sampling time affected the total number of bacteria in the water column ($P<0.05$) and total numbers, productivity and specific growth rate of bacteria in the sediment ($P<0.001$) (Table 4). Numbers of bacteria in the water column declined over time ($P<0.05$) (Table 5) while numbers, of bacteria in the sediment increased over time ($P<0.001$) (Table 5). Productivity of bacteria in the sediment was lower when measuring during Week 5 than when measured during Weeks 1 or 10 ($P<0.05$). Specific growth rate of bacteria in the sediment was highest when measured during Week 1 compared with measurements during Weeks 5 or 10 ($P<0.05$) was higher when measured during Week 10 than Week 5 ($P<0.05$).

There was a significant interaction between preparation and feed rate for specific growth rate of bacteria in the sediment ($P<0.05$) (Table 4). The significant interaction occurred because, although specific growth rates of bacteria in the sediment were higher in the higher feeding rate pools compared with the lower feeding rate pools for both prepared and unprepared pools, the difference was greater for unprepared pools compared with prepared pools. (Specific growth rate increased from 0.0025 to 0.0032 in unprepared pools and from 0.0021 to 0.0026 in prepared pools). This was the only significant two-way or three-way interactions for any of the indices of bacterial dynamics in the water column or sediment.

Meiobenthos and Macrobenthos

The meiobenthos in the sediment were grouped into five major categories; protozoans, nematodes, copepods, diatoms and 'others'; a category which included forams, ostracods, hydrozoans, bryozoans, amphipods and barnacles

(Figs 3a, 1b and 1c; Table 6). The number of nematodes and copepods found in the pools ($0.1x10^6$ - $1.7x10^6$ m^{-2} and $2.6x10^3$ - $237.2x10^3$ m^{-2} respectively; Figs 3a, b and c) were similar to those recorded during studies in large, commercially used penaeid farming ponds in Malayasia ($0.03x10^6$ - $1.4x10^6$ m^{-2} and $8.0x10^3$ - $978.0x10^3$ m^{-2} for nematodes and harpacticoid copepods respectively) (Moriarty et al., 1987).

At Week 1, all categories were more abundant ($P<0.05$) in the prepared pools except for diatoms which were found in similar numbers ($P>0.05$) in all pools (Fig. 3a; Table 7). Feeding rate had no effect ($P>0.05$) on any category except protozoans which were present in higher numbers ($P<0.05$) in the low feeding rate pools (Fig. 3a; Table 7). There was no interaction ($P>0.05$) between preparation and feeding rate on abundance of any category at Week 1.

At Week 5 the effects of preparation were less evident (Fig. 3b). Protozoans were the only category present in greater numbers ($P<0.01$) in the prepared pools, and nematodes were actually more abundant ($P<0.05$) in the unprepared pools (Fig. 3b; Table 7). Nematodes were also more abundant ($P<0.05$) in the low feeding rate pools although feeding rate did not affect ($P>0.05$) any other category. There was a significant interaction ($P<0.05$) between preparation and feeding rate on the abundance of only one category (protozoans) at Week 5.

At Week 10, although the abundance of protozoans in the unprepared pools had increased, they were still more abundant ($P<0.05$) in the prepared pools (Fig. 3c). Again, nematodes were more abundant ($P<0.001$) in the unprepared pools, although feeding rate had no effect ($P>0.05$) on their abundance. The only category which was affected by feeding rate was diatoms ($P<0.05$) which were more abundant in the low feeding rate pools. There was an interaction ($P<0.05$) between preparation and feeding rate for only one category (others) at Week 10 (Table 7).

When data from all sampling periods was analysed using three-factor ANOVA, neither preparation nor feeding rate affected abundance of any category of meiofauna, and time affected abundance of protozoa ($P<0.001$) and copepods ($P<0.01$) only. Protozoa and copepods were more abundant in Week 10 ($P<0.05$) (Fig. 3c). Two-factor interactions and the three-factor interaction were not significant ($P>0.05$) for any category of meiofauna.

Macrobenthos found in sediment samples taken during Week 10 were grouped into eight major categories; polychaetes, bivalves, copepods, amphipods, insect remains, algae, bryozoans and 'others', a category which included forams and barnacles (Table 6; Fig. 4). No category was found in all pools, or, with the exception of amphipods in the prepared, low feeding rate pools, in all ponds of any treatment combination. In general, amphipods and insect remains were more abundant in the prepared pools while polychaetes and copepods were more abundant in the unprepared pools (Fig. 4). Bivalves were not found in any pools fed at the high feeding rate. The large variation in the abundance of all the major categories of macrobenthos, both within and between treatments, precluded analysis of variance of these data.

Prawn stomach contents

Preparation, feeding rate and their interaction all had no effect ($P>0.05$) on the number of empty stomachs (expressed as a percentage of the total number of stomachs examined) (Table 8). Prawn stomach contents were grouped into six major categories; copepods, insect remains, algae, remains of prawn exuviae, artificial feed and others (a category which included forams, hydrozoans, bivalves, ostracods, barnacles and bryzoans) (Table 6). Preparation, feeding rate or their interaction all had no effect ($P>0.05$) on occurrence of any of these categories (Table 8). Despite their prevalence in the meiobenthos and macrobenthos, respectively, remains of nematodes and polychaetes were either not found (nematodes) or only found in one stomach (polychaetes) (Table 6). Similar results were recorded in previous work in model ponds (Allan and Maguire, in press). One of the major problems with stomach contents analyses is that only recently ingested material is easily identifable (Schroeder, 1983; Lilyestrom and Romare, 1987) and the importance of easily digested food items, especially those without any hard body parts like nematodes and sedentary polychaetes, are likely to be underestimated.

DISCUSSION

Pond preparation

Results from this study indicate that preparing (fertilising) ponds well in advance of stocking can increase prawn growth and production compared with that in unprepared ponds. Previous results indicating the beneficial effects of fertilisation in penaeid ponds have usually been for lower density culture (<10 prawns m^{-2}) (Rubright et al., 1981; Chakraborti et al., 1986; Garson et al., 1986; Lanari et al., 1989). where natural pond productivity migh be expected to make a greater contribution to the prawns nutritional requirement than in ponds stocked at higher densities (New, 1987).

The average results from bi-weekly sampling indicate that prawn growth increments in the prepared (fertilised) pools was greater than in unprepared pools at Week 2 and Week 4, although, after this time (at Weeks 6, 8 and 10), the average bi-weekly prawn growth increments were similar for all treatment combinations (Fig. 1a). Thus, pond preparation, as practiced here, was effective in increasing prawn growth during the first month of the experiment only. Rapid recruitment and colonization of the unprepared pools by meiobenthos, especially during the first five weeks (Fig. 3b), migh explain the similar prawn growth rates in prepared and unprepared pools after Week 4 (Fig. 1a).

In several other studies, where ponds were stocked at lower densities (<10 prawns m^{-2}), the natural food was capable of sustaining prawn growth for at least several weeks with little or no inputs of supplementary feed (Rubright et al., 1981; Lee and Schlesser, 1984; Wyban et al., 1987; Lanari et al., 1989). However, this may be related to stocking density and total biomass of prawns. Lanari et al. (1989) suggested that in fertilised ponds, natural productivity could sustain satisfactory

growth of *P. japonicus* stocked at 1.5 or 3.0 prawns m^{-2} for 50 or 40 days respectively. Similarly, Wyban et al. (1987) considered that manuring ponds stimulated the development of natural foods suitable for prawns up to a carrying capacity of about 1700 kg ha^{-1}.

During the present study, prawn growth in the prepared pools declined after Week 4 (Fig. 1a). This contrasts with some reports where prawn growth rates increase over time (Akiyama and Chang, 1990). In other studies a decline in growth over time, when total biomass of prawns increases, has been related to a reduction in the abundance of natural food organisms due to grazing by prawns (Rubright et al., 1981; Wyban et al., 1987; Gonzales, 1988; Lanari et al., 1989). In the study by Rubright et al. (1981) a decline in the abundance of several categories of meiofauna (including polychaetes, nematodes and copepods) associated with declining growth rates supported this hypothesis. Meiobenthos are actively preyed upon by prawns (Bell and Coull, 1978; Rubright et al., 1981; Das et al., 1982; Warwick, 1987; Moriarty et al., 1987; Gonzales, 1988). However, in the present study, although it was clear that the abundance of all categories of meiobenthos were greater in the prepared pools at Week 1 (Fig. 2a) there was no indication that abundance of any category of meiobenthos declined over time (Fig. 2b and c).

Other factors which might have explained the decline in growth in the prepared ponds over time are a lack of supplementary food or adverse water quality, e.g., low temperatures or sediment deterioration. However, although feed inputs, as a percentage of prawn biomass, were decreased over time (Table 1a), apparent food consumption was monitored twice daily and, as indicated by the deteriorating S values over time (Fig. 2), it is more likely that prawns were overfed than underfed. The major decrease in prawn growth in all treatments between Weeks 8 and 10 was probably related to water temperatures which declined by an average of more than 2.0 and 2.5 °C for mean minimum and mean maximum temperatures respectively (Fig. 1b). However, apart from decline in temperature there was no evidence that water quality deteriorated over time and, except for a problem with low DO on two occasions during the experiment, the concentrations of potentially toxic DO, ammonia and nitrite, were all well above (DO) or below those likely to affect growth (Wickins, 1976; Seidman and Lawrence, 1985; Allan et al., 1990; Allan and Maguire, 1991; Allan and Maguire, in press).

Sediment condition was not assessed quantitatively here although visual observation suggested that sediments were clearly reduced, especially in the high feeding rate pools, at the end of the experiment. Reduced sediments may have retarded prawn growth or affected meio- or macrobenthic communities. Hydrogen sulphide, produced by heterotrophic bacteria in anoxic sediments, is toxic to prawns at concentrations of only 0.1-4.0 mg l^{-1} (Tournier, 1972; Shigueno, 1975). In fish farming ponds, reduced sediments retarded grazing and consequently growth of tilapia (Avnimelech and Zohar, 1986). The effect of reduced sediments on prawn grazing has not been assessed, although the removal of anoxic sediment may prevent growth retarding conditions in intensive penaeid culture (Wyban and Sweeney, 1989).

During the present ten week study, 25-31 % of prawns examined at the end of the experiment had empty stomachs. This is considerably more than was recorded after a shorter, eight week experiment in the same facilities with the same species (7 % of prawns examined) (Allan and Maguire in press). This may be associated with a reduction in grazing over time as sediment condition deteriorates. Josefson and Widbom (1988) showed that meiofaunal communities were not affected by hypoxia (dissolved oxygen concentrations as low as 0.2 mg l^{-1} at the sediment - water interface) and meiofaunal communities did not decline over time during our study.

Feeding rates

Feeding rates had a significant but minor (4 %) effect on weight gain and no effect on final biomass or biomass gain. Consequently, S was much worse for pools managed with the higher feeding rate. At the lower feeding rate, preparation did not affect S (2.1±0.02 and 2.0±0.02 for prepared and unprepared pools respectively) while at the higher rate, S was worse in the unprepared pools (4.4±0.11) than in the prepared pools (3.8±0.1) (Table 2). This interaction between preparation and feed rate for S reflects the faster growth of prawns in the prepared pools as apparent food consumption was not affected by preparation (Table 2).

The deterioration in S for all treatments over time (Fig. 2) may have been due to a decline in the contribution of natural food items to the prawns nutrition or it may have reflected progressive overfeeding. Apart from studies with penaeid postlarvae and juveniles fed live earthworms, surprisingly little experimental work has been done to determine optimum feed rates for penaeid prawns in ponds (Sreekumaran Nair et al., 1982; Millamena, 1990). In aquaria, optimum feeding rates of 11 and 12 % of prawn biomass day^{-1} have been estimated for *P. monodon* and *P. merguiensis* respectively (Lee, 1971; Sedgwick, 1979). An experimental basis for the feeding regimes advocated in practical prawn farming manuals or reviews (Anon, 1984; Apud et al., 1985; Chiang and Liao, 1985; Clifford, 1985; Pascual, 1985; Robertson, 1988; Akiyama and Chwang, 1990) has not been established. Maguire and Leedow (1983) determined optimum feeding rates (using trout pellets with 39 % protein) for *Metapenaeus macleayi* during a seven week farming trial in netting enclosures with an earthen pond. Constant feeding rates, were used and the optimum rate was approximately 5 % of prawn biomass day^{-1}. This corresponds to the average food input used in the present study in higher feeding rate pools. Clearly, this rate was too high during the present experiment. The use of a higher feeding rate in tropical areas where water temperatures are closer to the optimum for growth of *P. monodon* (27-33 °C, Maguire and Allan, in press may result in better food conversion ratios than those recorded here. It should also be noted that the highest growth rates recorded here (0.17 g day^{-1}) are lower than some reported for *P. monodon* at lower stocking densities (0.23-0.39 g day^{-1}) (Liao, 1977; Apud et al., 1985; Chakraborti et al., 1986) but are similar to those reported in pond trials in NSW (0.17 g day^{-1}; Allan, 1990) and within range of those reported in pond trials in Taiwan (0.15-0.28 g day^{-1}; Chen et al., 1989).

Prawn farmers and research workers often use feed trays to indicate food consumption and as a guide for adjusting feeding rates (Apud et al., 1985; Chen et al., 1989). The presence of uneaten pellets on the trays in the present study was taken as an indication of overfeeding, and feeding rates were temporarily reduced. Even though the trays were supplied with an amount of feed that was proportional to the area of the pool covered by the tray (i.e., 6 %), they proved to be a very poor guide to food consumption. When pools were drained after the interruption to aeration during Week 9, uneaten pellets were observed on the bottom of many of the high feeding rate pools although no pellets were present on feeding trays. The very high S values recorded in the higher feeding rate pools indicate that overfeeding was a problem in these pools. Despite this, the feeding trays in these pools rarely indicated overfeeding. It was apparent that prawns were feeding preferentially from the trays, possibly using the trays on a refuge from reduced sediments.

Bacterial dynamics

Bacterial numbers were two orders of magnitude greater in the sediment than in the water column, and increased with time in the sediment. Water was regularly exchanged and bacterial numbers did not change with time in the water column. Bacterial productivity was also two orders of magnitude higher in the sediment compared with the water column. Moriarty (1986 a) also found bacterial numbers and production were two to three and one to two orders of magnitude respectively higher in the sediment than in the water column of penaeid prawn farming ponds in Malaysia (Table 9). However, the values for bacterial numbers for the present study were three to four times lower than those recorded by Moriarty (1986a) for the water column and ten to twelve times lower for sediments (Table 9). Similarly, productivities for both the water column and sediments were more than two orders of magnitude lower in the present study than Moriarty's values (Table 9). Bacterial numbers in the water column for present study were similar to those reported for coastal sediments or seagreass beds although productivities were higher (Table 9). In the sediment of pools numbers were lower than for other environments and productivities were similar to those for coastal sediments (Table 9).

In the Malaysian prawn ponds, feed was supplied in excess and, at the high temperatures which prevailed, the pellets were being rapidly degraded by bacteria (Moriarty, 1986a). In the present study, the low productivities and very low specific growth rates of bacteria in the pool sediment, and thus slow doubling times of 5 to 28 days indicate that the bacteria were limited by organic matter supply rather than by grazing by meiofauna. Doubling times in the water column of the pools were two to six days and productivity was also low. This indicated that although water column bacteria were not as limited as those in the sediment, by the supply of dissolved organic matter, growth was limited and numbers were being controlled, perhaps by the regular flushing of water from the pools.

In general these results indicate that little of the feed input was being degraded by bacteria, even though feed conversion ratios were high and, especially in the higher feeding rate pools, prawns were obviously overfed. It is possible that

meiofauna, and not microorganisms, were consuming more of the feed, however, this was not supported by any differences in abundance of meiofauna in the low and high feeding rate pools. Water quality was obviously not affected by bacterial activity in these pools, possibly due to the rapid response (i.e., water exchange) to reduced DO concentrations or elevated pH. Although organic matter was observed to build-up in the pools towards the end of the experiment, the low initial organic matter content of the sediment was probably low compared to commercial ponds and the relatively short duration of the experiment meant that the total amount of organic matter in the pools was probably considerably less than in commercial ponds. This may have contributed to the lower bacterial activity. Managing prawn farming ponds with low bacterial activity is desirable (Madenjian, 1990a) as rapid deterioration in water quality especially DO, is less likely.

Water quality

Excess feeding can result in a build-up of organic material, a reduction in DO following microbial decomposition and an increase in metabolic wastes (Boyd, 1982; Millanena, 1990). The deleterious effects of excess feeding on DO concentrations were apparent from the lower morning DO concentrations in the pools receiving the higher feeding rate (Table 2) and, more importantly, from the mass mortality of prawns in one pool receiving the higher feeding rate following the interruption to aeration during Week 1. Following the second interruption to aeration, during Week 9, DO concentrations were also lower ($P<0.05$) in pools receiving the higher feeding rate (average 1.8 mg oxygen l^{-1}) compared with the pools receiving the lower feeding rate (average 2.7 mg oxygen l^{-1}). The lower average morning and afternoon pH ($P<0.001$) in the high feeding rate pools followed the decomposition of large quantity of organic matter. A similar reduction in pH after feeding rates were increased was also recorded during studies with channel catfish (Boyd, 1982).

The pellets used in this study contained approximately 7.5 % nitrogen and 1.9 % phosphorus. Ammonia concentrations were much higher in the pools receiving the higher feeding rate. Millamena (1990) found that when more than 50 mg minced shrimp meat l^{-1} was added to post larval *P. monodon* tanks, DO declined as biological oxygen demand increased and metabolic waste products accumulated leading to a decline in prawn survival. Boyd (1982) recorded gradual increases in nitrate, total ammonia-nitrogen, organic nitrogen, filterable orthophosphate, total phosphorus, chemical oxygen demand, standard biological oxygen demand and chlorophyll a when feeding rates were increased in chemical catfish ponds.

During the study average chlorophyll a values were higher in prepared and compared with unprepared ponds lower in the high feeding rate pools than in the low feeding rate pools. The effect of feeding rates on chlorophyll a was also reflected in the significantly lower pH values recorded in low feeding rate pools. The result for chlorophyll a contrasts to Boyd's (1982) findings and indicates that the increased water exchange in the high feeding rate pools, compared with the low feeding rate pools, served to dilute the algal bloom as was noted in previous experiments (Allan and Maguire, unpublished data).

Conclusions

Preparation of ponds increases production of prawns in ponds stocked at 15 prawns m^{-2} although the benefits are noticable for a relatively short period only. The transfer of prawns into new, well prepared ponds during the culture cycle, as occurs where separate nursery ponds are used, could have commercial benefits. Such systems have been suggested for *P. monodon* culture at low densities (<5 prawns m^{-2}) (Tiro et al., 1986; Pudjiatro and Baliao, 1987). However, the commercial advantage of improved growth rates has to be balanced against the loss of production associated with ponds remaining unstocked during the pond preparation period. This may be significant when a two crops per year production schedule is planned (Hardman et al., in press).

When planning feeding strategies, growth rates as well as prawn biomass should be considered and increasing feeding rates, solely on the basis of observations from feed trays, is not recommended. Feeding rates should be related to estimated biomass and, especially during cooler periods, the use of an average feeding rate of approximately 2.5 % of prawn biomass day^{-1} for *P. monodon* stocked at 15 prawns m^{-2} may significantly lower feeding costs without affecting prawn production.

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REFERENCES

Allan, G. L., 1990. Results from commercial and experimental prawn farming ponds in New South Wales during 1988/89. In: J. Paynter and N. Preston (Editors), Proc. Fourth Ann. Conf. Aust. Mariculture Assoc., Brisbane, July 1989, pp. 51-57.

Allan, G. L. and Maguire, G. B., in press. Effects of stocking density on production of *Penaeus monodon* Fabricius in model ponds. Aquaculture.

Allan, G. L. and Maguire, G. B., in press. Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon*. Aquaculture.

Allan, G. L. and Maguire, G. B., 1988. Prawn farming research in model ponds. In: J. Paynter and N. Preston (Editors), Proc. Third Ann. Conf. Aust. Mariculture Assoc., Lismore, June 1988, pp. 88-96.

Allan, G. L. and Maguire, G. B., 1991. Lethal levels of low dissolved oxygen and effects of short term oxygen stress on subsequent growth of juvenile *Penaeus monodon*. Aquaculture, 94: 27-37.

Allan, G. L., Maguire, G. B. and Hopkins, S. J., 1990. Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved oxygen levels. Aquaculture, 91: 265-280.

Anderson, J. M., 1987. Production and decomposition in aquatic ecosystems and implications for aquaculture. In: D. J. W. Moriarty and R. S. V. Pullin (Editors), Detritus and Microbial Ecology in Aquaculture. ICLARM Conference Proceedings 14, International Centre for Living Aquatic Resources Management, Manila, Philippines, pp. 123-145.

Anderson, R. K., Parker, P. L. and Lawrence, A., 1987. A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp *Penaeus vannamei* in a pond growout system. J. World Aquaculture Soc., 18(3): 148-155.

Apud, F. D., Primavera, J. H. and Torres, P. L. (Jr)., 1985. Farming of prawns and shrimp. Aquaculture Extension Manual No. 5 (3rd Edition), Southeast Asian Fisheries Development Centre. Tigbauan, Iloilo, Philippines, 66 pp.

Anon., 1984. Pond culture. In: Prawn Industry Development in the Philippines. Proc. Natl. Prawn Industry Workshop, April 1984, Iloilo, Philippines, pp. 9-15.

Avnimelech, Y. and Zohar, G., 1986. The effect of local anaerobic conditions on growth retardation in aquaculture systems. Aquaculture, 58: 167-174.

Bratbak, G. and Dundas, I., 1984. Bacterial dry matter content and biomass estimations. Appl. Environ. Microbiol., 48(4): 755-757.

Bell, S.S. and Coull, B. C., 1978. Field evidence that shrimp predation regulates meiofauna. Oecologia (Berl.) 35: 141-148.

Boyd, C. E., 1982. Water Quality Management for Pond Fish Culture. Elsevier, Amsterdam, 318 pp.

Chakraborti, R. K, Halder, D. D., Das, N. K., Mandal, S. K. and Bhowmik, M. L., 1986. Growth of *Penaeus monodon* Fabricius under different environmental conditions. Aquaculture, 51: 189-194.

Chen, J. C., Liu, P. C. and Lin, Y. T., 1989. Culture of *Penaeus monodon* in an intensified system in Taiwan. Aquaculture, 77: 319-328.

Chiang, P. and Liao, I. C., 1985. The practice of grass prawn (*Penaeus monodon*) culture in Taiwan from 1968 to 1984. J. World Maricul. Soc., 16: 297-315.

Clifford, H. C., 1985. Semi-intensive shrimp farming. In: G. W. Chamberlain, M. G. Haby and R. J. Miget (Editors), Texas Shrimp Farming Manual - An Update on Current Technology. Texas A & M University System, Research and Extension Centre, Corpus Christi, TX. Chapter 4, pp. 15-43.

Coleman, J. A. and Edwards, P., 1987. Feeding pathways and environmental constraints in waste-fed aquaculture: Balance and optimization. In: D. J. W. Moriarty and R. S. V. Pullin (Editors), Detritus and Microbial Ecology in Aquaculture. ICLARM Conference Proceedings 14, International Centre for Living Aquatic Resources Management, Manila, Philippines, pp. 240-279.

Daley, R. J. and Hobbie, J. E., 1975. Direct counts of aquatic bacteria by a modified epifluorescence technique. Limnology and Oceanography, 20: 875-881.

Dal Pont, G., Hogan, M. and Newell, B., 1974. Laboratory techniques in marine chemistry. II. Determination of ammonia in seawater and the preservation of samples for nitrate analysis. Aust. CSIRO Div. Fish. Oceanogr. Rep., No. 55, CSIRO, Sydney, New South Wales, 11 pp.

Das, N. K., Ghosh, A. N. and Chakrabarti, N. M., 1982. Food of some penaeid prawns relative to the culture in brackish water ponds of Sunderbans. In: Proc. Symp. Coastal Aquaculture, Part 1., Cochin, India Jan, 1980, pp. 202-205.

Douglas, D. J., Novitsky, J. A. and Fournier, R. O., 1987. Microautoradigraphy - based enumeration of bacteria with estimates of thymidine - specific growth and production rates. Mar. Ecol. Prog. Ser., 36: 91-99.

Fallon, R. D., Newell, S. Y. and Hopkinso, C. S., 1983. Bacterial production in marine sediments: will cell - specific measures agree with whole - system metabolism? Mar. Ecol. Prog. Ser., 11: 119-127.

Folk, R. L., 1980. Petrology of Sedimentary Rocks. Hemiphill, Austin, TX, 184 pp.

Fry, J. C., 1987. Functional roles of the major groups of bacteria associated with detritus. In: D. J. W. Moriarty a R. S. V. Pullin (Editors), Detritus and Microbial Ecology in Aquaculture. ICLARM Conference Proceedings 14, International Centre for Living Aquatic Resources Management, Manila, Philippines, pp. 83-121.

Fry, J. C., 1988. Determination of biomass. In: B. Austin (Editor), Methods in Aquatic Bacteriology. John Wiley & Sons, 425 pp.

Fuhrman, J. A. and Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol., 66: 109-120.

Garson, G. I., Pretto, R. M. and Rouse, D. B., 1986. Effects of manures and pelleted feeds on survival, growth, and yield of *Penaeus stylirostris* and *Penaeus vannamei* in Panama. Aquaculture, 59: 45-52.

Gonzales, E. P., 1988. Contribution of natural food to the growth and survival of tiger prawn postlarvae/juveniles in brackishwater ponds. Unpublished MSc Thesis, Asian Institute of Technology, Bangkok, Thailand, 81 pp.

Hardman, J. R. P., Treadwell, R. and Maguire, G. B., 1990. Economics of prawn farming in Australia. In: (Editors), International Crustacean Conference, Brisbane, 2-6 July 1990, (in press).

Hyslop, E. J., 1980. Stomach contents analysis - a review of methods and their application. J. Fish Biol., 17: 411-429.

Jana, B. B., Patel, G. N., Roy, S. K. and De, U. K., 1980. Growth characteristics of the heterotrophic bacterial population of water and bottom sediments in tanks under different trophic conditions. Hydrobiologia, 75: 231-239.

Jeffrey, S. W., 1981. Phytoplankton ecology - with particular reference to the Australasian region. In: M. N. Clayton and R. J. King (Editors), Marine Biology: An Australasian Perspective. Longman Cheshire, Melbourne, pp. 241-291.

Jeffrey, S. W. and Vesk, M., 1981. The phytoplankton-systematics, morphology and ultra structure. In: M. N. Clayton and R. J. King (Editors), Marine Botany: An Australian Perspective. Longman Cheshire, Melbourne, pp. 138-179.

Josefson, A. B. and Widbom, B., 1988. Differential response of benthic macrofauna and meiofauna to hypoxia in the Gullmar Fjord basin. Mar. Biol., 100: 31-40.

Lanari, D., Ballestrazzi, R. and Tibaldi, E., 1989. Effects of fertilization and stocking rate on the performance of *Penaeus japonicus* (Bate) in pond culture. Aquaculture, 83: 269-279.

Lee, C. S. and Shlesser, R. A., 1984. Production of *Penaeus vannamei* in cattle manure-enriched ecosystems in Hawaii. J. World Maricult. Soc., 15: 52-60.

Lee, D. L., 1971. Studies on the protein utilisation related to the growth on *Penaeus monodon* Fabricius. Coll. Repr. Tungkang Mar. Lab., 1: 191-203.

Liao, I. C., 1977. A culture study on grass prawn, *Penaeus monodon*, in Taiwan - the patterns, the problems and the prospects. J. Fish. Soc. Taiwan, 5(2): 11-29.

Lilyestrom, C. G. and Romaire, R. P., 1987. Diet and food assimilation by channel catfish and Malaysian prawns in polyculture as determined by stomach content analysis and stable carbon isotope ratios. J. World Aquaculture Soc., 18(4): 278-288.

Madenjian, C. P., 1990. Patterns of oxygen production and consumption in intensively managed marine shrimp ponds. Aquaculture and Fisheries Management, 21: 407-417.

Maguire, G. B. and Allan, G. L., in press. Effects of temperature on growth, food consumption and food conversion for *Penaeus monodon, Penaeus plebejus* and *Metapenaeus macleayi*. In: G. L. Allan and W. Dall (Editors), Proc. Aquaculture Nutrition Workshop (ANW), Salamander Bay, April 1991, ANW, Salamander Bay, Australia.

Maguire, G. B. and Bell, J. D., 1981. The effects of fish on growth and survival of school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture, 24: 267-283.

Maguire, G. B. and Leedow, M. I., 1983. A study of the optimum stocking density and feeding rates for school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture, 30: 285-297.

Major, G. A., Dal Pont, G., Klye, J. and Newell, B., 1972. Laboratory techniques in marine chemistry. A manual. Aust. CSIRO Div. Fish. Oceanogr. Rep., No. 51, CSIRO, Sydney, New South Wales, 55 pp.

Millamena, O. M., 1990. Organic pollution resulting from excess feed and metabolic build-up: Effect on *Penaeus monodon* postlarvae. Aquaculture Engineering, 9: 143-150.

Moriarty, D. J. W., 1984. Measurements of bacterial growth rates in some marine systems using the incorporation of tritiated thymidine into DNA. In: J. E. Hobbie and P. J. LeB. Williams (Editors), Heterotrophic Activity in the Sea. Plenam Publishing Corporation, pp. 217-231.

Moriarty, D. J. W., 1986a. Bacterial productivity in ponds used for culture of penaeid prawns. Microb. Ecol., 12: 259-269.

Moriarty, D. J. W., 1986b. Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Adv. Microb. Ecol., 9: 245-292.

Moriarty, D. J. W., 1990. Techniques for estimating bacterial growth rates and production of biomass in aquatic environments. Methods in Microbiology, 22: 211-234.

Moriarty, D. J. W. and Pollard, P. C., 1981. DNA synthesis as a measure of bacterial productivity in seagrass sediments. Mar. Ecol. Prog. Ser., 5: 151-156.

Moriarty, D. J. W., Cook, H. L., Hassan, R. B. and Thanabal, M., 1987. Primary production and meiofauna in some penaeid prawn aquaculture ponds at Gelang Patah. Malay. Ag. J., 54: 37-51.

Nailon, R. W., 1985. Extensive grow-out system for shrimp. In: G. W. Chamberlain, M. G. Haby and R. J. Miget (Editors), Texas Shrimp Farming Manual: An Update on Current Technology. Texas A & M University Systems Research and Extention Centre, Corpus Christi, TX, Chapter 4, pp. 43-48.

New, M. B., 1987. Feed and feeding of fish and shrimp - a manual on the preparation and presentation of compound feeds for shrimp and fish in Aquaculture. Report ADCP/REP/87/26. United Nations Development Program Food and Agriculture Organisation of the United Nations, Rome, Italy, 275 pp.

Ordner, M. T. and Lawrence, A. L., 1987. Importance of polychaetes to penaeid pond culture. J. World Aquaculture Soc. (Aquaculture Communiques), 18(1): 36A-37A.

Parker, N. C., 1987. Feed conversion indices: Controversy or convention. Prog. Fish Cult., 49(3): 161-166.

Pascual, F. P., 1985. Nutrition and feeding of *Penaeus monodon*. Aquaculture Extension Manual No. 3 (Third Edition). Southeast Asian Fisheries Development Centre. Tigbauan, Iloilo, Philippines, 10 pp.

Pudjiatno and Baliao, D. D., 1987. Production of prawn *Penaeus monodon* Fabricius) using the modular pond system. Technical Report, SEAFDEC/FAO Network of Aquaculture Centres in Asia, Tigbauan, Iloilo, Philippines, 22 pp.

Reymond, H. and Lagardere, J. P., 1990. Feeding rhythms and food of *Penaeus japonicus* Bate (Crustacea, Penaeidae) in salt marsh ponds: Role of halophilic entomofauna. Aquaculture, 84: 125-143.

Robertson, C. H., 1988. Prawn farming in Queensland - Problems and progress so far. In: L. H. Evans and D. O'Sullivan (Editors), Proc. First Aust. Shellfish Aquaculture Conf. Perth Curtin Univ. of Technology, Perth, Australia, pp. 17-21.

Rosenberry, R., 1991. World Shrimp Farming 1990. Aquaculture Digest, San Diego, CA, 31 pp.

Rubright, J. S., 1978. An investigation into the role of meiofauna in the food chain of a shrimp mariculture pond system. Unpublished MSc Thesis, Texas A&M University, TX, 56 pp.

Rubright, J. S., Harrell, J. L., Holcomb, H. W. and Parker, J. C., 1981. Responses of planktonic and benthic communities to fertilizer and feed applications in shrimp mariculture ponds. J. World Maricul. Soc., 12(1): 281-299.

Schroeder, G. L., 1983. Stable isotope ratios as naturally occurring tracers in the aquaculture food web. Aquaculture, 30: 203-210.

Seidman, E. R. and Lawrence, A. L., 1985. Growth, feed digestibility, and proximate body composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. J. World Maricul. Soc., 16: 333-346.

Sreekumaran Nair, S. K., Krishna Iyer, H., Lalithambika Devi, C. B. and Krishnan Kutty, M., 1982. Studies on the growth of penaeid prawns: I. Length-weight relation and condition factor under different levels of feeding. Mahasagar, 15(2): 95-104.

Sreekumaran Nair, S. K., Krishna Iyer, H., Balasubramanian, T. and Krishnan Kutty, M., 1983. Studies on the growth of penaeid prawns: II Growth of *Penaeus indicus* under different levels of feeding. Magasahar, 16(1): 31-36.

Tiro, L. B., Pudadera, B. J., Potestas, I. O. and Kungvankij, P., 1986. Intensification techniques in the extensive culture of *Penaeus monodon*. In: J. L. Maclean, L. B. Dizon and L. V. Hosillos (Editors), The First Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines, pp. 93-95.

Warwick, R. M., 1987. Meiofauna: Their role in marine detrital systems. In: D. J. W. Moriarty and R. S. V. Pullin (Editors), Detritus and Microbial Ecology in Aquaculture. ICLARM Conference Proceedings 14, International Centre for Living Aquatic Resources Management, Manila, Philippines, pp. 282-292.

Wickins, J. F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9: 19-37.

Winer, B. J., 1971. Statistical Principles in Experimental Design. 2nd Edition. McGraw-Hill, New York, 907 pp.

Wyban, J. A. and Sweeney, J. N., 1989. Intensive shrimp growout trials in a round pond. Aquaculture, 76: 215-225.

Wyban, J. A., Lee, C. S., Sato, V. T., Sweeney, J. N. and Richards, W. K. Jr., 1987. Effect of stocking density on shrimp growth rates in manure-fertilized ponds. Aquaculture, 61: 23-32.

TABLE 1

Feeding rates used during the pond preparation/feeding rate experiment or recommended for commercial culture of *Penaeus monodon*.

	Average weight (g prawn^{-1})	Feeding rate (% of prawn biomass day^{-1})
Present study (high rate)	5-7	8
	7-10	7
	10-13	6
	13-16	5
	16-19	4
Present study (low rate)	5-7	4
	7-10	3.5
	10-13	3
	13-16	2.5
	16-19	2
Anon. (1984)	5-10	8
	10-15	6
	>15	4
Chiang and Liao (1985)	2-13	14-6
	13-30	6-4
	>30	4-3
Clifford (1985)	4	9.8
	8	6.4
	12	4.7
	16	3.8
	20	3.2
Pascual (1985)	1-10	8-10
	>10	3-5
Robertson (1988)	5-6	10
	6-8	8
	8-10	6
	10-12	4
	12-30	3
Akiyama and Chwang (1990)	3-15	4-2.5[1] 8-4[2]
	15-40	2.5-2[1] 4-2[2]
A commercial feed manufacturing company[3]	5-10	10-7
	10-20	7-5

1 For ponds with production of 0.8-2.5 t ha^{-1} crop^{-1}

2 For ponds with production of 2.5-20.0 t ha^{-1} crop^{-1}

3 A Taiwanese feed manufacturing company. Information printed on 20 kg bags of grower pellets purchased in 1988 and 1989

TABLE 2

Effects of pond preparation and two feeding rates on prawn performance indices in pools stocked with *Penaeus monodon* during a 71 day experiment[1].

	Unprepared(U)		Prepared(P)		Significance[2]		
Variable	Low feeding rate (L)	High feeding rate (H)	Low feeding rate (L)	High feed rate (H)	P	F	PxF
Prawn performance index							
Survival (%)[3]	92.7±1.1	88.2±1.5	89.0±1.4	89.3±1.8	ns	ns	ns
Weight gain (g prawn^{-1})	9.7±0.1	9.9±0.1	11.4±0.2	12.0±0.2	***	*	ns
Final biomass (g m^{-2})	198.7±1.2	196.7±5.9	218.5±2.1	226.4±6.5	***	ns	ns
Biomass gain (g m^{-2})	129.7±1.3	122.5±5.0	143.9±1.2	152.4±6.2	***	ns	ns
Apparent Food consumption (% of prawn biomass day^{-1})	2.5±0.03	5.0±0.1	2.6±0.1	5.0±0.03	ns	***	ns
S[4]	2.1±0.02	4.4±0.1	2.0±0.02	3.8±0.1	***	***	**

1 Values are means ± standard error (n=4 replicate pools for UL, UH and PL and n=3 replicate pools for pH). Salinity and minimum and maximum temperatures (mean [range] for all pools were 30.5 ‰ (28.7-34.5 ‰), 22.9 °C (17.2-26.5 °C) and 25.3 °C (18.6-28.7 °C) in that order

2 Results from two-factor ANOVA; P = preparation, F = feeding rate, P x F = preparation x feeding rate interaction; *** = P<0.001, ** = P<0.01, * = P<0.05, ns = not significant P>0.05

3 Data transformed (arcsine $x^{0.5}$) prior to statistical analysis

4 Data transformed (log x) prior to statistical analysis

TABLE 3

Effects of pond preparation and two feeding rates on pond water quality and pond management variables in pools stocked with *Penaeus monodon* during a 71 day experiment[1].

Variable	Unprepared(U)		Prepared(P)		Significance[2]		
	Low feeding rate (L)	High feeding rate (H)	Low feeding rate (L)	High feed rate (H)	P	F	PxF
Water quality variable							
DO morning (mg l^{-1})	6.3±0.1	5.8±0.1	6.2±0.04	5.7±0.1	ns	***	ns
DO afternoon (mg l^{-1})	10.1±0.1	10.5±0.1	10.8±0.3	10.2±0.1	ns	ns	*
pH morning	8.3±0.01	8.2±0.02	8.4±0.04	8.1±0.03	ns	***	*
pH afternoon	8.6±0.01	8.5±0.03	8.7±0.05	8.4±0.01	ns	***	*
Total ammonia-nitrogen (μg l^{-1})[3]	56.7±9.1	483.8±190.6	90.2±33.7	592.9±120.5	ns	***	ns
NO_2-N (μg l^{-1})	3.9±1.6	9.7±4.4	4.1±3.0	10.3±2.6	ns	ns	ns
NO_3-N (μg l^{-1})	1.5±0.1	1.1±0.1	1.4±0.1	1.3±0.04	ns	ns	ns
PO_4-P (μg l^{-1})[3]	97.8±13.8	208.6±58.9	170.4±6.4	168.8±7.6	ns	ns	ns
Chlorophyll a (μg l^{-1})	48.7±6.6	24.2±2.6	81.7±10.8	59.8±17.2	**	*	ns
Chlorophyll b (μg l^{-1})[3]	0.1±0.01	0.2±0.02	0.2±0.03	0.1±0.1	ns	ns	ns
Chlorophyll c (μg l^{-1})	24.5±5.5	10.8±2.6	46.5±12.3	25.6±9.7	*	ns	ns
Pheophytin (μg l^{-1})	13.5±3.2	15.1±8.1	26.4±7.8	18.1±2.2	ns	ns	ns
Pond management variable							
Total input of N (g m^{-2})	30.1±0.8	51.5±1.8	34.5±1.0	58.3±1.6	***	***	ns
Total input of P (g m^{-2})	8.6±0.3	14.5±0.6	9.9±0.3	16.6±0.5	***	***	ns
Water exchange (% d^{-1})	9.5±1.0	11.1±0.7	12.9±0.7	14.3±0.8	**	ns	ns

1 Values are means ± standard error (n=4 replicate pools for UL, UH and PL and n=3 replicate pools for pH). Salinity and minimum and maximum temperatures (mean [range] for all pools were 30.5 ‰ (28.7-34.5 ‰), 22.9 °C (17.2-26.5 °C) and 25.3 °C (18.6-28.7 °C) in that order

2 Results from two-factor ANOVA; P = preparation, F = feeding rate, P x F = preparation x feeding rate interaction; *** = P<0.001, ** = P<0.01, * = P<0.05, ns = not significant P>0.05

3 Data transformed (log x) prior to statistical analysis

4 Data were heterogenous after transformation

TABLE 4

Summary of three-factor analysis of variance results comparing the effects of pond preparation (P) feed rate (F), sampling time (T) and their interactions on bacterial dynamics in the water column and sediment of pools during a 71 day farming trial with *Penaeus monodon*[1].

	Treatment						
Index	P	F	T	PxF	PxT	FxT	PxFxT
Water column							
Total numbers	*	ns	*	ns	ns	ns	ns
Productivity	ns	ns	ns	ns	ns	ns	ns
Specific growth rate	ns	ns	ns	ns	ns	ns	ns
Sediment							
Total numbers	ns	ns	***	ns	ns	ns	ns
Productivity	ns	ns	***	ns	ns	ns	ns
Specific growth rates	ns	ns	***	*	ns	ns	ns

[1] *** = P<0.001; * = P<0.05; ns = not significant P>0.05

TABLE 5

Numbers, productivity and specific growth rates of bacteria in the water column and sediment of prepared and unprepared pools during a 71 day experiment with *Penaeus monodon* and receiving two different feeding rates[1].

	Treatment			
	UL	UH	PL	PH
Water column				
Week 1				
Number ($x10^9$ l^{-1})	5.8±1.1	4.5±0.7	4.4±0.6	4.1±0.9
Productivity ($x10^{-6}$ mg C ml^{-1} day^{-1})	16.6±3.9	25.1±5.6	44.9±15.6	15.9±5.1
Specific growth rate	0.003±0.001	0.006±0.001	0.013±0.006	0.005±0.003
Week 5				
Number ($x10^9$ l^{-1})	3.8±0.4	4.5±0.6	3.3±0.6	4.6±0.8
Productivity ($x10^{-6}$ mg C ml^{-1} day^{-1})	24.0±3.1	25.7±4.4	34.4±12.2	28.1±4.9
Specific growth rate	0.008±0.002	0.007±0.002	0.01±0.004	0.007±0.001
Week 10				
Number ($x10^9$ l^{-1})	3.6±0.1	4.4±0.1	2.9±0.4	3.2±0.03
Productivity ($x10^{-6}$ mg C ml^{-1} day^{-1})	30.2±6.5	27.3±8.5	28.8±3.9	31.9±6.3
Specific growth rate	0.009±0.002	0.007±0.002	0.01±0.002	0.01±0.002
Sediment				
Week 1				
Number ($x10^{12}$ m^{-2} cm^{-1})	5.8±0.3	4.9±0.3	8.5±1.7	5.1±0.5
Productivity ($x10^{-4}$ mg C cm^{-3} day^{-1})	26.9±2.7	33.3±2.5	22.6±6.7	21.2±4.5
Specific growth rate	0.004±0.0002	0.006±0.0001	0.003±0.001	0.004±0.0004
Week 5				
Number ($x10^{12}$ m^{-2} cm^{-1})	10.2±0.1	9.8±0.1	9.2±1.3	8.4±1.4
Productivity ($x10^{-4}$ mg C cm^{-3} day^{-1})	9.4±1.8	10.9±2.6	11.9±2.3	15.4±6.6
Specific growth rate	0.001±0.0002	0.001±0.0003	0.001±0.0003	0.002±0.002
Week 10				
Number ($x10^{12}$ m^{-2} cm^{-1})	8.8±0.4	10.0±0.4	10.1±1.0	9.4±0.5
Productivity ($x10^{-4}$ mg C cm^{-3} day^{-1})	25.1±8.5	28.7±5.9	25.4±5.0	24.9±4.5
Specific growth rate	0.003±0.001	0.003±0.001	0.002±0.001	0.002±0.003

[1] Values are means ± SE. U = unprepared, P = prepared; L = low feeding rate; H = high feeding rate. Treatment effects were not significant for any time period for either bacterial numbers or productivity ($P>0.05$) and there were no significant interactions between preparation and feeding rate ($P>0.05$).

TABLE 6

Major categories of benthos and their relative abundance in meiobenthos, macrobenthos and prawn stomach samples taken during or following a 71 day experiment with *Penaeus monodon* in pools[1].

	Relative abundance[2]		
	Meio-benthos	Macro-benthos	Prawn stomachs
Phylum Chlorophyta	N	A	C
Phylum Chryosphyta			
Class Bacillariophyceae	C	N	R
Super Phylum Protozoa			
Phylum Sarcodina			
Class Rhizopoda	A	R	C
Phylum Ciliophora			
Class Ciliatea	C	N	N
Phylum Cnideria			
Class Hydrozoa	R	N	R
Phylum Nematoda			
Class Adenophora	A	R	N
Phylum Mollusca			
Class Gastropoda	R	N	N
Class Bivalvia	R	C	C
Phylum Annelida			
Class Polychaeta	R	A	R
Phylum Arthropoda			
Class Ostracoda	A	N	R
Class Copepoda	A	N	A
Class Cirripedia	N	R	R
Class Malacostraca[3]	N	A	A
Class Insecta	N	R	C
Phylum Bryozoa			
Class Gymnolaemata	N	C	R

1 Organisms were usually only classified to class

2 N = none found, R = rare, C = common, A = abundant

3 Prawn exuviae were abundant in stomach contents and some prawn tissue was also found

TABLE 7

Summary of analysis of variance results comparing the effects of pond preparation (P), feed rate (F) and their interaction on the abundance of the five major categories of meiobenthos found in the sediment of pools during Weeks 1, 5 and 10 of a 71 day experiment with *Penaeus monodon*.

	Time of sampling[1]								
	Week 1			Week 5			Week 10		
Meiofauna Category	P	F	PXF	P	F	PXF	P	F	PXF
Protozoans	**	*	ns	**	ns	*	*	ns	ns
Nematodes	**	ns	ns	*	*	ns	***	ns	ns
Copepods	***	ns	ns	ns	ns	ns	ns	ns	ns
Diatoms	ns	ns	ns	ns	ns	ns	ns	*	ns
Others[2]	**	ns	ns	ns	ns	ns	ns	ns	*

1 P = preparation; F = feeding rate; PXF = interaction between preparation and feeding rate. *** = $P<0.001$; ** = $P<0.01$; * = $P<0.05$; ns = not significant $P>0.05$

2 Others include: Forams, ostracods, hydrozoans, bryozoans, amphipods and barnacles

TABLE 8

Number of stomachs examined, number of empty stomachs and occurence (%) of major food categories in the stomachs of prawns from each treatment combination in a 71 day experiment with *Penaeus monodon* in pools.

	Treatment Combination[1]			
	UL	UH	PL	PH
Number of stomachs examined	57	60	58	42
Number of empty stomachs	15	15	18	11
Occurence %[2]				
Copepods	14.2±7.7	26.7±8.6	8.7±1.9	2.4±2.4
Insect remains	5.1±1.7	11.7±3.2	8.7±3.3	2.4±2.4
Algae	23.3±6.6	15.0±6.9	8.6±3.3	26.2±9.5
Remains of prawn exuviae	35.3±9.0	30.0±4.3	16.8±7.9	45.2±16.7
Artificial feed	42.4±5.3	43.3±12.9	43.2±5.9	40.5±6.3
Others	8.7±5.3	0	8.7±5.3	9.5±4.8

1 U = unprepared, P = prepared, L = low feeding rate, H = high feeding rate.

2 Number of stomachs containing one or more individuals of each food category expressed as a percentage of the total number of stomachs examined (Hyslop, 1980). Values are means ± standard error (n=4 replicate pools for UL, UH and PL and n=3 replicate pools for PH). The effects of preparation, feeding rate or their interaction did not effect the number of prawns (expressed as a percentage) with empty stomachs or the occurrence of any category ($P>0.05$).

TABLE 9

Comparison of bacterial numbers and productivity in pools with some other marine environments

Site	Number[a] (l^{-1} or m^{-2} cm^{-1})	Productivity[a] (mg C m^{-3} day^{-1} or mg C m^{-2} day^{-1} cm^{-1})	Reference
Water			
Pools[b]	$2.3x10^9$ - $8.7x10^9$	5.4 - 79.0	Present study
Malaysian prawn ponds	$8.8x10^9$ - 2.6×10^{10}	888 - 2088	Moriarty, 1986a
Fertilised tank water	$44.9x10^7$ - $73.2x10^7$	-	Jana et al., 1980
Seagrass beds	$2.8x10^9$ - $6.8x10^9$	2.4 - 7.2	Moriarty and Rolland, 1981
Open ocean	$0.2x10^8$ - $5.8x10^8$	0.8-2.73	Douglas et al., 1987
Open ocean	$5x10^8$ - $2.5x10^9$	1.9 - 19.2	Fuhrman and Azam, 1980
Sediment			
Pools[b]	$4.0x10^{12}$ - $12.3x10^{12}$	6.2 - 44.0	Present study
Malaysian prawn ponds	$5.3x10^{13}$ - $2.1x10^{14}$	244 - 504	Moriarty, 1986a
Seagrass beds	$4.3x10^{13}$ - $1.7x10^{14}$	48 - 168	Moriarty and Rolland, 1981
Coastal sediments	$9.7x10^{12}$ - $4.1x10^{13}$	16.8 - 40.8	Fallon et al., 1983

[a] Units for water: l^{-1} and mg C m^{-3} day^{-1}. Units for sediment m^{-2} cm^{-1} and mg C m^{-2} day^{-1} cm^{-1}

[b] Range for all treatment combinations over all sampling periods (n=45)

Figure 10.1 Growth of *Penaeus monodon* (a) and average minimum and maximum water temperatures (b) in pools over two-week intervals of a 71 day experiment with different pond preparation and feeding rates. Values are means for n=4 replicate pools for UL, UH and PL and n=3 replicate pools for pH. Bars = standard error of the mean. W = week.

a) ■ = UL unprepared, low feeding rate

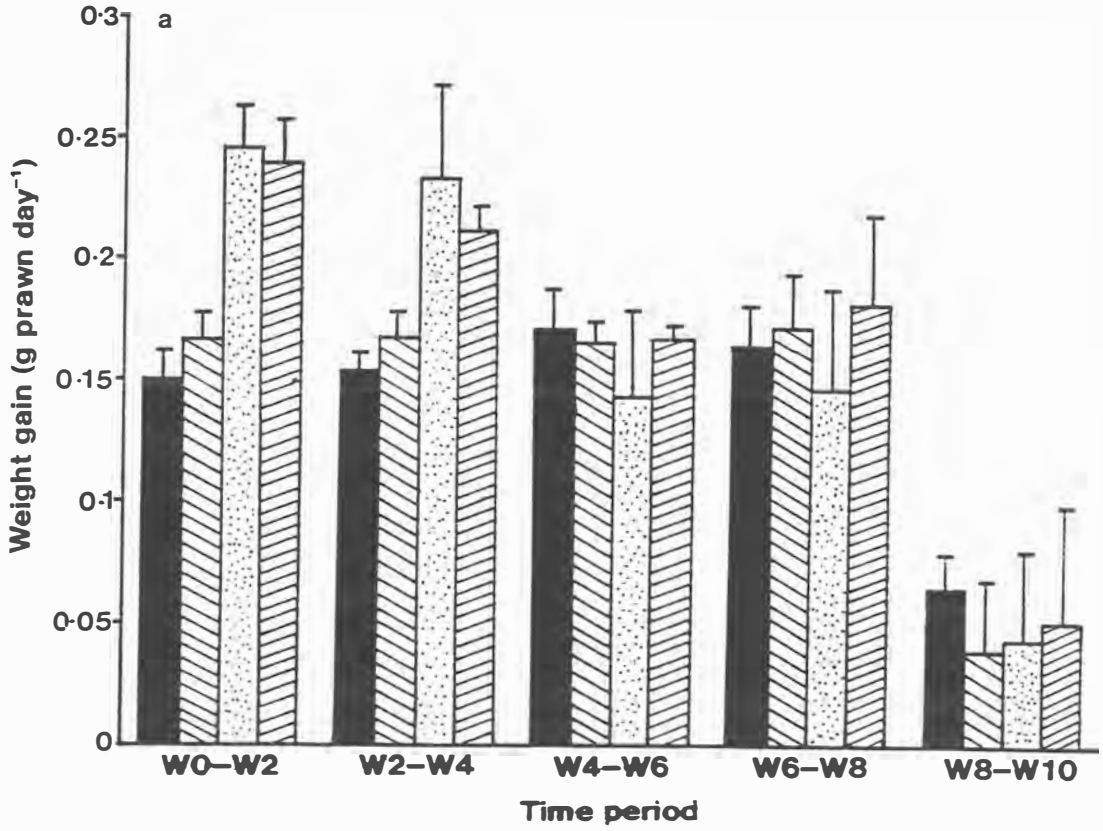
▧ = UH unprepared, high feeding rate

▨ = PL prepared, low feeding rate

▨ = PH prepared, high feeding rate

b) -▲- = average minimum water temperature

-●- = average maximum water temperature


a
0·3
0·25
0·2
0·15
0·1
0·05
0
Weight gain (g prawn day⁻¹)
W0–W2
W2–W4
W4–W6
W6–W8
W8–W10
Time period

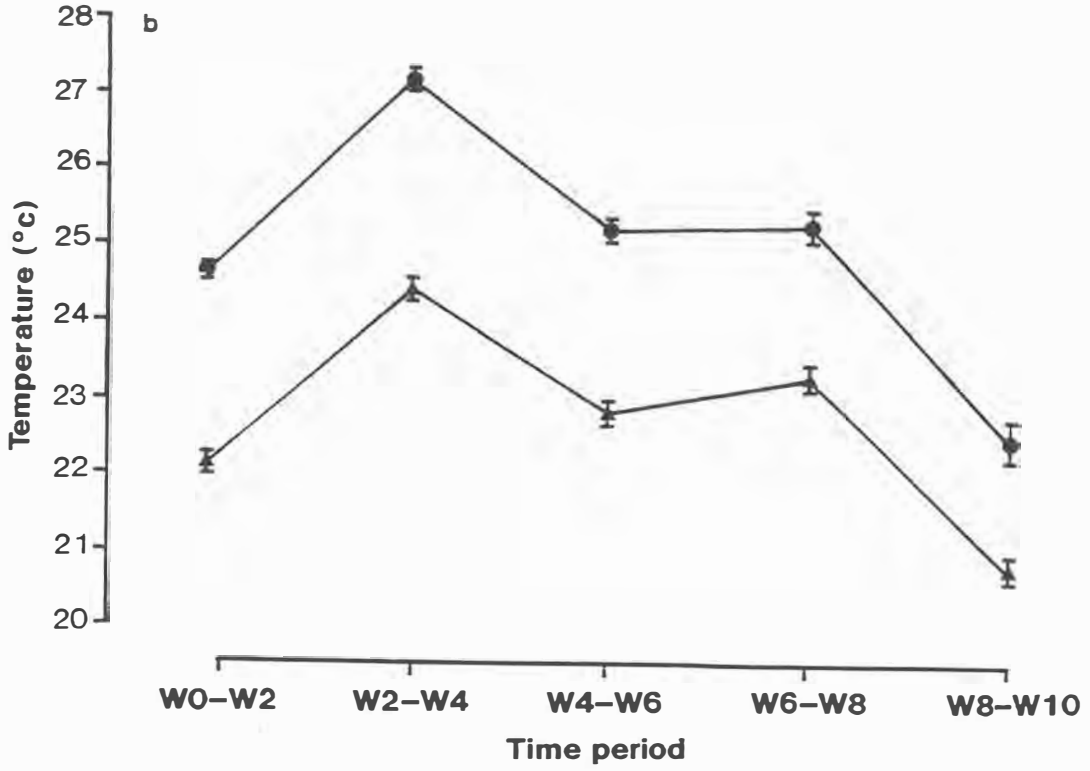

b
28
27
26
25
24
23
22
21
20
Temperature (°c)
W0–W2
W2–W4
W4–W6
W6–W8
W8–W10
Time period

Figure 10.2 Food conversion ratios (S) for *Penaeus monodon* in pools at two-week intervals of a 71 day experiment with different pond preparation and feeding rates. ● = unprepared pools with low feeding rates (UL); ■ = unprepared pools with high feeding rates (UH); ▲ = prepared pools with low feeding rates (PL); ⊙ = prepared pools with high feed rates. Symbols = means (n=4 replicate pools for UL, UH and PL and n=3 replicate pools for PH). Bars = standard errors.

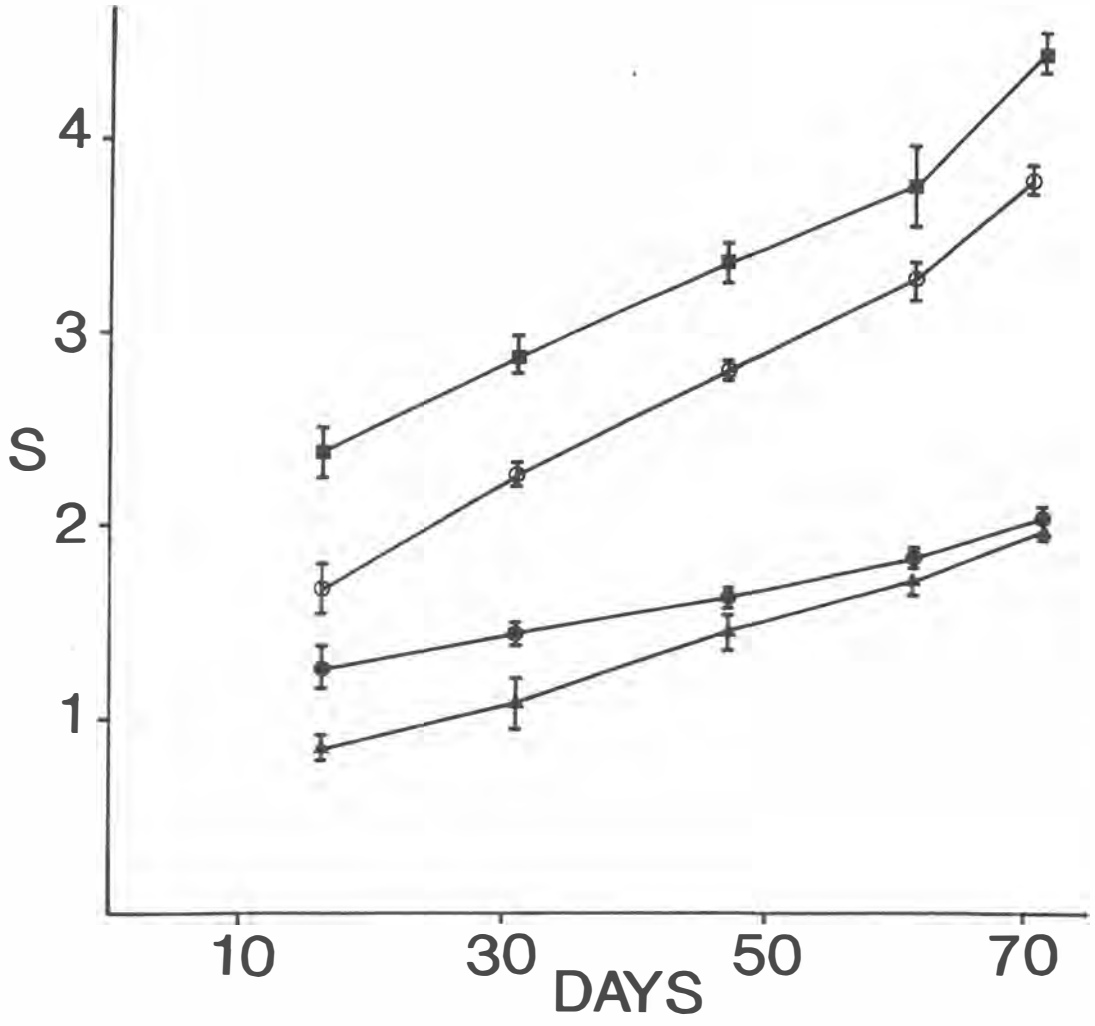

4
3
S
2
1
10
30
DAYS
50
70

Figure 10.3 Abundance of major categories of meiobenthos in pools stocked with *Penaeus monodon* during a 71 day experiment with different pond preparation and feeding rates. UL = unprepared pools with low feeding rates; UH = unprepared pools with high feeding rates; PL = prepared pools with low feeding rates; PH = prepared pools with high feeding rates. Values = (n=4 replicate pools for UL, UH and PL and N=3 replicate pools for PH). Bars = standard error. Where a standard error bar is broken the standard error of the mean is given. a = Week 1; b = Week 5; and c = Week 10.

■ = protozoans

▨ = nematodes

▩ = copepods

▧ = diatoms

▥ = others

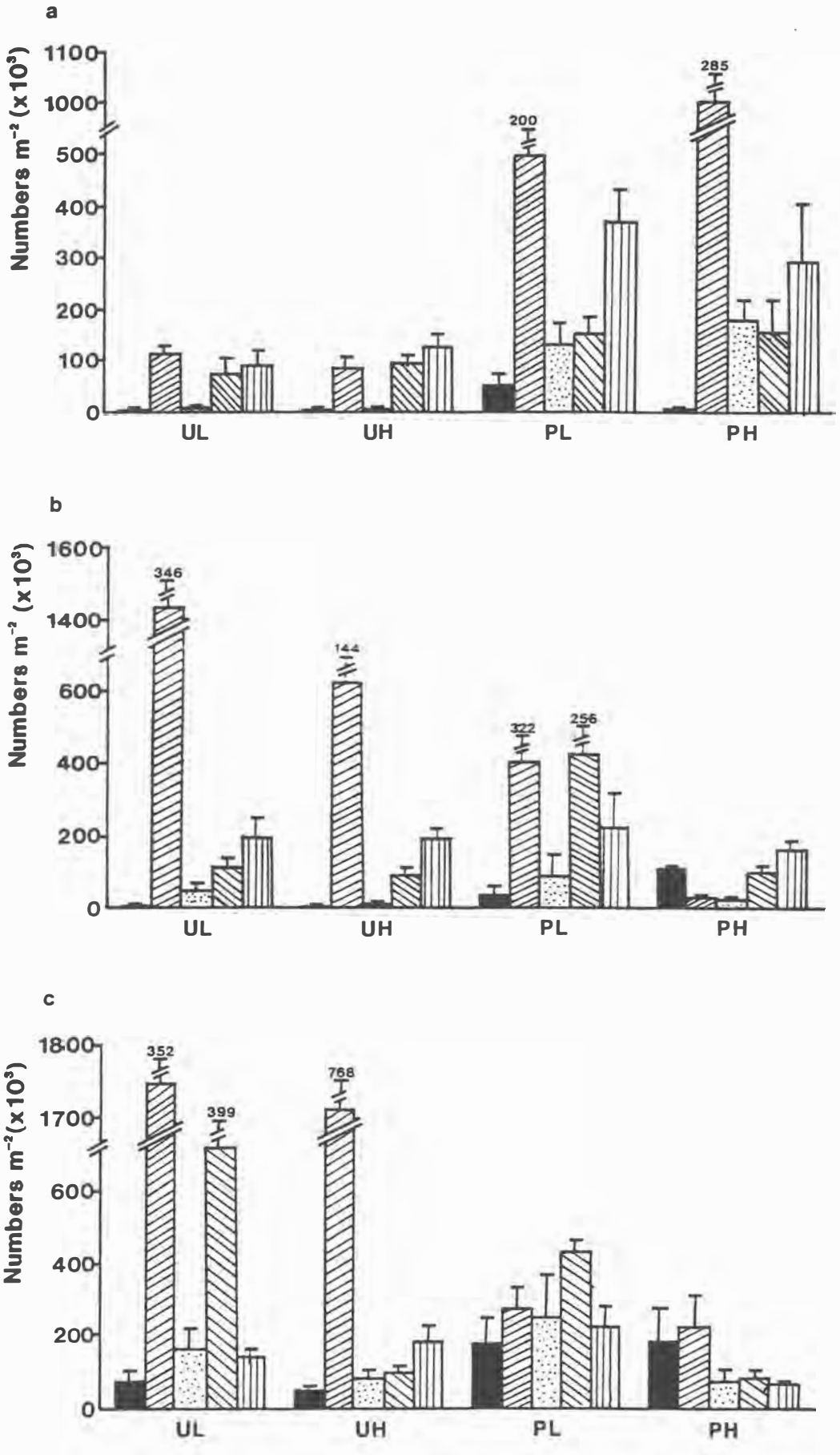

a
Numbers m⁻² (x10³)
1100
1000
500
400
300
200
100
0
200
285
UL
UH
PL
PH
b
Numbers m⁻² (x10³)
1600
1400
600
400
200
0
346
144
322
256
UL
UH
PL
PH
c
Numbers m⁻² (x10³)
1800
1700
600
400
200
0
352
399
768
UL
UH
PL
PH

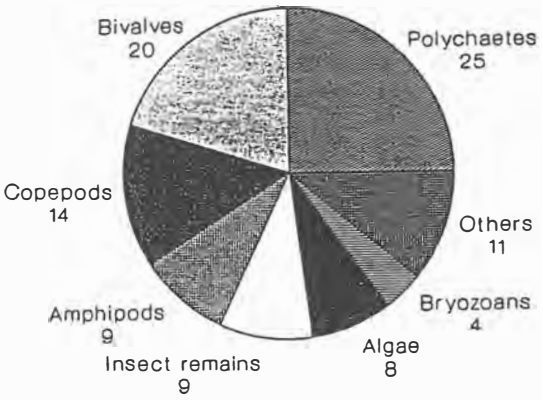
Figure 10.4 Abundance (%) of major categories of macrobenthos in pools with *Penaeus monodon* after a 71 day experiment with different pond preparation and feeding rates. Values are means (n=4 replicate pools for UL, UH and PL and n=3 replicate pools for PH).

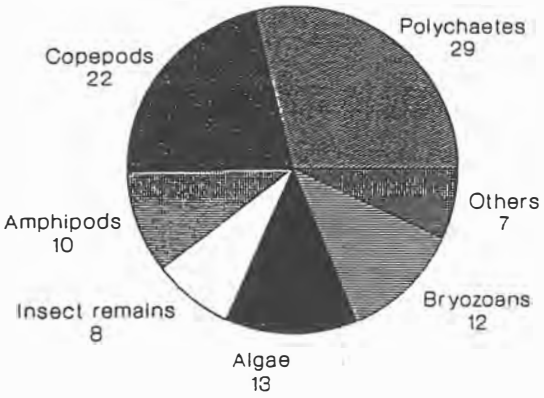
a = unprepared, low feeding rate (UL)

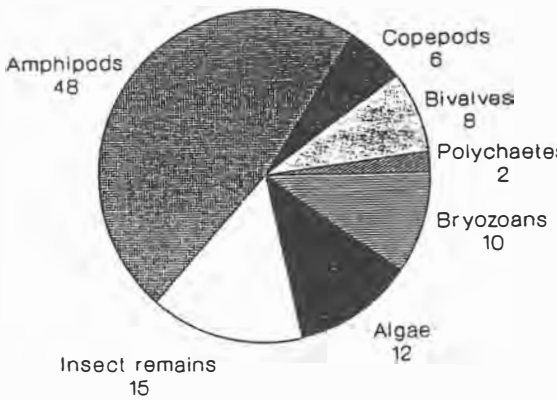
b = unprepared, high feeding rate (UH)

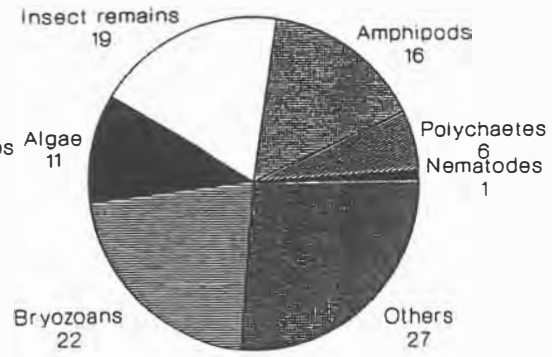
c = prepared, low feeding rate (PL)

d = prepared, high feeding rate (PH)


Bivalves
20
Polychaetes
25
Copepods
14
Others
11
Amphipods
9
Bryozoans
4
Insect remains
9
Algae
8


Copepods
22
Polychaetes
29
Others
7
Amphipods
10
Bryozoans
12
Insect remains
8
Algae
13


Amphipods
48
Copepods
6
Bivalves
8
Polychaetes
2
Bryozoans
10
Algae
12
Insect remains
15


Insect remains
19
Amphipods
16
Algae
11
Polychaetes
6
Nematodes
1
Bryozoans
22
Others
27

4 GENERAL DISCUSSION

The overall objective of this study was to improve prawn farming pond management. During a series of bioassays, conducted in aquaria, the acutely toxic (96 h LC_{50}) and growth reducing concentrations for a number of critical environmental variables were determined (Chapters 2.2-2.5). These concentrations provide a basis for managing water quality in prawn culture systems. During three separate longer-term experiments in 9-10 m^2 model ponds (pools) the effects of different fertilisation regimes, stocking densities, pond preparation and feeding rates on prawn production and water quality were evaluated (Chapters 3.2-3.4). The bioassay results were used to assess whether changes in water quality due to the use of different pond management regimes affected prawn growth or survival.

4.1 SPECIES

After a number of failed projects involving a range of species (Heasman, 1984), early commercial prawn farming in Australia was based on *M. macleayi*, as was much of the experimental prawn farming research in New South Wales (Maguire, 1980; Maguire and Allan, 1985), and this species was used in the early experiments here (Chapters 2.2 and 2.3). When these experiments were conducted, *P. monodon* juveniles were not available. However, as the commercial industry developed, hatcheries began producing *P. monodon* postlarvae and this species quickly dominated the infant prawn culture industry in Australia (Maguire and Allan, 1991). *P. monodon* accounts for 46 % of total aquaculture production from penaeids (Rosenberry, 1991) and this species was given priority for subsequent experiments. Although there is a lot of published information on *P. monodon* culture, there had been surprisingly little research on environmental requirements for juveniles and much of the pond management literature is orientated to extensive culture and/or is not applicable to Australian conditions and farming practices.

4.2 BIOASSAYS

4.2.1 Procedures

The acutely toxic and growth reducing concentrations of ammonia, DO and pH were assessed using procedures designed for aquatic pollutant toxicology (Sprague, 1969, 1970, 1971; APHA, 1971). The suitability of these methods for prawns had to be assessed before the major experiments could commence. Not feeding animals during acute bioassays and the avoidance of experimental conditions which might affect the toxicity of the test solution, were recommended (APHA, 1971). Sediment in aquaria may affect the concentration of ammonia by providing a substrate for nitrifying bacteria. However, as cannibalism among penaeids can be a problem, especially when sediment is not provided for burrowing (Subrahmanyam and Oppenheimer, 1969; Otazu-Abrill and Ceccaldi, 1981), or when prawns are not fed, the effects of absence of sediment on prawn mortality (and cannibalism) and growth had to be assessed. As aeration was essential for chronic bioassays where animals were being fed, the effects of aeration on ammonia concentrations also had to be determined.

Continuously-flowing water was advocated (Sprague, 1969; APHA, 1971) and was used for most bioassays. The first two experiments with *M. macleayi* (Chapter 2.2) were conducted before the facilities for continuous-flow were installed and static bioassays with daily water exchange were used. Accurate control of pH was not possible during continuous-flow bioassays, using the facilities available, and the experiments with *P. monodon* described in Chapter 2.5 were also conducted using static bioassays with daily water exchange. This provided the opportunity to compare growth of both species in continuous-flow and static bioassays. The average growth rate of *M. macleayi* in controls during the static bioassay described in Chapter 2.2 was 0.058 g day^{-1}, which was approximately 19 % slower than that recorded in the control aquaria during the continuous-flow bioassay described in Chapter 2.3 (0.071 g day^{-1}). Both experiments were conducted without sediment with similar temperature, salinity and food and feeding regimes. Differences in growth rates for the faster growing species, *P. monodon*, were even greater. The average growth rate for *P. monodon* in control aquaria during the static bioassay was 0.096 g day^{-1} (Chapter 2.5) which was 47 % and 39 % respectively slower than that recorded in control aquaria using continuous-flow bioassays (0.181 g day^{-1}; Chapter 2.3 and 0.157 g day^{-1}; Chapter 2.4). Wickins (1981) concluded that sensitivity to a pollutant was related to growth rate and that prawns growing maximally tended to show greater sensitivity. The use of continuous-flow rather than static bioassays is therefore preferable, at least for the two penaeid species studied here.

4.2.2 Acclimation

One of the limitations of standard toxicological bioassays is that critical concentrations, especially acutely toxic concentrations, are estimated following abrupt or rapid exposure of experimental animals to test solutions. The effects of increased tolerance following acclimation are generally not considered despite considerable evidence for crustaceans and fish that acclimation can significantly increase tolerance to adverse water quality variables (Shepard, 1955; Preston, 1985a, 1985b). The apparent tolerance of *P. pencillatus* to high ammonia concentrations described by Chen et al. (1988) may have been partly due to acclimation to gradually increasing ammonia concentrations.

This problem is not as great for chronic toxicity tests where responses to a range of concentrations are measured for a longer period (e.g., three weeks as used in the present study; see Chapters 2.3 and 2.5). Skidmore (1982) noted that chronic toxicity tests (of greater than seven days duration) to derive water quality criteria for toxic substances were preferred when framing regulations for northern hemisphere countries. Another method of deriving acutely toxic values is the use of experiments where concentrations of the test solution are changed gradually until a critical maxima (or minima) is reached (Kilgour and M^{c}Cauley, 1986). Clearly a standard technique is necessary to allow comparisons, e.g., between species and life stages, and to ensure results are reproducible. Critical values determined using gradual changes in the concentration of the water quality variable (to account for acclimation) would only be relevant to culture situations where that variable increased at a similar or slower rate. Critical values determined using these types

of experiments may overestimate tolerance under actual culture conditions.

4.2.3 Experimental design

All bioassays were designed with three, randomly allocated, replicate aquaria for each treatment. As most of the bioassays were used to generate response curves (Chapters 2.3, 2.4 and 2.5), it was considered preferable to have a large number of treatment levels, to cover as wide a range of concentrations as possible, and fewer replicates, rather than fewer treatment levels and more replicates.

The ability to detect differences between treatment means for prawn weight gain using the bioassay facilities with three replicate aquaria per treatment was estimated as described by Roberts (1983). This analysis required an estimation of the mean and standard deviation for experimentation error in aquarium experiments. During one experiment (Chapter 2.4), growth of *P. monodon* was not affected by treatment ($P>0.05$). The overall mean ± standard deviation for prawn weight gain (3.08 ± 0.39 g prawn^{-1}) for all aquaria in this experiment ($n=21$ aquaria) was calculated to provide the best available estimate of variation in the bioassay facility. Results showed that, using three replicate aquaria, a difference in prawn weight gain of 25 % could be detected at $P=0.05$, indicating that the experimental facilities and procedures were not particularly sensitive for separating treatment effects. However, eight replicates would have been necessary to detect a 15 % difference in prawn weight gain between treatments and it was not logistically possible to provide this many replicates for bioassays. Where two or more factors were being investigated (Chapters 2.2, 2.3 and 2.5), factorial designs were used as these are more powerful, especially where interactions between factors are not significant (Underwood, 1981).

4.2.4 Interaction between water quality variables

Critical concentrations for adverse water quality variables are very useful for prawn farmers or hatchery operators as they provide a basis for water quality management decisions. This is especially useful where only one variable is affected, e.g., through an aeration system failure. However, when water quality deteriorates, several variables often interact. For example, in ponds when algal blooms crash and large quantities of organic material are deposited on the pond bottom and are subsequently decomposed by microbial action, rapid reductions in DO and pH can occur simultaneously, as can the liberation of large quantities of ammonia and the production of other toxic substances such as H_2S (Boyd, 1982). It is therefore also important to consider the interaction of water quality variables at potentially adverse concentrations.

The increased toxicity of ammonia to *P. monodon* when DO concentrations were reduced (Chapter 2.3), demonstrates a synergistic relationship between these two water quality variables. A DO concentration of 2.2 mg l^{-1} did not reduce the growth of *P. monodon* (Seidman and Lawrence, 1985). However, when combined with ammonia concentrations of 1.60-1.63 mg TAN l^{-1}, mortality of *P. monodon* was 90.0 % compared with only 33.3 % in aquaria with similar ammonia concentrations but

higher DO (5.7 mg l^{-1}) (Chapter 2.3). The interactive effects of salinity and pH were also demonstrated in Chapter 2.5 where a reduction in pH from 7.8 to 5.5 was less growth-inhibiting at a lower salinity (15 ‰) than at a higher salinity (30 ‰).

Factorial experiments which assess several variables together provide toxicity information which can be more relevant to farming situations. For example, the acutely toxic effect of ammonia was reduced for *M. macleayi* when sediment was provided (Chapter 2.2), indicating that critical concentrations of ammonia determined in typical toxicity bioassays without sediment (Chapter 2.3; Wickins, 1976b; Chin and Chen, 1987; Jayasanker and Muthu, 1983a) will not necessarily extrapolate directly to culture situations. Fortunately, critical concentrations of ammonia determined by toxicity bioassays are likely to be conservative estimates and still have much application in the study of cultured and wild penaeids.

Interaction between environmental and water quality variables can also make interpretation of experimental results difficult. For example, in a study to assess the use of steel-making waste slags as substrates for *P. monodon*, Chien et al. (1989) concluded that prawn growth was reduced when no additional substrate was placed in aquaria. However, during that study water exchange was infrequent and ammonia concentrations were higher in aquaria without substrates, presumably due to less surface area for nitrifying bacteria in these aquaria. In their aquaria without substrate, ammonia concentrations may have reached growth reducing concentrations (Chapter 2.3) and contributed to an erroneous interpretation of results.

4.2.5 Applicability of bioassay results

During the course of the present study, results of research which determined the effect of reduced concentrations of DO on growth of *P. monodon* were published (Seidman and Lawrence, 1985). Rather than repeat this research, which was conducted using comparable methods to those used here, an experiment to assess the effects of short-term oxygen stress on subsequent growth was conducted. Prawn farmers had often asked whether prawns that survived an oxygen crisis in a pond would subsequently exhibit impaired growth or increased mortality. Results (Chapter 2.4) demonstrated that provided mass mortality was avoided, subsequent mortality and growth were unaffected by a single, major (0.5 or 1.0 mg oxygen l^{-1}), short-term (4-12 h), DO stress. These results have obvious implications for the planning of feeding and harvesting schedules. The applicability of the oxygen-stress experiment, which was conducted in aquaria, to large-scale culture facilities was demonstrated during the experiment described in Chapter 3.4. Here a short-term reduction in DO occurred in one pool (4-8 h; 0.6 mg l^{-1}) during the last week of the experiment. On the basis of the results from the oxygen-stress experiment (Chapter 2.4), a decision was made not to harvest this pool early. Ultimately, survival in this pool was high (86.0 %) and within the range for the other pools (84.7-95.3 %). Growth of prawns in this pool during the two week period when the crisis occurred was also similar to that recorded in the other pools subject to the same management regime.

4.3 POND MANAGEMENT EXPERIMENTS

4.3.1 Procedures

During pool experiments the effects of manipulating major pond management variables (fertilisation methods and stocking and feeding rates) on prawn production, water quality and natural food resources were used. An essential first stage in the pond management research was to develop an appropriate experimental facility.

For model pond experiments (Chapters 3.2-3.4), a set of fast draining, fiberglass pools was used. Prawn survival, growth and food conversion rates for *P. monodon* in the new pools were comparable with commercial results from NSW (Allan, 1989) and Taiwan (Chen et al., 1989) (Chapter 3.2). As only 16 pools were available it was also important to determine the variability between replicate pools and hence the minimum number required per treatment. Using the weight gain data from the phytoplankton and benthic pools (n=12) (Chapter 3.2) a mean and standard deviation (11.95 ± 0.76 g prawn^{-1}) were calculated. Using four replicate pools, a difference in weight gain of between 10 and 15 % could be detected at a probability level of P=0.05.

Later experiments were therefore conducted with four replicate pools per treatment (Chapter 3.3) or treatment combination (Chapter 3.4). The validity of this estimation was demonstrated during the stocking density experiment (Chapter 3.3). A difference in average weight gain of 9.3 % was not significant (P>0.05) but a difference of 14.7 % was significant (P<0.05). For the final experiment (Chapter 3.4) a more powerful factorial design (Underwood, 1981) was used to determine the interactive effects of pond preparation and feeding rate. Here the interaction between the two factors was not significant (P>0.05) and the effect of feeding rate on prawn weight gain was significant (P<0.05) with only 4 % difference in weight gain between prawns fed at the high and low feeding rate.

4.3.2 Water quality management

During all pool experiments (Chapters 3.2-3.4), pools were managed as individual units. For each pool, water exchange and aeration were used to maintain water quality variables above or below the growth reducing concentrations identified during the water quality bioassays. Following results from Chapter 3.2, water exchange and fertilisation were also used to stimulate and maintain phytoplankton blooms. Measurements of DO, pH and/or chlorophyll a concentration were used to indicate the intensity of phytoplankton blooms and when bloom 'crashes' were imminent. Managing pools within experiments as individual units is similar to the approach adopted by commercial prawn farmers and enabled an estimation of the effects of different management practices on water quality control procedures (i.e., aeration, water exchange and fertilisation. However, it was not always possible to maintain optimum water quality and when it did deteriorate, the results from the water quality bioassays helped assess whether the changes in water quality variables caused by different pond management practices were likely to affect

prawn survival or growth.

4.3.3 Natural food

Results from the literature emphasised the importance of natural food in the diet of pond cultured penaeids (Anderson, et al., 1987; Rubright et al., 1981). During the present study, the best growth results were recorded in pools which had been prepared for the longest period of time (Chapter 3.2). Even for prawns fed to excess, increasing the period of pond preparation from two-three days to one month, was associated with an increase in individual prawn weight gain of 21 % (Chapter 3.4).

During the experiment to determine optimum stocking density (Chapter 3.3), prawn growth declined as density increased. On the basis of bioassay results, reductions in growth could not be explained by water quality. Previous researchers had suggested that reduced growth following an increase in prawn biomass was due to reduced abundance of natural food items following increased grazing pressure (Hanson and Goodwin, 1977; Rubright et al., 1981; Maguire and Leedow, 1983; Ordner and Lawrence, 1987). However, this hypothesis was not supported by results from the present study. During the stocking density experiment, there was a reduction in macrobenthos, but surprisingly, meiobenthos was more abundant in pools stocked at higher densities. This suggested that factors other than the availability of natural food were affecting prawn growth. Reduced feeding activity, including a reduction in the consumption of supplementary pellets, occurred during the stocking density experiment. Similarly, although growth was reduced over time in the pond preparation and feeding rate experiment (Chapter 3.4), there was no indication that abundance of meiobenthos declined over time. The unexpected decline in growth over time during the experiments described in Chapters 3.3 and 3.4 may be associated with the buildup in reduced sediments over time in the same way that grazing activity of *Tilapia* in fish ponds declined as sediments became chemically reduced during farming trials (Avnimelech and Zohar, 1986). The higher number of prawns with empty stomachs after the longer experiment (10 weeks) described in Chapter 3.4 (25-31 % of prawns examined) compared with the eight week experiment described in Chapter 3.3 (7 % of prawns examined) may be associated with a reduction in grazing over time as sediment condition deteriorated.

4.4 FUTURE RESEARCH

There are a number of other water quality variables for which critical concentrations were not determined during this study and which could limit production of penaeids. These include nitrite, nitrate and hydrogen sulphide. Nitrite and nitrate concentrations recorded during early farming trials (Maguire and Allan, 1985) and pond management experiments (Chapters 3.2, 3.3 and 3.4) were much lower than critical values reported for juvenile penaeids (Wickins, 1976b; Chen et al., 1990), and further research on these variables is not considered a priority. However, very little information is available on the concentrations of hydrogen sulphide in prawn culture environments although prawns are susceptible to low levels (Shigueno,

1975; Tsai, 1990). The estimation of critical concentrations for this variable was beyond the scope of the present study, although it is a priority for future research. The effect of hydrogen sulphide, as well as other factors associated with reduced sediments, on prawn grazing and feeding should also be investigated.

The use of feeding rates which were approximately half those recommended in practical prawn farming manuals or by commercial prawn feed manufacturers did not result in any significant differences in prawn yield and only very minor differences in prawn weight gain (Chapter 3.4). Major improvements in food conversion efficiency and consequently a reduction in feed costs were however, apparent. There is a major need to experimentally determine optimum feeding rates for different size prawns, in both sub- and tropical conditions.

4.5 SUMMARY

The bioassay section of this study provides, for the first time, estimations of lethal and growth reducing concentrations for the major production-limiting water quality variables for *P. monodon*. These critical concentrations were assessed using comparable experimental facilities and procedures. Unlike the majority of previous studies, continuous-flow rather than static bioassays were generally used and growth reducing concentrations were established following growth experiments rather than using estimates of acutely lethal concentrations and an 'application' factor. The interactive effects of a number of variables were also assessed which provided relevant information for actual culture situations.

Prawn farming ponds are complex ecosystems which farmers attempt to manage by stimulating and maintaining rapid prawn growth. This study quantified the effects of some of the major management strategies, not only on prawn survival and growth but also on other aspects of the pond ecosystem including bacteria, meio- and macrobenthos, algae and water quality. The effects of the pond management strategies examined on prawn growth and aspects of the pond ecosystem are summarised in Table 4.1. All the management strategies assessed affected chlorophyll a concentrations and many affected other water quality variables. The effects of management strategies on these variables would have been even more pronounced if each model pond had not been managed on an individual basis to sustain algal blooms and maintain water quality.

A useful experimental system for pond trials was developed to assess the effects of major pond management strategies on survival, growth and food conversion efficiency for prawns, as well as many other aspects of the pond ecosystem.

The results and recommendations in this report should allow prawn farmers to manage their ponds on a less empirical basis, reduce the likelihood of water quality crises and help farmers to deal with them when they do occur and ultimately increase the production of cultured prawns.

TABLE 4.1

A summary of the relationships between pond management strategies and aspects of the prawn farming pond ecosystem[1]

Management strategy	Variable								
	Bacteria	Algae[2]	Meio-benthos	Macro-benthos	Prawns[3]	DO	pH	NH_3	PO_4
Water exchange rate[4]	na	*	na	na	ns[5]	*[6]	ns	ns	*
Fertilisation regime[7]	na	*	na	na	ns[8]	ns	*	*	*
Stocking density[9]	na	*	[10]	[10]	*	*	ns	ns[11]	ns
Pond preparation[12]	*[13]	*	*[14]	ns	*	ns	ns	ns	ns
Feed rate[12]	ns	*	*[14]	ns	*	*	*	*	ns

1 na = not assessed; ns = not significant ($P>0.05$); * = significant relationship ($P<0.05$)
2 Phytoplankton, as indicated by chlorophyll a concentration
3 Prawn weight gain
4 See Chapter 3.1
5 Mass mortality occurred in one pool where no water was exchanged
6 DO was affected by water exchange rate when stocking density was 47 prawns m^{-2} but not when 20 prawns m^{-2}
7 See Chapter 3.2
8 Prawn growth was not affected by fertilisation regime but was reduced ($P<0.05$) in ponds with benthic algae mats where filamentous algae blooms also developed
9 See Chapter 3.3
10 Treatment effects were obvious even though the data were not statistically analysed
11 Average NH_3 concentrations increased with stocking density but data were too variable to statistically detect differences ($P>0.05$)
12 See Chapter 3.4
13 Total numbers and productivity of bacteria in the water column were affected by preparation. Bacteria in the sediment were unaffected ($P>0.05$)
14 Some categories only

5 SUMMARY

Acutely toxic and growth-reducing concentrations of ammonia, dissolved oxygen (DO) and pH were determined for juvenile penaeids in 70 l aquaria. These results were then used during 8-12 week experiments where the effects of different water exchange rates, fertilisation regimes, stocking densities, pond preparation periods and feeding rates on prawn production indices and water quality were assessed.

For ammonia, the 96 h LC_{50} values were 1.69 and 1.39 mg un-ionised ammonia-nitrogen (UAN) l^{-1} for *Penaeus monodon* Fabricius and *Metapenaeus macleayi* (Haswell) respectively. Low DO for 96 h increased the acute toxicity of ammonia to *P. monodon*. Provision of a sediment layer reduced the acute toxicity of ammonia to *M. macleayi*. ' Maximum acceptable' concentrations, that reduced growth only by 5 % (EC_5 values) over three weeks, were 0.21 mg UAN l^{-1} for *P. monodon* and 0.35 mg UAN
l^{-1} for *M. macleayi*.

For DO, the 96 h LC_{50} to *P. monodon* was 0.9 mg 0_2 l^{-1}. Short-term, severe DO stresses did not reduce subsequent growth of *P. monodon*. For pH, the 96 h LC_{50} to *P. monodon* was 3.7 pH. The EC_5 for pH was 5.9 at 30 ‰ salinity. Interactive effects of pH and salinity on growth, moulting frequency and osmoregulation are described.

In pools (9-10 m^2) used as model prawn farming ponds, increasing regular water exchange rates from 0 to 20 or 40 % day^{-1} did not increase survival or growth of *M. macleayi*, however, nutrient and phytoplankton concentrations were reduced.

For *P. monodon*, weight gain decreased exponentially with increased stocking density in the range 5 to 40 prawns m^{-2}, whereas biomass gain and food conversion ratio increased and feeding activity and food conversion efficiency decreased. A longer period of pond preparation resulted in a 20 % increase in growth for *P. monodon*. Results indicated that a 50 % reduction in commercially used feeding rates can be achieved without affecting production of *P. monodon* in sub-tropical conditions. The dynamics of bacteria, algae, meio- and macrofauna and prawn feeding behaviour, in relation to pond management practices, are discussed.

These results provide a basis for objective farm pond management. They should assist farmers to manage prawn farming ponds on a less empirical basis, reduce the likelihood of water quality crisis and increase pond production.

6 TRANSFER OF RESULTS TO INDUSTRY

6.1 SCIENTIFIC PUBLICATIONS

a Effect of sediment on growth and acute ammonia toxicity for the school prawn *Metapenaeus macleayi* (Haswell).

Authors: G L Allan and G B Maguire.

Publication details: For submission to Aquaculture.

b Acute and chronic toxicity of ammonia to juvenile *Metapenaeus macleayi* and *Penaeus monodon* and the influence of low dissolved-oxygen levels.

Authors: G L Allan, G B Maguire and S J Hopkins, 1990.

Publication Details: Published in Aquaculture, 91: 265-280.

c Lethal levels of low dissolved oxygen and effects of short-term oxygen stress on subsequent growth of juvenile *Penaeus monodon*.

Authors: G L Allan and G B Maguire, 1991.

Publication details: Published in Aquaculture, 94: 27-37.

d Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon*.

Authors: G L Allan and G B Maguire.

Publication details: Accepted for publication in Aquaculture.

e The use of model prawn farming ponds to evaluate fertilisation strategies for pond culture of *Penaeus monodon*.

Authors: G L Allan and G B Maguire.

Publication details: Submitted to J Aquaculture and Fisheries Management.

f Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds.

Authors: G L Allan and G B Maguire.

Publication details: Accepted for publication in Aquaculture.

g The effects of food preparation and feeding rate on *Penaeus monodon* Fabricius production, pond water quality and the dynamics of bacteria and benthos in model prawn farming ponds.

Authors: G L Allan and G B Maguire.

Publication details: For submission to Aquaculture.

6.2 CONFERENCE PROCEEDINGS

a Pond management - Environment requirements. (Appendix 1.)

Authors: G L Allan, 1987.

Publication details: Summary papers of First Australian Prawn Farming Research Workshop, Brackish Water Fish Culture Research Station (BWFCRS) 17-18 August, 1987 - BWFCRS, Salamander Bay, pp. 18-21.

b Water quality and grow-out pond management. (Appendix 2a.)

Authors: G L Allan, 1987.

Publication details: In: G. B. Maguire (Editor), Proc. Prawn Farming Workshop, North Coast Agricultural Institute, Wollongbar, 11 December 1987. Brackish Water Fish Culture Research Station, Salamander Bay, pp. 40-48.

c Summary of results of prawn farming research at the Brackish Water Fish Culture Research Station, Salamander Bay, 1986-1987. (Appendix 2b.)

Authors: G B Maguire and G L Allan, 1987.

Publication details: In: G. B. Maguire (Editor), Proc. Prawn Farming Workshop, North Coast Agricultural Institute, Wollongbar, 11 December 1987. Brackish Water Fish Culture Research Station, Salamander Bay, pp. 54-58.

d Prawn farming research in model ponds. (Appendix 3.)

Authors: G L Allan and G B Maguire, 1988.

Publication details: In: J. Paynter and N. Preston (Editors), Proc. Third Ann. Conf. Aust. Mariculture Assoc., Lismore, June 1988, Aust. Mariculture Assoc., Brisbane, pp. 88-96.

e Results for commercial and experimental prawn farming ponds in New South Wales during 1988/89. (Appendix 4.)

Authors: G L Allan, 1990.

Publication details: In: J. Paynter and N. Preston (Editors), Proc. Fourth Ann. Conf. Aust. Mariculture Assoc., Brisbane, July 1989, Aust. Mariculture Assoc., Brisbane, pp. 51-57.

f Lethal effects of acidified seawater on *Penaeus monodon* and the interactive effects of salinity and pH on sub-lethal effects. (Appendix 5.)

Authors: G L Allan and G B Maguire, 1991.

Publication details: In: P. J. F. Davie and R. H. Quinn (Editors), Proc. 1990 International Crustacean Conf., Brisbane 2-6 July 1990, Mem. Qld. Museum, 31: 420.

6.3 POPULAR ARTICLES

a Water quality parameters to water in prawn farming. (Appendix 6.)

Authors: G L Allan and G B Maguire, 1987.

Publication details: Austasia Aquaculture Magazine, 2(5): 5-7.

b First Australian prawn farming research workshop. (Appendix 7.)

Authors: G L Allan and G B Maguire, 1987.

Publication details: Austasia Aquaculture Magazine, 2(6): 14-15.

6.4 PRAWN FARMER VISITS

During the course of this grant every prawn farmer in NSW and southern Queensland was visited at least three times by G Allan and G Maguire and most farmers in north Queensland were visited at least once. During these visits results of the research described in this grant report were communicated to the farmers as were the implications for farm management.

7 APPENDICES

APPENDIX 1.

Summary of paper presented at the first Australian prawn farming research workshop, Brackish Water Fish Culture Research Station 17-18th August, 1987, by Geoff Allan.

FIRST

AUSTRALIAN PRAWN FARMING

RESEARCH WORKSHOP

BRACKISH WATER FISH CULTURE

RESEARCH STATION

SALAMANDER BAY, N.S.W. 2301

AUGUST 17 - 18, 1987.

HOSTED BY THE N.S.W. DEPARTMENT OF AGRICULTURE.

POND MANAGEMENT - Environmental Requirements

Geoff Allan (N.S.W. Department of Agriculture) listed some of the major environmental variables which affect prawn growth and survival. He also summarised research results from the Brackish Water Fish Culture Research Station and other published information on the requirements of several species used by Australian prawn farmers. Although the environmental variables were discussed separately Geoff stressed that it was the interaction between variables which affected prawn growth and survival in a farming situation.

Temperature

Species	Upper lethal Limit T°C	Lower Lethal Limit T°C	Optimum Growth T°C	Optimum Food Conversion T°C
Metapenaeus macleayi (school prawn)	34-35	<6	21-27	18-27
Penaeus monodon (leader prawn)	39-42	7-9	27-33	24-33

The results for optimum temperature for growth and food conversion indicate that even at sub-optimum temperatures, reasonable production returns can be achieved provided feed inputs and consumption rates are accurately monitored and reduced during cooler weather.

Temperature requirements are of obvious use when selecting climatic regions to locate prawn farms and also when planning which species to farm during different seasons.

Salinity

One method which has been used to indirectly estimate optimum salinity for growth is to determine the isosmotic point (that point where the osmotic pressure in the blood equals that of the surrounding water).

Data for 11 species of penaeids were presented which showed that the isosmotic point was similar and relatively high (23-30‰) for a range of penaeid species. However prawn farmers from Taiwan and south-east Asia consider that the optimum salinity for growing the leader prawn is 10 - 25‰ which is c onsiderably lower than its isosmotic point. The need for more research on salinity requirements particularly in terms

of effect on other environmental variables and on biological processes in prawn grow-out ponds (e.g. on the natural productivity of ponds) was discussed.

Salinity data, preferably over several years, is very useful when selecting sites for prawn farming. As with temperature data, the salinity regime in a particular region (especially where there is a pronounced wet and/or dry season) should be considered when planning production strategies, e.g. the timing of stocking and harvest and also the choice of species. Rainfall data for several centres was presented and used to show the extent and severity of the dry season in many regions in northern Australia. During the dry season high salinities (often hypersaline) will be experienced at prawn farms which do not have access to abundant supplies of freshwater.

Dissolved oxygen

Dissolved oxygen is the major production limiting variable in prawn farms. Especially on hot still mornings when there is a high organic load in ponds or following the crash of an algal bloom, low dissolved oxygen levels leading to reduction in growths or even death of prawns can and do occur. The maintenance of a moderate algal bloom helps to maintain adequate dissolved oxygen levels. The lethal level of dissolved oxygen (95 LC_{50} - that level which kills 50% of the test organisms over a 96 hr period) was estimated as 0.86 ppm for leader prawns. For other penaeids lethal levels of 0.7-1.4 ppm (*P. japonicus*) and 0.9 ppm (*P. schmitti*) have been reported.

Physiological studies have been used to estimate critical levels of dissolved oxygen. The incipient limiting dissolved oxygen level (that level above which oxygen consumption of the prawn is unaffected by the ambient dissolved oxygen level) has been reported as being 4.0-4.3 ppm for leader prawns, however, in a separate study growth of juvenile (<1.0 g) leader prawns at four constant dissolved oxygen levels (1.0, 2.0, 3.0, and 4.0 ppm) was only reduced at the lowest, i.e., 1.0 ppm.

Further research is needed on the effect of dissolved oxygen levels on prawn growth, including the effects of supersaturation and diurnal fluctuations. Information on dissolved oxygen requirements is essential for prawn farmers to plan management strategies, e.g. aeration, water exchange and fertilization schedules.

pH

Changes in the pH of pond waters can affect:

1. acid-base status and ionic regulation,
2. cardiovascular performance and oxygen transport and

3. the toxicity of other physio-chemical variables, e.g. ammonia (almost 10 times more toxic at pH 8 than 7).

Acidic conditions can reduce prawn survival and growth and potentially harmful, low pH levels have been recorded in several New South Wales prawn farming ponds. A lethal limit of 3.8 (96 hr LC_{50}) was estimated for leader prawns. Sub-lethal pH levels have been reported to reduce growth and moulting frequency and to result in shell weight loss (presumably as a consequence of exoskeletal dissolution as CaC_{O3} is lost due to acidosis). This could effect the marketability of prawns.

pH can also be used as an indication of algal activity; increasing as algae remove CO_2 during phosynthesis and decreasing during respiration. pH measurements can therefore be used to help plan fertilization, water exchange and aeration schedules.

Where acid-sulphate soils have been used or where pond waters become acid for other reasons, e.g. acidic ground water seeping into ponds, lime can be used to reduce acidity. In some instances several tonnes of algricultural lime ($CaCO_3$) per ha have been used although applicaton rates will vary depending upon the severity of the problem.

Toxic Nitrogenous Wastes

In prawn farming pond ammonia is directly excreted by prawns and can accumulate following decomposition of organic matter. At sufficiently high concentrations ammonia may inhibit prawn growth and depress survival rates. In solution ammonia exists in two forms, the highly toxic unionised form (NH_3) and the much less toxic ionised form (NH_4). The proportion of each is dependant upon pH, temperature and salinity. Ammonia is depleted following assimilation by algae in the pond and following nitrification by bacteria. In grow-out ponds where prawns are stocked at low to moderate densities (<50 prawns/m^2) and where a moderate algal bloom is maintained high ammonia levels have not been recorded. However in densely stocked larval rearing tanks, nursery systems or intensively farmed grow-out systems the build-up of ammonia could lead to problems with reduced growth a m ortality of prawns. The lethal levels (96hr LC_{50}) of unionised ammonia for leader prawns and school prawns were estimated as 1.84 and 1.40 mg NH_3-N/l respectively. The critical level (EC_5 - that level at which growth is reduced by 5% compare with controls) was estimated as 0.20 and 0.33 mg NH_3-N/l respectively. Nitrite is another potentially toxic compound which can accumulate in aquaculture facilities. One study found that a concentration of 6.4 mg NO_2-N/l reduced growth of one penaeid species by 50% over 34 days. However, high concentration of nitrite have not been a problem to date in prawn farming pond trials in New South Wales.

Control of both ammonia and nitrite can be affected by stimulation and maintenance of an algal bloom in ponds or by water exchange. It is also desirable to prevent large amounts of organic matter from accumulating on the pond bottom.

Substrate

Although school prawns have been shown to prefer certain types of substrates, no significant differences in growth were found where school prawns and leader prawns were grown on a range of substrates including one treatment where no sediment was provided. However substrate is important for several other reasons including the following:

(1) engineering condsiderations, e.g. soil porosity, ease of pond construction;
(2) silt characteristics (excess silt in water columns can reduce light penetration);
(3) adsorbtion of chemical fertilizers e.g. phosphorous, to some sediment types;
(4) accumulation of pesticides in sediments, and
(5) problems occurring when acid-sulphate soils are used for pond construction.

Pond sediments may also become chemically reduced following accumulation and microbial degradation of organic material on the pond bottom. Inputs of organic material from excessive algal blooms, death or decay of prawns, detritus washed into pond, residual organic mater e.g. cane stubble etc., can all lead to problems firstly with low dissolved oxygen (partly as a result of microbial respiration) and as the oxygen is depleted the production of hydrogen sulphide, potentially a very toxic compound. From a management viewpoint close attention must be paid to monitoring input of organic material especially feed inputs.

APPENDIX 2.

a Allan, G., 1987b. Water quality and grow-out pond management. Paper presented at Prawn Farming Workshop, North Coast Agricultural Institute, Wollongbar, 11th December, 1987.

b Maguire, G.B. and Allan, G.L., 1987. Summary of results of prawn farming research at the Brackish Water Fish Culture Research Station, Salamander Bay, 1986-1987. Appendix to printed papers, Prawn Farming Workshop, North Coast Agricultural Institute, Wollongbar, 11th December, 1987.

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PRAWN FARMING WORKSHOP

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Department of Agriculture New South Wales

WATER QUALITY AND GROW-OUT POND MANAGEMENT

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The term water quality is used to encompass the whole gamut of physical, chemical and biological variables which interact to characterise a body of water. For prawn hatcheries, nursery systems and grow-out ponds the maintenance of adequate water quality is crucial to ensure optimum survival and growth of the species being cultured and is therefore one of the major components of good management.

Some of the major physical, chemical and biological water quality variables are listed in Table 1. Although the interactions among all these variables can be very complex, for most practical aquaculturists routine measurement of only a few variables is generally sufficent to assess the suitability of the pond water for prawn culture and to provide the prawn farmer with the necessary information to design pond management strategies. Those variables which are considered to be most useful to measure on a routine basis are indicated by bold type. In some locations of course, specific problems may arise for example where industrial or agricultural practices cause problems with heavy metal or pesticide contamination. In such cases monitoring of additional variables may be necessary.

To enable the prawn farmer to interpret the results from water quality measurements and to then devise appropriate pond management strategies, the farmer should firstly have some knowledge of the requirements of the species being cultured and secondly have some understanding of the major processes which are occurring in the pond.

TABLE 1. LIST OF VARIABLES WHICH INFLUENCE WATER QUALITY.

- **Temperature ***
- **Salinity ***
- **Dissolved oxygen ***
- Biological oxygen demand
- Chemical oxygen demand
- **pH ***
- Alkalinity
- Nutrient content
  - ammonia (NH_3) *
  - nitrite (NO_2)
  - nitrate (NO_3)
  - phosphate (PO_4)
  - silica (Si)
- Plant pigments
  - chlorophylls a, b and c
  - phaeophytin
- Primary productivity
- Total organic matter on pond bottom
- Suspended organic matter
- Suspended inorganic matter
- Heavy metal concentration
- Pesticide residues
- Soil characteristics
  - sediment composition
  - soil pH
  - minerals, e.g., iron (Fe)
  - humic compounds
- Sediment condition
  - oxidation reduction potential
  - hydrogen sulphide (H_2S)
- Bacteria
- Other pond fauna including the animals being cultured
- Wind/solar radiation

Some of the major water quality variables are discussed below. Where available the requirements for leader prawns, *Penaeus monodon*, are given as an example. These results are either from research carried out at the Brackish Water Fish Culture Research Station, (for a summary of recent prawn farming research results please see appendices), or from published information. Methods used to measure water quality variables and the application of the results to prawn farming pond management are also discussed briefly.

1. Temperature and Salinity

For *P. monodon* postlarvae upper and lower lethal temperatures have been reported as being 39-42°C and 7-9°C (Motoh 1981). The optimum temperature for maximum growth was determined as 27-33°C (Maguire and Allan unpublished data) while reported optimum salinities range from 10 - 25‰ (Maguire and Allan 1987). Temperature can be easily and accurately measured using a thermometer although many water quality meters have a built-in thermistor. Salinity may be measured by using a refractometer, salinometer or conductivity meter or by using a hydrometer with a temperature correction table.

Consideration of the local temperature and salinity regimes is essential when choosing a prawn farming site and when determining which species are to be cultured during different times of the year. Rainfall data is available from the Bureau of Meteorology and is very useful when assessing the severity and duration of both wet and dry seasons (Maguire and Allan 1987).

2. Dissolved Oxygen

This is the most critical, production limiting, water quality variable. A lower lethal level for *P. monodon* juveniles was estimated as approximately 0.9 ppm (Allan *et al*, unpublished data) with a critical level (that level below which growth is reduced) of between 1.2 and 2.2 ppm over 16 days (Seidman and Lawrence 1985). Dissolved oxygen may be measured using an oxygen meter although for reliable measurements to be taken it is essential that meters are regularly calibrated. Winkler's titration may be used as an alternative or for calibrating meters (APHA 1971). Dissolved oxygen test kits which employ this method are available, although it is more time consuming than using a dissolved oxygen meter and consequently less popular with prawn farmers. In a pond, dissolved oxygen is highest during the day and usually peaks in the late afternoon following photosynthesis by algae in the pond. The more dense the algal "bloom" the higher the afternoon dissolved oxygen readings. These increases are moderated by overcast weather. Dissolved oxygen will decline during the night as algae consume oxygen through respiration. Many other organisms in the pond also consume oxygen and the prawns themselves may be only minor consumers. When there is a large input of organic material, e.g., following prolonged

overfeeding or the deposition of a lot of dead algal material, consumption of oxygen by bacteria which decompose this material (microbial decomposition) can lead to a serious depletion in dissolved oxygen particularly when this situation coincides with hot still nights. Adequate facilities for artificial aeration, e.g., paddle wheel aerators, or rapid water exchange, can alleviate problems with reduced dissolved oxygen to some extent, however it is essential that ponds are managed to prevent build-up of excess organic matter. The stimulation or maintenance of a moderate algal bloom will help maintain adequate dissolved oxygen levels.

3. **Hydrogen Sulphide** (H_2S)

This is a gas produced when oxygen has been depleted and anaerobic microbial decomposition occurs. Even low levels (0.1 ppm) are reported to be stressful to prawns (Apud *et al* 1985). The presence of H_2S is easily detected by the characteristically black sediment and a strong "rotten egg" odour. Preventing the build-up of excess organic matter on the pond bottom is the best way to prevent problems with H_2S however draining the pond and allowing pond bottom sediments to completely dry out between farming trials will reduce problems. Applications of agricultural lime ($CaCO_3$) to the dry sediment are also considered useful.

4. **pH**

Problems with acidic conditions (low pH) in prawn farming ponds can occur especially where acid sulphate soils have been used. A lower lethal pH of approximately 3.7 was estimated (Allan *et al* unpublished data) for *P. monodon* while growth was reported to cease below a pH of 5 for penaeid aquaculture in acid sulphate soils in Costa Rica (Webber and Webber 1978). pH may be measured using commercially available pH meters or alternatively inexpensive pH papers may be used, however the reliability of pH papers should be checked.

Sediments can also become acidic if they contain large stores of slowly decaying organic matter. In ponds acidic conditions also reduce the availability of carbon dioxide and other nutrients, e.g., phosphorous, needed to stimulate an algal bloom. Applications of agricultural lime ($CaCO_3$) can be used to raise pH and therefore increase the availability of carbon dioxide and nutrients for photosynthesis.(Boyd 1982). Fertilisation can then be used to stimulate algal growth.

pH measurements also provide a very useful indication of algal activity in ponds. As algae photosynthesise during daylight hours, carbon dioxide (CO_2) is consumed and pH increases. When a very dense algal bloom exists in a pond pH can rise above 9.2 (Maguire and Allan 1985). Although

reduction in prawn growth at these high pH levels has not been recorded, very dense algal blooms can quickly deplete available nutrients and rapidly die leading to the deposition of excessive organic matter on the pond bottom which may in turn lead to a reduction in dissolved oxygen levels. Rapid water exchange can be used to dilute excessive algal blooms.

5. Ammonia

Prawns and other aquatic animals excrete ammonia directly and it can also accumulate following decomposition of organic matter. Ammonia exists in solution in two forms, a highly toxic unionised ("free") form and a less toxic ionised form. The proportion of each is determined chiefly by the pH and also by temperature and salinity. An upper lethal level of approximately 37 mg NH_3-N total ammonia/l (1.8mg NH_3-N unionised ammonia/l at pH = 8.0, temperature = 27.0°C and salinity = 33‰) was estimated with growth being reduced above about 4.1 mg NH_3-N total ammonia/l (0.2 mg NH_3-N unionised ammonia/l at pH = 8.0, temperature = 27.0°C and salinity = 33‰) over 3 weeks (Allan *et al*, unpublished data). Ammonia can be measured using ion-selective electrodes and a mV meter or by using test kits designed for salt water. In a laboratory more sophisticated spectrophotometric analysis is generally used. In natural systems ammonia is converted through nitrite to nitrate by certain groups of bacteria in the sediment and is also assimilated directly by algae. In a well managed pond it is unlikely that ammonia will build up to serious levels.

6. Pond water quality management

Some understanding of the processes which are occurring in the pond will help the prawn farmer interpret the relationship among the key variables and why the measurements change over time. This information can then be used to anticipate potential crisis situations, hopefully in time to prevent them occurring. The most important initial step which will influence pond management is site selection. This will determine the materials, soil, water etc. that the prawn farmer will have to work with. The second step is the design of farm facilities; ponds, water supply and drainage systems, aeration equipment, etc. This will influence the control the prawn farmer will have over the pond environment and his/her capacity to respond to crises. Once a site has been selected and the facilities installed the prawn farmer must manage the pond environment to optimise water quality. One of the major pond management strategies used by prawn farmers to control water quality is the stimulation and maintenance of a moderate algal bloom. A phytoplanktonic algal bloom, consisting predominantly of single celled plants suspended in the water column, serves several roles:

a) Maintenance of dissolved oxygen.
b) Assimilation of ammonia.

c) Reduction in light penetration restricting the growth of benthic (bottom dwelling) algae and reducing predation by wading birds.
d) Serving as the basis for a food chain involving bacteria and other pond fauna which could constitute natural food items for prawns.

An algal bloom is often initiated by a single large input of fertiliser, e.g., 60 kg/ha each of Nitram and DAP (Diammonium phosphate) and maintained by the addition of relatively small quantities of fertilisers, e.g., 15 kg/ha each of Nitram and DAP and through water exchange. The quantity of fertilisers required to stimulate and maintain an algal bloom will vary greatly at different prawn farming sites and during different seasons depending upon the nutrient characteristics of the pond sediment and the water supply.

If this management strategy is adopted it is crucial that regular measurements of dissolved oxygen and pH are made (preferably in the morning and again in the late afternoon). This allows early indications to be obtained of a decline in an algal bloom and hence an indication of when fertiliser needs to be added. Also this information will help prevent an excessive bloom from developing. Some typical measurements of dissolved oxygen and pH in a pond with a moderate algal bloom might be 5 ppm and 7.8 in the morning and 10 ppm and 8.5 in the late afternoon (Maguire and Allan 1985). Secchi-disc depth can also be a useful indication of the density of an algal bloom. Heavily stocked ponds usually require more intensive water quality management (Maguire and Allan 1986). The provision of emergency artificial aeration is recommended for all ponds, and is essential for more intensively stocked ponds, as an insurance against depletion of dissolved oxygen.

Table 2 summarises the major components of pond management. Good management is a process of information collection and response. By monitoring water quality regularly the prawn farmer can collect the necessary information to choose the most appropriate management strategy to optimise water quality and maximise production.

TABLE 2. MAJOR COMPONENTS OF POND MANAGEMENT.

WATER QUALITY MONITORING

- Choice of variables and methods
- Calibration and care of instruments
- Regular sampling
- Representative samples from pond
- Interpretation of results
- Visual observation

SPECIES AND STOCKING DENSITY

STOCKING AND HARVESTING SCHEDULE

FEEDING

- Natural food
- Type of artifical diet
- Feed rates
- Feeding times and frequency

FERTILIZATION AND LIMING

- Types
- Application rates
- Method of application
  Timing (period between fertilization and stocking)

WATER EXCHANGE

- Timing (tidal cycle, floods etc.)
- Quantity

AERATION

- Timing
- Duration

EXCLUSION OF PREDATORS

- Fish
- Birds
- Humans

POND PREPARATION BETWEEN FARMING TRIALS

- Drainage
- Drying
- Liming

7. Research

One of the major aims of the research being conducted at the Brackish Water Fish Culture Research Station is to provide information on the environmental requirements of prawns being cultured in New South Wales. In Table 1 an asterisk has been used to indicate which variables have been or are being investigated. Three approaches are being used to assess the effect of water quality variables on prawn growth and survival:

1. Constant levels of individual variables are maintained throughout the experiments to determine lethal levels as well as those "safe" levels at which growth is unaffected. For some experiments concentrations of several variables are varied together to assess interactive effects.

2. Variables are varied on a cyclical basis so that they are increased and decreased over daily intervals to simulate diurnal fluctuations which can occur in ponds, e.g.,low temperatures in the morning and higher temperatures in the afternoon.

3. High but sub-lethal concentrations are maintained for only short periods to stress prawns after which time conditions are returned to normal. These experiments will be run to determine the effect on growth rates of short term crisis situations where water quality has been temporarily reduced e.g. during an oxygen crisis.

A series of experiments is also being conducted in "model ponds" (3.5m diameter fiberglass tanks) to assess the impact of different management strategies, e.g., stocking density, on production of prawns, water quality and the dynamics of algae and bacteria within ponds. For a more detailed description of prawn farming being carried out at the Brackish Water Fish Culture Research Station please see Allan and Maguire (1988).

REFERENCES.

Allan G. L., and Maguire, G.B., 1988. Prawn farming research by the N.S.W. Department of Agriculture. **Australian Mariculture Association Newsletter, 1(2): 10-14.**

Allan, G. L., Hopkins, S.J. and Maguire, G. B. (unpublished data).

American Public Health Association (APHA), 1971. Standard methods, **13th Edition, APHA, Washington DC, 874 p.**

Apud, F. D., Primavera, J. H. and Torres, P. L. (Jr.), 1985. Farming of Prawns and Shrimp. **3rd Ed. Aquaculture extension manual No. 5. Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department, 67 p.**

Boyd, C.E., 1982. Water Quality Management For Pond Fish Culture. **Developments in Aquaculture and Fisheries Science 9, Elsevier. 318 p.**

Maguire, G. B. and Allan, G., 1985. Development of methods for growing juvenile school prawns, *Metapenaeus macleayi*, in estuarine ponds. **In: P. C. Rothlisberg, B. J. Hill and D. J. Staples (Editors), Second Aust. Nat. Prawn Sem., NPS2, Cleveland, Australia, pp 337 - 351.**

Maguire, G. B. and Allan, G., 1986. A Guide to Marine Prawn Farming in New South Wales. **Information Leaflet. Department of Agriculture, N.S.W., 26 p.**

Maguire, G. B. and Allan, G., 1987. Prawn farming in Australia - Status and Problems. **In: Prawn Farming for Profit. Potential for Success. Seminars presented by SCP Fisheries Consultants Australia Pty. Ltd., pp 13 - 28.**

Maguire, G. B. and Allan, G. L. (unpublished data).

Motoh, H., 1981. Studies on the fisheries biology of the giant tiger prawn, *Penaeus monodon* in the Philippines. **Tech. Report No. 7., SEAFDEC Aquaculture Department, Iloilo, Philippines, 128 p.**

Seidman, E. R. and Lawrence, A. L., 1985. Growth, Feed, Digestibility and Proximate Body Composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. **J. World Maricul. Soc. 16: 333-346.**

Webber, R. J. and Webber, H. H., 1978. Management of acidity in mangrove sited aquaculture. **Rev. Biol. Top. 26 (Supl. 1): 45-51.**

SUMMARY OF RESULTS OF PRAWN FARMING RESEARCH AT THE BRACKISH WATER FISH CULTURE RESEARCH STATION, SALAMANDER BAY, 1986-87

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All of the research summarised below involved the stocking of juvenile prawns (usually 1-5g) and the intention is to apply the results to the siting and management of grow-out ponds or intensive nursery units. For a more comprehensive discussion of the development and current directions of this research program see Allan and Maguire (1988).

A. WATER QUALITY BIOASSAY RESULTS.

Research has been conducted on different water quality parameters, e.g. dissolved oxygen, to find out at what constant but extreme levels these parameters will kill prawns or inhibit their growth. The 96hr LC_{50}, (that concentration which will kill 50% of the test animals over a 96 hour period), was used as the measure of acute toxicity while the EC_5, (that concentration which reduces growth by 5% over a period of 3-4 weeks), was used as a measure of chronic toxicity. It has also been possible to vary the levels of two water quality parameters in combination so that the interactive effects on prawns could be assessed. In addition, a study was made of the effects of a short but severe stress, i.e. a low dissolved oxygen level for up to 12 hours, on subsequent growth rates. Following the initial stress normal conditions were maintained, i.e. oxygen levels were kept at a constant high level for the three week period.

1. Acute toxicity of ammonia to school prawns, *Metapenaeus macleayi*.

Ammonia can be present in seawater in a very toxic (unionised) form or in a relatively nontoxic (ionised) form. The proportion of each form is chiefly determined by the pH level. The combined amount of ionised and unionised ammonia is known as total ammonia. Ammonia concentrations can be expressed as milligrams per litre (mg/l). The 96hr LC_{50} was estimated as 26.34 mg NH_3-N total ammonia/l (1.40 mg NH_3-N unionised ammonia/l at pH = 8.1 and Temperature = 25.0°C)

2. Acute toxicity of ammonia to jumbo tiger prawns, *Penaeus monodon*.

The 96hr LC_{50} was estimated as 37.32 mg NH_3-N total ammonia/l (1.84 mg NH_3-N unionised ammonia/l at pH = 8.0 and Temperature = 27.0°C)

3. Acute toxicity of acidified seawater to jumbo tiger prawns, *Penaeus monodon*.

The 96hr LC_{50} was estimated as 3.8 pH units.

4. Acute toxicity of low dissolved oxygen levels to jumbo tiger prawns, *Penaeus monodon*

The 96hr LC_{50} was estimated as 0.86 ppm.

5. Chronic toxicity of ammonia to school prawns, *Metapenaeus macleayi*.

An EC_5 was estimated as 8.84 mg NH_3-N total ammonia/l (0.40 mg NH_3-N unionised ammonia/l at pH = 8.0 and Temperature = 25.0°C). Problems with control of dissolved oxygen and ammonia levels were experienced with this experiment which may have affected this result. Consequently this experiment will be repeated.

6. Chronic toxicity of ammonia to jumbo tiger prawns, *Penaeus monodon*.

An EC_5 was estimated as 4.10 mg NH_3-N total ammonia/l (0.20 mg NH_3-N unionised ammonia/l at pH = 8.0 and Temperature = 27.0°C).

7. Effect of substrate on the acute toxicity of ammonia to school prawns, *Metapenaeus macleayi*.

At an ammonia concentration of 31-33 mg NH_3-N total ammonia/l (1.35-1.40 mg NH_3-N unionised ammonia/l) survival was significantly higher in aquaria provided with sediment (sand) compared with aquaria without sediment. This result demonstrated that a sediment can act as a refuge for burrowing prawns from potentially lethal ammonia concentrations.

8. Effect of low dissolved oxygen on the toxicity of ammonia to jumbo tiger prawns, *Penaeus monodon*

When the dissolved oxygen concentration was maintained at approximately 2.0 ppm, a 96hr LC_{50} of 1.4 mg NH_3-N as unionised ammonia /l was determined. This compares with a 96hr LC_{50} of 1.8 mg NH_3-N as unionised ammonia /l when the dissolved oxygen concentration was maintained at levels approaching saturation. The increase in toxicity of ammonia when the dissolved oxygen level was low was suprisingly slight. This further demonstrates the high tolerance of this species to unfavourable conditions.

9. Effect of a single severe oxygen stress for periods of up to 12 hours on subsequent growth of jumbo tiger prawns, *Penaeus monodon*.

Prawns exposed to a dissolved oxygen level of 0.5 ppm for up to 12 hours and then grown for 3 weeks at high (saturated) dissolved oxygen levels did not suffer any adverse effects in terms of growth. This was quite suprising as this stress was sufficient to kill a few of the prawns.

10. Methodology.

To provide this detailed information on the effect of a variety of water quality parameters on prawn survival and growth, a sophisticated bioassay system has been installed. Using this flow-through experimental facility, seawater is filtered, heated and the dissolved oxygen content adjusted by means of vacuum and nitrogen gas stripping. Peristaltic pumps are used to precisely control the concentration of the water quality parameters being tested in the 70 l experimental aquaria. To determine LC_{50}'s probit analysis was carried out using the Applesoft computer programme written by Mr A. Woods, University of N.S.W. Analysis of variance techniques were used for chronic toxicity experiments (one way ANOVAR) experiments, and for interactive experiments (two way ANOVAR). To estimate EC_5's regression analysis was used. Microstat computer programmes were used for ANOVAR and regression analysis.

B. **OTHER RESEARCH RESULTS**.

1. Effects of water temperature on school prawns (*Metapenaeus macleayi*), jumbo tiger prawns (*Penaeus monodon*) and eastern king prawns (*Penaeus plebejus*).

School prawns were grown in aquaria at constant temperatures from 15^0C to 30^0C while jumbo tiger prawns were grown at 18^0C to 33^0C for several weeks. For both species food consumption and moulting frequency increased as temperature increased but survival rates were unaffected by temperature. The optimum temperatures for growth and food conversion efficiency for school prawns were 21-27^0C and 18-27^0C respectively. The optimum temperatures for growth and food conversion efficiency for jumbo tiger prawns were 27-33^0C and 24-30^0C respectively. Eastern king prawns were grown at 18^0C to 33^0C and the optimum temperature for growth of eastern king prawns was 30^0C. In the eastern king prawn experiment several groups of prawns were also grown at temperatures which increased and decreased over a 24 hour period to simulate what happens in ponds where there is a daily water temperature cycle. Prawns grown in a daily 21-30^0C cycle suffered only a 17% growth reduction compared to prawns grown at a constant 30^0C. This 9^0C daily temperature range is much greater than that usually observed in a 1m deep pond, i.e., 3^0C.

2. Effects of sediment type on jumbo tiger prawns, *Penaeus monodon*.

In aquaria these prawns grew equally well on bare perspex, mud or coarse sand.

3. Effects on jumbo tiger prawns, *Penaeus monodon*, of dieldrin levels in sediment.

Dieldrin levels in the range 0-0.3 ppm of the dry weight of the sediment had no effect on prawn growth or survival rates. Results of testing for residual dieldrin levels in the prawns themselves are still awaited.

4. Effects of stocking density on school prawns, *Metapenaeus macleayi* in pens within ponds.

School prawns were grown at a range of stocking densities (20-100 per m^2) and the maximum increase in biomass of prawns was obtained at 60 per m^2. Survival rates were high at all densities. (Pens allow prawns access to pond fauna and as well a very nutritious diet was supplied.)

5. Effects of commmercial diet type on jumbo tiger prawns, *Penaeus monodon*, in pens.

Of the diets tested in the summer of 1986/87, the Taiwanese Lux brand diet was the most successful in terms of growth rates for prawns stocked at 15 juveniles per m^2. A control diet, chopped meat from pipis which are burrowing oceanic beach bivalves, was more physically stable in seawater than Lux pellets and produced better food conversion efficiency. Three Australian commercial prawn diets produced only 70% of the growth rate obtained with Lux pellets and as well these three diets had poorer water stability. Survival rates were very high for all groups of prawns regardless of which diet was supplied. The unfed controls which only had access to pond fauna in the pens grew poorly but survived well. Testing of diets available in the 1987/88 summer is currently under way.

6. Effects of pond management strategy on jumbo tiger prawns, *Penaeus monodon*, in model ponds.

A set of 16 large (3.5 m diameter) fiberglass tanks have been installed and are being used to simulate prawn ponds. The effects of two different management practices on prawn production, water and sediment quality and the dynamics of algae and bacteria within ponds were compared. The first management practice was the stimulation and maintenance of a phytoplanktonic algal bloom, as carried out by the authors during their pilot scale prawn farming program and by several other commercial prawn farmers in Australia and overseas, e.g., Taiwan. The second management practice was the stimulation and maintenance of a benthic algal bloom, often called "lab-lab", a practice used by traditional prawn farmers, e.g., in the Philippines. The trial was carried out at a density of 15 juveniles per m^2. Prelminary analyses from this recently completed trial indicate that growth rates

in the phytoplankton treatment were not much higher than in the benthic algal treatment. One major finding was that the benthic algal tanks could develop very dense filamentous algal blooms which made supplementary feeding and drain harveting much more difficult. The tanks supported growth, survival and food conversion rates that would have been highly acceptable in a commercial pond.

C. FUTURE PRIORITIES.

It is hoped that bioassay and pond management research will continue. Specifically, the chronic toxicity of ammonia to school prawns must be assessed. Other aquarium based research may include the testing of the effects of reduced sediment conditions and moulting stimulants on prawn growth rates. Assessing different feed input rates and stocking densities will be a high priority for the pool studies. A major task will be the writing of scientific publications describing in detail the above research and other studies.

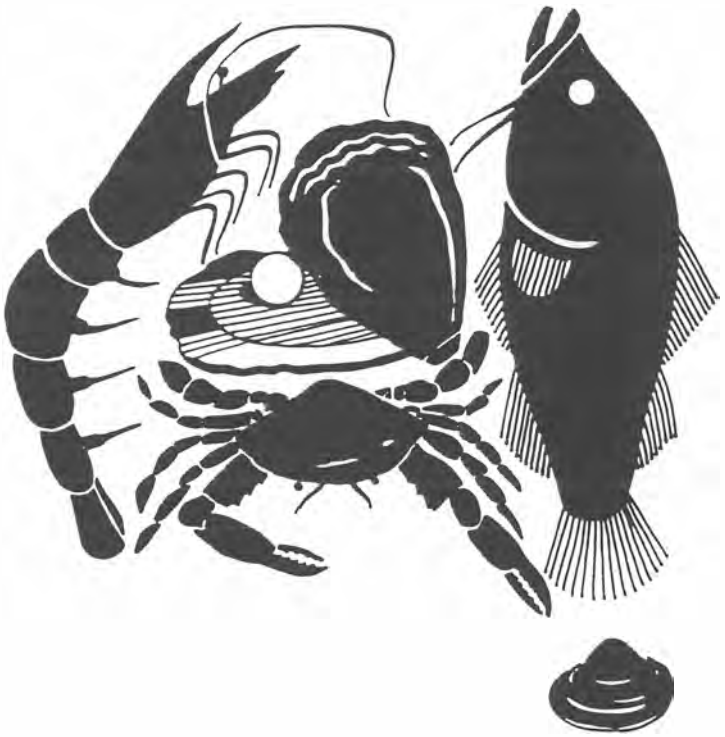
D. REFERENCES.

Allan, G.L. and Maguire, G.B., 1988. Prawn farming research by the N.S.W. Department of Agriculture. **Australian Mariculture Association Newsletter, 1(2): 10-14.**

APPENDIX 3.

Allan, G.L. and Maguire, G.B., 1988. Prawn farming research in model ponds. In: J. Paynter and N. Preston (Editors), Proc. Third Ann. Third Ann. Conf. Aust. Mariculture Assoc., Lismore, June 1988, Aust. Mariculture Assoc., Brisbane, pp. 88-96.

PROCEEDINGS OF THE AUSTRALIAN MARICULTURE ASSOCIATION THIRD ANNUAL CONFERENCE



LISMORE, NSW, JUNE, 1988

PRAWN FARMING RESEARCH IN MODEL PONDS

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The goal of the aquaculturist is to provide optimum conditions for the species being cultured in order to enhance survival and growth and maximise production. To do this an aquaculturist should have a good knowledge of the environmental requirements of the species being cultured as well as an understanding of how the environment can be regulated or managed to optimise water quality.

The objectives of this current research were to assess the effects of some major pond management variables on prawn survival and growth rates and on pond water quality. The results obtained have been used to help determine appropriate pond management strategies for the grow-out of penaeid prawns in ponds. This paper describes the experimental methodology used for this research and briefly outlines the major results and implications for pond management. The detailed experimental data from the research described are currently being used to prepare several manuscripts for scientific journals.

This research was carried out in model ponds and an appropriate design for these experimental units had to be developed. Ideally, the model ponds should be large enough to simulate earthen ponds so that the results can be confidently extrapolated to large commercial ponds and yet still be small enough to enable well replicated, controlled experiments to be conducted.

WATER EXCHANGE

For the first two experiments 4 m diameter, 1m high, above-ground, "Driclad" steel swimming pools with plastic liners were used at a site adjacent to Lake Wooloweyah, Clarence River, N.S.W. (Maguire and Allan, 1985a). These pools were provided with a water supply, continuous aeration via two airstones in each pool and a 100 mm deep layer of sand as a substrate. The pools were drained using 50 mm siphons. The first two experiments were designed to investigate the effects of different water exchange rates. As Australian prawn farms established in recent years rely upon pumping for filling ponds and for water exchange, it is important to reduce unnecessary pumping so as to minimise operational costs.

Both experiments were conducted with juvenile, 1.5-2.2 g, school prawns, *Metapenaeus macleayi*. The prawns were fed each evening using a commercially produced trout pellet (35-40% protein) at approximately 5% biomass/day. The experiments ran for 8 weeks after which time the prawns were harvested, counted and weighed in bulk (sexes separated). The first experiment was stocked at a

density of 20 prawn/m^2 and five water exchange rates, 0, 5, 10, 20 and 40%/day, were tested. For each water exchange rate there were three replicate pools. Water was exchanged every second day, e.g. for the pools receiving 10%/day, 20% of the pool volume was replaced every second day. The second experiment was stocked at a density of 50 prawns/m^2 and 4 water exchange rates, 0, 5, 10 and 20%/day, were tested with four replicate pools per water exchange rate. Water was exchanged every third day. During both experiments temperature, salinity, dissolved oxygen (DO), pH and ammonia were measured every three days and oxidised nitrogen (nitrite plus nitrate), reactive phosphorous, silica and plant pigments (chlorophyll a, b and c and phaeophytin) were measured every 8 days (first experiment) and every 6 days (second experiment). The methods used for the water quality analysis are described in Maguire and Allan (1985a,b).

The major findings from these two experiments were:

1. At 20 prawns/m^2 survival rates were high (>90%) in all pools and were unaffected by water exchange rate ($P>0.05$).

2. At 50 prawns/m^2 on the last day of the experiment mass mortality (>75%) occurred in one pool which had received no water exchange. In all other pools survival rates were above 87%.

3. Growth rates were low during both experiments with an average individual prawn weight increase of 2.5g for experiment 1 and 2.2g for experiment 2. Water exchange rates had no significant ($P>0.05$) effects on prawn growth in either experiment. However, during both experiments there was a trend for growth to be highest at 5%/day water exchange and to decrease as the water exchange rate increased.

4. During both experiments the concentration of phosphorous and plant pigments declined as water exchange rates increased ($P<0.001$) i.e., the algal bloom was being diluted. However exchange rate had no significant effects ($P>0.05$) on pH or on the concentrations of ammonia or oxidised nitrogen.

The major implications for pond management arising from these experiments were that even where DO levels can be maintained, ponds should not be managed for extended periods without water exchange. However increasing regular water exchange rates (e.g. above 5-10%/day) did not result in increased prawn survival or growth but rather served to flush out nutrients, algae and suspended organic matter. Generally this is undesirable as ponds are fertilized and algal blooms generated for a purpose. However, occasionally excessive algal blooms develop in prawn farming ponds and can quickly deplete available nutrients thereby increasing the risk of the bloom "crashing" and depositing large amounts of organic material on the pond bottom. Microbiological decomposition of excessive amounts of organic material can deplete DO levels and reduce prawn growth and survival. In these circumstances water exchange can be an effective management

strategy provided pumping capacity is sufficient to enable large volumes of water to be quickly exchanged. Water exchange has been used in a similar manner in freshwater ponds to reduce the ammount of algae and other forms of suspended organic matter in pond water (Boyd, 1982).

FERTILIZATION - ALGAL BLOOM MANAGEMENT

During the water exchange rate experiments, problems were experienced with corrosion of the steel swimming pools and with rapid deterioration of the plastic pool liners. Consequently a set of 16, 3.5m diameter, 1.2m high, fibreglass tanks was constructed for subsequent pond management experiments in model ponds at the Brackish Water Fish Culture Research Station, Port Sephens, N.S.W. The first experiment conducted in these tanks was designed to:

1. Assess whether the fibreglass tanks could be used to simulate prawn ponds in terms of survival and growth of prawns, and

2. Compare the effects of two commonly used methods of managing algal blooms on prawn survival and growth and on pond water quality.

For a discussion of the role of algal blooms in prawn farming pond management see Allan (1987).

For the purposes of this discussion the types of algae referred to can be described as follows:

> Phytoplanktonic algae - a mixture of single celled species which are usually suspended in the water column.
>
> Filamentous algae - multi-celled species which form long (often several meters) strands or filaments often attached to the pond bottom or other hard substrates.
>
> Benthic algae - species which occur on the pond bottom. The type of benthic algae referred to as "lab-lab" by traditional prawn farmers in Asia is a mixture of both single-celled and multi-celled benthic algae, (sometimes including filamentous algae) which combine together to form a thick mat on the pond bottom.

The first method of algal bloom management involved the stimulation and maintenance of a phytoplanktonic algal bloom as used by the authors during the pilot-scale prawn farming program on the Clarence River (Maguire and Allan, 1985a), and by many commercial farmers in Australia and overseas, e.g. Taiwan. Phytoplanktonic algal blooms were stimulated in eight of the 16 model ponds by the addition of Nitram and Di-Ammonium Phosphate (D.A.P.) at the rate of 100 kg/ha for each fertilizer. Smaller additions, (5-25 kg/ha of either Nitram and D.A.P. or Aquasol and D.A.P.), were added periodically to maintain the blooms. Aquasol

was used in an attempt to improve the stability of the phytoplankton blooms; it may offer advantages in terms of micronutrient levels.

The second method involved the stimulation of a benthic algal bloom as practiced by traditional milkfish and prawn farmers in the Philippines (Chen, 1972, Bardach *et al.*, 1972), and proposed by Cordover (1987) for Australian prawn farming ponds. Eight model ponds were partially filled to about 20cm and then fertilized, (using approximately 2 tonne/ha of relatively dry cow manure plus a smaller addition, approximately 40 kg/ha, of Nitram and D.A.P.), to allow colonization by benthic algal species which formed a dense mat over the pond bottom. When the benthic algal bloom was established the ponds were slowly filled over several days to maintain water clarity, and very little fertilizer was added during the experiment so as to avoid the growth of phytoplankton.

The total amount of nitrogen and phosphorous initially added to all ponds was similar whether it was added as inorganic fertilizer, e.g. Nitram and D.A.P., or as organic fertilizer, e.g. cow manure. However, as periodic additions of fertilizer were made throughout the experiment to the ponds managed with phytoplanktonic algal blooms but not to those managed with benthic algal blooms, the total input of nitrogen and phosphorous as fertilizer for the benthic algal ponds was about 70% less than for the phytoplanktonic algal ponds.

Juvenile leader prawns, *Penaeus monodon* (average size 2.2g) were stocked at a density of 15/m^2 and were fed twice daily (2/3 of their daily ration in the evening and 1/3 in the morning) using an imported Taiwanese prawn feed (42% protein). Feeding was *ad libitum* and feed trays enclosing approximately 0.5m^2 were checked twice daily to assist with the adjustment of feed rates. Temperature, DO and pH were measured twice daily and nutrients, (ammonia, oxidised nitrogen, reactive phosphorous and silicon), and plant pigments, (chlorophyll a, b and c and phaeophytin), were measured regularly. The methods for water quality analyses outlined in Maguire and Allan (1985a,b) were used except that ammonia was measured using the spectrophotometric methods described by Dal Pont *et al.* (1973). The experiment ran for 8 weeks after which time survival, growth and food conversion efficiency, (FQ), were recorded. FQ is the weight of dry feed added divided by the wet biomass gain of prawns (in the presence of natural food items) in ponds. Ponds were aerated during the night for an average period of 5.6 hours/day. Following the conclusions from the previous water exchange rate experiments, water exchange was only carried out when considered necessary to:

1. dilute excessive algal blooms,
2. reduce the concentration of other suspended organic matter, and
3. help prevent low DO levels from occurring.

Each pond was managed separately on the basis of DO, pH and chlorophyll measurements. The average daily water exchange rate for individual ponds was between 6 and 9.5%/day.

The major findings from this experiment were:

1. Excellent survival (>88% for each pond), growth (average individual weight gain of about 12g/prawn) and FQ's (approximately 1:1) were obtained. These results would have been very acceptable to a commercial farmer and indicated that the fibreglass tanks could be used to simulate prawn farming ponds.

2. Overall, the different methods of managing algal blooms had no significant ($P>0.05$) effects on prawn survival or growth. However, there were significant differences in growth rates among the benthic algal ponds.

   The eight benthic algal ponds were considerably less turbid than the phytoplanktonic ponds and in four of the benthic algal ponds excessive growth of filamentous algae occurred which was so dense that it made supplementary feeding and effective harvesting very difficult. In the more turbid phytoplanktonic ponds growth of filamentous algae was not a problem. Prawn growth in the benthic algal ponds where the growth of filamentous algae was excessive was significantly less than in the benthic algal ponds where very little filamentous algae grew ($P<0.001$).

   Latapie *et al.* (1972) also attributed a reduction in prawn growth (for the brown shrimp *Penaeus aztecus*) to reduced accessibility to artifical feeds caused by excessive aquatic vegetation (in this case an angiosperm *Ruppia martina*).

3. The method of managing the algal bloom had a significant effect ($P<0.001$) on water quality with elevated nutrient levels (ammonia, oxidised nitrogen and reactive phosphorous) and plant pigment concentrations being recorded in the phytoplanktonic algal bloom treatment where regular inputs of fertilizer were made.

The major implications of this experiment for pond managers were that the type of bloom maintained in ponds did not directly affect prawn survival or growth. However, the excessive growth of filamentous algae, common in the clear benthic algae ponds, reduced prawn growth (probably because of the reduced availability of supplementary feed) and made effective harvesting difficult. In commercial ponds this could dramatically reduce the production of marketable prawns.

STOCKING DENSITY

In order to achieve an adequate return on investment for land purchase and pond construction, it is important to make the most efficient use of the available ponds. As supplies of postlarval

prawns increase, prawn farmers are becoming more interested in raising stocking density to improve production per unit area. An experiment was designed to investigate the effects of different stocking densities on prawn survival and growth and on pond water quality. In this experiment four stocking densities were used, 5, 15, 25 and 40 prawn/m^2 with four replicate ponds at each density. Juvenile leader prawns (average size 3.4g) were used and the experiment was run for 8 weeks. A phytoplanktonic algal bloom was stimulated and maintained in all ponds. Similar methods to those described for the previous experiment were used for feeding, water quality monitoring and the determination of survival, growth and food conversion efficiency (FQ's).

Aeration was provided for 7.3 hours each night for ponds with stocking densities of 5, 15 and 25 prawns/m^2 and 12 hours each night for those stocked at 40 prawns/m^2. The increased rates were provided following consistently lower morning DO readings in those ponds with the highest stocking density during the first two weeks of the experiment. As in the previous experiment, water exchange was carried out when considered necessary and each pond was managed separately. As stocking density increased higher rates of water exchange were required. These average rates were as follows:

5 prawn/m^2 - 5.9% water exchange/day
15 prawn/m^2 - 6.8% water exchange/day
25 prawn/m^2 - 7.7% water exchange/day
40 prawn/m^2 -11.5% water exchange/day

The major findings from this experiment were:

1. In all ponds survival rates were high (>75%) and were unaffected by stocking density ($P>0.05$).

2. There was a significant ($P<0.001$) decreasing exponential relationship between stocking density and growth. However, biomass gain and food consumption as a percentage of biomass both increased significantly ($P<0.001$) as stocking density increased. FQ deteriorated significantly ($P<0.001$) with increasing density.

   Increasing stocking density produced similar results for other species, e.g. school prawns (Maguire and Leedow, 1983), the grooved tiger prawn *Penaeus semisulcatus*, (Issar *et al.*, 1987) and the white or blue prawn *P. vannamei* (Sandifer *et al.*, 1987).

3. As stocking density increased the total input of nutrients (from the increased amounts of supplementary feed) also increased. As a consequence algal growth was encouraged and even though increased water exchange rates were used at high densities, stocking density still had significant effects ($p<0.005$) on evening DO levels and the concentration of plant pigments. The management strategy, in particular the aeration regime, was sufficient to maintain minimum DO levels above 4.2 ppm in all ponds throughout the experiment.

Although there was a significant difference ($p<0.001$) between average minimum DO levels in the most densely stocked ponds and all others, the average minimum DO for all densities was relatively high. (7.4, 7.5, 7.3 and 6.9 ppm for ponds stocked at 5, 15, 25 and 40 prawns/m^2 in that order.) Stocking density had no significant effect ($p>0.05$) on pH levels or on the concentration of nutrients.

4. A simple economic model was used which considered the cost of prawns to stock ponds, the cost of feed and an estimate of returns based on harvest yields multiplied by projected market prices. As the price received for prawns is dependant upon their size, the market price was weighted to take this into account. This model suggested that the most favourable economic returns would have been received at the lower stocking densities (5 - 15 prawns/m^2).

5. At the same stocking densities growth and FQ's were poorer than those obtained during the preceding experiment when average temperatures were approximately 2°C higher and a longer pond preparation period was possible. Average individual weight gain and FQ results of 1.45 g/week and 1:1 respectively were obtained in the preceding (algal bloom management) experiment. The equivalent values in the stocking density experiment were 0.94 g/week and 1.8:1.

The major conclusions from this experiment were that although increased production was achieved by increasing stocking densities, prawn growth was slower and food conversion efficiencies were reduced. In commercial situations slower growth means either smaller, less valuable prawns at harvest or that prawns must be kept for longer periods in ponds. Reduced food conversion efficiencies result in increased feed costs. Although the economic model used was a very simple one, it does indicate that prawn farmers must consider cost/benefit effects when planning for increased production in ponds. When higher stocking densities are used the increase in prawn biomass makes maintenance of good water quality, especially in terms of disssolved oxygen levels, more difficult and increases the need for efficient aeration and adequate capacity for rapid water exchange.

USEFULNESS OF MODEL PONDS

The prawn farming research in model ponds described in this paper has yielded some interesting results with implications for the management of commercial prawn farming ponds. However, although the model ponds were designed to simulate commercial ponds, because of their smaller size there are differences and consequently caution must be exercised when extrapolating the results to larger ponds. The following points are relevant to this problem:

1. Model ponds have a higher wall area to pond bottom area ratio than large ponds. To help overcome "wall effects" during the experiments with the fiberglass tanks, the walls were scrubbed regularly to prevent a buildup of algae on the walls. Removable PVC panels were used to line the ponds during the last experiment to make this scrubbing operation more convenient.

2. Model ponds were relatively easy to manage, especially in terms of water exchange and aeration. This is not generally the case in large commercial ponds and if more intensive prawn farming methods, e.g. increased stocking densities, are used close attention will have to be paid to maintaining adequate water quality.

3. All the experiments described were run for 8 weeks. Although this was usually a long enough period to generate meaningful survival and growth data, commercial trials are generally run for a longer period so as to achieve the preferred commercial prawn size, e.g. >25g. During longer farming trials a greater potential exists for the buildup of organic matter, e.g. accumulated uneaten feed, algae and detritus. This increases the necessity for water quality monitoring, especially DO, and for adequate aeration and water exchange.

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REFERENCES

Allan, G.A., 1987. Water quality and grow-out pond management. In: Proceedings of the Prawn Farming Workshop, North Coast Agricultural Institute, Wollongbar, 11th December, 1987, pp 40-48.

Bardach, J.E., Ryther, J.H. and McLarney, W.O., 1972. Aquaculture - The Farming and Husbandry of Freshwater and Marine Organisms. Wiley-Interscience, 868 p.

Boyd, C.E., 1982. Water Quality Management for Pond Fish Culture. Developments in Aquaculture and Fisheries Science, 9. Elsevier, Amsterdam, 318 p.

Chen, T.P., 1972. Fertilization and feeding in coastal fish Farms in Taiwan. In: T.V.R. Pillay (Ed.) Coastal Aquaculture in the Indo-Pacific Region, Fishing News (Books) Ltd., London pp 410-416.

Cordover, R., 1987. Prawn farming the Australian way. Austasia Aquaculture, 1(10): 5-6.

Dal Pont, G., Hogan, M. and Newell, B., 1973. Laboratory techniques in marine chemistry. Determination of ammonia in sea water and the preservation of samples for nitrate analysis. Aust. CSIRO Div. Fish. Oceanogr. Rep., 11 p.

Issar, G., Seidman, E.R. and Samocha, Z., 1987. Preliminary results of nursery and pond culture of *Penaeus semisulcatus* in Israel. Bamidgeh, 39(3): 63-74.

Latapie, W.R., Broom, J.G. and Neal, D.A., 1972. Growth rates of *Penaeus aztecus* and *P. setiferus* in artifical ponds under varying conditions. Proc. Ann. Meet. World Mariculture Soc., 3: 241-254.

Maguire G.B. and Leedow, M.I., 1983. A study of the optimum stocking density and feed rate for school prawns *Metapenaeus macleayi* (Haswell) in some Australian brackish water farming ponds. Aquaculture, 30: 285-297.

Maguire G.B. and Allan, G., 1985a. Development of methods for growing juvenile school prawns, *Metapenaeus macleayi*, in estuarine ponds. In: P.C. Rothlisberg, B.J. Hill and D.J. Staples (Editors). Second Aust. Nat. Prawn Sem., NPS2, Cleveland, Australia, pp 337-351.

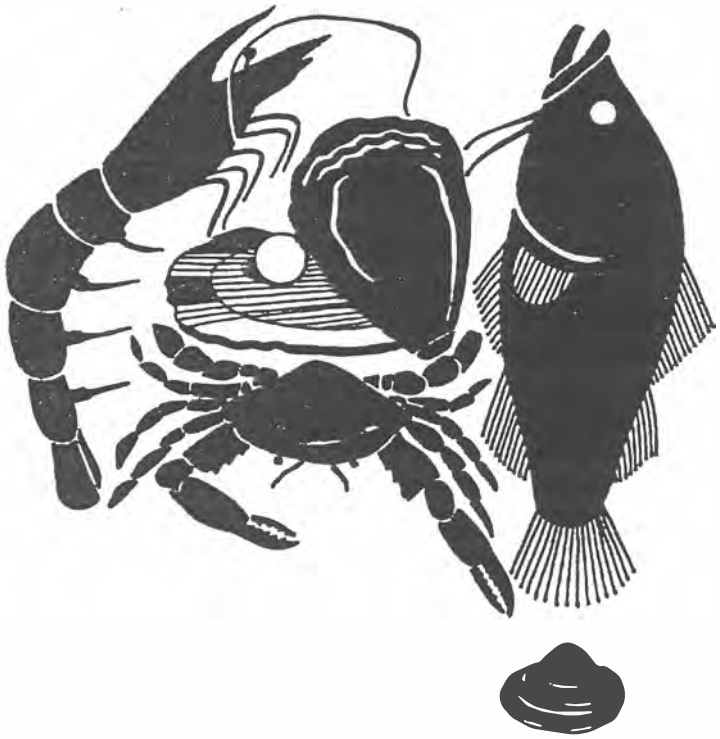
Maguire, G.B. and Allan, G,. 1985b. Clarence River Prawn Farming Program. Final Report to granting authorities. Chapter 3 - prawn pond water quality management. Unpublished. (Report available from the authors.)

Sandifer, P.A., Hopkins, J.S. and Stokes, A.D., 1987. Intensive culture potential of *Penaeus vannamei*. J. World Aquaculture Soc., 18(2): 94-100.

APPENDIX 4.

Allan, G.L., 1990. Results for commercial and experimental prawn farming ponds in New South Wales during 1988/89. In: J. Paynter and N. Preston (Editors), Proc. Fourth Ann. Conf. Aust. Mariculture Assoc., Brisbane, July, 1989. Aust. Mariculture Assoc., Brisbane, pp. 51-57.

The Australian Mariculture Association



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RESULTS FROM COMMERCIAL AND EXPERIMENTAL PRAWN FARMING PONDS IN NEW SOUTH WALES DURING 1988/89

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In Australia the prawn farming industry has been relatively slow to develop and despite several attempts prior to 1980 (Heasman, 1984) commercial scale production didn't eventuate until the mid 1980s. Since that time, however, there has been considerable development of the industry particularly in Queensland, northern New South Wales and to a lesser extent the Northern Territory (Maguire and Allan, 1988). Although prawn farming in northern NSW is disadvantaged by cooler temperatures, to date the production of cultured prawns from this state has been greater than from the other states in Australia combined (Curtis, 1988; Maguire and Allan, 1988; Curtis, 1989 personal communication, 1989).

This situation is likely to change as more farms are developed in Queensland. However, the absence of cyclones and pronounced wet and dry seasons, proximity to markets and technology and adequate supplies of brackish water in the areas that are available for prawn farming should ensure that production levels also continue to rise in NSW.

In this paper the commercial prawn farming production results from NSW during 1988/89 will be examined and related to national and international production results.

One way that research can assist with increasing yields from prawn farming grow-out ponds is by quantitatively comparing the effectiveness of different pond management strategies. The results of an experiment aimed at assessing the effectiveness of pond preparation and two different feeding regimes, on the production of prawns are summarised. This experiment was conducted by NSW Agriculture & Fisheries at the Brackish Water Fish Culture Research Station at Salamander Bay. The detailed experimental data from this research are currently being used to prepare manuscript for submission to scientific journals.

Commercial results

During 1988/89 there were three hatcheries in NSW which produced postlarval penaeid prawns. Total hatchery production was approximately 7 million postlarvae, the majority of which were produced from one hatchery. the major species cultured was the leader prawn, *Penaeus monodon*, however smaller batches of the Australian tiger prawn, *P. esculentus*, and the eastern king prawn, *P. plebejus* were also reared.

Major problems included: difficulty in obtaining suitable *P. monodon* broodstock at certain times, producing sufficient quantities of live food, diseases (including ciliates and bacteria) and inadequacies or breakdowns in equipment, e.g. heaters. One NSW hatchery located at a sub-optimal estuarine site has ceased operation and a larger hatchery, located with access to oceanic sea-water, is under construction.

At the end of 1988/89 there were approximately 160ha of prawn farming ponds constructed in NSW although approvals had been obtained for some 450ha.

Approximately 129ha of ponds were stocked and the production results are presented in Table 1. For comparative purposes results for 1986/87 and 1987/88 are included.

Table 1 Production of cultured prawns in NSW – all species

Year	Yield 1 (t/ha)	Survival[1] (%)	Total Prod'n (t/yr)	Pond Area	Harvest Weight[1]
86 – 87	0.23	18	15	NA	34
87 – 88	1.10	59	40	150	22
88 – 89	2.15	65	118	129	22

[1] Average per farm

The high average values for yield and survival were due in part to the excellent results from some of the prawn farms where smaller and more intensively managed ponds were used. the total production of 118 t (an increase of nearly 300% from 1987/88) was worth an estimated $1.2 million. *P. monodon* (89 t) and the school prawn, *Metapenaeus macleayi*, (29 t) were the major species produced. The juvenile school prawns, *Metapenaeus macleayi*, used in the pond trials were collected from the wild.

The production statistics for pond trials with the different species are presented in Table 2. Only those trials where accurate records were available are included, thus not all production is accounted for in this table. Although small numbers of eastern king prawns *Penaeus plebejus* were stocked into ponds in NSW, harvest of this species was negligible.

Table 2 1988/89 Production results – species separated[1]

Species	Weight gain[3] (g/wk)	Survival (%)	Total Prod'n (t/yr)	Yield (t/ha)	FQ[3,4]	Pond Area[3] (ha)	Harvest weight (g)
P. m	1.16	63.9	89.0	1.77	2.2:1	69.4	25.0
M. m	0.55	66.9	17.7	1.58	NA	21.5	7.8
P. e	0.73	11.7	0.2	0.15	17.8:1	1.8	13.5

[1] Only those trials where accurate records were available were included.

[2] P. m = *Penaeus monodon* – data from 39 pond trials.
M. m = *Metapenaeus macleayi* – data from 7 pond trials.
P. e = *Penaeus esculentus* – data from 2 pond trials.

3 Averages per pond.

4 FQ: Weight of dry feed divided by the increase in the wet weight of prawns.

5 Collected as juveniles from capture fisheries and on-grown in ponds.

At several farms the use of more intensively managed nursery ponds for postlarval and early juvenile stages improved production results. For *P. monodon* an average yield of 3.75 t/ha was recorded for the 9 ponds which were stocked with juveniles from nursery ponds compared with only 1.18 t/ha for the 30 ponds where postlarvae were stocked directly into growout ponds.

These encouraging production results from NSW were achieved despite the fact that postlarvae, especially *P. monodon*, were often unavailable in the quantities or at the times required. This was the major reason why a total pond area of only 129ha of ponds was stocked during 1988/89, a reduction of 14% from 1987/88 levels. Furthermore, some ponds were not stocked and one farm not operated in 1988/89 because of financial constraints or corporate restructuring.

In addition to the proble s with availability, the postlarvae which were received were often of very poor quality, and high initial post-stocking mortality was reported at several farms. Other problems related to pond management, for example, dissolved oxygen (DO) crises occurred in several ponds. This led to occasional major crop losses. Overfeeding was a problem at many farms, exacerbating problems with water quality management, especially DO and unnecessarily increasing production costs.

The prospects for a continued rise in production of cultured prawns in NSW in 1988/89 are good. Increased capacity at several local and interstate hatcheries should ensure that supplies of postlarvae are more abundant. In addition, the trend evident at several farms in NSW towards smaller ponds, with improved aeration and water exchange capacity, should alleviate problems with water quality management and, together with a tendency towards the use of nursery ponds, should ensure that production results continue to improve. A total harvest of approximately 250t for 1989/90 is achievable without further pond construction.

In Queensland in 1988 approximately 140ha of ponds were stocked producing an estimated 114.8t of prawns (Curtis, M. personal communication, 1989). However, there are several large prawn farms under construction or planned in Queensland, especially in the Cardwell region, which should result in significant increases in production during the next few years.

In the last ten years the annual world production of cultured prawns has increased dramatically. From 1981 to 1988 the total world production of cultured prawns, as a percentage of total prawns harvested, rose from 2.1% to 22.0%. In 1988 this represented some 450,000t (live weight) out of a 2 million t market (Rosenberry, 1989).

In Australia between 1975/76 and 1982/83 the average annual production of prawns from capture fisheries was nearly 22,000t with an average of approximately 2,500t from NSW (Alexander, 1984). In 1986/87 the catch of 2,100t in NSW was worth an estimated $17 million (Montgomery, 1988). At present the aquaculture component of total prawn production in NSW is relatively minor; 118t in 1988/89.

However, if production increases of the magnitude experienced over the last two years continue, aquaculture will quickly become a major source of production.

Research results

Results from earlier experiments (Allan and Maguire, 1988), as well as from commercial trials, indicated the importance of natural food items in the diet of prawns in ponds. Overseas studies have shown that from 53 to 77% of growth of *P. vannamei* was due to prawns grazing on pond biota during a seven week experiment (Anderson *et al.*, 1987).

The objectives of the study described here were to quantify the effects of pond preparation, using two different feeding regimes, on prawn growth, survival and food conversion efficiency; pond water quality, and the abundance of natural food items within model ponds.

Sixteen 3.5m diameter, 1.2m high fibreglass tanks, each with a bottom area of approximately $10m^2$, were used to simulate prawn farming ponds. All ponds were provided with a 100mm layer of sand, filled with sea – water and fertilized. Using regular inputs of fertilizer and periodic water exchange a phytoplanktonic algal bloom was maintained. After five weeks all ponds were drained and allowed to dry out. One month before stocking eight of the ponds were refilled and managed with regular fertilization and water exchange to maintain a phytoplanktonic algal bloom and to encourage the colonization of bacteria and marine invertebrates (e.g. meiofauna) which may be natural food items for prawns. The remaining eight (unprepared) ponds were filled only two days before stocking, at which time they were also fertilized, and from then on managed in a similar fashion to the prepared ponds.

Juvenile leader prawns. *Penaeus monodon* (average size 4.9g) were stocked at a density of $15/m^2$ and were fed twice daily using an imported Taiwanese prawn feed (42% protein). Feed trays enclosing approximately $0.6m^2$ were checked twice daily. The following feeding regimes were used:

Average prawn weight (g)	Feed added (% prawn biomass/day)	
	High	Low
5.0 – 6.9	8	4
7.0 – 9.9	7	3.5
10.0 – 12.9	6	3
13.0 – 15.9	5	2.5
16.0 – 19.0	4	2

The high regime was similar to that advocated by many of the practical prawn farming manuals (e.g. Pascual, 1985; Clifford, 1985) and was used by several NSW prawn farms during 1988/89. The second rate was similar to that used by the author during other experiments in model ponds. For each feeding regime 4 replicate prepared ponds and 4 replicate unprepared ponds were used. Temperature, DO and pH were measured twice daily and nutrients, (ammonia, oxidised nitrogen, reactive phosphorous and silicon) and plant pigments, (chlorophyll a, b, and c and phaeophytin), were measured frequently. The results from these measurements were used to plan management decisions, e.g. fertilization and water exchange, and each pond was managed separately. A sample of prawns (between 20 and 36) from each pond was weighed in bulk every 2 weeks to monitor growth. The experiment ran

for 10 weeks after which time survival, growth and food conversion efficiency, (FQ) were recorded. FQ is the weight of dry feed divided by the increase in the wet weight of prawns. Pond were aerated during the night for six hours/day. Water exchange was carried out when considered necessary to:

1. dilute excessive algal blooms,
2. reduce the concentration of other suspended organic matter, and
3. help prevent low DO levels.

During weeks 1, 6 and 10 samples of sediment and pond water were taken from each pond to investigate differences in bacterial biomass and productivity and the abundance of meiofauna between treatments.

The major findings of this experiment were:

1. Both prepared and unprepared ponds fed at the higher feeding rate were difficult to manage and, even though water was exchanged more frequently in these ponds, problems with low DO occurred. A DO crisis in one of the ponds resulted in 80% mortality. Survival in the rest of the ponds was excellent (85 – 90%).

2. Pond preparation had a highly significant effect on average prawn weight gain (11.6g/prawn compared with 9.8g/prawn in unprepared ponds). Measurements indicated that the differences in prawn growth (during each two week period) between prepared and unprepared ponds was greatest at two and four weeks, with negligible differences between treatments after this time.

3. Preparation had a significant effect on the abundance of meiofauna (including numbers of copepods, nematodes and protozoans) in the ponds during the first week after stocking. By the sixty week, However, differences between treatments were not so pronounced.

4. Although there was a significant difference between prawn weight gain at the different feeding regimes the increase at the higher rate was minor (10.8g/prawn compared with 10.6g/prawn at the lower rate).

5. Both pond preparation and feed rate significantly affected FQ values. Best results (2.1:1) were recorded for the prepared ponds, fed at the low rate with the worst values (4.7:1) recorded for unprepared ponds, fed at the high rate.

The major implications of this experiment for pond managers were:

1. Adequately preparing ponds well in advance of stocking, by fertilising and exchanging water regularly, can result in increases in prawn growth rates of around 18%.

2. Feed rates can be substantially reduced (by up to 50% in some cases) with little reduction in prawn growth or survival, and improvements in water quality management.

3. Where ponds have been well prepared feeding regimes could be changed. Initial recommended feeding rates could be reduced, when natural food items are abundant, with rates increasing as natural food items are progressively depleted.

4. Increasing feed rates on the basis of empty feed trays is likely to result in overfeeding. When water levels were lowered during the experiment described, uneaten pellets were commonly observed on the pond bottom even though the trays were empty.

Conclusion

The availability and quality of postlarvae has been one of the major restraints to development of the prawn farming industry in NSW. The situation will be alleviated as more hatcheries develop and overcome initial operational difficulties. Some excellent early results this season are already apparent from some hatcheries in southern Queensland and northern NSW. The seasonal shortages in supply of P. monodon broodstock are likely to force commercial hatcheries to invest more effort in stockpiling broodstock and controlling maturation. There is a role for research to assist with improving techniques for controlled maturation.

Over the last few years commercial production of cultured prawns has risen sharply. As farmers improve pond management techniques and increase their ability to manage water quality through aeration and water exchange, production levels should continue to rise. Prawn farming research has assisted with improving pond management techniques and can assist in the future development of the industry in NSW. Research priorities include the development and quantitative assessment of appropriate methods for intensive nursery culture. This is particularly relevant for NSW and southern Queensland, although even in northern Australia intensive nursery culture has some major advantages. The formulation of locally made, nutritionally adequate artificial diets remains a high priority, and further work needs to be done to optimise feeding strategies in ponds. There is also potential for investigating polyculture, especially in NSW prawn farming ponds.

Acknowledgements

These production statistics could not have been compiled without the co-operation of all the prawn farmers in NSW and their assistance is gratefully acknowledged. The part of this paper describing commercial production results will also appear in the magazine Austasia Aquaculture.

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References

Allan, G.L. and Maguire, G.B. 1988. Prawn farming research in model ponds. In: Proceedings of the Australian Mariculture Association, Third Annual Conference, Lismore NSW. June, 1988, pp.88 – 96.

Alexander, J. 1984. Prawns – Australia's most valuable fisheries product. Aust. Fish. 43(4):53 – 57.

Anderson, R.K., Parker, P.L. and Lawrence, A. 1987. A $^{13}C/^{12}C$ tracer study of the utilization of presented feed by a commercially important shrimp Penaeus vannamei in a pond growout system. J. World. Aquaculture Soc., 13(3):148 – 155.

Clifford, H.C.(III). 1985. Semi – intensive shrimp farming. In: Chamberlain, G.W., Haby, M.G. and Miget, R.J. Texas Shrimp Farming Manual, an Update on Current Technology. Texas Agricultural Extension Service, Texas A & M University System Research and Extension Centre.

Curtis, M. 1988. The production status of Queensland mariculture. In: Proceedings of the Australian Mariculture Association, Third Annual Conference, Lismore, NSW. June, 1988, p.16 – 27.

Heasman, M.P. 1984. Marine prawn farming in Australia: Lessons from the past and prospects for the future. In: Pollock, B.R. and Quinn, R.H. (Editors), The Potential of Aquaculture in Queensland. Queensland Department of Primary Industries, Brisbane, pp.1 – 14.

Maguire, G.B. and Allan, G.L. 1988. Progress in the Australian prawn farming industry. Aust. Fish. 47(4):35 – 38.

Montgomery, S. 1988. Trends in New South Wales prawn catch. Aust. Fish. 47(8):24 – 30.

Pascual, F.P. 1985. Nutrition and Feeding of Penaeus monodon. Aquaculture Department, Southeast Asian Fisheries Development Centre, Extension Manual No. 3 (Third Edition), Tigbauan, Iliolo, Philippines, 10p.

Rosenberry, R. 1989. World Shrimp Farming 1988. Aquaculture Digest, February 1989, 17p.

APPENDIX 5.

Allan, G.L. and Maguire, G.B., 1991. Lethal effects of acidified seawater on Penaeus monodon and the interactive effects of salinity and pH on sub-lethal effects. In: P.J.F. Davie and R.H. Quinn (Editors), Proc. 1990 International Crustacean Conference, Brisbane 2-6 July, 1990, Mem. Qld. Museum, 31: 420.

PROCEEDINGS

OF THE

1990 INTERNATIONAL CRUSTACEAN CONFERENCE

BRISBANE
1 SEPTEMBER,1991

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VOLUME 31

LETHAL EFFECTS OF ACIDIFIED SEAWATER ON *PENAEUS MONODON* AND THE INTERACTION OF SALINITY AND PH ON SUB-LETHAL EFFECTS

Acidification commonly occurs in marine aquaculture systems when ponds are built using acid-sulphate soils containing pyrite (Boyd, 1982). Low salinity can accompany low pH in situations where run-off from acid-sulphate sediments in pond dykes enters ponds during heavy rains.

Low pH can predispose prawns to disease and influence the toxicity of other toxins, eg. ammonia and aluminium, and in acid waters crustaceans may experience impaired ionic regulation (Morgan and McMahon, 1982). The aims of this study were to estimate lethal and 'minimum acceptable' levels of low pH for *Penaeus monodon* in acidified water and to investigate the interactive effects of low pH and salinity on prawn weight gain, moulting frequency, dry matter content and haemolymph osmotic pressure.

Static bioassays were conducted in 70 L aquaria with three replicate aquaria, each containing 10 prawns, for all low pH treatments. pH was adjusted using 10 N HCl and all aquaria were lightly aerated (100 mL min^{-1}) to maintain dissolved oxygen levels above 5.0 mg O_2 L^{-1}. The average individual initial prawn weight was between 4 and 6 g for all experiments. To estimate lethal levels a bioassay was run for 96 h with pH levels of 7.8, 7.0, 6.1, 5.1, 4.1, 3.8 and 3.0. Prawns survived well (>90%) at pH levels of 5.1 or above. The 96 h LC_{50} (95% confidence limits) estimated was 3.7 (3.4, 4.1).

To assess sub-lethal effects a longer term (23 d) growth experiment was conducted. Eight treatments were established: six at 30 ppt with average pH values of 7.8, 7.3, 6.7, 6.1, 5.5 and 4.9 and two others at 15 ppt with average pH values of 7.8 and 5.5. The effects of treatment on survival rate were not significant ($P>0.05$). Other performance data were analysed using single factor ANOVA (including all eight treatments) or using two factor ANOVA (including data from treatments at pH levels 7.8 and 5.5 for both salinities, 15 and 30 ppt. Growth was depressed at pH ≤ 5.5 at 30 ppt ($P<0.05$) and although salinity did not affect growth ($P>0.05$) there was a significant pH/salinity interaction ($P<0.05$). The absence of a significant difference in growth between 15 and 30 ppt was surprising as prawn farmers in Australia and overseas place great emphasis on maintaining low salinity levels in *P. monodon* ponds. This does not appear necessary in terms of the physiological requirements of *P. monodon* although low salinity could influence other biotic components of pond ecosystems.

The 'minimum acceptable' level was defined as that level which reduced growth by 5% (the EC_5) and was estimated, using two-phase linear regression analysis (Sedgwick, 1979), as being 5.9 pH units for *P. monodon* at a salinity of 30 ppt.

Moulting frequency was highest at pH 4.9 (30 ppt) and was inversely related to salinity, while the interaction was not significant ($P>0.05$). The dry matter content was depressed at pH 4.9 (30 ppt) but unaffected by salinity or the interaction ($P>0.05$).

Juvenile *Penaeus monodon* are efficient osmoregulators in the range 15 to 30 ppt with an isosmotic point of between 23 and 25 ppt (Cawthorne *et al.*, 1983). However, a reduction in internal osmolarity at reduced pH had been recorded for a number of freshwater crustaceans and fish (Morgan and McMahon, 1982; Hobe *et al.*, 1983). To investigate whether changes in osmotic pressure might explain the pH/salinity interaction prawns were exposed to combinations of two pH (7.8 and 5.6) and salinity (15 and 30 ppt) levels for three days; sufficient time for osmotic and ionic equilibrium to be reached. At the end of the experiment osmotic pressures in the water and prawn haemolymph were measured and the difference between these two values (Dop) calculated as an indication of osmoregulatory ability. At both salinities (15 and 30 ppt) haemolymph osmotic pressure was closer to ambient osmotic pressure at reduced pH (5.6). Both salinity ($P<0.001$) and pH ($P<0.01$) significantly reduced D_{OP}, and there was no interaction ($P>0.05$). Although the results of this experiment showed that reduced pH lowered osmoregulatory ability in *Penaeus monodon*, they did not confirm the hypothesis that differences in osmoregulatory ability were responsible for the interaction between pH and salinity on weight gain. The interactive effects of pH and salinity on ionic regulation may warrant further investigation.

The estimation of lethal and sublethal low pH levels for *P. monodon* (3.8 and 5.9 pH units respectively) should assist prawn farmers with the management of acidic ponds.

Literature Cited

Boyd, C.E. 1982. 'Water quality management for warm water fish culture'. Developments in Aquaculture and Fisheries Science, Vol. 9. (Elsevier: Amsterdam). 318p.

Cawthorne, D.F., Beard, T., Davenport, J. and Wickins, J.F. 1983. Responses of juvenile *Penaeus monodon* Fabricius to natural and artificial sea waters of low salinity. Aquaculture **32**: 165–174.

Hobe, H., Wilkes, P.R.H., Walker, R.L., Wood, C.M. and McMahon, B.R. 1983. Acid-base balance, ionic status, and renal function in resting and acidexposed white suckers *(Catostomus commercom)*. Canadian Journal of Zoology **61**: 2660–2668.

Morgan, D.O. and McMahon, B.R. 1982. Acid tolerance and effects of sublethal acid exposure on iono-regulation and acid-base status in two crayfish *Procambarus clarki* and *Orconectes rusticus*. Journal of Experimental Biology **97**: 241–252.

Sedgwick, R.W. 1979. Influence of dietary protein and energy on growth, food consumption, and food conversion efficiency in *Penaeus merguiensis* De Man. Aquaculture **16**: 7–30.

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APPENDIX 6.

Allan, G. and Maguire, G.B., 1987a. Water quality parameters to watch in prawn farming. Austasia Aquaculture Magazine, 2(5): 5-7.

WATER QUALITY PARAMETERS TO WATCH IN PRAWN FARMING

2.5.3 In marine prawn farming ponds, and indeed all other aquaculture installations, the maintenance of adequate water quality is essential to ensure optimum survival and growth of the animals being cultured. The water quality of a body of water is determined by a complex interaction of physical, chemical and biological parameters. Some of the more important of these are listed in Table 1.

Fortunately for most practical aquaculture situations, measurement of only a few of these parameters is sufficient to give the farmer some indication of the processes which are occurring in the pond, the suitablility of the pond water for the animals being cultured and the information necessary to devise water quality management strategies. Some of these parameters and methods used for their measurement are discussed below. Emphasis has been placed where possible on methods which can be used by aquaculturists without access to sophisticated laboratory equipment.

Different species often have quite dissimilar lethal limits and optimum ranges for water quality parameters. In this article, the levels determined for the jumbo tiger prawn *(Penaeus monodon)* are given where available. Implications for pond management are briefly discussed.

A list of some suppliers of water quality analytical products is given in 2.5.4. This list is not intended to be exhaustive and as few of the products have been tested by the authors, no endorsements of any product can be inferred.

1. Temperature & Salinity

For *P. monodon* postlarvae upper and lower lethal temperatures have been reported as being 39-42°C and 7-9°C [1*]. The optimum temperature for maximum growth was determined as 27-33°C [2] whilst reported optimum salinities range from 10-25% [3].

Temperature can be easily and accurately measured using a thermometer although many water quality meters have a built in thermister. Salinity may be measured using a refractometer, salinometer or conductivity meter or using a hydrometer with a temperature correction table.

Information on temperature and salinity regime is essential when choosing a prawn farming site and when determining which species are to be cultured during different times of the year. Rainfall data is available from the Bureau of Meteorology and is very useful when assessing the severity and duration of both wet and dry seasons [3].

2. Dissolved Oxygen

This is the most critical, production limiting, water quality parameter. A lower lethal level for *P. monodon* juveniles was estimated as approximately 0.9 ppm [4] with a critical

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level (that level below which growth is reduced) of between 1.2 and 2.2 ppm over 16 days [5]. Dissolved oxygen may be measured using an oxygen meter although for reliable measurements to be taken it is essential that meters are regularly calibrated. Winkler's titration may be as an alternative or for calibrating meters [6]. Dissolved oxygen test kits which employ this method are available, although it is more time consuming than using a dissolved oxygen meter and consequently less popular among prawn farmers.

In a pond, dissolved oxygen is highest during the day, usually peaking in the late afternoon, following photosynthesis by algae in the pond. The more dense the algal "bloom" the higher the dissolved oxygen readings. These increases are moderated by overcast weather. Dissolved oxygen will decline during the night as algae consume oxygen through respiration. Many other organisms in the pond also consume oxygen and the prawns themselves may be only minor consumers.

When there is a large input of organic material, e.g., following prolonged overfeeding or the deposition of a lot of dead algal material, consumption of oxygen by bacteria which decompose this material (microbial decomposition) can lead to a serious depletion in the dissolved oxygen particularly when this situation coincides with hot still nights. The provision of artificial aeration, e.g., paddle wheel aerators, or rapid water exhange, can alleviate problems with reduced dissolved oxygen to some extent, however it is essential that ponds are managed to prevent build-up of excess organic matter. The stimulation or maintenance of a moderate algal bloom will help maintain adequate dissolved oxygen levels.

3. Hydrogen Sulphide (H^2S)

This is a gas produced when oxygen has been depleted and anaerobic microbial decomposition occurs and even low levels (0.1 ppm) are reported to be stressful to prawns [7]. The presence of H^2S is easily detected by the characteristically black sediment and a strong 'rotten egg' odour. Preventing the build-up of excess organic matter on the pond bottom is the best way to prevent problems with H^2S however between farming trials draining the pond and allowing pond bottom sediments to completely dry out will reduce problems. Applications of agricultural lime ($CaCO^3$) to the dry sediment are also considered useful.

4. pH

Problems with acidic conditions, (low pH), in prawn farming ponds can occur especially where acid supihate soils have been used. A lower lethal pH of approximately 3.7 was estimated [4] for *P. monodon* while growth was reported to cease below a pH of 5 for penaeid aquaculture in acid sulphate soils in Costa Rica [8]. pH may be measured using commercially available pH meters or pH paper strips although the reliability of pH paper should be checked.

Sediments can also become acidic if they contain large stores of slowly decaying organic matter. In ponds acidic conditions also reduce the availability of carbon dioxide and other nutrients, e.g., phosphorous, needed to stimulate an algal bloom. Applications of agricultural lime ($CaCO^3$) can be used to raise pH and therefore increase the availability of carbon dioxide and nutrients for photosynthesis [9]. Fertilization can then be used to stimulate algal growth.

pH measurements also a very useful indication of algal activity in ponds. As algae photosynthesise during daylight hours, carbon dioxide (CO^2) is consumed and pH increases. When a very dense algal bloom exists in a pond pH can rise above 9.2 [10]. Although reduction in prawn growth at these high pH levels has not been recorded, very dense algal blooms can quickly deplete available nutrients and rapidly die leading to deposition of excessive organic matter on the pond bottom which may lead to a dissolved oxygen crisis. Rapid water exchange can be used to dilute excessive algal blooms.

5. Ammonia

Prawns and other aquatic animals excrete ammonia directly and it can also accumulate following decomposition of organic matter. Ammonia exists in solution in two forms, a highly toxic unionised (free) form and a less toxic ionised form. The proportion of each is determined chiefly by the pH and also by temperature and salinity. An upper lethal level of approximetely 37mg NH^3-N total ammonia/l (1.8mg NH^3-N unionised ammonia /l at pH = 8.0, temperature = 27.0°C and salinity 33%⁰) was estimated with growth being reduced above about 4.1mg NH^3-N total ammonia/l (1.8mg NH^3-N unionised ammonia/l at pH =8.0, temperature = 27°C and salinity = 33%⁰) over three weeks [4].

Ammonia can be measured using ion-selective electrodes and a mV meter or using test kits designed for salt water. In a laboratory more sophisticated spectrophotometric analysis generally used. In natural systems ammonia is converted through nitrite to nitrate by certain groups of bacteria in the sediment and is also assimilated directly by algae. In a well managed pond it is unlikely that ammonia will build up to serious levels.

As pH rises the proportion of ammonia as toxic unionised ammonia increases but this should pose few problems for aquaculturists. This is because pH rises in response to increased algal activity which should ensure that the total amount of ammonia declines rapidly as it is assimilated by the algae.

Conclusions

In conclusion it is important that a prawn farmer have some understanding of the complex interactions that can occur in ponds. However, by measuring only a few parameters on a regular basis it is possible to obtain a reliable indication of the water quality and to formulate appropriate pond management strategies designed to prevent crisis situations occuring. The most important task is to prevent adversely low dissolved oxygen levels from occurring.

One of the major strategies used by prawn farmers to control water quality is the stimulation and maintenance of a moderate algal bloom. A phytoplanktonic algal bloom, consisting predominantly of single celled plants suspended in the water column, serves several roles:

a) Maintenance of dissolved oxygen
b) Assimilation of Ammonia.
c) Reduction in light penetration restricting the growth of benthic (bottom dwelling) algae and reducing predation by wading birds.
d) Serving as the basis for a food chain involving bacteria and other pond fauna which could constitute natural food items for prawns.

An algal bloom is often initiated by a single large input of fertilizer, e.g. 100kg/ha each of Nitram and DAP (diammonium phosphate) and maintained by the addition of relatively small quantities of fertilizers, e.g., 2 kg/ha each of Nitram and DAP and through water exchange. If this management strategy is adopted it is crucial that regular measurements of dissolved oxygen and pH are made (preferably in the morning and again in the late afternoon). This allows early indications to be obtained of a decline in an algal bloom and hence an indication of when fertilizer needs to be added. Also this information will help prevent an excessive bloom from

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developing. Some typical measurements of dissolved oxygen and pH in a pond with a moderate algae bloom might be 5 ppm and 7.8 in the morning and 10 ppm and 8.5 in the later afternoon [10].

Secchi-disc depth can also be a useful indication of the density of an algal bloom. Heavily stocked ponds usually require more intensive water quality management [11]. The provision of emergency artificial aeration is recommended for all ponds, and is essential for more intensively stocked ponds, as an insurance against depletion of dissolved oxygen.

Source: Article by Geoff L. Allan (Biologist) and Greg B. Maguire (Research Scientist), Department of Agriculture, NSW (Division of Fisheries), Brackish Water Fish Culture Research Station, Salamander Bay, NSW 2301.

***REFERENCES**

1. **Motoh, H. 1981.** Studies on the fisheries biology of the giant tiger prawn, *Penaeus monodon* in the Philippines. Tech. Report No. 7, SEAFDEC Aquaculture Department, Iloilo, Philippines, 128 pp.

2. **Maquire, G.B. and Allan, G.L.** (Unpublished data)

3. **Maguire, G.B. and Allan, G.L. 1987** Prawn farming in Australia. Status and Problems. In: Prawn Farming for Profit. Potential for Success. Seminars presented by SCP Fisheries Consultants Australia Pty Ltd, pp 13 - 28.

4. **Allan, G.L., Hopkins, S.J. and Maguire, G.B.** (Unpublished data).

5. **Seidman, E.R. and Lawrence, A.L., 1985.** Growth, Feed, Digestibility and Proximate Body Composition of juvenile *Penaeus vannamei* and *Penaeus monodon* grown at different dissolved oxygen levels. J. World Maricul. Soc. 16: pp 333-346.

6. **American Public Health Assoc. (APHA), 1971.** Standard methods, 13th Edition, APHA, Washington DC, 874 pp.

7. **Apud, F.D., Primavera, J.H. and Torres, P.L. (Jr.)**, 1985. Farming of Prawns & Shrimp. 3rd Ed. Aquaculture extension manual No. 5. Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department, 67 pp.

8. **Webber, R.J. and Webber, H.H., 1978.** Management of acidity in mangrove sited aquaculture. Rev. Biol. Top 26 (Sulp. 1): pp 45-51.

9. **Boyd, C.E.** Water Quality Management for Pond Fish Culture. Developments in Aquaculture & Fisheries Science, 9 Elsevier. 318 pp.

10. **Maguire, G.B. and Allan G., 1985.** Development of methods fro growing juvenile school prawns, *Metapenaeus macleayi*, in estuarine ponds. In P.C. Rothlisberg, B.J. Hill and D.J. Staples (Editors), Second Aust. Nat. Prawn Sem., NPS2, Cleveland, Australia, pp 337-351.

11. **Maguire, G.B. and Allan, G., 1986.** A Guide to Marine Prawn Farming in New South Wales. Information leaflet. Department of Agriculture, N.S.W., 26 pp.

Table 1

LIST OF PARAMETERS WHICH INFLUENCE WATER QUALITY

- Temperature
- Salinity
- Dissolved oxygen
- Biological oxygen demand (BOD)
- Chemical oxygen demand
- pH
- Alkalinity
- Nutrient content, e.g.,
  - *ammonia (NH^3)*
  - *nitrite (NO^2)*
  - *nitrate (NO^3)*
  - *phosphate (PO^4)*
  - *silica (Si)*
- Plant pigments, e.g.,
  - *chlorophylls a, b and c*
  - *Phaeophytin*
- Primary productivity
- Suspended organic matter
- Sediment condition, e.g.
  - *oxidation reduction potential*
  - *hydrogen sulphide (H^2S)*
  - *minerals, e.g., iron (Fe)*
  - *humic compounds*
- Micro-organisms
- Other pond fauna inc. animals being cultured
- Wind/solar radiation

SOME SUPPLIERS OF ANALYTICAL PRODUCTS

2.5.4. The following list should not be taken as an endorsement of these companies or their products by the authors or by the editor of AustAsia Aquaculture Magazine.

Activon Scientific Products Co Pty Ltd
2A Pioneer Avenue
PENNANT HILLS, NSW 2120.
Tel: (02) 875 2799

B.D.H. Chemicals
242 Beecroft Road
EPPING NSW, 2121.
Tel: (02) 869 7055
Suppliers of Merck products.

CHK Engineering Pty Ltd
1 Jordon Street
(PO Box 174)
GLADESVILLE NSW, 2111.
Tel: (02) 816 2611
Suppliers of CHK pH & conductivity meters.

Doobie Instruments
PO Box 87
SANDRINGHAM, VIC 3191.
Tel: Vic (03) 598 8244; NSW (02) 789 6533; Qld (07) 394 1355; NT (089) 81 5822; SA (08) 352 2000.
Suppliers of Doobie Instruments and Solomat portable instruments.

Extech Equipment Pty Ltd
8 Stirling Cresent
SURREY HILLS, VIC 3127.
Tel: (03) 830 1588
Agents for Cole Palmer equipment.

Foss Electric (Aust.) Pty Ltd
14 William Street
BROOKVALE, NSW 2100
Tel: (02) 938 3244
Suppliers of Radiometer products.

I.C.I. Australian Operations Pty Ltd
Scientific Instruments Division
117 Victoria Street
WEST END, QLD 4101
Tel: (07) 840 5444
Suppliers of I.C.I. pH & Electro-chemistry meters.

Imbros Pty Ltd
GPO Box 917
HOBART, TAS 7000
Tel: (002) 34 9899
Suppliers of W.T.W. products

Instrument Engineering Pty Ltd
PO Box 168
WEST RYDE, NSW 2114.
Tel: (02) 852 457
Agents for Horiba scientific instruments.

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APPENDIX 7.

Maguire, G.B., 1987. First Australian prawn farming research workshop. Austasia Aquaculture Magazine, 2 (6): 14-15.

FIRST AUSTRALIAN PRAWN FARMING RESEARCH WORKSHOP

2.6.9 This workshop was held at the New South Wales Department of Agriculture's Brackish Water Fish Culture Research Station at Salamander Bay in August 1987. Approximately 70 research scientists, biologists from commercial farms, nutritionists from feed companies and representatives from farmer organisations accepted invitations to this inaugural workshop. Several sessions each dealing with a separate area of research were held and the format involved an address from a leading researcher in that particular field followed by up to 45 minutes of lively discussion among participants.

The research carried out by several of the speakers was primarily aimed at understanding the requirements and dynamics of natural prawn populations but frequently these studies contributed information relevant to aquacultural situations. Each session was chaired by another scientist whose work was relevant to the specific topic and these participants played a major role in contributing to and directing the discussion arising out of the overview papers.

Dr Greg Maguire from the Research Station presented an introductory address which indicated that while significant progress had been made in Australian hatcheries, postlarval quality and supply were still often inadequate. These factors were thought to be a major cause of the disappointing results for the farming of leader prawns *(Penaeus monodon)* in NSW during the 1986/87 summer. Summaries of hatchery and pond results are given in Table 1. He also indictaed that there was a major need for research workers to rapidly increase their knowledge of aquaculture if they were to be of major assistance to the industry or were to evaluate aquaculture research grant applications. The need for cooperative projects which would often involve more than one research group or organisation as well as input from the industry itself was emphasized. The fact that many farms are now employing graduate biologists could be seen as extremely conductive to cooperative research.

The address on maturation was given by Dr Peter Crocos (CSIRO Marine Laboratories, Cleveland), who battled against a tropical downpour with conditions resembling Noah's flood. He emphasized that captive maturation involved the interaction of hormonal, environmental and nutritional factors as well as species specific patterns. Reports of hormonal injections inducing maturation were noted however in the short term eyestalk ablation was considered likely to be the major maturation technique used in commercial hatcheries. Crustacean hormonal systems are very different indeed to vertebrate systems and maturation is usually controlled by a gonad inhibiting hormone release from the eyestalk. In the longer term research may lead to the development of an analogue for this hormone which would take away the need for eyestalk ablation. The need for more research on male maturation was also indicated. There was considerable discussion of the cost effectiveness of alternative systems for heating the large, e.g. 5m diameter, shallow, indoor maturation tanks that are often used.

The topic of larval rearing was covered by Dr Peter Rothlisberg of CSIRO. Again it was emphasised that the different species of penaeid prawns often had different environmental requirements but that in general high water temperatures (27-29°C) and high salinity levels (32-34°/oo) were preferable. Food requirements warrant considerable attention both as a research topic and because of the high workload in hatcheries associated with larval food production. The suitability of food items is affected by both physical (size) and nutritional characteristics. Interestingly mysis stage larvae can be very active algal consumers. Ms Gay Marsden of South Pacific Hatcheries at Yamba, NSW, reported that simple nutrient media seemed to be adequate for large scale algal production and that photoperiod manipulation shows promise. Effective methods for sorting algae for later use, e.g., slurrying, freezing, cryopreservation and freeze drying need to be investigated. Microparticulate diets seem likely to become more widely used in the future. It was suggested that hatcheries need to use larval size rather than age when assessing the rate of development of postlarvae.

Ms Jan Paynter of the University of Queensland discussed the importance of diseases in aquaculture. In general she considered that the incidence of prawn diseases was usually related to inadequate management or environmental conditions. A range of diseases and causative organisms were discussed including bacteria *(Vibro, Leucothrix, Micrococcus)*, fungi *(Lagenidium)*, ciliates (peritrich and apostome ciliates) and viruses. The only common prawn viral problem in Australia, commonly termed Plebejus Baculovirus, has been associated with mortality of larval and postlarval prawns on several occasions. Extended discussion took place in relation to the most appropriate policy for responding to and containing this disease but not a great deal of concensus was achieved on these issues. More data was obviously required on the mode of transfer and pathogenicity of this virus.

Prawn nutrition was the topic of a paper presented by Dr Bill Dall of CSIRO. He indicated that the common impression of prawns as detritus consumers is being revised as more evidence becomes available on the predominantly carnivorous nature of at least several species. The protein requirements of prawns are relatively high and although carbohydrate can be useful in sparing protein it is not possible to include high levels of fat in the diet to reduce the amount of protein used for energy production. Prawns have quite unusual lipid requirements in terms of essential lipids and it is usually necessary to include a marine lipid source e.g., fish oil, to satisfy these requirements. Starvation trials indicated that some amino acids which are generally considered as non-essential seem to play important metabolic roles. However supplementation of diets with one of these amino acids, proline, has not so far increased prawn growth rates. His team has had some success with inhibiting the leaching of soluble nutrients from prawn diets at least on an experimental scale. The nutritional superiority of some Taiwanese and Japanese diets may well be due to the inclusion of very expensive marine mollusc or crustacean meals, e.g. squid meal.

Two sessions dealt with marine ponds as habitats for prawns and in the first of these Mr Geoff Allan, Dept. of Agriculture NSW, discussed the environmental requirements of a range of species with emphasis on leader prawns. He presented the results of numerous bioassay and physiological studies which indicated the optimum or

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acceptable levels of several water quality parameters including temperature, salinity, dissolved oxygen, pH, and dissolved nitrogenous compounds, e.g. ammonia. The importance of sediment condition and composition was also considered in terms of acidity, redox potential and pesticide content. These numerous factors were discussed in relation to pond management practices.

In the next session considerable interest was generated by Dr David Moriarty of CSIRO who used partly hypothetical models to indicate how ponds function in terms of providing energy (carbon) for prawn production. One interpretation of the models is that two general pathways could be followed although these did interact. These were: (1) phytoplankton to zooplankton, meiofauna and macrofauna and finally to prawns and (2) phytoplankton to benthic detritus to bacteria to meiofauna and finally to prawns. His model suggested that the former may be more important and that the conditionswhich favour high bacterial densities e.g., large amounts of benthic organic matter (high carbon to nitogen ratio), could well cause excessive demand for oxygen and hence prawn mortality. As a great deal of carbon is lost as carbon dioxide during respiration there is a considerable energy loss for each step in a food chain, so clearly the efficiency of a pathway will depend on the number of steps in that pathway from phytoplankton or detritus to the final consumer (preferably prawns but possibly also certain predatory fish).

Dr Jim Stoddart of the Australian Institute of Marine Science (AIMS) at Townsville outlined that organisation's plans for a prawn genetics research program and emphasised the role that commercial farmers could play in ensuring that after different genetic strains were identified, sufficient numbers of prawns from these different genetic strains could be maintained and evaluated.

The final session of the workshop was aimed at identifying research priorities and this was chaired with considerable wit by Dr John McIntyre of the University of NSW. It was obvious that many commercial operators had a short term need for labour saving devices and improved technology, e.g., automated larval counters, while research workers were often more interested in longer term goals arising out of an understanding of how biological systems function, e.g., larval cultures and ponds. This is not to say that the importance of both types of work was not appreciated by both groups. Some 25 technical, research or advisory topics were suggested as requiring greater attention and the following were considered by many participants to be of major importance.

1. Bacterial dynamics in larval tanks.

2. Development of methods and advisory material for improving yields from prawn farming ponds. (Improved dissemination of prawn farming information in general was seen as a major goal).

3. Prawn nutrition.

4. A national prawn diseases policy.

In general the response of the participants to the workshop was very encouraging and planning has commenced for a second workshop to be held at Griffith University in Brisbane during winter 1988. The organisers for that workshop will be Dr Bill Dall (co-organiser of the inaugural workshop) and from Griffith University, Dr Angela Alkington and Dr Nigel Preston.

This summary was compiled by Dr Greg Maguire (co-organiser of the inaugural workshop) and Mr Geoff Allan. The reports provided by the various reconteurs for the individual sessions and by Mr Kevin Tarbey, Tasmanian State Institute of Technology, Launceston, were most useful. More comprehensive summaries will be distributed to the workshop participants.

Source: Article written by Greg Maguire, NSW Dept. ofAgriculture, Brackish Water Fish Culture Research Station, C/- P.O., Salamander Bay, NSW 2301. Tel: (049) 82 1232, Fax: (049) 82 1107.

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Jeffery, S.W. and Garland, C.D. 1987. Mass culture of micro-algae essential for mariculture hatcheries. Australian Fisheries, Vol. 46 No. 5, May 1987.

Lewis, T.E., Garland, C.D. and McMeekin, T.A. 1986. Manual of hygiene for shellfish hatcheries. University of Tasmania, Hobart, 45pp.

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Fisheries now undertake routine but comprehensive sampling programs in southern Tasmania (see figure). This program was partially funded by a Fishing Industry Research Trust Account (FIRTA) grant.

Unacceptably high toxin concentrations have been detected in commercial mussels *(Mytilus edulis planulatus)* and Pacific oysters *(Crassostrea gigas)*, while high concentrations have been also detected in the tissues of wild queen scallops *(Equichlamys bifrons)*, doughboy scallops *(Mimachlamys asperrimus)* and commercial scallops *(Pecten fumata)*.

Shellfish Farm Closures

Earlier this year several shellfish farms were closed in the southern waters of Tasmania (see maps), due to the presence of the toxic dinoflagellate, *Gymnodinium catenatum*. However after a few months they were reopened as the toxin had completely disappeared from the shellfish.

A tempory ban on the transfer of shellfish stocks from one area to another was introduced to prevent the spreading of the dinoflagellate or its benthic resting spore.

These programs will ensure that health risks are avoided and the quality of the Tasmanian shellfish product guaranteed.

Sources: Extracts from Gustaaf Hallegraeff and Colin Sumner, "Toxic plankton bloom affect shellfish farms", Australian Fisheries, Vol. 45 No. 12, December 1986; and 2. Gustaaf Hallegraeff, "Red tides in the Australasian region", CSIRO Marine Laboratories Report, 187, 1987.
Maps and figures courtesy of Gustaaf Hallegraeff, CSIRO Marine Laboratories, GPO Box 1538, Hobart Tas. 7001. Tel: (002) 20 6311.