MARICULTURE NUTRITION

FINAL REPORT

TO

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G B MAGUIRE, JOHN A NELL & IAN R SMITH

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FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT (FIRTA 84/67)

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FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

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SECTION 1

INTRODUCTION

1.1 Scope of project

Virtually all of the research proposed in the original "Mariculture nutrition" grant application (1984-1986) and in the application to extend the grant (1986-1987) has been undertaken. (One experiment proposed in the 1986/1987 progress report was not conducted after a cooperating feed company failed to supply the promised International Crustacean Nutrition Project diets.)

The major objectives of the grant were as follows:

(a) The development and evaluation of supplementary prawn diets to be used in prawn farming ponds.

(b) The development of microencapsulated artificial oyster diets for larval and juvenile oysters.

In addition, several related topics have been investigated. Considerable attention has been given to utilising soluble nutrients (amino acids and minerals) when feeding oysters, particularly planktonic larvae approaching the settling phase when they become attached to a hard substrate. More emphasis has been placed on optimising environmental conditions for prawns and oysters than originally proposed in the 1984/1985 application. For oysters we chose to assess these environmental parameters, e.g., temperature and salinity, in combination with another important environmental factor, i.e., fluoride concentration. As indicated in the proposal to extend the grant for 1986/1987, it was possible to utilise the Technical Officer employed on this grant to investigate nutritional factors in oysters on a commercial, estuarine oyster farming lease rather than only in experimental systems. Again we chose to conduct this work in combination with another variable of interest to the oyster industry, i.e., intertidal growing height and hence the incidence of an oyster disease (winter mortality).

As a result of these initiatives, this research grant report encompasses a wide variety of research. In addition to direct nutritional studies involving artificial diets fed to prawns and oysters, research has been carried out on a range of associated topics. These include:

(a) The effects of dissolved nutrients and toxins in seawater on oysters.

(b) The interactive effects of the type of diet and stocking density of prawns in ponds.

(c) The effects of water quality factors on prawns and oysters particularly in relation to feed intake at different temperatures.

(d) Changes in indices of the nutritional condition of oysters in relation to intertidal growing height and winter mortality.

The relevance of these individual components of the total project to the overall goal of increasing our understanding of oyster and prawn feeding and nutrition is quite clear. However, as this is a diverse set of topics, we have decided to discuss below how each component relates to the research station's overall program for examining the factors which affect oyster and prawn growth and survival rates. Furthermore, this overall program includes several separate projects that have been funded through F.I.R.T.A. The interrelationships among these F.I.R.T.A. projects have been emphasised.

1.2 Overview of research station's prawn and oyster research programs

1.2.1 Temperature

Oysters and prawns are farmed commercially in situations where growth and survival rates can be affected by a range of environmental factors. To interpret growth data and to improve production results it is necessary to have some understanding of the effects of these environmental variables. As both prawns and oysters are ectotherms, water temperature is a key environmental variable. Previous research by one of the authors indicated that water temperature had a major effect on the uptake by Sydney rock oysters (*Saccostrea commercialis*) of a soluble amino acid, L-methionine from seawater (Nell and Dunkley, 1984). In the present study the effects of water temperature on the growth rates of Sydney rock and flat oysters (*Ostrea angasi*) were investigated (Section 3.3.1 of this report). As some species of oysters are usually grown intertidally, air temperature can also be an important consideration. The tolerances of Sydney rock oysters and Pacific oysters (*Crassostrea gigas*) to a range of air temperatures was studied as part of the F.I.R.T.A. funded "Pacific oyster" project.

Observations made during experimental prawn farming trials in ponds at this research station indicated that food consumption rates are affected by the water temperatures that prawns are exposed to. Studies were undertaken in aquaria (Section 2.2) to more precisely describe the effects of different water temperatures on food consumption, growth and food conversion efficiency for school (*Metapenaeus macleayi*), eastern king (*Penaeus plebejus*) and leader prawns (*Penaeus monodon*).

Growth studies in estuaries and in ponds are of major interest to industry but experiments in aquaria are far more easily controlled and interpreted. For these latter studies it is preferable to provide favourable environmental conditions at least for control groups. This is particularly important for feeding studies as the nutritional requirements of slow growing prawns may differ from those of faster growing individuals. Hence information is again required on environmental requirements and in the case of eastern king prawns it was even necessary to define the water temperatures which would simply allow high survival rates and prevent serious disease problems (Section 2.2).

1.2.2 Salinity

As oysters and prawns are farmed commercially in estuarine areas, salinity can be another key environmental variable. Again salinity has been shown to affect soluble nutrient uptake (Nell and Dunkley, 1984).

In this project the tolerances and osmotic responses to a range of salinity levels were investigated for several bivalves including Sydney rock and flat oysters (Section 3.2). In addition, the effects of salinity on the growth rates of these oysters were measured (Section 3.3). Similarly the osmotic and growth responses of school prawns to a range of salinities were also studied (Section 2.3).

1.2.3 Trace minerals and contaminants

Growth and other physiological parameters are not only affected by natural fluctuations in environmental variables but also through contamination of estuaries by potentially toxic substances. The effects of high levels of copper (Section 3.8) and fluoride (Section 3.3.1) on some of these parameters (growth, survival and accumulation of potential toxins) have been investigated for Sydney rock or flat oysters. At low concentrations, trace minerals can be considered as nutrients and in particular, potassium has been identified as a useful nutrient for encouraging Sydney rock oyster larvae to undergo the desirable process of settling and attaching to oyster spat collecting materials (Section 3.8).

Other aspects of oyster larval settlement in hatcheries and in estuaries are being covered by research station staff in the ongoing F.I.R.T.A. funded "Nursery culture" project and in privately funded research (McOrrie, 1986). This latter research has now attracted F.I.R.D.C. funding as "Oyster Settlement and Recruitment Study".

In relation to prawn farming a more pressing concern is the accumulation of organochlorine pesticides by prawns grown in ponds constructed using soils previously used for agriculture. That study was conducted as another component of the research station's overall approach to research on prawn and oyster biology and husbandry.

1.2.4 Other water quality factors in ponds

A range of water quality factors other than temperature, salinity and pollutants can affect prawns and oysters, particularly when grown in nutrient enriched ponds. The changes which occur in water quality during prawn farming pond trials have been documented in an earlier F.I.R.T.A./Reserve Bank grant report ("Clarence River pilot scale prawn farming"). Subsequently detailed investigations using a bioassay system were undertaken to assess the effects of different levels of key water quality parameters, either singly or in combination, on prawns. This work is a part of the ongoing F.I.R.T.A./F.I.R.D.C./Reserve Bank funded "Prawn farming pond management" project. The other part of that project involves setting up different pond management treatments (including stocking density and feed input treatments) in replicated pools and determining treatment effects on prawn growth and survival rates, water quality and pond biota. Some of the initial work which led to the development of this experimental system was conducted during the "Mariculture nutrition" project (Section 2.6.2).

Some initial work on the production of oysters in ponds was carried out by two of the authors (Maguire et al., 1981; Nell and Wisely, 1984). However, a considerable amount of work remains to be done on the effects of water quality variables on oysters, e.g., tolerance to high pH levels during algal blooms. Moreover, it is likely that nutrient enriched estuarine ponds will be important nursery sites for ongrowing hatchery spat to a size at which commercial oyster farmers can place them on estuarine leases (Holliday, 1985). Further development of this and other nursery systems in being conducted in the ongoing F.I.R.T.A. funded "Nursery culture" project and as a part of the operational procedures for the research station's large scale oyster hatchery.

1.2.5 Stocking density

The growth rates of animals in aquacultural systems are usually sensitive to the density of animals in the system. Earlier research by one of the authors led to a mathematical description of a density-growth relationship for school prawns (Maguire and Leedow, 1983) and the hypothesis that this inverse relationship was largely due to competition for natural food items in the ponds. The relationship was further investigated during this project using factorial-type studies involving combinations of supplementary diet types and stocking densities (Section 2.5). The appropriateness of a diet may depend on the density at which a particular species is stocked. This work was carried out in netting enclosures within ponds (pens). In contrast to pool trials (Section 2.6.2), the effects of different treatments on water quality tend to be negated in that type of experimental system. Promising results at high density treatments led to an additional density experiment involving a range of high stocking density treatments (Section 2.5). Following communication of these results to industry (Maguire and Allan, 1987), commercial high density trials with school prawns were undertaken by farmers.

The effects of stocking density on the growth of oyster spat in estuarine nursery trays was the subject of research conducted by Holliday and Nell (1988).

1.2.6 Natural productivity and food intake

One of the most influential factors affecting the growth rates of cultured aquatic animals is the supply of food. Food items can arise from the natural productivity of the farming environment, i.e., chiefly in natural, estuarine areas for oysters and in man-made ponds for prawns, or from direct inputs of supplementary feed, e.g., pelleted prawn diets. The potential for natural productivity in ponds to contribute to the food intake of prawns was demonstrated in earlier research (Maguire and Bell, 1981; Maguire and Leedow, 1983). Some of the nutrient pathways involved in the transfer of carbon and nitrogen from algae within ponds to prawns should be elucidated in the "Prawn farming pond management" project mentioned previously. The residual organic content of filtered seawater in hatcheries has proved to be a quite significant source of nutrients for Sydney rock oyster larvae (Section 3e).

1.2.7 Supplementary feeding

A considerable number of dietary studies involving supplementary diets for oysters and prawns were conducted by research station staff both prior to and during this project. While dietary composition has been the major research topic, the appropriate quantity and form of presentation of the feeds are also important factors and these were investigated by Maguire and Leedow (1983) for prawns and Nell and Wisely (1984) for oysters.

Initial research on the formulation of artificial diets for Sydney rock oysters focussed on nutrients which were not encapsulated. Soluble nutrients, e.g., amino acids and vitamins, are taken up readily by oysters (Nell and Dunkley, 1983; Nell et al., 1984) and other bivalves (Section 3.2). However, supplementation of an algal diet with a mixture of soluble amino acids regretably did not improve juvenile Sydney rock oyster growth rates (Section 3.4). Less soluble nutrients were also supplied to oysters in an unencapsulated form as an artificial diet containing single cell proteins from bacteria or yeast (Nell and Wisely, 1983; Nell, 1985). It was concluded that encapsulation of the diets was desirable. Various published methods for producing encapsulated or microparticulate diets were evaluated (Section 3.5) but regretably various artificial diets produced using some of these methods did not prove to be as effective a diet as live microalgae (Section 3.6).

The research was redirected towards supplementary feeding of oyster larvae as this is likely to be more economically feasible than supplementary feeding of adults in a grow-out situation. Oysters, if given a supplementary diet in a commercial farming trial, would usually be fed microalgae. Sydney rock oyster larvae were fed various species of microalgae either singly or in combination (Section 3.6). The most recent research was aimed at using encapsulated diets in combination with microalgae to feed oyster larvae (Sections 6.1 and 6.2).

Research station staff involved in nutritional research on prawns have given high priority to assessing the suitability of commercial formulations (Maguire and Hume, 1982) and this type of work continued (Section 2.6). More basic research on specific nutritional requirements using experimental diets was conducted both previously (Maguire and Hume, 1982) and during this project (Section 2.4). An

overview of international trends in prawn feeding and nutrition was prepared for industry (Maguire, 1987).

1.2.8 Growth rates in commercial systems

Ultimately all of this research is aimed at improving and interpreting growth and production results in commercial scale farming systems. Prior to the setting up of commercial prawn farms in New South Wales, growth and production data had to be generated by research station staff mostly as part of the F.I.R.T.A./Reserve Bank funded "Clarence River pilot scale prawn farming" project (Maguire and Allan, 1985). Subsequently, it became possible to obtain this type of data from the commercial farms (Maguire and Allan, 1988) and hence prawn farming research was redirected towards experimental scale work at the research station (Allan and Maguire, 1988). Commercial scale oyster hatchery and nursery facilities have been developed at the research station and growth and production data for these early stage oysters will continue to be generated. Some growth data is available from commercial subtidal leases (Wisely, et al., 1982) but very little growth data has been obtained for juvenile and adult oysters on intertidal leases in estuaries. This type of information has become available through research conducted in this project (Section 4) and in the ongoing F.I.R.T.A. funded "Pacific oyster" project.

1.2.9 Diseases

The growth data presented in Section 4 arises out of a study of growth, survival and nutritional condition of Sydney rock oysters in relation to growing height and the incidence of the disease known as winter mortality (Wolf, 1977a).
Another major oyster disease "QX" (*Marteilia sydneyi*) (Wolf, 1977b), was investigated during the F.I.R.T.A. funded "Sydney rock oyster genetics" project. Survivors of "QX" outbreaks have been used as broodstock and progeny placed on oyster leases as part of an ongoing attempt to establish whether resistance to "QX" has a genetic base. Bacteriological characteristics of edible oysters and oyster larval rearing systems have also been investigated by research station staff (E. Capps, unpublished data).

Research on prawn diseases has chiefly been carried out by the University of Queensland under a F.I.R.T.A. funded program. Research station staff have cooperated with the program and N.S.W. Department of Agriculture veterinary staff are taking an increasing interest in prawn diseases.

1.3 **Summary of research strategies**

Overall, it can be seen that the work covered by the various sections of this report fits into a general program of prawn and oyster research carried out utilising a team approach so that individual projects tend to interact rather than being discrete units.

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SECTION 2

THE EFFECTS OF SOME ENVIRONMENTAL, NUTRITIONAL AND POND MANAGEMENT FACTORS ON MARINE PRAWNS

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Results presented to Industry in:

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, 1986-87. In: G. B. Maguire (Editor), Proc. Prawn Farming Workshop, Wollongbar. Dept of Agriculture NSW, Sydney, pp. 54-58.

2.1 Maguire, G. B. and Allan, G. L. Effects of temperature on growth, food consumption and food conversion for *Penaeus monodon*, *Penaeus plebejus* and *Metapenaeus macleayi*.

Effects of temperature on growth, food consumption and food conversion for *Penaeus monodon*, *Penaeus plebejus* and *Metapenaeus macleayi*

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INTRODUCTION

In addition to feed quality and price, feed costs are greatly affected by feed inputs which are usually varied in relation to biomass and water temperature in aquacultural systems. Water temperature has been shown to influence metabolic rate, food consumption, moulting frequency, growth, food conversion efficiency and survival of a range of crustaceans including several carid and penaeid species farmed experimentally or commercially (Choe, 1971; Venkataramiah et al., 1974, Farmanfarmaian and Moore, 1978; Kurmaly et al., 1989). The leader prawn (*Penaeus monodon*) is the major farmed penaeid world-wide including Australia and recommended feed rates in ponds for this species are provided by feed companies in many countries (Maguire and Allan, in press). However, these

usually relate to water temperatures perceived to be optimum for this species and, in Australia, this species is often farmed at water temperatures considered by farmers to be suboptimal. Adequate data on the effects of temperature on food consumption and conversion efficiency for *P. monodon* are not available. The need for research on alternative species to *P. monodon* for farming in cooler months in Australia has also been recognised. School prawns (*Metapenaeus macleayi*) and eastern king prawns (*Penaeus plebejus*) have been proposed as having potential for this role and have been trialled by several farmers (Maguire and Allan, 1991). In the present study the effects of temperature on growth, food consumption and food conversion efficiency were determined in separate experiments for *P. monodon*, *M. macleayi* and *P. plebejus*.

MATERIALS AND METHODS

Juveniles of each species, collected from aquacultural facilties or NSW estuaries, were grown in 60 l aquaria. For each species replicate aquaria (n=3) were used for each of the experimental temperatures which were approximately 3 °C apart (Table 1). Two additional treatment levels for *P. plebejus* involved diurnal temperature cycles of 21.0 - 27.1 °C and 20.9 - 29.7 °C. The average temperatures for these two cycles (based on two hourly readings) were 24.5 °C and 28.3 °C. Prawns were progressively acclimated to experimental temperatures using 3 °C increments every 2 - 3 days. *P. monodon* and *M. macleayi* were individually tagged (see Allan et al., 1990) but, to minimise stress, *P. plebejus* were not tagged. Temperature control was effected using 200W submersible aquarium heaters. For

P. monodon and *M. macleayi* about 50 l was removed from each tank and replaced with preheated seawater (*P. monodon* 1 - 2 times daily; *M. macleayi* once daily). To overcome the mortality of *P. plebejus* at lower water temperatures in static aquaria recorded in earlier trials, continuous flow aquaria (180 ml min^{-1} $tank^{-1}$) were used for that species. Dissolved oxygen and pH levels were above, and unionised ammonia and nitrite plus nitrate levels well below, growth reducing levels for penaeid prawns (Allan and Maguire, 1991, in press; Allan, et al., 1990).

Prawns were fed bivalve flesh (pipi, *Plebidonax deltoides*) *ad libitum* each afternoon and for *P. plebejus* this diet was supplemented with a pelleted prawn feed imported from Taiwan (41.8 % crude protein [Nx6.25] dry basis). Average ratio pipi: pellets was 4.5:1 on a dry weight basis for the experiment with *P. plebejus*.

Average weight gain values (g $prawn^{-1}$) were based on mean values for each aquarium; for *P. monodon* and *M. macleayi* individual tags allowed for exclusion, from initial weight estimates, of prawns which died. Food consumption data were based on the equivalent weight for a diet containing 92 % dry matter and were expressed as a % of wet prawn biomass [0.5 (initial biomass + final biomass)] on a per day basis. Food conversion ratio (FCR) data (feed input on a 92 % dry matter basis: wet biomass gain) involved initial biomass estimates corrected for mortality. Moulting frequency data were expressed as moults $prawn^{-1}$ day^{-1} based on daily counts of moults and number of prawns present in each tank on each day. These data may be conservative as penaeids, especially *P. monodon*, consume moults. Survival rate data were transformed using arcsine ([% survival x

$0.01]^{0.5}$) and other data were transformed as required to ensure homogeneity of variance as indicated by Cochran's test. Homogeneity was only achieved for food consumption and FCR data for *P. monodon* by deleting the 33 °C treatment from the analyses. Treatment effects were compared using one-way ANOVA and regression, and least significant difference values (LSD) were used for comparing individual means.

RESULTS

The optimum temperature ranges (°C) for growth of *P. monodon, P. plebejus* and *M. macleayi* were 27.2 - 33.1, 30 and 21.3 - 26.8 in that order (Fig. 1a, 2a, 3a). Significant growth reductions of 28.2 % and 17.8 % were recorded with *P. plebejus*, for 21.0/27.1 °C and 20.9/29.7 °C temperature cycles, compared to 26.8 °C and 30.0 °C respectively (Fig. 2a). Moult frequency increased with temperature for *P. monodon* ($P<0.001$), *M. macleayi* ($P<0.05$) and *P. plebejus* ($P<0.01$) (Fig. 1b, 2b, 3b). Food consumption, as a percentage of average biomass, increased with temperature ($P<0.001$) over the entire range for each species (33.0 °C excepted for *P. monodon*) (Fig. 1c, 2c, 3c). These regression analyses indicated increases in food consumption, for a 10 °C temperature difference, of 4.76 % of average biomass d^{-1} (*P. monodon*) and 2.1 % of average biomass d^{-1} (*M. macleayi* and *P. plebejus*).

Temperature had a significant effect on FCR for *P. monodon* (33.0 °C excluded) and *M. macleayi* ($P<0.001$) but not for *P. plebejus* ($P>0.05$) (Fig. 1d, 3d). The

optimum temperature range for FCR was 24.1 - 30.1 °C for *P. monodon* and 18.2 - 26.8 °C for *M. macleayi* (Table 2). The average FCR for *P. plebejus* was 1.5:1.

DISCUSSION

The increase in moulting frequency and food consumption at higher temperatures could be interpreted as a response to increased metabolic rate for a poikilothermic animal. Similarly Kurmaly et al. (1989) found that respiration rate of *P. monodon* (postlarvae to adult) increased with temperature in the range 15 - 30°C. These trends may be interpreted as enhanced opportunity for growth but at excessively high temperatures growth can be disrupted. The optimum temperature range for *P. monodon* was not exceeded in this present study but growth results were adversely affected at 30.3 °C and 33.0 °C for *M. macleayi* and *P. plebejus* respectively. Clearly food consumption (in relation to average biomass) and moulting frequency were unreliable guides to growth in these cases.

The optimum temperature range for growth was quite broad for *P. monodon* (5.9 °C) and *M. macleayi* (5.6 °C) but a single optimum temperature was identified for *P. plebejus* (30.0 °C). Similar differences in responses to water temperature have been recorded for other crustaceans (Jones, 1988). However, growth data for *P. plebejus* should be interpreted with caution as gill melanisation, associated with the presence of apostome ciliates, possibly *Synophrya* sp. (Johnson and Bradbury, 1976) within the gills, became evident as the experiment progressed. Dense melanisation was not observed at 30.0 - 33.0 °C but occurred in 41.8 - 48.8 % of

prawns at 18.6 - 24.2 °C and 16.2 % at 27.1 °C indicating an inverse relationship between temperature and melanisation (Fig. 2d). Growth depression at 33.0 °C in the absence of gill melanization indicated that growth results were not necessarily determined by the presence of apostome ciliates.

The other major difference among the species was the absence of a significant effect of temperature on FCR for *P. plebejus* ($P>0.05$). Farmanfarmaian and Moore (1978) found that growth increased with temperature for *Macrobrachium rosenbergii* but that FCR was not significantly affected ($P>0.05$) while Venkataramiah et al. (1974) concluded that growth and FCR for several penaeids were affected by temperature. The poorer FCR values for *P. monodon*, compared to *M. macleayi* and *P. plebejus*, could have been influenced by high food consumption levels and unfavourably high salinities.

The major finding of the study was that, through appropriate inputs of feed, optimum food conversion could be obtained at temperatures suboptimal for growth of *P. monodon* and *M. macleayi* (24.1 °C and 18.2 °C respectively). The relatively minor reductions in growth caused by 6.1 °C and 8.8 °C daily temperature fluctuations were promising when considered in relation to diurnal temperature fluctuations of only 2-3 °C recorded in some prawn farming ponds (Maguire and Allan, 1985).

While data for *P. plebejus* should be interpreted with caution, this species grew as well as *P. monodon* at optimum temperatures (on a g prawn^{-1} week^{-1} basis) but,

based on the results of this study, could not be considered as appropriate for farming in cooler seasons. *M. macleayi* was relatively well suited to cooler temperatures but other factors such as postlarval availability and market value of prawns harvested from ponds should also be considered.

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TABLE 1

Experimental conditions for aquarium experiments on effects of water temperature on three penaeid species

Variable	Species		
	Penaeus monodon	*P. plebejus*	*Metapenaeus macleayi*
No of prawns per aquarium	10	15	10
Average initial prawn weight (g prawn^{-1})			
x	1.9	1.1	2.4
Range[a]	1.6-2.3	0.9-1.3	2.1-2.9
Duration of experiment	4	3.5	6
Experimental temperatures (°C)[b]	18.4-33.1	18.6-33.0	15.7-30.3
Salinity range (‰)	34.0-36.5	26.1-33.2	30.5-34.8

[a] Based on mean values for individual tanks

[b] Experimental temperatues were arranged in increments of approximately 3°C

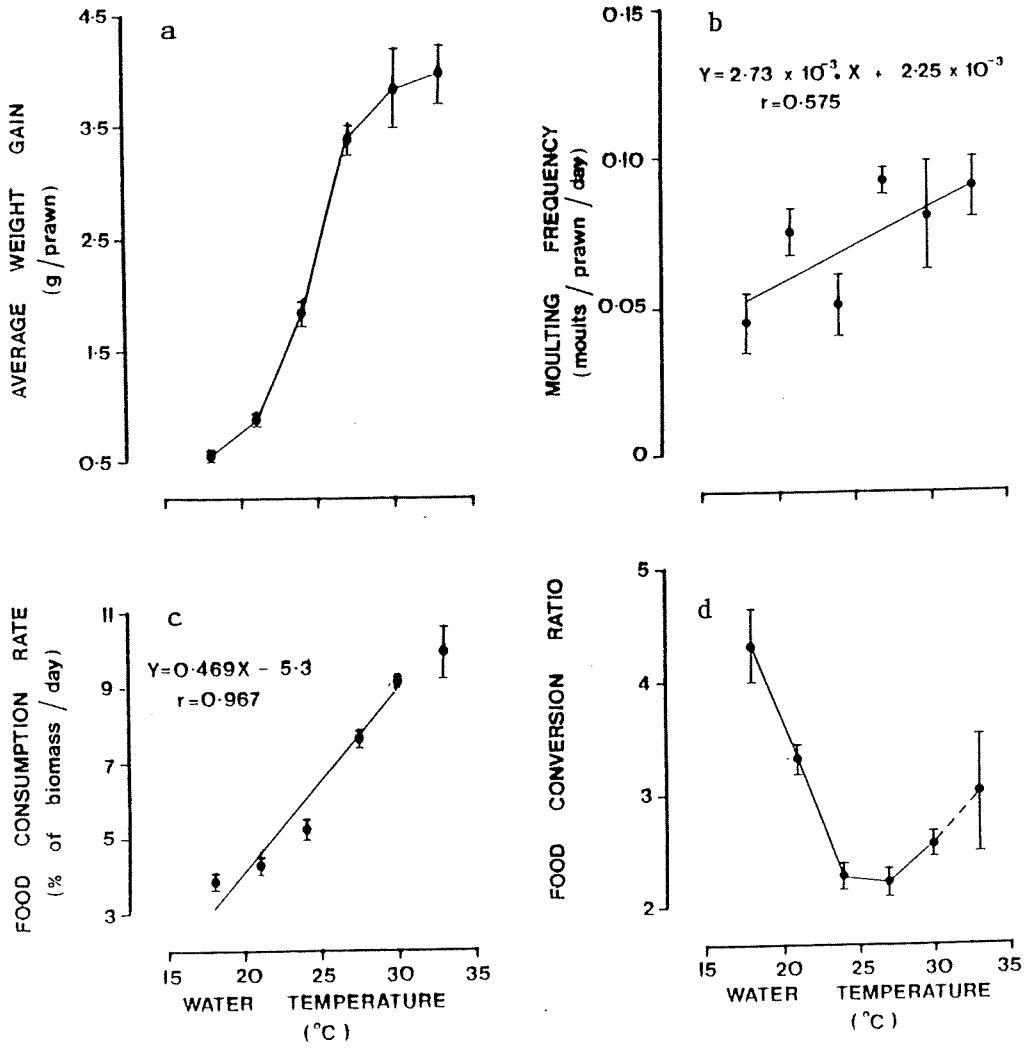

Figure 1. Effects of water temperature in aquaria on a) growth, b) moulting frequency, c) food consumption, and d) food conversion ratio for juvenile leader prawns, *Penaeus monodon*. (Values are mean ± S.E., n = 3.)

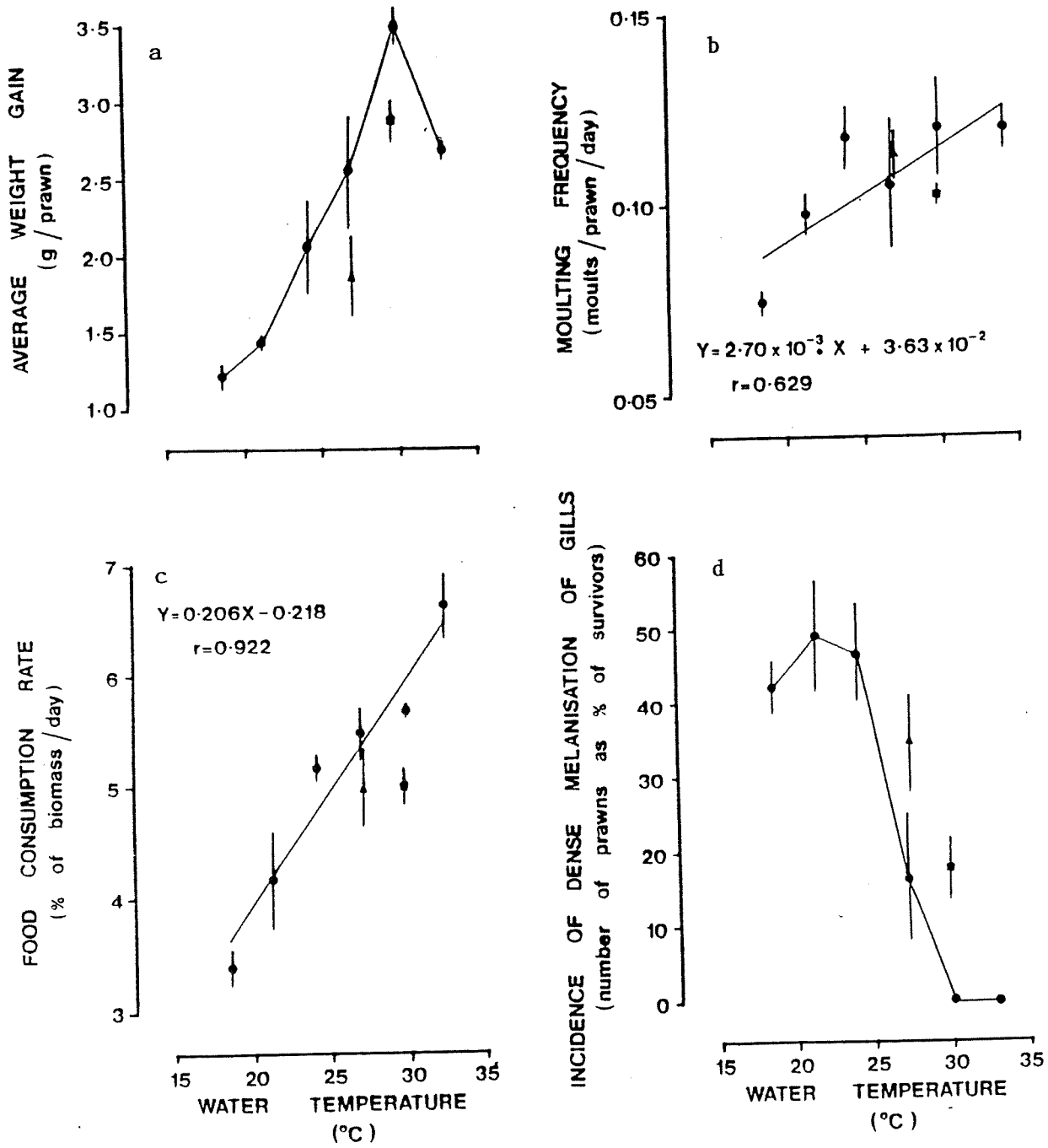

Figure 2. Effects of water temperature in aquaria on a) growth, b) moulting frequency, c) food consumption, and d) incidence of gill melanisation, due to apostome ciliates, for juvenile eastern king prawns, *Penaeus plebejus*. (Values are mean ± S.E., n = 3. The symbols ▲ and ■ indicate results for prawns grown under cyclic daily temperature regimes of 21.0/27.1^0 C and 20.9/29.71^0 C respectively.)

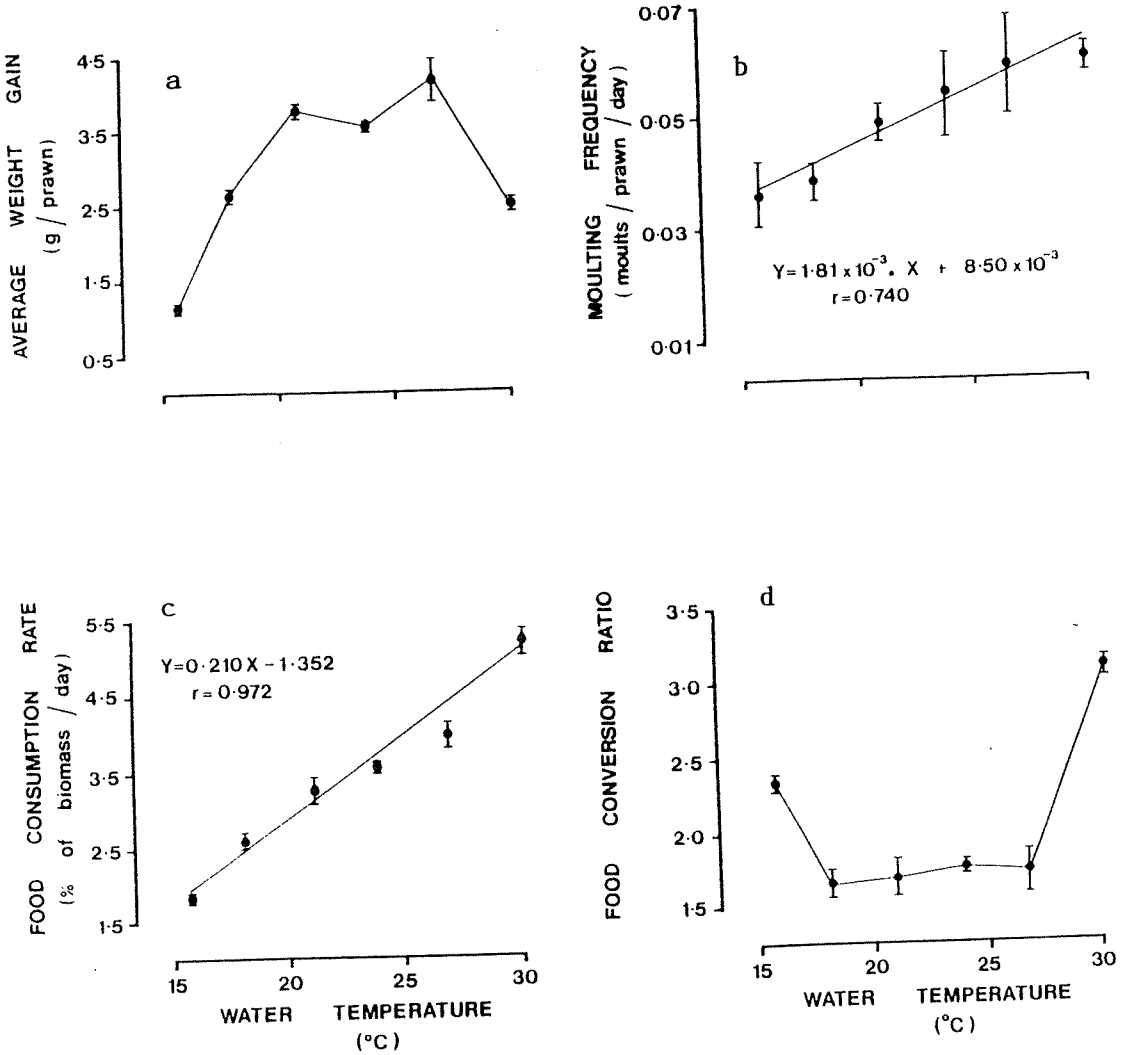

Figure 3. Effects of water temperature in aquaria on a) growth, b) moulting frequency, c) food consumption, and d) food conversion ratio for juvenile school prawns, *Metapenaeus macleayi*. Values are mean ± S.E., n = 3.)

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EVALUATION OF THE SUITABILITY OF SOME AUSTRALIAN AND TAIWANESE DIETS FED TO LEADER PRAWNS (*Penaeus monodon*) IN PONDS.

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ABSTRACT

Leader prawns (*Penaeus monodon*) performed better under pond conditions (higher weight gains and better food conversion efficiency) when fed Taiwanese prawn diets rather than Australian diets during the summers of 1986/87 and 1987/88. Proximate, fatty acid and amino acid composition of these diets are discussed. Apart from unusually high and low lipid levels in two Australian diets, the differences in growth results for prawns fed the various diets could not be explained by differences in chemical composition, food consumption rates or pellet stability in seawater. The importance of improving locally manufactured diets and the need for farmers to use appropriate feed rates are emphasized.

INTRODUCTION

Feed costs are a major component of operating costs in many aquacultural industries. These can be minimised through use of appropriate feed rates and by improving the nutritional quality and reducing the cost of artificial diets. The farming of penaeid prawns in ponds usually involves the use of artificial diets unless very low stocking densities are used. As prawn farming began to expand rapidly throughout the world during the 1970's, a considerable amount of research on penaeid nutrition was conducted (see reviews by New 1976 and Maguire 1980). While this nutritional research has continued (Kanazawa 1985), much of the progress towards the development of cost-effective prawn diets has been made through the efforts of feed companies who progressively improved their commercial rations. The formulations for these feeds are usually proprietary information. However, comparisons of the weight gains of prawns fed a range of commercially available feeds may prove to be a useful direction for nutritional research. This is particularly so when growth data can be related to the results of chemical analyses of the diets (Ako and Dominy 1987). In addition to helping to indicate relationships between growth and feed composition, this approach is of considerable interest to farmers who wish to know which of the available diets are more effective.

Diets can be evaluated in aquaria, where prawns are thought to obtain most of their nutritional requirements from the artificial feeds, or under pond conditions in which natural food items can make a major contribution to the diet of the prawns (Anderson *et al.* 1987). In the present study, netting enclosures (pens) within fertilised ponds were used to allow comparisons of growth rates of leader prawns (*Penaeus monodon*) when fed a range of Australian or Taiwanese prawn diets. Comprehensive analyses of the composition of these diets were also carried out.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND FACILITIES

Diets available to commercial farmers in Australia in the summers of 1986/87 and 1987/88 were compared in Experiments 1 and 2 respectively. Juvenile leader prawns were obtained from experimental or commercial ponds that had been stocked with hatchery reared postlarvae. The prawns were harvested from the ponds with seine nets and in Experiment 2 they had to be transported about 700 km by road in aerated seawater. The prawns were stocked into replicate pens within tidal, 0.11 ha ponds at the Brackish Water Fish Culture Research Station, Salamander Bay, New South Wales. These pens were as

described by Maguire and Bell (1981) except that they enclosed an average of 2.7 m^2 of pond bottom per pen and the lids, walls and subsurface floors were constructed using 7 mm plastic mesh.

The ponds were initially fertilised with 20 kg N/ha and 2 kg P/ha using single superphosphate and ammonium nitrate. Periodic additions of fertilisers (10% of initial amount) were made in an attempt to maintain phytoplankton blooms. The pond used in Experiment 1 developed a serious leak so that daily refilling of the pond was required to help maintain a depth of 1 m in the pond. This was only partially successful and this shallow pond did not usually have an obvious phytoplankton bloom. As a result filamentous algae and the seagrass *Halophila decipiens* grew on the pond bottom within the pens. In Experiment 1 feed was supplied twice per day (morning and afternoon) and visual observation of uneaten feed on the sediment layer within each pen was used to adjust feed rates on an *ad libitum* basis.

In Experiment 2 pond leakage problems were much less serious but daily replacement of water losses was considered to have provided an adequate water exchange rate. During this experiment it was possible to maintain a sufficient phytoplankton density to prevent the growth of benthic algae and seagrass. To allow for more precise adjustment of feed rates in Experiment 2, a 0.6 m diameter feed tray, made of 18 mm electrical conduit and 2.0 mm vinylon mesh netting, was placed in each pen. Feed was supplied four times daily in this experiment but the relative amounts provided on a morning:afternoon basis were similar to those used in Experiment 1 i.e, 1:2. On weekends feed was only supplied twice per day but this did not have an obvious effect on daily food consumption rates.

While pond leakage problems did not allow for optimum pond management, water samples taken from each pen at the end of each experiment indicated that ammonia and nitrite levels were much lower than the levels found to adversely affect penaeid growth rates (Wickins 1976). Ammonia and nitrate were measured using the methods described by Dal Pont *et al.* (1973) and Major *et al.* (1972). Each pen was continuously aerated with compressed air released through adjustable plastic valves suspended near the bottom sediment. Temperature and salinity readings were taken using a thermometer, a thermograph and a Yeokal salinity/temperature conductivity meter.

EXPERIMENT 1

Five diets and an unfed control were tested using four replicate pens per diet. The diets included a Taiwanese diet (A), three Australian diets (A-C) and freshly shucked and chopped meats from pipis, *Plebidonax deltoides*, which are a type of burrowing bivalve collected from oceanic beaches.

Despite the fact that it would be too expensive to use pipi meat in commercial ponds, it was included as a control because prawns in aquaria grow well on this diet. Apart from pipi meat and Australian diet C in Experiment 1, which was a crumble, all diets in both experiments were pelleted (2-4 mm diameter pellets). In Experiment 1 the diets were fed for six weeks during which time average daily water temperature and salinity levels (mean, range) were 24.1°C (20.6-26.0°C) and 33.9‰ (26.0-40.0‰) respectively. Prawns were stocked at 15 juveniles/m^2 and the average initial weight was 6.32±0.11 g/prawn (mean±S.E, n=24 for groups of 39-47 prawns).

EXPERIMENT 2

Two diets (Australian D and Taiwanese B) were each fed to prawns stocked at 15 and 30 juveniles/m^2. Two other diets (Australian E and Taiwanese C) were only tested using 15 juveniles/m^2. The higher density treatments were included because it was not possible to obtain other Australian diets. An increased number of replicates (5 pens/diet) were used in the experiment. The average initial weight of the prawns was 2.59±0.04 g/prawn (mean±S.E, n=31 for groups of 39-86 prawns). The diets were fed for seven weeks during which time the average daily minimum and maximum water temperatures (mean, range) were 24.4°C (19.8-27.5°C) and 27.2°C (22.5-31°C) respectively. Average daily salinity levels (mean, range) were 30.1‰ (28.7-31.5‰).

PERFORMANCE INDICES

Average weight gain values (g/prawn) were determined for each pen. For Experiment 1, the combined weight of all prawns stocked into a pen was recorded. After being harvested, the prawns from a pen were sexed prior to bulk weighing. A similar procedure was used in Experiment 2 except that groups of about 15 prawns were added sequentially to the 35 pens until the appropriate number had been stocked into each pen. The total weight values for each subgroup were combined to provide estimates of initial prawn biomass and average initial prawn weight for each pen. This procedure was adopted to reduce variation among replicate pens in average initial prawn weight.

To minimise handling stress for the large number of prawns involved (1850 prawns in Experiment 2), individual prawns were not sexed prior to stocking. There was no significant effect of diet type on sex ratio at harvest ($P>0.05$). This was important because females were consistently larger than males at harvest.

Only a few dead or moribund prawns were observed in the pens during the first week after stocking. In each experiment additional prawns of equivalent size were added to compensate for these initial losses. Differences between the number of

prawns in a pen at the beginning and end of a trial were used to estimate mortality. In four of the pens in Experiment 2, it was suspected that prawns had escaped and these pens were not included for performance estimates or statistical analyses.

The term food quotient (F.Q.) has been used to indicate the apparent efficiency of food conversion (feed input:biomass gain) in the presence of natural food (Maguire and Leedow 1983). Feed consumption rates (feed input:biomass, expressed as a percentage) were estimated for the concluding two weeks of Experiment 2 using prawn biomass at harvest as the biomass estimate. All feed input values were adjusted to a standard of 92% dry matter to allow for differences in moisture content among the diets.

An economic index was used to compare the cost efficiency of each diet within Experiment 2. The estimates of relative cost efficiency were derived by ranking the calculated Economic Return (ER) for each diet. Using this method the best diet in terms of economic return had a Relative Cost Efficiency (RCE) of 1.0 while the equivalent value for the poorest diet was zero.

$$RCE = \frac{(ER \text{ for a diet} - ER \text{ for poorest diet})}{(ER \text{ for best diet} - ER \text{ for poorest diet})}$$

$$\underset{(\$/m^2)}{ER} = \underset{(\$/m^2)}{\text{Value of crop}} - \underset{(\$/m^2)}{\text{Cost of stock}} - \underset{(\$/m^2)}{\text{Feed costs}}$$

$$\underset{(\$/m^2)}{\text{Value of crop}} = \underset{(kg/m^2)}{\text{Weight of crop}} \times \underset{(\$/kg)}{\text{Price of crop}}$$

Size dependent price estimates were used because market prices for prawns generally increase with average prawn size. The size price model used was:

$$\underset{(\$/kg)}{\text{Price of crop}} = \underset{(g/\text{prawn})}{\text{Average prawn weight}}$$

This relationship was only applied to groups of prawns whose average sizes ranged from 9-15 g. For very large prawns e.g. 30 g, this simple linear relationship would overestimate the value of the prawns for the domestic market

$$\underset{(\$/m^2)}{\text{Cost of stock}} = \underset{(\text{prawns}/m^2)}{\text{Density}} \times A\$0.075 \text{ per juvenile}$$

Feed prices were provided by the Australian feed companies at the beginning of Experiment 2 (A$1,100 - 1,300/tonne) or were obtained from prawn farmers who had imported or obtained quotes for the importation of Taiwanese feeds prior to this experiment (A$1,600 - 1,660/tonne).

Feed costs ($/m²) = Total weight of feed used (kg/m²) x Feed price ($/kg)

STATISTICAL ANALYSES

All treatments were randomly allotted and homogeneity of variance was confirmed using Cochran's test. One way ANOVA was used to assess treatment effects and the least significant difference (LSD) method was used for multiple comparisons of means (Steel and Torrie, 1960). Survival rate data were subjected to an arc sin square root transformation.

Covariance analyses were used in Experiment 1 to examine the most influential covariates when assessing weight gain, biomass gain and food conversion results. The general linear models procedure (Freund *et al.* 1986) was used for these analyses. The covariates examined were: average initial prawn weight; pen size; survival rate; sex ratio; occurrence of other species of prawns in the pens, and average coverage of sediment within pens by seagrass and filamentous algae. The aim was to identify ways of improving the methodology used in Experiment 1 so that variation in performance indices among replicates in Experiment 2 could be reduced.

DIETARY ANALYSES

Chemical analyses were usually carried out in accordance with methods specified by the Association of Official Analytical Chemists (1980). Detailed methodology for amino acid and fatty acid analyses is available from one of the authors (Dr. R. Baigent).

The physical stability of diets was determined by estimating the percentage of dry matter retained on 550-600 µm plankton mesh arranged as a concave seive submerged in seawater in a 1 litre beaker. The beakers were positioned in a water bath at 27°C and each of four replicate beakers per diet received similar metered air flow which was released through a 5 mm glass tube at the bottom of each beaker. Up to 30 g of a diet was placed in each seive and these samples were aerated for five hours prior to dry matter determinations. The method was adapted from Forster (1972).

RESULTS

In both experiments leader prawns grew much better on Taiwanese diets than on Australian diets. In Experiment 1 average weight gains for prawns fed pipi meat were similar to those fed Australian diets. Small increases in average weight for unfed controls were recorded in each experiment. In Experiment 2 Taiwanese diet B produced a significantly greater weight gain ($P<0.01$) than Taiwanese diet C in pens stocked at 15 prawns/m². However, in both experiments there were no significant differences among Australian diets in average weight gain ($P>0.05$). An increase in stocking

density from 15 to 30 juveniles/m2 depressed weight gains for both Taiwanese diet B and Australian diet D in Experiment 2 ($P<0.01$). Survival rates were very high in most pens and were not significantly affected by diet type ($P>0.05$).

More efficient food conversion (low F.Q. values) was obtained with Taiwanese diets than with Australian diets. However, there were some differences between the average weight gain and food conversion results. In Experiment 1, Australian diet A produced a significantly poorer food conversion result than the other Australian diets ($P<0.01$). While weight gain on pipi meat was no better than for the Australian diets in Experiment 1, this diet produced the best food conversion efficiency (Table 1). This was largely due to the fact that feed consumption rates for prawns fed pipi meat are usually relatively low. Although there was a significant difference in weight gain results for the Taiwanese diets (B and C) at 15 juveniles/m^2 in Experiment 2, there was no significant difference ($P>0.05$) in the food conversion results. In contrast, Australian diet E produced a similiar weight gain as Australian diet D, yet the food conversion results for diet E were significantly poorer than for diet D ($P<0.01$).

Taiwanese diets were not necessarily more physically stable in water than Australian diets. In Experiment 1, Taiwanese diet A was the most stable diet while in Experiment 2 Taiwanese diet B was the poorest and Taiwanese diet C equal best in terms of stability (Tables 1,2). The stability results should be interpreted with caution as coarsely ground diets can tend to fall apart but larger particles are still retained on the plankton mesh seives. This factor may have influenced the results for Australian diet D in Experiment 2. Overall, the poorest stability result was obtained for Australian diet C in Experiment 1. This was not suprising as the diet was supplied as a crumble.

There was no significant effect of diet type on food consumption rate over the last two weeks of Experiment 2 when expressed as a percentage of biomass ($P>0.05$). The data from this experiment were used because it was considered that feed consumption could be much more accurately measured when feed trays were used. The final period was used rather than the initial period because at the beginning of an experiment there is a tendancy to underfeed as feed inputs are progressively raised until excess feed is eventually supplied. By the end of the experiment feed inputs would have more closely reflected demand for feed by the prawns.

The economic analysis used for Experiment 2 (Table 2) indicated that although the Australian diets tested were less expensive per tonne than the Taiwanese diets, they were also less cost-effective.

Analysis of a covariance in Experiment 1 indicated that average initial prawn weight was the only covariate which was consistently significant ($P<0.05$) for all the dependant covariates: weight gain, biomass gain and food conversion efficiency. However, considered individually, pen area and the presence of other species of prawns inside the pens (from natural recruitment) were also significant covariates ($P<0.05$) for weight gain. Sex ratio was a significant covariate for both biomass gain and food conversion efficiency and pen area was also a significant ($P<0.05$) covariate for food conversion efficiency. This information was useful for designing Experiment 2; the aim being to minimise the variation among replicates in that experiment.

The proximate analysis of the diets used in Experiment 1 and or Experiment 2 are presented in Tables 3 and 4 respectively. The result for amino acid and fatty acid analyses are presented in Tables 5 and 6 respectively.

DISCUSSION

There were a few differences in chemical composition among the diets (Tables 3-6) which could help explain the differences in weight gains achieved with the various diets. The most obvious difference between Taiwanese and Australian diets was in Experiment 2 where Australian diets D and E had unusually low and high total lipid levels respectively (Table 4). The optimum dietary lipid level for another penaeid *Penaeus japonicus*, is 6% and high lipid levels can depress growth rates (Deshimaru *et al.* 1979).

The amino acid composition of all of the diets were quite similar when expressed as a percentage of dietary protein (Table 5). However, the best diet in Experiment 1, Taiwanese diet A, had a higher combined level of methionine and cystine than two of the Australian diets (A,B) and a higher lysine level than Australian diet A. It is difficult to explain differences among the performances of the diets in Experiment 2 on the basis of amino acid composition. This is particularly so for the two Taiwanese diets with diet B having a lower level of all of the essential amino acids than diet C which produced a lower weight gain. Some caution should be expressed when comparing these diets as diet B was air freighted to Australia while diet C was sent by sea. Differences in storage conditions could have affected the relative nutritional value of the Taiwanese diets. Furthermore there was no significant difference between these two diets in terms of food conversion efficiency ($P>0.05$). In general, all of the diets had low levels of several essential amino acids, especially methionine (+ cystine), when compared to the composition of prawn tail muscle.

In Experiment 2 the Taiwanese and Australian diets had similar fatty acid composition (Table 6). The levels of C20:5ω3 and C22:6ω3 fatty acids were, however, generally

higher in the Taiwanese diets. Kanazawa *et al.* (1977) emphasized the importance of these fatty acids for *P. japonicus*. Taiwanese diet B and Australian diet D contained considerably higher levels of linoleic (18:2ω6) and linolenic (18:3ω3) fatty acids than Taiwanese diet C, Australian diet E or squid meal, a widely used ingredient in prawn diets. Given the importance attached to supplying adequate levels of ω3 fatty acids in prawn diets (Kanazawa 1985), the low overall ω3:ω6 ratio in all of the diets was suprising. The results indicate that terrestrial ingredients make up a significant proportion of the diets as ingredients of aquatic origin e.g. squid meal, tend to have a much higher ω3:ω6 ratio (Table 6).

The testing of these diets under pond conditons created considerable interest among feed companies and commercial farmers. The results indicate that further development of the Australian diets is required and this problem is currently being addressed by several feed companies. However, apart from the differences noted in the total lipid levels, the poor relative performance of the Australian diets could not be adequately explained by differences in protein, mineral, fatty acid or amino acid content. However, other variables can affect the usefulness of individual ingredients e.g. the presence of growth inhibitory substances. An investigation of the relative suitability of potential feed ingredients should be given a high priority when attempts are made to improve Australian prawn diets.

The pen system used to evaluate the diets has major advantages in that the testing is carried out under pond conditions where rapid growth rates can be obtained and large juveniles easily maintained. As such, the results are generally applicable to commercial prawn farms and consequently are of more interest to farmers than those from experiments conducted in aquaria. However, it may be more cost effective to use aquaria to evaluate potential ingredients and formulations. Pens or model ponds could then be used to compare the cost efficiency of commercial diets when feed companies are confident, on the basis of aquarium trials, that the diets are nutritionally adequate.

Partly as a result of this research, many prawn farmers in Australia have chosen to use Taiwanese diets. However, as noted earlier in this paper, the availability of cost-effective nutritionally adequate diets is not the only factor involved in determining feed costs. Appropriate feed rates must also be chosen. In Table 7 encouraging production results from New South Wales prawn farms are presented. Unfortunately, the food conversion results from these farms were generally not satisfactory. While farmers are improving their pond management skills, there is still a tendancy to overfeed. Feed trays can be very useful as indicated by the food conversion results from Experiment 2. However, feed rates should still be considered in relation to estimations

of prawn biomass. Some feed manufacturers provide recommended feed rates (as a percentage of biomass) and these should be considered as the maximum amounts that could be used under ideal conditions in ponds stocked at a high density. It is usually better to use more conservative feed rates. Overfeeding will not only increase feed costs but will also contribute to problems in maintaining adequate water quality, especially for dissolved oxygen.

A major factor in pond management, particularly in temperate areas is the need to reduce feed rates during cooler weather. In Experiment 2, water temperatures were unfavourable for two of the seven weeks of the experiment ie, maximum daily water temperatures were less than 25°C. By reducing feed inputs during this cooler period, satisfactory food conversion results were obtained. Maguire and Allan (unpublished data) found that in aquaria optimum food conversion efficency could be obtained for leader prawns at a low temperature at which growth rates were depressed. This was achieved by reducing feed inputs. It should also be noted, however, that it is far easier to manage aquaria and pens than commercial-scale ponds.

If marine prawn farming is to prosper in Australia it will probably be necessary for cost-effective, locally made feeds to be developed. Farmers will continue to purchase imported feeds if local diets do not improve or do not maintain a price advantage. However, farmers can also have a major influence on feed costs by monitoring feed consumption rates.

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TABLE 1

Performance indices for leader prawns fed commercial diets during 1986/87 in Experiment 1 (mean±S.E.).

Diet	Weight gain[1] (g/prawn)	Survival[2] (%)	F.Q.[1]	Instability[1] (% weight loss)
Taiwanese A	8.8±0.4[a]	97.0±3.1	3.7±0.2[a]	21.5±0.6[a]
Australian A	5.9±0.2[b]	97.0±1.8	6.4±0.4[b]	33.5±0.5[b]
B	6.2±0.5[b]	98.9±0.7	5.1±0.4[c]	35.2±0.8[b]
C	6.6±0.4[b]	97.4±3.2	5.3±0.3[c]	66.2±0.6[c]
Pipi meat[3]	6.1±0.3[b]	97.6±1.0	2.8±0.2[a]	-
Unfed	2.2±0.4[c]	94.3±4.2	-	-

1 Diet type had a significant effect on this variable ($P<0.05$). Means, within a column, which share a common superscript are not significantly different ($P>0.05$).

2 Data subjected to an arc sin square root transformation prior to analysis. Diet type had no significant effect on this variable ($P>0.05$).

3 Freshly shucked and chopped meats of the bivalve *Plebidonax deltoides*.

TABLE 2

Performance indices for leader prawns fed commercial diets during 1987/88 in Experiment 2 (mean±S.E.).

Diet	Stocking density (no./m²)	Wt gain[1] (g/prawn)	Survival[2,3] (%)	FQ[1]	Instability[1] (% wt loss)	FC[1,3,4] (%/day)	RCE[5]
Taiwanese							
B	15	11.6±0.2[a]	91.0±3.5	1.26±0.03[a]	24.5±1.8[a]	3.0±0.1	1.00
B	30	9.5±0.2[bc]	94.5±1.4	1.43±0.01[b]	-	2.8±0.6	0.98
C	15	10.0±0.6[c]	88.2±3.3	1.26±0.03[a]	11.2±0.8[bc]	2.8±0.2	0.65
Australian							
D	15	7.7±0.4[d]	89.7±3.1	1.62±0.04[c]	9.1±1.4[b]	3.6±0.1	0.35
D	30	6.5±0.1[e]	85.4±3.0	1.78±0.03[d]	-	3.7±0.1	0.00
E	15	7.1±0.3[de]	86.0±4.5	1.90±0.06[d]	14.9±2.0[c]	3.4±0.4	0.22
Unfed	15	1.2±0.2[f]	82.7±4.0	-	-	-	-

1 Diet had a significant effect on this variable ($P<0.05$). Means, within a column, which share a common superscript are not significantly different ($P>0.05$).

2 Data subjected to an arc sin square root transformation prior to analysis.

3 Diet type did not have a significant effect on this variable ($P>0.05$).

4 Average food consumption per day during last two weeks (FC). FC = feed input (92% dry matter)/ final biomass (expressed as a percentage).

5 Data from replicates have been pooled to calculate economic return values which have been ranked (0 = poorest, 1 = best diet) as relative cost efficiency (RCE).

TABLE 3

Composition of diets in Experiment 1 (expressed on a dry weight basis).

Diet	Protein (%)	Lipid (%)	Ash (%)	Ca (%)	P (%)	Fe (mg/kg)
Taiwanese A	42.5	5.7	15.0	3.2	1.6	1003
Australian A	42.0	8.6	11.1	2.8	1.8	477
B	36.8	5.6	10.7	3.3	1.3	802
C	41.1	6.0	7.8	1.8	1.2	399
Pipi meat	72.4	6.3	15.2	0.48	0.97	n/a[1]

1. Data not available.

TABLE 4

Composition of diets in Experiment 2 (expressed on a dry weight basis).

Diet	Protein (%)	Lipid (%)	Ash (%)	Ca (%)	P (%)	Fe (mg/kg)
Taiwanese B	41.8	6.5	15.2	2.8	1.8	689
C	46.7	6.4	11.9	2.7	2.1	234
Australian D	40.8	3.9	11.0	3.1	1.3	658
E	42.4	13.9	13.6	3.1	2.0	880

TABLE 5

Amino acid (AA) composition of diets expressed as a percentage of the concentration found in prawn tail muscle[1].

Essential amino acids	Diets								
	Experiment 1					Experiment 2			
	Taiwanese	Australian			Pipi	Taiwanese		Australian	
	A	A	B	C		B	C	D	E
Arginine	101	123	120	126	131	96	99	108	104
Histidine	84	97	114	104	55	70	88	86	60
Isoleucine	66	79	59	69	69	73	87	80	70
Leucine	62	73	69	74	62	65	73	73	69
Lysine	99	84	131	121	90	104	116	104	102
Methionine (+ Cystine)	43	32	31	41	49	48	53	56	51
Phenylalanine (+ Tyrosine)	78	76	75	67	74	69	81	87	77
Threonine	65	74	67	75	75	71	76	75	71
Tryptophan	–	–	–	–	–	–	–	–	–
Valine	68	73	70	79	65	75	80	81	83

1. The amino acid composition data were based on analyses expressed as g AA/16g N for the diets and for prawn tails (Shewbart *et al.* 1972). This is equivalent to AA content as a percentage of dietary protein.

TABLE 6

Major fatty acids present in diets in Experiment 2 (expressed as % of total fatty acids).

Fatty acid	Diets				Squid meal[1]
	Taiwanese		Australian		
	B	C	D	E	
C14:0	5.16	7.10	4.74	8.67	7.21
C16:1ω7	5.24	6.94	4.93	9.51	6.94
C16:0	23.25	27.83	24.92	23.02	21.79
C18:4ω3	1.54	2.05	0.00	1.67	2.85
C18:2ω6	21.30	12.00	23.84	11.28	5.05
C18:3ω3	3.05	1.72	3.14	1.86	1.39
C18:1ω9	12.12	12.76	13.95	12.43	11.68
C18:1ω7	3.36	3.93	3.15	3.01	3.82
C18:0	3.66	3.80	4.56	4.15	2.89
C20:4ω6	0.59	0.80	0.00	0.73	0.67
C20:5ω3	6.96	8.92	4.19	6.77	9.89
C20:4ω3	0.00	0.00	0.00	0.52	0.64
C20:1	1.50	1.95	1.44	1.15	4.17
C20:0	0.00	0.00	0.00	0.21	0.27
C22:6ω3	4.51	5.88	3.99	3.18	7.09
C22:5ω3	0.46	0.00	0.00	0.64	0.70
C22:1	0.43	0.00	0.00	0.00	1.61
ω3:ω6 ratio	0.75	1.45	0.47	1.22	3.94

1. Included for comparative purposes.

TABLE 7

Production results from commercial prawn farming ponds in New South Wales stocked at >8 prawns/m² (as postlarval or juvenile leader prawns) during the 1987/88 growing season[1].

Stocking density mean (range)	Production (kg/ha) mean	Average weight at harvest (g)	Survival (%) mean	F.Q. mean (range)
13.8 (8-22)	1530	21.7	58.5	3.1 (1.2-4.2)

1. Based on results from eleven ponds.

2.3 Maguire, G. B. and Allan, G., 1989. Nutritional studies on school prawns *Metapenaeus macleayi* and leader prawns *Penaeus monodon*. In: E. Chauvez (Editor), Proceedings of the Mexico-Australia Workshop on Marine Sciences, Merida, pp. 469-478.

Proc. Workshop Mexico-Australia Mar. Sci.

NUTRITIONAL STUDIES ON SCHOOL PRAWNS METAPENAEUS MACLEAYI AND LEADER PRAWNS PENAEUS MONODON

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ABSTRACT

Two types of nutritional experiments have been conducted by the authors. Aquaria are used to test experimental diet in a situation where the diets are the only major source of nutrients for prawns. Alternatively, swimming pools or netting enclosures within ponds (pens) allow comparison between commercially available diets in a situation where prawns also have access to natural food organisms within ponds. Result of aquarium trials are presented which show that school prawn growth rates are improved when bivalve meat is included in a compounded diet fed to prawns. This improvement was attributed to an improved amino acid balance rather than to changes in the lipid composition of the diet. Pen and pool trials are discussed in relation to trends in the formulation of commercial prawn farming diets in Australia.

RESUMEN

Los autores han desarrollado dos clases de experimentos nutricionales. Se utilizaron acuarios para probar dietas experimentales en una situación donde éstos son la única fuente de nutrimentos para los camarones. Alternativamente, los encierros en jaulas dentro de estanques permitieron hacer comparaciones entre dietas comercialmente disponibles, en una situación donde los camarones también tienen acceso a alimento natural. Se presentan los resultados de las pruebas en los acuarios y muestran que las tasas de crecimiento en las agrupaciones de camarones incrementaron cuando carne de bivalvo fue incluída en una dieta balanceada para alimentarlos. Este incremento fue atribuído más bien a un aumento en el balance de los aminoácidos que a cambios en la composición de lípidos en la dieta. Los experimentos de jaulas y estanques se discuten en relación a una orientación en la formulación de dietas comerciales para el cultivo de camarones en Australia.

INTRODUCTION

Feed costs are a major factor in determining the profitability of prawn farming operations, particularly when high stocking densities are used. Farming of school prawns (Metapenaeus macleayi) may well have to be carried out at relatively high densities to be of continuing interest to commercial growers (1). Al low densities, school prawns can be grown on simple inexpensive diets but at higher densities more nutritionally complete diets are required (2,3).

As commercial prawn farming progressed in Japan the emphasis shifted from using crushed bivalve in farming ponds to using compounded diets, i.e., based on a combination of feed ingredients (4). During the development of thes Japanese commercial diets, bivalve meat was used as a control diet in experimental feeding trials. Also, supplementation of experimental diets with various components of bivalve meat proved to be useful for identifying growth-limiting inadequacies in dietary formulations (5).

In the present study various components of bivalve meat were obtained by solvent extractions of pipi meat (Plebidonax deltoides). These components were used to supplement an experimental, compounded diet fed to school prawns. Pipi meat which had not been treated with solvents was also used as a control diet. One of the aims of this study was to identify wheter protein quality of the types of lipids used were major limiting factors for the development of nutritionally adequate, commercial prawn diets. The status of Australian commercial marine prawn diets is discussed in relation to diet evaluation studies involving school prawns and another species, the leader prawn Penaeus monodon.

MATERIALS AND METHODS

Experimental Systems

The pipi supplementation experiment with school prawn was conducted in 60 l aquaria. The school prawn and leader prawn trials for evaluating commercial diets were conducted in swimming pools and pens, respectively. Pens are netting enclosures surrounding sections of pond bottom. These experimental systems have previously been described (1).

Juvenile school prawn (2-6 g individual prawn weight) were collected by otter trawling in the William River, New South Wales, Australia. They were transported in aerated tanks to the Brackish Fish Culture Research Station at Port Stephens (32°42'S, 152°12'E) and separated into male and female groups, only the latter of which were used for the aquarium experiment.

Into each aquarium were added ten individually weighed and tagged prawns (fluorescent pigment in petroleum jelly injected into tail musculature). The aquaria contained coarse sand covering a subsurface biofilter plate which was connected to a continuous source of compressed air. Water recirculated through the sand bed which had been conditioned prior to the experiment to encourage the growth of nitrifying bacteria. Daily water exchange ranged from an initial rate of 50% per day by the time the experiment was terminated after four weeks. Seawater (215-31%) was preheated prior to water exchange in the aquaria which were maintained at 25 ±0.6°C. Dissolved unionized ammonia and nitrite levels measured in all aquaria were below those reported to inhibit penaeid growth rates (6). All diets were supplied on an ad libitum basis and were fed once per day just prior to the onset of the dark phase within the artificially controlled 12:12 day: night light cycle. There were three replicate aquaria per diet.

Preparation of Diets

Pipis, a type of burrowing marine bivalve, were collected from the intertidal surf zone on a sandy beach near the research station and kept alive in aerated seawater before being shucked. The lipid component of pipi meat was extracted using the following method (7): Six volumes of chooped pipi meats were homogenized with six volumes of rain water. After filtering the homogenate, the residue was rinsed with six volumes of solvent mixture and two volumes of rain water. The filtrate was allowed to seprate overnight into three phases: water/methanol; water/protein and chloroform/lipid. Four preparations of pipi meat were used: freshly shucked and chooped meat which was held on a sieve to drain off excess liquid (chooped pipi); chooped, drained pipi meat homogenized in a domestic blender and frozen (whole pipi component); the insoluble residue collected after the extraction process and frozen (insoluble pipi component), and the fraction which was obtained by evaporation the chloroform phase (pipi lipid component). This last component was subsequently stablished with Endox antioxidant (approximately 250 mg Endox/kg oil).

Five compounded diets were prepared for the experiment (Table 1). These involved a basic experimental diet (A) and various modifications of this diet (B-E). This basic diet involved some of the more common feed ingredients available to commercial prawn feed producers in Australia. It included a range of protein sources, high and low fibre carbohydrate sources, fish oil (the protein meals also contain lipid), cholesterol, glycine (as a food attractant), minerals, vitamins and a binder. The methods used by the authors to prepare this type of compounded diet have previously been described (2). Diet (A) contained no pipi components and was prepared as an alginate-bound, 50% moisture content, firm past which was extruded through a meat grinder (3 mm die), frozen or presented as a compounded diet.

The actual nutrient composition of the diets is given in Table 2. Tabulated composition values (8,9) were used for some ingredients and the actual composition of the batches obtained from commercial suppliers may have differed from these values. The major discrepancy between actual composition and that aimed for when the diets were designed was for diet (C), where the protein content was substantially lower than planned. This could have resulted from variations between groups of chooped, drained pipis in protein or moisture content. Differences between protein levels in the range 400-450 g protein/kg dry weight should not affect shool prawn growth rates (2).

Statistical Analysis

Homogeneity of variance, overall treatment effects and differences among treatment means were assessed using Cochran's Test, Analysis of Variance ANOVA and the Least Significant Difference Method (10).

Five compounded diets were prepared for the experiment (Table 1). These involved a basic experimental diet (A) and various modifications of this diet (B-E). This basic diet involved some of the more common feed ingredients available to commercial prawn feed producers in Australia. It included a range of protein sources, high and low fibre carbohydrate sources, fish oil (the protein meals also contain lipid), cholesterol, glycine (as a food attractant), minerals, vitamins and a binder. The methods used by the authors to prepare this type of compounded diet have previously been described (2). Diet (A) contained no pipi components and was prepared as an alginate-bound, 50% moisture content, firm paste which was extruded through a meat grinder (3 mm die), frozen and then broken up into individual pellets. Before being frozen the extruded diet was sprayed with a fine mist of a 3% $CaCl_2$ solution to improve binding. Diets B-E were prepared in the same manner except than before being frozen the extruded pastes were sprayed with a 1.5% sodium alginate solution followed by a 3% $CaCl_2$ solution to coat the pellets. This resulted in an estimated 2.75 g dry sodium alginate per kg dry diet being retained on the surface of the pellets.

Diet (B) differed from diet (A) only in being coated with alginate. Diets (C-E) contained pipi components with diet (C) containing the equivalent of 300 g of dry whole pipi component per kg dry diet. The pipi was in fact added to the diet in an undried form after its moisture content was estimated. Diets (A-E) were designed to have the same total protein and total lipid contents. Hence in diet (C) the levels of fish and soybean meals, torula yeast and fish oil were reduced. The level of maize meal was increased to retain the same total amount of dry matter. The amounts of the insoluble component and the lipid component in 300 g dry whole pipi component were estimated to be 196.8 g and 15.0 g, respectively on a dry weight basis. These amounts were incorporated into diets (D) and (E), respectively and appropriate adjustments made to other protein sources, fish oil and maize meal (Table 1). Besides diets (A-E), an additional treatment was included in the experimental design as a control. Prawns in these aquaria were fed chopped pipi which was not homogenized, treated with solvents, frozen or presented as a compounded diet.

The actual nutrient composition of the diets is given in Table 2. Tabulated composition values (8,9) were used for some ingredients and the actual composition of the batches obtained from commercial suppliers may have differed from these values. The major discrepancy between actual composition and that aimed for when the diets were designed was for diet (C), where the protein content was substantially lower than planned. This could have resulted from variations between groups of chopped, drained pipis in protein or moisture content. Differences between protein levels in the range 400-450 g protein/kg dry weight should not affect school prawn growth rates (2).

Statistical Analysis

Homogeneity of variance, overall treatment effects and differences among treatment means were assessed using Cochran's Test, Analysis of Variance ANOVA and the Least Significant Difference Method (10).

RESULTS

The type of diet provided had no significant effect on prawn survival rates ($p>0.05$) which were relatively high for all treatments (Table 4).

School prawns grew significantly better on chopped pipi than on diets (A-E) (Table 4) ($p<0.01$). Coating the pellets with sodium alginate did improve their physical stability in sea water, but there was no significant difference between the weight gains for prawns fed diets (A) or (B) ($p>0.05$). The supplementation of the basic formulation used in diets (A) and (B) with pipi lipid (diet (E) did not significantly improve growth ($p>0.05$). Inclusion of the whole pipi component (diet (C) and the insoluble component (diet (D) did significantly improve the weight gains ($p<0.01$). There were no significant differences between prawns fed diets (C) and (D) ($p>0.05$).

DISCUSSION

Aquarium Experiment

In the present study supplementation of the basic experimental diet (A,B) with whole pipi and insoluble pipi components (diets (C) and (D), respectively), did substantially improve school prawn weight gains whereas supplementation with pipi lipid (diet (E) did not (Tables 1,4). In a similar study involving supplementation of an experimental diet with insoluble and lipid components of the short-necked clam (Venerupis philippinarum), both components improved P. japonicus weight gains (4,5). However, in that study the insoluble component had a larger growth promoting effect than the lipid component when added to the basic diet which was devoid of clam components. Deshimaru (5) described the insoluble component as the protein fraction. He concluded that the nutritive value of the clam should be primarily attributed to its protein content and that the low nutritive value of the basic experimental diet was because of its unappropriate amino acid balance.

When a complex nutrient source, e.g., insoluble pipi meat fraction, is added to a diet several nutrient levels are likely to be altered. The development of techniques for retaining purified soluble nutrients, e.g., amino acids, in experimental diets would allow for more precise control of nutrient levels than can be achieved by incorporating bivalve components. However, the low rate of intake of soluble nutrients by prawns due to leaching of these nutrients from diets is a major constrain (5). In the present study inclusion of whole and insoluble pipi components not only altered the amino acid balance but also increase Copper and Zinc levels and reduced Calcium, Phosphorous and Potassium levels (Table 2). Some of these minerals have been shown to affect prawn growth rates (11). Regardless it is probably reasonable to attribute the growth promoting effects of these components to an improved amino acid balance. In both the present study and the one conducted by Deshimaru (4,5) there was evidence that inclusion of bivalve meat can cause a major increase in the arginine level. There were also some increases in the levels of tyrosine and methionine in the present study (Table 3).

None of the compounded diets produced growth increments as large as that obtained with chopped pipi meat. This suggests that the design of the basic experimental diet (A,B) would have to be changed before it could be used commercially to feed school prawns in a situation where natural food items made only a minor contribution to the diet of the prawns. When a moderate stocking density was used, i.e., 15 prawns/m^2, natural food items comprised more than 40% of the stomach (proventriculus) contents of school prawns (12). At higher stocking densities this contribution could decline greatly. It should be noted however, that trials in aquaria are likely to provide a far more stringent test for compounded diets than pond trials would. It is interesting to note that when one commercial diet which was widely used on commercial Penaeus japonicus farms in Japan was fed to school prawns in aquaria, poor growth results were obtained despite the fact that quite rapid growth rates were obtained when chopped pipi meat was used in that experiment (13).

OVERVIEW OF AUSTRALIAN COMMERCIAL PRAWN FARMING DIETS

Two types of commercial diet evaluation trials have been carried out by the authors. In the first, school prawns were stocked into replicated 3.4 m diameter swimming pools at a density of 15 prawns/m^2. The feed types used were as follows: unfed; a commercial trout diet (398 g protein/kg dry diet); a commercial school prawn diet based on a poultry ration supplemented with fish meal (228 g protein/kg dry diet); and an experimental prawn diet (401 g protein/kg dry diet) designed for a commercial manufacturer. This last diet was formulated to have an amino acid balance similar to that of pipi meat. The results of this trial confirmed the results of commercial pond trials and earlier pen trials (2,3), i.e., that a relatively simple, low protein, pelleted diet was adequate for school prawns grown at a moderate density in the presence of a natural food supply. Subsequently, the only Australian commercial prawn farming diet specifically designed for school prawns has been upgraded and should be more appropriate for commercial school prawn farming trials at higher stocking densities.

The second type of commercial diet evaluation trial was conducted using leader prawns (P. monodon) sotcked at a density of 15 prawns/m^2 in replicated 3 m^2 pens. This much larger species is the one which at least initially wil be used by the majority of Australian commercial prawn farmers (14). The dietary treatment were as follows: unfed; frozen pipi meat and four commercial diets designed for leader prawns. One of these was an imported Taiwanese diet (425 g protein/kg dry diet) and three were Australian diets (368-419 g protein/kg dry diet). All diets supported high survival rates but the Australian diets produced either much poorer growth or food conversion results than the Taiwanese diet or the pipi meat. These Australian diets are being redesigned and several other commercial feed companies are also likely to produce diets for leader prawns. The emphasis seems to be on including marine protein meals, e.g., squid meal, in addition to fish meal. This strategy would also change the lipid component of the diet. However, if the results of the school prawn aquarium trials presented in this paper and the bivalve supplemntation results with P. japonicus diets (4,5) are a guide, the major impact could well be an improvement in the amino acid balance. Some marine protein

meals can also be a source of a type of food attractant and an unidentified growth factor (15). Regretably, except for some locally made fish meals, marine protein meals are not produced in Australia and their inclusion will increase feed costs.

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TABLE 1 Composition of experimental diets (ingredients expressed as g/kg dry weight)

Ingredient	Diets			
	A,B[a]	C	D	E
Fish meal (640g/kg protein)	230.70	65.70	124.90	230.70
Soybean meal	316.10	90.00	171.20	316.10
Torula yeast	164.00	46.70	88.80	164.00
Glycine[b]	10.00	10.00	10.00	10.00
Maize meal	69.00	282.40	190.40	69.00
Wheat bran	94.45	94.45	94.45	94.45
Vitamin mix[c]	20.00	20.00	20.00	20.00
Mineral mix[d]	30.00	30.00	30.00	30.00
Cholesterol	5.00	5.00	5.00	5.00
Cod liver oil	45.00	40.00	52.80	30.00
Endox[e]	0.25	0.25	0.25	0.25
Binder[f]	15.00	15.00	15.00	15.00
TSPP[g]	0.50	0.50	0.50	0.50
Whole pipi component	–	300.00	–	–
Insoluble pipi component	–	–	196.80	–
Pipi lipid component	–	–	–	15.00

a Diet B contained the same ingredients as diet A but the pellets were coated with sodium alginate.

b Added as a food attractant (4).

c Vitamins mixed with wheat starch. Dietary vitamin levels (as mg active vitamin/kg dry diet) were retinol (A) 2.00, cholecalciferol (D) 0.03, alpha-tocopherol acetate (E) 50.00, menaphthone dimethylpyrimidinol bisulphite (K) 10.00, thiamin (B_1) 20.00, riboflavin 30.00, pyridoxine (B_6) 20.0, pteroylmonoglutamic acid (folic acid) 5.00, ascorbic acid (C) 3,000.00, calcium pantothenate 60.00, myo-inositol 4,000.00, biotin 2.00, choline chloride 3,000.00, nicotinic acid (niacin) 200.00 and cyanocobalamin (B_{12}) 0.20.

d Dietary mineral levels (as mg/kg dry diet) were $CaHPO_4.2H_2O$ 16380, K_2HPO_4 11000, $Al_2(SO_4)_3.18H_2O$ 69, $ZnSO_4.7H_2O$ 900, $CuCl_2$ 85, KI 50 and $CoCl_2.6H_2O$ 250.

e Anti-oxidant.

f Manugel GMB (sodium alginate).

g Tetrasodium polyphosphate (TSPP) is a sequestrant for the binding process.

TABLE 2 Nutrient composition of experimental diets and pipi components (nutrient content expressed as g/kg dry weight unless otherwise stated)

Component	Diet					Pipi component	
	A	B	C	D	E	Whole	Insoluble
Crude protein[a]	460.0	464.0	408.0	453.0	462.0	724.0	762.0
Ash	120.3	125.5	115.0	109.7	125.3	151.6	125.8
Lipid	102.0	101.0	87.5	93.1	102.9	62.6	8.3
Calcium	12.8	13.5	7.9	9.9	13.2	4.8	4.8
Phosphorus	15.3	15.2	12.1	13.4	15.8	9.7	9.5
Potassium	16.5	17.7	13.8	12.2	16.4	12.6	7.4
Magnesium	3.1	3.2	2.7	2.9	3.0	3.1	3.2
Sodium	6.5	6.5	9.6	7.7	6.9	19.1	10.2
Manganese (mg/kg)	45.5	42.0	27.1	33.1	40.5	6.3	8.4
Copper (mg/kg)	55.8	60.0	115.0	76.5	68.6	60.6	130.0
Zinc (mg/kg)	259.0	275.0	341.7	338.2	290.0	243.5	421.0

a Crude protein determined as N x 6.25.

TABLE 3 Amino acid composition of experimental diets and pipi components (expressed as a percentage of total protein)

Amino acid	Diet					Pipi component	
	A	B	C	D	E	Whole	Insoluble
Aspartic acid	8.0	8.8	8.2	9.7	9.0	8.9	8.8
Threonine	3.6	4.3	4.0	4.1	4.0	4.5	4.0
Serine	5.0	5.7	4.3	4.8	5.5	4.5	4.2
Glutamic acid	12.6	13.4	13.2	13.1	13.1	13.1	12.2
Proline	6.7	7.3	6.1	6.5	7.0	4.9	4.5
Glycine	8.3	8.9	9.0	8.3	8.7	6.2	7.3
Alanine	5.2	5.6	5.3	5.7	5.7	5.5	5.7
Valine	4.6	4.9	4.1	4.9	4.6	4.3	3.9
Cystine	3.6	3.7	3.4	3.3	3.3	2.8	2.9
Methionine	1.0	1.2	2.0	1.6	1.4	2.5	2.5
Isoleucine	3.5	3.7	3.7	4.1	3.7	3.8	3.7
Leucine	6.2	6.5	6.8	7.8	7.0	7.1	6.5
Tyrosine	2.6	2.7	3.7	3.5	2.8	3.2	3.0
Phenylalanine	3.6	3.7	3.7	3.3	4.2	3.2	2.9
Histidine	2.3	3.6	2.8	2.6	2.6	1.9	1.9
Lysine	5.0	5.0	5.1	5.7	4.9	6.4	6.2
Arginine	6.3	6.1	7.1	7.4	6.6	7.6	7.8
% amino acid recovery	91.4	98.8	95.8	94.7	97.5	93.0	90.8

TABLE 4 Weight gain and survival results for school prawns fed experimental diets (X ± S.D.)[1]

Diet	Weight gain (g/prawn)	Survival[2] rate (%)
A	0.43 ± 0.11 [a]	80.0 ± 10.0
B	0.43 ± 0.09 [a]	90.0 ± 17.3
C	1.10 ± 0.04 [b]	100.0 ± 0
D	1.00 ± 0.27 [b]	90.0 ± 10.0
E	0.51 ± 0.10 [a]	93.3 ± 5.8
Chopped pipi	2.42 ± 0.29 [c]	100.0 ± 0

1 Means sharing a common superscript within each column are not significantly different ($p>0.05$).

2 Data transformed (arc sin square root) prior to statistical analysis. Survival rate not significantly affected by diet type ($p>0.05$).

SECTION 3

THE EFFECTS OF SOME ENVIRONMENTAL AND NUTRITIONAL FACTORS ON OYSTER LARVAE AND SPAT

JOHN A. NELL

3.1 Introduction

Very little was known about the effect of temperature and salinity on Sydney rock oyster (*Saccostrea commercialis*) and flat oyster (*Ostrea angasi*) spat. Oyster farmers have thought salinity to be an important factor in oyster growth and spat recruitment, but have not been able to distinguish between the effects of salinity and the generally higher nutrient levels associated with low salinity estuarine water as compared to oceanic water (Ward and Twilley, 1986). A lot of interest has been shown (King, 1977) in the cultivation of Pacific oysters (*Crassostrea gigas*) in hypersaline ponds. However, no data was available on the salinity range for maximum growth of the introduced Pacific nor the native Sydney rock nor the flat oyster. There was therefore a considerable need to do some salinity studies. Temperature is another important factor in oyster growth and farming that was not well understood. Water temperature tends to increase as light intensity (sunshine) increases on a seasonal basis. However both factors can directly influence algal production and hence the supply of food for oysters. Therefore a series of experiments were conducted to investigate the effects of temperature on oyster growth rates under conditions where excess food was provided and where light levels did not differ between treatments.

Copper is known to be toxic to oysters (Calabrese et al., 1977), but has also been known to stimulate the settlement of oyster larvae (Prytherch, 1931, 1934). There have been large increases in oyster meat copper concentrations in the Georges River, NSW, from 37 mg/kg in 1973 to 65 mg/kg in 1985 (Chvojka, personal communication, 1987). The reasons for this increase are not known, but the high copper concentrations are of great concern. For these reasons an experiment was conducted on the effect of copper concentrations on the settling rate of oyster larvae.

Potassium has been used to stimulate the settlement of abalone larvae (Baloun and Morse, 1984). Because of the need for rapid settlement of oyster larvae in hatcheries, a number of experiments were conducted to test the use of potassium as a stimulant for the settling of oyster larvae.

Fluoride is a potential environmental hazard (Anonymous, 1983) for Port Stephens, Australia's premier oyster growing area. A series of experiments were conducted to assess the possible effect of increased fluoride concentrations in seawater on oysters.

Cultured unicellular algae have been used for decades as the sole food source in oyster hatcheries. Because of the high cost and unpredictability of production, development of artificial diets for oysters has been attempted by several investigators. The best of the artificial oyster diets were compared against a standard algal diet consisting of *T. Isochrysis* aff. *galbana* and *Pavlova lutheri*. Oysters readily absorb dissolved amino acids from seawater (Nell et al., 1983) but no attempt had been made to use dissolved amino acids as a dietary supplement. The effect of amino acids on the growth rate of juvenile oysters was investigated.

The evaluation of the relative value of various algal species as food sources for larval oysters has been given a new impetus, because of the F.I.R.T.A. funded work by Drs. S. W. Jeffrey and C. D. Garland. Their fatty acid composition data are a useful guide in explaining why oyster larvae grow better on some algal species and not so well on others. The findings by Enright et al. (1986a, b) that the 20:5ω3 and 22:6ω3 fatty acids are of great importance to the food value of algal species, has given oyster larval nutrition a new direction. However, algae size and algal cell wall digestibility are other important factors to be considered. It is also likely that larval size and age will have an effect on the choice of algal species. Some experiments were conducted to evaluate the food value of some algal species for 1-day old Sydney rock oyster larvae.

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Salinity Tolerance and Absorption of L-Methionine by Some Australian Bivalve Molluscs

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Abstract

The salinity tolerance range of the scallop *Pecten fumatus* Reeve was 25–40 g l^{-1}, of the pipi (clam) *Plebidonax deltoides* (Lamarck) and the flat oyster *Ostrea angasi* Sowerby, 20–45 g l^{-1}, and of the blue mussel *Mytilus edulis planulatus* Lamarck and the Sydney cockle *Anadara trapezia* (Deshayes), 15–45 g l^{-1}. All of these bivalves absorbed substantial amounts of the amino acid L-methionine directly from seawater.

Introduction

The salinity tolerance of bivalves is species-dependent and habitat related (Pierce 1970; Bedford and Anderson 1972; Vernberg and Vernberg 1972; Anderson and Anderson 1975). For mussels of the genus *Modiolus*, Pierce (1970) showed that estuarine species have the widest and oceanic species the narrowest salinity tolerance range.

Active absorption of free amino acids dissolved in seawater has been demonstrated for many marine bivalves (Stephens 1972, 1982; Stewart 1979). The absorbed amino acids partially meet their nutritional requirements (Stephens 1972; Manahan and Crisp 1982; Wright 1982). Dissolved amino acids were used to supplement an artificial oyster diet (Nell and Wisely 1984) following a study of conditions necessary to optimise the accumulation of the essential (Harrison 1976) amino acid L-methionine and its metabolites in oysters (Nell and Dunkley 1984).

The salinity tolerance of five Australian bivalves with aquaculture potential were determined to aid in the selection of hatchery and field culture sites with a suitable salinity regime. The ability of bivalves to absorb dissolved L-methionine from seawater also was determined for possible amino acid supplementation of artificial diets in tanks or raceways.

Materials and Methods

Salinity Tolerance

The collection details and habitat descriptions for the experimental animals are shown in Table 1. All animals were collected from localities with relatively stable seawater salinities of approximately 35 g l^{-1} and transported in seawater with a salinity of 35 g l^{-1}. The experiment commenced the day after collection and the animals were transferred directly from a salinity of 35 g l^{-1} to the experimental salinities of 5–55 g l^{-1} with 5-g l^{-1} intervals. Mortality rates were recorded over a range within which most animals survived. When all individuals of a species held at the extreme salinities had died, mantle fluid samples were taken from all survivors for osmolarity determination. Hence, there were different experimental periods for the species, depending on the period of survival (Table 1).

Four aerated aquaria (40 litres) were used for each salinity. Each aquarium contained four adult animals of the following species: the scallop, *Pecten fumatus* Reeve, 1852, the pipi (clam), *Plebidonax deltoides* (Lamarck, 1818), the flat oyster, *Ostrea angasi* Sowerby, 1871, the blue mussel, *Mytilus edulis planulatus* Lamarck, 1819, and the Sydney cockle, *Anadara trapezia* (Deshayes, 1840). Sufficient sand was placed

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in one-third of the floor area of each aquarium to allow the pipis and scallops to bury themselves. All animals were randomly allocated to aquaria. Salinities were measured with a temperature-compensated refractometer. Precise salinities were obtained by adding an artificial sea salt mixture (Wood and Ayres 1977) or rainwater as required to sand-filtered seawater with a salinity of 35 g l^{-1}. Temperatures were kept at 22°C and the water was changed each day.

Pooled mantle fluid samples of all animals of a species from individual aquaria were used to measure osmolarities. Animals were taken out of water 20 min before opening and dried off to avoid mantle fluid mixing with seawater. Mantle fluid was chosen for osmotic pressure determination because of the difficulty encountered in obtaining sufficient volume of either pericardial fluid or blood from *Plebidonax deltoides*. Any of these fluids can be used to determine the salinity tolerance range of bivalves as the osmolarity of all three is very similar (Pierce 1970; Wada 1984). To ensure that mantle fluid could be used instead of pericardial fluid to determine the salinity tolerance range of bivalves, this relationship was established experimentally with non-acclimatised Sydney rock oysters, *Saccostrea commercialis* (Iredale & Roughley, 1933). Experimental techniques were as described previously. Oysters were held in experimental salinities for only 2 days before fluid samples were taken. Collected mantle fluid samples were stored in capped vials and frozen until the end of the experiment. Fluid samples for osmolarity determination can be stored in this manner for at least 1 week without a change in osmolarity (Nell and Dunkley 1984). The osmolarity of mantle fluid and water samples from the aquaria was determined on a vapour pressure osmometer (Wescor 5100B). Salinity tolerance range in this study was defined as the salinity ranges in which bivalves survived and their mantle fluid osmolarities conformed with the osmolarity of the seawater that they were in.

Table 1. Determination of the salinity tolerance range for five bivalve species

Scientific and common names	Collection details Habitat	Place	Latitude (°S.)	Salinity tolerance range (g l^{-1})	Survival[A] (days)
Pecten fumatus, scallop	Sand bottom, deep water	Jervis Bay	35	25–40	2
Plebidonax deltoides, pipi (clam)	Ocean beach	Stockton Bight	32	20–45	7
Ostrea angasi, flat oyster	Mud bottom	Jervis Bay	35	20–45	8
Mytilus edulis planulatus, blue mussel	Rope culture	Jervis Bay	35	15–45	10
Anadara trapezia, Sydney cockle	Seagrass beds	Jervis Bay	35	15–45	11

[A] Longest survival time at salinities outside the salinity tolerance range.

L-Methionine Absorption

The ability of the bivalves to absorb L-methionine directly from seawater was investigated with tritium-labelled L-methionine (Radiochemical Centre, Amersham, England) at 555 kBq l^{-1} as a tracer. Non-labelled L-methionine was added to obtain the required L-methionine concentration of 0·7 mg l^{-1} in seawater. This concentration of L-methionine produced the highest level of accumulation (Nell *et al.* 1983) in *Saccostrea commercialis*. This oyster was used again in this experiment, together with the five species of bivalve used in the salinity tolerance study.

Four aquaria each containing three randomly allocated animals in 3 litres of water with a salinity of 30 g l^{-1} and a temperature of 22°C were set up for each species. Seawater was filtered through a 0·22-μm membrane filter. All animals were acclimated to the experimental conditions for 10 days. After a 4-h experimental period in the L-methionine solutions, the bivalves were opened and the tissues were washed with water and homogenised in 5 volumes of 10% (w/v) trichloroacetic acid. The homogenates were centrifuged and 0·5-ml aliquots of the supernatants treated with 1 ml of solubiliser (Radiochemical Centre, Amersham, England). Radioactivity was counted by liquid scintillation spectrophotometry (Packard model 6892) with 10 ml of scintillation fluid (4 g of 2,5-diphenyloxazole, 0·2 g of 1,4-bis[5-phenyloxazolyl-2-]benzene, 330 ml of Teric and 670 ml of toluene per litre). Quenching was reduced

by the addition of acetic acid to the scintillation fluid. The data in Table 3 were checked for homogeneity of variance using the Cochran test and the variances were within acceptable limits ($P = 0{\cdot}05$) to compare the data statistically. They were then submitted to analysis of variance and mean values were compared by least-significant differences (Sokal and Rohlf 1981).

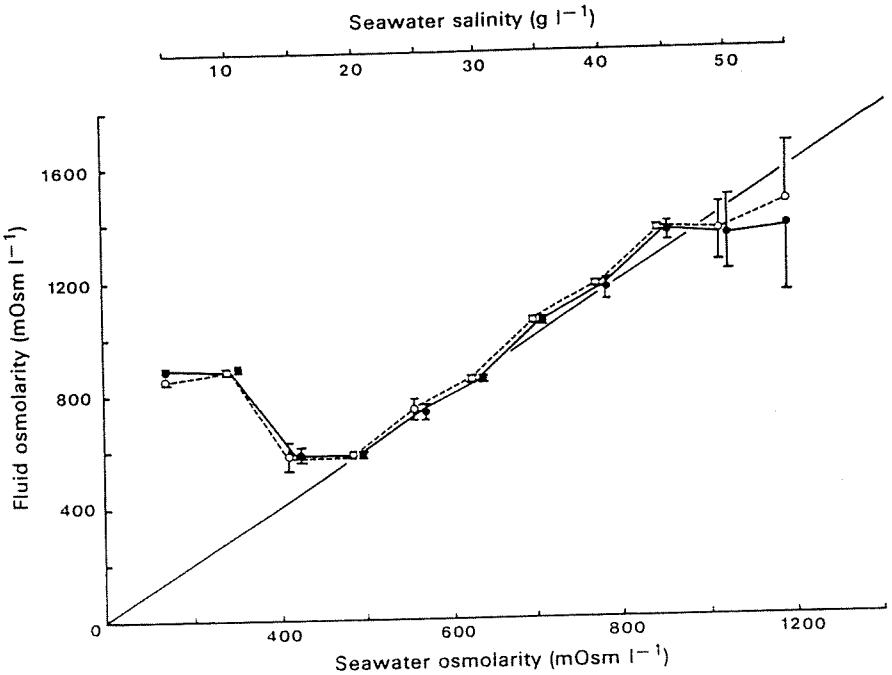

Fig. 1. Relationship between mantle fluid (○---○) and pericardial fluid (●—●) osmolarities for *Saccostrea commercialis*. Means ± 1 s.d. Diagonal line indicates isosmolarity.

Table 2. Bivalves surviving within the salinity tolerance ranges
Sixteen bivalves of each species were used for each salinity

Salinity (g l^{-1})	Number surviving				
	Pecten fumatus	*Plebidonax deltoides*	*Ostrea angasi*	*Mytilus edulis planulatus*	*Anadara trapezia*
5	0	0	0	0	0
10	0	0	0	0	0
15	0	0	0	13	16
20	0	14	16	16	16
25	14	15	16	16	16
30	15	16	15	16	16
35	15	15	16	16	16
40	16	16	16	16	16
45	0	16	16	13	16
50	0	0	0	0	0
55	0	0	0	0	0

Results

Salinity Tolerance

There was a good relationship between mantle and pericardial fluid osmolarities in *Saccostrea commercialis* (Fig. 1). There was no difference in the fluid osmolarities at the salinities from

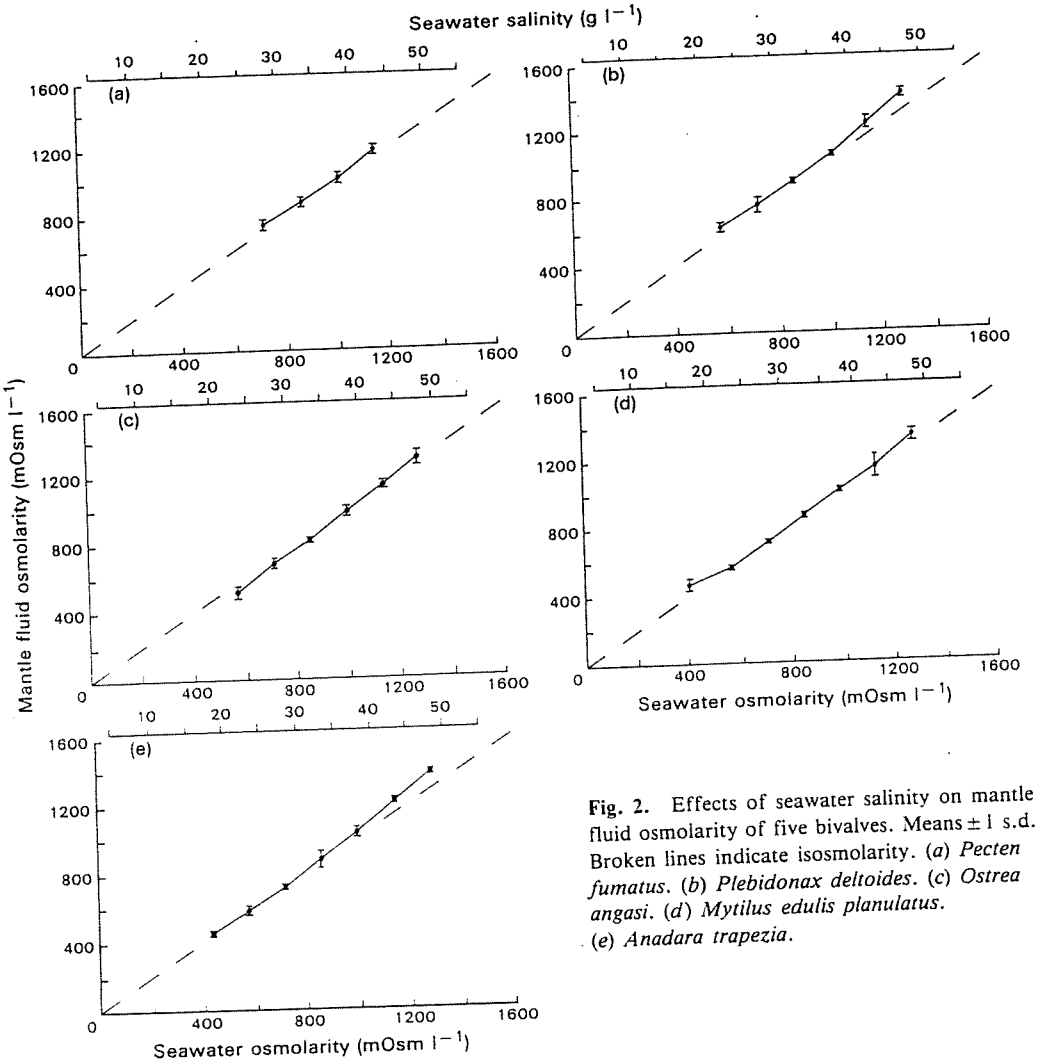

Fig. 2. Effects of seawater salinity on mantle fluid osmolarity of five bivalves. Means ± 1 s.d. Broken lines indicate isosmolarity. (*a*) *Pecten fumatus*. (*b*) *Plebidonax deltoides*. (*c*) *Ostrea angasi*. (*d*) *Mytilus edulis planulatus*. (*e*) *Anadara trapezia*.

Table 3. Comparison of the accumulation of L-methionine and its metabolites

Values are means ± 1 s.d. Within each column, means that do not share a common superscript differ significantly ($P < 0{\cdot}05$)

Species	Total wet tissue weight (g)	Amount of L-methionine accumulated by bivalve (μg)	Concentration of L-methionine in bivalve (μg g^{-1} wet tissue)
Pecten fumatus	7·2 ± 0·7	286 ± 42^{bc}	40 ± 6^{a}
Plebidonax deltoides	7·8 ± 1·1	364 ± 55^{c}	47 ± 6^{a}
Ostrea angasi	3·9 ± 1·2	217 ± 73^{ab}	55 ± 7^{a}
Mytilus edulis planulatus	7·6 ± 1·7	365 ± 75^{c}	50 ± 12^{a}
Anadara trapezia	3·5 ± 0·3	136 ± 42^{a}	38 ± 9^{a}
Saccostrea commercialis	4·7 ± 0·5	281 ± 86^{bc}	62 ± 22^{a}

20 to 45 g l^{-1} but, even outside this range, mantle fluid osmolarity was a good indicator of salinity tolerance. A linear regression through the origin gave a relationship for pericardial fluid (y) on mantle fluid (x) osmolarity of $y = 0{\cdot}99x$ ($r = 0{\cdot}99$).

None of the species of bivalve tested was an effective osmoregulator, as they tolerated unregulated changes in mantle fluid osmolarity within their ranges of salinity tolerance (Fig. 2). Very few bivalves died within the salinity tolerance ranges (Table 2) and survival time outside these ranges was correlated to the width of the salinity tolerance ranges (Table 1). The stenohaline bivalves had the shortest and the euryhaline bivalves the longest survival times, at salinities outside the salinity tolerance range.

L-Methionine Absorption

All bivalves accumulated large amounts of L-methionine and its metabolites. There were no significant ($P > 0{\cdot}05$) differences among the bivalves in the tissue concentrations (Table 3). The average concentration of L-methionine and its metabolites in the bivalves was 49 μg g^{-1} tissue. The actual amounts accumulated were greatly dependent on the tissue weight of the bivalves (Table 3).

Discussion

Both mantle and pericardial fluids may be used to determine the salinity tolerance range of bivalves. This finding is in agreement with the reports of Pierce (1970) and Wada (1984), that the osmolarities of mantle fluid, pericardial fluid and blood of bivalves are very similar.

Bivalves from an oceanic habitat had the narrowest and those from an intertidal estuarine habitat the widest salinity tolerance range. Pierce (1970) found the salinity tolerance range for mussels of the species *Modiolus modiolus* L., 1758 collected from a rocky coast to be 22–41 g l^{-1}, and for mussels of the species *Modiolus demissus demissus* Dillwyn, 1817 collected from a salt marsh to be 8–48 g l^{-1}. In this study, *Mytilus edulis planulatus* had a salinity tolerance range of 15–45 g l^{-1} and this lower salinity tolerance level was very similar to the 13 g l^{-1} reported by Almada-Villela (1984) for *Mytilus edulis edulis* L., 1758 collected in England. *Pecten fumatus* had a salinity tolerance range of 24–40 g l^{-1}. Thus, it is far less tolerant of low salinities than *Argopecten irradians* (Lamarck, 1819), which has a lower salinity tolerance level of 10 g l^{-1} (Mercaldo and Rhodes 1982).

The salinity tolerance range of the intertidal estuarine *Saccostrea commercialis* was found to be 15–45 g l^{-1} by Nell and Dunkley (1984), but this range was subsequently shown to extend to 50 g l^{-1} (J. A. Nell, unpublished data); however, it grows best at a salinity range of 25–35 g l^{-1}, as does *Ostrea angasi* (J. A. Nell and G. Livanos, unpublished data). The lower salinity tolerance level of *Ostrea edulis* L., 1758 of 15 g l^{-1} (Chanley 1957), is lower than that of *O. angasi* in this study (20 g l^{-1}), but higher than that of the American oyster *Crassostrea virginica* Gmelin, 1791 (5–6 g l^{-1}: Castagna and Chanley 1973; Anderson and Anderson 1975). The salinity tolerance range of the Pacific oyster, *Crassostrea gigas* Thunberg, 1793, was 5–55 g l^{-1} (J. A. Nell, unpublished data) and this oyster grows well at salinities as high as 40 g l^{-1} (Hughes-Games 1977; King 1977). Thus, *C. gigas* could be cultivated successfully in hypersaline ponds (Hughes-Games 1977; King 1977), whereas this is not likely to be successful with either *S. commercialis* or *O. edulis* (J. A. Nell and G. Livanos, unpublished data).

This study showed that the wider the salinity tolerance of the bivalve investigated, the longer its survival time at salinities outside the salinity tolerance range. Survival time at salinities below the salinity tolerance range decreases with increasing temperatures (Mercaldo and Rhodes 1982). Bivalves held at the extreme salinities of 5 and 55 g l^{-1} died before those held at 10 and 50 g l^{-1}, which in turn generally died before those held at 15 and 45 g l^{-1}. This suggests that the five species tested were unable to remain totally inactive and to keep the valves tightly closed. Rather, the animals became as active as necessary to provide minimal water exchange, thereby lowering their tissue fluid osmolarity and water balance levels

until these became critical and killed them. This is supported by the findings of Nell and Dunkley (1984), who found that *S. commercialis* held at salinities outside their salinity tolerance range for 10 days were hyperosmotic at 5 g l^{-1} and hypo-osmotic at 55 g l^{-1} and dying. Bivalves, especially those from intertidal habitats, need not exchange water over short periods of time to maintain tissue fluid oxygen levels. They may instead revert to anaerobic metabolism (Bayne *et al.* 1976; Zwaan 1977; Gilles and Jeuniaux 1979). Water exchange, however, may be required to reduce the concentration of excretory products such as ammonia in the tissue fluids.

The variations from the isosmotic line that occur in molluscs and are evident in Fig. 1 of this study have been discussed by Vernberg and Vernberg (1972). The deviations from the isosmotic line are a result of the animal's ability to hyper-regulate, at least over some part of their normally encountered salinity range. In the case of *Plebidonax deltoides* the response is very similar to that of some gastropods that maintain internal body fluids slightly hyperosmotic but parallel to the isosmotic line (Vernberg and Vernberg 1972).

The concentration of L-methionine and its metabolites in the tissue after exposure for 4 h was very high for all species tested. This suggests that all species could benefit from dissolved free amino acids in seawater to help meet their nutritional requirements for essential amino acids (Harrison 1976), as well as in maintaining their fluid osmolarities (Gilles 1972; Hoyaux *et al.* 1976; Powell *et al.* 1982). There was surprisingly little difference in the L-methionine absorption of the six species tested, despite the large differences in their natural habitats.

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EFFECTS OF FLUORIDE CONCENTRATION IN SEAWATER ON GROWTH AND FLUORIDE ACCUMULATION BY SYDNEY ROCK OYSTER (*SACCOSTREA COMMERCIALIS*) AND FLAT OYSTER (*OSTREA ANGASI*) SPAT

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Abstract—Fluoride concentrations in Sydney rock oyster spat increased linearly from 45 to 204 μg^{-1} dry spat with increasing seawater fluoride additions from 0 to 30 mg l^{-1}. Over this fluoride range, weight gains decreased linearly with a 20% growth depression at the highest concentration. Higher concentrations of fluoride were found in spat of both species held at a salinity of 15‰ in 30 mg F l^{-1} than in those held at salinities of 25, 35 and 45‰ at the same seawater fluoride concentration. Spat of both species grew fastest at salinities of 25 and 35‰. Mortalities of flat oyster spat were higher at 15‰ than at salinities of 25, 35 and 45‰. Weight gains for Sydney rock oysters increased with increasing temperatures from 12 to 30°C. Fluoride concentrations in Sydney rock oyster spat held in seawater containing 50 mg F l^{-1} at 24 and 30°C (2282 and 2130 $\mu g\, g^{-1}$ dry spat) were much higher than in those held at 12 and 18°C (1116 and 1140 $\mu g\, g^{-1}$ dry spat).

Key words—accumulation, concentration, fluoride, growth rates, mortalities, oysters, salinity, spat, temperature, weight gains

INTRODUCTION

Fluoride is readily accumulated in the shell and meats of marine bivalves (Hemens and Warwick, 1972; Wright and Davison, 1975) Exposure of the brown mussel (*Perna perna*) to seawater containing as little as 7.2 mg F l^{-1} caused increased mortality (Hemens and Warwick, 1972). Increases in mortality rates were also recorded among blue mussels (*Mytilus edulis*) when exposed to a level of 10 mg F l^{-1} for 30 days (Wright and Davison, 1975).

Oysters (species not mentioned) held for longer than 5 days in seawater containing 128 mg F l^{-1} or for more than 30 days at 32 mg F l^{-1} suffered high mortalities (Moore, 1969). In addition to adversely affecting oysters, elevated fluoride levels in seawater can lead to undesirable fluoride levels in oyster meats for human consumption. Any major increase in oyster meat fluoride levels above that found in meats from unpolluted waters could be considered to be undesirable. Specifically fluoride levels $\geqslant 30\, \mu g\, g^{-1}$ dry meat are a potential health risk if oysters are consumed in large quantities (Moore, 1969). Fluoride concentrations of 18, 30 and 100 $\mu g\, g^{-1}$ dry adult oyster meat were observed for oysters held for 30 days in seawater supplemented with 2, 8 and 32 mg F l^{-1} in that order (Moore, 1969).

This study was initiated in response to a proposal to dump fluoride-containing aluminium smelter wastes at Wallaroo (Anonymous, 1983) near Port Stephens, New South Wales, a major Australian oyster growing estuary. The effects of fluoride concentration, salinity and temperature on the growth, survival and fluoride accumulation rates for two oyster species were investigated.

MATERIALS AND METHODS

Unless otherwise stated, all experiments were conducted in 8 l. aquaria containing lightly aerated seawater with a salinity of 30‰ at 24 ± 2°C for 3 weeks. Salinities were measured with a temperature compensated refractometer and were adjusted by adding artificial sea salt mixture (Wood and Ayres, 1977) or rainwater as required to sand filtered seaswater with a salinity of approx. 30‰.

Four samples of 100 juvenile oysters (spat) were taken at random at the start of each experiment from the same population as the experimental spat to determine initial dry weights. All live oyster spat were counted at the end of each experiment, dried at 110°C and weighed. Each of the four replicates per treatment consisted of 100 spat held on a nylon mesh tray in an aquarium.

Water in the aquaria was changed daily and excess food in the form of the algae *Isochrysis galbana* and *Pavlova lutheri* added to the aquaria to a final concentration of 150,000 cells ml^{-1} for each species. Fluoride was added as sodium fluoride (NaF) dissolved in distilled water at 10 g F l^{-1}. All fluoride concentrations in this study are additions to the background level of 0.7 mg F l^{-1}.

Experiment 1

The effects of fluoride concentration in seawater on the growth rate and fluoride accumulation of Sydney rock oyster spat were determined using fluoride additions to seawater of 0, 5, 10, 15, 20, 25 and 30 mg F l^{-1}.

Experiment 2

Sydney rock oyster and flat oyster spat were held at salinities of 15, 25, 35 or 45‰ with or without a fluoride addition of 30 mg F l^{-1} seawater. Growth and fluoride accumulation rates were assessed in a separate experiment for each species. Samples taken from each pair of replicates for analysis of the fluoride content of Sydney rock oyster spat at the end of the experiment were pooled because of the small sample weights.

Experiment 3

Growth and fluoride accumulation rates were measured for Sydney rock oyster spat grown at 12, 18, 24 and 30°C with or without a fluoride addition of 50 mg l^{-1} seawater. The fluoride concentration in oyster shell and meat of 4 samples of 6 adult oysters from Port Stephens were determined for comparison with juvenile spat. All oysters and spat were held in filtered seawater for 24 h and were washed before the preparation of samples for analysis. Whole oyster spat were used for analysis, because of the difficulty in separating the meat from the shell in large numbers of spat. The shell content in whole dry spat is approx. 89% (J. A. Nell, personal observation, 1987).

Fluoride analysis

All oyster fluoride concentrations were calculated on a dry weight basis. Whole spat, adult oyster shells and flesh samples were dried at 110°C and ground to a fine uniform powder. Samples of dried, powdered adult shells and whole juvenile oysters (0.1 g) were extracted with 5 ml of 1 M HCl by stirring mechanically in polypropylene beakers for 20 min followed by ultrasonic agitation for a further 10 min. The solution was brought to pH 5–6 with 1M NaOH, 20 ml of total ionic strength adjustment buffer (TISAB) were added (Anonymous, 1973) and the volume was adjusted to 30 ml with deionised water. For adult oyster flesh, 1–2 g of dry, ground sample was treated in the same way as the juvenile oyster samples, the final solution was filtered and the fluoride concentration determined by standard addition.

Standards of 0.2, 1.0, 2.0 and 10.0 μg F ml^{-1} were prepared in polypropylene beakers, containing 0.3 g NaCl, 0.15 g $CaCl_2 \cdot 2H_2O$, 20 ml TISAB and adjusted to a total volume of 30 ml with deionised water. Depending on the levels of fluoride in the samples, two standards which differed in concentration by a factor of 10 were selected and used for calibration.

Fluoride concentrations were measured using an ion selective electrode (Frant and Ross, 1966) coupled with a double junction reference electrode connected to a high impedance mV meter with direct concentration readout. Standards and samples were measured by immersing the electrodes and stirring until a stable response was obtained (about 3 min). The meter calibration was checked after every 3 samples and adjusted as required.

Previous investigations (Singer and Armstrong, 1968; Stewart *et al.*, 1974) have shown that ion selective electrode procedures compare favourably with older, slower methods for the determination of fluoride in biological samples. The major drawback of this technique is the effect of total ionic strength on electrode response. In this procedure we have minimised the problem by using small sample weights where possible and adjusting the ionic matrix of standards to match that of the samples by addition of specified quantities of NaCl, $CaCl_2$ and TISAB.

A number of extraction procedures have been investigated to establish a pretreatment technique which was both rapid and reliable. Dry ashing of the samples was found to be unsatisfactory as it gave inconsistent results. Ashing after wetting with 1M NaOH improved reproducibility and gave results comparable to those obtained by KOH fusion. Simple acid extraction, similar to that employed by Tusl (1970), Wright and Davison (1975) and Torma (1975) was found to be most efficient for this type of sample and had the added advantage of reduced analytical time. Recoveries of fluoride (NaF) added to oyster shell at levels ranging from 14 to 3600 mg kg^{-1} were consistently between 89 and 98% (mean 94.8%, coefficient of variation 3.5%).

Statistical analysis

Linear regression analyses were used to show the effect of fluoride additions to seawater on spat. Other data were analysed by analysis of variance after testing for homogeneity of variance using the Cochran test. Arcsin $\sqrt{x}$ transformations were used for the percentage mortalities because the data covered a wide range of values. Log_{10} transformations were used for the fluoride concentrations in oysters to achieve homogeneity of variance. Least significant differences were calculated to compare mean values. All statistical analyses were carried out according to Sokal and Rohlf (1981).

RESULTS

Experiment 1

Whole Sydney rock oyster spat fluoride concentrations increased linearly ($P < 0.001$) from 45 to 204 μg g^{-1} dry spat with increasing seawater fluoride additions from 0 to 30 mg l^{-1} (Fig. 1). Weight gains decreased linearly ($P < 0.01$) with a 20% growth depression being recorded at the highest concentration. Overall mortality was 1.9% and the seawater fluoride concentration had no significant effect ($P > 0.10$) on mortality.

Experiment 2

Both the Sydney rock and the flat oyster spat grew much faster at salinities of 25 and 35‰ (Table 1) than at 15 and 45‰ ($P < 0.05$). The highest fluoride concentrations in spat were found at 15‰ in seawater containing 30 mg F l^{-1} (Table 2). The flat oyster spat showed an increased mortality at the lowest salinity of 15‰ (Table 3). Sydney rock oyster spat had a much higher mortality rate (17.7%) than the flat oyster but their mortality rate was not significantly affected by salinity ($P > 0.10$). There were no significant effects on mortality rate caused by fluoride level on either species ($P > 0.10$).

Experiment 3

Weight gains of Sydney rock oyster spat were very low at 12°C (Table 4) and increased with increasing temperatures up to 30°C. There was no effect of seawater fluoride concentration on weight gains at 12°C. A trend towards reduction in growth rate became apparent at 18 and 24°C. However, the reduction in growth rate with added fluoride was only significant ($P < 0.05$) at 30°C. Fluoride concentrations in Sydney rock oyster spat held in seawater containing 50 mg F l^{-1} at 24 and 30°C (2282 and 2130 μg g^{-1} dry spat) were much higher than in those held at 12 and 18°C (1116 and 1140 μg g^{-1} dry spat). By the end of the experiment, the mean mortality was 3.5% and there were no significant effects of temperature or fluoride concentration on mortality ($P > 0.10$).

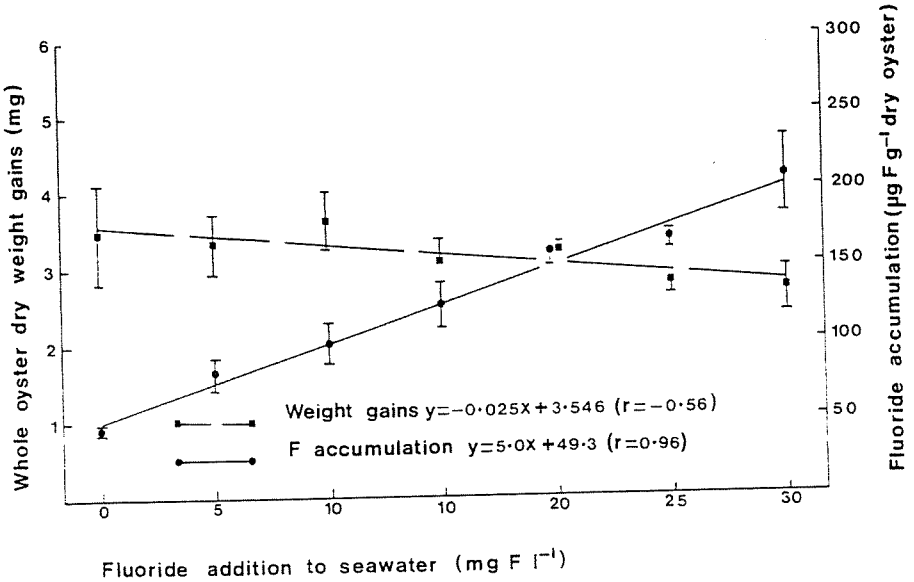

Fig. 1. Effects of fluoride concentration in seawater on growth and fluoride accumulation by Sydney rock oyster (*Saccostrea commercialis*) spat over 3 weeks. Means ± SD Experiment 1. Initial average dry weight of spat was 2.65 mg.

The concentration of fluoride found in dry adult oyster shell and meat from Sydney rock oysters collected from a commercial intertidal oyster lease in Port Stephens, New South Wales, Australia, was 111 and 1.8 $\mu g\, g^{-1}$, respectively.

DISCUSSION AND CONCLUSION

Sydney rock and flat oyster spat readily accumulate large amounts of fluoride. The dry shell and meat fluoride concentrations of 111 and 1.8 $\mu g\, g^{-1}$, respectively, in adult Sydney rock oysters grown in unpolluted seawater show that under natural conditions, fluoride accumulation occurs primarily in the shell of oysters. However, prolonged exposure of oysters to high fluoride concentrations greatly increases the fluoride concentrations in oyster meats (Moore, 1969).

The reduction in growth rates at the extreme salinities of 15 and 45‰ confirmed the finding of Nell and Holliday (1988) that these salinities are outside the range for maximum growth of both oyster spe-

Table 1. Effects of salinity and fluoride concentration in seawater on growth of Sydney rock oyster (*Saccostrea commercialis*) and flat oyster (*Ostrea angasi*) spat over 3 weeks. Growth is expressed as dry weight gain (mg) per spat*. Experiment 2

Salinity (‰)	Sydney rock oyster		Flat oyster	
	0 mg F l^{-1}	30 mg F l^{-1}	0 mg F l^{-1}	30 mg F l^{-1}
15	0.49 ± 0.26^{a}	0.39 ± 0.15^{a}	2.5 ± 0.3^{ab}	2.1 ± 0.7^{a}
25	1.33 ± 0.40^{b}	1.02 ± 0.07^{b}	9.4 ± 1.0^{c}	10.5 ± 2.9^{c}
35	1.29 ± 0.42^{b}	1.02 ± 0.57^{b}	10.4 ± 1.5^{c}	8.5 ± 2.5^{c}
45	0.51 ± 0.36^{a}	0.38 ± 0.15^{a}	4.8 ± 0.4^{b}	4.6 ± 1.1^{b}

*Means ± SD. For each species, means which do not share a common superscript differ significantly ($P < 0.05$). Initial average dry weights of Sydney rock and flat oyster spat were 0.12 and 2.77 mg, respectively.

Table 2. Effects of salinity and fluoride concentration in seawater on fluoride accumulation by Sydney rock oyster (*Saccostrea commercialis*) and flat oyster (*Ostrea angasi*) spat over 3 weeks. Fluoride accumulation data are expressed as $\mu g\, F\, g^{-1}$ dry spat*. Experiment 2

Salinity (‰)	Sydney rock oyster		Flat oyster	
	0 mg F l^{-1}	30 mg F l^{-1}	0 mg F l^{-1}	30 mg F l^{-1}
15	27 ± 3^{a}	834 ± 92^{e}	63 ± 26^{c}	386 ± 68^{f}
25	36 ± 1^{b}	574 ± 53^{d}	16 ± 2^{a}	179 ± 29^{dc}
35	46 ± 1^{bc}	525 ± 127^{d}	18 ± 6^{a}	145 ± 52^{d}
45	51 ± 4^{c}	652 ± 20^{dc}	36 ± 2^{b}	218 ± 56^{c}

*Means ± SD. For each species, means which do not share a common superscript differ significantly ($P < 0.05$). For statistical analysis a $\log_{10}$ transformation was used.

Table 3. Effects of salinity on the mortality of flat oyster (*Ostrea angasi*) spat over 3 weeks. Experiment 2

Salinity (‰)	Mortality* (%)
15	6.6 ± 5.4^{b}
25	2.3 ± 2.5^{a}
35	2.6 ± 2.1^{a}
45	1.4 ± 1.3^{a}

*Means ± SD. Means which do not share a common superscript differ significantly ($P < 0.05$). For statistical analysis an Arcsin $\sqrt{x}$ transformation was used.

cies. Growth rates of Sydney rock oyster spat is increased with increasing temperatures, presumably because of increased filter-feeding activity (Nell and Dunkley, 1984). The increase in oyster spat fluoride concentrations, when temperatures increased from 12 and 18°C to 24 and 30°C, was probably also caused by increased filter feeding activity at higher temperatures.

Fluoride was not very toxic to either Sydney rock or flat oyster spat. Significant reductions in growth were only obtained at very high seawater fluoride concentrations of 30 and 50 mg F l^{-1}. Even at these fluoride concentrations no effect of fluoride on mortality was observed, whereas Moore (1969) reported high mortalities for oysters (species not mentioned) held for longer than 30 days in seawater containing 32 mg F l^{-1}.

It has been demonstrated that increased dissolved calcium levels can have a detoxifying effect on fluoride (Neuhold and Sigler, 1960). Increasing the salinity from 15 to 45‰ would have increased the calcium concentration from 0.17 to 0.51 g Ca l^{-1}. However, any detoxifying effect if operative in the present study must have been minor compared to the deleterious effects of hypersaline conditions on Sydney rock and flat oyster spat. Only the flat oyster spat suffered increased mortalities at the salinity of 15‰. This indicates that they are less tolerant of low salinities than Sydney rock oysters (Nell and Gibbs, 1986).

This study indicates that fluoride accumulation, rather than a reduction in growth is the major effect of high seawater fluoride concentrations on Sydney rock and flat oysters. Fluoride is toxic to man (Underwood, 1977) and any major increase in oyster meat fluoride levels above that found in oyster meats from unpolluted waters must be considered undesirable. Specifically, fluoride concentrations should be kept below 30 $\mu g\ g^{-1}$ dry oyster meat. The greatest danger of increased oyster meat fluoride concentrations would occur in summer, the main oyster marketing period in Australia, as fluoride accumulation in oysters is much higher at summer water temperatures of 24–30°C than at winter water temperatures of 12–18°C.

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Table 4. Effects of temperature and fluoride concentration in seawater on growth and fluoride accumulation by Sydney rock oyster (*Saccostrea commercialis*) spat over 3 weeks. Experiment 3

Temperature (°C)	Dry weight gains		Fluoride accumulation	
	0 mg F l^{-1}	50 mg F l^{-1}	0 mg F l^{-1}	50 mg F l^{-1}
12	0.25 ± 0.07^{a}	0.33 ± 0.09^{a}	71 ± 24^{a}	1116 ± 218^{b}
18	1.00 ± 0.25^{b}	0.98 ± 0.08^{b}	72 ± 6^{a}	1140 ± 202^{b}
24	2.04 ± 0.32^{c}	1.63 ± 0.35^{c}	59 ± 21^{a}	2282 ± 706^{c}
30	2.80 ± 0.65^{d}	2.10 ± 0.65^{c}	58 ± 14^{a}	2130 ± 357^{c}

*Means ± SD. For dry weight gains and fluoride accumulation, means which do not share a common superscript differ significantly ($P < 0.05$). Initial dry weight of spat was 1.53 mg. For statistical analysis a $\log_{10}$ transformation was carried out on fluoride accumulation data.

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Fluorine danger to oysters

by Dr. J. A. Nell

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THE SITING of an aluminium smelter waste dump on a tributary of Port Stephens, north of Newcastle, threatens the local oyster industry.

The risk, though remote, could raise estuarine fluorine levels in the oysters, reducing their growth rate and rendering them unsuitable for human consumption.

Oysters are cold-blooded estuarine animals which are immobile as adults — hence their immediate environment necessarily affects their survival, growth and reproduction.

That environment is subject to widely fluctuating salinity and temperature, yet pollution from residential, agricultural and industrial developments may threaten them.

With the proposal to site the waste dump approximately 1200 m from Twelve Mile Creek, a tributary to Swan Bay in Port Stephens, we began a series of three-week experiments to assess the effects of temperature, salinity and fluorine concentration.

Juvenile oyster spat (1 mg dry weight each) were fed as much unicellular algae as they could filter, and the effects of controlled conditions monitored.

Temperature

The effects of temperature on the growth rates of Sydney rock *(Saccostrea commercialis)* and flat oysters *(Ostrea angasi)* spat are shown in Table 1. Both species of oysters showed increasing weight gains with increasing temperatures up to 28-30 °C.

However, substantial weight gains were obtained at 12 °C indicating that winter may be a suitable time for the culture of hatchery and naturally caught spat.

Growth rates of both species were maximum at 28-30 °C, however these temperatures could be stressful during long-term exposure.

Temperatures of 28 °C and higher may be experienced north of the Hastings River in New South Wales, during short periods in summer (Wolf and Collins, 1979).

Although both species appear to prefer similar temperatures for growth (Table 1), they have quite different reproductive cycles.

In New South Wales, the Sydney rock oyster fattens, ripens and spawns from October to May, whereas the flat oyster does this from June to February (J. E. Holliday, personal communication, 1985). Flat oysters, however are not marketed when they are ripe, because they hold their larvae in their mantle cavity for approximately two weeks before releasing them.

A different growing technique for flat oysters is essential if they are to be cultivated intertidally, as they do not tolerate high temperatures out of water as well as Sydney rock oysters.

They should be shaded and cultivated approximately 200 mm below the normal growing height for Sydney rock oysters (J. E. Holliday, personal communication, 1985). This could be achieved by suspending trays of flat oysters below trays of Sydney rock oysters.

Salinity

The effects of salinity on the growth and survival as indicated by the salinity tolerance range are shown in Table 2.

Both the Sydney rock oyster and the flat oyster grow best at a salinity of 25-35 parts per thousand.

However the Sydney rock oyster tolerates low salinities better and this is important after heavy rain.

The Sydney rock oyster can survive for seven days or more at salinities below its tolerance range

Table 1. The effects of temperature on the growth rate of juvenile Sydney rock oysters *(Saccostrea commercialis)* and flat oysters *(Ostrea angasi)*.

	Whole oyster dry weight gains over three weeks (%)*	
Temperature (°C)	*Sydney rock oyster*	*Flat oyster*
12	16	128
16	—	191
18	65	—
20	—	520
24	133	690
28	—	806
30	183	—

*Initial whole oyster dry weight was 1 mg.

*Salamander Bay, NSW 2301.

Table 2. The salinity preference of some oysters.

Species	*Salinity tolerance range (ppt)*	*Salinity range for maximum growth (ppt)*
Flat oyster *(Ostrea angasi)*	20-45	25-35
Sydney rock oyster *(Saccostrea commercialis)*	15-50	25-35
Pacific oyster *(Crassostrea gigas)*	5-55	??-40

whereas flat oysters survive only for about two days in such conditions. For example, if salinities dropped to 10 parts per thousand or less for one week, most Sydney rock oysters, but few flat oysters, would be expected to survive.

The preferred salinity range for maximum growth of Pacific oysters *(Crassostrea gigas)* is not known, although good growth rates have been reported for salinities as high as 40 parts per thousand (King, 1977; Hughes-Games, 1977). The salinity tolerance range of this oyster is somewhat wider than that of the Sydney rock oyster (Table 2).

Neither the Pacific nor the flat oyster keeps as well out of water as the Sydney rock oyster (J. A. Nell, personal observation, 1985). This is a factor to be considered when marketing oysters, because it determines their shelf life.

The Pacific oyster has a subtropical nature (Korringa, 1976). Spawning takes place at water temperatures of 20 °C and higher, each female producing millions of eggs. Most oyster hatcheries culture Pacific oyster larvae in seawater with a salinity of 35 parts per thousand at 26-28 °C (Holliday, 1985) but they can also be reared successfully at a salinity as low as 25 parts per thousand (W. Guse, personal communication, 1985).

Fluorine

Growth rates of juvenile Sydney rock oysters decreased linearly with increasing estuarine water fluorine levels from the port's normal 0.7 ppm to 30 ppm under experimental laboratory conditions (J. A. Nell and G. Livanos, unpublished data, 1985).

At the experimental level of 30 ppm growth rates were reduced by 20 per cent compared with the control oysters kept at 0.7 ppm. (Oysters accumulate fluorine in their shells as well as their meats).

Fluorine levels in whole dry spat increased linearly from 45-204 ppm over the range of experimental fluorine levels from 0.7-30 ppm. Normal day adult oyster shell and meat fluroine levels are 111 and 2 ppm respectively. This indicates that flourine is primarily deposited in oyster shell.

The greatest potential damage to the oyster industry, if estuarine water fluorine levels were to rise, would not be in reduced growth rates of oyster spat, but in rendering oyster meats unsuitable for human consumption.

When adult oysters were exposed to only 2 and 8 ppm additional fluorine, dry oyster meat fluorine levels increased from 7 to 18 and 30 ppm respectively (Moore, 1969). Fluorine levels of 18 and 30 ppm in human foods would be highly undesirable (Underwood, 1977).

It is difficult (Moore, 1969) to keep oysters alive for longer than 30 days at 32 ppm and for longer than five days at 128 ppm fluorine in water.

This indicates that prolonged exposure to water fluorine levels as low as 32 ppm could be disastrous to oyster populations in the affected area.

Because of the oyster's ability to accumulate fluorine in its meats and the toxicity of fluorine to oysters, it is imperative that the water fluorine level in Port Stephens is not allowed to rise beyond the normal levels found in estuarine and seawater.

The Department of Agriculture, NSW is concerned about the potential problems associated with fluorine pollution of Port Stephens. However, providing all conditions laid down in the report by the Commissioner of Inquiry (Simpson, 1985) are met, the Department does not object to the establishment of the aluminium smelter waste dump at Wallaroo.

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3.4 Supplementation of an algal diet for juvenile Sydney rock oysters (Saccostrea commercialis) with a mixture of dissolved amino acids

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Abstract

When juvenile Sydney rock oysters (*Saccostrea commercialis*) were supplied with 0. 1.5 and 3.0 mg essential amino acids per litre, with or without algae, there was no effect of amino acid supplementation on growth rates.

Introduction

Active absorption of free amino acids dissolved in seawater has been demonstrated for many marine bivalves (Stephens, 1972, 1982; Stewart, 1979) and for Sydney rock oysters (Nell et al., 1983). The absorbed amino acids partially meet the nutritional requirements of bivalves (Stephens, 1972; Manahan and Crisp, 1982; Wright, 1982). A feeding experiment was conducted to investigate the effect of essential amino acid supplementation of an algal diet for juvenile oysters.

Materials and Methods

Oysters

Small hatchery-reared Sydney rock oysters (*Saccostrea commercialis*) were held on nylon mesh trays in 8 L aerated aquaria (one tray of 50 spat per aquarium). Filtered seawater with a total of volatile suspended solids (> 0.7 μm - < 35 μm) content of 1.2 and 0.3 mg/L respectively was used. The seawater had a salinity range of 29-34‰, it was maintained at 24°C and was changed daily. The average whole dry weight of four samples of 50 of the small spat at the start of the experiment was 0.7 $\pm$ 0.08 mg. There were four replicates per treatment and the experiment was terminated after three weeks.

Feeding

Juvenile oysters were supplied with 0. 1.5 and 3.0 mg essential amino acids (Table 1) per litre, with or without the algae *T. Isochrysis* aff. *galbana* and *Pavlova lutheri* in equal weight proportions at a combined rate of 3 mg dry weight per litre per day.

Statistical Analysis

Homogeneity of variance was confirmed using the Cochran test. The data were then submitted to analyses of variance and mean values were compared by least significant differences (Sokal and Rohlf, 1981).

Results and Discussion

There was no effect of essential amino acid supplementation on the growth rate of juvenile oysters. Oysters supplied with the microalgae *T. Isochrysis* aff. *galbana* and *Pavlova lutheri* grew much faster than the unfed oysters (Table 2). There were no mortalities in this experiment. Although Sydney rock oysters readily absorb dissolved amino acids from seawater (Nell et al., 1983) they must not have been a growth limiting factor in this study.

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TABLE 1 Essential[1] amino acid composition of Sydney rock oyster (*Saccostrea commercialis*) and a crystalline amino acid mixture

Amino acid	Sydney rock oyster[2] (mg/g protein)	Crystalline amino acid mixture[3] (g/100g)
Arginine	45	11.5
Histidine	15	3.8
Isoleucine	35	8.9
Leucine	53	13.5
Lysine	57	14.5
Methionine	18	4.6
Cystine	5	1.3
Phenylalanine	30	7.7
Tyrosine	29	7.4
Threonine	36	9.2
Valine	34	8.7
Proline	35	8.9

1 Harrison, 1976.
2 Nell, 1985.
3 The proportions of the essential amino acids in the crystalline amino acid mixture were the same as those in the Sydney rock oyster meat.

TABLE 2 Effects of essential amino acid concentration in seawater on the growth rate of juvenile Sydney rock oysters (*Saccostrea commercialis*) over 3 weeks.

	Dietary composition (mg/L)		Whole spat dry weight gains[1] (mg)
	Amino acids	Algae[2]	
1.	0.0	0	0.31 ± 0.22 [a]
2.	1.5	0	0.32 ± 0.08 [a]
3.	3.0	0	0.37 ± 0.19 [a]
4.	0.0	3	0.81 ± 0.33 [b]
5.	1.5	3	0.82 ± 0.26 [b]
6.	3.0	3	0.79 ± 0.43 [b]

1 Values are mean ± S.D. Means with a common superscript do not differ significantly ($p > 0.05$). Initial average whole dry weight of the spat was 0.70 ± 0.08 mg.

2 The algae *T. Isochrysis* aff. *galbana* and *Pavlova lutheri* were fed at equal proportions on a dry weight basis.

3.5 Nell, J. A., 1985. Microparticulate diets for aquaculture evaluated. Feedstuffs, 57(22): 16-17.

MICROPARTICULATE DIETS FOR AQUACULTURE EVALUATED

Reprinted from Feedstuffs 57(22): 16-17 (June 3, 1985)

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Abstract

Although the culture of shellfish and finfish has been practiced for a long time, the larval rearing of these animals in hatcheries still relies on feeding live phytoplankton and zooplankton. The production of these live food organisms is both costly and labor intensive. However, with the development of a wide range of microparticulate artificial diets, alternative feeding practices are developing. These diets may allow reductions in feeding costs and greater consistency in food composition. This paper reviews recent developments in microparticulate diets for aquaculture, with emphasis on prawn larval rearing.

Types of microparticulate diets

Microparticulate diets for aquaculture must not only provide a complete diet, but also the particles should be of a suitable size for the animals to ingest. Food particles should be neutrally buoyant in water and have impermeable or semi-permeable walls (Table 1). A proper balance of positively buoyant lipid components (>250g/kg dry diet), together with carbohydrates and protein can give the required particle density.

Aeration of water to aid suspension of larvae will assist the suspension of food particles, so that slightly negatively buoyant diets may be acceptable (Jones and Gabbott, 1976). Good aeration to keep food particles suspended is best achieved in cone shaped containers (McVey and Fox, 1983) to prevent food particles from settling out on the bottom.

With molluscan larvae and adults, which ingest food whole, the particle wall should be impermeable and stable in seawater, but readily broken down by a change in pH or by the action of digestive enzymes (Jones *et al.*, 1974). Alternatively, the particle wall may be broken mechanically by larvae or adults such as prawns which masticate food.

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The coating or binding materials used in preparation of microparticulate diets often constitute a large proportion of the particle and this should be taken into account when formulating a diet. Microparticulate diets may be grouped as follows (Table 2) according to Teshima *et al.* (1982).

1 Micro-encapsulated diets are microparticulate diets made by enclosing a solution, colloid or suspension of dietary ingredients within a membrane.

2 Micro-binding diets are powdered diets with a binder such as propylene glycol alginate (Forster, 1972) and calcium alginate (Levine *et al.*, 1983).

3 Micro-coating diets are prepared by coating micro-binding diets with some materials such as wheat gluten and cholesterol-lecithin.

Micro-encapsulation of diets commenced with the development of a nylon-protein capsule (Table 2) by Chang *et al.* (1966). This type of capsule is very stable and has been used successfully to feed marine prawn larvae (Jones *et al*,. 1979; Kanazawa *et al*,. 1982; Maugle *et al*,. 1983a). However, care must be taken that the toxic solvents used in the preparation are removed.

A protein-walled capsule is produced commercially by Frippak Feeds.[1] This capsule has a protein wall, which is insoluble in water. When fed to marine prawn larvae, it produced good growth and survival rates (N. Preston, personal communication, 1984).[2] The gelatin-acacia (Langdon, 1981; Langdon and Waldock, 1981; Chu *et al.*, 1982), lipid-walled microcapsule (Langdon, 1981 and Langdon and Siegfried, 1984) and the microgel particulate diet (Langdon and Siegfried, 1984; Langdon and Bolton, 1984) have all been used successfully to feed juvenile oysters or larvae.

The whole-egg diet (Chow, 1980) utilizes the property of egg albumin to coagulate at high temperatures. Besides being a useful binder, egg albumin is a relatively complete protein source and the inclusion of whole-egg into diets improves their buoyancy.

1 Frippak Feeds, Armstrong Road, Daneshill Industrial Estate, Basingstoke, Hants., RG24, ONU, England.

2 N. Preston, Department of Zoology, The University of Sydney, Sydney, NSW, 2006.

The calcium alginate micro-binding diet preparation was used successfully to feed brachyuran crab larvae (Levine *et al.*, 1983) on dry powdered *Artemia nauplii* and appears suitable for the binding of other ingredients and for feeding to prawn and fish larvae. The binder propylene glycol alginate (Forster, 1972) may also be used to prepare micro-binding diets. A wide variety of binders was investigated by Forster (1972) and it was concluded that they had little effect on the digestibility of diets for juvenile prawns.

Zein (modified maize gluten) micro-coating diets have also been used successfully to feed marine prawn larvae (Kanazawa *et al.*, 1982) and when fed to marine finfish larvae (Teshima *et al.*, 1982) produced growth and survival rates which were only slightly less than those achieved with *Brachionus*. Other micro-binding diets have been used successfully to feed marine prawn larvae (Teshima *et al.*, 1982) and have produced growth and survival rates equal to those achieved on live *Artemia*.

Table 1 Some properties of microparticulate diets used in aquaculture

Property	Filter feeders	Particle feeders
Particle size	5 to 150μm	40 to 1000μm
Particle wall	Nontoxic impermeable	Nontoxic impermeable or semi-permeable
Easily ruptured, irregular shape, rough surface	Enzyme soluble, pH labile	easily ruptured
Buoyancy	Neutral in seawater	Neutral in seawater
Internal phase	Aqueous or oil based diet	Aqueous or oil based diet

After Jones and Gabbott, 1976

Discussion

If microparticulate diets are to substitute for *Brachionus* or newly hatched *Artemia*, both of which are commonly used prawn larval foods (Liao *et al.*, 1983), particle sizes of 45 and 450mm, respectively, should be aimed for. Feeding level and frequency are also important (Teshima and Kanazawa, 1983) and should be investigated for each set of conditions to achieve optimum results.

If particles are dried, the drying method is also important because drying at high temperatures for a long time is likely to damage vitamins. Teshima and Kanazawa (1983), found that freeze dried particles produced better growth rates in marine prawns than oven dried (60°C) particles.

The choice of ingredients used will depend on availability and convenience, but care must be taken to formulate complete diets containing all the major and minor nutrients in adequate quantities and suitable proportions. A sound knowledge of nutrition is required before formulating a prawn diet. Salt and freshwater prawn nutrition was reviewed by New (1976). Penaeid prawn nutrition was reviewed by Maguire (1980) with particular emphasis on practical farming application. The nutrition of the freshwater prawn *Macrobrachium rosenbergii* was reviewed by Biddle (1977) and Sick and Millikan (1983).

Leaching of water soluble nutrients (Goldblatt *et al.*, 1979; Langdon, 1981) from diets is a problem in aquaculture, but it is a particularly serious problem with microparticulate diets. Great care should be taken that the vitamin premix contains adequate amounts of all vitamins. It may also be advisable to increase the level of the premix into the diet to make up for losses due to leaching. This may be avoided by including single cell proteins such as yeasts and bacterial proteins, which are usually good sources of B vitamins (Thomas and Corden, 1977). The fat soluble vitamins are no problem as they dissolve in oil instead of water and they are also present in substantial quantities in fish oils.

Minerals supplied in the premix will be difficult to contain within a permeable membrane. However, requirements for the major inorganic elements might be met simply by the diffusion of salts from seawater into the particles. This benefit is only available to marine animals and dietry inclusions must always be made for freshwater species. In the case of oysters, minerals are absorbed directly from seawater (Nell and Wisely, 1984), eliminating the need for their inclusion in artificial diets.

Microcapsules may also be used to supply medicinal compounds to prevent or cure internal diseases in marine organisms (Jones and Gabbott, 1976). The enzyme amylase has been encapsulated in a nylon-protein membrane successfully to improve starch digestion and growth rates of marine prawns (Maugle *et al.*, 1983a,b). This procedure could also be used to encapsulate drugs for release within the digestive tract and it would widen the range of disease treatments available to aquaculturists.

The development of water-stable microparticulate diets allows accurate determination of the nutrient requirements of marine organisms. In general, minerals and vitamins are added to artificial diets without adequate data on the quantitative requirements of the nutrients. Aquaculture nutrition is now entering a new and exciting period of research. New feeding techniques are becoming available, which will enhance commercial aquaculture. The development of simple microparticulate diets for prawn larvae will improve the commercial viability of prawn farms and hatcheries.

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Table 2 Microparticulate diets used in aquaculture

	Composition	Size (μm)	Comment	References
Micro-encapsulated diets				
Nylon-protein	Carbohydrate protein, oil	5-300	Toxic solvents used	1,2,3,4,5,6,7
Gelatin-acacia	Oil	5-10	Used to supply fat soluble vitamins and fatty acids	6,8,9,10
Lipid-walled	Oil, vitamins	5-20	Encapsulates water soluble vitamins and supplies fat soluble vitamins in the oil	8,11
Chitosan	Complete diet		Chitosan not available in Australia	6
Glycopeptide	Protein	5>30	Difficult to prepare	2
Whole egg	Protein, fat	100-400	Unstable	12
Micro-binding diets				
Agar	Complete diet	40-1000		6
Gelatin	Complete diet	40-1000		6
Carrageenan (edible purple seaweed)	Complete diet	<5-125	Carrageenan not available in Australia	6,13
Calcium alginate	Complete diet	>44		14
Microgel	Carbohydrate Protein, lecithin	1->20	Contains cellulose	8,11
Micro-coating diets				
Cholesterol-lecithin	Complete diet	40-1000	High cholersterol level	6
Zein (modified maize gluten)	Complete diet	40-1000	Zein not available in Australia but wheat gluten may be used instead	6,15

1) Chang *et al.*, 1966; 2) Gabbott *et al.*, 1975; 3) Jones *et al.*, 1975; 4) Teshima *et al.*, 1981; 5) Sakamoto *et al.*, 1982; 6) Teshima *et al.*, 1982; 7) Maugle *et al.*, 1983a; 8) Langdon, 1981; 9) Langdon and Waldock, 1981; 10) Chu *et al.*, 1982; 11) Langdon and Siegfried, 1984; 12) Chow, 1980; 13) Teshima *et al.*, 1983; 14) Levine *et al.*, 1983; 15) Kanazawa *et al.*, 1982.

3.6 Effect of several artificial diets on the growth of juvenile Sydney rock oysters (*Saccostrea commercialis*)

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Abstract

Juvenile Sydney rock oysters (*Saccostrea commercialis*) fed an artificial diet exhibited a growth rate equal to 60% of that obtained on an algal diet. The artificial diet was based on starch, bacterial protein (Pruteen) and lipid-walled capsules. The growth rate for that artificial diet was higher than for three other artificial diets but the differences were not significant ($p > 0.05$).

Introduction

Cultured unicellular algae have been used for decades as the sole food source in oyster hatcheries. Because of the high cost and unpredictability of production, development of artificial diets for oysters has been attempted by several investigators (e.g., Castell and Trider, 1974; Chu et al., 1982, 1987; Langdon and Waldock, 1981; Nell and Wisely, 1983, 1984; Langdon and Siegfried, 1984; Laing, 1987). All of these attempts assist the future development of commercial oyster diets, yet none of the diets are without problems. They either do not support good growth or they cause bacterial fouling of oysters, tanks and water. This study was conducted to assist in the development of commercial diets for oysters.

Materials and Methods

Oysters

Small hatchery-reared Sydney rock oysters (*Saccostrea commercialis*) were held on nylon mesh trays in 8 L aerated aquaria (one tray of 50 spat per aquarium). Sand filtered water with a salinity of 32‰ was used in the aquaria. The water in the aquaria was maintained at 24°C and changed daily. The average whole dry weight of four samples of 50 small spat at the start of the experiment was 2.95 ± 0.38 mg. There were four replicates per treatment and the experiment was terminated after three weeks.

Feeding

The oysters were fed once a day at the rate of 6 mg dry matter per litre. They were either unfed or fed a combination of the algae T. Isochrysis aff. galbana and Pavlova lutheri, a bacterial protein (Pruteen) and lipid-walled capsule diet (Table 1), a gelatin-acacia cod liver oil capsule diet (Table 2), a protein-walled capsule (CAR) diet from Frippak Feeds, Basingstoke, England, or a microgell and lipid-walled

capsule diet (Table 3). All algal cell, particles or capsules were in the size range of 5-20 μm.

Statistical Analysis

Homogeneity of variance was confirmed using the Cochran test. The data were then submitted to analyses of variance and mean values were compared by least significant differences (Sokal and Rohlf, 1981). The larval survival data were transformed by arcsin $x^{0.5}$ before analysis of variance, because the percentage covered a wide range of values.

Results and Discussion

The best growth rates were obtained with the algal diet. The starch, bacterial protein (Pruteen) and liped-walled capsule diet was the best of all the artificial diets tested. This diet is very similar to the oyster "fattening" diet that was developed by Nell and Wisely (1983, 1984). The growth rates on all the other artificial diets were substantial and much better than that of the unfed oysters. There was no significant ($p > 0.10$) dietary effect on mortalities. The average mortality was 15%.

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TABLE 1 Starch, Pruteen and lipid-walled capsule diet

Composition	g/kg dry weight
Wheat starch	445
"Pruteen" plankton grade [1]	319
Lipid-walled capsules [2]	236
Wheat starch	445
Pruteen	319
Cod liver oil	200
Acacia	32
Fat soluble vitamin premix (Table 4)	1
Water soluble vitamin premix (Table 5)	3
ω_3 fatty acids	56
ω_6 fatty acids	6
Cholesterol	1
Calculated composition:	
Crude protein (N x 6.25)	250
Carbohydrate	482
Total lipids	229
ω_3 fatty acids	56
ω_6 fatty acids	6
Cholesterol	1
Fat soluble vitamin premix	1
Water soluble vitamin premix	3

1 Bacterial protein (*Methylophilus methylotrophus*), Pruteen.

2 Langdon and Siegfried, 1984.

TABLE 1 Starch, Pruteen and lipid-walled capsule diet

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1 Bacterial protein (*Methylophilus methylotrophus*), Pruteen.

2 Langdon and Siegfried, 1984.

TABLE 2 Gelatin-acacia microcapsule[1] composition

Calculated composition	g/kg dry weight
Cod liver oil	811
Carbohydrate (acacia)	92
Protein (gelatin)	93
Fat soluble vitamins (Table 4)	4
ω_3 fatty acids	226
ω_6 fatty acids	4
Crude protein (N x 6.25)	92

1 Langdon and Waldock, 1981; Chu et al., 1982, 1984.

TABLE 3 Microgell and lipid-walled capsule diet

Composition	g/kg dry weight
Microgell[1]	764
Lipid-walled capsule	236
Ingredients	
Wheat starch	386
Egg albumin	253
Soy lecithin	115
Carboxymethyl cellulose	9
Cod liver oil	200
Acacia	32
Fat soluble vitamin premix (Table 4)	1
Water soluble vitamin premix (Table 5)	3
Cholesterol	1
Calculated composition:	
Crude protein (N x 6.25)	213
Carbohydrate	427
Total lipids	315
ω_3 fatty acids	67
ω_6 fatty acids	65
Cholesterol	1
Fat soluble vitamin premix	1
Water soluble vitamin premix	3

[1] Langdon and Siegfried, 1984.

TABLE 4 Fat soluble vitamin premix

Vitamin	g vitamin/100 g premix
Retinol (A)	1.1
Cholecalciferol (D_3)	0.02
α-Tocopherol acetate (E)	26.4
Menadione	5.6
2,6-Di-t-butyl-p-cresol (Butylated hydroxytoluene; BHT)	2.0

TABLE 5 Water soluble vitamin premix

Vitamin	g vitamin/kg premix
Thiamin.HCl (B_1)	20
Riboflavin (B_2)	20
Pyridoxine.HCl (B_6)	20
Folic acid	20
Ascorbic acid (C)	100
Ca-pantothenate	40
Myoinositol	60
Biotin	2
Choline bitartrate	160
Nicotinamide	80
Cyanocobalamin (B_{12})	0.2
2,6-Di-t-butyl-p-cresol (Butylated hydroxytoluene; BHT)	20

TABLE 6 Comparison of artificial diets for juvenile Sydney rock oysters (*Saccostrea commercialis*) over 3 weeks

Diets	Whole dry spat weight gains[1] (mg)
1. Unfed control[2]	0.07 ± 0.37
2. *T. Isochyrsis* aff. *galbana* /*Pavlova lutheri*	2.02 ± 0.16 [b]
3. Starch, bacterial protein (Pruteen) and lipid-walled capsules	1.27 ± 0.80 [ab]
4. Gelatin-acacia cod liver oil capsules	0.86 ± 0.57 [a]
5. Protein-walled capsules (CAR)	0.60 ± 0.56 [a]
6. Micro-gell and lipid-walled capsules	0.69 ± 0.33 [a]

1 Values are mean ± S.D. Means with a common superscript do not differ significantly ($p > 0.05$). Initial average whole dry weight of the spat was 2.95 ± 0.38 mg.

2 Some of the unfed replicates lost weight and were therefore excluded from statistical analysis.

3.7 The evaluation of some algal species as food for 1-7 day old Sydney rock oyster (*Saccostrea commercialis*) larvae

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Abstract

The algae Pavlova lutheri, *T. Isochrysis* aff. *galbana* and *Chroomonas salina* produced the highest 6-day length increase in Sydney rock oyster (*Saccostrea commercialis*) larvae fed a single algal species. When larvae were fed *Pavlova lutheri* in combination with other algal species, several species improved the growth rate of larvae compared to those fed a diet of *Pavlova lutheri* alone.

Introduction

Sydney rock oyster (*Saccostrea commercialis*) larvae have been reared in hatcheries for over 9 years, but no studies had been carried out to evaluate the value of algal species as food for these larvae. The two algal species Pavlova lutheri and *T. Isochrysis* aff. *galbana* which are widely used in overseas hatcheries (Holliday, 1985) have been used routinely for feeding these larvae. The findings by Enright et al. (1986a, b) that the 20:5W_3 and 22:6W_3 fatty acids are of great importance to the food value of algal species, has given oyster larval nutrition a new direction. However, algae size and algal cell wall digestibility are other factors to be considered. In the present study a wide range of algal species were fed to Sydney rock oyster larvae either singly or in combination with *Pavlova lutheri*.

Materials and Methods

Oyster larvae

One day old D-stage oyster larvae were stocked in 8 L non-aerated aquaria at a density of 5 larvae/mL. Water in the aquaria was maintained at 26°C and changed every 48 hours with the larvae being retained on a 45 um diagonal screen. Algae (Table 1 and 2) were added to the aquaria at the rate of 0.95 mg dry weight per litre at the start of the experiment and after every water change. When a combination of algal species was used (Table 2), they were fed on a calculated equal dry weight basis. There were four replicates per treatment. The experiments were terminated after 6 days and the larvae were preserved in a solution containing 10% formalin in seawater. The percentage of live and dead larvae at the time of preservation was assessed by microscopic examination. The term survival in the text describes the percentage of larvae alive at the end of the experiments as not all larvae stocked could be accounted for. The length (greatest distance parallel to the hinge) of > 50 preserved larvae per aquarium were measured using a microscope and a micrometer slide. Seawater with a salinity of 35‰ was filtered down to 1 um before use to reduce the background level of potential oyster food

experiment and after every water change. Care was taken to use cultures that were in logarithmic growth phase. When a combination of algal species was used (Experiment 2), they were fed on an equal dry weight basis.

For all experiments there were four replicates per treatment. The experiments were terminated after 6 days and the larvae were preserved in seawater containing 10% formalin. The length (greatest distance parallel to the hinge) of >50 preserved larvae per aquarium were measured using a microscope and a micrometer slide ($\pm 0.5\ \mu m$). Oceanic seawater (35‰) was filtered using 1 μm (nominal) cartridge filters before use to reduce the background level of potential oyster food items in the water. Levels of suspended solids in seawater were $\leq$ 0.6 mg l^{-1} (dry weight), equal to 63% of the weight of the algal supplements.

Algal culture techniques

Algal cultures were grown in 20 l polyethylene bags. All species were batch cultured in oceanic water (salinity 34-35 ‰) using f/2 beta growth medium (Guillard, 1983) at 21 ± 1°C with a 16:8 h light:dark cycle and were illuminated with cool white fluorescent tubes to an intensity of 4000 Lux at the container surface.

Dry weight determination of algal cells

Algal cultures were filtered through a 35 μm (diagonal) mesh screen to remove any debris or clumps of algal cells and the cell concentration determined with a Coulter counter. A known volume (50 - 150 ml) was filtered through a 0.7 μm pore size glass fibre filter to collect the algal cells. The filter was washed with 100 ml of 0.5 M ammonium formate to remove seasalts (Epifanio, 1979) and dried at 100°C. The dry weight of suspended solids in the seawater used for the experiments was also determined in this way.

Experimental design

For Experiment 1, larvae were supplied with a diet consisting solely of one of a range of algal species (Table 2). For Experiment 2, *P. lutheri* (which promoted the greatest growth in Expt. 1) was fed in combination with the same range of species as in Experiment 1 (Table 3). Both experiments had an unfed control.

Statistical analysis

Homogeneity of variance was confirmed using the Cochran test (Winer, 1971). The data in Experiments 1 and 2 were subjected to a single factor analysis of variance. In both experiments mean values were compared using Tukey's *w* (Sokal and Rohlf, 1981).

Results

The average dry weight of algal cells ranged from 8 pg for *Th. pseudonona* to 96 pg for *Tetraselmis chui* Butcher (Table 1). The length/breadth ratio of all species measured had a narrow range from 1.1-1.7 except for *Phaeodactylum tricornutum*

Bohlin which had a ratio of 6.4 (Table 1).

Experiment 1

When used as the sole food source the algae *P. lutheri*, Tahitian *Isochrysis* and *Chroomonas salina* (Wislouch) Butcher produced the highest length increases in larvae. Length increases of larvae supplied with the algae *Dunaliella tertiolecta* Butcher were significantly higher ($P<0.05$) than those of the unfed larvae, whereas length increases of larvae supplied with *T. suecica, Ch. calcitrans* and *T. chui* were higher, but not significantly different ($P>0.05$) from those of the unfed larvae (Table 2). Length increases of larvae supplied with *N. atomus* were lower, but not significantly different ($P>0.05$) from those of the unfed larvae (Table 2), whereas those of larvae supplied with *Ch. gracilis* Schütt, *Ph. tricornutum* and *Th. pseudonana* were significantly lower ($P<0.05$) than those of unfed larvae.

Experiment 2

When larvae were supplied with the alga *P. lutheri* in combination with other algal species, six diets produced higher length increases in larvae (Table 3) than those supplied with a diet of *P. lutheri* alone, however, the differences were not significant ($P>0.05$). The addition of Tahitian *Isochrysis* or *N. atomus* to *P. lutheri*, produced some of the highest length increases. Length increases of the unfed larvae were lower, but not significantly different ($P>0.05$) from those supplied with a combination of *P. lutheri* and *Ch. gracilis* (Table 3), and significantly less ($P<0.05$) than those from larvae supplied with any of the other algal diets.

Discussion

Practical experience in the rearing of Sydney rock oysters has, over a period of ten years, selected three species, *P. lutheri*, Tahitian *Isochrysis* and *Ch. calcitrans* as superior larval feeds (Frankish et al., in press). Our experimentation supported the practical observations as one, or a combination of, these species produced the greatest length increase in larvae whether fed singly, or in combination with *P. lutheri*. These three species contain high levels of one or both of the long chain unsaturated fatty acids, 20:5n3 and 22:6n3 (Brown et al., 1989), which individually or in combination have been shown to be important in obtaining high growth in Pacific oyster spat (Langdon and Waldock, 1981) and in flat oyster spat (Enright et al., 1986a, b). However, it has been suggested (Whyte et al., 1990) that oyster larvae have a relatively low requirement for 20:5n3 and 22:6n3 fatty acids and once met, carbohydrate level is the most important factor governing the food value of an algal species. The similarities between algal species of protein levels (Enright et al., 1986a) and amino acid levels (Webb and Chu, 1983) suggested that they are unlikely to be important factors.

Several algal species (*N. atomus, Ph. tricornutum, T. suecica* and *T. chui*) when used as the sole food did not produce growth increases significantly greater ($P>0.05$) than unfed controls (Table 2). However, when fed in combination with *P. lutheri*, they all produced higher length increases than larvae fed *P. lutheri* alone (Table 3). *Chr. salina,* a species found to be of value to flat oyster spat (Laing and

Millican, 1986), and *D. tertiolecta* produced high length increases in larvae when supplied singly (Table 2), but did not improve length increases in larvae when fed in combination with *P. lutheri* (Table 3). Two algal species reported to have produced good growth and survival in juvenile American oysters *Crassostrea virginica* (Gmelin), *Th. pseudonana* (Romberger and Epifiano, 1981) and *Ch. gracilis* (Enright et al., 1986a), failed to produce good growth either as a single feed or in combination with *P. lutheri* (Tables 2 and 3).

The performance of various algal species in relation to one another varied from one experiment to another. Growth rates of algae produced under routine culture conditions, differ from time to time and this may have altered their food value. Differences in nutrient reserves of the 1-day-old larvae used may also have affected the relative performance of the algal species in different experiments. The amount of natural food present in filtered oceanic water (0.6 mg l^{-1} dry weight of suspended solids) is also an important, but often overlooked factor influencing the growth of oyster larvae.

In summary, when algae were supplied to 1-7 day old Sydney rock oyster larvae, a wide range of algal species in combination with *P. lutheri* produced high length increases (Tables 3 and 5). However, in the case of stored concentrated algae, a diet of *P. lutheri* with *Ch. calcitrans* produced the best results (Table 5).

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TABLE 1

Algal cell dry weight and size

	Dry weight (pg[1])		Size (μm)[2]		Length/ breadth ratio
	n	x±SD	Length x±SD	Breadth x±SD	
Diatoms					
Chaetoceros gracilis Schütt	4	20±3.8	7.4±1.2	5.4±1.1	1.4
Phaeodactylum tricornutum Bohlin	4	19±3.0	23.8±2.9	3.7±0.8	6.4
Chaetoceros calcitrans (Paulsen) Takano	4	15±3.3	4.0±0.9	3.1±0.6	1.3
Thalassiosira pseudonana Hasle and Heimdal	4	8±1.3	5.7±1.0	4.6±1.0	1.2
Flagellates					
Tertraselmis chui Butcher	6	96±17.9	13.8±1.3	9.1±1.3	1.5
Dunaliella tertiolecta Butcher	6	83±21.1	10.2±1.5	8.3±1.8	1.2
Chroomonas salina (Wislouch) Butcher	4	60± 1.4	11.4±1.5	6.9±1.2	1.7
Tetraselmis suecica (Kylin)	6	52±11.0	9.7±1.4	7.1±0.9	1.4
Pavlova lutheri (Droop) Green	5	23± 4.1	6.9±1.4	4.9±0.8	1.4
Nannochloris atomus Butcher	5	21± 4.8	5.0±1.1	4.5±0.9	1.1
Tahitian *Isochrysis* aff. *galbana* Green	4	19± 4.3	8.5±1.6	5.4±1.1	1.6

[1] 1pg = 1 x 10^{-12}g

[2] n = 30

TABLE 2

Comparison of the food value of some algal species fed to 1-day-old Sydney rock oyster (*Saccostrea commercialis*) larvae for six days. Experiment 1.

Algal species	Average length increase after 6 days (μm)[1]
Pavlova lutheri	32.2±1.2[a]
Tahitian *Isochrysis*	31.4±1.6[a]
Chroomonas salina	31.3±1.4[a]
Dunaliella tertiolecta	24.8±1.7[b]
Tetraselmis suecica	22.7±2.6[bc]
Chaetoceros calcitrans	22.5±1.1[c]
Tetraselmis chui	20.7±0.4[cd]
Unfed control	20.6±2.1[cd]
Nannochloris atomus	18.3±0.7[de]
Phaeodactylum tricornutum	15.8±0.9[e]
Chaetoceros gracilis	11.8±0.6[f]
Thalassiosira pseudonana	8.9±0.6[f]

[1] Values are means ± SD. Means with a common superscript do not differ significantly (P>0.05). Initial average length of the 1-day-old larvae was 72.0 ± 0.2 μm.

TABLE 3

Comparison of the food value of some algal species in combination with *Pavlova lutheri* fed to 1-day-old Sydney rock oyster (*Saccostrea commercialis*) larvae for six days. Experiment 2.

Algal diets	Average length increase after 6 days (μm)[1]
Pavlova lutheri/Tahitian *Isochrysis*	41.3 ± 2.5^{a}
Pavlova lutheri/Nannochloris atomus	40.8 ± 2.5^{a}
Pavlova lutheri/Tetraselmis suecica	38.2 ± 6.6^{ab}
Pavlova lutheri/Phaeodactylum tricornutum	37.8 ± 1.1^{abc}
Pavlova lutheri/Chaetoceros calcitrans	37.4 ± 3.9^{abc}
Pavlova lutheri/Tetraselmis chui	37.3 ± 3.7^{abc}
Pavlova lutheri	35.5 ± 2.0^{abc}
Pavlova lutheri/Dunaliella tertiolecta	35.2 ± 3.6^{abc}
Pavlova lutheri/Chroomonas salina	33.5 ± 4.4^{abc}
Pavlova lutheri/Thalassiosira pseudonana	30.8 ± 2.9^{bc}
Pavlova lutheri/Chaetoceros gracilis	28.6 ± 5.4^{cd}
Unfed control	20.9 ± 1.8^{d}

1 Values are means ± SD. Means with a common superscript do not differ significantly ($P > 0.05$). Initial average length of the 1-day-old larvae was 74.7 ± 0.6 μm.

3.8 Nell, J. A. and Holliday, J. E., 1986. Effects of potassium and copper on the settling rate of Sydney rock oyster (*Saccostrea commercialis*) larvae. Aquaculture, 58: 263-267.

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Effects of Potassium and Copper on the Settling Rate of Sydney Rock Oyster (*Saccostrea commercialis*) Larvae

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ABSTRACT

Nell, J.A. and Holliday, J.E., 1986. Effects of potassium and copper on the settling rate of Sydney rock oyster (*Saccostrea commercialis*) larvae. *Aquaculture*, 58: 263–267.

Larvae of the Sydney rock oyster (*Saccostrea commercialis*) were stimulated to undergo metamorphosis and settle in concentrations of 5–12 mM potassium chloride in seawater. At higher potassium chloride concentrations settling rates were reduced. A concentration of $3.14 \cdot 10^{-7}$ mM $CuCl_2$ increased the settling rate on the first day, but after 5 days, settling rates were lower than the control and remaining larvae had died.

INTRODUCTION

For efficient hatchery operation it is important that oyster larvae settle rapidly. Hence an extensive number of studies on settlement have been undertaken. Eyed photosensitive larvae of the American oyster, *Crassostrea virginica*, prefer the darker underside of settling surfaces (Ritchie and Menzel, 1969). These larvae are also stimulated to settle by increases in temperature (Lutz et al., 1970; Hidu and Haskin, 1971) but not salinity (Hidu and Haskin, 1971). Larvae of the American oyster are stimulated to settle by the addition of shellfish glycogen (Keck et al., 1971) and a proteinaceous substance in mantle fluid (Veitch and Hidu, 1971).

Copper concentrations of 0.15–0.50 mg Cu/l in seawater immediately initiated the settling process of larvae of the American oyster (Prytherch, 1931, 1934). Low copper concentrations are, however, toxic to oyster larvae (Calabrese et al., 1977). Elevated potassium levels in seawater were shown to induce and stimulate metamorphosis in *Haliotis rufescens* larvae (Baloun and Morse, 1984). Therefore, a series of experiments were conducted to study the effect

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TABLE 1

Effects of copper concentration in seawater on the settling rate (means ± SD) of Sydney rock oyster (*Saccostrea commercialis*) larvae

Copper concentration (10^{-3} mM $CuCl_2$)	Live spat settled (%)	
	Day 1	Day 5
0	2.0 ± 0.5 a	23.7 ± 6.0 c
1.57	9.2 ± 2.1 c	11.0 ± 1.8 b
3.14	5.4 ± 2.9 b	5.2 ± 1.6 a
4.71	4.0 ± 1.3 ab	3.0 ± 0.7 a
6.28	2.3 ± 0.8 a	1.9 ± 0.1 a
7.85	1.9 ± 0.6 a	2.1 ± 0.9 a

Within each column, means which do not share a common superscript differ significantly ($P < 0.05$).

of $CuCl_2$ and KCl on the settling rate of Sydney rock oyster (*Saccostrea commercialis*) larvae.

MATERIALS AND METHODS

All the oyster larvae used in these investigations were reared at the Brackish Water Fish Culture Research Station at Port Stephens, N.S.W., Australia. Experiments were conducted on "eyed" larvae retained on a 300-μm (diagonal measurement) screen. There were four randomised aquaria (8 l) per treatment, each painted black and containing 2000 larvae. The settling surfaces were grey polyvinyl chloride sheets (300 × 150 × 0.8 mm) held 5 mm off the bottom of the aquaria. Sand-filtered seawater from the Port Stephens estuary with a salinity of 30‰ was used and preheated to and maintained at 28 ± 1 °C. The aquaria were dimly lit (100 lux) and lightly aerated. Water in the aquaria was changed daily and the algae, *Isochrysis galbana* and *Pavlova lutheri*, added to the aquaria once a day at a concentration of 75 000 cells/ml for each species.

The pH in all aquaria with algae and cupric chloride or potassium chloride added remained between 7.0 and 8.5, the range for optimal survival and development of oyster larvae (Calabrese and Davis, 1966). The number of live settled spat that could not be washed off the sheets by gentle rinsing with seawater was counted and the viability of the larvae checked daily. The number of live spat settled was expressed as a cumulative percentage of "eyed" larvae stocked.

The effects of copper as $CuCl_2$ on the settling rate of larvae were investigated at concentrations of 0, 1.57, 3.14, 4.71, 6.28 and 7.85 × 10^{-3} mM $CuCl_2$. The effects of potassium on the settling behaviour of larvae were determined in two separate experiments. The initial experiment used concentrations of 0 and 12 mM KCl to determine the effect on the settling rate over 7 days. In the second experiment concentrations of 0, 4, 8, 12, 16 and 20 mM KCl were used to determine the optimum concentration in seawater over a 7-day settling period.

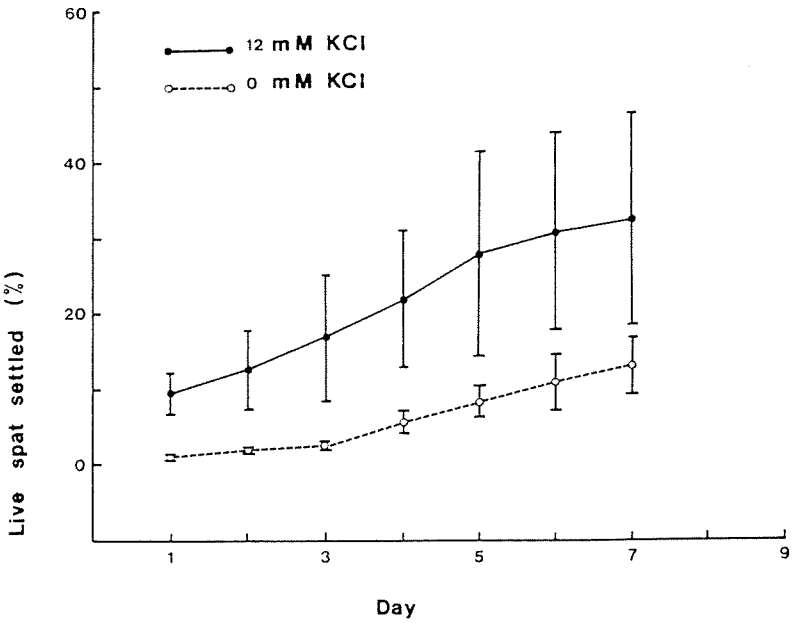

Fig. 1. Effects of potassium concentration in seawater on the settling rate of Sydney rock oyster (*Saccostrea commercialis*) larvae. Means ± SD of cumulative percentage of live spat settled.

One-way analysis of variance was used to assess the effects of copper and potassium on settling rates after testing for homogeneity of variance using the Cochran test. Least significant differences were used to compare mean values. All statistical analyses were carried out according to Sokal and Rohlf (1981).

RESULTS

There was an initial settling stimulus at day 1 in copper concentrations of 1.57 and 3.14×10^{-3} mM $CuCl_2$ (Table 1). However, by day 5 the copper additions were having an adverse effect. The experiment was terminated on day 5 when >90% of the larvae treated with copper at concentrations $\geq 3.14 \times 10^{-3}$ mM $CuCl_2$ had died, while those in the control treatment were alive.

Potassium as 12 mM KCl had an immediate and continued effect on settling rates (Fig. 1). The average settling rates on day 7 for 0 and 12 mM KCl were 13 and 32%, respectively, and were significantly different ($P < 0.05$).

The optimum potassium concentration for the settling of oyster larvae was found to range from 8 to 12 mM KCl (Fig. 2). Settling rates at higher concentrations were significantly reduced ($P < 0.05$). Larvae at 16 and 20 mM KCl began to die after day 2 and most had died by day 7.

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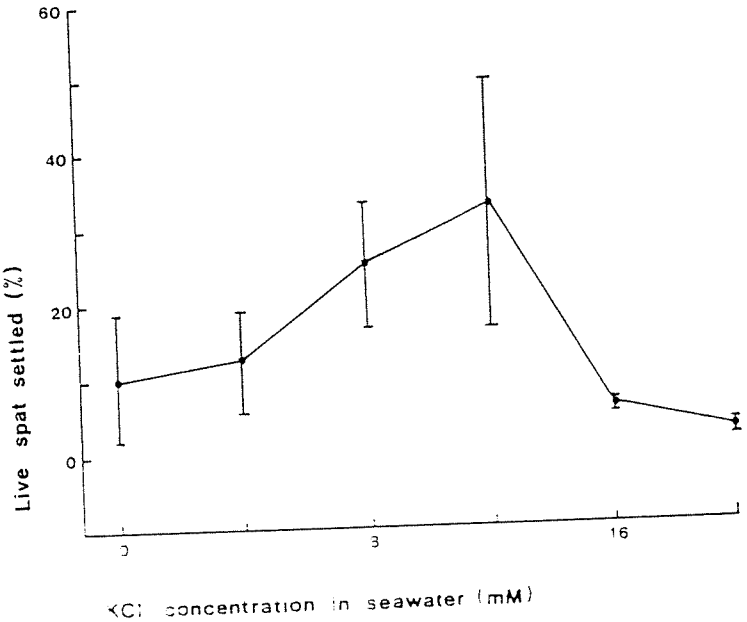

Fig. 2. Determination of the optimum potassium concentration in seawater for the settling of Sydney rock oyster (*Saccostrea commercialis*) larvae. Means ± SD of cumulative percentage of live spat settled.

DISCUSSION

Copper appeared to be too toxic to be used as a settling stimulant for Sydney rock oyster larvae. This confirms the finding by Calabrese et al. (1977), that low concentrations of copper ($\geq 0.05 \times 10^{-1}$ mM $CuCl_2$) are highly toxic to larvae of the American oyster (*Crassostrea virginica*) although it was shown by Prytherch (1931, 1934) to increase the settling rate of larvae of the same oyster. In the present study, potassium as KCl at 8–12 mM proved to be a suitable stimulant and could be used in hatcheries. The effect of potassium and the concentration required for this effect were very similar to those reported for the metamorphosis of larvae of the abalone *Haliotis rufescens* (Baloun and Morse, 1984). The use of settling stimuli could be of great benefit to oyster hatcheries; however, if cultchless spat are required, it would be preferable to achieve metamorphosis without settlement. The use of 10^{-1} mM adrenalin (epinephrine) for the induction of settlement in Pacific oyster, *Crassostrea gigas*, larvae was reported by Coon and Bonar (1985). They reported that 95% of the larvae metamorphosed, with more than 90% of these being unattached.

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3.9 Conclusion

The salinity ranges for survival and growth of some oyster species are given in this study. These data should be considered before selecting any new oyster hatchery or growing site. It is interesting to note that growth rates of juvenile Sydney rock oysters increased with increasing temperatures from 12-30°C. This was somewhat surprising as the Sydney rock oyster is farmed at latitudes from 27-37°S, where water temperatures of 30°C rarely occur. Similar results, however, were obtained by Nell (1986) with flat oyster spat, a species of oyster which is generally found in cooler water than the Sydney rock oyster. The geographic range of these oysters is probably depended on their reproductive success rather than on maximum growth.

Increased copper and fluoride levels in water in oyster growing areas are not likely to be high enough to affect the mortality rates of larvae or spat. Oysters however, are significant accumulators of copper and fluoride and relatively small increases of the element in water could make oysters unsuitable for human consumption.

Oyster larval nutrition is a new field of research that deserves more attention. It is intended to do more research at the Brackish Water Fish Culture Research Station on the evaluation of the feeding value of algal species for 2-week old Sydney rock oyster larvae. The use of gelatin-acacia capsules (Langdon and Waldock, 1981; Chu et al., 1987) for the supplementation of algal diets with cod liver oil as a source of essential fatty acids will also be investigated. The harvesting of algal cultures by centrifugation and the storage of algal paste is another technique that could assist the hatchery rearing of oyster larvae.

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SECTION 4

THE EFFECTS OF GROWING HEIGHT ON GROWTH, CONDITION AND WINTER MORTALITY OF SYDNEY ROCK OYSTERS (*SACCOSTREA COMMERCIALIS*, IREDALE & ROUGHLEY)

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4. The effects of growing height on growth, condition and winter mortality of Sydney rock oysters (*Saccostrea commercialis*, Iredale & Roughley).

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ABSTRACT

Mortality in Sydney rock oysters *(Saccostrea commercialis)*, exposed to Australian winter kill disease showed an inverse relationship with growing level for the range of 300 mm below normal intertidal growing level (52% mortality) to 300 mm above normal growing level for trays (9% mortality).

Whole weight, dry meat condition index, lipid content and glycogen content of the oyster meats were not significantly different between oysters in the different height treatments.

Oysters moved between high and low risk areas in a reciprocal habitat exchange experiment differed in mean cumulative mortality. In the 1986 season in the Georges River, NSW, the mean cumulative mortality of oysters left in a low risk area for winter mortality was 17%, whereas those left in a high risk area experienced 35% during the same period. October was the month of greatest mortality during the experimental period.

These results indicated that raising the growing level by 300 mm can confer on market sized Sydney rock oysters at least as much protection against winter mortality losses as does the current practice of transferring them to a low risk area upstream. It also does this without a reduction in condition. No growth differences were recorded between height treatments but this could be expected as growth was unusually poor for all treatments of oysters during the experimental period.

INTRODUCTION

The Sydney rock oyster *(Saccostrea commercialis)* is cultured intertidally by the stick and tray method in estuaries along the eastern seaboard of Australia (Malcolm, 1987) from about 27°S to 37.5°S latitude. For the southern half of this range, i.e. from Port Stephens to the NSW/Victorian border, the species is susceptible to the economically important disease Australian winter kill (Lauckner, 1983), known locally as "winter mortality". Losses attributed to this disease can range to 70 to 80% of marketable oysters in some areas (Lauckner, 1983, Malcolm, 1987).

Roughley (1926) first studied the disease and described symptoms which included abscesses or pustules in gill, mantle, gonadal and adductor muscle tissues, followed by gaping and death. Histological examination revealed massive haemocytic infiltration around focal necroses but no aetiological agent was apparent. Farley *et al* (1988) attributed the disease to a small (1-2 μm) intracellular parasite of the recently erected phylum Ascetospora (Levine *et al*, 1980). They described the organism from tissue of *S. commercialis* specimens from Woolooware Bay, Georges River, NSW and named the new genus *Mikrocytos* and species *M. roughleyi*. The life cycle outside of the host, and mechanism of infection of this organism are unknown as is the case for other members of this phylum.

Several environmental factors have been associated with the severity of winter mortality and knowledge of these may be useful in developing management techniques for controlling the severity of the disease.

Sydney rock oysters affected with winter mortality typically die just after winter, and the disease is experienced only in the colder part of the geographic range of these oysters. Roughley (1926) concluded, in the absence of evidence for a causative organism, that low temperature stress was the probable cause of the disease. He qualified this with the observation that oysters higher in the tidal range are more exposed to low air temperatures longer so these could not be solely responsible for the disease. He had also observed lower mortalities among naturally caught Sydney rock oysters on mangrove trunks and pneumatophores which occur higher in the tidal range than farmed oysters, and consequently suggested raising oyster growing levels as a means of reducing the severity of the disease.

Wolf (1976, unpublished data/ personal communication) found that raising the growing level of oysters on the leases could be used to reduce winter mortality losses, and stated that this had been successfully applied by an oyster farmer in Port Stephens, NSW. Wolf (1967) had already added high salinity as a contributing factor to the disease. He based this on oyster farmers' experience of greater losses at the salty seaward ends of estuaries than upstream and from their general belief that winter mortality is worse in years with low rainfall in autumn and early winter.

Farmers with oysters in areas with a history of severe winter mortality generally sell their largest oysters, which they believe to be the most vulnerable, before winter's end. Trayed oysters which will not be marketed before the end of winter are relocated upstream in the estuary where experience has shown that there is a lower risk from the disease. To be sure of saving them they must be moved upstream by mid to late May (the last month of autumn). Sticks of younger oysters which would be ready for market the following year are often left in the high risk downstream areas. These are on racks set at about 150 mm higher than the level of the trays and usually escape severe mortality at this level.

Some oyster farmers believe that oysters which do not spawn in autumn, and thus enter winter with a high condition index, are more susceptible to the winter mortality disease than oysters in poor condition.

This study quantifies the consequences of the growing level manipulation suggested by Roughley (1926) and Wolf (1976). Growth, meat composition, and condition index were measured to determine if a loss in production was associated with raising oyster growing levels, and to determine the relationship between meat condition and mortality.

The effect on mortality of relaying oysters in a lower risk area was also determined to allow this disease management method to be compared with height manipulation. A reciprocal habitat transfer experiment was set up in which plots of oysters were exchanged between high and low risk areas for winter mortality. It was designed to identify the risk period in Woolooware Bay and to measure the degree of protection that the transfer of oysters to a low risk area offered the oyster farmers during the 1986 winter season on the Georges River, NSW.

MATERIAL AND METHODS

Experiment 1: the effect of growing height on cumulative mortality.

Space on a commercial oyster lease in Woolooware Bay, Georges River, NSW (lat.34°S). was provided by local oyster farmers. The tarred wooden rails used to support trayed oysters in the traditional growing method (Malcolm, 1987) were modified to provide five growing levels at intervals of 150 mm, centred on the intertidal growing height normally used by oyster farmers in Woolooware Bay. Oyster sticks in Woolooware Bay are grown at the second highest level used in this experiment, i.e. normal tray level plus 150 mm.

Commercial 1.8 x 0.9 m, tarred, timber and galvanised wire mesh oyster trays were divided into two equal compartments. Two trays at each level provided four replicate compartments at each of the five growing heights.

Sydney rock oysters of approximately plate grade (45 to 50 g, for the half-shell trade) and 3 years old were obtained from two oyster farmers, and were well mixed before they were placed in the tray compartments at the rate of 190 oysters per compartment (235/m^2). The trays were covered with a light plastic netting to minimise oyster losses resulting from wave action.

The experiment ran from 23 April, 1986 (mid autumn) to 12 January, 1987 (mid summer). Oysters which died at the start as a result of culling damage were replaced with live oysters which had been held under similar conditions.

Mortality was determined monthly and samples of six oysters from each replicate were weighed and analysed for glycogen, lipid content, and their condition index determined.

Glycogen content was assayed by the enzymatic method of Kepler and Decker (1974). Lipid content was determined by the chloroform-methanol-water extraction method of Bligh and Dyer (1959). The condition index (CI) was calculated as:-

$$CI = \frac{\text{dry weight of oyster meat (g)} \times 100}{\text{shell cavity volume (mL)}}$$

Cavity volumes were determined by subtracting the weight in air of the oyster's shell from the weight in air of the intact oyster (Lawrence and Scott, 1982). This is a valid method because the effective density of cavity contents is close to 1 g/mL. Dry meat weights were determined by drying to a constant weight at 90°C.

Experiment 2: The effect of reciprocal movement of oysters between high and low risk growing areas.

Oyster trays and oysters in this experiment were the same as those used in experiment 1 with the exception that the trays were divided into eight compartments (plots) each of which was stocked with 100 oysters. Oysters which died at the start as a result of culling were replaced after a two week acclimatisation period. A set of six trays with numbered plots were deployed on a commercial oyster growing lease at Woolooware Bay, (WWB) near the mouth of the Georges River. This area has a history of high losses from winter mortality. A second set of oyster trays with plot numbers paired with those at WWB was deployed at Lime Kiln Bar (LKB), about 15 km further upstream, a location considered by oyster farmers to be of low risk for the disease.

The experiment ran from April 23 (mid autumn) to December 14 (early summer), 1986. Each month all oysters in each of four plots in the low risk area were exchanged with the oysters from their matched numbered plots in the high risk area. The plot numbers of pairs to be exchanged were determined using random number tables, and plots were not exchanged more than once during the experiment.

Dead oysters were recorded and removed at exchange times and cumulative mortality calculated.

STATISTICAL ANALYSIS

Homogeneity of variance was confirmed using the Cochran test (Winer, 1971). The data for each of these experiments were then subjected to a single factor analysis of variance, (Sokal and Rohlf, 1981).

RESULTS

Experiment 1: The effect of growing height on cumulative mortality.

At the termination of the experiment an inverse relationship was apparent between cumulative mortality and growing level (Table 1, Fig. 1). The mean cumulative mortality of oysters at the highest level (9.3 ± 0.9%) was significantly lower ($P<0.05\%$) than normal tray level (35.1 ± 8.4%) and at the lowest level (52.4 ±

6.2%) was significantly higher ($P<0.05\%$) than for oysters at the normal tray growing level. The mean cumulative mortalities of oysters at intermediate levels were consistent with this trend for all monthly counts after the August count (Fig.1) when an increase in mortality rate was recorded for all but the topmost level.

The cumulative mortality of oysters in trays at the normal tray growing level reached 30% by mid-October (Fig 1), and increased slowly thereafter.

Measurements of whole weight (Table 2; Fig.2), dry meat condition index (Table 3), glycogen content (Table 4) and lipid content (Table 5) were not significantly different between the height treatments ($P<0.05$). Data for the five height treatments were therefore pooled for dry meat condition index, glycogen content, and for lipid content (Fig.3), although the variance of the lipid content data was not homogeneous between treatments.

Experiment 2: The effect of reciprocal movement of oysters between high and low risk growing areas.

Oysters left in Woolooware Bay for the whole term of the experiment exhibited a significantly greater mean cumulative mortality of 35.4 ± 6.4% (mean ± SD) than those left upstream at Lime Kiln Bar (16.8 ± 6.1%) during the same period.

Oysters shifted from LKB to WWB in the months May (end of autumn) to October (mid spring) inclusive, exhibited mortalities which were not significantly different from those oysters left at WWB (Fig.4).

Oysters moved from LKB to WWB after this time experienced a lower average cumulative mortality similar to oysters which had remained for the whole experimental period at LKB.

Oysters moved upstream from WWB to LKB in May to October inclusive, experienced low mortalities which were not significantly different to those of oysters left in LKB for the whole of the experiment. Oysters moved up river after this time showed high mortalities similar to those remaining in WWB for the whole experimental period.

DISCUSSION

The inverse relationship between winter mortality and growing height (Roughley, 1926) was demonstrated clearly in the growing level experiment. Oysters on the highest trays (raised 300 mm), suffered a mortality of about 9% over the nine month period of the experiment. Oysters die at around this rate in areas where no winter mortality occurs (Nell & Mason, 1991). The second highest level was identical to normal stick height in WWB (normal tray level + 150 mm) and here the cumulative mortality was half that for normal tray height (35%). Such a loss of marketable oysters could not be sustained regularly by farmers.

The lower levels used in the experiment showed greater mortalities (up to 52%) but these levels would not normally be used for growing oysters because of the risk of losses from mud worm attack in summer (Roughley, 1922) as well as from winter kill.

The winter and spring of 1986 were considered by the local oyster farmers to be "normal" with respect to winter mortality. In worse years for the disease, younger oysters and those higher in the intertidal range can be expected to die (Roughley, 1926) so data across several years are needed to determine the most useful winter growing height for commercial use.

No significant increase ($P<0.05$) in whole oyster weight occurred for all treatments of oysters in the experiment (Table 2, Fig.2) so it was not possible to find differences, if any, between treatments. This lack of growth was also experienced by Woolooware Bay commercial oyster farmers who described it as atypical. The lack of growth is consistent the growth reduction caused by bis-tributyl tin oxide (Nell & Chvojka, 1992) later found to be a problem in Gwawley Bay, not far from Woolooware Bay in the Georges River (Batley, *et al*, 1989).

There were no differences between the height treatments in measurements of condition index, which supports Roughley (1926) in his view that condition is a not major factor in determining winter mortality losses. Oyster farmers, however, claimed in Roughley's time (Roughley, 1926) and still do believe that the "fattest" oysters die first and most when winter mortality occurs. The dry cold autumns which are associated with winter mortality are most likely also to prevent late spawning in oysters, which means they will normally be in a fat condition during those winters which are worst for winter mortality.

Glycogen and lipid content also showed no indication of difference between height treatments. In the data pooled for all height treatments (Fig.3) dry meat condition index was seen to drop quickly from April to May indicating that at least some oysters spawned. Their condition remained constant at a dry meat condition index of just above 12 (about the minimum condition suitable for marketing) until November. A rapid rise to December's reading followed by another fall in January indicates further spawning. Spawning is commonly associated with the spring tides close to the summer solstice. The glycogen content showed a sharp drop to the December reading consistent with conversion of glycogen into gonadal material prior to spawning. The oysters' reproductive capacity would therefore appear to be functioning normally in spite of the low growth recorded during the experiment.

Oyster farmers who, for several decades, have transferred oysters from susceptible downstream leases for over-wintering on leases upstream, recognise a critical time before which oysters must be moved. They typically justify this by quoting situations in which a patch of oysters was moved from a downstream lease to an upstream lease over a week or two. They found afterwards that oysters on trays which had been moved before a particular date "all" survived, whereas those moved later than this time showed a consistently high mortality.

This critical time is said to become progressively later from around the end of April (mid autumn) at Quibray Bay near the entrance to Botany Bay, to around the beginning of June (first month of winter) at Oyster Bay, approximately 20 km upstream. Winter mortality is not usually experienced upstream from Oyster Bay except in very bad years.

The reciprocal transfer experiment designed to measure this phenomenon showed surprising results. Oysters which were moved out of Woolooware Bay from May until August 20, 1986 to Lime Kiln Bar and without suffering any greater mortality to those oysters which had been left at LKB for the whole of the experiment. Oysters coming into WWB from LKB all suffered about the same high mortality (about 30%) as those left in WWB for the whole experiment up to and including the September exchange. After September their mortality was no greater than those left in the low risk area (around 15%). These results are not consistent with the 2.5 month incubation period suggested by Wolf (Farley *et al*, 1988) nor the concept that oyster farmers hold of a critical time early in winter for moving the oysters out of the area of risk. Instead a relatively short period of infection was recorded in September and October. The period of infection and incubation may be much more variable than previously believed.

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TABLE 1

Mean cumulative mortality* of Sydney rock oysters (*Saccostrea commercialis*) at five growing levels on Woolooware Bay, Georges River, NSW, from May 1986 to January 1987.

	Cumulative mortality (%)							
Month	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Growing level								
+ 300 mm	3.9 ±0.7	4.3 ±0.8	4.7[a] ±1.1	5.7[a] ±1.2	6.8[a] ±0.9	7.6[a] ±0.9	8.4[a] ±1.1	9.3[a] ±0.9
+ 150 mm	3.6 ±0.5	4.6 ±1.2	6.4[ab] ±1.8	7.4[a] ±2.2	13.0[a] ±3.3	15.0[a] ±3.5	15.5[a] ±3.8	16.8[a] ±4.2
0 mm†	1.7 ±0.5	3.4 ±0.3	6.7[ab] ±1.1	11.8[ab] ±1.3	30.3[b] ±7.2	33.0[b] ±8.4	33.6[b] ±8.5	35.1[b] ±8.4
- 150 mm	1.4 ±1.4	4.1 ±2.1	9.6[bc] ±3.8	21.4[bc] ±9.1	41.7[bc] ±8.7	44.9[bc] ±9.7	45.7[bc] ±9.8	47.2[bc] ±9.6
- 300 mm	3.7 ±1.0	6.3 ±1.0	11.8[c] ±1.6	25.5[c] ±5.4	44.5[c] ±7.9	48.9[c] ±6.7	50.0[c] ±6.8	52.4[c] ±6.2

* Data are expressed as (mean ± SD) with the latter shown below the mean.

† Growing level 'O mm' is the normal growing level for oyster trays in Woolooware Bay which is a little below mid-tide.

Within colums, means which do not have a common superscript differ significantly ($P<0.05$). For analysis of variance an arcsin $x^{0.5}$ transformation was used and Tukeys *w* was used to compare mean values.

TABLE 2

Whole weights* of Sydney rock oysters (*Saccostrea commercialis*) at five growing levels on Woolooware Bay, Georges River, NSW, from April 1986 to January 1987.

	Whole weight (g)									
	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Growing Level										
+ 300 mm	48.5 ±2.6	51.3 ±3.1	50.0 ±2.6	51.3 ±5.2	52.2 ±1.1	47.3 ±2.3	48.3 ±3.1	49.6 ±1.9	54.7 ±3.0	52.0 ±4.0
+ 150 mm	48.5 ±2.6	53.4 ±3.0	59.5 ±2.4	51.2 ±2.4	49.4 ±4.8	56.1 ±10.4	50.8 ±4.1	53.7 ±3.8	52.6 ±4.9	53.2 ±5.9
0 mm†	48.5 ±2.6	50.6 ±1.2	55.4 ±4.0	53.6 ±2.4	54.3 ±4.9	56.3 ±3.0	52.3 ±3.5	59.0 ±4.7	54.9 ±3.8	53.1 ±2.6
- 150 mm	48.5 ±2.6	55.2 ±5.7	53.8 ±5.7	53.2 ±6.6	51.2 ±4.0	57.9 ±5.8	51.1 ±3.5	52.7 ±4.1	55.8 ±7.4	60.6 ±7.4
- 300 mm	48.5 ±2.6	54.1 ±6.3	56.0 ±2.1	51.4 ±5.4	53.6 ±3.0	54.5 ±4.4	53.4 ±5.4	54.7 ±1.4	54.9 ±4.4	51.4 ±6.2

* The data are expressed as (mean ± SD), with the latter shown below the mean.

† Growing level '0 mm' is the normal growing level for oyster trays in Woolooware Bay, and is a little below mid-tide.

TABLE 3

Condition index* of Sydney rock oysters (*Saccostrea commercialis*) at five growing levels on Woolooware Bay, Georges River, NSW, from April 1986 to January 1987

	Condition Index*									
	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan
Growing Level										
+ 300 mm	16.6 ±1.7	12.8 ±1.2	12.3 ±1.4	12.1 ±0.6	11.8 ±1.2	12.0 ±1.2	12.3 ±0.8	13.9 ±1.0	14.9 ±1.1	15.1 ±0.7
+ 150 mm	16.6 ±1.7	13.8 ±1.7	12.5 ±0.3	13.5 ±0.7	12.8 ±1.6	13.4 ±1.0	12.7 ±1.1	12.9 ±0.8	16.2 ±1.1	15.3 ±1.4
0 mm†	16.6 ±1.7	12.5 ±1.7	11.9 ±2.0	12.0 ±0.5	11.7 ±0.7	12.4 ±1.1	12.8 ±1.0	12.6 ±0.2	15.5 ±0.9	14.0 ±1.6
- 150 mm	16.6 ±1.7	13.9 ±1.2	12.6 ±1.0	12.8 ±1.2	12.3 ±1.0	12.8 ±0.8	13.2 ±0.7	11.2 ±1.5	14.8 ±1.8	12.9 ±1.4
- 300 mm	16.6 ±1.7	13.6 ±0.3	12.5 ±1.6	12.9 ±1.0	13.2 ±1.0	12.6 ±1.2	11.7 ±1.6	12.2 ±0.9	15.1 ±2.9	14.0 ±1.8

* The data are expressed as mean dry meat condition index (mean ± SD), with the latter shown below the mean.

† Growing level '0 mm' is the normal growing level for oyster trays in Woolooware Bay, and is a little below mid-tide.

TABLE 4

Glycogen* levels of Sydney rock oysters (*Saccostrea commercialis*) at five growing levels in Woolooware Bay, Georges River, NSW, from April 1986 to January 1987.

	Glycogen content*									
	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Growing Level										
+ 300 mm	21.3 ±1.2	18.1 ±1.1	19.7 ±1.2	19.0 ±1.9	20.0 ±0.9	22.2 ±0.9	22.5 ±0.3	22.9 ±1.7	18.8 ±1.2	21.2 ±0.6
+ 150 mm	21.3 ±1.2	18.9 ±1.5	18.7 ±1.6	19.9 ±3.2	19.8 ±1.0	23.0 ±0.6	22.3 ±0.8	23.9 ±1.3	18.9 ±1.1	21.7 ±2.0
0 mm†	21.3 ±1.2	16.2 ±1.1	18.9 ±0.8	21.8 ±1.1	20.3 ±0.6	23.1 ±1.9	20.9 ±1.3	23.6 ±0.8	19.3 ±1.2	21.6 ±0.9
- 150 mm	21.3 ±1.2	18.3 ±2.8	18.6 ±0.3	21.6 ±0.5	19.0 ±2.5	22.1 ±0.7	22.0 ±0.8	21.8 ±2.9	22.1 ±0.6	21.2 ±0.7
- 300 mm	21.3 ±1.2	16.3 ±0.2	18.9 ±1.1	19.1 ±0.7	21.6 ±1.0	22.4 ±0.4	21.9 ±0.9	18.8 ±1.1	22.7 ±1.0	20.3 ±1.3

* Data are expressed as mean glycogen level (g/100 g dry meat weight) ± SD (xbar ± SD), with the latter shown below the mean.

† Growing level '0 mm' is the normal growing level for oyster trays in Woolooware Bay, and is a little below mid-tide.

TABLE 5

Lipid content of Sydney rock oysters (*Saccostrea commercialis*) grown at five growing levels on Woolooware Bay, Georges River, NSW, from April 1986 to January 1987.

	Lipid content*									
	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Growing Level										
+ 300 mm	19.2 ±1.0	15.4 ±0.9	11.9 ±1.4	12.8 ±0.5	13.2 ±2.0	12.5 ±7.0	9.8 ±1.9	18.2 ±3.9	9.9 ±0.9	17.2 ±1.9
+ 150 mm	19.2 ±1.0	15.6 ±2.0	14.0 ±1.9	22.1 ±1.5	15.2 ±2.0	7.5 ±3.2	16.6 ±8.4	15.5 ±0.9	11.9 ±2.0	16.2 ±0.9
0 mm†	19.2 ±1.0	13.5 ±1.5	14.0 ±0.3	18.4 ±2.0	15.0 ±3.2	11.6 ±1.8	17.5 ±0.7	16.3 ±1.3	10.5 ±1.5	16.9 ±2.6
- 150 mm	19.2 ±1.0	14.1 ±0.4	16.3 ±2.6	16.5 ±1.8	12.4 ±1.9	10.5 ±4.0	16.6 ±1.5	13.9 ±3.9	10.1 ±0.4	16.6 ±1.9
- 300 mm	19.2 ±1.0	14.0 ±2.5	15.2 ±2.0	15.1 ±2.3	13.4 ±1.8	7.1 ±0.6	11.2 ±1.7	9.5 ±0.9	12.4 ±2.9	16.3 ±0.8

* Data are expressed as mean lipid content (mean± SD), with the latter shown below the mean.

† Growing level '0 mm' is the normal growing level for oyster trays in Woolooware Bay, and is a little below mid-tide.

Fig 1 The effect of growing height on cumulative mortality (%) of the Sydney rock oyster *Saccostrea commercialis* in Woolooware Bay, NSW, from April 1986 to January 1987.

Fig 2 Whole weight of Sydney rock oysters *Saccostrea commercialis*, at five growing heights during the winter mortality season from April 1986 to January 1987.

Fig 3 Dry meat condition index (A), glycogen content (B) and lipid content (C) of Sydney rock oysters *Saccostrea commercialis*, pooled data, during the winter mortality season from April 1986 to January 1987.

Fig 4 The effect on cumulative mortality (%) of time of reciprocal transfer of Sydney rock oysters *Saccostrea commercialis* between a high risk winter mortality area, Woolooware Bay, and an area of low risk, Lime Kiln Bar.

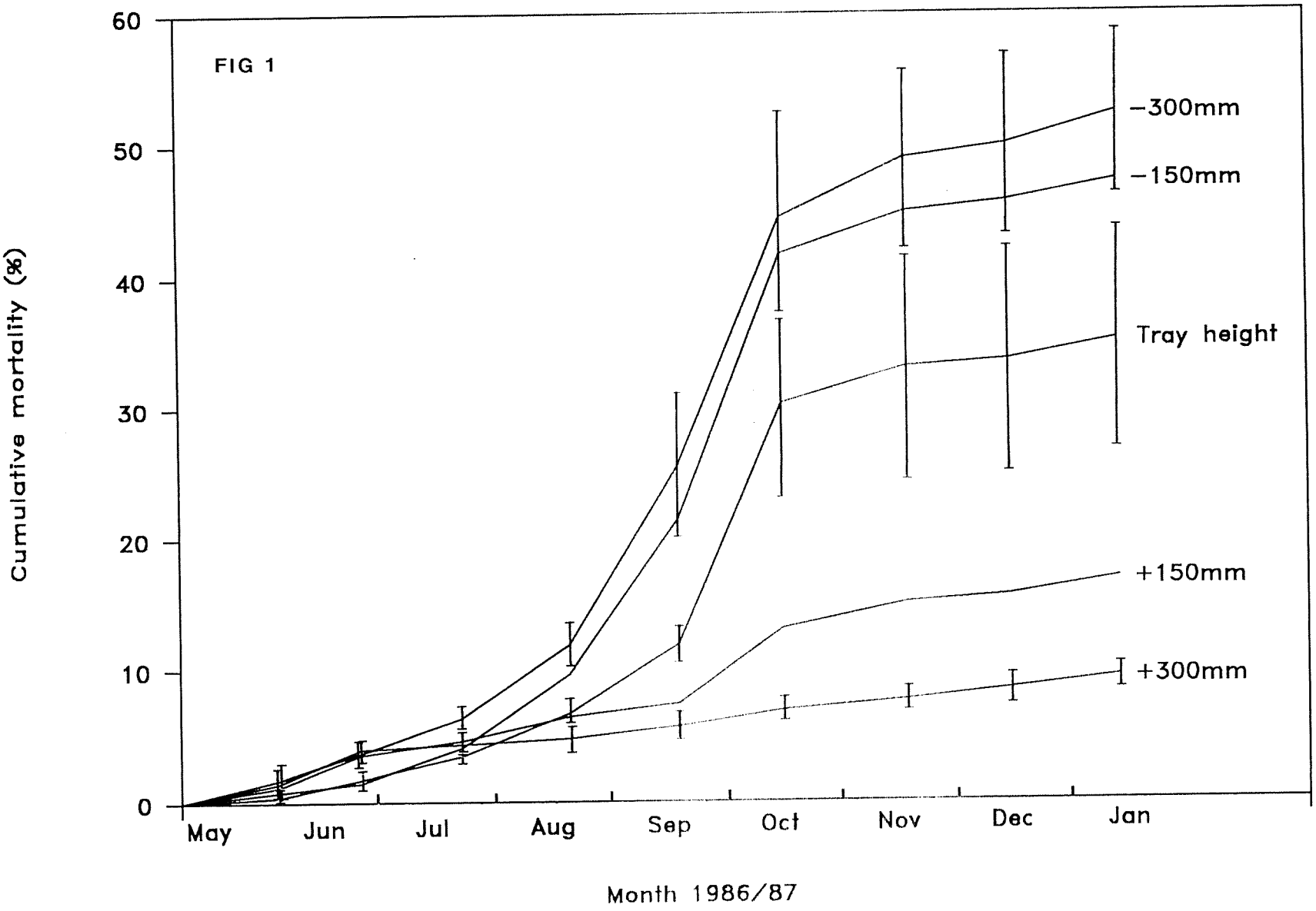


FIG 1
Cumulative mortality (%)
60
50
40
30
20
10
0
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Month 1986/87
-300mm
-150mm
Tray height
+150mm
+300mm

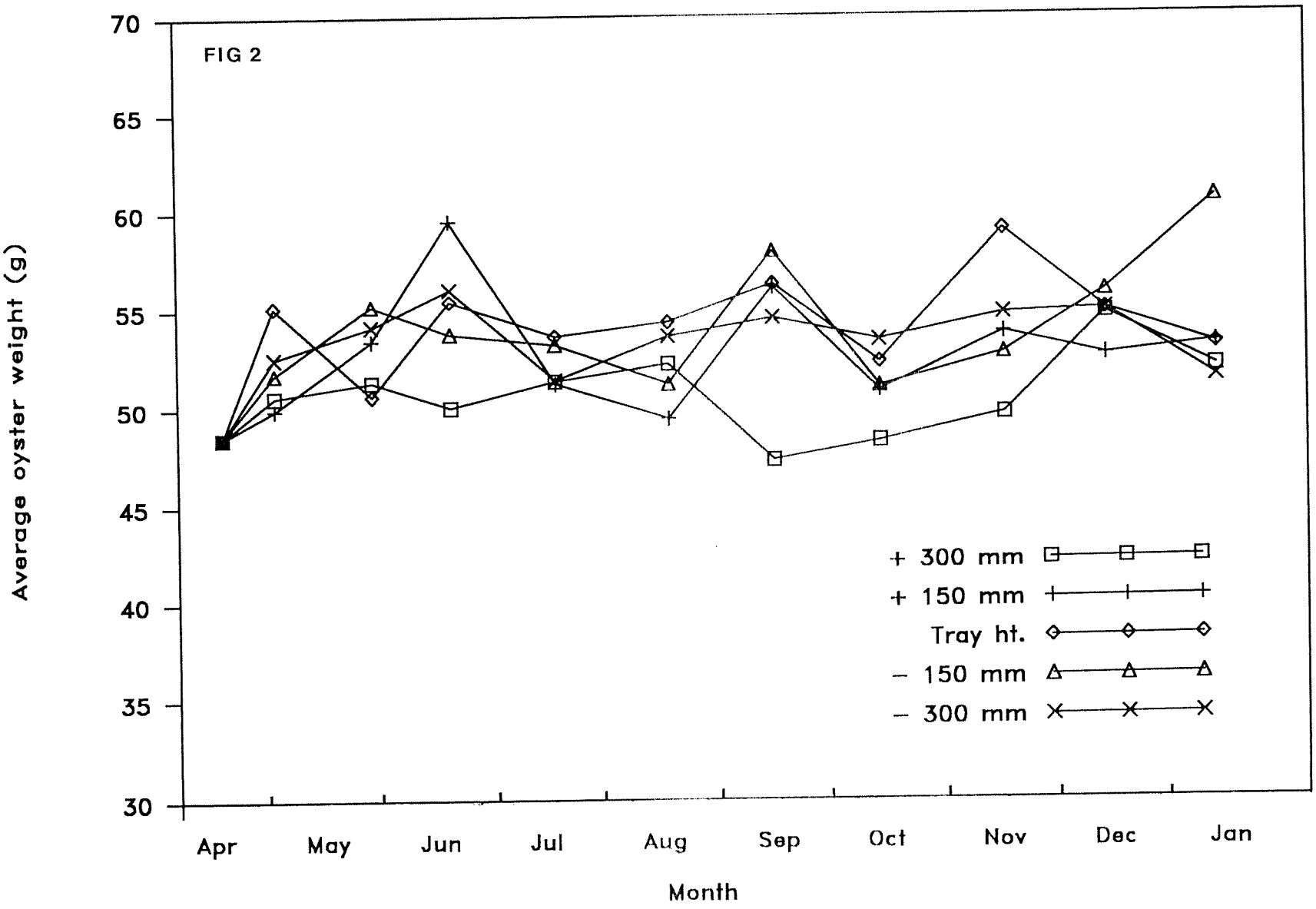


FIG 2
Average oyster weight (g)
70
65
60
55
50
45
40
35
30
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Month
+ 300 mm
+ 150 mm
Tray ht.
– 150 mm
– 300 mm

FIG 3A

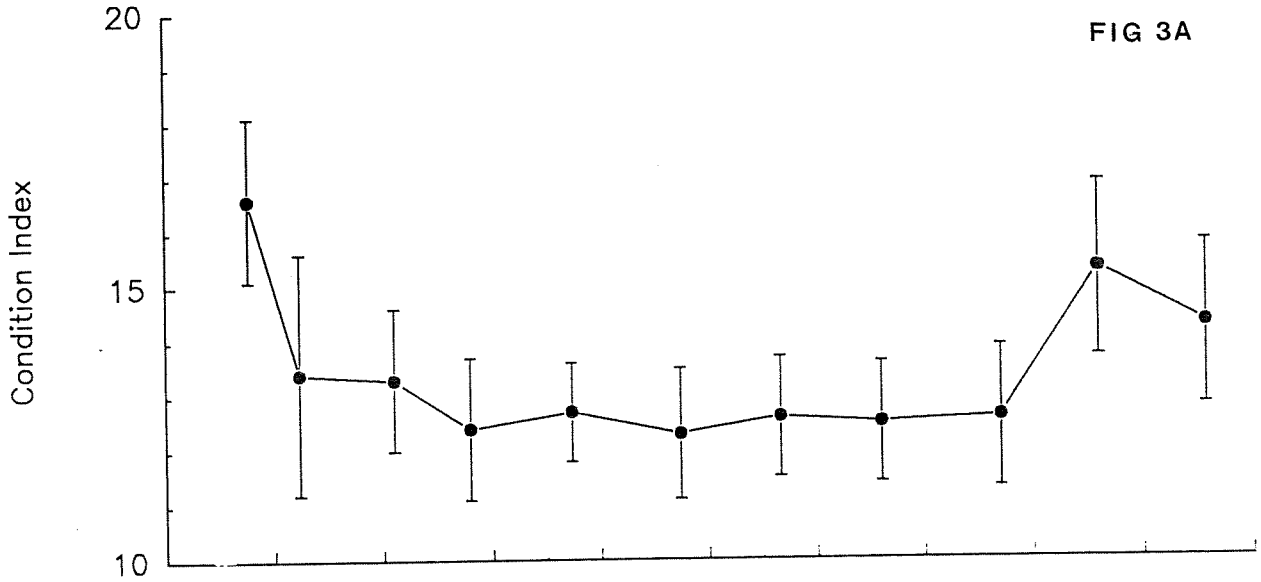


FIG 3B

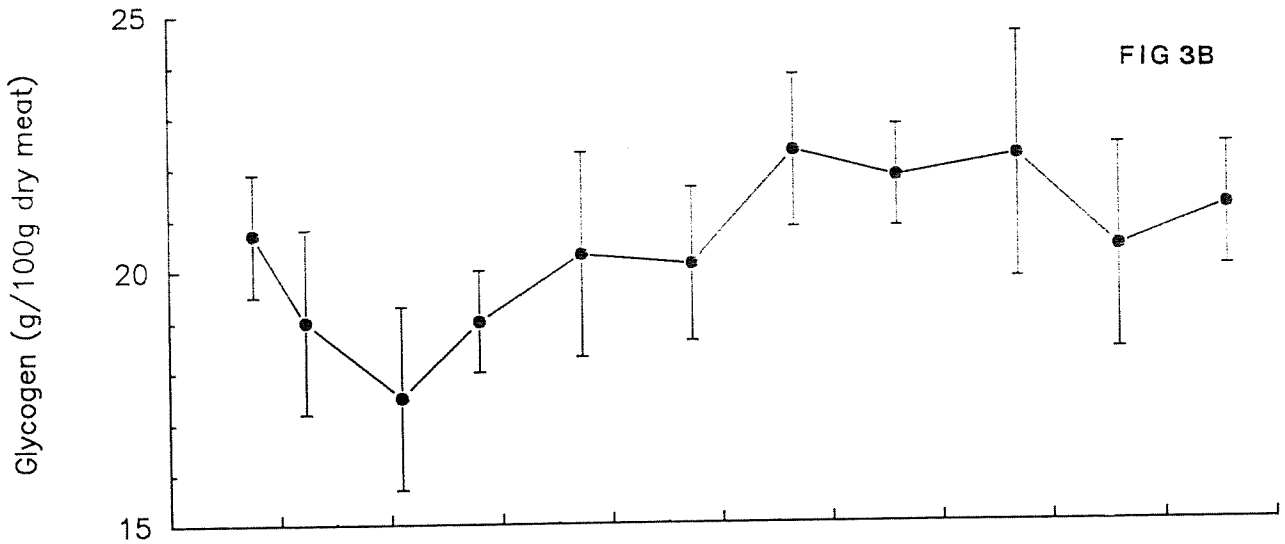
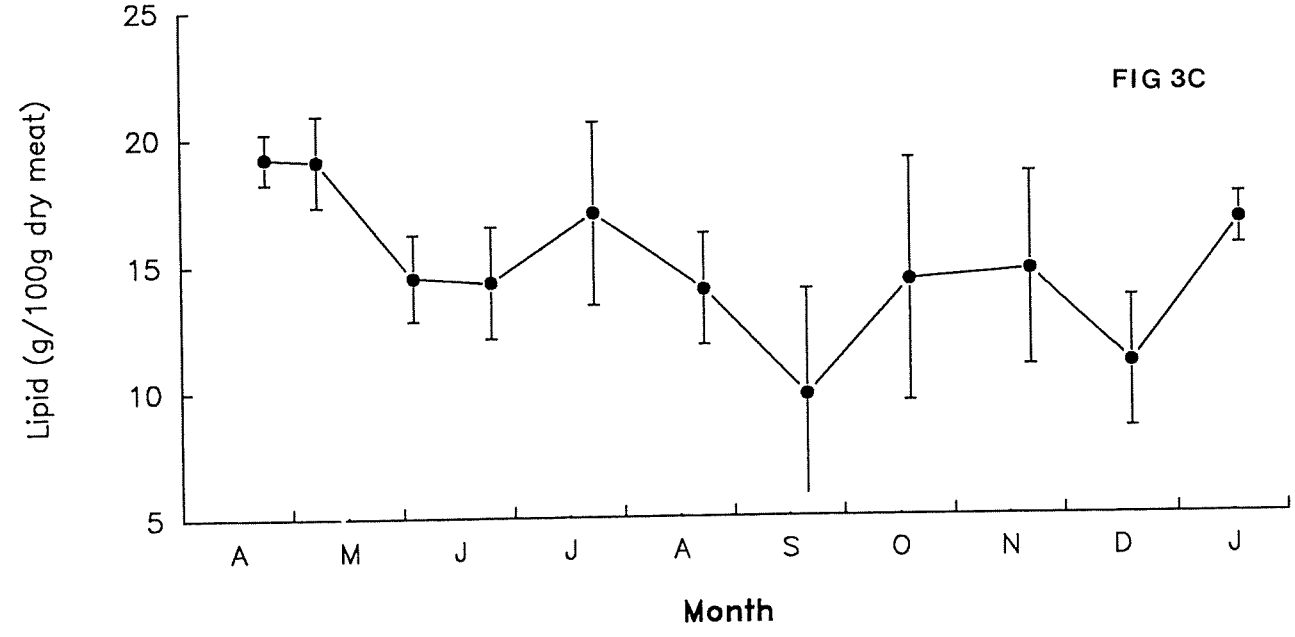


FIG 3C



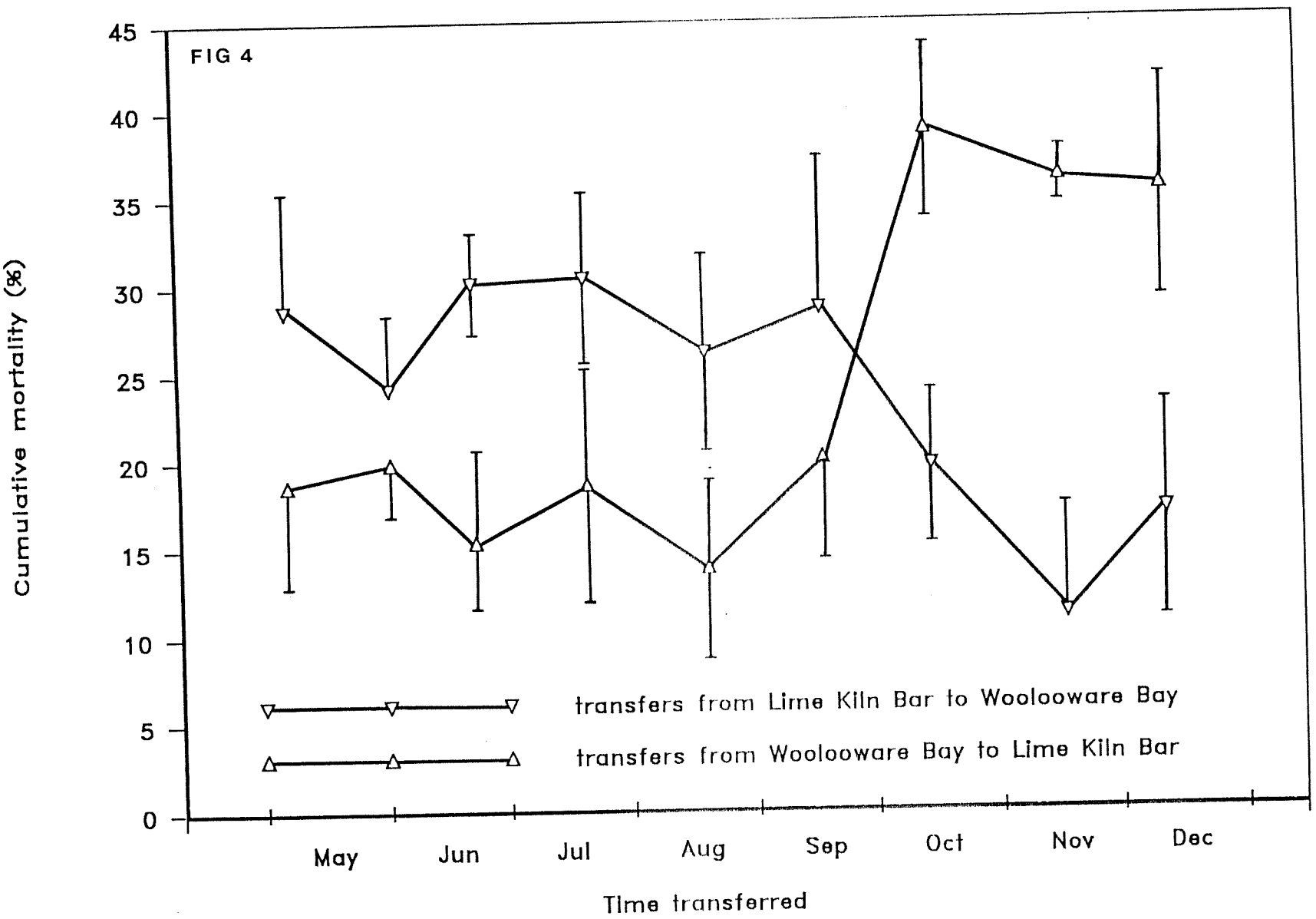


FIG 4
Cumulative mortality (%)
45
40
35
30
25
20
15
10
5
0
transfers from Lime Kiln Bar to Woolooware Bay
transfers from Woolooware Bay to Lime Kiln Bar
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Time transferred

SECTION 5

CONCLUSION

This study showed good progress in the fields of prawn and oyster nutrition. The effects of temperature on growth, food consumption and food conversion on some commercially farmed species were demonstrated and the suitability of some commercially available (1986-88) Australian and Taiwanese diets evaluated. Results of diet evaluation in aquaria swimming pools or netting enclosures in ponds were discussed.

The effects of range of environmental factors (salinity, fluoride, potassium and copper) and nutritional factors (dissolved amino acids) on oysters were demonstrated. Several artificial diets and a range of algal species were evaluated. Subsequent studies demonstrated the benefits of supplementation of algal diets with cod liver oil containing gelatin-acacia capsules and the potential use of stored algal concentrates as alternative foods for commercial hatcheries.

SECTION 6

APPENDICES

6.1 Numaguchi, K. and Nell, J. A., 1991. Effects of gelatin-acacia microcapsule and algal meal supplementation of algal diets on growth rates of Sydney rock oyster *Saccostrea commercialis* (Iredale & Roughley) larvae. Aquaculture, 94: 65-78.

Aquaculture, 94 (1991) 65–78
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Effects of gelatin–acacia microcapsule and algal meal supplementation of algal diets on growth rates of Sydney rock oyster, *Saccostrea commercialis* (Iredale & Roughley), larvae

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ABSTRACT

Numaguchi, K. and Nell, J.A., 1991. Effects of gelatin–acacia microcapsule and algal meal supplementation of algal diets on growth rates of Sydney rock oyster, *Saccostrea commercialis* (Iredale & Roughley), larvae. *Aquaculture*, 94: 65–78.

Gelatin–acacia microcapsules were not adequate as a complete diet for *Saccostrea commercialis* larvae but were useful as a dietary supplement at a concentration of 0.05 mg/l (1330 microcapsules/ml), and reduced the requirement for microalgae. The inclusion of cod liver oil in the microcapsules was found to produce better growth rates than squid or modified fish oil, both of which contained higher concentrations of the C22:6ω3 and C20:5ω3 fatty acids. Algal meal, a dried extract of *Dunaliella salina* (Teodoresco), fed at 0.123 mg/l was equal to the microcapsules fed at 0.05 mg/l as a dietary supplement.

INTRODUCTION

Spat production of bivalves in hatcheries has usually been dependent on microalgae for food. Algal culture is rather expensive (Bolton, 1982) and not always reliable as cultures may fail to grow. Artificial dietary supplements and substitutes for algae would be very beneficial for spat production. The diets should be neutrally buoyant, stable in water and cheaper to provide than algae. Jones et al. (1974) emphasized that bivalve larvae and spat, which ingest their food whole, require particles with a capsule wall that is impermeable and stable in seawater but readily broken down by a change in pH or by the action of digestive enzymes. Gabbott et al. (1976) investigated the feeding of microencapsulated diets to blue mussel, *Mytilus edulis* (L.), and Pa-

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cific oyster, *Crassostrea gigas* (Thunberg), spat and demonstrated the importance of capsule size and digestibility.

The importance of the essential fatty acids C22:6ω3 and C20:5ω3 for *Ostrea edulis* (L.) was clearly demonstrated by Enright et al. (1986a,b). The need for the C22:6ω3 fatty acid in larval development was indicated by the effectiveness of including oyster lipid extract in gelatin–acacia microcapsules fed to oyster spat by Langdon and Waldock (1981). A comprehensive artificial diet consisting of microgel particles, lipid-walled microcapsules and kaolin showed promise when fed to American oyster, *Crassostrea virginica* (Gmelin), spat (Langdon and Siegfried, 1984). This kind of diet, however, tends to produce high bacterial numbers (Langdon and Bolton, 1984) and is therefore less suitable for larvae. Gelatin–acacia microcapsules are a useful artificial diet as American oyster larvae can be reared to metamorphosis on this diet alone, although growth rates were lower than on a live microalgae diet (Chu et al., 1987).

Adult Sydney rock oysters, *Saccostrea commercialis* (Iredale & Roughly), were successfully "fattened" on non-encapsulated artificial diets (Nell and Wisely, 1983, 1984; Nell, 1985). These diets were shown to be unsuitable for oyster larvae, because of the high numbers of bacteria growing on the particles in the larval rearing tanks (K. Numaguchi, personal observation, 1988). We therefore decided to investigate the use of gelatin–acacia microcapsules and algal meal as a partial substitute for algae in the diet of Sydney rock oyster larvae.

MATERIALS AND METHODS

Gelatin–acacia microcapsules

Gelatin–acacia microcapsules were prepared using 160 bloom, beef skin gelatin (Langdon and Waldock, 1981). The mean diameter of the microcapsules was 4.5 ± 1.1 μm ($\bar{x} \pm$ s.d., $n=50$). The average dry weight of the microcapsules was estimated to be 37.6 ± 5.4 pg ($\bar{x} \pm$ s.d., $n=9$), using a method for the determination of the dry weight of algal cells (Epifanio, 1979). The average oil content of the microcapsules was estimated to be $81.1 \pm 5.4\%$ ($\bar{x} \pm$ s.d., $n=5$), by total lipid extraction (Bligh and Dyer, 1959).

Algal meal

The algal meal was a dried extract of *Dunaliella salina* (Teodoresco) supplied by Betatene Ltd.; Cheltenham, Vic., Australia. It is a dry reddish brown powder (Table 1) and is a by-product of β-carotene production. The algal meal was analysed for ash by heating at 550°C for 4 h, for crude protein (N×6.25) by nitrogen estimation, and for total lipid contents by ether extraction.

TABLE 1

Dry weight composition of algal meal[1]

Nutrients	%
Ash	72.7
Carbohydrate (calculated)	12.1
Protein (N×6.25)	10.3
Total lipids (ether extract)	4.9

[1]The algal meal was a dried extract from *Dunaliella salina*.

Feeding and maintenance of oyster larvae

One-day-old D-stage Sydney rock oysters were stocked at a density of 5 larvae/ml, in lightly aerated aquaria (8 l) maintained at 25 ± 1 °C. All experiments were randomised with three replicates per treatment. Oceanic water (salinity 35‰), filtered through 5- and 1-μm filter cartridges was used in all experiments. The amounts of total suspended and volatile (particulate organic matter) solids > 0.7 μm were 2.4 and 0.8 mg/l, respectively. Water was changed every 48 h and all larvae were retained on a 45-μm (diagonal) mesh screen.

Except for experiment 5, the algae, *Pavlova lutheri* (Droop) and *Isochrysis* aff. *galbana* (Parke) clone T-Iso., were used for the feeding experiments. Algal rations were composed of a 50/50 mixture (dry weight) of *Pavlova lutheri* and *Isochrysis galbana*. The average dry weights of individual cells for *Isochrysis galbana* and *Pavlova lutheri* were determined to be 19 ± 4.3 and 23 ± 4.1 pg ($\bar{x}$ ± s.d., $n = 5$), respectively, using the method of Epifanio (1979). Oils were encapsulated in gelatin–acacia microcapsules, and cod liver oil was used unless stated otherwise. No antioxidants were used in the preparation of the microcapsules, although the modified fish oil (ω60 methyl ester) contained 0.05% ethoxyquin.

The experiments were conducted over 8 days, unless stated otherwise, and the larvae were preserved in a solution containing 0.2% buffered formalin in seawater. The length (greatest distance parallel to the hinge) of ≥ 30 preserved larvae per aquarium was measured using a microscope and micrometer slide. The term survival in the text describes the percentage of larvae stocked which were alive at the end of the experiments. Not all larvae stocked could be accounted for.

Experiment 1

Larvae were either not fed or fed microcapsules at 0.013, 0.025, 0.05, 0.10 or 0.15 mg l^{-1} day^{-1} or algae at 0.05 or 0.25 mg l^{-1} day^{-1} (Table 2). Algae at 0.05 or 0.25 mg/l were equivalent to a total of 2.4×10^3 and 1.2×10^4 cells/ml, respectively.

Experiment 2

Larvae were either not fed or fed algae at 0.10 or 0.25 mg l^{-1} day^{-1}. The larvae fed algae at 0.10 mg l^{-1} day^{-1} received either only algae or microcapsules as an algal supplement at 0.013, 0.025, 0.05, 0.10 or 0.15 mg l^{-1} day^{-1} (Table 3). Algae at 0.10 mg/l were equivalent to a total of 4.8×10^3 cells/ml.

Experiment 3

Larvae were either not fed or fed algae at 0.025, 0.05, 0.1, 0.175, 0.25, 0.375 or 0.5 mg l^{-1} day^{-1}. The algal diets were either fed as above or supplemented with microcapsules at 0.05 mg l^{-1} day^{-1} (Fig. 1). There was an additional treatment of larvae fed algae at 0.0125 mg l^{-1} day^{-1} supplemented with microcapsules at 0.05 mg l^{-1} day^{-1}. Algae at 0.025, 0.05, 0.1, 0.175, 0.25, 0.375 or 0.5 mg/l were equivalent to a total of 1.2×10^3, 2.4×10^3, 4.8×10^3, 8.4×10^3, 1.2×10^4, 18×10^4 and 24×10^4 cells/ml, respectively.

Experiment 4

Larvae were either not fed or fed algae at 0.05 or 0.25 mg l^{-1} day^{-1} (Table 4). The low density algal diet (0.05 mg l^{-1} day^{-1}) was either fed alone or supplemented with microcapsules at 0.05 mg l^{-1} day^{-1}. Different treatments received microcapsules containing one of the following oils: modified fish oil (ω60 methyl ester made from sardine oil by Nippon Chemical Feed Co Ltd., Hokkaido, Japan): olive oil, soybean oil, corn oil, squid oil, or cod liver oil (Table 4). The fatty acid composition of the oils is shown in Table 5. A sample of 800 ml was taken from the (8 l) aquaria for length measurements of larvae on day 8, and the experiment was terminated on day 14.

Experiment 5

Larvae were either not fed or fed diets A, B, C or D (Table 6) which involved various combinations of algae (*Isochrysis galbana*) and microcapsules. Samples of 800 ml were taken from the (8 l) aquaria on days 5, 9, 13 and 17 for length measurements of larvae, and the experiment was terminated on day 21.

Experiment 6

Larvae were either not fed or fed live algae at 0.05 or 0.25 mg l^{-1} day^{-1}. Larvae fed live algae at 0.05 mg/l included treatments (Table 8) with live algae only or live algae supplemented with algal meal at 0.123 mg/l, gelatin–acacia microcapsules at 0.05 mg/l or both algal meal (0.123 mg/l) and gelatin–acacia microcapsules (0.05 mg/l). The feeding level of 0.123 mg l^{-1} day^{-1} for the algal meal was chosen, because this level was previously shown to promote the best growth rates in Sydney rock oyster larvae. Higher levels of algal meal also caused problems with bacterial contamination (K. Numaguchi, personal observation, 1988).

Lipid analyses

Fatty acid composition was determined by a transesterification method based on that of Welch (1977). Oil samples (50 mg) were heated in 3% methanolic sulphuric acid for 75 min at 105°C. The methyl esters so formed were quantified using a Hewlett Packard (Avondale Pennsylvania, USA) 5890 gas chromatograph and HP 7673A autosampler fitted with a 30 m × 0.25-mm BP1, non-polar capillary column (Scientific Glass Engineering Pty. Ltd., Ringwood, Vic., Australia). A fish oil reference sample (C.S.I.R.O. Division of Oceanography, Hobart, Tasmania, Australia) was used to aid identification of the fatty acid methyl esters.

Statistical analyses

Homogeneity of variance was confirmed using Cochran's test (Winer, 1971). The data were examined statistically by analysis of variance and mean values were compared by least significant differences (Sokal and Rohlf, 1981). The larval survival data were transformed by arcsin $x^{0.5}$ before analysis of variance, because the percentages covered a wide range of values. Throughout the text means ± standard deviation (s.d.) are used.

RESULTS

Experiment 1

Growth rates of larvae fed only with microcapsules increased with increasing (0.013–0.15 mg/l) microcapsule concentrations (Table 2). Growth rates of the larvae fed microcapsules at 0.15 mg/l were similar to those fed algae at

TABLE 2

The effects of gelatin–acacia microcapsule (GAM) concentration on the growth rates of Sydney rock oyster larvae over 8 days[1] (experiment 1)

Diets (mg/l)	Length increase[2] (μm)
Unfed control	13.9 ± 1.1^{a}
0.013 GAM	14.7 ± 1.9^{a}
0.025 GAM	15.7 ± 2.2^{a}
0.05 GAM	17.4 ± 2.6^{b}
0.10 GAM	20.2 ± 2.9^{bc}
0.15 GAM	22.4 ± 2.5^{c}
0.05 Algae[3]	23.4 ± 1.0^{c}
0.25 Algae	32.5 ± 1.1^{d}

[1]Values are means ± s.d. Means with a common superscript do not differ significantly ($P > 0.05$). There were no significant dietary effects ($P > 0.10$) on survival rate and the average was 40 ± 7%.
[2]Initial average length of the 1-day-old D-stage larvae was 68.0 ± 0.2 μm.
[3]The algae *Isochrysis galbana* and *Pavlova lutheri* were fed on an equal dry weight basis; 0.25 mg algae/l was equivalent to a total of 1.2×10^{4} cells/ml.

0.05 mg/l (2.4×10^3 cells/ml). Larvae fed with algae at 0.25 mg/l (1.2×10^4 cells/ml) had by far the highest growth rate. There were no significant dietary effects ($P>0.10$) on larval survival rate and the average was $40\pm7\%$ ($\bar{x}\pm$s.d.).

Experiment 2

For larvae fed the low concentration (0.10 mg/l) of algae supplemented with microcapsules, growth rates increased with microcapsule concentration up to 0.05 mg/l (Table 3). At this feeding rate the growth rate of the larvae was similar to that of larvae fed the high concentration (0.25 mg/l) of algae without supplementation. The survival rates of larvae on all diets was high ($65\pm9\%$, $\bar{x}\pm$ s.d.) and although there was a significant effect ($P<0.05$) of diet type on survival, there was no clearly discernible survival pattern among larvae fed the different diets (Table 3).

Experiment 3

The addition of microcapsules at 0.05 mg/l to algal diets produced a significant ($P<0.001$) improvement in growth rates at all algal concentrations (Fig. 1). There were no significant dietary effects ($P>0.10$) on survival rate and the average was $32\pm9\%$ ($\bar{x}\pm$s.d.). In this experimental system, an algal density maintained at 0.175 mg/l is adequate for growing larvae until they are 8 days old.

TABLE 3

The effects of gelatin–acacia microcapsule (GAM) supplementation of an algal diet on the growth and survival rates of Sydney rock oyster larvae over 8 days[1] (experiment 2)

Diets (mg/l)	Length increase[2] (μm)	Survival[3] (%)
Unfed control	14.8 ± 0.7^{a}	64.9 ± 7.7^{bc}
0.10 Algae[4]	24.1 ± 2.3^{b}	76.1 ± 4.2^{d}
0.10 Algae+0.013 GAM	26.1 ± 3.2^{bc}	54.8 ± 4.6^{a}
0.10 Algae+0.025 GAM	27.6 ± 2.0^{bcd}	68.5 ± 4.8^{bc}
0.10 Algae+0.05 GAM	30.4 ± 0.7^{d}	70.6 ± 8.0^{cd}
0.10 Algae+0.10 GAM	28.8 ± 0.8^{cd}	55.5 ± 12.2^{a}
0.10 Algae+0.15 GAM	29.3 ± 3.3^{cd}	65.8 ± 3.0^{bc}
0.25 Algae	30.5 ± 2.3^{d}	62.3 ± 8.7^{ab}

[1]Values are means$\pm$s.d. Within columns, means with a common superscript do not differ significantly ($P>0.05$).
[2]Initial average length of the 1-day-old D-stage larvae was 68.0 ± 0.2 μm.
[3]For statistical analyses an arcsin $x^{0.5}$ transformation was used.
[4]The algae *Isochrysis galbana* and *Pavlova lutheri* were fed on an equal dry weight basis; 0.25 mg algae/l was equivalent to a total of 1.2×10^4 cells/ml.

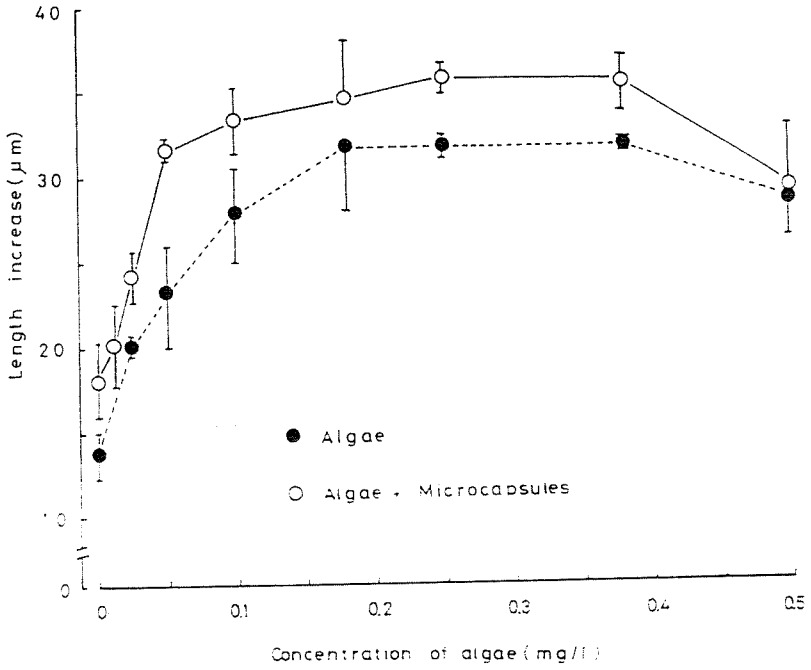

Fig. 1. Eight-day growth responses of Sydney rock oyster larvae when fed gelatin–acacia microcapsules (GAM) at 0.05 mg per l of aquarium water per day with algae over a range of concentrations (experiment 3). Values are means ± s.d. The algae *Isochrysis galbana* and *Pavlova lutheri* were fed on an equal dry weight basis: 0.25 mg algae/l was equivalent to a total of 1.2×10^5 cells. There were no significant dietary effects ($P > 0.10$) on survival rate and the average was 32 ± 9%. Initial average length of the 1-day-old D-stage larvae was 71.1 ± 0.7 μm.

Experiment 4

Supplementing an algal diet with microcapsules which contained cod liver oil produced by far the best growth rates and a high survival rate (Table 4). This dietary combination (0.05 mg cod liver oil GAM/l + 0.05 mg algae/l) produced significantly better ($P < 0.05$) growth rates after 14 days than the larvae fed only algae at 0.25 mg/l. Squid oil was the second best oil supplement but the other oils (modified fish oil, olive, soybean and corn oil) produced no significant improvement ($P > 0.05$) in growth rates when compared to the 0.05-mg algae/l treatment.

Fatty acid composition of the oils and algal meal

Modified fish oil, squid and cod liver oil contain (Table 5) large amounts of the essential fatty acids C22:6ω3 (31, 16 and 9% of the total fatty acid content in that order) and C20:5ω3 (17, 14 and 10%, respectively). The

TABLE 4

The effects of different oil supplements in gelatin–acacia microcapsules (GAM) on the growth and survival rates of Sydney rock oyster larvae[1] (experiment 4)

Diets (mg/l)	8-Day length increase[2] (μm)	14-Day	
		Length increase (μm)	Survival[3] (%)
Unfed control	18.2 ± 2.2^{a}	24.9 ± 2.1^{a}	22.6 ± 7.4^{a}
Algae[4] only 0.05	25.1 ± 4.3^{b}	36.6 ± 8.0^{ab}	34.4 ± 7.0^{cd}
Algae only 0.25	32.5 ± 3.5^{cd}	51.9 ± 14.9^{bc}	45.3 ± 7.2^{f}
Algae (0.05) + GAM (0.05)			
Modified fish oil	24.6 ± 3.4^{ab}	40.9 ± 9.3^{bc}	32.8 ± 3.0^{bc}
Olive oil	25.7 ± 4.1^{b}	42.2 ± 8.0^{bc}	38.9 ± 5.2^{de}
Soybean oil	26.4 ± 4.4^{bc}	38.7 ± 11.7^{abc}	45.7 ± 2.3^{f}
Corn oil	30.1 ± 5.0^{bcd}	45.8 ± 6.4^{bc}	27.9 ± 5.4^{ab}
Squid oil	33.7 ± 2.4^{c}	52.5 ± 5.5^{c}	37.8 ± 3.5^{cde}
Cod liver oil	35.6 ± 4.0^{d}	71.1 ± 10.8^{d}	43.3 ± 5.5^{ef}

[1]Values are means ± s.d. Within columns, means with a common superscript do not differ significantly ($P > 0.05$).
[2]Initial average length of the 1-day-old D-stage larvae was 67.5 ± 0.7 μm.
[3]For statistical analyses an arcsin $x^{0.5}$ transformation was used.
[4]The algae *Isochrysis galbana* and *Pavlova lutheri* were fed on an equal dry weight basis: 0.25 mg algae/l was equivalent to a total of 1.2×10^{5} cells/ml.

vegetable oils contain little or none of these important fatty acids. Corn and soybean oil contain large amounts of the shorter chain unsaturated fatty acids (C18:2ω6 and C18:1ω9). Olive oil contains largely the C18:1ω9 fatty acid (74%). The algal meal contained a large amount (45%) of the C16:0 fatty acid, only small amounts of the C18 saturated and unsaturated fatty acids and no longer chain fatty acids (Table 5).

Experiment 5

Diet A with the high algal concentrations (0.25, 0.5 and 1.0 mg/l) produced the best growth rate and the highest percentage of "eyed" larvae, i.e., larvae that are competent to metamorphose and settle (Fig. 2, Tables 6 and 7). However, the larvae on this diet had a significantly lower ($P < 0.05$) survival rate than those fed diet B with the low algal concentrations (0.05, 0.1 and 0.2 mg/l) or the low algal concentration diets supplemented with microcapsules (diets C and D).

Supplementation of the low algal concentration (0.05, 0.1 and 0.2 mg/l) diets with microcapsules at 0.05 mg/l or more significantly improved ($P < 0.05$) the growth rates and promoted development of the larvae. Supplementation of the low concentration algal diet with more microcapsules (diet D) than 0.05 mg/l (diet C) produced no further improvement in larval development (Table 7).

TABLE 5

Fatty acid composition (%) of oils[1]

Fatty acid	Soybean oil	Corn oil	Olive oil	Cod liver oil	Squid oil	Modified fish oil	Algal meal
C10:	–	–	–	–	–	–	–
C12:0	–	–	–	–	–	0.30	10.81
C14:0	–	–	–	6.60	4.88	0.24	2.97
Unknown[2a]	–	–	–	0.78	–	–	0.94
Unknown[b]	–	–	–	0.47	–	–	1.80
C16:1ω7	–	–	0.76	6.82	5.07	0.69	4.28
C16:0	9.76	10.95	10.66	14.97	15.16	2.65	45.17
C17:0	–	–	–	–	0.51	–	–
C18:4ω3	–	–	–	4.09	3.34	1.38	0.67
C18:2ω6	54.52	48.81	6.72	2.81	1.54	0.85	1.51
C18:3ω3	6.49	1.14	–	1.56	1.15	0.55	1.81
C18:1ω9	20.77	33.08	73.99	10.87	11.19	6.69	3.64
C18:1ω7	1.97	1.47	2.25	2.67	3.54	1.84	1.03
Unknown[c]	–	–	–	0.40	0.45	0.22	0.92
C18:0	4.13	2.24	3.21	1.87	1.88	1.32	1.41
Unknown[d]	0.53	0.46	–	–	–	–	0.76
C20:4ω6	–	–	–	0.28	0.76	0.98	–
C20:5ω3	–	–	0.33	9.54	13.94	17.06	–
C20:4ω3	–	–	–	0.70	0.80	1.33	–
Unknown[e]	–	–	–	0.92	–	0.62	–
C20:1	0.23	0.40	0.26	8.57	5.64	8.27	–
C20:0	0.38	0.49	0.39	0.41	0.52	0.24	–
C22:6ω3	–	–	–	8.77	15.82	30.83	–
C22:5ω3	–	–	–	1.15	1.26	2.66	–
Unknown[f]	–	–	–	–	–	1.14	–
C22:1	0.44	–	–	12.02	4.13	9.19	–
Unknown[g]	–	–	–	1.36	1.38	0.89	–
C22:0	–.	–	–	1.36	1.88	0.26	–
C24:0	0.09	–	–	0.78	0.61	2.07	–
C26:0	–	–	–	–	1.00	0.52	–
Total	99.31	99.04	98.57	99.77	96.45	92.79	77.72

[1]Data expressed as percentage of total fatty acids present.
[2]Unknown indicates unidentified components; other fatty acids at concentrations <0.2% are not listed.

Experiment 6

Growth rates of larvae fed live algae at 0.05 mg/l were significantly increased ($P<0.05$) when supplemented with algal meal at 0.123 mg/l, microcapsules at 0.05 mg/l or algal meal and microcapsules at the rates mentioned before (Table 8). There were no significant dietary effects ($P>0.10$) on survival and the average rate was $37\pm8\%$ ($\bar{x}\pm$s.d.).

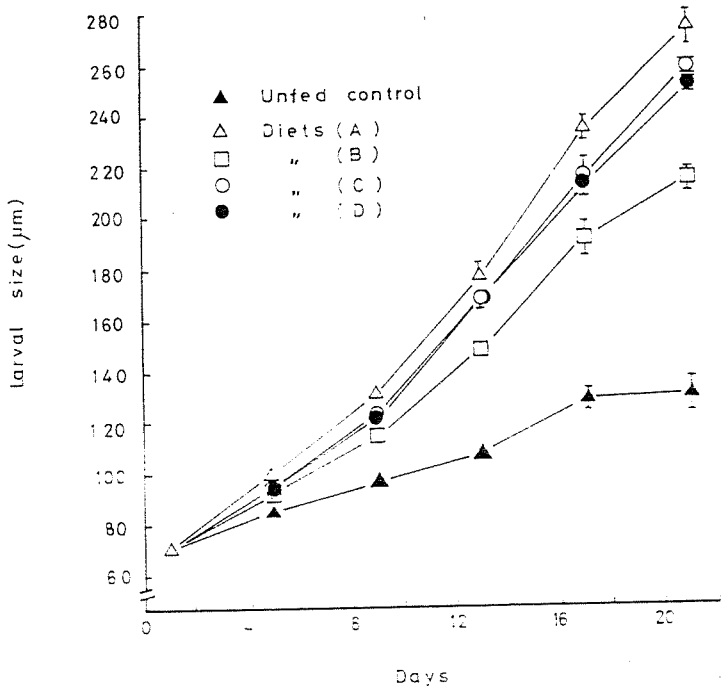

Fig. 2. The effects of diets on the growth of Sydney rock oyster larvae over 20 days (experiment 5). Values are means ± s.d. The alga *Isochrysis galbana* was fed as in Table 6.

TABLE 6

Concentrations of algae *Isochrysis galbana* and gelatin–acacia microcapsules (GAM) fed to Sydney rock oyster larvae (Experiment 5)

Diets	Dry weight of diets (mg/l)		
	Days: 1–7	8–14	15–20
(A) Algae	0.25 (12.5×10^3)[1]	0.5 (25×10^3)	1.0 (50×10^3)
(B) Algae	0.05 (2.5×10^3)	0.1 (5×10^3)	0.2 (10×10^3)
(C) Algae	0.05 (2.5×10^3)	0.1 (5×10^3)	0.2 (10×10^3)
GAM	0.05 (1.3×10^3)	0.05 (1.3×10^3)	0.05 (1.3×10^3)
(D) Algae	0.05 (2.5×10^3)	0.1 (5×10^3)	0.2 (10×10^3)
GAM	0.05 (1.3×10^3)	0.1 (2.6×10^3)	0.2 (5.2×10^3)

[1]Parentheses show the number of algal cells or gelatin–acacia microcapsules per ml.

TABLE 7

The effects of diet on length increase, percentage of "eyed" larvae and survival of Sydney rock oyster larvae over 20 days[1] (experiment 5)

Diets	Length increase[2] (μm)	"Eyed" larvae (%)	Survival[3] (%)
Unfed control	60.0 ± 6.8^{a}	0	27.8 ± 3.3^{a}
(A) Algae	203.1 ± 6.6^{d}	94.0 ± 3.6^{b}	26.8 ± 3.6^{a}
(B) Algae	144.1 ± 5.1^{b}	75.0 ± 4.4^{a}	49.6 ± 9.2^{b}
(C) Algae + GAM	187.7 ± 5.8^{c}	81.3 ± 2.3^{b}	45.5 ± 14.5^{b}
(D) Algae + GAM	181.5 ± 0.6^{c}	81.0 ± 3.0^{b}	50.5 ± 19.1^{b}

[1]Values are means ± s.d. Within each column, means with a common superscript do not differ significantly ($P > 0.05$).
[2]Initial average length of 1-day-old D-stage larvae was 70.1 ± 0.4 μm.
[3]For statistical analyses an arcsin $x^{0.5}$ transformation was used.

TABLE 8

The effects of algal meal (AM) and gelatin–acacia microcapsule (GAM) supplementation of a live algal diet on the growth rates of Sydney rock oyster larvae over 8 days[1] (Experiment 6)

Diets (mg/l)	Length increase[2] (μm)
Unfed control	13.9 ± 1.1^{a}
0.05 Algae[3]	23.4 ± 1.0^{b}
0.05 Algae + 0.123 AM	29.5 ± 2.1^{c}
0.05 Algae + 0.05 GAM	30.4 ± 1.6^{c}
0.05 Algae + 0.123 AM + 0.05 GAM	28.7 ± 3.5^{c}
0.25 Algae	32.5 ± 1.1^{c}

[1]Values are means ± s.d. Means with a common superscripts do not differ significantly ($P > 0.01$). There were no significant dietary effects ($P > 0.10$) on survival rate and the average was $37 \pm 8\%$.
[2]Initial average length of the 1-day-old D-stage larvae was 68.2 ± 0.4 μm.
[3]The algae *Isochrysis galbana* and *Pavlova lutheri* were fed on an equal dry weight basis; 0.25 mg algae/l was equivalent to a total of 1.2×10^{4} cells/ml.

DISCUSSION

Gelatin–acacia microcapsules which contained cod liver oil were a most effective supplement for algal diets fed to Sydney rock oyster larvae. In the present study the optimum concentration (Table 2) for microcapsules as the sole dietary component is 0.10–0.15 mg/l (2660–3990 microcapsules/ml), which is considerably more than the 500 microcapsules/ml recommended by Chu et al. (1982). The optimum concentration (Table 3) for microcapsules as a supplement to algal diets was found to be 0.05 mg/l (1330 microcapsules/ml). When supplementation was provided at 0.05 mg/l, over a range of algal concentrations from 0.0125 to 0.5 mg/l (600–24 100 cells/ml), growth rates at all algal concentrations were increased (Fig. 1).

Although microcapsules were shown to be an excellent dietary supplement or partial substitute for algae, growth rates of larvae fed only microcapsules were less than that for larvae fed only algae (Table 2). The microcapsules used in this study contained 81% oil, the remainder was gelatin (protein) and acacia (carbohydrate). The optimum level of dietary protein for juvenile American oysters *C. virginica* was found to be 21% (Flaak and Epifanio, 1978), which is considerably more than that provided by the gelatin in the microcapsules. Gelatin has a rather unusual amino acid composition (Lentner, 1981) and is possibly not as good a source of protein for the larvae as algae.

Of all oils tested, cod liver oil produced the highest growth rates (Table 4), although the squid and modified fish oil had much higher concentrations of two important (Langdon and Waldock, 1981; Enright et al., 1986a,b) long chain unsaturated fatty acids (C22:6ω3 and C20:5ω3). This implies that the concentrations of these two very important essential fatty acids in cod liver oil were adequate for optimum larval development. The amount of C22:6ω3 fatty acid in the modified fish oil was 38% according to the supplier, but on analysis it was found to be only 31%, so it is possible that some oxidation may have occurred, although the oil contained 0.05% ethoxyquin. Oxidation of some of the unsaturated fatty acids may have reduced the food value of the modified fish oil. The low growth rates on the vegetable oils were in accordance with the findings by Trider and Castell (1980), who also compared the food value of different oils (not encapsulated). Trider and Castell (1980) found cod liver oil to be better than corn and hydrogenated coconut oil for adult American oysters. Although the relatively high essential fatty acid content of the cod liver oil is likely to have enhanced larval growth rates, this oil may also be an important energy source for oyster larvae.

The use of gelatin–acacia microcapsules containing cod liver oil in combination with a low input of algae (0.05, 0.1 and 0.2 mg/l) significantly ($P<0.05$) increased the percentage of "eyed" larvae (i.e., larvae that are competent to metamorphose and settle) compared to the low concentration algal diet without supplementation (Table 7); however, the growth rates of even the larvae fed algae at a high rate (Table 7), would not have been acceptable in commercial hatcheries where a length increase of 240 μm (total length 310 μm) would be expected by day 20 for Sydney rock oyster larvae. The slower growth rates in this study may have been caused by the small size of the aquaria (8 l) and/or by a less than optimum food concentration. Commercial hatcheries try to maintain the optimum concentration by feeding several times a day, whereas in this study, for logistical reasons, larvae were only fed once a day. Higher growth rates may have been produced by the microcapsule-supplemented diets C and D, if higher algal feeding rates had been used or if the larvae had been fed more frequently.

This study shows that microcapsules may be used successfully as a partial

substitute for algae in oyster hatcheries. This would appear to be preferable to using them as an alternative diet (Chu et al., 1987). Algal meal fed at 0.123 mg/l could be an alternative dietary supplement to microcapsules fed at 0.05 mg/l. The above level of algal meal would have supplied rather insignificant levels of protein and total lipids (12.7 and 6.0 μg/l, respectively). It appears unlikely, therefore, that the growth promotion of the algal meal was due to its macronutrient content, but more likely due to some unidentified trace nutrient. This could not have been the C20:5ω3 and C22:6ω3 essential fatty acids (Enright et al., 1986a,b), because the algal meal did not contain any of these fatty acids (Table 5).

Recently gelatin–acacia microcapsules were used successfully in our laboratory to supplement the algae *Dunaliella tertiolecta* for the rearing of Sydney rock oyster larvae in two (2000 l) tanks (J.E. Holliday, personal communication, 1988). This was done out of necessity as the laboratory was experiencing some algal production problems. *D. tertiolecta* is a good algal supplement but a poor complete diet (J.A. Nell, unpublished data, 1988) for Sydney rock oyster larvae. This finding was supported by Walne (1970), who reported that *D. tertiolecta* fed alone is not a good diet for bivalve larvae. Growth rates of Pacific oyster spat, however, were greatly improved when *D. tertiolecta* was supplemented with encapsulated 22:6ω3 fatty acid (Langdon and Waldock, 1981). Although microcapsules appeared to be unsuitable as an alternative diet for Sydney rock oyster larvae, they proved to be a very useful partial substitute or supplement for live algal diets.

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

R E S E A R C H

A comparison of commercial diets for Leader prawns (*Penaeus monodon*)

Feed bills can constitute up to 60% of operating costs for Australian prawn farms de-

Feed bills can constitute up to 60% of operating costs for Australian prawn farms depending on feed rates and feed price. Naturally enough then, farmers wish to make sure they get maximum benefit from their feed dollar. However, faced with a choice of imported and Australian feeds, it is difficult to evaluate their comparative performance, as production results from similarly managed ponds tend to vary greatly. In 1988, four researchers * compared a range of *P. monodon* feeds under pond conditions and found that the Australian diets were consistently less effective than equivalent Taiwanese diets. The present trial, which had to be conducted in aquaria, was undertaken to see if this situation had improved.

During June, 1990 four pelleted grower diets for leader prawns were compared in 40 litre aerated aquaria (five tanks per diet, each containing eight juvenile prawns, average weight 6.30-6.95 g per prawn). Three of the diets were imported feeds (a Thai diet CP, Taiwanese Fong Leng and Taiwanese President) and were obtained from Australian farmers. These diets were, according to bag labels, produced around the same time (February-March, 1990). The other diet was Australian (Aquafeed) and was dispatched by the feed company in late May. The prawns were fed twice daily to slight excess and water quality in the recirculating systems used was adequate (temperature 26.7°C, salinity 30.4 ppt, pH 7.6-7.9 and negligible ammonia and nitrite).

The growth, survival and physical stability data are summarised in the table. Statistical analyses indicated some clear differences between feeds, with CP and Fong Leng supporting considerably larger weight gains than President and Aquafeed. However, there were no significant differences between CP and Fong Leng or President and Aquafeed. Prawns fed with CP did survive better than those fed the other diets. All diets were very water stable in the absence of prawns but the use of a recirculating system, in which water is reused after passing through a physical filter and a biofilter, indicated how wasteful prawns are with artificial feeds.

The growth rates obtained were quite reasonable for aquarium trials especially as an electrical problem caused depressed water temperatures for the last week of this four week trial. However, it should be noted that aquarium trials pose a severe test for a diet as the prawns do not have access to natural feed, as in ponds, which could help make up for any nutritional inadequacies. The differences in survival rates may not have been repeated under pond conditions . The density used in the trial (about 50 prawns per square metre) was also very high. The salinity was higher than is used in most commercial ponds but other aquarium trials showed that salinity (15-30%) did not affect growth rates of juvenile *P. monodon*.

Given the above cautionary comments, what can be concluded from the trial?

It is noteworthy that the Australian feed (Aquafeed), which was designed to be as good as President feed, satisfied this goal. This represents a great improvement over the earlier trials. However, President, a widely used prawn feed, did not prove to be as good as Fong Leng, again confirming an earlier conclusion that Taiwanese diets differ in performance depending on brand. CP and Fong Leng both rated highly.

I would urge Australian farmers to continue to make purchases of Australian feeds. This trial involved only one local diet. Also, it would not be in the interest of industry to rely totally on imported feeds. Australian prawn farmers need to foster the development of a local feed industry which is responsive to their needs. Furthermore, the results apply only to the batches tested and further improvement in local diets can be expected in the future. Nevertheless, based on this trial there is still a place for imported feeds in the Australian market even though they may be more expensive and not 'as fresh' as locally manufactured feeds. What are needed now are more observations from pond trials.

Performance of juvenile *Penaeus monodon* on a range of commercial feeds.*

Brand	Feed	Origin	Price (A$ tonne) ex - Brisb.	Av. Weight Gain (g/prawn)	Survival (%)	Feed Stability (% loss)***
CP	4005 ** Grower 2	Thailand	1485-1575	2.80 a	100	13.0a
Fong Leng	Grower	Taiwan	1450	2.81a	90	11.3a
President Enterprises	Grower	Taiwan	1380 -1460	2.03b	83	8.4b
Aquafeed	Grower	Australia	1350	1.85b	85	13.4a

* Average values, within a column, which do not share a common letter (a,b) are significantly different.

** This is a high performance grower for 6-20g prawns stocked at > 20 prawns per square metre. Price varies with quantity.

*** % loss of dry matter for pellets held on a gently aerated sieve for 5 hours in the absence of prawns.

This magazine article has been prepared prior to the completion of chemical analyses and further data analyses because of the interest the trial created among farmers and feed suppliers. A more comprehensive scientific report should be published later.

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* Maguire, G.B., Allan, G.L., Baigent, R. and Frances, J., 1988. Evaluation of the suitability of some Australian and Taiwanese diets fed to leader prawns (Penaeus monodon) in ponds.

6.3 Maguire, G. B. Issues associated with the evaluation of commercial prawn farming diets.

Issues associated with the evaluation of commercial prawn farming diets

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ABSTRACT

Maguire, G. B., 1991. Issues associated with the evaluation of commercial prawn farming diets. In: G. L. Allan and W. Dall (Editors), Proc. Aquaculture Nutrition Workshop, (ANW), Salamander Bay, 15-17 April 1991, ANW, Salamander Bay, Australia, pp. 00.

Comparisons of artificial feeds, conducted with appropriate replication and statistical analyses, provide useful information for pond managers attempting to optimise their supplementary feeding strategies in ponds. The experimental systems which can be used to evaluate prawn feeds are discussed and it is suggested that aquaria and pens within ponds are useful systems for developing new feeds and evaluating existing feeds respectively.

INTRODUCTION

Pond managers usually have to choose from a range of commercially-available, artificial diets, with the aim of maximising the cost effectiveness of their

supplementary feeding strategies. Given the large scale of commercial ponds and the inherent variability in results from individual ponds (Wee, 1989), they are rarely in a position to evaluate conclusively alternate brands of feed. The need for comparative feed trials of commercial diets has been recognised in agricultural industries (Batterham et al., 1988).

The evaluation of feeds is complicated by the fact that ingredient profiles are usually proprietary information and that diet performance as assessed by feed trials is only one of the factors that influence pond managers along with costs, reliability of supply, credit arrangements, extension services provided by feed companies, information from other users, proposed stocking densities and natural food levels in ponds. An adverse result for a particular brand in a feed trial may evoke a very strong reaction from the supplier of that feed (Maguire, 1990b). However, given the commercial sensitivity of the work, researchers must give due consideration to the impact on their scientific reputation, particularly if the work cannot be conducted in a way that limits avenues for criticism.

The aim of this paper is to consider the factors which may enhance or detract from the goal of useful and objective research and is based on comparisons of commercial diets for *Penaeus monodon* conducted by the author during recent years (Maguire et al., 1988; Maguire and Allan, 1989; Maguire, 1990a; Maguire, unpublished data, 1991).

Experimental systems

A variety of systems can be used for comparisons of pond management and feeding strategies (Maguire and Allan, 1985). Four major systems have been used for comparing prawn diets. These are aquaria (New, 1976; Maguire, 1990a), netting enclosures within ponds (Maguire et al., 1988; A. Lawrence, personal communication, 1987), model ponds (Maguire and Allan, 1989; Freeman and Duerr, 1991) and larger ponds e.g., 0.1 - 0.2 ha (Elam and Green, 1974).

The 'ideal' system allows for cost-effective replication, elimination of confounding variables other than diet type, and applicability of results to commercial ponds. In general, farmers would prefer results obtained from replicated larger ponds but these facilities are not readily available, produce variable results and are very labour intensive. Model ponds provide results which seem applicable to larger ponds but are again very labour intensive (Allan and Maguire, 1988). Netting enclosures (1-3 m^2 pens) allow for the establishment of pond fauna and are only labour intensive for establishment and harvesting but not for operational aspects other than physical maintenance of the netting pens. All of these systems will yield results which are sensitive to pond characteristics especially pond productivity.

Aquaria also allow generous replication and are usually temperature controlled. However, they are not usually considered to contain significant natural food levels and may be prone to poor water quality. Growth rates may be lower in static aquaria receiving periodic water exchange, than in flow through systems (Allan and Maguire, in press). The use of recirculating systems for aquarium trials should

minimise variation among replicates and, in the author's most recent feed trial with commercial diets at the National Key Centre for Teaching and Research in Aquaculture, nested analysis of variance, based on results with individually tagged prawns, indicated that differences among replicates were not a significant source of variation ($P>0.05$). The relative absence of environmental fluctuations and natural food items in aquarium systems does make application of the results to commercial ponds more tenuous and performance indices such as food conversion ratio should be interpreted with caution (Maguire, 1990b). However, they do overcome the concern that the results are only applicable to the specific ponds used for pen trials. Indeed it has been argued that research workers should use aquaria and let pond managers apply the data to specific ponds, based on prior experience with their production systems.

On balance, aquarium systems are probably most appropriate for development trials with commercial rations, while replicated trials conducted under pond conditions are preferable for comparisons of different brands of commercial feeds, particularly if the data are to be used directly by farmers. It is quite possible that, with appropriate assistance on data analysis, pond managers could use simple experimental systems such as pens to validate cost effectively the results of aquarium trials for their own ponds.

Sourcing of diets and experimental animals

Commercial feed trials create a dilemma in that to obtain feeds of the same manufacturing date and to minimise storage time before the research worker

receives the feed, the diets should be obtained directly from the feed manufacturers. The Australian prawn farming industry still relies heavily on imported feeds and hence direct liaison with feed manufacturers overseas would be necessary. As the results of comparative feed trials can greatly influence feed purchases, the risk of obtaining an unrepresentative feed sample from a company should be considered seriously. A trial based on feed samples of the same age, held under the same ideal storage conditions, will address the question of which product at point of manufacture is the best under the conditions provided for the experiment i.e., under pond aquarium conditions. This is not necessarily the question most farmers would be interested in as they usually wish to know which diet will perform most cost effectively, not at the point of manufacture, but as commercially available within Australia and after being stored under farm conditions. Trials conducted by the author (Maguire et al., 1988; Maguire, 1990a) reflect the latter approach, although where possible the imported diets have been of similar age (Maguire, 1990b). However, a major limitation is that if diets are not obtained from manufacturers but from individual farmers, there can be differences in storage conditions prior to the research worker receiving the diets and this could confound the feed trial. Of these two somewhat imperfect approaches, the latter seems to be the more appropriate, especially if the data are to be made available to farmers.

Ideally the animals used in a feed trial should be obtained from the wild or from aquacultural facilities where the stock results from several spawners so that a small gene pool is avoided. As far as possible the stock should be disease free as this factor may have depressed growth rates in feed trials (Freeman and Duerr, 1991).

Frequency of trials

One legitimate concern is that a single feed trial provides results which are applicable to the particular batch tested and may not reflect the average performance of sequential batches of feed for a given brand (Evans, 1990). As feed trials require substantial resources it is generally not possible to conduct numerous trials before reaching a conclusion, unless the work is externally funded e.g., by feed companies. It is unlikely that a competitive research grant could be obtained for commercial diet testing. Another factor is the need for rapid feedback to farmers so that the information relates to current formulations. The onus is on feed companies to regularly conduct or contract out feed trials so that, as part of a general quality control program, diet testing is conducted prior to the commercial release of feed resulting from a major change in formulation strategy.

Statistical techniques

An experiment may lead to a conclusion that there is no significant difference between two diets ($P>0.05$). The choice of the 0.05 critical level as opposed to a 0.10 value will influence the likelihood of 'finding' significant differences. Similarly, if analysis of variance (ANOVA) indicates a significant effect due to diet type for a group of diets, the subsequent choice of multiple comparison technique for comparing average results for pairs of diets will be influential. A conservative technique such as 'Tukey's Honestly Significant Difference Test' will often result in fewer significant differences for the same data set than less conservative techniques such as 'Fisher's Least Significant Difference Test' (Klockars and Sax,

1986). A useful approach is to conduct a power analysis (Cohen, 1988) so that a 'no significant difference' result can be further examined to give an estimate of the power of the experiment i.e., 1-β, where β is the probability of falsely accepting an incorrect null hypnoses (Type II error) i.e., reaching a conclusion of no significant difference when a genuine difference existed. Ideally, there should be sufficient replication to give a power value of 0.8 for a specified minimum effect of diet type. Power analyses have been used in Australian aquacultural nutrition research (Wee et al., in press). A minimum size of effect is important because industry may not be interested in minor growth differences even though these can be detected with very generous replication. In general, emphasis should be placed on the assumptions inherent in the statistical techniques used and on evaluation of the appropriate level of replication (Underwood, 1981, 1990). One of the assumptions for ANOVA is homogeneity of variance but variation among replicates may depend on the quality of the diet used for those replicates (Freeman and Duerr, 1991).

Analysis of covariance may also be a useful approach, particularly for pond trials, as it allows the influence of other variables that differ between replicates to be examined e.g., natural food levels or survival rates (Maguire et al., 1988).

Most of the feed trials discussed or referred to in this paper have involved a group of diets compared under the same conditions, with the effect of diet type being analysed by one-way ANOVA. It should be emphasised that this is a much more robust approach than a series of individual t-tests as a much better estimate of random variation is obtained using ANOVA and the probability of a Type I error is reduced (falsely rejecting a true null hypothesis i.e., concluding that there is a

significant difference when there is not). However, instead of relying on one-way ANOVA, factorial experiments could be conducted so that diet type is examined in combination with other relevant variables, e.g., temperature, salinity (Shiau et al., 1991) or perhaps more importantly, feed rate. Factorial experiments have inherent statistical power and also allow examination of interactions between the two or more factors being studied. In the most recent feed trial conducted by the author, there was a significant interaction ($P<0.05$) between diet type and feeding strategy (i.e., daily feed input in contrast to only feeding on three days out of every four days; see Courtney, 1989). Relative growth responses to different fish diets may be influenced by feeding strategy (*ad libitum* in contrast to restricted daily inputs of feed; Lovell, in press). In feed trials by the author, *ad libitum* feed strategies have generally been used as these probably reflect the most common commercial practice.

Histological analyses of prawns may also be a useful initiative (Vogt et al., 1985) as, in the above prawn experiment, both feed type and feeding strategy influenced hepatopanceas structure. Feed trials, particularly in aquaria, are often restricted to relatively short periods (3-6 weeks) and histological changes could be indicators of longer term trends.

Impact of feed trials

The purpose of commercial feed trials is to provide relative performance data to help pond managers improve the cost effectiveness of their supplementary feeding practices and to provide information for feed companies and their agents so that

diets can be redesigned where necessary. Feed trials conducted by the author have influenced farm management decisions at Australian prawn farms. There are, however, some genuine concerns about the impact of such trials. Adverse results may inhibit the development of the much needed Australian aquacultural feeds industry (Evans, 1990) and reduce the number of brands marketed in Australia, thereby reducing future options for pond managers. From the point of view of the research worker the value of this type of research, in relation to other possible uses of research resources, must be weighed against potential damage to scientific reputation given that it is probably impossible to eliminate potential confounding factors such as feed storage differences or unrepresentative samples of commercial feeds.

Given the difficulties of funding this type of work and the unwelcome controversy it generates, it is likely that the publications may only be available for data based on developmental work for diets. Increasingly, the trend is for evaluation of commercially-available, agricultural diets to be done on a contract basis with companies having the right to suppress unfavourable information. Some companies will publicise the results of trials which indicate that their diets perform well (Subramanyam, 1991). However, pond managers should consider doing their own trials either individually of collectively.

The results of feed trials may also be of value in a research sense as nutrient analysis data may correlate with growth data and suggest future directions for more detailed research with experimental diets (Ako and Dominy, 1987; Maguire et al., 1988). However, unless a large number of diets are compared, the multiplicity

of nutritional variables inherent in the design of a diet may make the link between growth results and diet composition difficult to establish. As the ingredient profiles are usually proprietary information, it is even more difficult for the research to interpret growth and other performance data in relation to specific nutrients or ingredients.

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