

Thesis
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**The nursery culture and nutrition of
post-larval black tiger shrimp
Penaeus monodon Fabricius.**

by

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DEDICATION

In loving memory of Paul Solomon

DECLARATION

I hereby declare that this thesis has been composed by myself and is the result of my own investigations. It has neither been accepted nor submitted for any other degrees. All the sources of information have been duly acknowledged.

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ABSTRACT

An evaluation was made of the role of secondary nursery rearing within the intensive shrimp culture industry. A series of concrete nursery tank trials were conducted rearing post-larval *Penaeus monodon* Fabricius for 35 days from PL₁₅ to PL₅₀. Shrimp production was directly related to stocking density at up to 2,000 shrimp m⁻², but growth, survival, FCE and size range were negatively density dependant. Water exchange and aeration were necessary to maintain shrimp production at high density. The use of mesh habitats and sand substrates and the effects of dietary formulation and feeding regime also influenced shrimp production. The production of shrimp nursed in net cages was comparable to that of shrimp nursed in concrete tanks at densities of 500 shrimp m⁻². An economic analysis of secondary nursing in tanks suggested that it may be profitable if the value of the juvenile shrimp produced is > 2.5 times that of post-larvae. A cage on-growing trial showed that the stunting of shrimp during high density secondary nursing was not permanent, but was rapidly compensated for by increased growth rate during on-growing at lower density.

A stress test was developed which enabled quantification of the stress tolerance (vigour) of post-larval shrimp. This entailed direct transfer of post-larvae into water at reduced or increased temperature and salinity for one hour. The tests facilitated discrimination between batches of post-larvae produced from a single hatchery. The stress resistance of juvenile shrimp following secondary nursing was significantly better than that of ex-hatchery post-larvae, suggesting that older or secondary-nursed juveniles are more likely to survive transfer and subsequent on-growing than are primary-nursed post-larvae. Low vigour shrimp are suggested to have poor growth potential, although further research into the effects of shrimp vigour on shrimp production during pond on-growing is required.

A series of nutritional trials were conducted in laboratory and outdoor nursery tanks to study the optimal levels of, and relationship between, protein, lipid, carbohydrate and energy in diets for post-larval *P. monodon*. Lipids including cod liver oil:soybean oil (3:1), soy lecithin (3-6 %) and cholesterol (0.5 %) were necessary to optimise shrimp production. Dietary lipid levels of 8-12 % in nutrient balanced diets maximised shrimp survival and production. Carbohydrate (starch) levels of 21-38 % were optimal in

nutrient balanced diets at carbohydrate:lipid ratios of 2-5:1. Both carbohydrate and lipid were shown to be able to spare protein for growth. Protein levels were decreased from 44 % to 38 % in nutrient balanced diets without compromising shrimp production. Further reductions in protein levels may be possible in nutrient balanced diets. The level of protein supporting optimal shrimp production was shown to be directly related to the total energy and protein:energy ratio of the diet. Models are proposed for estimating the level and proportions of protein, lipid and carbohydrate which are best able to supply the nutrient and energy requirements of post-larval *P. monodon*. The benefits of reducing the protein level of shrimp diets are discussed in terms of minimising diet cost and feed wastage.

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- 9.3 Relationship between protein, lipid and carbohydrate in diets promoting optimum growth of post-larval *P. monodon*.
- 9.4 Relationship between energy provision from protein, lipid and carbohydrate in diets promoting optimum growth of post-larval *P. monodon*.

CHAPTER 1. Background

1.1 RATIONALE AND PROBLEMS

Shrimp culture is currently a large industry, particularly in tropical 'developing' nations of the world, and has great potential for generation of both income and employment for large numbers of people. The recent trend for intensification of a long-standing extensive industry however, has brought with it many problems which are currently threatening the sustainable socio-economic and environmental basis of the industry.

Of the species of marine penaeid shrimp currently cultured intensively worldwide, none is more important than the giant or black tiger shrimp *Penaeus monodon* Fabricius. The post-larval/juvenile stage of culture of this species was studied as a model in this thesis in order to address some of the problems currently encountered with two interlinking areas of intensive shrimp culture: *i.e.* those of the 'secondary' nursery rearing and nutrition of post-larval/juvenile tiger shrimp.

1.1.1 Nursery culture

The nursery phase of shrimp culture is an integral part of the culture cycle of shrimp under current farming practices. Nursery culture is that part of the intensive culture cycle that takes wild-caught, or more usually, hatchery-reared post-larval shrimp and rears them for a short time at high density in a nursery prior to on-growing. The shrimp are commonly stocked into the nursery at 5-15 days post-metamorphosis to post-larvae (PL₅₋₁₅) and nursed for 1-6 weeks before release into the production ponds for on-growing (Fegan, 1992; Hopkins & Villalon, 1992; Samochoa & Lawrence, 1992; Stern & Letellier, 1992; Sturmer *et al.*, 1992).

The current trend in intensive shrimp culture is to stock shrimp into the production ponds when they reach PL₁₂₋₂₀ (Lee & Wickins, 1992; Olin & Fast, 1992). The 'primary' nursing phase between the hatchery rearing of larval and the pond on-growing of juvenile shrimp is necessary for a number of reasons. These include: the poor

tolerance of un-nursed shrimp to the stressful transfer from the hatchery to the on-growing ponds; the need for a period of time over which the young post-larvae can be weaned from the usually live hatchery diets to formulated, pelleted diets; and the susceptibility of young post-larvae to environmental extremes and predation in the production ponds (Charmantier *et al.*, 1988; Tseng, 1988; Lee & Wickins, 1992; Olin & Fast, 1992; Chanratchakool *et al.*, 1994).

In addition, the 'secondary' nursing (beyond PL₁₂₋₁₅) of shrimp may be a desirable stage in the shrimp culture industry for a number of reasons. These include: perhaps most importantly, an improvement in stock quality through the stocking of larger, older, more stress tolerant post-larvae/juveniles (for *P. monodon* at least); a reduction in the grow-out period by stocking larger seed, resulting in increased pond production; more accurate estimations of mortality early in the culture cycle; increasing the efficiency of resource utilisation through the maintenance of high stocking densities through each phase of the culture cycle; increasing the diversification and efficiency of the industry and creating opportunities for businesses conducting nursery culture only (Motoh, 1985; Lee & Wickins, 1992; Primavera, 1992; Samocha & Lawrence, 1992; Sturmer *et al.*, 1992; Chanratchakool *et al.*, 1994).

The use of secondary nursery systems however, does have a number of potential and recognised disadvantages. These include: the higher cost to on-growers for older post-larvae; the larger size-range of post-larvae following secondary nursing; the difficulties encountered in transporting older post-larvae; and the slower growth rate of post-larvae in nurseries than in on-growing ponds (Clifford, 1992; Lee & Wickins, 1992; Stern & Letellier, 1992; Sturmer *et al.*, 1992; Chanratchakool *et al.*, 1994).

Both the hatchery and on-growing phases of shrimp culture have been investigated thoroughly since the advent of commercial-scale intensive shrimp culture systems in the early 1980s. The nursery phase however, despite the growing recognition of its importance, has until recently received scant attention. The positive and negative aspects of secondary nursery rearing have not been fully investigated. Secondary nursery rearing is in need of further research in order to ascertain its role in intensive shrimp culture systems (Samocha & Lawrence, 1992; Sturmer *et al.*, 1992).

1.1.2 Nutrition

Under intensive pond culture conditions, shrimp growth relies to a large extent on applied formulated, pelleted feeds. The management of feeds and feeding is thus a major concern in successful intensive shrimp culture.

The most important aspect of feed management is the cost of the feeds which accounts for 55-60 % of the operational costs in intensive systems and 40 % in semi-intensive systems. With the increasingly competitive global market for marine shrimp (Csavas, 1990, 1993), in order to operate a shrimp farm profitably, it is therefore important to minimise feed costs as much as possible (Chanratchakool *et al.*, 1994). In order to achieve this, knowledge of the nutritional requirements of the species being cultured at each stage of its life cycle is of primary importance. Research into the nutritional requirements of shrimp has lagged behind that of many finfish species due to the relatively recent adoption of intensive shrimp farming techniques (Cuzon, 1993). Shrimp nutrition research is thus in its infancy compared to that of humans and domestic animals. Nevertheless, research conducted into shrimp nutrition over the past 20 years has resulted in diets able to support profitable shrimp production. Further improvements in the profitability of shrimp farming are likely to result from research in the areas of husbandry and feed technology, but largely in that of nutrition (Akiyama, 1992; Akiyama *et al.*, 1992; Hopkins & Villalon, 1992; Cuzon, 1993).

The aim of shrimp culture is to produce shrimp sustainably with the largest margin of profit (Chanratchakool *et al.*, 1994). Currently, the components of the diet supplying protein account for the largest percentage of the cost of the diet. Thus, cost-efficiency improvements in shrimp nutrition will probably result from more accurate identification of dietary protein requirements and their relationships with dietary non-protein energy sources and levels of total dietary energy (Cowey & Sargent, 1979; Jobling, 1983; Cuzon, 1993). These areas of the protein and energy requirements of shrimp, particularly with regard to the differences between purified and practical formulations, tank and pond trials, results from different laboratories, the various life stages of the shrimp and the species of shrimp studied, are all in need of further study.

Beyond the cost of formulated diets, nutrition plays a central role in profitable shrimp farming due to its intimate relationships with shrimp health and disease prevention, and many aspects of the culture system and environment in general. Rationalization of the use of dietary proteins in particular, is important for reasons including: the role of waste feed (especially nitrogen from protein) in the deterioration of the pond environment (Kaushik & Cowey, 1991; Lin *et al.*, 1991; Briggs & Funge-Smith, in press); the current wasteful over-reliance on fish meal in shrimp diets (Tacon & Jackson, 1985; New & Wijkstrom, 1990; Cuzon, 1993); and the wastage of byproducts from shrimp culture and elsewhere which could be used effectively in shrimp diets (Briggs and Funge-Smith, in press).

1.2 OBJECTIVES OF THE PRESENT WORK

In view of the lack of knowledge regarding the benefits or disadvantages of 'secondary' nursing of penaeid shrimp and the importance of nutrition, particularly in terms of protein:energy requirements in the future profitability of intensive shrimp culture, the objectives of this thesis are:

- Firstly, to investigate and evaluate the role of 'secondary' nursing of penaeid shrimp within the intensive shrimp culture industry.
- Secondly, to investigate the potential of stress testing (determining shrimp vigour by subjecting them to environmental shocks) shrimp seed prior to stocking on-growing ponds and to determine whether secondary nursing of post-larvae can increase shrimp vigour.
- Thirdly, to study the nutritional requirements of post-larval/juvenile tiger shrimp, particularly with regard to optimising protein:energy ratios, enhancing the contribution of non-protein energy sources, reducing dietary cost and waste production.

CHAPTER 2. Introduction

What does a fish know about the water in which he swims all his life ?

Albert Einstein, 1935

2.1 INTRODUCTION .

This review outlines the biology and culture of penaeid shrimp, with particular reference to the black or giant tiger shrimp *Penaeus monodon* Fabricius. Emphasis is placed on the nursery and nutrition of the tiger shrimp, identifying areas where research work is required in order to assist the development of the current intensive culture industry for this species.

2.2 SOME ASPECTS OF THE BIOLOGY OF PENAEID SHRIMP OF RELEVANCE TO THEIR CULTURE.

2.2.1 Introduction

An understanding of the biology of a species being cultured is essential in order to adapt the culture system to suit that species and to anticipate any problems that may arise due to its behaviour. Some aspects of the natural history of *P. monodon* of relevance to their culture will be discussed under the following headings: taxonomy, distribution, habitat, life history and moulting and growth.

2.2.2 Taxonomy

The full classification for *Penaeus monodon* is given below:

Phylum: Arthropoda

Class: Crustacea

Order: Decapoda

Suborder: Natantia

Infraorder (section): Penaeidea

Superfamily: Penaeoidea

Family: Penaeidae

Genus: *Penaeus* (*Penaeus*)

Species: *monodon*

Authority: Fabricius, 1798.

Source: Holthuis (1980)

In this thesis, *Penaeus monodon* has been referred to as a "shrimp", rather than as a "prawn", since it is a marine species, in line with the latest view of the FAO (Csavas, 1988).

2.2.3 Distribution

Penaeus monodon is a widespread tropical and sub-tropical shrimp, from about 40 °N to 40 °S latitude. It is particularly common in south east Asia from eastern India, through Malaysia and Thailand to the Philippines, Indonesia, Taiwan and northern and eastern Australia, where it forms an important commercial fishery. It also occurs in the eastern Indian Ocean to east and south-east Africa and east to Japan and Korea where it is of less commercial importance (Holthuis, 1980; Tseng, 1988).

2.2.4 Habitat

Most shrimp species live for one or occasionally two years after reaching maturity at 6-12 months old. Penaeid shrimp such as *P. monodon* live in shallow water at depths of 0 to 162 m in the sea for adults and in brackish-water estuarine and wetland *i.e.* mangrove areas for juveniles. They usually bury themselves beneath the bottom mud or sand during the day and emerge to feed at night (Holthuis, 1980; Tseng, 1988; Bailey-Brock & Moss, 1992).

2.2.5 Life history

Like all closed thelycum penaeid prawns, *P. monodon* are dioecious and mating generally occurs in deep water offshore when the female has just moulted and has a soft shell. This allows the male, generally during the night, to deposit one or more spermatophores (containing many sperm) within or close to the genital opening of the female. *P. monodon* is a closed-thelycum (or pouch) species meaning that the spermatophores are kept in the thelycum for days or even weeks during the inter-moult period and may go on to fertilize more than one batch of eggs (Lin & Ting, 1986; Bailey-Brock & Moss, 1992).

Spawning is the release of the eggs directly into the sea in the case of penaeid shrimp where they are simultaneously fertilized by the sperm stored in the thelycum. The eggs hatch into the first of 6 free-swimming, non-feeding nauplius larvae after 12-24 hours. After 2-3 days they then metamorphose into the first of 3 phytoplankton-filtering protozoa larvae. After 3-4 days the shrimp metamorphose into the first of 3 zooplankton-feeding mysis stages. The mysis larvae metamorphose after 3-5 days into post-larvae (megalopa), which then settle to the bottom and begin to move inshore to rich intertidal feeding grounds (Motoh, 1981, 1985; Tseng, 1988; Bailey-Brock & Moss, 1992; Bray & Lawrence, 1992). At this stage, the seedstock may be regarded as being juvenile rather than post-larval shrimp. This distinction however, has yet to be agreed upon. Motoh (1981, 1985) suggested that the juvenile stage begins with the completion of the gill system (at approximately PL₁₃), when the shrimp have a carapace length of 2.2 to 11.0 mm, they have the full complement of rostral spines and the length of the abdominal segments is only 65 % of the carapace length. At this stage in the wild the shrimp show benthic behaviour, are present in brackishwater areas and are approximately one to two months of age. However, the gut enzyme system may not be completed until PL_{35,45}, by which stage they may be termed adolescent, where the body proportions have stabilised to the abdomen being 58 % of the carapace length. The adolescent stage may also be characterised by the development of the outer genitalia, and a carapace length of 11-34 cm. After the 4 month adolescent stage, the shrimp may be termed subadults at the onset of sexual maturity when they begin their migration from the nursery to the spawning grounds offshore. At this stage they may have a

carapace length of 37-47 mm and an age of approximately 8 months. The shrimp become adult at about 10 months on completion of sexual maturity. At this stage the shrimp are located at depths of up to 160 m and are ready for spawning (Figure 2.1) (Motoh, 1981, 1985).

2.2.6 Moulting and growth

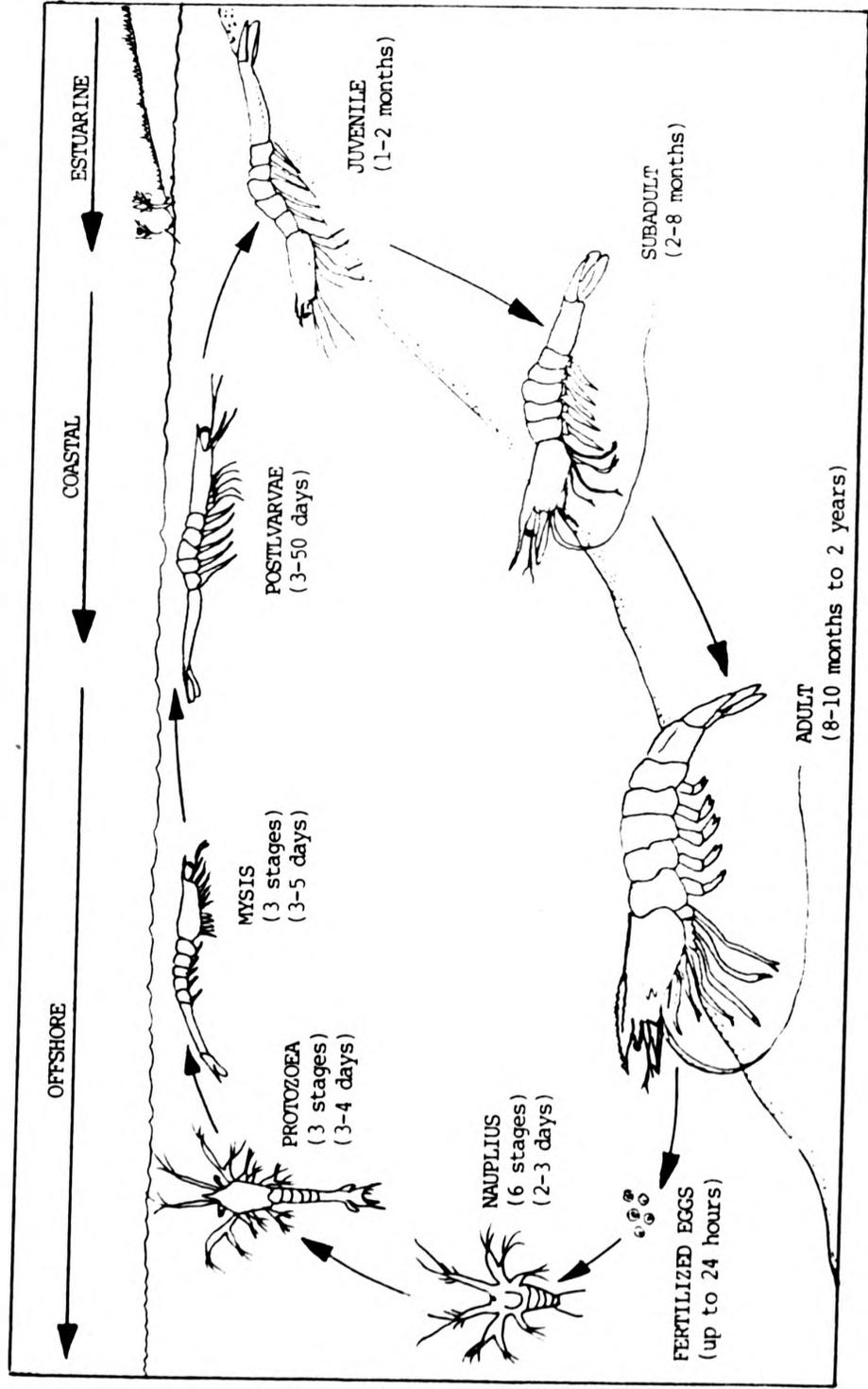
Penaeid shrimp, like all crustacea have an external shell or exoskeleton which is capable only of limited expansion. In order to grow, they must shed the old exoskeleton (ecdysis), take up large quantities of water to swell to the largest size possible, before the newly laid down exoskeleton hardens. This water is then gradually replaced by somatic growth before the process must be repeated. Growth rate of shrimp therefore depends upon the frequency of moulting and the size or weight increase at each moult. Both of these functions may be affected by numerous factors including nutrition, environment, species, size and age. Moulting is generally more rapid during the early life cycle at 2-3 times daily for nauplii, decreasing to once every 3-4 weeks under optimal temperature during adulthood, equating to the pond on-growing phase of culture (Bray & Lawrence, 1992; Lee & Wickins, 1992).

Moulting is thus a critical time for shrimp and is an energy demanding process. Soft, newly moulted animals are more susceptible to predation (particularly cannibalism for *P. monodon* under intensive cultivation) and poor environmental quality. During culture, it is therefore important to maintain environmental quality and not to stress shrimp during moulting. It is also important that the shrimp are allowed to consume their cast exoskeletons so that the minerals contained within the exuvium, essential for formation of the new exoskeleton, are not lost (Bray & Lawrence, 1992; Lee & Wickins, 1992).

P. monodon is the largest of all penaeid shrimp, growing to a maximum total length of 336 mm and weight of 60-130 g (Holthuis, 1980). Under culture conditions, they are rarely grown to above 40 g in weight, taking a total of 5-6 months from the egg. Broodstock animals are usually wild-caught, between 100 and 200 g in weight and may spawn between 100,000 and one million eggs per spawning, depending upon size

Figure 2.1

The life cycle of *Penaeus monodon*, indicating habitats and length of various stages in the life cycle (modified after Motoh, 1981).



(Tseng, 1988; Bray & Lawrence, 1992). However, males of about 60 g and females of about 90 g have been recommended for spawning by Bray & Lawrence (1992). The reproductive biology of shrimp both in the wild and in captivity are not of direct relevance to this thesis but have recently been reviewed elsewhere (Bliss & Mantel, 1985; Laufer & Downer, 1988; Bray & Lawrence, 1992; Browdy, 1992; Chang, 1992)

2.3 THE SHRIMP CULTURE INDUSTRY

2.3.1 Historical development

The origins of shrimp culture may go back hundreds or even thousands of years to the first brackish-water and marine ponds (Schuster, 1952; Fast, 1992; Menasveta, 1992). These large ponds served as fertile areas for the growth of fish, shrimp and a mixture of other animals which entered them through their own behaviour patterns or on the tides. After some time, the ponds were then harvested and the edible animals retained. These extensive culture methods are still practised profitably today where land and labour costs are low and pond improvement costs have been depreciated. Although this culture system can be profitable, with low risk, yields are low and unpredictable (usually less than 200 kg ha⁻¹ year⁻¹) and the species composition is uncontrolled (Fast, 1992). For these reasons, the system has been improved by screening the pond inlets, manually stocking seed of the desired species (often both shrimp and fish at up to 20 animals m⁻² in polyculture) and often by employing some form of fertilization or supplementary feeding (often chicken feed) in order to increase yields (Bardach *et al.*, 1972; Fast, 1992). These semi-intensive techniques are now used in countries including India, Bangladesh, the Philippines, Indonesia and South/Central America. Problems are encountered however, with intermittent seed availability and the difficulty of distinguishing the seed of desirable species (Fast, 1992). In addition, relatively low yields are obtained per unit area with this form of culture (up to 1 t ha⁻¹ year⁻¹).

The current trend worldwide is now for intensification of the shrimp culture industry. This has come about for many reasons including the:

1. low yield of traditional extensive systems,
2. increasing population and wealth of developed nations leading to increased demand for shrimp,
3. worldwide shrimp capture fisheries stagnating at 2 million t year⁻¹ in 1985 due to overexploitation and increased fuel prices,
4. high land costs and increasing pressures on limited coastal resources,
5. development of commercial scale hatchery technology in the 1960s,
6. initial high profitability of intensive shrimp culture,
7. advent of refrigeration and improved transportation broadening marketing possibilities,
8. high potential of shrimp to earn foreign exchange for developing countries,

Intensive shrimp farming involves the construction of relatively small, deep, more easily-managed ponds (usually > 1 m deep and < 1 ha in size), stocking with large numbers (30-100 shrimp m⁻²) of either wild-caught, but more usually hatchery-reared seed and supplementary feeding with high-quality pelleted diets (Briggs & Funge-Smith, In press a). In addition, close management of water quality and aeration is necessary in order to maintain the environmental quality of the pond. Using these techniques, production has risen to 2-10 t ha⁻¹ year⁻¹, with yields of over 20 t ha⁻¹ year⁻¹ sometimes being recorded (Briggs & Funge-Smith, in press).

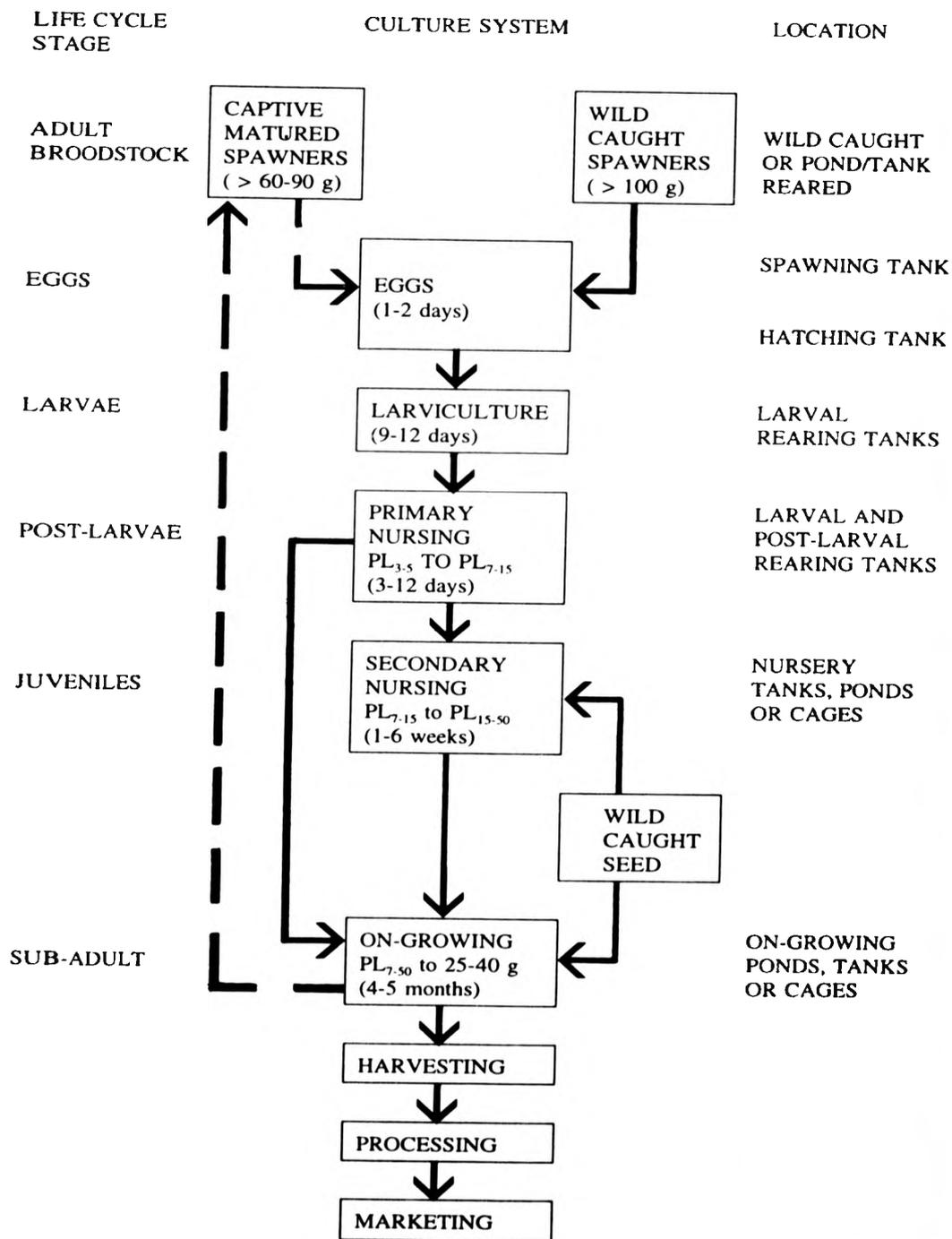
The intensive shrimp culture systems can be divided into a number of discrete areas which are summarised in figure 2.2.

In some cases, all of these operations are carried out in one location by a single farming operation. In many areas, such as Taiwan and Thailand, these operations have become specialised, comprising entire businesses in their own right.

2.3.2 Current status of the intensive shrimp culture industry

Intensive shrimp culture technology has only been developed over the past 15-20 years as a result of the above mentioned factors. It has now developed into a diverse and financially successful industry and is still expanding, with new countries rapidly

Figure 2.2 Schematic diagram of an intensive shrimp culture system, indicating life cycle stages, culture systems and holding facilities.



adopting these techniques. In 1984, the world production of cultured shrimp was approximately 160,000 t worth £ 670 million, accounting for 12 % of the total production of shrimp (Table 2.1, Figure 2.3). The respective figures for 1992 reached 815,212 t, worth £ 5,507 million, accounting for over 30 % of the total production of shrimp (Table 2.1, Figure 2.3) (Fast, 1992; Anon., 1992 a,b,c; Anon., 1993a,b; FAO, 1994). Of the current cultured shrimp production, *P. monodon* is the most important single species, accounting for approximately 50 % or 388,565 t in 1992 (Table 2.1, Figure 2.3)(FAO, 1994). The intensive culture of this species originated in Taiwan, but is now produced mostly in Thailand, Indonesia and the Philippines (Csavas, 1992; Menasveta, 1992; Anon., 1993a; FAO, 1994).

2.3.3 Current and future problems and research requirements

The intensive shrimp culture industry has recently shown signs of entering the mature phase of its development, characterised by increasing competition and falling prices and profit margins (Csavas, 1988, 1992; Fast, 1992). The expansion in intensive shrimp farming is thus likely to decelerate due to problems including:

1. intense competition resulting in market price fluctuation and decline
2. lack of expertise of operators
3. pollution
4. disease
5. poor infrastructure
6. shortages of suitable farming areas

In addition, a number of worrying environmental and socio-economic problems have recently arisen. These problems have resulted largely from the mis-management and overexploitation of natural resources by intensive shrimp farming, in competition with other coastal resource users. The problems are manifested through production crashes, declining productivity (Briggs and Funge-Smith in press; NACA, 1994), land dereliction (Chua, 1993, Phillips *et al.*, 1993), mangrove destruction (Phillips *et al.*, 1993), salination of agricultural land (Liao, 1990; Anon, 1992a; Primavera, 1992; Anon, 1993c), pollution of marine and freshwater resources (Briggs & Funge-Smith,

Table 2.1 The production of cultured shrimp from 1984 to 1993.

STATISTIC : DATE	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Total world shrimp production by culture in metric tonnes (t)	162,646	210,088	297,893	482,174	548,562	567,231	599,807	690,000	815,212	744,000*
World production of <i>P. monodon</i> (t)	47,672	55,726	89,384	147,856	154,564	197,896	223,040	300,000*	388,565	370,000*
Thailand production of <i>P. monodon</i> (t)	170	106	897	10,544	40,774	81,492	105,028	137,241	172,300	161,500*
Proportion of world <i>P. monodon</i> production from Thailand (%)	0.4	0.2	1.0	7.1	26.4	41.2	42.0	45.7	44.3	43.7*
Taiwanese production of <i>P. monodon</i> (t)	10,755	16,715	44,387	78,548	30,603	16,672	8,570	12,000	19,500	17,550*
Proportion of world <i>P. monodon</i> production from Taiwan (%)	22.6	30.0	49.7	53.1	19.8	8.4	3.4	4.0	5.8	4.7*

* = estimate (Anon., 1993a)

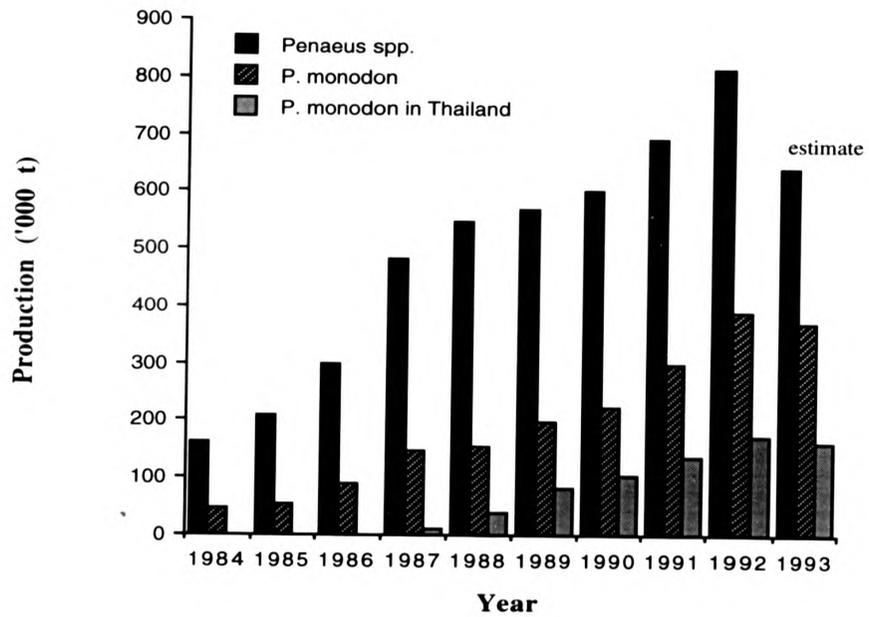


Figure 2.3 Production of cultured penaeid shrimp and *P. monodon* worldwide and in Thailand.

in press; Primavera, 1992; Masae & Rakkheaw, 1992), and the marginalisation of farmers, fishermen and other coastal inhabitants (Chong, 1990; Masae & Rakkheaw, 1992; Primavera, 1992; Anon, 1993d; Chua, 1993).

These problems were most dramatically revealed in the recent declines of the industries in Taiwan and central Thailand and increasing problems elsewhere including China, Indonesia, the Philippines, Sri Lanka and Ecuador (Chong, 1990; Primavera, 1992; Anon, 1993d; Chua, 1993). The shrimp culture industry of Taiwan has collapsed by 70-90 % on three occasions since 1987 (Table 2.1), causing huge losses to many areas of the industry from broodstock supply through the various stages of the culture cycle to marketing and transport of the final product (Lin, 1989; Chua, 1993). Similarly, Taiwanese intensive culture technology, extended to central Thailand, was capable of supporting production for only 3-4 years before the collapse of the industry there in 1990, causing an estimated loss of £ 18.3 million. These collapses have ultimately been attributed to viral disease losses due first to the monodon baculovirus and recently the yellowhead virus which was estimated to have caused £ 20.8 million worth of losses in Thailand alone in 1992 (T. Flegal, pers. comm., 1993). The underlying reasons however are multi-causal, but are primarily a result of mismanagement of the pond environment, particularly with regard to overstocking.

With the increasing competition for shrimp markets from the currently expanding global shrimp culture industry, the industry in the future will have to increase its efficiency by reducing production costs to maintain profitability. There are many aspects of the current intensive shrimp culture systems which are in need of research in order to improve the efficiency of the industry. The main fields requiring further research are detailed in Table 2.2 and will provide the researchers currently working on them and any future investigators with years of work. This thesis, however, concentrates on just two of the areas mentioned, *i.e.* those of secondary nursery culture, with particular attention on seed quality, and nutrition, with emphasis on protein-sparing and waste discharge. The general principles behind these two areas will be discussed in the following two sections, nursery culture in section 2.4, and nutrition in section 2.5.

Table 2.2 Research requirements for the intensive shrimp culture industry.

Area	Field	References
Maturation/ Hatchery	broodstock maturation and management, seed quality, genetic selection, nutrition, disease control, water treatment	Browdy (1992); Fast & Lester (1992); Fegan (1992); Kawahigashi (1992); Lee & Wickins (1992); Wilkenfeld (1992); Wyban <i>et al.</i> (1992)
Nursery	primary and secondary nursery systems, seed quality and size variation, economics, nutrition, harvesting and transfer	Fegan (1992); Hopkins & Villalon (1992); Samocha & Lawrence (1992); Stern & Letellier (1992); Sturmer <i>et al.</i> (1992)
On-growing	nutrition, stocking density, water quality control, polyculture, integration, grow-out technology, pond dynamics, system economics	Boyd (1992); Boyd & Fast (1992); Boyd & Musig (1992); Chien (1992); Clifford (1992); Fast (1992); Fast & Boyd (1992); Fast & Lannan (1992); Fast & Lester (1992); Hirono (1992); Lin (1989); Peterson & Daniels (1992); Pruder (1992)
Nutrition	feed cost, protein:energy level and FCR reduction, fish meal alternatives, optimal feeds for all species and life stages, waste reduction, contribution of natural productivity, difference between tank and pond trials	Akiyama (1992); Akiyama <i>et al.</i> (1992); Briggs & Funge-Smith (in press); Cuzon (1993); Fast & Lester (1992); Fox <i>et al.</i> (in press); Kanazawa (1981, 1985); Tacon (1990); Hopkins & Villalon (1992)
Environmental quality	waste reduction, integration, recirculation, static water ponds, pond liners, rehabilitation of ex-shrimp farming areas, mangrove destruction	Briggs & Funge-Smith (in press); Chua (1993); Fast & Lester (1992); Folke & Kautsky (1992); Lin (1989); Lin <i>et al.</i> (1991); Macintosh & Phillips (1992); NACA (1994); Phillips <i>et al.</i> (1993); Pullin (1993)
Disease	treatments, immunization, antibiotic use, diagnostic techniques, relationships with nutrition, culture techniques and environment	Bell (1992); Brock & LeaMaster (1992); Carpenter (1992); Fast & Lester (1992); Lee & Wickins (1992); Lightner (1992); Lightner & Redman (1992); Lin (1989)
Marketing	expanding domestic and overseas markets, value added products, quality control of product	Fast & Lester (1992); Larson (1992); Rackowe (1992); Shang (1992); Wayland (1992)
Regulation and policy	role of government, NGOs and coastal planners in coastal development, effluent discharge limits, chemical abuse, wetland protection, wild stock preservation, transfer of exotic species, education and training, sociological impacts	Barg (1992); Fast & Lester (1992); Lee & Wickins (1992); Lin (1989); Masae & Rakkheaw (1992); Primavera (1992)

2.4 NURSERY CULTURE

The nursery phase is an integral part of the intensive shrimp culture system that has developed over the past 15-20 years. Nursery culture may be subdivided into two stages which may run seamlessly together in one operation or may be distinct and separate operations under other circumstances. For the purposes of this thesis, and to clarify the role of nurseries within the shrimp culture industry generally, it has been considered appropriate to make a subdivision into 'primary' and 'secondary' nursing.

2.4.1 Primary nursing

2.4.1.1 Description of primary nursing

Primary nursing may be considered that part of the culture cycle that takes young post-larvae one to seven days post-metamorphosis (PL₁₋₇) and rears them for up to two weeks or so (until PL₁₂₋₂₀) before direct stocking into the on-growing (production) ponds.

2.4.1.2 Function of primary nursing

Primary nursing is an obligatory phase in the culture cycle which is invariably used in shrimp culture operations using hatchery-reared seed (*i.e.* usually intensive culture operations). The main functions of the primary nursery phase are summarised in Table 2.3, and are mainly concerned with stock improvement and feeding habits.

Table 2.3 The function of the primary nursery stage in the intensive shrimp culture system.

Area	Field	References
Stock improvement	Enhanced tolerance to transfer from hatchery to on-growing ponds; Protect stock from predation and competition; Improve post-larval pigmentation, moulting and growth; More care for stock over critical period in life cycle; Post-larvae still pelagic until PL _{5,7}	Charmantier <i>et al.</i> (1988); Tseng (1988); Lee & Wickins (1992); Olin & Fast (1992); Chanratchakool <i>et al.</i> (1994)
Feeding habits	Wean post-larvae off larval diets; Stimulate acceptance of pelleted diets	Tseng (1988); Lee & Wickins (1992)

Primary nursing thus allows a period of time over which the sensitive young post-larvae can be weaned from the highly controlled hatchery conditions to those of the on-growing ponds (Tseng, 1988; Lee & Wickins, 1992). Without such a phase, direct stocking of young post-larvae will result in heavy mortality (Apud *et al.*, 1979; de la Pena *et al.*, 1985). This is a result of many factors. Modern shrimp hatcheries facilitate the survival of large numbers of seed which in nature, would normally die (Primavera, 1992). This is often a result of enforced multiple spawnings of broodstock and the maintenance of the larvae in conditions of high sterility with optimal water quality and food availability. Direct transfer of early post-larvae to ponds may expose them to bacterial pathogens which they have previously not encountered. Also, their digestive physiology may not be developed well enough to adapt immediately to formulated rations (Fegan, 1992; Lee & Wickins, 1992). Young post-larvae are not as tolerant to environmental stress as older, larger shrimp (Fegan, 1992). In addition, they are still pelagic until PL_{5,7}, before changing to a benthic lifestyle. Thus behavioural and physiological changes tend to enhance their susceptibility to predation and competition from other organisms within on-growing ponds (Tseng, 1988; Lee & Wickins, 1992; Olin & Fast, 1992).

The primary nursing phase is thus vital and is usually carried out either in the larval rearing tank within the hatchery, or by transfer of the post-larvae at PL₁₋₇ to primary nursing tanks within, or outside of the hatchery building. Primary nursing is usually conducted in tanks, but occasionally utilises ponds or cages. In some of the more developed intensive shrimp culture industries (*i.e.* in Taiwan and Thailand), this phase of the cycle is completed by operators buying PL₅₋₇ from hatcheries and nursing them in tanks for 1-2 weeks until PL₁₂₋₂₀, before selling them on to pond on-growers. This operation is still regarded as primary nursing for the purposes of this thesis.

When hatchery-reared seed are stocked into on-growing ponds, the most common age to stock is when they have reached PL₁₂₋₂₀. Thus, primary nursing is commonly the only form of nursing conducted. Under some circumstances however, this nursing period is extended within the same tanks for a further 1-3 weeks. This then is where the distinction between primary and secondary nursing becomes confused. The reasons for this extension in the nursery period are the same as those that will be discussed for secondary nursing in the next section.

2.4.2 Secondary nursing

2.4.2.1 Description of secondary nursing

Secondary nursing is considered here to be that part of the culture cycle that takes post-larvae immediately following primary nursing (PL₁₂₋₂₀) and instead of stocking them directly into the on-growing ponds, rears them for a further 2-6 weeks (until PL₂₅₋₆₀) before stocking into the on-growing ponds.

2.4.2.2 Function and benefits of secondary nursing

Secondary, unlike primary nursing, is an optional phase in the culture cycle, using either hatchery-reared and primary-nursed or wild-caught post-larvae. The main reasons for incorporating a secondary nursery phase are summarised in Table 2.4, and are mainly concerned with stock improvement, management of the culture system, economics, efficiency of use of the culture system and increasing feeding efficiency.

Table 2.4 The reasons for incorporating a secondary nursery phase into the intensive shrimp culture system.

Area	Field	References
Stock improvement	<p>Larger, hardier, disease-resistant seed; High survival and survival predictability; Hold post-larvae if quality in doubt; Improve pigmentation; Enhanced osmoregulatory capability due to gill development</p>	<p>Pantastico & Oliveras (1980); AQUACOP (1985b); Motoh (1985); Tseng (1988); Cordover (1989); Clifford (1992); Lee & Wickins (1992); Olin & Fast (1992); Primavera (1992); Samocha & Lawrence (1992); Stern & Letellier (1992); Sturmer <i>et al.</i> (1992); Chanratchakool <i>et al.</i> (1994)</p>
Management	<p>Enhanced predictability, consistency and programming of system; Headstart shrimp so reduced grow-out time and more cycles per year; Better predator and competitor control; Easier to count seed stocked; Bridge between hatchery and on-growing ponds; Easier disease treatments; Seed holding area for stockpiling and overwintering; Diversify industry; Easier acclimation of shrimp to ponds; Aid maintenance of pond water quality</p>	<p>Hirono (1983); Malca (1983); Smith <i>et al.</i> (1983); AQUACOP (1984, 1985a); Juan <i>et al.</i> (1988); Cordover (1989); Samocha <i>et al.</i> (1990); Clifford (1992); Lee & Wickins (1992); Olin & Fast (1992); Primavera (1992); Samocha & Lawrence (1992); Stern & Letellier (1992); Sturmer & Lawrence (1992); Sturmer <i>et al.</i> (1992); Chanratchakool <i>et al.</i> (1994)</p>
Economics	<p>Reduced risk; Higher value stock; Increased harvest weight; Maximize profits</p>	<p>Mock (1983); Primavera (1983); Juan <i>et al.</i> (1988); Sandifer <i>et al.</i> (1988); Cordover (1989); Lee & Wickins (1992); Samocha & Lawrence (1992); Sturmer & Lawrence (1992)</p>
System usage	<p>Maximize facility usage by maintaining high stocking density; Extend growing season in temperate climate and facilities</p>	<p>Tseng (1988); Samocha & Lawrence (1992); Stern & Letellier (1992); Sturmer <i>et al.</i> (1992)</p>
Feeding	<p>Weaning from hatchery diets; Minimize feed wastage and increase feeding efficiency; Maximize natural productivity</p>	<p>Malca (1983); Sandifer <i>et al.</i> (1988); Tseng (1988); Lee & Wickins (1992); Samocha & Lawrence (1992); Stern & Letellier (1992); Sturmer <i>et al.</i> (1992); Chanratchakool <i>et al.</i> (1994)</p>

There are thus many reasons for the incorporation of a secondary nursery phase into the culture system (particularly intensive culture) for penaeid shrimp. Recent surveys on the use of such systems has shown that 52-87 % of farms worldwide used nurseries including a 'secondary' phase at least occasionally (Hopkins & Villalon, 1992; Stern & Letellier, 1992). In addition, their use will probably increase as the trend for intensification in the shrimp culture industry continues.

The main reason for using secondary nursery systems concerns improving the quality of seedstock. A period of secondary nursing can enhance seed quality. It has been considered that a combination of stressors, including inadequate nutrition and poor water quality in the hatchery, although not apparent through microscopic examination, lead to larger, older, secondary nursed post-larvae being more tolerant to the transfer to, and the fluctuating environmental conditions within the on-growing ponds (Lee & Wickins, 1992; Olin & Fast, 1992). This is most probably due to their enhanced osmoregulatory capabilities because of their better developed branchial gill systems (Charmantier *et al.*, 1988; Clifford, 1992; Fegan, 1992), and enhanced disease resistance (Clifford, 1992). They are also more able to survive predation by birds, fish, insect larvae *etc.* within the ponds (Malca, 1983; Clifford, 1992; Lee & Wickins, 1992). Survival, notoriously difficult to estimate in ponds (due to the benthic nature of shrimp), particularly in the first few weeks, is thus increased and its predictability enhanced. This in turn allows more accurate estimations of feeding and feed consumption, important in the economics of the intensive culture system (AQUACOP, 1985b; Cordover, 1989; Samocha & Lawrence, 1992; Sturmer *et al.*, 1992). Holding shrimp in nurseries also allows the farmer more time to assess the quality of his stock, delaying stocking until such time that he feels confident of their on-growing potential. This is a very important point for intensive farmers since it is extremely difficult to assess the performance of the shrimp during the first period of on-growing. Indeed, a recent survey of shrimp pond managers worldwide revealed that by far the most important impediment to increasing production output and/or profitability was seed quality, with seed availability being placed second (Hopkins & Villalon, 1992). Similarly, one of the main people responsible for the initial success of the Taiwanese shrimp culture industry, I-Chiu Liao, Director General of the Taiwan Fisheries Research Institute has stated that stock enhancement is a major area requiring future

research and development (Liao, 1992).

The use of secondary nurseries are thus an important management tool in the repertoire of intensive shrimp culturists. They are thought to increase the predictability, profitability and consistency of the culture operation, enable easier disease treatments due to confining the shrimp in a small volume of water, enable easy counting, stocking and acclimation of the seed and aid in pond water and sediment quality maintenance through reducing the grow-out period (Hirono, 1983; Malca, 1983; AQUACOP, 1984, 1985a; Cordover, 1989; Primavera, 1992; Samocha & Lawrence, 1992; Sturmer *et al.*, 1992). This reduced grow-out time also has relevance in temperate/semi-tropical areas such as the U.S.A. and Israel where, by stockpiling and nursing shrimp in covered tanks in the spring, the growing season can be extended by two months to encompass two crops (where usually only one crop per year can be harvested) without adverse effects on the shrimp (Smith *et al.*, 1983; Clifford, 1985; Sturmer & Lawrence, 1987b, 1988a; Juan *et al.*, 1988; Seidman & Issar, 1988; Samocha *et al.*, 1990; Samocha & Lawrence, 1992; Sturmer *et al.*, 1992). Even in tropical climates, more cycles can be completed per year, allowing more profit and greater control over the pond environment. More efficient feed usage can also result from enhanced natural productivity and the maintenance of high stocking densities, simplifying feeding procedures (Malca, 1983; Clifford, 1992; Lee & Wickins, 1992; Chanratchakool *et al.*, 1994).

These advantages may, at least in some circumstances, increase the profitability and reduce the risk of the industry by increasing stock value, primarily through the maintenance of high densities of rapidly growing shrimp during on-growing (Lee & Wickins, 1992; Samocha & Lawrence, 1992; Stern & Letellier, 1992; Sturmer *et al.*, 1992). Increased diversification of the industry is also accomplished, further promoting the effective management of each stage in the culture cycle (Chanratchakool *et al.*, 1994).

2.4.2.3 Disadvantages of secondary nursing

Unfortunately, the use of secondary nursery systems also has some potential and

recognised disadvantages which have to be surmounted before they can be profitably employed. These disadvantages are shown in Table 2.5, and are largely concerned with aspects of management, productivity and economics.

Table 2.5 The disadvantages of incorporating a secondary nursery phase into the intensive shrimp culture system.

Area	Field	References
Management	Transportation difficulties; Increased staff skills required; Harvesting difficulties; More complex technology	Licop (1984); Wyban <i>et al.</i> (1989); Clifford (1992); Lee & Wickins (1992); Olin & Fast (1992); Samocha & Lawrence (1992); Stern & Letellier (1992); Chanratchakool <i>et al.</i> (1994)
Productivity	Slow growth rate; Increased size range; Increased stress on transfer; Decreased survival	Clifford (1992); Lee & Wickins (1992); Samocha & Lawrence (1992); Stern & Letellier (1992); Chanratchakool <i>et al.</i> (1994)
Economics	Increased cost of production; High risk; Capital intensive	Samocha & Lawrence (1992); Stern & Letellier (1992); Sturmer <i>et al.</i> (1992)

Management difficulties which may be encountered include the more complex technology and thus skills that are needed to run a secondary nursery operation (Samocha & Lawrence, 1992). The other major problems revolve around the cannibalism, stress and difficulty of harvesting and transporting the larger shrimp produced following the secondary nursery period (Licop, 1984; Clifford, 1992; Lee & Wickins, 1992; Olin & Fast, 1992; Stern & Letellier, 1992; Chanratchakool *et al.*, 1994). Harvesting is often accomplished by draining and/or capture of the shrimp seed with netting which may stress the shrimp and makes estimation of the number stocked difficult (Wyban *et al.*, 1989; Lee & Wickins, 1992; Chanratchakool *et al.*, 1992). Similarly, transportation of the larger seed may be difficult due to the larger biomass of the shrimp and their state of development, particularly of the rostrum, leading to problems with standard techniques of transport in plastic bags. Both harvesting and transportation problems however, may be minimised through development of suitable techniques and the location of nursery units adjacent to the on-growing ponds. All of

these difficulties however, may be overcome through more thorough research and experience such as is currently being developed by the commercial units now in operation. Indeed, recent research has shown that transfer of 0.5-1 g juveniles from intensive tank nurseries to on-growing ponds can be achieved without excessive mortality (Sturmer *et al.*, 1992).

Problems with productivity include again, increased stress on transfer to the on-growing ponds, following on from stressful hatchery conditions, which may lead to decreased survival (Olin & Fast, 1992; Stern & Letellier, 1992). In addition, the slow growth rate and increased population size range obtained due to holding the post-larvae at high density is also considered undesirable (Chanratchakool *et al.*, 1994). The current lack of research however, has yet to demonstrate the validity of these ideas, and even suggests that these features may not be permanent, but may instead be compensated for by increased growth rate on introduction into the ponds with high quantities of available feed. Indeed, Sandifer *et al.* (1988) state that although survival is not different, mean weight of shrimp on harvest is substantially higher when juvenile, rather than post-larval shrimp, are stocked. Similarly, in China it is recognised that seed quality has a significant bearing on the size and yield of harvested shrimp following on-growing (Cao & Jiang, 1990).

The final problem with secondary nursery culture involves the economics of such operations. This is particularly manifested in the increased risk of the operation, its capital intensive nature, the requirement for skilled labour and the increased cost of production, leading to higher seed prices to the on-growers (Lee & Wickins, 1992; Samochoa & Lawrence, 1992; Stern & Letellier, 1992; Sturmer *et al.*, 1992). One of the few economic studies conducted on nursery systems has shown that direct stocking of on-growing ponds with post-larvae and producing one crop per year is more profitable than stocking nursed juveniles and producing one crop per year in southern U.S.A. (Juan *et al.*, 1988). Although few studies have been conducted regarding the economics of secondary nursing, it is certain that higher priced seed are produced. The increased quality of nursed seed however, have been demonstrated to command a higher value for on-growers, who, in the Philippines for example, are willing to pay 30 % more for them (Bauman & Jamandre, 1990). Additionally, from a recent survey of

shrimp nursery operators, mainly in South America, ex-hatchery post-larvae were currently estimated to cost £ 2-3 (rarely £ 4) per thousand, whilst nursed juvenile shrimp were worth more at £ 4-5 (occasionally £ 3) per thousand (Stern & Letellier, 1992). Similar figures are quoted by Fegan (1992) from Thailand, while Nielsen (1991) reports that cage nursed juveniles command 2-3 times the price of post-larvae in India. This increased value may therefore negate the increased risk associated with, and the investment necessary for their production. Indeed, economic analyses conducted on shrimp nursery systems have shown that ponds (Tabbu, 1985), cages (Agbayani *et al.*, 1985; Nielsen, 1991) and tanks (Juan *et al.*, 1988) can be run as profitable operations, primarily due to the increased value of the nursed juveniles. In addition, a recently conducted survey of shrimp farmers worldwide revealed that the majority of farmers did not put seed costs high on the list of impediments to increasing production, but were more concerned with seed quality and availability (Hopkins & Villalon, 1992). In terms of production costs, seed purchase has been shown to account usually for 20 %, occasionally as low as 15 %, of the total costs for on-growing shrimp (Hopkins & Villalon, 1992).

What is necessary, in order to ascertain the commercial viability of a secondary nursery phase is more research. This should particularly be focused on an economic analysis of various types of secondary nursery and whether the benefits derived from their use outweigh their disadvantages. These investigations will have to take account of the various situations in which secondary nurseries are used, their various types and performances, and establish their worth in each case.

2.4.2.4 Secondary nursery culture systems

There are three main types of secondary nursery culture systems currently employed by farmers and under investigation by researchers. These three systems comprise ponds, cages and tanks. The main characteristics of these nursery types are shown in Table 2.6 in terms of stocking age and density, rearing period, survival and production.

Table 2.6 Characteristics of pond, cage and tank secondary nursery systems.

System	Stocking age (d)	Rearing period (d)	Stocking density (shrimp m ⁻²)	Growth rate (mg d ⁻¹)	Survival (%)	Production (g m ⁻² d ⁻¹)
Ponds	PL ₄₋₁₂	25-60	50-500 (15-1,200)	8-50	45-90	2-8
Cages/ Pens	PL ₅₋₂₁	14-30	200-3,000 (10-17,000)	16-36	60-80	2-8
Tanks	PL ₁₋₁₂	15-45	500-6,000 (25-12,500)	20-45	55-90	2-50

(Data abstracted from Tables 2.7, 2.8 and 2.9)

2.4.2.4.1 Pond nurseries

Earthen nursery ponds have been used successfully for some time as an intermediate stage in the intensive culture of penaeid shrimp and freshwater prawns. This type of nursery takes two main forms consisting of either separate nursery ponds or areas of larger production ponds which are sectioned off into a nursery area. The total area of nursery ponds commonly comprises 5-10 % of the area of the on-growing ponds (Stern & Letellier, 1992; Chanratchakool *et al.* 1994).

The separate nursery ponds are usually 0.1-1 ha in area, up to 1 m in depth and are stocked with hatchery-reared or more rarely, wild-caught post-larvae from PL₃₋₅ onwards. The post-larvae are then usually reared for 4-8 weeks at densities of 15-500 (commonly 50-200) shrimp m⁻². The best results achieved to date (Wyban *et al.*, 1991) involved rearing *P. vannamei* at 800-1,200 PL m⁻² for up to 50 days and achieved maximum production rates of 26 g m⁻² d⁻¹ (Tables 2.6 & 2.7). Harvesting and transfer is accomplished either by netting or by draining directly into the on-growing ponds. The advantages of this system of nursing are in the low cost of the systems and the optimisation of pond usage, such that with proper management, all the pond facilities on the farm are in constant usage at all times. The disadvantages of this system include the relatively low stocking densities, survival and production possible, the requirement

Table 2.7 A review of results with secondary nursery pond trials.

Species	Seed stage	Rearing period (d)	Stocking density (no. m ⁻²)	Growth rate (mg d ⁻¹)	Survival (%)	Production (g m ⁻² d ⁻¹)	Reference
<i>P. monodon</i>	PL _{4,5}	25-29	15-150	16-52	20-93	0.3-1.4	Apud <i>et al.</i> (1979)
	PL _{4,5}	28	100	15	46-69	0.7-1.0	Apud & Camacho (1980)
	PL	28-31	200	21-35	30-68	0.1-0.2	Ravichandran <i>et al.</i> (1982)
	PL _{4,5}	45	50-150	15-34	42-67	0.7-1.7	Tabbu (1985)
	PL	30	400	4	53-79	0.2	Hamid (1986)
	PL ₁₅	14	200-300	?	?	?	Liao (1992)
<i>P. vannamei</i>	PL	32	200	?	84	?	Parker <i>et al.</i> (1974)
	PL	30-45	150-200	6-8	?	?	Ducnas <i>et al.</i> (1983)
	PL	45-60	150-200	8-22	?	?	Malca (1983)
	PL	30-60	50-200	16-67	> 60	2-3	AQUACOP (1984)
	PL	45	166	36	?	?	Davis <i>et al.</i> (1985)
	PL _{5,10}	30-60	50-200	17	70-80	2-5	Escobar (1985)
	PL _{10,12}	30-45	3,500	14	46-70	2.7-11.8	Sturmer & Lawrence (1986)
	PL _{10,12}	28	125-500	14-36	?	4-7	Sturmer & Lawrence (1987a)
	PL _{10,12}	42	250-500	31-50	?	4-5.5	Sturmer & Lawrence (1987a)
	PL	45	?	11-33	70	?	Hirono (1989)
	PL	30-42	125-500	13-50	75-95	6-8	Sturmer <i>et al.</i> (1991)
	PL	45	150-200	13-18	67-75	2	Villalon (1991)
	PL	30-50	800-1200	20-40	80-90	11-26	Wyban <i>et al.</i> (1991)
	PL	15-45	50-150	?	60-80	?	Stern & Letellier (1992)
<i>P. semisulcatus</i>	PL	38	400	90	65	1.4	Issar <i>et al.</i> (1987)
	PL	45-69	109-194	33-78	55-85	4-6	Seidman & Issar (1988)
<i>P. stylirostris</i>	PL	15-45	50-150	?	60-80	?	Stern & Letellier (1992)
	PL _{10,12}	28	250	18	88	3.9	Sturmer <i>et al.</i> (1992)

for additional pond facilities on the farm which require close management of water and sediment quality and natural food production and possible difficulties with harvesting stress (AQUACOP, 1985b).

Where secondary nursery ponds are part of the main on-growing ponds, a section (commonly 5-10 %) of the on-growing pond is partitioned off using fine mesh screens forming a pen. This type of nursery also stocks young post-larvae as above. The post-larvae are also nursed in these pens for 4-8 weeks at densities as above before the restraining netting is removed and the shrimp allowed access to the whole pond area (Tables 2.6 & 2.7). The advantages of this type of pond nursery are in terms of stress reduction on transfer of the shrimp and in the closer control of shrimp feeding and monitoring in such nurseries. The disadvantages of this system are again the low densities possible, the reliance on pond productivity, problems with benthic algae and phytoplankton levels in the water column, the possibly low survival and production obtained and the fact that large areas of the ponds will be unproductive over the nursery period (AQUACOP, 1985b).

2.4.2.4.2 Cage nurseries

Cages have been used with some success for the secondary nursing of penaeid shrimp. They comprise of either floating or fixed cages and sometimes cages open to the pond bottom where they are more accurately known as pens. The cages are fixed in either ponds (on-growing or separate), canals, estuaries or the sea. They are stocked as for the pond nurseries at PL₅₋₂₅ at densities of 10-10,000 (commonly 100-3,000) shrimp m⁻² for 2-4 weeks. The maximum production achieved in cages was 40 g m⁻² d⁻¹ by Walford & Lam (1987), who reared *P. indicus* at up to 1,380-3,450 PL₂₀ m⁻² for 22 days (Tables 2.6 & 2.8). The shrimp are harvested from the cages by netting them out, transferring the whole cage or simply releasing the post-larvae/juveniles directly into the ponds, if they are situated within the on-growing ponds. The advantages of this system are the low cost of cage equipment, the ability to utilise otherwise unproductive areas. The disadvantages are problems with high density nursery culture if aeration or water flow are not provided and the possibility of stock escape through net damage.

Table 2.8 A review of results with secondary nursery cage trials.

Species	Seed stage	Rearing period (d)	Stocking density (no. m ⁻²)	Growth rate (mg d ⁻¹)	Survival (%)	Production (g m ⁻² d ⁻¹)	Reference
<i>P. monodon</i>	PL ₁₁₋₂₁	30	200	?	73-74	?	Primavera (1976)
	0.15 g	30	200-400	33-36	65-75	4.6-7.2	Siddharaju <i>et al.</i> (1982)
	PL ₅	15-25	3,000	?	80	?	de la Pena & Prospero (1984)
	PL ₅₋₁₆	14-21	4-16,895	?	41-60	?	de la Pena <i>et al.</i> (1985)
	PL	?	200-350	?	73	?	Singh & Mattai (1988)
	PL ₂₀	63-81	150-600	18-24	44-83	1.9-8.2	Reyntjens (1989)
	PL ₂₀₋₂₈	30	72-432	8-26	92-97	0.6-7.3	Rodriguez <i>et al.</i> (1993)
<i>P. indicus</i>	0.05 g	30	300-500	27-31	60-70	6.1-7.6	Siddharaju <i>et al.</i> (1982)
	PL ₅	14-22	1,380-3,450	16-31	76-83	8.4-40	Walford & Lam (1987)
	0.25 g	40	67-100	52	55-80	2.9-4.3	Bose (1988)
<i>P. vannamei</i> <i>P. stylirostris</i>	PL	?	10-60	?	?	?	Inszczyński <i>et al.</i> (1988)

2.4.2.4.3 Tank nurseries

Tank or raceway nurseries are attracting a considerable amount of research activity currently and have produced good results both under research and commercial conditions. The tanks or raceways are constructed of a wide variety of materials including concrete, brick, lined wood or metal, plastics and fibreglass and range in size from 10-60 m² and up to 1 m in depth (Stern & Letellier, 1992). This type of nursery may be located either within the hatchery complex, adjacent to the ponds or in areas remote from other facilities. They are stocked with young seed from PL₁₋₂₅ at high densities of 100-12,000 (commonly 500-6,000) shrimp m² for 2-4 weeks.

The best results achieved in tanks to date were from Sturmer & Lawrence (1988a), who stocked up to 6,000 *P. vannamei* PL₃ m⁻² for 47 days and obtained production of up to 65 g m⁻² d⁻¹ (Tables 2.6 & 2.9). The shrimp are then harvested by either netting or draining the tanks directly into the on-growing ponds. The advantages of this system include the high densities and hence productivity possible, optimised use of on-growing ponds through constant maintenance of high stocking densities, easier harvesting and the better stock and environmental control possible (AQUACOP, 1985b). Disadvantages include the additional, high costs associated with their construction, the need for aeration, water flow and high quality formulated diets, increasing the cost of production and the slower growth rates achieved at high density (Issar *et al.*, 1989; AQUACOP, 1985b). With the recent developments in intensive tank nurseries however, it has been postulated that early post-larvae can be nursed at high stocking density without negatively affecting their future performance (Robertson *et al.*, 1992). This theory however, remains debatable and is in need of further clarification. Furthermore, research results on the intensive tank rearing of *P. monodon* have only rarely been published (Forster & Beard, 1974; AQUACOP, 1985b; Juario & Benitez, 1988), despite the fact that it is being carried out commercially in many areas of Southeast Asia.

Table 2.9 A review of results with secondary nursery tank trials.

Species	Seed stage	Rearing period (d)	Stocking density (no. m ⁻²)	Growth rate (mg d ⁻¹)	Survival (%)	Production (g m ⁻² d ⁻¹)	Reference
<i>P. monodon</i>	0.15 g	14-28	25-166	61-80	100	2-10.2	Forster & Beard (1974)
	PL ₅	35-85	50-200	12-29	< 50	0.7-1.1	AQUACOP (1984)
	PL ₃	30	1-10,000	3	70	2.3-23	AQUACOP (1985b)
	PL ₅	30-40	50-100	33-50	60	1-3	Juario & Benitez (1988)
	PL ₆₋₂₅	15-20	5-30,000	?	?	?	Parado-Esteva (1988)
<i>P. vannamei</i>	0.01 g	20-47	500-3,500	2.5-10	58	2-6.8	Sturmer & Lawrence (1986)
	PL ₄₋₅	16	4,000	0.2	58	0.4	Sumeru & Nur (1986)
	0.01 g	68	500	25-32	58-82	9.3-10.1	Sandifer <i>et al.</i> (1987)
	PL ₁₀₋₁₂	28	550-4,400	25-40	60-70	14-48	Sturmer & Lawrence (1987b)
	PL ₁₀₋₁₂	30-45	3-6,000	19	55	31-65	Sturmer & Lawrence (1988a)
	0.01 g	35	3,100-3,300	14-22	77-99	41-54	Samocha <i>et al.</i> (1990)
	PL	?	7,800	?	> 92	?	Sturmer <i>et al.</i> (1992)
<i>P. indicus</i>	0.01 g	112	25-166	118-174	27	0.8-7.8	Forster & Beard (1973)
<i>P. setiferus</i>	0.07 g	18-49	625-12,500	0.4-21	89-97	4.5-17.6	Mock <i>et al.</i> (1973)
<i>P. merguensis</i>	PL ₁	15-20	8-12,000	?	62-85	?	Ruangpanit <i>et al.</i> (1985)
<i>P. semisulcatus</i>	0.07 g	41	400	44	74	13	Issar <i>et al.</i> (1987)
<i>P. japonicus</i>	0.01 g	47	74-536	6.4-19	?	1.4-3.7	Canavate & Sanchez (1989)
<i>P. stylirostris</i>	PL	42	280-680	29-45	?	11-17	Sturmer <i>et al.</i> (1992)

2.4.2.5 Important parameters for optimal nursery production

2.4.2.5.1 Stocking density

One of the most important parameters affecting the production rate of juvenile shrimp in secondary nurseries is stocking density. It is generally true that there is an inverse relationship between stocking density and growth and production. Thus, intensively stocked tank nurseries are usually acknowledged to increase production, but at the cost of individual weight gain. This is particularly true at high stocking densities in tank nurseries, although in some instances, such correlations have not been found.

In nursery ponds, growth rates of post-larval *P. vannamei* have been shown to be independent of stocking density (at densities of 125-500 shrimp m²) for the first 21 days, but are inversely proportional thereafter. Survival rates however, were shown to be unaffected by stocking density (Sturmer & Lawrence, 1987a, Sturmer *et al.*, 1992). Similarly, increasing stocking density in earth ponds from 50-150 shrimp m² significantly decreased growth by 48-118 % after 45 days (Tabbu, 1985). In net cages, an inverse relationship has also been found between stocking density and growth rate at densities of 72-432 shrimp m² (Rodriguez *et al.*, 1993). Likewise, in aquarium trials simulating tank nurseries, stocking density was found to have an inverse relationship to shrimp growth and survival rates, even at low densities of 74-144 shrimp m² (Ray & Chien, 1992). These authors concluded that this was primarily due to deteriorating water quality at higher densities. In high density tank nursery trials, production rates increased from 14 to 48 g m² d⁻¹ as stocking densities increased from 550-4,400 shrimp m², but mean final weight decreased from 1.7 to 0.7 g respectively (Sturmer & Lawrence, 1987b). In contrast, in intensive concrete nursery tanks, with high levels of aeration, but minimal water exchange (5 % d⁻¹), stocking density (from 1,000-10,000 shrimp m²) was found to have little effect on growth and survival. This was probably due to the use of high feeding rates within highly controlled systems (AQUACOP, 1985b).

The choice of nursery system and stocking density thus depends both upon the demand for the seed produced in terms of numbers and size required and the level of skill and

investment available for development of the nursery systems. Economic analyses of each system, so far little studied, will then be necessary to determine optimal profitability.

2.4.2.5.2 Rearing period

In low density pond nursery rearing, shrimp growth rate is generally high, but may become limited after some time as the biomass increases. Thus rearing periods not exceeding 25-35 days have been recommended for pond-based nurseries (Sturmer & Lawrence, 1987a; Stern & Letellier, 1992; Sturmer *et al.*, 1992). In tank-based intensive nursery systems, no such restrictions apply provided adequate water quality control through aeration and water exchange is achieved.

2.4.2.5.3 Habitats

Published literature on the effects of a wide range of artificial habitats, increasing the wetted surface area of nurseries has received some attention, but generated conflicting results. Habitat inclusion is thought to stimulate natural productivity, provide shelter for moulting shrimp and hence increase growth, survival, feeding and production. In earthen nursery ponds, coconut palm leaves have been used with some success to provide shelters for post-larvae (Sriraman & Sathiyamoorthy, 1988). In raceway tanks at densities of 500 PL m², habitat inclusion (vertically-placed seine netting or fibreglass screens) doubling the effective surface area of the raceways improved survival from 58-82 %, but not growth (Sandifer *et al.*, 1987). Good FCRs resulting from the use of habitats were suggested to be due to "fouling" communities associated with the habitats (Sturmer & Lawrence, 1988a). More recently however, no beneficial effects were observed when the area of habitats in these raceways was increased by 67 % (Samocho *et al.*, 1990). Furthermore, nursing post-larvae without any habitats did not reduce growth or survival. The use of habitats is thus an area deserving further investigation under a range of conditions.

2.4.2.5.4 Substrates

Mud/earth substrates are thought to be responsible for the increased natural productivity of earth pond and pen cage nurseries. This may be due either to increased natural productivity or reduced stress to young shrimp which burrow into the sediments. Although no evaluation of substrate preference or type has been published in the literature, commercial concrete tank nurseries in Thailand often utilise a 5-10 cm layer of coarse-grained riverine sand, suggesting that it increases productivity (Fegan, pers. comm., 1991). Additionally, polyethylene-lined ponds with a 20-30 cm layer of sand substrate have been tested (Issar *et al.*, 1987; Sandifer *et al.*, 1987). In net cages, feeding nets were used successfully for feeding and as substrates/habitats (de la Pena *et al.*, 1985). In aquarium trials, simulating tank-based nurseries however, simulations of sediments of deteriorating quality were found to have no effect on shrimp growth or survival rates at low stocking density (72-144 shrimp m⁻²) (Ray & Chien, 1992). The functions and benefits of substrate use in nurseries are thus little studied.

2.4.2.5.5 Aeration/water flow rate

Although, earthen nursery ponds do not normally require aeration, some research has shown that stocking densities can be increased profitably if mechanical aeration (10 hp ha⁻¹) and water exchange (5-40 % pond volume d⁻¹) are used in the ponds, particularly for young post-larvae (Apud & Camacho, 1980; AQUACOP, 1985b; Sturmer *et al.*, 1992). These benefits accrued from water flow and aeration are thought to be due to enhanced water quality in terms of oxygen supply and improved nutrient distribution within the ponds (Sturmer *et al.*, 1992).

In nursery tanks stocked at relatively low density (up to 500 shrimp m⁻²), water exchange once weekly is thought to be sufficient (Somalingam & Murthy, 1978), while AQUACOP (1985b) have developed an intensive tank nursery for penaeids utilising low water exchange rates of 5 % d⁻¹. The system depends instead upon high levels of aeration and the development of a phytoplankton and bacterial medium with both nutritive and purifying qualities. This system worked well for shrimp stocked at PL₃ nursed for 30 days at densities of up to 10,000 m⁻², with high rates of growth and

survival. Without such aeration, water exchange rates of up to 400 % d⁻¹ were found to be necessary to sustain production. Again however, little information has been published regarding optimal water flow or aeration rates for tank nurseries.

2.4.2.5.6 Feeding

2.4.2.5.6.1 Dietary formulation

Most nutritional studies with shrimp have been conducted using young (post-larval or juvenile) animals in tank-based facilities. This is the case largely for two reasons. Firstly, young shrimp grow relatively quicker than older shrimp, enabling results to be derived more quickly. Secondly, a greater degree of control can be exercised in tank, rather than pond or cage trials, enabling the researcher to be certain that dietary differences are the only variable existing during the trial. The results from tank-based nutritional trials may therefore be applied with more confidence to the nutritional requirements of young shrimp in intensive secondary nursery tank systems than to those at any other stage in the culture cycle. However, literature on the nutritional requirements of shrimp tested in secondary nursery systems of any type under field conditions is very limited and consists largely of 'trial and error' results (Sturmer *et al.*, 1992).

Differences between the nutritional requirements (and hence production) of young shrimp reared in outside nursery systems and those reared under laboratory conditions may be expected for a number of reasons. The most important of these include firstly, the increased access to natural feeding, particularly in pond and cage nurseries (AQUACOP, 1985b), and secondly, the growth-enhancing effects (> 62 % increase) of suspended particles in the water derived from shrimp ponds, even when they are lined (Leber & Pruder, 1988; Moss, 1992). The possibility exists that microalgae may be responsible for this effect since enhanced growth of post-larval shrimp in Israel has resulted from their culture in raceways seeded with up to 500,000 cells ml⁻¹ of various micro-algal species (quoted in Sturmer *et al.*, 1992). Under commercial conditions, ponds are often used either directly in the case of pond or cage nurseries, or as a water source for tank nurseries (Issar *et al.*, 1987). In addition, researchers who have used

formulated feeds have rarely quoted the dietary formulations used or tested different formulations under field conditions. Thus, optimal nutritional and management practices for nursery systems are in need of development, particularly in terms of growth, survival and production rates at minimum operational cost (Hopkins & Villalon, 1992; Liao, 1992; Stern & Letellier, 1992; Sturmer *et al.*, 1992).

In low-density pond and cage nurseries, supplementary feeding may not be necessary, other than the use of organic and inorganic fertilizers to stimulate natural productivity (Ravichandran *et al.*, 1982; Zhang & Li, 1985; Sturmer *et al.*, 1992). In the Philippines, the culture of lab-lab, a biological complex of algae, bacteria and various animals is stimulated by fertilization to supply feed for the nursed shrimp (Jumalon, 1978). Similarly in China, techniques have been successfully developed for the transplantation and propagation of small benthic crustaceans and polychaetes in earthen nursery ponds in order to increase natural productivity (Liu, 1985).

As stocking densities increase, particularly in tank nurseries with limited natural production, supplementary feeding gains more importance in order to maintain growth and survival rates and hence increase productivity (Apud *et al.*, 1979; Sturmer *et al.*, 1992). For example, supplementing natural productivity with applied feeds increased growth rates of PL_{4,5} stocked at 50-150 shrimp m⁻² by 8-59 %, with greater differences at the higher stocking densities (Tabbu, 1985). The relative contributions of pond productivity and supplementary feeds to the nutrition of young shrimp in nurseries is an area which is still little understood (AQUACOP, 1985b).

Successful fresh or frozen natural feeds commonly used at higher intensities have included combinations of clam or mussel meat, raw ground fish, shrimp head meal, chopped shrimp, boiled squid offal, frozen *Artemia*, groundnut cake and rice bran (Somalingam & Murthy, 1978; Apud *et al.*, 1979; Khannapa *et al.*, 1980; de la Pena *et al.*, 1985; Issar *et al.*, 1987; Bose, 1988; Singh & Mattai, 1988; Parado-Esteva, 1988; Sriraman & Sathiyamoorthy, 1988).

In intensive nursery tanks, formulated pellets containing 30-50 % protein, up to 8 % lipid and vitamin and mineral supplements, as well as *Artemia* nauplii in the initial

stages are commonly used. This is in order to satisfy the nutritional requirements of the shrimp, reduce water quality problems encountered with fresh feeds and help promote moulting (Issar *et al.*, 1987; Sandifer *et al.*, 1987; Sturmer & Lawrence, 1986, 1987a,b, 1988a,b; Parado-Estepa, 1988; Stern & Letellier, 1992; Sturmer *et al.*, 1992; Fegan, pers. comm.). Further discussion of the precise nutritional requirements of young shrimp will be included in section 2.5 and forms a large part of the work included in this thesis.

2.4.2.5.6.2 Feeding regime

The effects of feeding regime (rate and frequency) on shrimp nursery production is perhaps less understood than the nutritional requirements of the shrimp. Feeding rates for juvenile shrimp have generally been estimated from expected growth, assumed feed conversion, and rates used for other species and ages of shrimp. Indeed, no specific studies have been conducted to determine optimum feeding rates for young shrimp under pond culture (Sturmer *et al.*, 1992). This situation exists despite the significance of feeds and feeding to the success and profitability of nursery systems, particularly the more intensive systems.

In terms of feeding rate, in low density nurseries, fertilization can be carried out weekly, or as needed, depending on phytoplankton density, leading to virtually constant feed availability. In more intensive systems however, supplementary feeding with fresh feeds is usually necessary at approximately 100 % of the total wet body weight d^{-1} for PL₄₋₅, decreasing to 20 % d^{-1} for PL₂₀₋₃₀ (Apud *et al.*, 1979). Formulated, usually pelleted or crumbled rations are fed at various rates, with no clear guidelines currently being available. Commercial tank nurseries in Thailand feed pelleted diets at 25 % of wet body weight d^{-1} between PL₅ and PL₁₅₋₂₀. AQUACOP (1985b) suggest using a rate of 30 % for penaeid PL₃₋₃₀. Mock *et al.* (1973) fed Tetra Marin feed once or twice daily at 94 %, decreasing to 37 % of wet body weight d^{-1} as the shrimp grew. Sandifer *et al.* (1987) fed a pelleted diet once daily at 10 % wet body weight d^{-1} , supplemented with fresh squid three times per week. Sturmer & Lawrence (1988a) and Sturmer *et al.* (1992) have conducted some research, based on observations of post-larval feeding habits which suggested that feeding rates should be 10-20 % of the total wet body

weight d^{-1} throughout 5 weeks of nursing post-larval *P. vannamei* at a mean initial weight of 1.58 mg. To date however, very little information has been published on the optimal feeding rates for the nursery phase of the shrimp culture cycle, despite its importance in the production of healthy seed for stocking the on-growing ponds (Sturmer *et al.*, 1992).

Feeding frequency is also important, primarily because during the hatchery and primary nursing phases, food is almost continuously available since feeding is carried out 5-6 times per day and live feeds are usually used. The post-larval shrimp are thus adapted to high feed availability and frequent feeding, rather than the common once daily regime in nursery systems (Stern & Letellier, 1992), which is likely to result in low growth rates and high FCRs.

In the laboratory, frequent feeding has been shown to increase the growth of shrimp, prawns and fish (Sedgwick, 1979; Marian *et al.*, 1982). Sampath (1984) however, showed that over a range of feeding frequencies from once in five days to four times daily, that there was no benefit in feeding 13 g *P. monodon* more than twice daily. More frequent feeding may however, be beneficial at smaller body (stomach) size (Marian *et al.*, 1982). Subramanian & Krishnamurthy (1986) showed that juvenile *P. indicus* held in the laboratory displayed better growth and feeding efficiency when fed three times daily than when fed only once. In the field, various authors have used differing feeding regimes, but comparisons between regimes are scarce. Issar *et al.* (1987) fed a combination of pellets and fresh/frozen feeds to juvenile *P. semisulcatus* in concrete nurseries 5-8 times d^{-1} *ad libitum* and achieved good growth and FCRs as low as 1.6. Sturmer *et al.* (1992) describe successful pond nursing of penaeid post-larvae, where feeding is conducted several times daily, unless low dissolved oxygen conditions are encountered. In contrast, Sturmer & Lawrence (1988a) and Sturmer *et al.* (1992) detail research showing that there were no benefits in terms of growth to feeding post-larval shrimp in intensive tank nurseries continuously, rather than once daily. The automated, continuous feeding regime however, proved more cost effective under American conditions.

2.4.3 Seed quality

As mentioned previously, one of the major impediments to the success of any shrimp culture operation is the lack of knowledge regarding the quality of seed being cultured. The definition of high quality seed is difficult, but may be considered in terms of animals which exhibit characteristics including high growth and survival rates, disease and predator resistance and low variability in size distribution. In traditional, more extensive forms of shrimp culture, seed are most frequently derived from the wild and are generally considered to be of high quality. With the increasing dependence on hatchery-reared seed however, particularly in the more intensive forms of culture, seed quality has become more variable. This is because the young shrimp are mass-reared in sub-optimal environments, often from enforced multiple spawnings of captive broodstock.

Inclusion of a nursery phase in the culture cycle is one technique whereby seed quality is thought to be improved, resulting in enhanced and more predictable production during the on-growing phase. What is unclear however, is how the quality of seed produced, either from the wild, ex-hatchery or following nursing, can best be judged. Numerous techniques for the establishment of seed quality have been proposed and are summarised in Table 2.10. Most of these techniques however, have failed to show any correlation with subsequent on-growing performance. There is a need therefore, for a simple, rapid, repeatable and objective measure of seed quality which can be used to predict viability during on-growing.

With the lack of such a test, the current trend of thought is that larger, older shrimp are more likely to be able to survive the transfer from the controlled conditions of the hatchery to the more variable ones encountered within production ponds. Indeed, many authors consider that *P. monodon* seed should be at least PL₁₅₋₃₀ before stocking, particularly if ponds have low salinity (Tseng, 1988; Chiang *et al.*, 1989; Clifford, 1992). Nursing is therefore considered to increase seed quality or viability. Thus nursed, or at least larger seed, commonly have a higher value than younger, smaller seed (Bauman & Jamandre, 1990; Fegan, 1992). One drawback of nursing, particularly with intensive systems is thought to be the increased variation in size of the seed

Table 2.10 Parameters used in determining seed quality.

Stage in cycle	Measure	Drawback	Reference
Broodstock	Female serum protein level, male sperm quality	Tedious and dependant on moult-stage, no indication of larval quality	AQUACOP (1983); Clark, Jr. <i>et al.</i> (1984); Leung-Trujillo & Lawrence (1987); Trujillo (1990)
Egg	Spawn size, egg diameter, hatch rate	Poor predictor of larval quality, unproven correlation with viability	Simon (1982); Bray & Lawrence (1992)
Larvae	Positive phototaxis, survival, rearing period	Unproven correlation with viability	Bray & Lawrence (1992)
Post-larval/ Juvenile	Weight/length	Tedious and unproven correlation with viability	Wilkenfeld <i>et al.</i> (1984); Kuban <i>et al.</i> (1985); Samocha <i>et al.</i> (1989); Bray <i>et al.</i> (1990)
	Condition (fouling, colour, deformities)	Unproven correlation with viability	Skinner (1985); Lightner (1988a,b); Parado-Esteba (1988); Yunker (1990)
	Swimming behaviour	Unproven correlation with viability	Chanratchakool <i>et al.</i> (1994)
	Biochemical composition	Unproven correlation with viability	Araujo (1991); Fegan (1992)
	Muscle:gut ratio (> 4:1)	Unproven correlation with viability	Bauman & Jamandre (1990); Bauman & Scurra (1990); Wilkenfeld (1992)
	Weight frequency distribution	Difficult with young post-larvae, may disappear on on-growing	Kalagayan <i>et al.</i> (1991); Holloway <i>et al.</i> (1990); Clifford (1992); Fegan (1992); Stern & Letellier (1992)
	Stress tests	Unproven correlation with viability	Maugle (1988); Charmantier <i>et al.</i> (1989); Baybay (1989); Tackaert <i>et al.</i> (1989); Arellano (1990); Bauman & Jamandre (1990); Nietes (1990); Briggs (1992); Clifford (1992); Wilkenfeld (1992)

produced. Thus if the coefficient of variation (CV = standard deviation/mean) is > 15 %, a significant variation in size is indicated (Clifford, 1992). There is some evidence that a high degree of size variation may indicate the presence of disease (Holloway *et al.*, 1990; Kalagayan *et al.*, 1991). There are also fears by the farmer that this may continue during on-growing to produce harvested shrimp of a wide size range which have a lower market value (Fegan, 1992). This theory however, is open to considerable debate and has yet to be confirmed.

The other most commonly used criterion for judging quality is larval performance during the hatchery phase and post-larval size, appearance and behaviour (Olin & Fast, 1992; Wilkenfeld, 1992). Particular attention is placed on microscopic examination of the midgut gland for viral inclusions (Wilkenfeld, 1992) and the colouration of the post-larvae. Well pigmented, dark coloured shrimp with distinct and well-defined chromatophores located in the mid-ventral axis are the most highly regarded (Olin & Fast, 1992). A simple test, commonly performed is stirring the water in a bowl containing the post-larvae and ensuring that they swim rapidly against the current with straight bodies. When the current subsides they should cling to the bottom of the bowl oriented parallel to the water flow. Unhealthy seed tend to be lethargic, unresponsive, have arched bodies and are swept into the middle of the container (Chanratchakool *et al.*, 1994). Finally, the development of the muscle in the sixth abdominal segment to a muscle:gut ratio of > 4:1 is considered to be a sign of good quality. This is because this ratio has invariably been recorded in wild-caught seed and has shown signs of being correlated with high rates of growth and survival (Bauman & Jamandre, 1990; Bauman & Scurra, 1990).

A recently developed technique which seems to hold some promise in the prediction of grow-out viability is the stress test. This test involves the exposure of post-larvae to some form of environmental stress in order to reveal any sub-clinical signs of poor health (Tackaert *et al.*, 1989; Arellano, 1990; Durán Gómez *et al.*, 1991). It is assumed for these tests that healthy shrimp will be able to withstand such stress, while diseased or less viable shrimp will not. Indeed, some shrimp farmers in the Philippines will pay a 30 % premium for post-larvae which withstand such tests (Bauman & Jamandre, 1990), while many pond operators in Ecuador will only buy post-larvae which they

have seen undergoing a stress test (D. Macintosh, pers. comm., 1992).

Various types of environmental stress have been imposed upon post-larvae, but the tests usually involve the abrupt transfer of the shrimp from water at optimal salinity and temperature into water at extreme (high or low) combinations of temperature, salinity and/or pH, or into water containing chemicals such as formalin (Table 2.11). The shrimp are then left for a period of time after which their survival or state of health is recorded.

Shrimp post-larvae of various species were subjected to salinity shocks by Tackaert *et al.* (1989), who showed that those fed with n-3 highly unsaturated fatty acid (HUFA) enriched *Artemia* were more able to tolerate such stress. Similarly, Arellano (1990) found enhanced stress resistance to pH shock in post-larvae with high levels of HUFA in their tissues. These results suggest that conditions during the larval rearing phase may have some bearing on subsequent stress resistance. Similarly, Preston (1985) observed that the nutritional status of shrimp during the larval stages had more effect on survival than either temperature or salinity stress. The use of salinity shocks in combination with temperature stress have been found necessary with *P. monodon* since they tend to be extremely euryhaline (Valencia 1976; Catedral *et al.*, 1977; Pantastico & Oliveros, 1980). The use of formalin at 100-150 mg l⁻¹ to provide an environmental stress has been tested with some success (Bauman & Jamandre, 1990). Such stress tests should ideally be conducted over a period of 1-2 hours to enhance the practicability of the test under commercial conditions, the precise level of salinity/temperature/pH reductions, or chemical concentrations, being dependant upon the species tested and its age or size. The ideal conditions for each species and age class have yet to be identified, but simultaneous reductions in temperature and salinity of 10 °C and 20 ‰ salinity over four hours have been suggested (Clifford, 1992). It has been shown however, that the tolerance to salinity shocks is directly proportional to post-larval age, with PL₁ only withstanding a 1-10 ‰ decrease, while PL₂₀ can tolerate decreases of 20 ‰ (Olin & Fast, 1992). This result confirms that tolerance to environmental stress is increased with age, probably due to the rapidly developing osmoregulatory capacity of these post-larvae (Charmantier *et al.*, 1988).

Table 2.11 Stress tests used for determining post-larval quality.

Species	Post-larval stage	Time (hours)	Stressor	Results	Reference
<i>P. monodon</i>	PL ₁₇	24	Salinity/temperature increase/decrease	Wide salinity tolerance range (10-40 ‰)	Valencia (1976)
	PL ₁₅	1-3	Salinity/temperature increase/decrease	When decrease salinity 30-5 ‰ gives 50 % survival in 3 hours	Baybay (1989)
	PL ₁₀₋₂₀	1-2	Salinity decrease	n-3 HUFA-fed PL better stress resistance	Tackaert <i>et al.</i> (1989)
	PL ₁₃₋₂₃	0.5-2	Salinity decrease of 15-20 ‰ or exposure to 100-150 mg l ⁻¹ formalin	Significant differences between batches from different hatcheries	Bauman & Jamandre (1990)
	PL ₁₁₋₃₅	1	Salinity/temperature decrease/increase	5 ‰ salinity and 20 °C combination causes significant mortality in 1 hour, older PL better stress resistance	Nietes (1990)
	PL ₁₅₋₅₀	1	Salinity/temperature decrease/increase	Significant differences between batches of PL from same hatchery, older PL better stress resistance	Briggs (1992)
<i>P. japonicus</i>	PL ₁₋₁₀	24-96	Salinity decrease	24 h LS50 at 25 °C 10 ‰ at PL ₁₋₆ , 7 ‰ at PL ₁₀	Charmantier <i>et al.</i> (1988)
	5,9,17 & 22 mg	2	Salinity decrease	Older PL better stress resistance	Durán Gómez <i>et al.</i> (1991)

Problems with interpretation of the results of stress tests include difficulties with assessing whether the post-larvae are dead or merely become moribund as a technique of surviving these stresses (Fegan, 1992). Also, the osmoregulatory capacity develops rapidly during the early post-larval stages, making objective comparisons between different batches or different aged post-larvae difficult unless the precise age is known. Nevertheless, these techniques can be used to determine when a particular batch of shrimp is able to withstand a set environmental shock and hence is able to survive conditions likely to occur in the on-growing ponds (Charmantier *et al.*, 1989; Fegan, 1992). Finally, although these tests have been used to show differences in stress tolerance between batches of shrimp both at the laboratory scale and in commercial situations, the relationship between stress tolerance and subsequent on-growing performance is still unknown. Further detailed investigations into these relationships, and tests designed for different ages of post-larvae are thus warranted (Bauman & Jamandre, 1990).

2.5 SHRIMP NUTRITION

2.5.1 Introduction

Feeding of shrimp with formulated diets in commercial aquaculture is confined to semi-intensive or intensive systems where stocking densities are approximately 5-20 and > 20 shrimp m⁻² respectively (Lee & Wickins, 1992). Extensive systems (< 5 shrimp m⁻²) rely on the natural productivity of the pond, often stimulated with organic and inorganic fertilizers. This aspect of shrimp nutrition will not be covered here but is described in more detail elsewhere (Clifford, 1992; Boyd & Fast, 1992; Primavera, 1992). Larval rearing commonly involves the use of live feeds including phytoplankton and *Artemia* (Fegan, 1992; Léger & Sorgeloos 1992), and sometimes rotifers (Fukusho, 1989; Samocha *et al.*, 1989) or nematodes (Fontaine *et al.*, 1982; Biedenbach *et al.*, 1989). Alternative feeds include crustacean tissue suspensions (Tacon, 1986) and micro-encapsulated diets (Jones, 1985; Jones *et al.*, 1974, 1979a,b, 1987; Kurmaly *et al.*, 1988, 1989a,b; Amjad & Jones, 1992). This aspect of nutrition is not covered in this thesis, but is described in detail elsewhere (Kanazawa *et al.*, 1982, 1985; Mock &

Fontaine, 1980; Sandifer & Williams, 1980; Wilkenfeld *et al.*, 1984; Fegan, 1992; Léger & Sorgeloos, 1992). Similarly, the specialized post-commercial culture phase of broodstock nutrition is not relevant here, but is comprehensively covered elsewhere (Harrison, 1990; Bray & Lawrence, 1992; Browdy, 1992). This thesis addresses only those aspects of nutrition of relevance to intensive nursery and pond culture of shrimp, and particularly those of energy requirements as met by the supply of dietary proteins, lipids and carbohydrates.

Similar to fish, the culture of shrimp has a history stretching back many hundreds or even thousands of years. The original culture systems relied on the natural productivity of the ponds or enclosures to supply all of the nutritional requirements of the shrimp. As shrimp culture technology advanced, particularly that of artificial propagation, there has been a trend for increasingly intensive systems. This trend is likely to continue since the land available for aquaculture is declining drastically due to over-population (FAO, 1981). As intensity levels increased, it became necessary to supply nutritionally complete, prepared diets which were able to support maximum shrimp growth and survival at minimum cost (Hepher, 1979; Lovell, 1989; Tacon, 1990). Indeed, it has been stated that much of Taiwan's early success in intensive shrimp production could be attributed to the development of formulated feeds (Liao & de Oliveira Gomes, 1989).

The nutrition of shrimp is still in its infancy, research being initiated only in the 1970s as advances in technology permitted intensification of the culture systems (Deshimaru & Shigeno, 1972; Liao & de Oliveira Gomes, 1989). Furthermore, most nutritional research has been conducted by feedstuff manufacturers and the results are therefore proprietary (New, 1980). In comparison with shrimp, fish nutrition has a slightly longer history, but itself lags far behind that of terrestrial animals. Many of the diets currently formulated for shrimp are thus based on research conducted on fish or terrestrial livestock, supplemented with intuition and "unknown growth factors", rather than on scientific studies on shrimp nutrition itself (Akiyama *et al.*, 1992). Indeed, the first diets used for shrimp culture included pellets formulated for broiler chickens, pigs, gamebirds and fish (Sandifer & Smith, 1985). Nevertheless, complete formulated rations are now available that, when fed to shrimp under intensive (up to 100 shrimp

m²) culture in ponds, result in the economic production of large quantities of shrimp (up to 15 t ha⁻¹ cycle⁻¹) at food conversion ratios (FCRs) of between 1.2:1 and 2:1 (Akiyama, 1992).

Despite the undeniable recent progress in shrimp nutrition, many authorities have suggested that one of the major constraints to commercial shrimp culture is the requirement for the development of nutritionally complete, digestible, water stable and cost-effective rations (Akiyama, 1992; Akiyama *et al.*, 1992; Cuzon, 1993). A recent worldwide survey on shrimp pond management (Hopkins & Villalon, 1992) placed nutrition, including feed quality, price and application technology, as the third most important area requiring research and development effort. Stock quality/disease and pond dynamics/water quality maintenance were considered to be the most important areas. Feed quality was also placed fourth, behind seed quality, seed availability and disease, in terms of the most important impediments to increasing production and/or profitability (Hopkins & Villalon, 1992).

2.5.2 Importance of nutrition in shrimp culture

2.5.2.1 Feed cost

Feed costs amount to as much as 60 % of operational costs in intensive shrimp culture systems (FAO, 1983; Csavas, 1990; Chamberlain, 1992; Primavera, 1992). Reducing feed costs thus provides more opportunity for reducing production costs than any other single expense (Lovell, 1983). In Thailand, currently the largest producer of *P. monodon* in the world, the cost of intensive shrimp production is approximately £ 2.4-3.0 kg⁻¹. The cost of formulated feeds is currently £ 0.8 kg⁻¹ (pers. observation, 1993; NACA, 1994), which, at the commonly achieved wet weight FCR of 1.8:1 (NACA, 1994), accounts for 48-60 % of the production cost. Thus, if feed costs or FCRs can be reduced by optimisation of the dietary formulation and feeding regime, significant reductions in the cost of producing shrimp can be achieved. Feed ingredients may represent up to 80 % of the final cost of shrimp diets (Chamberlain, 1992). Since protein sources such as fish meal (see section 2.5.2.3), are the most expensive sources of energy, minimisation of protein levels (so that protein is not catabolized for energy),

maximisation of protein retention and replacement of fish meal by cheaper protein sources may have the greatest potential in reducing feed cost. Proximate analysis (by this author) of diets which are currently used in intensive pond culture of *P. monodon* in Thailand may be seen to contain approximately 45 % protein derived mainly from fish meal (Table 2.12). Fish meal is currently included in shrimp diets at levels of 30-40 % (AQUACOP & Cuzon, 1989; New & Wijkstrom, 1990; Akiyama *et al.*, 1992) and can account for up to 60 % of the cost of the dietary ingredients (Aquastar, pers. comm., 1993).

There is considerable potential to reduce these high protein levels since diets tested in laboratory tank trials (with no access to supplementary feeding), containing protein levels as low as 30-35 %, have been shown to promote growth of *P. monodon* equal to that of diets containing 40-45 % protein (see section 2.5.5).

Table 2.12 Proximate analysis of commercial feeds used in Thailand for *P. monodon* pond culture (mean % of dry weight \pm sd).

Nutrient	Feed (n=9)
Protein	45.43 \pm 2.62
Lipid	6.08 \pm 0.51
Ash	12.76 \pm 0.80
Fibre	3.07 \pm 0.36
Carbohydrate	22.98 \pm 2.40
Dry matter	90.32 \pm 1.10
Nitrogen	7.08 \pm 0.59
Phosphorus	1.34 \pm 0.20
Carbon	43.16 \pm 1.71

2.5.2.2 Shrimp growth

The field of nutrition is large and primarily involves the supply of essential dietary nutrients to the cultured animals (Tacon, 1990). This encompasses all of the chemical

and physiological processes which provide nutrients to the animal for normal maintenance, functioning and growth, involving ingestion, digestion, absorption and transport of nutrients and waste removal (Akiyama *et al.*, 1992). Many of these processes and requirements are similar for all animals, although the quality and quantity of nutrients required will vary between species and for numerous other reasons. As stated previously, the nutritional requirements of shrimp are far from being confirmed. Although scientific experiments may yet be able to determine diets that will maximize shrimp growth (to the levels achieved with fresh feeds), in the practical sense it may be more important to define diets which produce optimal growth in terms of profitability and cost-effectiveness. In order to optimise shrimp growth and reduce costs, further research is required into areas including specific nutritional requirements and their inter-relationships, feed ingredient quality and sourcing, feed processing technology, the cost-effectiveness of various dietary formulations, nutrient stability and loss, and the contribution of natural productivity.

2.5.2.3 Feed ingredients

Because shrimp naturally consume a wide variety of marine proteins (see section 2.5.4.3), formulated feeds were first developed based upon fish and shrimp meals. Lacking evidence of specific dietary requirements, these feeds were probably over-formulated and contained the essential nutrients in abundance. That this remains true is probably because feed margins are high and feed brand reputations are still being established (Akiyama, 1992; Akiyama *et al.*, 1992). Recently however, much concern has been shown regarding the wasteful conversion of fish, shrimp and other marine meals into shrimp flesh. This may be particularly important in light of the high requirement for protein in shrimp diets, the declining catches of fish and shrimp from the world's oceans and the inevitable fluctuations in supply, quality and cost of marine meals (Tacon & Jackson, 1985; New & Wijkstrom, 1990; Chamberlain, 1992). A report by Charoen Pokphand Group, the largest shrimp farming company in Thailand (Anon., 1993b), stated that rising prices for feed ingredients, and particularly fishmeal, were resulting in Thai shrimp becoming less competitive on world markets (see section 2.5.2.1).

Attempts are now being made to reduce reliance on fish meal in shrimp diets through increased use of other conventional feed ingredients, particularly soybean meal and other vegetable proteins. Soybean meal for example, has been included successfully at up to 40 % of shrimp diets (Akiyama, 1988). These ingredients often have their nutritional value optimised through the use of new processing technology, enzyme digestion techniques and supplementation with limiting amino acids (Tacon & Jackson, 1985). These techniques may be necessary for plant proteins since their use may result in decreased food utilisation efficiency and hence increased waste production, placing limits on fish meal substitution. Unconventional feed ingredients including single cell proteins, plant and animal protein concentrates, whole food organisms and animal and food processing wastes are also receiving increased attention (Tacon & Jackson, 1985; Akiyama *et al.*, 1992). The use of alternative marine meals such as squid, shrimp and bivalve meals has also gained in importance, often producing superior performance to fish meals due to their better amino acid profiles (Cruz-Rique *et al.*, 1986, 1987, 1989; Cruz-Suarez *et al.*, 1987; AQUACOP & Cuzon, 1989; Fox *et al.*, 1994). Shrimp farming was estimated to consume 180,000 t of fishmeal in 1988 and this may increase to 400-450,000 t by the year 2000 if the current levels of fish meal in diets are maintained (Phillips *et al.*, 1988; New & Wijkstrom, 1990). Although alternative ingredients may not entirely replace fish meal in shrimp diets, their development may reduce the reliance on this increasingly expensive and scarce resource (New & Wijkstrom, 1990).

2.5.2.4 Waste discharge

The environmental pollution from intensive aquaculture facilities, particularly salmon and shrimp farms, is becoming an increasing concern in many countries (Akiyama, 1992; Bergheim & Asgard, in press). Most of the wastes exported from intensive shrimp ponds are derived from the feed applied. Intensive shrimp farming requires high feeding rates due to the high density of shrimp stocked and the over-riding importance of pelleted diets to supply their nutritional requirements (New, 1990; Wang, 1990; Boyd & Musig, 1992; Clifford, 1992; Fast & Lannan, 1992). Pelleted feed application rates of up to 450 kg ha⁻¹ d⁻¹ in intensive Thai shrimp farms (Briggs & Funge-Smith, 1994; Clifford, 1992) and 670 kg ha⁻¹ d⁻¹ towards the end of ultra-intensive culture in

America (Wyban *et al.*, 1989) have been reported. Feeds supplied to intensive shrimp ponds in Thailand have been shown to account for 91-95 % of the nitrogen, 38-60 % of the phosphorus and 5 % of the solids entering the ponds (Briggs & Funge-Smith, 1994). Similar figures have been reported for warm-water marine fish ponds, with 88-99 % of the nitrogen and 75-94 % of the phosphorus being derived from the feed (Daniels & Boyd, 1989; Krom & Neori, 1989).

Since only 14 % of the pelleted feed applied is incorporated into shrimp biomass, 86 %, *i.e.* the vast majority of the feed entering the pond, is either metabolised by the shrimp or lost to the pond directly (Briggs & Funge-Smith, 1994; Phillips *et al.*, 1993). Similarly, 75-82 % of the feed fed to marine fish during pond culture has been shown to be wasted (Daniels & Boyd, 1989; Krom & Neori, 1989). Because this level of waste production exceeds the capacity of the pond to process the organic material from feed and faeces, it tends to accumulate in the pond sediments and water column (Boyd, 1992). If this is permitted, the excess nutrients and metabolites may increase to toxic levels, reduce dissolved oxygen concentrations, stress the shrimp and reduce both growth and survival (Costa-Pierce *et al.*, 1983; Millamena, 1990; Funge-Smith & Briggs, *in press*). Removal of these wastes is therefore required. Although this is attempted through water exchange, uptake by phytoplankton, intercropping with milkfish and sediment removal, none of these techniques is entirely effective at such high feed rates (Briggs & Funge-Smith, 1994; Boyd, 1992). In addition, flushing the ponds and removing sediments leads to problems with waste disposal and eutrophication of the immediate coastal environment. Boyd & Musig (1992) therefore postulate that the only effective way to reduce such pollution is to regulate the feed input to the ponds. This can only be achieved by increasing the feeding efficiency and/or reducing stocking densities. Increased feeding efficiency resulting in improved FCE and reduced wastage may be achieved through improvements in feeding management practices and diet quality.

Improvements in diet quality that are likely to help increase food conversion efficiency and reduce waste nitrogen (N) and phosphorus (P) production primarily involve optimisation of dietary protein (nitrogen) and energy levels, minimisation of dietary phosphorus levels and increasing overall diet digestibility (Akiyama, 1992; Barg, 1992;

Boyd & Musig, 1992; Phillips *et al.*, 1993). Figures 2.4 and 2.5 show theoretical calculations of the reductions in N and P loadings possible when protein (fishmeal) levels are decreased and/or FCRs decreased. Thus, if the protein:energy (Pr:E) ratio of diets can be optimised by using lipids and carbohydrates to spare protein, and protein levels reduced from 45 to 35 % without sacrificing growth or FCR, a 25 % reduction in N wastage can be achieved. Similarly, by reducing dietary fishmeal levels from 30 to 10 %, a 45 % reduction in P wastage is possible. These calculations also demonstrate the benefit of reducing the FCR of diets. Reducing the FCR of 45 % protein (30 % fishmeal) diets from 2:1 to 1:1 (as is routinely achieved with salmon diets) can result in a 60 % reduction in N wastage and a 55 % reduction in P wastage. Similar calculations of shrimp feed wastage have been performed by Lin *et al.* (1991) who estimate that waste production will be reduced by 65-75 % if the FCR can be reduced from 2:1 to 1.5:1. These calculations were partially confirmed by research in Thailand (Briggs & Funge-Smith, 1994), which recorded non-assimilated N wastage of 91 kg t⁻¹ shrimp fed a 45 % protein (30 % fishmeal) diet at an FCR of 1.9:1, and 105 kg t⁻¹ shrimp fed the same diet at an FCR of 2.1:1. Mean non-assimilated P wastage of 21 kg t⁻¹ shrimp from the 1.3 % P diet fed was recorded at an FCR of 2:1. In addition, the direct relationship between dietary protein level or the total amount of protein fed and ammonia and nitrite nitrogen production has recently been reported in intensive catfish ponds (Li & Lovell, 1992).

Reductions in dietary protein levels from 53 to 40 % (Colvin, 1976; Bautista, 1986) 35-36 % (Bages & Sloane, 1981; Shiau & Chou, 1991), or even 31 % (Shiau & Peng, 1992), while maintaining dietary energy levels constant, have been shown to result in non significant changes in shrimp growth rate during laboratory trials (see section 2.5.5.1, Table 2.13). Protein requirements may be expected to decline further under pond conditions with access to natural feeding and bacterial enhancement of applied feeds (Chamberlain, 1988). Hunter *et al.* (1987), analysing the biochemical composition of pond biota, shrimp ingesta and applied feeds in semi-intensive shrimp ponds, showed that the percentage dry weight of available protein in the formulated feed used increased from 14.4 % to 35.1 % after just 24 hours pond "conditioning". This, they suggested, was due to the microbial metabolisation of feed carbohydrates. Additionally, the protein level promoting maximum growth rate will not be the same as that resulting in

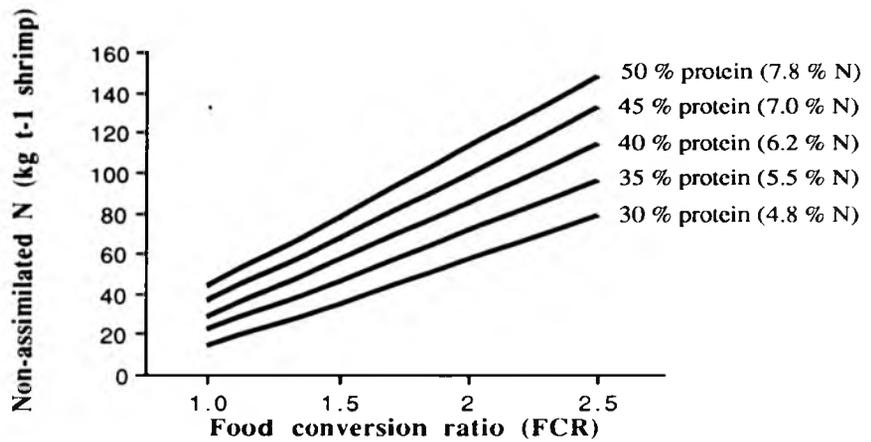


Figure 2.4 Relationship between dietary protein (nitrogen) level, food conversion ratio and non-assimilated nitrogen waste.

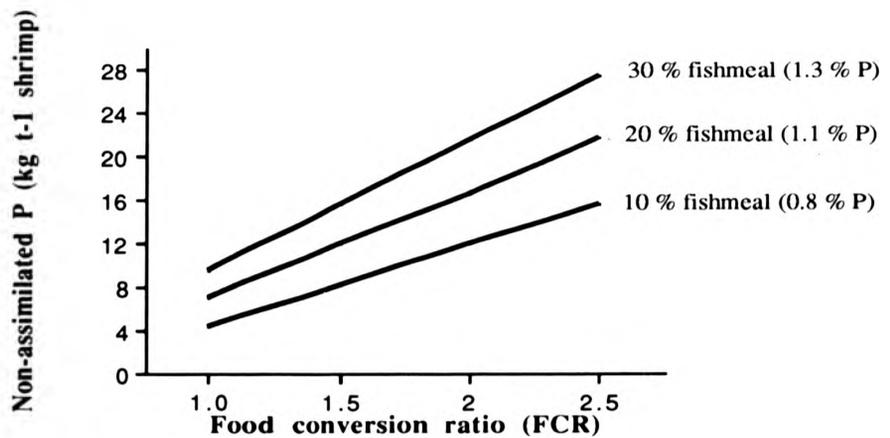


Figure 2.5 Relationship between dietary fishmeal (phosphorus) level, food conversion ratio and non-assimilated phosphorus waste.

minimum waste or maximum cost-effectiveness (Akiyama *et al.*, 1992). Reducing the level of dietary protein will reduce pollution since it has been shown to be directly related to total ammonia and nitrite nitrogen levels in fish ponds (Li & Lovell, 1992; Pruder, 1992). Optimised feeding management through monitoring and maximisation of shrimp feeding (*i.e.* the use of feeding nets) is also highly effective at improving food utilisation efficiency and reducing waste discharge from shrimp ponds (Chanratchakool *et al.*, 1994). Thus, the scope for reducing waste and increasing profit is clearly heavily dependant upon the feeding regime and the nutritional profile of the diet, particularly regarding protein and energy levels, phosphorus levels and digestibility (Akiyama, 1992). Currently little researched, identification of the most efficient dietary protein:energy ratios will allow better utilisation and reduced wastage of dietary nitrogen, the major component of feed waste (Akiyama, 1992).

2.5.2.5 Integration

Because the majority of the feed applied to intensive shrimp ponds is not incorporated into shrimp biomass, integration of other species has considerable potential to increase the utilisation efficiency of applied feeds, reduce waste discharge and increase income. Integrated shrimp culture systems have been proposed employing either polyculture with fish, bivalves and/or seaweeds, or the additional use of settlement ponds and biological treatment of waste water in order to reduce waste loadings (Ryther *et al.*, 1975; Wang, 1990; Wang *et al.*, 1990; Chandkrachang *et al.*, 1991; Lin *et al.*, 1991, 1992; Folke & Kautsky, 1992; Zhang & Wang, 1992; Briggs & Funge-Smith, 1993; Pullin, 1993). Such integration techniques are in need of further development to more fully assess their potential in maximising food use, alleviating environmental problems and promoting sustainable shrimp culture (Macintosh & Phillips, 1992; Phillips *et al.*, 1993; Briggs & Funge-Smith, in press).

2.5.2.6 Shrimp health

The relationships between the cultured shrimp, its environment and stress and disease are closely linked but poorly understood (Chanratchakool *et al.*, 1994). Some nutritional deficiency diseases and the effects that they have on the shrimp have been identified.

For example, various types of lipid including the highly unsaturated fatty acids eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), cholesterol and phosphatidylcholine are all known to be essential for penaeid shrimp (see section 2.5.5.4). Similarly, numerous amino acids, vitamins and minerals have been shown to be required for normal growth and survival of all shrimp species (Tacon, 1990). In addition, the presence of anti-nutritional factors, for example the trypsin-inhibitor lysinoalanine in alkali-treated soybeans, may give rise to nutritional pathologies (Tacon, 1990). Specific nutritional diseases that have been identified include blue shrimp, probably caused by carotenoid pigment deficiency, soft shell disease, possibly related to calcium and phosphorus nutrition, and moult death syndrome, probably resulting from a lack of lecithin in casein-based diets (Bowser & Rosemark, 1981; Conklin *et al.*, 1980, 1981; Kean *et al.*, 1985)(see section 2.5.5.4).

Identification of all of the essential, as well as the potentially harmful, dietary components is therefore imperative in order to optimise dietary formulations. However, diets and their nutritional quality may affect shrimp health in many other ways. For example, incorrect storage of feeds can lead to fat rancidity or fungal aflatoxins which may result in shrimp disease (Chanratchakool *et al.*, 1994). Underfeeding shrimp may also reduce production due to cannibalism, whilst overfeeding may result in pollution of the water by the uneaten food. Alternatively, the feeding of fresh feeds or animal meals containing bacterial or viral spores may result in shrimp health problems. Thus, ingredient selection, the correct treatment and storage of feeds and appropriate feeding management practices are all essential for optimising shrimp health and production.

2.5.3 Problems with nutritional research

The shrimp (and fish) nutrition research conducted to date has suffered from a number of problems. Regarding fish nutrition studies, Jobling (1983) has said that the wide range of experimental designs and methodologies employed has meant that comparisons of results obtained by various research groups is often difficult and that realistic interspecific comparisons are almost impossible. Some of the major problems encountered with the shrimp nutrition research conducted to date are examined in this section.

2.5.3.1 Species-specific differences

Although most shrimp species have similar nutritional requirements, specific differences exist between species. Most of the initial work on shrimp nutrition was carried out in Japan on *P. japonicus* starting in the 1970s. Later, during the 1980s, nutritional research was initiated on *P. monodon* and *P. vannamei* as their culture expanded in Southeast Asia and central/south America respectively. Species such as *P. japonicus* are mostly carnivorous and have protein requirements of 46-64 % of the diet. In contrast, omnivorous species such as *P. monodon*, have a lower requirement for dietary protein at 35-45 % (see section 2.5.5.1, Table 2.13). Thus, the requirements of one species cannot necessarily be inferred from those of other species.

2.5.3.2 Age-specific requirements

The requirements of each species also change through the life cycle and thus vary with age. This is largely due to the changing feeding habits, digestive physiology and growth rate of the shrimp through their life cycle (Tseng, 1988; Elliot *et al.*, 1989; Macdonald *et al.*, 1989; Lovett & Felder, 1990a,b). For example, lipid and protein requirements typically decrease as shrimp age and their growth rate declines (Colvin & Brand, 1977; Hunter *et al.*, 1987; Tacon, 1990). However, nutritional requirements may increase during the broodstock phase due to the heavy investment necessary for egg production. In order to investigate and ultimately optimise dietary nutrient profiles throughout the culture cycle of shrimp, it is therefore necessary to determine the nutritional requirements of each species at each stage from larvae to adult broodstock.

2.5.3.3 Source and physiological state of the shrimp

The source of shrimp used in nutrition trials and their physiological state is also important in terms of the results generated. Wild-caught shrimp generally perform better than those reared in a hatchery due largely to the lower quality of shrimp reared under artificial and often stressful hatchery conditions (Lin, 1989). The nutritional responses of the test animals will also depend upon the genetics and background of the shrimp used, particularly with regard to their past feeding history. Quality testing (*i.e.*

performing a stress test) of shrimp used during nutritional trials may therefore assist standardisation of research methodology (see section 2.4.3 and chapter 4). Stress tests have already been shown capable of determining differences between shrimp fed differently during the larval rearing phase (Preston, 1985; Tackaert *et al.*, 1989; Arellano, 1990; Durán Gómez *et al.*, 1991; see chapter 4). This is important because pre-experimental rearing conditions including nutrition clearly influence subsequent shrimp performance (Castell, 1989). Akiyama (1992) has suggested that growth and survival rates of shrimp during nutritional trials should be at least 85 % of an acceptable standard, with a coefficient of variation between replicates of < 10 %. If seed quality can be confirmed, performance below this standard may be assumed to be due either to water quality (which may be easily checked) or the quality of the diet fed. Ultimately, it would be desirable to establish standard strains of experimental crustaceans for use in nutritional trials (Castell, 1989).

2.5.3.4 Reference diets

The requirement for standard reference diets has been recognised as being essential for comparing results between different researchers and experimental systems (New, 1976a,b; Castell, 1989). Efforts have been made in this regard with the establishment of the International Working Group on Crustacean Nutrition in 1989 (Castell, 1989).

This group has formulated standard reference diets for use in nutritional trials with various species of crustaceans, but based on studies with lobster nutrition. However, species and age specific diets have yet to be developed, the diets have yet to be used by many of the researchers working in this field and, of course, these diets were not used prior to 1987.

2.5.3.5 The relationship between tank and pond trials

It is well known that the growth rates of shrimp and prawns fed formulated feeds in laboratory tank trials are below those of shrimp fed similar diets in ponds or those fed fresh or live foods (Biddle *et al.*, 1977). There are many possible reasons for this phenomenon including the lack of sources of natural productivity within tanks, sub-

optimal dietary formulations, the presence of growth-promoting factors in live feeds and pond water, general stress under captive conditions, and poor acceptance and palatability of purified or semi-purified diets.

The controlled conditions which can be maintained in laboratory-based tank systems are commonly used to determine the precise nutritional requirements of shrimp without access to any other source of nutrition. However, even under intensive pond culture conditions, the shrimp always have some access to the natural productivity of the pond ecosystem (Burgett, 1989; Castille & Lawrence, 1989; Moss *et al.*, 1992). The extent to which applied feeds contribute to the nutrition of the shrimp directly, or merely add to the fertilization and hence natural productivity of the ponds and act as feed in that way has long been an area requiring research (Stahl, 1979; Fair & Fortner, 1981; Malecha *et al.*, 1981; Anderson *et al.*, 1987; Hunter *et al.*, 1987). Recent data has suggested that the shrimp can derive between 42 and 77 % of their nutrition from natural food sources (see section 2.5.4.3) within semi-intensive (17-20 shrimp m⁻²) shrimp ponds (Maguire & Bell, 1981; Anderson *et al.*, 1987; Hunter *et al.*, 1987). More impressively, work with stable carbon isotopes has showed that *M. rosenbergii* in pond polyculture with fish (at 5-15 prawns m⁻²) demonstrated feeding patterns and weight gain independent of the presence or absence of applied feed pellets (Cohen & Ra'anan, 1983; Schroeder, 1983 a,b). There have also been indications that unfed *P. monodon* will grow as well as those that are fed for up to six week (Gonzales, 1988). Similarly, Moriarty (1985, 1986) suggested from his studies on bacterial biomass, that most of the supplemental feed applied to shrimp ponds supported bacterial growth rather than being directly utilized by the shrimp. Hunter *et al.* (1987) meanwhile, showed that shrimp ingesta in semi-intensive ponds resembled the composition of detrital aggregates more than that of the pelleted diet applied.

In addition to the contribution of these feed sources, use of pond water (even from lined ponds) rather than recirculated seawater in tank trials has been found to increase shrimp growth by up to 89 % (Leber & Pruder, 1988; Moss & Pruder, 1991; Moss, 1992; Moss *et al.*, 1989, 1992). This effect is probably due to the nutritional value of suspended particles (particularly those of 0.5-5 microns in size) in the water, including faeces, bacteria and microalgae, particularly diatoms (the microbial-detrital complex)

(Stahl & Ahearn, 1978; Moss *et al.*, 1992). In contrast, solids of < 0.5 microns, including dissolved organic carbon, were not found to contribute to shrimp growth (Moss *et al.*, 1989, 1992). The results of nutrition trials in tanks may therefore be expected to bear little resemblance to those obtained under commercial conditions in ponds (unless under very intensive culture) and thus should be applied with caution.

Ultimately, the production of diets which supply only what is unavailable from the natural productivity of the ponds will result in the most cost effective and low pollution diets (Hunter *et al.*, 1987). It has been suggested that (at least in the early stages of the culture cycle) the natural productivity of shrimp ponds may supply only the micronutrients and some critical, but unidentified growth factors while applied feeds may supply the macronutrients necessary for shrimp growth (Fair & Fortner, 1981). Such reductions of feed quality (and possibly feeding rate) may thus greatly reduce feed costs whilst having little impact on the total nutrition of the shrimp (Anderson *et al.*, 1987; Burgett, 1989; Castille & Lawrence, 1989). The economic advantage of feeding nutritionally incomplete rations is clear from the data of Hunter *et al.* (1987), who state that chicken feeds containing only 25 % protein may be purchased for only 17 % of the cost of nutritionally complete shrimp diets. Research into this area of shrimp nutrition is still lacking and emphasis should perhaps be placed upon optimising the relationship between the pond soil type, detrital food chains and applied feed (Stahl, 1979), with attention also focused on the effects of culture intensity (Castille & Lawrence, 1989). Nevertheless, in order to quantify the value of applied feeds to shrimp, laboratory scale tank trials are still required in order to determine the total nutrient requirements of the shrimp.

2.5.3.6 Dietary formulation and manufacture

Dietary formulations used in trials conducted by the various researchers working on shrimp nutrition have varied widely. Many shrimp nutritionists have opted for diets based on purified or semi-purified ingredients including casein/gelatin/albumen or purified amino acid mixtures as protein sources, purified fatty acids as lipid sources and purified carbohydrate sources of α starch, sucrose or glucose. The use of such diets has advantages in terms of precise definition of dietary constituents, reduced interference

between the dietary ingredients and enhanced replicability. However, disadvantages include reduced acceptance of purified ingredients, amino acid deficiencies and unknown applicability to practical dietary formulation (New, 1980; Boghen & Castell, 1981; Maugle *et al.*, 1983b).

Ingredient selection, particularly in more practical style diets, is important. This is because the storage and processing of dietary ingredients can affect their nutritional quality (New, 1980; Tacon & Jackson, 1985). For example, some ingredients may contain anti-nutritional factors which may have more affect on shrimp performance than the nutritional composition of the diet (Akiyama, 1992). The pre-treatment of these ingredients, such as heat processing soybean meal (Akiyama *et al.*, 1985) or soaking ipil ipil (*Leucaena leucocephala*) meal (Chamberlain, 1992) may be able to reduce the levels of these anti-nutritional factors without compromising digestibility.

There is also a lack of standardisation of diet manufacturing techniques. The pellet processing techniques used differ between researchers (*i.e.* expansion or pelletisation) and are reflected in the properties of the diet, particularly with regard to its stability in water, level of moisture (softness) and homogeneity. Differences between results may then be due more to the form of the diet than its nutritional profile. All of these differences should be quoted in research papers and standardised wherever possible.

2.5.3.7 Feeding regime

The feeding regime used during nutritional trials may also affect the performance of shrimp fed a common diet. Feeding regimes should be standardised in order to assist comparison between studies. The feeding rate varies depending largely upon shrimp age. Some researchers feed to demand (*ad libitum*), while others feed at rates varying from 10 to 100 % of the total shrimp biomass daily. The frequency of feeding is also important in shrimp nutrition studies. This is because shrimp (unlike fish) are slow feeders, break up the food pellets outside of their bodies (leading to feed losses) and may wait for some time before ingesting the feed by which time it may have become colonised by bacteria and its nutritional profile changed. These habits tend to increase the leaching of nutrients, particularly water soluble vitamins, from the feed, making

estimations of feed ingestion and nutritional requirements very complicated. Feeding small quantities of feed frequently may therefore increase feed ingestion and reduce problems with diet stability. Indeed, frequent feeding, offering diets three to four times daily rather than only once has been shown to increase shrimp growth rates during laboratory trials (Sedgwick, 1979; Sampath & Srithar, 1987; Robertson *et al.*, 1993). During commercial pond on-growing in Thailand, formulated feeds are usually fed four to five times at up to 100 % of shrimp biomass daily and often to satiation towards the end of the culture period (NACA, 1994). The time of feeding may also be important. Species such as *P. monodon* tend to be nocturnal and feed most frequently at night (Boddeke, 1983). Attempts have been made to improve dietary utilisation by manipulating feeding times to coincide with shrimp activity. Thus, increased growth of *P. japonicus* has been achieved by feeding three hours after dark when the secretion of digestive enzymes reached its peak (Cuzon *et al.*, 1982).

Ideally, shrimp should be fed *ad libitum* in order not to limit growth through restricted access to food. This is particularly important in investigations of energy requirements because if, as is thought, shrimp regulate food consumption on the basis of energy intake (Adolph, 1981; Cho & Kaushik, 1985), the feeding of diets with low levels of energy or low digestibility will result in increased food consumption if the food is available. Thus, restricting the ration leads to low consumption and poor growth on low energy/digestibility diets. However, true *ad libitum* feeding is difficult without automatic feeders and will always result in food wastage making accurate calculations of feeding efficiency and growth rate impossible (Jobling, 1983; Gusman & Leber, 1989). Thus, frequent feeding is a compromise solution and is used by most workers in studies of shrimp nutrition.

Shrimp are able to absorb nutrients, particularly minerals, directly from the water as well as from ingested material. Akiyama (pers. comm., 1991) found that significant growth (200 % increase over two weeks) of unfed shrimp in laboratory tank trials was eliminated and death resulted within six to eight days of the water being filtered to 50 microns. Indeed, it was found that by filtering the water in this way, the variability between replicates was reduced due to the elimination of this source of nutrition. Filtration and UV sterilisation of recycled water has been used to eliminate non-dietary

sources of nutrition by other authors (D'Abramo *et al.*, 1988; Castell, 1989). In addition, shrimp are coprophagous, cannibalistic and are able to reject fibrous or indigestible portions of the food before ingestion. All of these factors tend to complicate the estimation of nutritional requirements (New, 1980) and efforts should thus be made to reduce non-dietary sources of nutrition. This may be achieved through the above water treatment methods, the daily removal of uneaten food, exuviae and faeces and regular cleaning of tanks to remove algae, fungi and bacteria (Castell, 1989).

2.5.3.8 Experimental design

The design of nutrition trials may vary widely between different researchers. The holding facilities themselves, even in tank-based laboratory trials, may vary in terms of tank colour, shape and size (area and depth), water quality control (particularly temperature and salinity), photoperiod, light intensity, water exchange, substrate and habitat presence and so on. In addition, the animals may be held either communally or individually. Individual housing tends to reduce growth rates but is necessary to prevent cannibalism in carnivorous species and allows more accurate interpretation of results and may be used for all species to standardise results from different authors. The feeding regime employed is also of importance in this regard. The stocking density of shrimp during nutritional trials also varies widely between researchers. Another important factor is whether mortalities and/or the exuviae of recently moulted shrimp are removed from the tanks or not. This is important in nutritional trials since considerable nutritional benefit can be derived from such sources and their consistent removal from the tanks presents considerable practical difficulties. In many instances some of these details of experimental design are not reported. Such features are thus in need of standardisation towards optimal conditions for each species to be able to compare the results of different investigators (Castell, 1989).

2.5.3.9 Analytical techniques

There are a range of parameters which are used to determine the performance of shrimp during nutritional trials. Commonly, growth and survival rate data are used, but different measures are used by the various researchers leading to some confusion

between results. It is still far from clear which are the best indices of shrimp growth. Gain in weight, length (shell size) and dry body tissue have all been used. All of these parameters are complicated in shrimp nutrition studies since growth in length and wet weight is not continuous, but occurs through rapid periods of expansion immediately after moulting. This is then followed by long intermoult periods where very little wet weight is gained as water is gradually replaced by protein (see section 2.2.6). Final proximate analysis of shrimp produced from the trials is therefore necessary in order to quantify true growth which must be on a dry weight basis. The development of growth models for crustaceans that allow comparisons between variations in weight gain per moult at each developmental stage and the length of the inter-moult period in response to dietary differences may thus assist evaluation of shrimp growth responses (Castell, 1989).

Biochemical growth indices including measures of metabolic rate (*i.e.* oxygen consumption and nitrogen excretion rates) (Nelson *et al.*, 1977; Clifford & Brick, 1978, 1979; Dall & Smith, 1986; Kurmaly *et al.*, 1989c; Hewitt & Irving, 1990), digestive enzyme activity (Lee *et al.*, 1980, 1984; Van Wormhoudt *et al.*, 1980; Lee & Lawrence, 1985; Leung *et al.*, 1990), nutrient assimilation (Fenucci *et al.*, 1982; Pierce & Laws, 1982; Brown *et al.*, 1986) and histopathological changes (Pascual *et al.*, 1983; Storch *et al.*, 1984; Vogt *et al.*, 1985, 1986; Vogt, 1990) have also been used to measure nutritional responses. In many instances these indices may be quicker and more sensitive than changes in weight gain and survival in determining differences between treatments (Cuzon *et al.*, 1980; Vogt *et al.*, 1985; Vogt, 1990; Fox *et al.*, in press).

In the practical sense, the optimum diet is that which produces the largest biomass of shrimp for minimum cost. Thus, total biomass gain (yield or production), taking into account both growth and survival, together with some estimation of feed conversion and diet cost will be the most important parameters.

In order to establish the nutritional requirements of any species, the digestibility of the feed ingredients and the diet as a whole is vital. Although some work on nutrient digestibility has been conducted (see section 2.5.4.2), difficulties have been encountered with measuring digestibility in terms of the techniques used and variation due to factors

including shrimp feeding habits, size, and dietary ingredient source and level.

The first problem involved with digestibility determination with shrimp (and fish) is that it is necessary to collect faeces from an aqueous environment. The collection of naturally produced faeces either directly from the tanks or following filtration has been used, but leaching of nutrients from the faeces tends to overestimate digestibility (Hepher, 1988). Alternatively, techniques used on fish have involved manual stripping of faeces (Nose, 1960) and anal suction and fish dissection (Windell *et al.*, 1978). These methods however, are difficult with shrimp and may result in the addition of undigested physiological fluids and intestinal epithelium to the rectal contents which may underestimate nutrient digestibility.

The most common method for determining digestibility in both shrimp and fish involves the use of indigestible markers. Although a range of markers including chromic oxide (Cr_2O_3), polyethylene, acid-insoluble ash and crude fibre have been tried (Tacon & Rodrigues, 1984), chromic oxide has been the indicator most widely used. Relative changes in the percentage of chromic oxide present in the feed and faeces are used to represent the percentage of the feed that has been digested. By proximate analyses of both feed and faeces, the digestibility of each nutrient and/or the diet can be determined (Cho, 1987).

There are, however, numerous problems encountered with the use of chromic oxide as a digestibility marker for shrimp. Firstly, chromic oxide is thought to pass through the digestive tract at a different rate to the digesta (Leavitt, 1981). This variability in excretion pattern however, may be minimised by collecting faeces continuously over a period of days (Tacon & Rodrigues, 1984). More importantly, unless the faeces are removed quickly from the water, leaching of nutrients from the faeces will provide erroneous results. In addition, and in contrast to fish, shrimp tend to break up the food outside of their body and are able to reject large or other undesirable particles before ingesting the diet (Forster & Gabbott, 1971). Shrimp also break up their cast exuviae, consuming some and leaving other portions within the tank. Thus, it is extremely difficult to separate uneaten food and exuvium particles from faeces and regurgitated food and chromic oxide (which must also be collected), leading to underestimations of

nutrient digestibility. This is particularly the case with the small faecal strands produced by young shrimp commonly used in nutritional trials. Thus, although finely milled chromic oxide particles may not be regurgitated by shrimp (Forster & Gabbott, 1971), gravimetric techniques on total faecal collections may be preferable for use with crustaceans (Leavitt, 1981). Despite these problems, chromic oxide was used in this thesis for the sake of comparability between this and previous work, and because of similar problems with other markers for use with crustaceans.

The description of energy contents and flows is a major problem in nutritional studies with both fish and shrimp (Jobling, 1983). Since much of the work conducted has been on protein requirements (due to its over-riding importance in shrimp nutrition), the term protein:energy ratio has been used extensively (Bautista, 1986; Hajra *et al.*, 1988; Shiau & Chou, 1991; Cuzon *et al.*, 1993; see section 2.5.5.2). There is however, little agreement on whether the protein content (either total or digestible) should be expressed relative to the total, digestible or metabolisable energy content of the diet. Additionally, there is little agreement on how these energy values should be measured. Thus, direct comparison between results of different authors is difficult.

Gross or total energy is relatively easy to determine, but does not give any indication of the amount of energy available for metabolism and growth which is of most interest to nutritionists. Digestible energy (total energy minus that portion of the food energy voided in the faeces) must be measured through the use of digestibility estimates for each trial (rather than from 'standard' digestibility values). Metabolisable energy values for diets (total energy of the diet minus total energy of the faeces, urine and branchial waste) are currently unknown for shrimp and difficult to estimate as they vary with many factors including feed composition and feeding level (Jobling, 1983). Since the regulation of food consumption is adequately explained on the basis of a response to the digestible energy content of the diet (Hunt, 1980), it is perhaps best to express the concentration of specific nutrients relative to this parameter (Jobling, 1983). However, most of the studies conducted with shrimp to date have used total energy values. This is probably due to the difficulty of accurately measuring digestibility in shrimp as detailed above. For the sake of comparison between different authors, total energy values may thus be used until further work on digestibility of the various ingredients

used is available. The relationship between nutrient concentration and total energy is thus used in this thesis.

For determination of energy content in feeds, three methods are commonly used. These are chemical wet oxidation using dichromate, bomb calorimetry and chemical composition (proximate) analysis followed by the use of caloric (joule) conversion terms. Wet oxidation is rarely used in nutrition and bioenergetic studies since it is time consuming and tends to underestimate the gross energy content (Henken *et al.*, 1986). The bomb calorimeter measures the heat of combustion during the burning of a known (usually 0.9-1.0 g) amount of sample. Thus the energy content of the material is measured directly. Proximate analysis followed by the use of energy conversion terms measures the energy content indirectly. A number of errors can arise using this method. These include differences in the techniques used for quantifying levels of the three energy substrates of protein, lipid and carbohydrate. In addition, the most commonly used physiological fuel values (4.1, 9.45 and 5.65 kcal g⁻¹ or 17.2, 39.5 and 23.6 kJ g⁻¹ for carbohydrate, lipid and protein respectively) may vary between authors and are not appropriate for all biological materials (Cho *et al.*, 1982; Jobling, 1983; Henken *et al.*, 1986). It is important therefore, to standardise the analytical techniques used, to adjust the conversion terms used for the materials analysed, and to compare the results obtained with those from bomb calorimetry (Jobling, 1983).

Henken *et al.* (1986), in comparing the methods used for estimating the energy content of feeds, fish tissues and faeces found that total energy is most accurately determined by bomb calorimetry and that the other methods underestimate dietary and faecal energy contents. The proximate analysis techniques used for the calculation of energy content are usually modified Kjeldahl nitrogen x 6.25 for protein, Soxhlet ether or methanol/chloroform extraction for lipid, and the difference between dry matter content and the sum of protein, fat and ash in the dry matter for carbohydrate. The modified Kjeldahl technique measures the nitrogen content of the sample which is then converted to protein by multiplying by a factor of 6.25. However, the implicit assumptions that protein contains 16 % nitrogen and that all nitrogen is bound in protein are unsound (Jobling, 1983). For example, conversion factors of 6.38 should be used for casein due to the amino acid profile of this milk protein (Osborne & Voogt, 1978). Additionally,

non-protein nitrogen may be present only seasonally, or in various concentrations depending on protein source (Jobling, 1983). Henken *et al.* (1986) found that using hexane extraction, some lipids were not extracted and were grouped with carbohydrate leading to underestimations of energy content in the feed and faeces. Craig *et al.* (1978) and From & Rasmussen (1984) however, used methanol/chloroform extraction and estimated that conversion factors of 8.5 kcal g⁻¹ (35.5 kJ g⁻¹) respectively should be used for lipid to obtain comparable results with bomb calorimetry. Problems with carbohydrate content analysis may also arise since the usual "by difference" method tends to compound errors from analysis of the other dietary components. In addition, the energy value of different carbohydrate sources varies considerably (Jobling, 1983). Thus, bomb calorimetry seems to be the most accurate method of determining energy value and should be used to confirm calculated values wherever possible. Where bomb calorimetry has not been conducted by previous authors, comparison between results is possible through calculation provided that the techniques of proximate analysis and the dietary ingredients used are known.

The techniques used for proximate analysis of diets and shrimp must clearly be standardised and referenced to assist comparison of data generated by different workers. Additionally, it has been suggested that amino acid and fatty acid analyses should be performed rather than protein and lipid analyses since shrimp, like fish and other animals do not have true protein and lipid, but amino acid and fatty acid requirements (see sections 2.5.5.1 & 2.5.5.4).

Comparison of the results generated by different authors may also suffer from the large number of different statistical analyses that the data are subjected to. This aspect of nutritional research is another that is in need of further attention.

2.5.4 Feeding biology, digestion and the natural diet

In order to improve the nutritional value and cost-effectiveness of diets used in intensive shrimp culture, an understanding of the feeding biology, digestion and natural diet of the shrimp is required. This knowledge is essential for the development of low-cost, nutritionally complete and digestible diets which are appropriate for shrimp at each

stage of the culture cycle.

2.5.4.1 Feeding biology

In the wild, shrimp feed mainly nocturnally and vision plays a minor role in food detection (Hindley, 1975). Instead, food detection is usually accomplished by chemosensory means. Cuticular chemoreceptors (usually associated with hairs and setae) are located primarily at the anterior end of the body on antennules, mouthparts, chelae, antennae and maxillipeds (Heinen, 1980; Bailey-Brock & Moss, 1992). During foraging, the first three pairs of chelate pereopods are used to detect, seize and convey the food items to the mouthparts. Chemoreceptors in the mouthparts are thought to mediate ultimate acceptance or rejection of the food (Schmitt & Ache, 1979). Feeding in penaeids is stimulated by a range of chemicals. These chemicals are generally low molecular weight organic compounds including amino acids (particularly mixtures), nucleotides, quaternary ammonium compounds, yeast extracts and highly unsaturated fatty acids (Hindley, 1975; Carr, 1978; Heinen, 1980; Bryant *et al.* 1989; Hartati, 1991; Hartati and Briggs, 1993). The food seeking response is stimulated by concentrations of these substances as low as 10^{-5} - 10^{-6} molar, while stimulation of the mouthparts requires concentrations of 10^{-1} - 10^{-2} molar (Hindley, 1975). Small food particles may be placed directly in the pre-oral cavity and larger ones held by the third maxillipeds and further broken down by the gastric mill of the foregut before ingestion (Alexander *et al.*, 1980; Gibson, 1981). Sand grains, fibrous material and other inedible items can be sorted in the pre-oral cavity and either rejected (Alexander & Hindley, 1985) or ingested to aid in food grinding (Suthers, 1984).

2.5.4.2 Digestion

Digestion begins as the food passes rapidly through the elastic foregut, or proventriculus. This is of ectodermal origin and has a chitinous cuticle where some enzymes may be secreted, trituration and storage occur (Dall & Moriarty, 1983). On entry into the endodermal midgut, including the digestive gland, most of the digestive enzyme secretion, digestion and absorption occurs (Dall & Moriarty, 1983; Lovett & Felder, 1990a,b). The midgut digestive gland is the main site of this activity, and it

may also be involved in excretion, lipid and carbohydrate metabolism and the storage of inorganic reserves (Gibson & Barker, 1979). No true hepatic function is known however, and the term hepatopancreas is therefore considered inappropriate (Phillips *et al.*, 1977; Dall, 1981). Indigestible portions of the food then pass into a short, tubular, chitinous hindgut of ectodermal origin. Here, smooth, longitudinal, muscular pads grip the faecal mass, bound by a peritrophic membrane, and expel it through rhythmical, contractions through a distal sphincter (Dall & Moriarty, 1983; Bailey-Brock & Moss, 1992; Fox *et al.*, 1994).

Much work has been done recently on the digestive enzymes of crustacea and is reviewed by Dall & Moriarty (1983), Lovett & Felder (1990a,b) and Fox *et al.* (1994). The main endogenous digestive enzymes identified have included proteinases, carboxypeptidases, esterases, lipases, amylases, chitinases, cellulases, elastase and collagenase. Protein digestion is mainly through the action of trypsins and possibly chymotrypsins, whilst pepsin activity has been largely unrecorded (Lee *et al.*, 1980; Maugle *et al.*, 1982a; Tsai *et al.*, 1986). Little is known regarding the digestion of lipids by shrimp, but both esterase and lipase activity have been recorded, with a range of dietary lipids being assimilated efficiently (Lee *et al.*, 1980; Lee & Lawrence, 1982; Teshima & Kanazawa 1983).

A wide range of carbohydrases capable of digesting carbohydrates, have been reported including α and β amylase, maltase, sucrase, chitinase and cellulase (Lee & Lawrence, 1982; Maugle *et al.*, 1982b; Lovett & Felder, 1990a,b). The production of these latter two enzymes however, have usually been attributed to gut microflora, although their relative importance is not well understood (Hood & Meyers, 1973; Fair *et al.*, 1980; Ninawe & Banik, 1987). Chitin, entering the gut of penaeids through the addition of feed ingredients such as shrimp meals or the consumption of cast exuviae, is not well utilised by shrimp (Fox, 1993). Chitin may however, be partially digested through the action of gut or sediment bacteria, particularly under pond culture (Fox, 1993). Cellulose, a common ingredient in experimental shrimp diets, is considered to be indigestible and is thus used as a non-nutrient filler. The action of gut flora in digesting cellulose, particularly in pond trials, is thus worthy of further investigation in order to quantify its digestive capacity.

Work has also been conducted with some success in attempting to supplement diets with exogenous enzymes from either live diets or isolated and microencapsulated enzymes, in order to enhance the digestive capacity of shrimp. Live diets of short-necked clams, containing high levels of amylase and protease activity, were shown to promote better growth and survival in *P. japonicus* than freeze-dried clam diets (Maugle *et al.*, 1982b). In addition, supplementation of formulated feeds with microencapsulated amylase or bovine trypsin has been shown to improve growth and carbohydrate mobilisation and digestion in *P. japonicus* (Maugle *et al.*, 1983a,b). These results suggest that these enzymes may have improved shrimp growth and may be a possible reason for the improved performance of shrimp fed on live rather than formulated rations.

There is also evidence that some species are able to alter their endogenous digestive enzyme profiles depending upon their previous dietary history (Bailey-Brock & Moss, 1992). It has been suggested that dietary formulations may be predicted based on the enzyme spectra of each species (Fair *et al.*, 1980; Lee *et al.*, 1980). However, the level of digestive enzyme activity also depends on a number of other factors including shrimp age, size, species, stage in the moult cycle, diet and trophic status (Fox *et al.*, 1994). Thus, increased digestive enzyme activity may result for example from the development of the animals digestive apparatus as it grows (Elliot *et al.*, 1989; Macdonald *et al.*, 1989; Lovett & Felder, 1990a,b), or due to low levels of nutrients in the diet (Lee & Lawrence, 1985). When this is allied to the fact that little correlation has been shown between maximum digestive enzyme activity and growth (Lee *et al.*, 1984; Van Wormhoudt *et al.*, 1980; Chen & Lin, 1992), the enzyme activity of post-larval shrimp may be incapable of providing a practical indication of their nutritional requirements or digestive capabilities.

The techniques of and problems with measuring digestibility or assimilation (digestibility and absorption) efficiency in shrimp have been considered in section 2.5.3.9. Despite the problems encountered, digestion efficiencies for penaeid shrimp have been widely reported. Proteins, particularly of animal origin, are generally well digested, with apparent digestion efficiencies of 73-94 % being recorded independent of species, size or dietary level (Fenucci *et al.*, 1982; Teshima & Kanazawa, 1983; Lee & Lawrence, 1985; Smith *et al.*, 1985; Akiyama *et al.*, 1988, 1989; Akiyama, 1991;

Catacutan, 1991b; Shiau & Peng, 1992). Dietary lipids are also well digested, as is cholesterol in the presence of other lipid sources, with the apparent digestibility of a variety of oils being 75-93 % for adult *P. japonicus* (Teshima & Kanazawa, 1983) and *P. monodon* (Catacutan, 1991b), but only 45-70 % for juvenile *P. vannamei* (Smith *et al.*, 1985) and *Pleoticus muelleri* (Fenucci *et al.*, 1992). In contrast, carbohydrates are generally poorly digested, with apparent digestibilities of 36-76 % for the omnivorous *P. vannamei* (Akiyama *et al.*, 1989; Akiyama, 1991) and *P. stylirostris* (Fenucci *et al.*, 1982).

As is the case with digestive enzymes, little evidence has emerged for correlations between diet digestibility and growth (Fenucci *et al.*, 1982; Lee *et al.*, 1984; Smith *et al.*, 1985). This may however, be due more to dietary fibre levels and the difficulties encountered with measuring digestibility than its relationship with growth in shrimp (see section 2.5.3.9). It may be logical to consider therefore, that the inclusion of ingredients which are considered to be highly digestible may lead to diets which are more efficient nutritionally and which result in less waste.

2.5.4.3 The natural diet

The natural diet of a species can provide useful information on what its nutritional requirements might be at each particular stage of the life cycle. During the planktonic larval stages at sea shrimp will consume green algae, rotifers, other microplankton and microdetritus. The epibenthic post-larval and juvenile stages are spent in estuaries and coastal wetlands including mangrove forests. At this stage their preferred diet consists of plant matter including microalgae and macrophytes and animal matter including small crustaceans, molluscs, nematodes and polychaetes (Chong & Sasekumar, 1981; Gleason & Wellington, 1988; Tseng, 1988). When adult, living on the sea bed, they will eat larger benthic crustaceans, bivalves, polychaetes, fish and the larvae of other benthic animals such as echinoderms (Chong & Sasekumar, 1981; Stoner & Zimmerman, 1988; Tseng, 1988).

Shrimp at all stages of the life cycle beyond larvae will also consume large amounts of detrital aggregates (Chong & Sasekumar, 1981; Cockcroft & McLachlan, 1986),

although the relative importance of the attached microbial organisms (bacteria, protozoa and fungi), rather than the detritus itself, is unclear. Bacteria may be an important source of nutrition for shrimp, but it is thought that they are unable to supply sufficient quantities of some of the essential components necessary in formulated shrimp diets (Burgett, 1989; Castille & Lawrence, 1989). These essential components include polyunsaturated fatty acids, sterols, sulphurous amino acids including methionine, mineral supplements, and energy (Phillips, 1984; Castille & Lawrence, 1989; Cammen, 1980). Bacteria and other microorganisms however, have a more complex value to shrimp nutrition. Internally, they assist the uptake of dissolved organic matter and actively aid the elaboration of digestive enzymes such as chitinase. Chitinase is essential for the digestion of chitin which is ingested in the form of exuviae or other prawns (Hood & Meyers, 1973; Stahl, 1979; Maugle *et al.*, 1982a,b, 1983 a,b). Further, it has been reported that 85 % of penaeid gut bacteria are able to produce chitinase and that glucosamine, an end product of chitin degradation, is a growth factor required by shrimp (Hood & Meyers, 1973; Stahl, 1979; Maugle *et al.*, 1982a,b, 1983a,b). Additionally, it has been suggested that the midgut gland of shrimp contains a bacteriolytic agent enabling bacterial protein to be released for utilisation (Hood & Meyers, 1973; Stahl, 1979). The nutritional value and importance of protozoans, fungi and the detritus itself, largely composed of structural polysaccharides and other polymers from plant matter (Fenchel & Jorgensen, 1977; Lee, 1980), is even less known (Phillips, 1984; Bailey-Brock & Moss, 1992).

Studies conducted on shrimp using stable carbon isotope ratio and ingesta analysis techniques both in their natural habitats and in culture ponds, have assisted identification of the food webs utilised by penaeid shrimp. Studies in coastal mangroves and saltmarshes have shown that although little carbon is derived from macrophytes or macrophyte-derived detritus or associated heterotrophic microbes, major nutritional pathways may originate from planktonic microalgae (Gleason & Wellington, 1988), epiphytic algae (Fry, 1984) and/or benthic algae (Stoner & Zimmerman, 1988). Studies using these techniques in ponds, meanwhile have revealed food sources consisting mainly of detritus, phyto- and zoo-plankton and larger animals including polychaete worms, molluscs and crustaceans (Maguire & Bell, 1981; Hunter *et al.*, 1987). These food sources enter the ponds during water exchange or are stimulated by the

fertilization of the ponds by the applied feeds and fertilizers and shrimp faeces. In extensive and semi-intensive culture at least, these food sources may provide a larger proportion of the shrimp's nutritional requirements than the applied feeds (Moriarty, 1985; Anderson *et al.*, 1987; Hunter *et al.*, 1987) and hence are of considerable importance in the management of feeding in shrimp ponds. The contribution of natural food to the nutrition of shrimp in ponds should thus be considered when applying the results of laboratory-based nutrition trials to the formulation of least-cost, low protein diets.

2.5.5 Nutritional requirements of *Penaeus monodon*

This thesis concentrates upon the dietary energy requirements of *P. monodon* and how these requirements can be met through optimising the sources and levels of the energy-yielding substrates of protein, lipid and carbohydrate in the diet. This section therefore reviews the published data (mostly from laboratory-based trials) on these aspects of nutrition in penaeid shrimp. Wherever possible, data for *P. monodon* has been used, but where that data is unavailable, information derived from work with other penaeid species, freshwater prawns, lobsters and fish has been included. Information on the requirements for other nutrients can be found in a number of reviews of shrimp nutrition published over the past ten years (Kanazawa, 1985a; Teles, 1986; Liao & Liu, 1990; Tacon, 1990; Akiyama, 1992; Akiyama *et al.*, 1992; Cuzon, 1993; Fox *et al.*, 1994).

2.5.5.1 Protein

2.5.5.1.1 Structure and function

Proteins constitute the major proportion (65-75 %) of the total dry weight of animal tissues and are particularly high in fish and crustaceans (NRC, 1983; Walton, 1985; Hepher, 1988). Proteins are complex organic compounds of high molecular weight containing (in common with lipids and carbohydrates) carbon, hydrogen and oxygen. In addition however, they also contain approximately 16 % (12-19 %) nitrogen, and

sometimes phosphorus and sulphur (Tacon, 1990). When proteins are hydrolysed with acid or alkaline solutions or enzymes, 20 commonly found amino acids are obtained from animal tissues. These amino acids are linked together through peptide bonds to form polypeptides or polymerised units of amino acids (Shepherd & Bromage, 1988).

Physical and chemical differences between the amino acids and their polymers give rise to proteins with varying structural configurations. These differences allow the proteins to assume a wide range of functions within the animal body. These functions include roles in structure (collagen, chitin, elastin, glyco- and muco-proteins), storage (casein, ferritin), transport (haemo- and myo-globin, haemocyanin, serum albumin, β -lipoprotein), contraction (myosin, actin), protection (fibrinogen, thrombin, antibodies), and as enzymes, hormones and toxins. Proteins are also used as substrates for energy metabolism (Walton, 1985).

Due to the fact that the configuration, and hence function, of each protein can be changed by subtle differences in amino acid patterns, the successful synthesis of proteins relies on the simultaneous availability of all of the required amino acids. In shrimp, as in other animals, there is therefore a requirement for a certain quantity of specific amino acids, rather than for protein *per se* (Cho *et al.*, 1985; Walton, 1985). The ability to synthesise amino acids from simpler precursors varies with species, but for most animals (including fish and shrimp), some cannot be synthesised (at least at a sufficient rate) and must be supplied pre-formed in the diet (NRC, 1983; Steffens, 1989). Amino acids in this group are termed essential, whilst those which can be synthesised from other amino acids or their precursors are known as non-essential (NRC, 1983). These terms however, refer only to the dietary requirement since they are all essential within the body of the animal. In common with terrestrial vertebrates, fish and shrimp require the same ten essential amino acids within their diet. These are threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine and tryptophan. In addition, inter-conversion of certain amino acids (for example methionine to cystine and phenylalanine to tyrosine) is possible, so that the requirement for these two essential amino acids is partially dependant upon the corresponding non-essential amino acids in the diet (Tacon & Cowey, 1985).

2.5.5.1.2 Qualitative amino acid requirements

In order to establish the quantitative requirement for dietary amino acids, their qualitative need must first be established (Biddle *et al.*, 1977). Knowledge of a species' amino acid requirements and the essential amino acid profile of the protein sources used in the diets for that species are of primary importance in determining the requirements for dietary protein (amino acids). Results of previous estimations of dietary protein requirements have probably been confused due to the lack of this information and may thus be responsible for the reports of very high dietary protein requirements (New, 1980; Corbin *et al.*, 1983; Kanazawa, 1985a).

The complete qualitative essential amino acid requirements of prawns were first established by Miyajima *et al.* (1977) for *Macrobrachium ohione* by the oral administration of radioactively labelled U-C¹⁴ glucose. These authors found similar results to previous studies with fish and other animals, except that tryptophan was destroyed by the assay and its essentiality could not be assessed, and that tyrosine was essential. The requirement for tyrosine was also suggested for other prawn and shrimp species including *P. aztecus* (Shewbart *et al.*, 1972) and *M. rosenbergii* (Watanabe, 1975; Stahl & Ahearn, 1978). Using similar techniques, Coloso & Cruz (1980) with *P. monodon*, Kanazawa & Teshima (1981) with *P. japonicus* and Kanazawa & Teshima (1985) with *P. monodon*, found similar results for penaeid shrimp, but confirmed that tryptophan was essential. They also suggested that tyrosine could be considered as being semi-essential since it could be synthesised only slowly (low rate of radioactivity uptake) from phenylalanine, as has been shown for crustaceans in the Dungeness crab, *Cancer magister* (Lasser & Allen, 1976). Cystine, by contrast, was not considered semi-essential since it was found to incorporate an equally large amount of radioactivity as the non-essential amino acids in *P. japonicus* (Kanazawa & Teshima, 1981).

Other work on the qualitative requirements for essential amino acids has been more equivocal. Analysing the serum amino acid concentrations of shrimp (Dall & Smith, 1978) and prawns (Fair & Sick, 1982) during starvation, these authors assumed that the depletion of some amino acids previously regarded as non-essential (*e.g.* proline, glutamic acid and aspartic acid), were indeed critical for tissue metabolism during

periods of high metabolic rate. Whether these amino acids are truly essential has yet to be confirmed, but variations in the requirements may result from many factors including metabolic rate, moult cycle, age and salinity (Fair & Sick, 1982; Dall & Smith, 1987; Smith & Dall, 1991). The ability of gut or tank-dwelling microflora to supply essential amino acids (and other substances) to shrimp and prawns may also confuse the results of these studies (Hood *et al.*, 1971; Hood & Meyers, 1973). Stahl & Ahearn (1978) suggested that microorganisms may have contributed to results of their trials which disputed the essentiality of lysine, arginine, methionine and tryptophan in purified diets for *M. rosenbergii*. This fact may further complicate application of these results to the requirements of shrimp under pond culture conditions. This is because access to microbial/detrital aggregates may supply a large percentage of the essential amino acid requirements of the shrimp or prawns (Stahl & Ahearn, 1978; Castille & Lawrence, 1989). The role of each of the above mentioned factors in the amino acid requirements of shrimp are thus in need of further investigation.

The qualitative amino acid requirements of shrimp and prawn species are now at least partially known. The existing discrepancies in reported qualitative requirements are probably not a major problem in practical terms since most multi-ingredient formulated diets should not be qualitatively deficient in any essential amino acid (New, 1976a). The largely undefined quantitative essential amino acid requirements of shrimp thus pose a more important constraint on the formulation of cost-effective and nutrient-balanced shrimp feeds.

2.5.5.1.3 Quantitative amino acid requirements

The quantitative essential amino acid requirements of fish have traditionally been carried out by feeding graded levels of each amino acid within a crystalline amino acid test diet so as to elicit a dose-response curve (Ketola, 1982; Tacon & Cowey, 1985). The dietary requirement is then equated to the 'break-point' based on the observed growth response. This technique to date however, has established the complete quantitative requirements of only a few fish species and has failed with shrimp due to their poor assimilation of crystalline amino acids (Deshimaru, 1981; Deshimaru & Kuroki, 1974, 1982; Biddle *et al.*, 1977; Chen *et al.*, 1992a,b). The poor growth

obtained in these trials with shrimp may be due to excessive leaching of amino acids from the diet due to the feeding habits of the shrimp (Kanazawa & Teshima, 1981; Fortner & Sick, 1982; see section 2.5.3.9). Alternatively, it may result from disturbances to the normal metabolism of the shrimp, possibly involving gut pH balance (Deshimaru, 1976, 1982). In addition, and in common with fish, this technique has other disadvantages. These include, variable choice of reference protein (Tacon & Cowey, 1985), sub-optimal growth and FCE on diets containing crystalline amino acids (Wilson *et al.*, 1978), rapid and differential assimilation of amino acids (Plakas *et al.*, 1980), the unquantified interconversion of some amino acids (Ketola, 1982), and problems with interpretation of the dose-response curve (Tacon & Cowey, 1985).

Growth trials with diets based on purified proteins such as casein, and supplemented by set quantities of crystalline amino acids, rather than feeding graded levels in amino acid-based diets have been conducted with both prawns and shrimp. Using this technique, Watanabe *et al.* (1976) was able to show that although the prawn, *M. rosenbergii* was able to grow on a diet deficient in lysine, 1.4 % (of dry weight of the diet) resulted in optimum growth. Similarly, Stahl & Ahearn (1978) suggested that the same species required 1.4 % lysine, 1.3 % arginine, 3 % methionine and 0.6 % tryptophan for optimum growth. Work conducted on penaeid shrimp using these techniques has yielded conflicting results. Hew & Cuzon (1982) suggested a dietary requirement in casein and/or gelatin based diets (on a percentage of the diet basis) of 6.3 % lysine and 3.2 % arginine for *P. japonicus*. Deshimaru (1981) however, showed that growth of this species was not improved by supplementation of arginine to casein/albumin based diets. To add to the confusion, Teshima *et al.* (1986a) showed that supplementation of arginine to casein based diets improved their nutritive value to that of the live food control, and that half of the casein could be successfully replaced with a mixture of crystalline amino acids balanced to mimic the amino acid profile of whole larval shrimp (*P. japonicus*) bodies.

Problems encountered with this technique include the poor acceptance, rapid leaching and low assimilation rate of crystalline amino acids, leading to the variations and possibly overestimations in the reported requirements. Other problems include the time-consuming nature of these trials, the high cost and the poor applicability of these results

to practical dietary formulation. This method however, has recently been extended to feeding trials supplementing graded levels of microencapsulated crystalline arginine to casein-based diets to juvenile *P. monodon* (Chen *et al.*, 1992a,b). These authors used the broken line model analysis of weight gain and found that arginine requirements were only 0.25 % of the dry weight of the diet when arginine was microencapsulated in cellulose acetate phthalate which seems to be more readily assimilated than pure crystalline arginine (Chen *et al.*, 1992a). Other methods of supplementing arginine including glycerol monostearate microencapsulation and the inclusion of salmine, a natural protein high in arginine were also successful (Chen *et al.*, 1992b). This technique thus shows considerable potential for estimating amino acid requirements in shrimp which cannot effectively utilize pure crystalline amino acids.

Other techniques such as measuring the free amino acid levels within specific tissue pools such as haemolymph or muscle (Torres, 1973; Fair & Sick, 1982; Cho *et al.*, 1985) and measuring the oxidation rate of radio-actively-labelled amino acids (as above) have also been used in shrimp and prawns. These techniques however, also have drawbacks which include their high requirements for time and labour and the marked differences between, and the sometimes poor results of, various authors.

An alternative approach for estimating quantitative amino acid requirements is that of feeding graded levels of high quality protein and examining the daily deposition of individual amino acids in the carcass (Ogino, 1980). Results for fish species using this technique have been similar to those using the amino acid test diet technique, but studies on shrimp or prawns have yet to be conducted. Interestingly however, results using different fish species have revealed similar requirements, probably due to the similar amino acid profile of all fish muscle protein. The pattern of requirement between different fish species should thus be similar (Cowey & Tacon, 1983). Whether this will also hold for shrimp is unknown, but may be unlikely due to some unique aspects of their metabolism, particularly involving moulting and the synthesis of chitin (Sick & Millikin, 1983). This technique however, may more accurately reflect the true amino acid requirements of fish and shrimp than those derived from feeding amino acid test diets for a number of reasons. These include the greater likelihood of obtaining optimum growth from feeding 'whole' protein sources, the ability to determine

requirements for all ten essential amino acids simultaneously, and the suitability of this technique for all stages of the life cycle (Cho *et al.*, 1985; Tacon & Cowey, 1985).

Using the carcass deposition method of determining the essential amino acid requirements, it has been found that the relative proportions of essential amino acids in the fish carcass are the same as those required in the diet (Tacon & Cowey, 1985). Prior to this, it had already been suggested that the use of artificial diets combining a variety of protein sources to mimic the amino acid profile of the natural food or the muscle of target animal itself may result in optimum growth and food conversion efficiency (FCE) (Phillips & Brockway, 1956; Deshimaru & Shigeno, 1972; Kanazawa, 1985; Dy-Penaflorida, 1989). Deshimaru & Shigeno (1972) using these techniques with *P. japonicus* suggested that diets containing amino acid profiles similar to those found in its natural prey, the short-necked clam, *Venerupis philippinarum* or the shrimp itself, resulted in optimum growth and FCE. They found that the following proportions of amino acids in relation to total body weight apply to both clams and shrimp: leucine, lysine and arginine 7-9 %, methionine and cystine 2-3 %, and all other amino acids 3-6 %. They also showed that diets containing a high proportion of squid meal (with an amino acid profile similar to shrimp), or soybean meal also gave good results, whilst those based on fish meal (deficient in phenylalanine and basic amino acids) were inferior. A similar technique has also been used on the prawn, *M. rosenbergii*, where supplementation of sub-optimal diets with 1 % of the limiting amino acids (in relation to the profile in prawn muscle) in crystalline form, together with an alginate binder (to reduce leaching of the amino acids), resulted in improved growth and FCE (Farmanfarmaian & Lauterio, 1979, 1980). In diets containing 50 % total protein however, supplementation of crystalline amino acids to casein/albumin or whole egg protein-based diets was not found to increase the growth of *P. japonicus* (Deshimaru, 1982).

Although seemingly effective, supplementation of sub-optimal diets with crystalline amino acids is expensive and will not be a practical solution over the longer term. The establishment of the quantitative requirements for essential amino acids will ultimately result in the formulation of optimal diets for each species. In the absence of this data however, the most cost-effective and practical method of producing diets with balanced

amino acid profiles will probably be that of combining various defined and practical-style protein sources in order to match the profile found in the shrimp species or its natural prey. In order to assist this technique, the formulation of amino acid ratios (E/A ratios) has been introduced. The E/A ratio is calculated based on the amino acid composition of the whole body (or sometimes muscle) of the target species by dividing the weight of each essential amino acid present by the total weight of all amino acids present (including cystine and tyrosine) and multiplying by 100. The E/A ratios for the whole body protein of various species of fish have been shown to be highly correlated with the essential amino acid requirement pattern generated by growth studies for that species (see Wilson, 1985 for review). This method has also been used with shrimp and can be used to assess the potential of novel and existing protein sources for inclusion in dietary formulations where the requirements have yet to be confirmed (Arai, 1981; Dy-Penaflorida, 1989). Deshimaru *et al.* (1985) found that formulated diets for *P. monodon* based on shrimp head meal, fish meal and brewers yeast had a lower E/A ratio than clam meat for methionine and arginine. Protein sources rich in these amino acids, or supplementation of diets with the appropriate amino acids may thus be expected to enhance the quality of these diets.

2.5.5.1.4 Quantitative protein requirements

Based on techniques used initially with terrestrial animals, growth rate is the most common criteria used to assess the dietary protein requirement of fish and shrimp (De Long *et al.*, 1958; Hephher, 1988). Other techniques have also been used occasionally instead of, or in addition to, growth (and survival) as the criterion of requirement. These include maximum tissue protein retention or nitrogen balance (Ogino, 1980; Hewitt, 1992), protein efficiency ratio (Alava & Lim, 1982; Hajra *et al.*, 1988), and examination of changes in the metabolic rate (*i.e.* oxygen consumption, carbon dioxide excretion, nitrogen excretion and specific dynamic action) (Clifford & Brick, 1978, 1979; Dall & Smith, 1986, 1987; Hewitt & Irving, 1990) (see section 2.5.3.9). The optimal dietary protein requirement has usually been defined as the minimum amount of protein (expressed as a percentage of the diet) needed to supply adequate amino acids and produce maximum growth (NRC, 1983). Alternatively, it may be referred to as the protein level which tends to maximise simultaneously growth and protein deposition

(Ogino, 1980; Weatherley & Gill, 1987). However, since a considerable proportion of dietary amino acids are catabolized as an energy source, dietary protein requirements should more accurately be expressed in terms of the energy content of the diet. That this has rarely been done in studies on the protein requirements of shrimp is, at least in part, responsible for the wide discrepancies between, and usually relatively high requirements reported from, the results obtained.

Numerous studies have been conducted using a wide range of purified, semi-purified and practical-style diets on many species of shrimp and prawns. The reported protein requirements generally range from 30 to > 60 % (Table 2.13), but these requirements are all estimated from tank trials in the laboratory and may not represent the level required in commercial diets. In addition, the results of these trials are difficult to compare and are subject to variation (and often over-estimation) due to one or more of a large number of factors. One of the most important of these is the relationship between dietary protein and energy levels as mentioned above. This relationship will be discussed in detail in section 2.5.5.2.2 and is a major theme of this thesis. The other reasons are discussed in section 2.5.3, but the most important in terms of protein requirements include the following:

1. Lack of standardised test conditions and reference diets

Problems in this area include the different methodologies employed by the various workers, the lack of reference diets with which to compare results, variations in water quality conditions, the variable stocking densities employed, the feeding regime (rate, frequency and time) employed, dietary stability variations, unquantified diet consumption and lack of knowledge on the role of feeding attractants in the diets used.

2. Protein quality (source)

Many different protein sources, some of which may have been nutritionally sub-optimal (e.g. amino acid mixtures and pure proteins such as casein) have been employed (see section 2.5.5.1.2 and Table 2.13). The protein requirements of shrimp may thus reflect the quality of the protein source rather than its dietary level since higher quantities of

Table 2.13 Dietary protein requirements of penaeid shrimp species from laboratory tank trials.

<i>Penaeus</i> spp.	Body weight (g)	Major dietary protein source	Dietary protein level (%)		Reference
			Tested	Optimal	
<i>P. monodon</i>	0.5, 1.8	casein, fish meal	2-62	45.8	Lee (1971)
	0.01-0.7	-	-	34-40	Khannapa (1977)
	1-3	-	-	40	AQUACOP (1977)
	0.002	shrimp, squid, fish eggs, egg	25-55	35	Bages & Sloane (1981)
	1.32	casein	25-60	40.4	Alava & Lim (1983)
	0.65	shrimp, fish, yeast meals	44-51	49	Deshimaru <i>et al.</i> (1985)
	0.6-0.8	casein	40-50	40	Bautista (1986)
	0.51	shrimp meal	45-47	46.3	Hajra <i>et al.</i> (1988)
	0.90	casein	32-52	40-44	Shiau <i>et al.</i> (1991a)
	0.82	casein	35.5-40	35.5	Shiau & Chou (1991)
0.54	casein	30-41	30	Shiau & Peng (1992)	
<i>P. japonicus</i>	5.3	squid meal	63-76	> 60	Deshimaru & Shigeno (1972)
	-	shrimp, fish, soybean meals	-	> 40	Balazs <i>et al.</i> (1973)
	0.6	casein, albumin	-	46	Deshimaru & Kuroki (1974)
	-	casein, albumin	-	50-54	Deshimaru & Kuroki (1975)
	0.8	casein	2-66	52-57	Deshimaru & Yone (1978)
<i>P. vannamei</i>	0.03	-	25-40	30	Colvin & Brand (1977)
	4-20.8	shrimp meal	21-38	37-38	Smith <i>et al.</i> (1985)
	1.7	crab protein conc., casein	19.5-38	30-34.7	Cuzon <i>et al.</i> (1993)
<i>P. merguensis</i>	3-8	-	-	50-55	AQUACOP (1977)
	0.3	mussel meal	17-51	34-42	Sedgwick (1979)
<i>P. setiferus</i>	4	menhaden meal	14-52	28-32	Andrews <i>et al.</i> (1972)

protein are required if the source is of low quality. Additionally, novel (and potentially sub-optimal) protein sources have been used in many shrimp diets (reviewed in Pascual, 1983; Tacon & Jackson, 1985 and Fox *et al.*, in press), although not usually in trials designed for establishing dietary protein requirements. Pascual (1983), working with *P. monodon*, reviewed the sources of protein suitable for shrimp growth and concluded that combinations of a range of protein sources resulted in optimum growth. She also suggested that some plant proteins such as rice bran, corn meal, gluten and soybean meal, when mixed with animal proteins including fish, shrimp and earthworm meals, resulted in improved amino acid ratios. However, she suggested that a 2:1 mixture of fish to shrimp meals was the optimum combination for this species.

3. Shrimp species and age and physiological state

As can be seen from Table 2.13, there appear to be large differences in the protein requirements of various species of penaeid shrimp. These are likely to be due to the trophic status (natural feeding habits) of the individual species. Thus, the carnivorous shrimp, *P. japonicus* is estimated to have dietary protein requirements of 46-64 %, whilst the more omnivorous *P. monodon* has been reported to require 35-45 % in laboratory tank trials (Table 2.13). These results however, may also be complicated by other factors including differences in experimental methodology (see section 2.5.3). Shrimp age also has marked effects on dietary protein quality requirements, probably reflecting changes in their feeding habits and the development of their digestive physiology (Fenucci *et al.*, 1982). Chen *et al.* (1985) suggests that the decreasing requirement for animal as opposed to plant proteins as shrimp age may reflect a change in feeding habits from carnivory to omnivory or detritivory. In addition, the ratio of dietary protein used for metabolic activity:body tissue synthesis increases and the importance for protein decreases with age, leading to a decrease in growth rate and protein requirements with age (Hysmith *et al.*, 1972; Chen *et al.*, 1985; Cho *et al.*, 1985; Fauconneau, 1985; Kanazawa, 1985). Thus, protein requirements for commercial omnivorous shrimp diets are reported to decrease from approximately 55 to 35 % as they grow from larvae to adult (Tacon, 1990; Akiyama *et al.*, 1992). A series of diets with different protein levels of different qualities may thus be necessary for obtaining optimum growth and cost-effectiveness for shrimp at various stages of their life cycle

(Chen *et al.*, 1985). It is also likely that the quality or physiological state of the test shrimp themselves may influence their growth rate, further confusing estimations of their nutritional requirements. High health, quality tested, disease free and/or wild shrimp may thus outperform their hatchery-reared and highly stressed counterparts.

4. Natural feed availability

The majority of the trials investigating the protein requirements of shrimp have been conducted in the laboratory where access to alternative food sources is considered to be negligible. As discussed in section 2.5.3.5, this may not be true and in addition, this requirement will bear little relationship to the requirements under pond culture except under highly intensive conditions (Castille & Lawrence, 1989). For example, diets containing as little as 14 % protein have been found to support good growth and survival of *M. rosenbergii* cultured in ponds (Boonyaratapalin & New, 1982), whilst in tank trials protein levels of 30-40 % are considered to be optimal (Ashmore *et al.*, 1985; Millikin *et al.*, 1980). There is considerable potential for reducing the current level of protein (approximately 45 %, see Table 2.12) included in commercial diets for *P. monodon* during pond culture in Thailand. Optimal growth of juvenile *P. monodon* has been achieved in laboratory-based tank trials (without access to natural feeding) when fed diets containing as little as 35 % protein (see section 2.5.5.1). It should therefore be possible to reduce dietary protein levels by 10 % or more in commercial diets without loss of performance with the added nutritional contribution of natural feeds.

5. Undefined protein palatability, digestibility and assimilation rates

There has been limited work carried out on determining the digestibility and assimilation rates of different protein sources for penaeid shrimp, although for fish it is known that these may vary widely (Page & Andrews, 1973; Tacon & Cowey, 1985; Wilson, 1985). With the lack of a standard protein source in the trials conducted to date, estimations of protein requirements using various protein sources will depend upon the ability of the shrimp to digest and assimilate that source. The apparent digestibility of amino acids from both casein and gelatin for *P. vannamei* for example, have been

shown to be very high (> 95 %), whilst those of amino acids from other protein sources including fish meal (approximately 80 %) and soybean meal (approximately 90 %) are much lower (Akiyama *et al.*, 1988). Similarly, the palatability or attractiveness of those sources will vary leading to different rates of feed ingestion and hence apparent variations in protein requirements. This may be particularly important in plant-based diets which tend to be unpalatable and/or indigestible (Tacon & Jackson, 1985).

6. Analytical techniques

As detailed in section 2.5.3.9, the range of analytical techniques used by the various researchers studying the protein requirements of shrimp may also lead to poor comparability between results. This may be particularly significant in the estimation of protein:energy requirements, where numerous different techniques for the analysis of dietary energy content have been used. In addition, the use of growth rate data alone is not sufficient for estimating the true protein requirement since diets promoting good growth do not necessarily result in high survival or FCE. The inclusion of biomass gain (yield or production) data, in combination with estimations of FCE and/or protein deposition may thus assist standardisation of results.

2.5.5.2 Energy

2.5.5.2.1 Energy metabolism

Energy may be defined as the capacity to do work and is required for the maintenance of life processes including cellular metabolism, growth, reproduction and physical activity (Tacon, 1990). Energy metabolism involves the catabolism (breakdown) and oxidation of the energy yielding substrates and the consequent release and utilisation of this energy. Anabolism describes those metabolic processes in which complex compounds are synthesised from simpler substances.

Shrimp and fish are known to have a lower requirement for dietary energy than terrestrial animals due to three principal differences in their energy metabolism (Brett & Groves, 1979; NRC, 1983; Cho & Kaushik, 1985). Firstly, shrimp and fish are

poikilothermic and do not have to expend energy in maintaining a constant body temperature. Secondly, they exert less energy in maintaining their posture since they are supported by their aquatic medium. Thirdly, nitrogenous wastes, largely from amino acid catabolism, are excreted mostly as ammonia rather than uric acid or urea, allowing them to extract 10-20 % more energy from protein catabolism.

Shrimp, like other animals, are believed to eat primarily in order to satisfy their energy requirements (Adolph, 1981; Cho & Kaushik, 1985). These energy requirements are derived from the catabolism of energy yielding substrates in the diet, *i.e.* proteins, lipids and carbohydrates. These nutrients however, are also essential for the construction of living tissues. Thus, if the proportion of energy yielding substrates in the diet is unbalanced, nutritional deficiencies and hence reduced growth and survival may result (NRC, 1983; Robinson & Wilson, 1985; Tacon, 1990). For example, if the diet contains an excess of non-protein energy, once the energy requirements are met the shrimp will cease feeding and insufficient quantities of protein for optimal growth will be consumed. Excessive levels of dietary lipids (see section 2.5.5.4) and/or carbohydrates (see section 2.5.5.3) may also have pathological effects on shrimp. In addition, excess dietary energy can lead to fat deposition in the carcass which may reduce the dress-out yield and shorten the shelf-life of frozen shrimp (Clifford & Brick, 1979; NRC, 1983; Cho *et al.*, 1985). Conversely, if the dietary energy density is too low, the shrimp will utilise dietary energy substrates including protein for fulfilling their maintenance energy requirements rather than for growth. This may be compensated for by increased ingestion within the physical limits of the digestive tracts of the shrimp and the amount of feed offered. Optimal growth and dietary efficiency may therefore be achieved by feeding small quantities of diets containing high levels of both energy and protein, or large quantities of diets containing low levels of both energy and protein. Gallacher *et al.* (1979) showed that this was true for adult lobsters (*Homarus sp.*), with the critical parameter being the protein:energy ratio rather than the absolute level of either nutrient in the diet. The cost-effectiveness of these two strategies has yet to be examined, but will depend largely on the relationship between diet cost and shrimp growth rates.

2.5.5.2.2 Protein:energy (Pr:E) ratio and protein-sparing

It is thought that in fish and shrimp, protein is the preferred substrate for energy production (Cowey, 1979, 1980; Walton, 1985; Tacon, 1990; Zhou, 1990). This may in part explain the higher requirement for dietary protein in shrimp and fish than in terrestrial animals. Energy production from protein catabolism however, is inefficient both nutritionally and economically and should be avoided if possible. The preference for protein as an energy source may be due to the inability of shrimp to store protein (Maynard & Loosli, 1969) and may be responsible for the higher protein requirements of shrimp than terrestrial vertebrates (Cho & Kaushik, 1985). The ratio of protein to energy, as well as the ratios of non-protein energy sources of lipid and carbohydrate are therefore crucial, but remain largely unstudied (Guillaume, 1990; Kompiang, 1990). From a practical standpoint, New (1990) suggests that one of the most important areas for applied research is in examining the protein-sparing role of alternative sources of dietary energy (lipids and carbohydrates). Sparing involves the utilisation of carbon chains from carbohydrates and lipids for energy production, sparing dietary protein solely for growth and tissue maintenance (Clifford & Brick, 1978; NRC, 1983). It is thought that sparing occurs as a result of the modification of certain key metabolic pathways, precipitated by shifts in enzymatic activity in response to changes in the levels of energy yielding substrates (Freedland & Szepesi, 1971; Clifford & Brick, 1978). Determination of the optimal balance of energy yielding substrates in dietary formulations may therefore permit improved utilisation efficiency of dietary nutrients (Capuzzo, 1981; Corbin *et al.*, 1983).

Most of the studies conducted on shrimp nutrition to date have concentrated on the requirements for proteins, lipids, carbohydrates and other nutrients, without considering possible synergistic interactions (*i.e.* sparing) between the major nutrient classes (Clifford & Brick, 1978; Sandifer, 1981). Similarly, the requirements for total (gross), digestible and/or metabolisable energy in shrimp diets have been largely unstudied (see section 2.5.3.9). Since, as pointed out above, shrimp will eat until their energy requirements have been satisfied, the requirements for other nutrients depend on the energy density of the diet and should more correctly be referred to as a percentage of dietary energy (Cowey & Sargent, 1979; Jobling, 1983; Weatherley & Gill, 1987;

Cuzon, 1993).

Because of the energy-dependant consumption of diets, the use of isoenergetic diets in nutritional trials with shrimp is important. As discussed in section 2.5.3.9 however, the formulation of truly isoenergetic diets is difficult. Ideally, when estimating the energy content of shrimp diets, metabolisable or even digestible energy values should be used. Unfortunately, these are unknown for shrimp and total energy values are more commonly used. In this case, the palatability, digestibility and assimilation of the diets may vary. Deshimaru & Yone (1978) for example, showed that food consumption was inversely proportional to dietary protein content in diets considered isoenergetic in terms of total energy.

The few studies conducted to date on the energy and protein:energy ratio (Pr:E) requirements of shrimp have mainly been carried out on *P. monodon* and have yielded conflicting results (Bautista, 1986; Hajra *et al.*, 1988; Shiau & Chou, 1991). These differences have probably been due to two main reasons. Firstly, the use of varying sources and levels of the energy-yielding nutrients of protein, lipid and carbohydrate in the test diets; and secondly, differences between the methods used for calculating the energy density of the diets. Recalculating the results of these authors (and those authors investigating requirements for proteins, lipids and carbohydrates) using total energy (TE) conversion terms of 23.6 kJ g⁻¹ for protein, 39.5 kJ g⁻¹ for lipid and 17.2 kJ g⁻¹ for carbohydrate has facilitated comparisons between results of the different workers (Table 2.14). When the results are expressed in this way, the total energy level of the diets supporting maximum growth of *P. monodon* can be seen to range from 14.7 to 20.8 kJ g⁻¹ TE, generally increasing with the level of protein in the diet. Similarly, the optimum protein:energy ratio can be seen to range from 18.5-29.4 mg protein kJ⁻¹ TE, again increasing with dietary protein level. A similar trend can be seen in the requirements of other penaeid species, with little difference between the requirements of the various omnivorous species studied. These results are in contrast to the reported differences between protein requirements of various species of penaeid shrimp (see section 2.5.5.1) and suggest that the protein requirements of shrimp may be a) more accurately expressed in relation to the energy density of the diet and b) minimized in diets containing the correct quantities and balance of energy-yielding substrates.

Table 2.14 Dietary requirements for total energy (TE) and protein:energy ratio (Pr:E) of penaeid shrimp species from laboratory tank trials*.

<i>Penaeus</i> spp.	Body weight (g)	Optimum protein level (%)	Optimum lipid level (%)	Optimum carbohydrate level (%)	Optimum TE level (kJ g ⁻¹)	Optimum Pr:E ratio (mg Pr kJ ⁻¹ TE)	Reference
<i>P. monodon</i>	0.002	35-45	9.9-10	20-30	17.3-18.0	20.2-25.0	Bages & Sloane (1981)
	1.32	40.4	7	39.1	19.0	21.2	Alava & Lim (1983)
	0.62	45.5	10.3	33.2	20.5	22.2	Alava & Pascual (1987)
	0.6-0.8	39	5	20	14.7	26.7	Bautista (1986)
		46	5	20	16.3	28.3	
		52	5	20	17.7	29.4	
	0.52	46.3-46.7	9.9-10.6	31.7-33	20.4-20.8	22.3-22.9	Hajra <i>et al.</i> (1988)
	0.62	51	12.8	19	20.0	25.4	Catacutan (1991a)
	0.14	40	11.9	22.3	18.0	22.2	Catacutan (1991b)
	0.82	35.5	8.9	35.3	18.0	19.8	Shiau & Chou (1991)
		39.2	8.7	26.7	17.3	22.7	
	0.90	39.9	9.9	34.7	19.3	20.7	Shiau <i>et al.</i> (1991a)
		44.0	9.6	30.6	19.4	22.6	
0.54	30.8	8.1	35.7	16.6	18.5	Shiau & Peng (1992)	
	34.5	8.8	32.5	17.2	20.0		
	40.8	8.7	26.9	17.7	23.1		
<i>P. vannamei</i>	4.0	36.9	5.9	32.2	16.6	22.3	Smith <i>et al.</i> (1985)
		38.3	6.1	30.3	16.7	23.0	
	1.7	35	5.2	30	15.5	22.6	AQUACOP <i>et al.</i> (1991)
juvenile	30.4	5.1	37.5	15.6	19.4	Cuzon <i>et al.</i> (1993)	
	32.7	5.3	41	16.9	19.4		
	34.7	5.8	45	18.2	19.0		
<i>P. indicus</i>	0.4-0.7	42.8	6	28.5	17.4	24.6	Colvin (1976a)
		53.1	7	12.6	17.5	30.4	
<i>P. merguensis</i>	0.3	28	2.3	50	16.1	17.4	Sedgwick (1979)
		28	6.3	40	16.0	17.5	
		39.5	8.4	28.9	17.6	22.4	
		50.9	4.1	18.2	16.8	30.4	
<i>P. setiferus</i>	4.0	14	2	30	9.3	15.1	Andrews <i>et al.</i> (1973)
		23	3	30	11.8	19.5	
		32	4	30	14.3	22.4	

* TE levels and Pr:E ratios of these diets have been recalculated for comparability between authors by multiplying the level of energy-supplying substrates by the following conversion terms: protein 23.6, lipid 39.5, carbohydrate 17.2 kJ g⁻¹.

Using these recalculated figures, optimum growth and survival of *P. monodon* may thus be achieved on diets containing 40 % protein at a total energy level of 17.3-19.3 kJ g⁻¹ and at a Pr:E ratio of 20.2-23.1 mg protein kJ⁻¹ TE (Bages & Sloane, 1981; Alava & Lim, 1983; Catacutan, 1991b; Shiao & Chou, 1991; Shiao *et al.*, 1991a; Shiao & Peng, 1992). However, a non-significant decrease in growth and survival of *P. monodon* has resulted from a reduction in dietary protein levels to 35-36 % where diets contain 17.2-18 kJ g⁻¹ TE at a Pr:E ratio of 19.8-20.2 mg protein kJ⁻¹ TE (Bages & Sloane, 1981; Shiao & Chou, 1991; Shiao & Peng, 1992) (Table 2.14).

Manipulation of the protein and energy levels in these diets in order to optimise shrimp production at low levels of dietary protein requires the formulation of diets containing appropriate sources and levels of the non-protein energy substrates of carbohydrates and lipids. The next two sections discuss the studies performed to date on these areas and the potential of these nutrients to spare dietary protein in shrimp diets.

2.5.5.3 Carbohydrate

2.5.5.3.1 Structure and function

Carbohydrates are a group of compounds containing mainly carbon, hydrogen and oxygen, with the ratio of the last two elements being present in the same ratio as in water. Some derivatives however, also contain nitrogen (*e.g.* chitin in the shrimp exoskeleton) and sulphur (Tacon, 1990). Carbohydrates may be divided into two main groups depending on their structure; the sugars and the non-sugars. The sugars are themselves sub-divided into monosaccharides (those which cannot be hydrolysed into simpler compounds) and di-, tri-, and tetra-saccharides (those that can be hydrolysed into two, three or four monosaccharide units respectively). The most common monosaccharides of interest in shrimp nutrition include glucose, galactose and fructose. These sugars are not used directly by shrimp, but are first transformed into monosaccharide derivatives before being involved in biochemical reactions. The most important naturally-occurring disaccharides include sucrose, lactose, maltose and trehalose. The non-sugars by contrast, are termed polysaccharides and are composed of many monosaccharide units either all the same (homopolysaccharides) or different

(heteropolysaccharides). The homopolysaccharides generally function as energy reserves (*e.g.* starch in plant tissues and glycogen in fish and shrimp) or as structural components (*e.g.* cellulose in plants and chitin in shrimp). The heteropolysaccharides perform a range of roles including structural (bacterial cell walls), wound sealants (gums in plants), lubricants (mucilages) and blood anti-coagulants (mucopolysaccharides such as heparin).

No specific dietary requirement for carbohydrates has yet been established in diets fed to either fish or shrimp. This is probably due to three main reasons. Firstly, the natural prey of shrimp generally contains very little carbohydrate, particularly for the more carnivorous species. Secondly, glucose can be synthesised from dietary protein and lipids by gluconeogenesis by shrimp. Thirdly, shrimp tend to satisfy their energy requirements through the preferred catabolism of proteins and then lipids (Tacon, 1990; Zhou, 1990). Despite the non-essentiality of dietary carbohydrates, they do have many functions in the shrimp body including, use as energy substrates, and as metabolic intermediates for the synthesis of many biologically important compounds including chitin, RNA and DNA and mucous secretions (Tacon, 1990). In addition, reduced growth has been shown to result from feeding diets lacking a source of carbohydrate to both fish (Garling & Wilson, 1977; NRC, 1983) and shrimp (Andrews *et al.*, 1973). The inclusion of very high levels (> 35-40 %) of carbohydrate in shrimp diets however, has also led to poor growth, survival and histopathological changes in the body tissues of shrimp (Pascual *et al.*, 1983; Alava & Pascual, 1987; Catacutan, 1991b). Successful diets formulated for penaeid shrimp have therefore usually included 5-40 % carbohydrate in order to act as a cheap source of dietary energy and assist protein sparing, and to help bind the diet, increasing pellet water stability and decreasing leaching of water soluble dietary components (Millikin, 1982; Tacon, 1990).

2.5.5.3.2 Carbohydrates as dietary energy sources

The ability of carbohydrates to spare dietary protein for growth depends upon their ready and efficient assimilation from the diet. This in turn varies according to the source of carbohydrate, its complexity, pre-treatment, inclusion level and the level of other energy substrates in the diet (Capuzzo, 1982). There are also species specific

differences in the ability to utilise carbohydrate, primarily related to the trophic status of the different species.

Carbohydrate source (or complexity) is an important factor in its utilisation for energy. This is because, as with omnivorous fish species (Tacon, 1990), monosaccharides such as glucose are not utilised as efficiently by shrimp as disaccharides (Deshimaru & Yone, 1978; Abdel-Rahman *et al.*, 1979; Alava & Pascual, 1987) or polysaccharides, particularly precooked or gelatinised starch (Andrews *et al.*, 1972; Aquacop, 1976; Abdel Rahman *et al.*, 1979; Shiau & Chou, 1991; Shiau & Peng, 1992) (see Table 2.15). The poor growth and survival of shrimp fed glucose is due to their diabetic-like nature, as in salmonid fish. Glucose is absorbed quickly into the blood in the midgut gland, whilst insulin levels rise only slowly, limiting the regulation and metabolism of glucose and resulting in its rapid excretion (Abdel-Rahman *et al.*, 1979; Furuchi & Yone, 1982; Shiau & Peng, 1992). This process is slowed dramatically when complex carbohydrate sources are fed as the cleavage of glucose units proceeds slowly, enhancing the utilisation of such carbohydrate sources. In addition, the greater binding properties of starches over sugars may contribute to their better performance in shrimp diets (New, 1976a,b; Tacon, 1990). In terms of energy provision, the metabolism of the relatively simple sugars glucose, sucrose, trehalose and dextrin has been shown to lead to the storage of energy for metabolism in the form of glycogen, lipids or non-essential amino acids (Capuzzo & Lancaster, unpublished data in Capuzzo, 1981; Alava & Pascual, 1987). In contrast, the metabolism of complex starches leads to utilisation of these carbohydrates for immediate energy requirements (due to the difficulty of converting starches into lipids or non-essential amino acids) and produces a concomitant protein-sparing effect (Capuzzo & Lancaster, unpublished data in Capuzzo, 1981).

Glycolysis, the conversion of glucose to pyruvate, leading to the generation of energy via adenosine triphosphate (ATP) is the major route of glucose catabolism in shrimp and fish (Walton & Cowey, 1982; Cowey & Walton, 1989). However, the phosphorylation of glucose in glycolysis is catalyzed by enzymes such as hexokinase, the activity of which is very low in shrimp and fish, even when they are fed high levels of dietary glucose (Abdel-Rahman *et al.*, 1979; Cowey & Walton, 1989). Additionally, it is probable that the energy demand of the brain and other nervous tissue, which

Table 2.15 Optimum dietary carbohydrate sources for penaeid shrimp species from laboratory tank trials.

<i>Penaeus</i> spp.	Body weight (g)	Carbohydrate sources tested	Optimum carbohydrate source	Reference
<i>P. monodon</i>	1.76	maltose, sucrose, dextrin, molasses, cassava starch, corn starch, sago palm starch	sucrose	Pascual <i>et al.</i> (1983)
	0.62	glucose, sucrose, trehalose	trehalose	Alava & Pascual (1987)
	0.02	glucose, corn starch, rice starch, soluble starch, potato starch, dextrin, chitin	corn & rice starch	Briggs (1991)
	0.65	wheat flour, brown rice	wheat flour	Sheen & Chen (1991)
	-	starch, wheatflour (3 grades)	starch & wheat flour	Shiau <i>et al.</i> (1991b)
	0.5-0.6	glucose, dextrin, corn starch	corn starch	Shiau & Peng (1992)
<i>P. japonicus</i>	0.9	glucose, sucrose, dextrin, starch, glycogen	sucrose	Deshimaru & Yone (1978)
	0.4-0.7	glucose, sucrose, soluble starch, dextrin, potato starch, glycogen, maltose, lactose, fructose	maltose	Abdel-Rahman <i>et al.</i> (1979)
	juvenile	glucose, sucrose, dextrin, potato starch, glycogen, maltose	maltose	Kanazawa (1985)
<i>P. vannamei</i>	juvenile	wheat flour, broken rice, millet, sorghum, biscuit byproducts	wheat flour	Cruz-Suarez <i>et al.</i> (1991)
	8.8	wheat starch, whole wheat, sorghum	whole wheat	Davis & Arnold (1993)
<i>P. indicus</i>	0.04	glucose, fructose, galactose, maltose, sucrose, glycogen, starch	starch	Ali (1993)
<i>P. setiferus</i>	4.0	glucose, starch	starch	Andrews <i>et al.</i> (1973)
<i>P. duorarum</i>	3.4	glucose, starch	starch	Sick & Andrews (1973)

catabolize glucose, are met by gluconeogenesis (from amino and fatty acids), rather than by glycolysis. Glucose may thus be expected to have a limited role in energy-provision in shrimp and prawn diets.

Other carbohydrate sources such as fibres *i.e.* celluloses, lignin and pentosans are thought to form insoluble fractions in the feed and are often included as pellet binders. Although conflicting evidence exists regarding the utilisation of fibre by shrimp (Fair *et al.*, 1980; Diaz *et al.*, 1988), excessive levels may reduce diet gut passage time, block diffusion of digestive enzymes and digestion products, and may chelate metal ions required as enzyme co-factors (Millikin, 1982). Reduced palatability of diets containing high levels of dietary fibre and consequent poor growth rates of both shrimp (Diaz *et al.*, 1988) and fish (NRC, 1983) suggest that fibres are not utilised and probably have no role in energy provision (Tacon, 1990). For this reason, when estimations of dietary energy are made by calculation, carbohydrate values should be based on either direct methods of carbohydrate measurement or "by difference" using nitrogen free extract (NFE), not including the fibre component. In this case NFE will be 100 - % protein - % total lipid - % fibre - % ash, with all measurements being on a dry matter basis.

Data currently available from tank trials suggest that for *P. monodon*, as well as other shrimp and fish species, 20-35 % dietary carbohydrate (particularly di- and polysaccharides) can be used for energy production and spare protein for growth (Tables 2.14 & 2.16). Colvin (1976a) showed that substitution of protein by potato starch, involving only a small change in total energy, had no significant effect on growth of *P. indicus* despite a reduction in dietary protein level from 53 to 43 %. Teshima & Kanazawa (1984) also showed that protein sparing was dependant upon the carbohydrate content of diets fed to larval *P. japonicus*. Optimum survival was obtained on diets containing 45 % protein and 25 % of either sucrose or starch. Alava & Pascual (1987) fed isonitrogenous (45 % protein), isolipidic (10 %) semi-purified diets containing 10, 20 or 30 % of various carbohydrates (trehalose, sucrose and glucose) to juvenile *P. monodon*. These authors showed that shrimp growth and survival was optimum when fed diets containing 20 % and poorest with 30 % trehalose, and suggested that limited amounts of suitable carbohydrates can lead to increased protein assimilation efficiency. Bautista (1986) successfully reduced the protein level of diets for *P. monodon* from 50

Table 2.16 Dietary carbohydrate requirements of penaeid shrimp species from laboratory tank trials.

<i>Penaeus</i> spp.	Body weight (g)	Optimum carbohydrate source	Optimum carbohydrate level (%)		Reference
			Carbohydrate source	Total carbohydrate	
<i>P. monodon</i>	0.002	corn starch	20-30	20-30	Bages & Sloane (1981)
	1.76	sucrose	10	13.4	Pascual <i>et al.</i> (1983)
	0.6-0.8	sucrose	20	20	Bautista (1986)
	0.62	trehalose	20	33.2	Alava & Pascual (1987)
	0.004	soybean meal	40	33.9	Akiyama & FSGP (1989)
	0.14	gelatinized breadflour	5-25	22-33	Catacutan (1991b)
	0.65	wheat flour	30	30	Sheen & Chen (1991)
	0.82	dextrin	10-20	26-35	Shiau & Chou (1991)
<i>P. japonicus</i>	juvenile	glycogen	6-12	6-12	Deshimaru & Kuroki (1974)
	0.9	sucrose	10	10	Deshimaru & Yone (1978)
	0.4-0.7	maltose	19.5	19.5	Abdel-Rahman <i>et al.</i> (1979)
	0.64	α starch	28.5	28.5	Maugle <i>et al.</i> (1983b)
	larvae	starch, sucrose	25	25	Teshima & Kanazawa (1984)
	-	maltose	19.5	19.5	Kanazawa (1985)
<i>P. indicus</i>	0.1	corn starch	40	40	Ali (1982)
	0.04	corn starch	30	33.4	Ali (1993)
<i>P. setiferus</i>	4.0	starch	30	30	Andrews <i>et al.</i> (1972)
<i>P. duorarum</i>	3.4	starch	40	40	Sick & Andrews (1973)

to 40 %, when diets were maintained isoenergetic by increasing dietary carbohydrate (sucrose) levels from 0 to 10-20 %. Shiau & Chou (1991) also demonstrated the protein-sparing role of carbohydrate in diets for juvenile *P. monodon*. These authors showed that protein levels could be reduced even further, from 40 to 36 %, in diets including up to 20 % dextrin (35 % total carbohydrate) in order to maintain total dietary energy levels (re-calculated) at 18 kJ g⁻¹. The greater protein-sparing effect of starches was confirmed by Shiau & Peng (1992), who demonstrated enhanced protein deposition in *P. monodon* fed diets containing up to 30 % starch as compared to those fed dextrin or particularly glucose. Further, protein levels could be decreased from 41 to 31 % with no loss in performance of shrimp fed the 30 % starch diet.

Complex carbohydrates may thus play an important role in protein-sparing at levels of up to 35 % in shrimp diets. Further increases in carbohydrate may lead to histopathological changes in the prawn and eventually mortality. The contribution of energy-dense dietary lipids may therefore have more potential in protein-sparing than carbohydrates, and this will be considered in the next section.

2.5.5.4 Lipid

2.5.5.4.1 Structure and function

The lipids comprise a heterogeneous group of substances which are relatively insoluble in water, but soluble in organic non-polar solvents. This classification includes pigments and fat-soluble vitamins, but these substances are generally excluded in reports on the lipid requirements of crustaceans. The most important lipids in terms of shrimp nutrition include fatty acids and their esters, phospholipids and sterols. There are more than 40 different fatty acids that occur in nature and which may be represented by the general formula: CH₃ (CH₂)_n COOH. Fatty acids are aliphatic monocarboxylic acids and are liberated from fats and oils by hydrolysis. The most recent nomenclature for fatty acids is as follows. Fatty acids are referred to by a short-hand notation (*e.g.* linolenic acid, 18:3(n-3)). The first number represents the total number of carbon atoms present in the backbone of the molecule. The second number represents the total number of double bonds, and the 'n' (or omega) specifies the location of the first

double bond counting carbon atoms from the methyl terminus. Those fatty acids containing no double bonds are termed saturated and abbreviated to, for example, 16:0 (palmitic acid). Those fatty acids containing double bonds are termed unsaturated. Polyunsaturated fatty acids (PUFAs) are those containing more than two double bonds, whilst highly unsaturated fatty acids (HUFAs) contain more than four double bonds. Examples of HUFAs which are known to be essential in diets for penaeid shrimp include eicosapentaenoic acid (20:5(n-3)) and docosahexaenoic acid (22:6(n-3)). The degree of unsaturation of these fatty acids has a marked influence on the physical properties of the lipid since unsaturated fatty acids are generally more chemically reactive and have lower melting points than the corresponding saturated fatty acids.

Fatty acids are normally found in nature as esterified compounds. These may be classified into those that are glycerol-based and those in which glycerol is absent. The former group includes the triacylglycerides or simple fats (semi-solid at room temperature) and oils (liquid at room temperature) which are important as fatty acid stores (see section 2.5.5.4.2) and energy stores (see section 2.5.5.4.5) in shrimp. This group also includes compound lipids such as the glycolipids and phospholipids (see section 2.5.5.4.3). Those lipids that do not contain glycerol include sterols (see section 2.5.5.4.4), waxes, cerebrosides, terpenes and sphingomyelins. In addition to their structural and metabolic roles, lipids are important sources of metabolic energy through ATP. Lipids have a higher energy value than either proteins or carbohydrates, are highly digestible and hence can be catabolized for energy and may be used for protein-sparing in shrimp diets (Tacon, 1990; Zhou, 1990) (see section 2.5.5.4.5). It is thought that fatty acids from the triglyceride fats and oils are the major aerobic fuel source for energy metabolism in the tissue of aquatic animals (Tacon, 1990).

2.5.5.4.2 Essential fatty acid requirements

The ability to synthesise *de novo* saturated fatty acids from C¹⁴-labelled acetate has been demonstrated in many crustacean species including the freshwater crayfish *Astacus astacus* (Zandee, 1966), homarid lobsters and penaeid prawns (Zandee, 1967; Kanazawa & Teshima, 1977; Kanazawa *et al.*, 1979b). The bioconversion of saturated C¹⁴ palmitic and stearic acids to the corresponding monoenoic fatty acids has also been

demonstrated in *P. japonicus* (Kanazawa *et al.*, 1979a). In both cases some evidence of radioactivity was detected in n-3 and n-6 PUFA (Kanazawa *et al.*, 1979a), probably resulting from the addition of C¹⁴ acetate units, removed from palmitic acid and used to elongate the carboxyl end of n-3 essential fatty acids of dietary origin (Castell, 1982). On the other hand, the *de novo* synthesis of fatty acids of the n-3 (linoleic) and n-6 (linolenic) series is either very limited or non-existent in most shrimp and animal species. Simpler fatty acids obtained from the diet can be elongated and desaturated in a number of fish and shrimp species to n-3 and n-6 PUFAs and HUFAs. Some species however (including *P. monodon* and *P. japonicus*), are not able to perform this bioconversion quickly enough to satisfy their essential fatty acid requirements and must therefore have these PUFAs and HUFAs supplied pre-formed in the diet (Cowey & Sargent, 1977; Kanazawa *et al.*, 1979d; Kayama *et al.*, 1980). Thus, the fatty acid pattern of shrimp lipids represents a combination of fatty acids synthesised *de novo* and those obtained from dietary sources. The variable ability to elongate and desaturate fatty acids therefore affects the lipid requirements of shrimp and has yet to be fully investigated in most species (Castell, 1982; Tacon, 1990). The dietary fatty acid requirements of shrimp will also be affected by many other factors including species, temperature, salinity, season, age, sex, and the stage in the moult cycle.

The fatty acid composition and requirements of shrimp are complex and may, as mentioned above, be influenced by many different factors. Much as the fatty acid composition of shrimp raised under different conditions may be correlated with variable dietary fatty acid requirements, the wide range of fatty acid patterns found in different shrimp species are probably indicative of their dietary requirements (Castell, 1982). Insight into the dietary requirements may therefore be gained by comparison of the fatty acid patterns in the whole body or muscle tissue of the species under investigation, by inferences drawn from radioisotopic studies of fatty acid synthesis, or by dietary trials using known quantities of fatty acids and observing the effects of these upon growth, survival, FCR, moulting, body lipid and fatty acid levels (Castell, 1982). A range of studies have been conducted on shrimp species (primarily *P. japonicus*) using all of these techniques. Table 2.17 shows data from analyses of the fatty acid profiles of penaeid shrimp species and Table 2.18 contains the known fatty acid requirements of penaeid shrimp from radioisotopic studies and nutrition trials.

Table 2.17 Fatty acid profiles (% of total lipid in whole body) of penaeid shrimp species.

Fatty acid	<i>Penaeus</i> species								
	<i>P. japonicus</i>			<i>P. indicus</i>	<i>P. setiferus</i>	<i>P. merguensis</i>	<i>P. aztecus</i>		
Reference	1	2	3	4	5	6	7	8	9
14:0	2.4	1.8	-	1.3	1.8	0.7	3.8	4.2	-
14:1	0.5	0.1	-	-	-	-	-	-	-
16:0	15.4	16.1	16.1	14.1	12.6	18.3	17.4	15.4	17.6
16:1(n-7)	6.9	8.3	8.3	7.2	6.3	6.0	10.3	7.7	13.5
18:0	6.5	6.2	9.2	7.3	6.7	9.4	10.2	10.8	9.3
18:1(n-9)	9.0	11.3	19.5	10.0	11.9	10.7	14.2	13.4	14.9
18:2(n-6)	2.0	1.5	6.6	2.3	2.5	1.7	2.1	2.9	2.9
18:3(n-6)	7.9	5.4	-	-	-	1.0	-	-	2.6
18:2(n-3)	0.4	0.5	0.5	1.0	1.0	0.9	1.4	2.9	1.5
18:4(n-3)	2.0	2.4	-	1.5	0.2	0.5	-	-	-
20:1(n-9)	-	-	5.4	2.5	4.1	0.9	3.8	3.5	-
20:2(n-6)	1.2	1.2	-	-	1.9	0.7	0.4	0.3	1.7
20:3(n-3)	0.5	0.3	-	-	-	0.2	-	-	0.2
20:4(n-6)	3.3	3.3	9.3	6.5	4.0	4.7	0.4	0.3	6.4
20:4(n-3)	1.8	1.5	-	-	-	0.2	-	-	-
20:5(n-3)	13.1	12.7	14.4	11.2	16.9	12.6	20.6	20.4	15.5
22:1(n-9)	-	-	-	2.6	2.0	0.1	1.8	1.5	-
22:3(n-6)	0.4	0.4	-	-	-	-	2.1	2.1	-
22:4(n-6)	2.2	2.2	-	-	-	0.5	-	-	0.8
22:5(n-6)	-	-	-	3.2	-	0.3	-	-	1.2
22:5(n-3)	3.0	1.3	-	-	-	0.9	-	-	1.5
22:6(n-3)	7.6	10.3	11.7	9.3	15.7	14.6	12.0	10.0	10.3
24:1(n-9)	-	6.6	-	3.2	-	0.2	-	-	-
Total n-3	27.9	27.2	26.6	22.9	33.8	29.9	34.0	33.3	28.8
Total n-6	17.5	17.9	15.9	12.0	8.4	8.9	5.0	5.6	15.8
n-3/n-6 ratio	1.6	1.5	1.7	1.9	4.0	3.4	6.8	5.9	1.8

¹ Guary *et al.* (1974)(male); ² Guary *et al.* (1976)(maximum); ³ Teshima *et al.* (1977)(maximum); ⁴ Colvin (1976); ⁵ Joseph & Meyers (1975); ⁶ Clarke & Wickins (1980); ⁷ Shewbart *et al.* (1973)(spawned female); ⁸ Shewbart *et al.* (1973)(juvenile); ⁹ Chanmugan *et al.* (1983).

Table 2.18 The fatty acid requirements of penaeid shrimp species from laboratory tank trials.

<i>Penaeus</i> spp.	Fatty acid type	Requirement (% of diet)	Reference
<i>P. monodon</i>	20:5(n-3) & 22:6(n-3) > 18:3(n-3) > 18:2(n-6)	-	Ono <i>et al.</i> (1978)
	20:5(n-3) > 22:6(n-3) > 18:3(n-3) > 18:2(n-6)	-	Kanazawa <i>et al.</i> (1979b)
	n-3 HUFA	-	Millamena & Qunito (1985)
<i>P. japonicus</i>	20:5(n-3) > 22:6(n-3) > 18:3(n-3) > 18:2(n-6)	1.0	Kanazawa <i>et al.</i> (1978, 1979a,c,e)
	18:3(n-3) > 18:2(n-6)	1.0	Kanazawa <i>et al.</i> (1979d)
	n-3 and n-6 series	-	Deshimaru <i>et al.</i> (1979); Castell (1982)
	20:5(n-3) & 22:6(n-3) > 18:3(n-3) > 18:2(n-6)	0.5-1.0	Jones <i>et al.</i> (1979b); Teshima & Kanazawa (1984); Kanazawa <i>et al.</i> (1985)
<i>P. merguensis</i>	20:5(n-3) & 22:6(n-3) > 18:3(n-3) > 18:2(n-6)	-	Kanazawa <i>et al.</i> (1979b)
	18:3(n-3)	1.0	AQUACOP (1978)
	n-3 series HUFA	-	Clarke & Wickins (1980)
<i>P. indicus</i>	n-3 series HUFA	-	Colvin (1976b)
	20:5(n-3) & 22:6(n-3) > 18:3(n-3) > 18:2(n-6)	-	Read (1981)
<i>P. aztecus</i>	18:3(n-3)	1.0-2.0	Shewbart & Mies (1973); Shewbart <i>et al.</i> (1973)
<i>P. duorarum</i>	n-3 > n-6, 18:3(n-3)	1.0	Sick & Andrews (1973)
<i>P. stylirostris</i>	18:3(n-3)	-	Fenucci <i>et al.</i> (1981)

Note: > = better than

From the data available it can be seen that marine penaeid shrimp species generally have a higher requirement for n-3 series PUFAs and HUFAs than n-6 series fatty acids. This is in contrast to terrestrial animals which tend to have a higher requirement for n-6 series fatty acids, probably due to the preponderance of these fatty acids in the terrestrial environment. The n-3 series fatty acids are generally believed to have a higher essential fatty acid activity in aquatic animals because they permit a greater degree of membrane fluidity, flexibility and permeability at low temperatures (Cowey & Sargent, 1977). The n-3 series PUFAs and HUFAs are therefore predominant in the aquatic prey of penaeid shrimp. This may explain why penaeid shrimp must have these fatty acids supplied in the diet, since their presence in the natural prey has led to the shrimp losing the ability to chain elongate and desaturate linolenic and linoleic acids to the essential HUFAs 20:5(n-3) and 22:6(n-3) (Kanazawa, 1985; Kanazawa *et al.*, 1979b; Castell *et al.*, 1986). In summary, most penaeid shrimp including *P. monodon* (see Table 2.18), show optimum growth and survival on diets containing approximately 1 % of the n-3 series HUFAs 20:5(n-3) or 22:6(n-3). In addition, both of these HUFAs have a greater essential fatty acid activity than either of the PUFAs 18:3(n-3) or 18:2(n-6). D'Abramo (1990) however, suggests that only 0.075 % of any particular fatty acid species may be required. Despite this low requirement, higher quantities of dietary HUFAs may be beneficial in shrimp diets due to their function as feeding attractants (Bryant *et al.*, 1989). These requirements should therefore be reflected in both the quality and quantity of lipid supplied in the diets for these species. Several studies have shown that optimum growth and survival of penaeid shrimp resulted when they were fed diets containing a range of fatty acids (from pollack or cod liver oil, soybean oil, corn oil and/or clam lipids and lecithin), rather than feeding specific purified fatty acids of any type (See Tables 2.19 & 2.20).

2.5.5.4.3 Phospholipid requirements

There is convincing evidence that the inclusion of phospholipids into diets for marine crustaceans results in increased rates of growth and/or survival. The growth rate of *P. japonicus* has been shown to increase when 1 % of the lecithin (phosphatidylcholine) fraction of short-necked clam lipids was added to casein-based diets containing 7 % pollack liver oil (Kanazawa *et al.*, 1979f). These authors went on to suggest that since

Table 2.19 Optimum dietary lipid sources for penaeid shrimp species from laboratory tank trials.

<i>Penaeus</i> spp.	Body weight (g)	Lipid sources tested	Optimum lipid source	Reference
<i>P. monodon</i>	0.09	cod liver oil, crude degummed soybean oil, purified soybean oil + lecithin	crude degummed soybean oil + lecithin	Pascual (1986)
	juvenile	pollack liver oil, 18:1 n-9, 18:3 n-3, 20:5 n-3	pollack liver oil and fatty acids	Catacutan & Kanazawa (1985)
	0.62	cod liver oil, soybean oil, coconut oil, corn oil, beef tallow, pork lard	cod liver oil	Catacutan (1991a)
	0.03	cod liver oil, capelin oil, rape seed oil, soybean oil, peanut oil, lecithin	cod liver oil + lecithin or cod liver oil:soybean oil (3:1)	Briggs (unpublished data)
<i>P. japonicus</i>	0.3-0.6	Soybean oil, linseed oil, tripalmitin, sardine oil, clam oil	clam oil or sardine oil	Guary <i>et al.</i> (1976)
	juvenile	pollack liver oil, clam oil, soybean oil	pollack liver oil or clam oil	Kanazawa <i>et al.</i> (1977)
	0.4-1.9	pollack liver oil, soybean oil, cuttlefish liver oil	pollack liver oil: soybean oil (1:1-3:1)	Deshimaru <i>et al.</i> (1979)
<i>P. vannamei</i>	1.0	canola oil, coconut oil, cod liver oil, corn oil, cotton seed oil, lard, linseed oil, soybean oil, tallow	cod liver oil	Dominy & Lin (1989)
<i>P. indicus</i>	0.4-0.7	sunflower oil, linseed oil, peanut oil, soybean oil	peanut oil	Colvin (1976b)
	0.25-0.7	fish oil, linseded oil, sunflower oil	fish oil:sunflower oil (1:2)	Read (1981)
	juvenile	cod liver oil, prawn head oil, sardine oil, soybean lecithin	prawn head oil and mixtures of all oils	Ali (1990)
<i>P. merguensis</i>	2.8	soybean oil, linseed oil, shark oil, cod liver oil	cod liver oil	AQUACOP (1978)
<i>P. setiferus</i>	3.4	beef tallow, linseed oil, menhaden oil, corn oil	beef tallow	Sick & Andrews (1973)

Table 2.20 Dietary lipid requirements of penaeid shrimp species from laboratory tank trials.

<i>Penaeus</i> spp.	Body weight (g)	Optimum lipid source	Dietary lipid level (%)		Reference
			Tested	Optimal	
<i>P. monodon</i>	1.32	cod liver oil & cholesterol	-	7	Alava & Lim (1983)
	0.65	Soyabean oil & others	4.3-10.5	7.7	Deshimaru <i>et al.</i> (1985)
	0.6-0.8	cod liver oil:corn oil (1:1)	5-15	5-10	Bautista (1986)
	0.09	crude degummed soybean oil & lecithin (2 %)	9.4-10.8	9.6-10.8	Pascual (1986)
	0.51	palm oil:cod liver oil (1:1)	10-14	9.9-10.6	Hajra <i>et al.</i> (1988)
	0.62	cod liver oil	0.8-12.8	12.8	Catacutan (1991a)
	0.82	cod liver oil:corn oil (1:5)	-	8.7-8.9	Shiau & Chou (1991)
	juvenile	cod liver oil:corn oil (2:1)	0-12	8-12	Sheen & Chen (1992)
	0.03	cod liver oil & lecithin (3-6 %)	8.6-9.5	9.5	Briggs (unpublished data)
<i>P. japonicus</i>	juvenile	pollack liver oil & soybean oil	-	8.8	Deshimaru & Shigeno (1972)
	0.3-0.6	clam oil or sardine oil	2-8	4	Guary <i>et al.</i> (1976)
	juvenile	clam oil	6-9	8	Kanazawa <i>et al.</i> (1977)
	0.4, 1.9	pollack liver oil: soybean oil (3:1)	3-12	6	Deshimaru <i>et al.</i> (1979)
	juvenile	pollack liver oil, clam lecithin & cholesterol	-	8.5	Kanazawa <i>et al.</i> (1979)
	larvae	Pollack liver oil, lecithin % n-3 HUFA	6.5-16.5	6.5	Teshima & Kanazawa (1984)
<i>P. indicus</i>	0.4-0.7	peanut oil	8-9.9	9.8	Colvin (1976b)
	0.25-0.7	fish oil:sunflower oil (1:2)	3-7.4	5.9	Read (1981)
	juvenile	prawn head oil	0-10	6	Ali (1990)

the fatty acids of clam, soybean or egg lecithin were unable to produce this effect alone, it was the molecular form of the clam lecithin that was responsible. They also suggested that this effect occurred concomitantly with the growth-promoting effect of the naturally high n-3 HUFA content of clam lecithin. Teshima *et al.* (1982; 1986b,c) confirmed this requirement for phospholipids in both larval and juvenile *P. japonicus*, suggesting that optimal growth and survival was obtained on a casein-based diet containing 3 % soy lecithin (24 % phosphatidylcholine) and 6 % pollack liver oil. Kanazawa *et al.* (1985) later reported that phospholipids containing either choline or inositol and particularly high proportions of PUFAs and HUFAs as fatty acid moieties, were the most effective. Further, they suggested that the effectiveness of the phospholipids seemed dependant upon the nature of the fatty acids in the α and β positions of the molecule. Finally, they concluded that the optimum level of soy lecithin in larval *P. japonicus* diets varied with the quality of the basal lipid source, with 3.5 % being optimum when pollack liver oil was used, rising to > 6 % in diets containing inferior basal lipid sources.

Soy lecithin requirements were also shown to be related to other dietary ingredients in the American lobster (*Homarus americanus* Milne Edwards). Conklin *et al.* (1980, 1981) and Bowser & Rosemark (1981) estimated that juvenile lobsters required 6-8 % soy lecithin to prevent 'moult-death syndrome' when fed casein-based diets. However, using crab-protein instead of casein or casein/albumin protein sources, Kean *et al.* (1985) showed that the addition of soy lecithin was unnecessary, although increases in dietary lecithin tended to enhance weight gain when 0.2-0.5 % cholesterol was added to the diet. A growth and survival-enhancing effect has also been shown for soy lecithin with *P. monodon* at levels of 2 % of the diet, regardless of basal lipid source (Pascual, 1986). Chen (1993) later suggested that 1 % dietary phosphatidylcholine was required for optimum growth and survival of *P. monodon*. The exact requirements for dietary lecithin in this species however, have yet to be established. In contrast, Hilton *et al.* (1984) & Briggs *et al.* (1988) have found that the freshwater prawn, *M. rosenbergii* does not require lecithin in semi-purified diets. This result is probably due to differences between the fatty acid requirements of marine and freshwater crustaceans.

It is thought that the requirement of phospholipids in diets for marine crustaceans is due to their role in the transport and effective utilisation of lipids, particularly triglycerides

and cholesterol, in the shrimp body (Teshima & Kanazawa, 1979; D'Abramo et al., 1982, 1985; Teshima *et al.*, 1986b,c; D'Abramo, 1990; Baum *et al.*, 1990). Some growth-enhancing effects of phospholipids may also be due to the provision of choline and inositol, which may be deficient in semi-purified diets (D'Abramo, 1990). The requirements for phospholipids in practical diets however, may be reduced to 1-2 % due to their presence in ingredients such as shrimp, fish and soybean meals (Bordner *et al.*, 1986). However, lecithin may also be useful in shrimp diets as emulsifiers, helping to bind the diets and prevent leaching of water soluble nutrients (Chen & Jenn, 1992).

2.5.5.4.4 Sterol requirements

Many marine crustaceans have been shown to require a source of dietary sterols for optimum growth and survival. Despite the presence of relatively large amounts of sterols in crustacean tissues (23 % cholesterol in *P. esculentus* muscle, Chandumpai *et al.*, 1991), they, like other arthropods, lack the ability to synthesise sterols *de novo* (Van den Oord, 1964; Zandee, 1966, 1967; Whitney, 1970). Several estimates of the sterol requirement of penaeid shrimp have been reported. Most studies agree that cholesterol has the most value for penaeid shrimp, but that other sterols including β -sitosterol, 24-methylcholesterol and desmosterol were almost as effective and could be converted (dealkylated) to cholesterol by the shrimp (Kanazawa *et al.*, 1976; Teshima, 1982; Teshima *et al.*, 1983; Kanazawa, 1985; Teshima & Kanazawa, 1986). The quantity of cholesterol required by various species of crustaceans has been researched by many authors, with the results depending largely on ingredient composition, particularly with regard to the other lipid sources in the diet (Teshima & Kanazawa, 1983; D'Abramo, 1990). *P. japonicus* has been shown to require approximately 0.5-1 % (Kanazawa *et al.*, 1971, 1976; Teshima *et al.*, 1983; Teshima & Kanazawa, 1986; Teshima *et al.*, 1989), *Homarus* sp. 0.1-0.5 % (Castell *et al.*, 1975; D'Abramo *et al.*, 1984; Kean *et al.*, 1985), *P. kerathurus* 1 % (Bianchini, 1985), *P. penicillatus* > 0.5 % (Chen & Jenn, 1991), and *P. monodon* 0.5 % (Chen, 1993). The cholesterol requirements of the freshwater prawn, *M. rosenbergii* were not found to exceed the endogenous 0.12 % level present in the semi-purified diets used (Briggs *et al.*, 1988).

The requirement for cholesterol in shrimp diets is thought to be due to its essential role in biomembranes. Cholesterol is also a precursor for the synthesis of several steroid hormones and vitamin D₃. In addition, cholesterol esters play an important role, in combination with fatty acids (particularly phospholipids), in the absorption and transport of fatty acids from the gut into the haemolymph of shrimp (Tacon, 1990).

2.5.5.4.5 Lipids as dietary energy sources

Once the essential fatty acid, phospholipid and sterol requirements are met, lipids are useful in shrimp diets as highly digestible and rich sources of energy. The absolute requirements for dietary fatty acids, as suggested above, may be very low since purified crustacean diets lacking a source of lipids have been shown to result in reasonable growth and survival (Guary *et al.*, 1976; Davis & Robinson, 1986). Further, D'Abramo (1990) has suggested that levels as low as 0.075 % of the diet may be sufficient to satisfy the requirements for any particular fatty acid. Increased levels of lipid in shrimp diets may thus be assumed to contribute to the animals energy requirements, although a thorough understanding of the lipid requirements of shrimp is still lacking (D'Abramo, 1990). The importance of lipids in protein-sparing however, may be particularly marked in carnivorous species, for which the availability of dietary carbohydrate for energy is low (NRC, 1983; Cho *et al.*, 1985).

Some fish species are thought to be capable of utilising lipid at levels as high as 20-30 % of the diet (Halver, 1976). This ability to efficiently metabolize high levels of dietary lipid has a concomitant sparing effect on the dietary protein required for maximum growth (Biddle *et al.*, 1977). The optimum dietary lipid level for maximum protein sparing in most fish species has been estimated at between 12 and 24 % (Millikin, 1982), the main variation being species-specific. Crustaceans in contrast, are thought to be unable to tolerate such high dietary lipid levels and may be less adaptable to utilising lipids as energy sources than most fish species (Biddle *et al.*, 1977; Lovell, 1978; New, 1980). To date, relatively few studies have been conducted on the lipid requirements of penaeid shrimp, but the general consensus appears to indicate optimum performance on diets containing 5-10 % lipid of animal (marine fish or bivalve) or animal and plant origin (see Table 2.20). Lipid levels of approximately 6 % are thus

generally included in commercial diets for *P. monodon* pond culture (Table 2.12) In most of these studies however, the optimum dietary lipid level is influenced by a number of factors (see section 2.5.3), but particularly the lipid quality, the quality and quantity of protein, and the quality, quantity and availability of other dietary energy sources (D'Abramo, 1990; Sheen & D'Abramo, 1991).

Increasing the levels of dietary lipid above 10-12 % in crustacean diets has usually been reported to reduce growth and survival rates (Forster & Beard, 1973; Deshimaru *et al.*, 1979, 1985; Teshima & Kanazawa, 1984; Bautista, 1986) (see Table 2.20). High levels of dietary lipid may also increase the deposition of lipid in the carcass (particularly the midgut gland), leading to reduced storage time and consumer acceptability (Sedgwick, 1979; Chanmugan *et al.*, 1983). The inability of shrimp and prawns to tolerate such high lipid levels is probably a reflection of the low lipid level in their natural diet (Dall *et al.*, 1991) and their consequent low tissue levels. The total lipid content of cultured prawns, *M. rosenbergii* is 3.2 % (Chanmugan *et al.*, 1983), whilst that of penaeid shrimp has been reported to range from 3.3 to 9.3 % on a dry weight basis (Shewbart *et al.*, 1973; Guary *et al.*, 1974; Colvin, 1976; Teshima *et al.*, 1977; Clarke & Wickins, 1980; Chanmugan *et al.*, 1983). However, the lipid contents of larval and juvenile shrimp may often be elevated (Clarke *et al.*, 1990; O'Leary & Matthews, 1990) which may lead to oxidation of lipids in the shrimp in the absence of suitable antioxidants (*e.g.* vitamin E and selenium). Other problems with high dietary lipid levels include inhibition of appetite due to excessive levels of dietary energy (Church & Pond, 1982), decreased water stability (Akiyama, 1992) and difficult pelletisation at levels of over 6-8 % (D'Abramo, 1990; Akiyama, 1992). Finally, if there is a deficiency of antioxidants in stored diets open to atmospheric oxygen and containing high levels of PUFA-containing lipids, lipid auto-oxidation may result in rancidity which can seriously damage the nutritional value of the diet (Tacon, 1990).

It is likely however, that high levels of dietary lipid produce many of these effects mainly due to imbalances in the energy level of the diets being tested. Thus, diets containing suitable quantities of energy, protein and carbohydrate have been shown to result in good growth, survival and body lipid content in *P. monodon* when dietary lipid levels have been raised to between 10 and 12 % (Bautista, 1986; Pascual, 1986; Hajra

et al., 1988; Catacutan, 1991a,b; Sheen & Chen, 1992). In addition, previous limits placed on the lipid content of shrimp diets imposed due to the processing technology available are no longer valid since extrusion technology currently allows the production of fish diets containing lipid levels of 24 % or more (Akiyama, 1992). The protein-sparing ability of lipids, currently thought to be minimal in shrimp diets, may be enhanced through achieving an optimal balance between the energy-supplying substrates of protein, lipid and carbohydrate in shrimp diets (New, 1976a; Clifford & Brick, 1978; Akiyama, 1992).

2.5.5.4.6 Lipid:carbohydrate ratio

Initial work on lipid and carbohydrate requirements of shrimp and prawns suggested that the best-utilised diets were those containing relatively high levels of digestible carbohydrates and low levels of lipids. This tended to confirm suspicions that carbohydrates are more readily utilised than lipids as dietary energy sources. Due to the low requirements for both lipid and carbohydrates in shrimp, the dietary carbohydrate:lipid ratio (C:L) may thus be as important as the absolute level of either nutrient in shrimp diets. Initial research on freshwater prawns, *M. rosenbergii* (Clifford & Brick, 1978, 1979; Millikin *et al.*, 1980) and crayfish, *Procambarus acutus acutus* (Davis & Robinson, 1986), revealed that optimum growth and protein-sparing was obtained by feeding isonitrogenous, isoenergetic diets containing the lowest levels of lipid and highest levels of carbohydrate tested at C:L ratios of 3-4:1. Similar results have subsequently been found for *P. monodon* (Bautista, 1986; Shiau & Chou, 1991; Shiau & Peng, 1992), and other penaeid species (Colvin, 1976; Sedgwick, 1979; Cuzon *et al.*, 1993) (see Table 2.14). These effects have been attributed to the capacity of omnivorous shrimp and prawn species to utilise dietary carbohydrates more efficiently than lipids (Clifford & Brick, 1978, 1979). However, diets containing high levels of lipids (10-12 %) at C:L ratios of 1.6-3.0:1 have shown some indications of protein sparing (Bages & Sloane, 1981; Alava & Pascual, 1987; Catacutan, 1991a,b; Sheen & Chen, 1992)(see Table 2.14). To date, no work has been conducted looking specifically at the relative merits of carbohydrate versus lipid (C:L ratios) in diets for penaeid shrimp.

2.5.6 Aims of the present study

2.5.6.1 Nursery culture

Some of the questions raised from the deficiencies and inconsistencies in the reviewed information regarding the intensive nursery rearing of penaeid shrimp will be examined in this thesis. These include:

1. Is it possible to rear *P. monodon* in high density concrete secondary nursery tanks and how do tanks compare to cages as secondary nursing systems ?
2. What parameters are critical during this secondary nursing phase to promote optimal production of juvenile shrimp ?
3. What effect does the diet have on shrimp production during the secondary nursing period ?
4. What are the effects of high density secondary nursing on the subsequent performance of the juvenile shrimp during on-growing ? and
5. Is it possible to determine the quality (vigour) of the seed produced from hatcheries and nurseries ?

2.5.6.2 Nutrition

Much work is still required in the field of penaeid shrimp nutrition. One of the most important areas requiring research is that of the dietary energy requirements and how these may be most effectively supplied whilst sparing protein for growth. Research conducted during this thesis has been aimed at elucidating the following areas:

1. What are the optimal sources and levels of the non-protein energy substrates of lipids and carbohydrates in diets for juvenile *P. monodon* ?
2. What is the optimal ratio between protein, lipid and carbohydrate in diets for juvenile *P. monodon* in terms of protein-sparing and dietary energy level ? and
3. Can the results of laboratory-based tank trials be applied to the nutritional requirements of *P. monodon* under intensive secondary nursery culture ?

CHAPTER 3. Nursery rearing trials

We are like thistle-down blown about by the wind - up and down, here and there - but not one in a thousand ever getting beyond seed-hood.

Samuel Butler, 1912

The information contained in Chapter 3 was originally presented in a summarized form at the 22nd World Aquaculture Society Conference, San Juan, Puerto Rico, June 1991, 19 pp.

3.1 INTRODUCTION

The intensive shrimp culture industry that has developed in Asia over the past 15 years currently dominates the worldwide production of farmed shrimp. The culture system employed commonly includes three stages. These are the hatchery, nursery and on-growing phases of the culture cycle. The hatchery phase involves spawning of broodstock and a period of larval rearing which raises shrimp from the egg, through a number of larval stages, to the post-larval stage of development. Following larval rearing, the shrimp enter the nursery phase where they are cultured intensively in primary and then occasionally secondary nurseries for a total of 1-8 weeks. Following the primary or secondary nursing phase, the shrimp are stocked into ponds for the final phase in the cycle, that of on-growing to market size.

Of the three stages, the nursery phase has received the least amount of attention, but will become increasingly important as the global trend for intensification of the shrimp culture industry continues. The current trend is to rear ex-hatchery post-larvae 4-7 days post-metamorphosis (PL₄₋₇) for 1-2 weeks until reaching PL₁₂₋₂₀ in tank nursery systems before they are stocked into the on-growing ponds. This is known as primary nursing.

This primary nursing phase is essential since it provides a period of time over which the post-larvae can become adapted from the controlled conditions found within the hatchery to the more variable conditions of the grow-out ponds. In particular, the primary nursing phase allows close management of the shrimp over a critical period in

their life cycle. It is at this stage that their physiology is most rapidly metamorphosing, the shrimp are adapting from a pelagic to a benthic lifestyle and their diet is changing from live feeds of phyto- and zoo-plankton to larger, benthic prey items including molluscs and crustaceans.

Direct transfer of post-larvae from the primary nursery however, has often been found to result in high and unpredictable mortality at the beginning of the on-growing phase of culture (de la Pena *et al.*, 1985). This is probably due to a number of factors, but primarily to the low tolerance of young post-larvae to environmental stress and predation within the grow-out ponds. This results in problems with the estimation of feeding rates and the economics of the on-growing culture operation. For these reasons, some farmers include a further or 'secondary' nursing period in the culture cycle. This involves the rearing of primary nursed, or more rarely, wild-caught, post-larvae for a further period of 2-6 weeks, before transfer of the juvenile shrimp to the on-growing ponds. This secondary nursing has been conducted in ponds, cages or tanks of various sizes and at densities ranging from 50 to 17,000 PL m².

Research into secondary nursing of post-larvae was initiated in the early 1980s, but has only been the subject of detailed investigations over the past seven years. The amount of research work conducted however, has not been great and has concentrated mainly on the American species *P. vannamei*. Nevertheless, at least 50 % of American (north, central and south) farmers now utilize secondary nurseries, with a lesser (unknown) number using them for the predominant Asian species, *P. monodon* (Hopkins & Villalon, 1992; Stern & Letellier, 1992).

The use of secondary nursery systems has a number of potential benefits and disadvantages over the direct stocking of young primary-nursed or wild-caught post-larvae. The advantages include improvement of stock, the ease and predictability of management operations, the chance to headstart shrimp in order to increase the number of crops possible per year, particularly in temperate zones, the maximisation of system use, and the increased feeding efficiency possible, all resulting in economic benefits. The potential disadvantages include management problems in terms of harvesting and transport difficulties, and reduced stock productivity due to low and variable growth and

survival rates.

Due to the paucity of information on the use of secondary nursery systems (particularly highly-intensive tank nurseries for *P. monodon*), the advantages, problems, constraints and commercial viability of such systems have yet to be evaluated. Particular problems in need of research include:

1. the optimal methods for management of secondary nursery systems with particular regard to nursery system type, stocking density, duration of rearing period, water quality control, nutritional requirements, feeding regime and harvesting and transfer of the seed produced,
2. the potential of secondary nursing to enhance the quality of seed for stocking on-growing ponds, particularly in terms of seed quality and subsequent on-growing performance, and
3. the economic benefit to the inclusion of a secondary nursery phase in the intensive shrimp culture system.

There is therefore, a need for research on simple, low-cost outdoor nursery techniques which can be adopted rapidly and easily in tropical and subtropical regions of the world (Mulla & Rouse, 1985). For these reasons, a concrete nursery tank system was constructed and maintained over a two year period in order to investigate factors relating to the management of an intensive tank secondary nursery for *P. monodon*. The present study involves a series of five secondary nursery trials conducted in concrete tanks, one secondary nursery trial conducted in net cages and one on-growing trial conducted in net cages. Many interconnected variables may effect the growth, survival, feeding efficiency, productivity and profitability of the secondary nursing period. Separate trials were therefore conducted to study some of these variables, and the aims of these trials are listed below:-

1. to investigate parameters involved in the management of a concrete secondary nursery tank system for *P. monodon*. Particular emphasis was placed on a)

stocking density (trials 1 & 3), b) aeration (without air, trials 1 & 2, and with aeration, trials 3-5), c) mesh panel habitats (trials 2 & 4), d) sand substrates (trial 4), e) dietary formulation (trials 3 & 5), and f) feeding rate (trial 5),

2. to compare the use of concrete tank and net cage secondary nursing on shrimp performance (net cage nursery trial),
3. to investigate the economics of secondary nursery rearing in concrete tanks, and
4. to investigate the effects of secondary nursery rearing at various densities on the subsequent on-growing performance of juvenile shrimp in net cages (net cage on-growing trial).

In addition to an analysis of the secondary nursing of post-larval shrimp, the concrete nursery tanks were subsequently used for a further study on the nutritional requirements of the shrimp. This trial was conducted using diets developed and tested in laboratory-based studies at Stirling in order to compare results obtained in the laboratory with those obtained in the nursery tanks. This trial is detailed in Chapter 8.

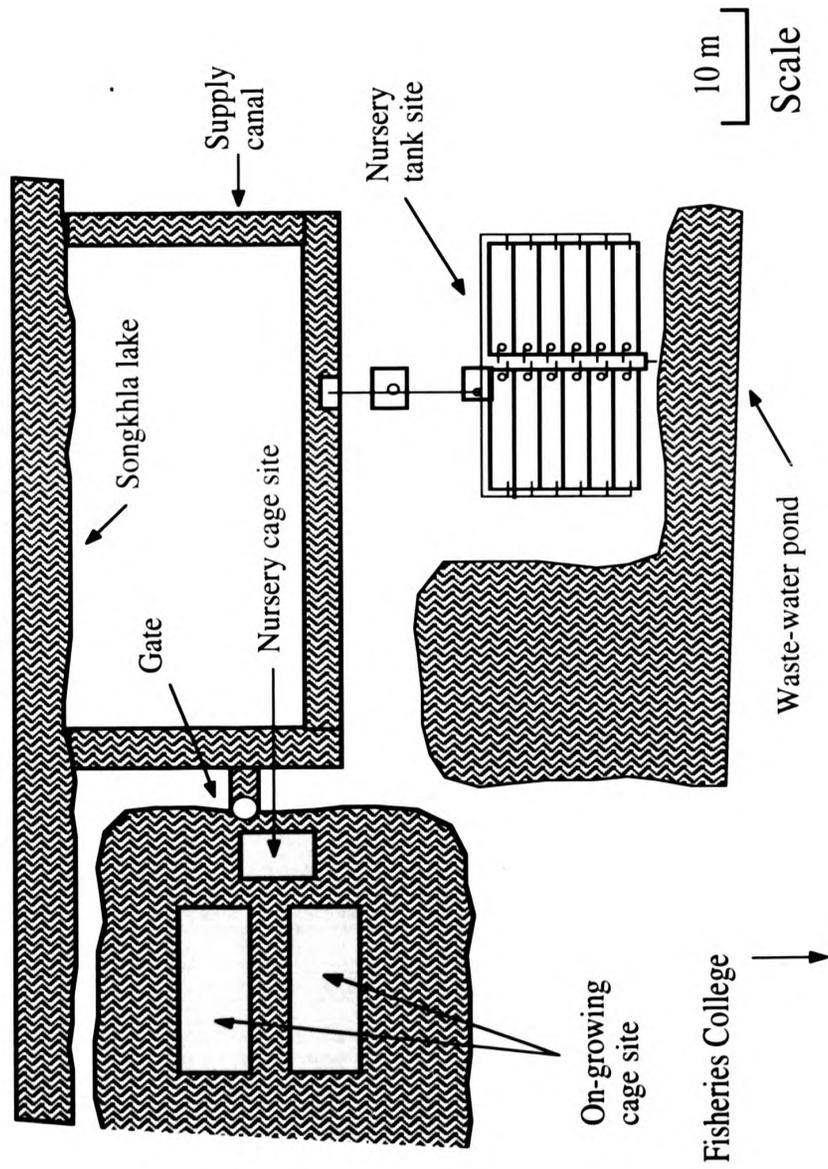
3.2 MATERIALS AND METHODS

3.2.1 Concrete tank nursery trials

The concrete nursery tank system was designed for this study and built at the field station of the Institute of Aquaculture at the Tinsulanonda Songkhla Fisheries College on Songkhla lake, southern Thailand. The tanks were located outside, adjacent to the canal supplying water to the college ponds from the nearby Songkhla Lake. The location of the nursery tank system on the fisheries college site is shown in Figure 3.1.

In total five separate trials were completed in the concrete nursery tanks. Each trial utilised four treatments, with three replicate tanks per treatment. The tanks for each replicate were selected at random for each trial in order to negate any effects of tank

Figure 3.1 Layout of experimental facilities in Songkhla.



position within the system. The trials were each run for 35 days, rearing post-larval *P. monodon* from PL₁₅ to PL₅₀. The tanks were scrubbed clean between trials using a hose and long-handled brush.

The five trials investigated shrimp performance and water quality in relation to the following parameters:

- Trial 1: **Stocking density** (125, 250, 500 & 1,000 PL m⁻²)
- Trial 2: **Habitats** (increase wetted surface area WSA by 0, 40, 80 & 160 %)
- Trial 3: **Stocking density** (1,000, 1,000, 2,000 & 3,000 PL m⁻²) and **dietary formulation** (reference and test) with aeration and water filtration
- Trial 4: **Habitats** (increase WSA by 0 & 120 %) and **substrates** (0 & 10 cm sand) with aeration and water filtration
- Trial 5: **Feeding rate** (low and *ad libitum*) and **dietary formulation** (reference and test) with aeration and water filtration

The treatments used in each trial are shown in Table 3.1.

3.2.1.1 Experimental system

3.2.1.1.1 Concrete nursery tanks

The reinforced concrete nursery tank system constructed comprised 12 tanks each 10 m in length, 2.5 m in width and 1.3 m deep (Figure 3.2). These tanks were constructed in two rows of six tanks, separated by a communal harvesting channel 1 m in width. A small area (5 %) at the outflow end of the tanks and the harvesting channel was covered by a sloping roof, with the remainder of the tank area being uncovered. The tanks had sloping bottoms to facilitate harvesting and the mean water depth was 1 m (range 0.9-1.2 m). The tanks were each thus 25 m² in bottom area, 50 m² in wetted surface area (WSA) and contained 25 m³ of water.

Table 3.1 Summary of concrete nursery tank trial treatments.

Trial No.	Treatment No.	Stocking density (shrimp m ⁻²)	Habitats (no. of panels)	Substrate (sand cm)	Aeration (l sec ⁻¹)	Diet type	Feeding Rate (% body wt d ⁻¹)
1	1	125	0	0	0	ref 1	normal
	2	250	0	0	0	ref 1	normal
	3	500	0	0	0	ref 1	normal
	4	1,000	0	0	0	ref 1	normal
2	1	500	0	0	0	ref 1	normal
	2	500	1	0	0	ref 1	normal
	3	500	2	0	0	ref 1	normal
	4	500	4	0	0	ref 1	normal
3	1	1,000	0	0	12	ref 1	normal
	2	1,000	0	0	12	test 1	normal
	3	2,000	0	0	12	ref 1	normal
	4	3,000	0	0	12	ref 1	normal
4	1	2,000	0	0	12	ref 1	normal
	2	2,000	0	10	12	ref 1	normal
	3	2,000	3	0	12	ref 1	normal
	4	2,000	3	10	12	ref 1	normal
5	1	2,000	3	0	24	ref 2	low
	2	2,000	3	0	24	ref 2	<i>ad libitum</i>
	3	2,000	3	0	24	test 2	low
	4	2,000	3	0	24	test 2	<i>ad libitum</i>

Notes:

Reference diets 1 & 2: see Table 3.2

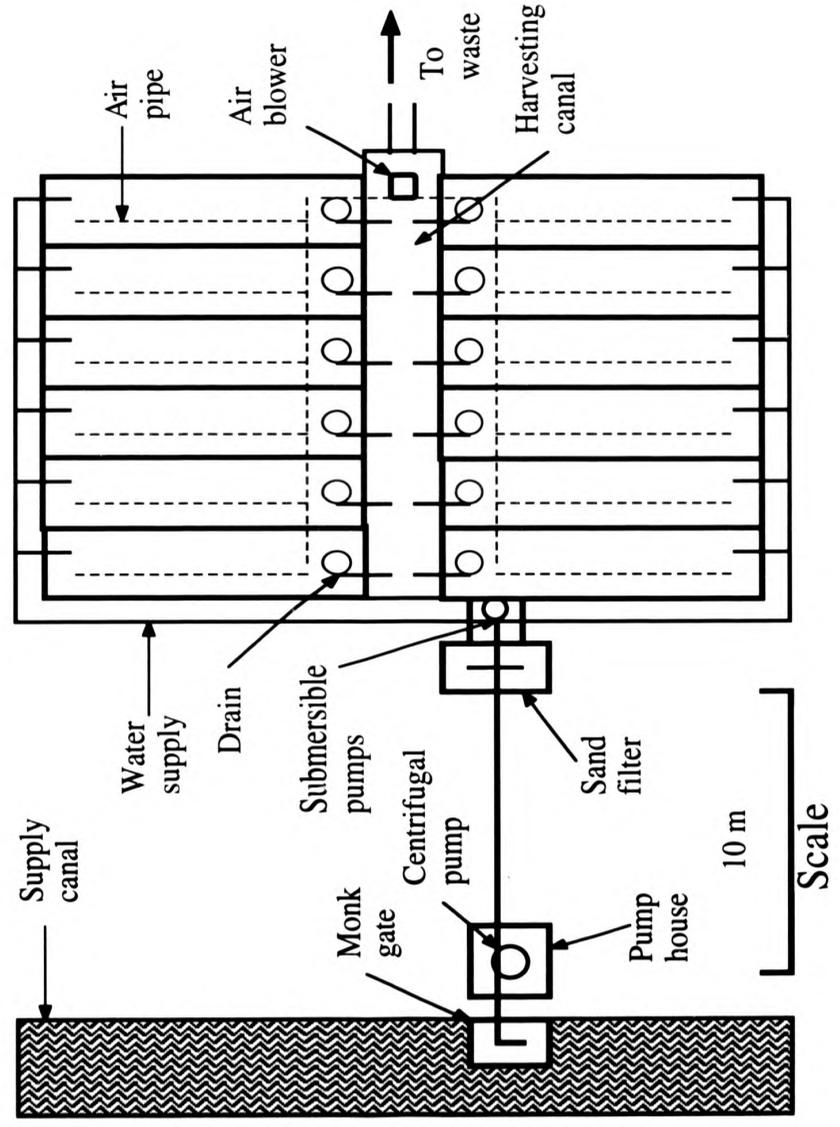
Test diets 1 & 2: see Table 3.2

Normal feeding rate: 35, 30, 25, 20 & 15 % wet body wt d⁻¹ for weeks 1-5 respectively,

Low feeding rate: 30, 25, 20, 15 & 10 % wet body wt d⁻¹ for weeks 1-5 respectively,

Ad libitum feeding rate: 45, 60, 42, 42 & 30 % wet body wt d⁻¹ for weeks 1-5 respectively.

Figure 3.2 The concrete nursery tank system at Songkhla.



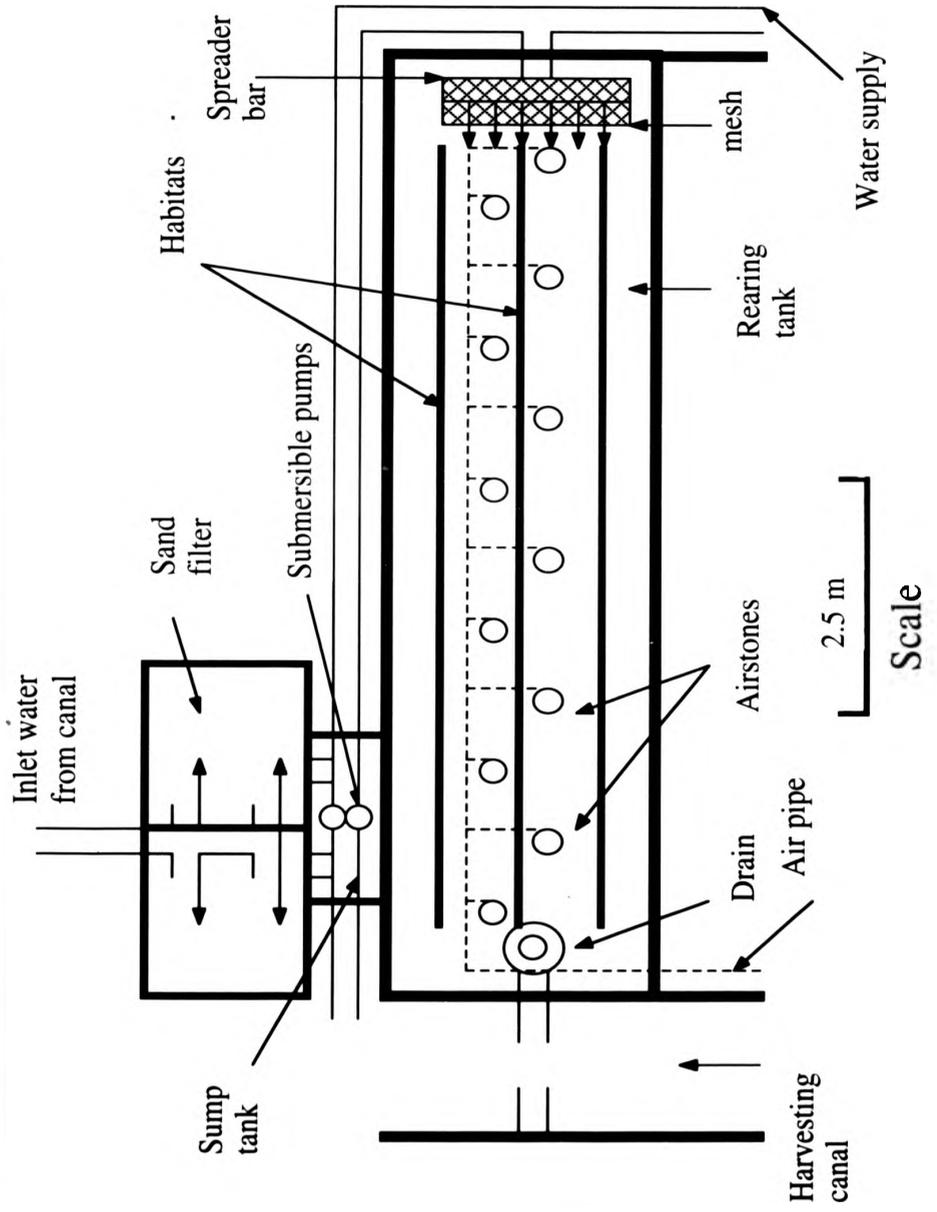
3.2.1.1.2 Water source, filtration, exchange rate and drainage

Water for the trials was pumped with one of two 2.2 kW electrically operated centrifugal pumps (Desmi Ltd.) from a screened (1 mm nylon mesh) monk gate in a canal supplying water to the main pond facility at the Tinsulanonda Songkhla Fisheries College. This water originated from the tidal Songkhla Lake and varied in salinity from 3 to 30 ‰ salinity depending upon season. Water was supplied via a foot valve in 50 mm PVC piping located within the monk gate, 30 m over a head of 3 m to the concrete tank system. For trials 1-2, water was introduced directly into the tanks via 32 mm PVC piping spreader bars to assist oxygenation of the water. The spreader bars were 2 m in width, punctured liberally with 10 mm holes and screened with 1 mm nylon mesh to prevent the introduction of fish or large debris (Figure 3.3).

For trials 3-5, a gravity sand filter was constructed and installed due to problems with excessive levels of suspended solids in the water supply (Figure 3.3). The sand filter had the following dimensions - width 4 m, length 4 m, height 1.5 m and was divided into two equal halves which could be operated either independently or in conjunction. The filter media in the sand filter rested upon a layer of hollow concrete blocks (30 x 10 x 5 cm) covered by a sheet of 2 mm plastic mesh. Three layers of media covered the mesh. The first layer was a 30 cm depth of 1 cm diameter coarse gravel, the second was a 30 cm depth of 5 mm fine gravel, and the third layer was a 30 cm depth of 2 mm coarse sand. In total there was 7.2 m³ of filter media in the sand filter. Water was pumped into the top of the sand filter from the monk gate, descended through the filter media by gravity into a sump and was then pumped into the tanks by two 1 kW electrically operated submersible pumps (Fluval Ltd.) as for trials 1-2. When the sand filter became blocked (approximately weekly), canal water was pumped into the bottom of the tanks so that it up-welled throughout the filter media and the resulting water and sediments were discharged to waste.

Water was supplied continuously (except during power cuts) to the tanks on a flow-through basis at 20 % tank volume d⁻¹ (3.5 l min⁻¹) per tank initially, rising to 40 % tank volume d⁻¹ (7.0 l min⁻¹) during the fifth week of all trials. Each tank was provided with its own water control valve on the inflow pipe so that the water flow could be

Figure 3.3 Details of concrete nursery tanks and sand filter.



adjusted to the desired rate. Water flow-through was necessary in order to maintain water quality within tolerable limits for the shrimp. Each of the water pumps had the capacity to supply 200 l min^{-1} which enabled the complete filling of all tanks within 24 h. This capacity also enabled each days' water supply to be pumped within 6 h if water of the correct salinity was only available at a particular stage of the tidal cycle. The excess capacity also facilitated rapid flushing of the tanks if poor water quality conditions (*i.e.* low dissolved oxygen) were encountered and back-flushing of the sand filter when dirty.

At the opposite end of each tank from the inlet, water exited from the bottom of the tanks via a double standpipe outlet system (Figure 3.3). The outlet system comprised a 100 mm hole in the bottom of the tank 15 cm from the end wall. Into this hole was placed a 1.2 m length of 100 mm diameter PVC pipe. A second 125 mm diameter PVC standpipe was placed over the internal pipe. The bottom 8 cm of this outer pipe was bored with numerous 10 mm holes over which was glued a section of 1 mm nylon mesh to prevent escape of the post-larvae. The outlet water plus any fine sediments then flowed through this mesh, up between the standpipes and out through the hole into the harvesting channel between the two rows of tanks before discharge. The outlet pipe into the harvesting channel was also covered with 1 mm nylon mesh to prevent escape of smaller post-larvae immediately following stocking.

3.2.1.1.3 Aeration

Trials 1 and 2 in the series did not utilise aeration due to the relatively low stocking densities used. When stocking densities were increased for trials 3-5, aeration was found to be necessary. Aeration was provided continuously with the use of a 1.5 kW, electrically operated air blower installed on a raised platform in the harvesting channel between the tanks (Figure 3.3). The air blower supplied air via a ring main of 2.5 cm PVC piping to which 10 m lengths of 2 cm flexible PVC hoses were attached. Each tank was supplied with one such hose which supplied air via 5 mm air hose to 4 cm diameter airstones located at regular intervals along the bottom of each tank. Trial 3 used 6 airstones tank^{-1} , providing an air volume of $0.04 \text{ l sec}^{-1} \text{ m}^{-2}$. Due to problems with low levels of dissolved oxygen in trial 3, trials 4 and 5 used 12 airstones tank^{-1} ,

providing an air volume of $0.07 \text{ l sec}^{-1} \text{ m}^{-2}$.

3.2.1.1.4 Habitats

Habitats were used in trials 2, 4 and 5 (Figure 3.3). The habitats consisted of 10 m lengths of 1 m wide 1 mm nylon mesh netting. Each habitat used thus added 20 m^2 of WSA to the tanks. The mesh panel habitats were fixed with 10 large (12 x 5 cm) fishing net floats spread at 1 m intervals along the upper edge and 10 half brick weights at 1 m intervals along the lower edge. The habitats were secured longitudinally along the length of the tanks so that they reached from the surface to the bottom of the tanks.

3.2.1.1.5 Substrates

Substrates were used only in trial 4. Substrates used consisted of a 10 cm layer of coarse grained (2 mm) riverine sand completely covering the bottom of the concrete nursery tanks.

3.2.1.2 Experimental animals

The *P. monodon* PL₁₅ used in these trials were obtained from the Aquastar shrimp farm in Ranod, southern Thailand. The shrimp were reared from eggs originating from wild-caught (Andaman sea) broodstock. Larval rearing was conducted using the "Taiwanese method", incorporating 50 % daily water exchange after mysis stage, feeds of *Skeletonema* sp. algae, *Artemia* nauplii and microencapsulated diets, and antibiotic treatment of bacterial diseases. The shrimp were transferred from the hatchery tanks at PL₅₋₇ to concrete primary nursery tanks where they were fed on *Artemia* nauplii until delivery to the experimental facility. Differences between the treatment of the larvae/post-larvae prior to the five nursery rearing trials could not be monitored due to lack of control over the hatchery and primary nursery periods. Although it is believed that there were no major differences between the treatments used for the various batches, there were size differences between the PL₁₅ used for the various nursery trials, possibly due to variations in the broodstock quality, or feeding regime used during the hatchery and/or primary nursery phases.

The PL₁₅ were delivered by truck in the cool of the morning (6-7 am) in oxygenated, double plastic bags, part-filled with 5 l of seawater at 30 ‰ salinity. For the trials where the initial salinity of the secondary nursery tanks was lower than 30 ‰, the salinity of the water in the primary nursery was adjusted to that in the secondary nursery tanks before post-larval transfer. Each bag contained 3-4,000 PL₁₅, which were delivered within one hour from the hatchery in Ranod, 70 km distant from the experimental nursery tank site. Three randomly selected bags were counted on arrival and the required number of shrimp gradually acclimated to the nursery tanks. Acclimation was achieved according to the following procedure. The number of bags corresponding to the correct number of shrimp were floated on top of the water in each tank. The bags were then opened and allowed to stand in the tanks for 30 min for temperature acclimation. The bags were then half-filled with water from the tanks and allowed to stand for a further 30 min for salinity acclimation. At this time, the bags were gently emptied into the tanks.

Trials were initiated on the day following introduction of the shrimp. Shrimp excess to requirements were always ordered, and a representative sample of 500 shrimp were blotted dry with tissue paper and counted. Three samples of 500 shrimp were then batch weighed (± 0.001 g) using a top pan balance (Mettler AJ100) and 100 shrimp individually measured (total length ± 0.5 mm) on a measuring board with 1 mm divisions to establish the mean initial weight and length of the shrimp. The PL₁₅ used had mean weights of 0.002-0.005 g and mean total lengths of 9-12 mm.

3.2.1.3 Feeds and feeding

3.2.1.3.1 Diet formulation and size

Fertilization of the tanks was not performed, but the tanks were filled one week prior to the start of each trial in order to settle out any solids present in the incoming water. Animals were fed only formulated cold-extruded pellets. The reference (control) and test diets used in all trials were supplied by the Aquastar fed mill in Ranod, southern Thailand. The reference diet was the commercial post-larval diet produced by Aquastar, whilst the test diet was a new formulation under test by Aquastar. Both diets cost £

0.86 kg⁻¹. The diets were purchased at the start of each trial and stored in a freezer until required in order to maintain freshness.

Trials 1-4 used reference diet 1, with one treatment in trial 3 using test diet 1. Two treatments in trial 5 used reference diet 2 and two treatments used test diet 2. Three replicate tanks were used for all treatments. The proximate composition of these diets are shown in Table 3.2. All diets were manufactured in two particle sizes. Size 1 was a crumble of 0.5-1 mm particle size and was fed for weeks 1-3, and size 2 was a pellet of 1-2 mm particle size and was fed for weeks 4-5. Both sizes of feed had the same proximate composition.

3.2.1.3.2 Feeding regime

The shrimp were fed six days wk⁻¹ (not feeding on the sampling day), four times d⁻¹ (25 % of the daily ration per feeding) at the following times: - 0700, 1100, 1500 and 1900. In trials 1-4, the shrimp were fed (on a wet shrimp/dry feed basis) at the normal rate (estimated as optimum during preliminary trials) of 35, 30, 25, 20 and 15 % of body weight d⁻¹ for weeks 1-5 respectively, allowing for an estimated mortality of 5 % wk⁻¹. In trial 5, the reference and trial diets were each fed a low feeding rate in one treatment and *ad libitum* (to satiation) in one treatment. Feeding was at 30, 25, 20, 15 and 10 % of body weight d⁻¹ for weeks 1-5 respectively at the low feeding rate. Feeding to satiation resulted in feeding rates of 46, 58-62, 42, 40-43 and 28-30 % of body weight d⁻¹ for weeks 1-5 respectively. In trial 5, the mortality, for purposes of feeding rate estimation, was based on that found in trial 4 (9 % wk⁻¹).

Feed was broadcast evenly over the entire surface of each tank by hand to allow all shrimp an equal opportunity to feed in trials 1-4 and in the low feeding rate for trial 5. In trial 5, feeding trays were used to establish when the shrimp were being fed to satiation. Two 1 m² feeding trays tank⁻¹, constructed from 1 mm nylon mesh on metal frames, suspended 5 cm off the tank bottom were used. Feed was broadcast evenly over the tanks and the trays, and the trays inspected prior to each feeding. If a large quantity of feed remained on the trays uneaten, the feeding rate was decreased by 5 % and *vice versa*.

Table 3.2 Proximate analysis of the diets used in nursery and on-growing trials.

Proximate analysis ¹	Reference diet 1	Reference diet 2	Test diet 1	Test diet 2
Protein ²	50.5	49.1	48.0	52.6
Total lipid ³	3.9	7.3	10.0	11.2
Carbohydrate ⁴	25.7	25.3	23.9	17.4
Fibre ⁵	2.5	2.5	2.0	1.6
Ash ⁶	17.5	15.9	16.1	17.2
Moisture ⁷	11.7	9.6	10.1	10.3
Total energy (kJ g ⁻¹) ⁸	17.8	18.7	19.3	19.8
Pr:E ratio (mg protein kJ ⁻¹ TE)	28.4	26.2	24.8	26.6
Carbohydrate:lipid ratio	6.6	3.5	2.4	1.6

¹ All results based on the mean of three replicates

² Analysed by macro-Kjeldahl analysis (AOAC, 1980)

³ Analysed by method of Folch-Lees (Folch *et al.*, 1957)

⁴ By difference in dry weight (NFE)

⁵ Measured by fibretec

⁶ Measured by combustion at 450 °C for 12 h

⁷ Measured by hot air oven drying at 105 °C for 24 h

⁸ Calculated using mean gross energy values of 23.4 kJ g⁻¹ for protein, 39.8 kJ g⁻¹ for lipid and 17.2 kJ g⁻¹ for carbohydrate.

3.2.1.4 Water quality measurement

A range of water quality parameters were monitored according to the following schedule:- twice daily (before the first and third feeds of the day), water from the inlet canal and the outlet of the tank was measured for dissolved oxygen (DO), temperature, pH, salinity and transparency. Weekly, water from the same sources was collected in one litre plastic bottles, filtered and measured for suspended solids, total ammonia nitrogen (TAN), nitrite nitrogen (NO₂-N) and dissolved reactive orthophosphate (DRP).

Dissolved oxygen and temperature were measured using a WTW microprocessor OXY 96 salinity-adjusted oxygen/temperature meter ($\pm 0.1 \text{ mg l}^{-1}$ and $\pm 1 \%$ saturation DO and $\pm 0.1 \text{ }^\circ\text{C}$). pH was measured using a WTW microprocessor pH 95 pH meter ($\pm 0.01 \text{ pH units}$). Salinity was measured using an Atago refractometer ($\pm 0.5 \text{ }^\circ\text{‰}$). Transparency was measured by Secchi depth readings and expressed as depths in m ($\pm 0.01 \text{ m}$) (Stirling, 1985). Weekly water samples were filtered under vacuum in a Buchner flask through preweighed and washed $1.2 \text{ } \mu\text{m}$ nominal pore size, 7 cm Whatman GF/C glass fibre filters. The filter papers were dried overnight at $75 \text{ }^\circ\text{C}$ and re-weighed to calculate the load of suspended solids ($\pm 0.1 \text{ mg l}^{-1}$) (Strickland & Parsons, 1972). Filtered water samples were immediately frozen ($-4 \text{ }^\circ\text{C}$) for a maximum of 2 days prior to colorimetric analysis for TAN and NO₂-N (Strickland & Parsons, 1972) and DRP (Eisenreich *et al.*, 1975) using a JASCO UVIDEK-430B double beam spectrophotometer. The results were expressed in $\mu\text{g l}^{-1}$.

3.2.1.5 Shrimp performance measurement and harvesting

Shrimp performance was measured once weekly (on the non-feeding day) for growth in length and weight by taking three random samples of 500 shrimp tank⁻¹ using a standardized netting procedure. This involved rapidly pushing a long-handled net along the entire length of the bottom of each tank and collecting random samples of shrimp from the net on surfacing. This procedure was repeated until the required number of shrimp were obtained. The shrimp were weighed and measured as in section 3.2.1.2. The shrimp were then replaced in their respective tanks. These weekly samplings were used to estimate growth rate and total biomass (assuming 5 % mortality wk⁻¹) in order

to adjust the daily feeding rate.

At the end of each trial on day 35, all the remaining shrimp were harvested. The water supply to each tank in turn was stopped and all but the last 20 cm of water drained from the tanks through the harvesting channel. This was achieved by replacing the two standpipes with a 100 mm diameter perforated standpipe covered with 1 mm nylon mesh to prevent shrimp loss. This had the effect of reducing water pressure on harvesting the shrimp so that they would not be damaged. Then a 50 cm long, 7.5 cm wide bag constructed from 1 mm nylon mesh was fastened (with a drawstring) to the outflow pipe leading into the shaded harvest channel between the two rows of tanks. The perforated standpipe was then withdrawn and the water allowed to drain out through the bag nets. Approximately 1 kg batches of shrimp were harvested, transferred to large (50 l) aerated bins, netted out, blotted dry and weighed with a top pan balance. This procedure was repeated until each tank was completely drained, using a final flush of clean water to harvest any remaining shrimp, before proceeding to the next tank. Three subsamples of shrimp were weighed and measured as described in section 3.2.1.2. The total and mean weight and mean length of shrimp in each treatment were measured and the survival, food conversion ratio (FCR), food conversion efficiency (FCE) and production calculated.

3.2.1.6 Data analysis

Shrimp performance data from the nursery trials was analysed as described below:-

1. Growth rate was measured as specific growth rate (SGR)

$$\text{SGR (\%)} = \frac{(\ln W_f - \ln W_i)}{(T_f - T_i)} \times 100$$

where:

SGR (%) = specific (relative) growth rate as a percentage of the wet body weight
d⁻¹ (assuming exponential growth)
W_i = mean initial wet weight in grammes on the day T_i
W_f = mean final wet weight in grammes on the day T_f

ln = natural logarithm (base e)
T_i = days (initial)
T_f = days (final)

2. Apparent food conversion ratio (FCR)

$$\text{FCR} = \frac{\text{Dry food fed (g)}}{\text{Wet weight gain (g)}}$$

3. Apparent food conversion efficiency (FCE)

$$\text{FCE} = \frac{\text{Wet weight gain (g)}}{\text{Dry food fed (g)}}$$

4. Survival

$$\text{Survival (\%)} = \frac{\text{Number of shrimp } T_f}{\text{Number of shrimp } T_i} \times 100$$

5. Production (yield)

$$\text{Production (g m}^{-2} \text{ d}^{-1}) = \frac{W_f - W_i}{A \times (T_f - T_i)}$$

where:

W_i = total wet weight in grammes on the day T_i
W_f = total wet weight in grammes on the day T_f
A = area of the tank in m²

6. Coefficient of variation (CV) of shrimp length

$$\text{Coefficient of variation} = \frac{S}{x} \times 100$$

where:

S = standard deviation of length
x = mean length

The effect of stocking density on the coefficient of variation in weight and length of PL₁₅ prior to the nursery trials, PL₃₀ following the nursery trials and juvenile shrimp following cage on-growing was investigated. This was analysed using a non-parametric analysis of variance (Kruskal-Wallis test) after a Bartlett test revealed that in all cases the data were heteroscedastic (not normally distributed) (Sokal & Rohlf, 1981).

7. A one way analysis of variance with equal sample size was used to investigate the effect of the single variable in each of tank trials 1 (including the net cage trial) and 2 on shrimp growth, survival (arcsin transformed), FCR, FCE and production after 35 days. Equality of variances was confirmed using a Barlett test and normality could be demonstrated graphically (Sokal & Rohlf, 1981).
8. The effect of stocking density for treatments 1, 3 and 4 in tank trial 3 on shrimp growth, survival (arcsin transformed), FCR, FCE and production after 35 days was analysed using a non-parametric analysis of variance (Kruskal-Wallis test) after a Bartlett test revealed that in all cases the data were heteroscedastic (Sokal & Rohlf, 1981). Treatment 2 in this trial was compared to treatment 1 (at a common density) for the effects of dietary formulation on shrimp growth, survival (arcsin transformed), FCR, FCE and production after 35 days, using the non-parametric Wilcoxon paired sample test after a Bartlett test revealed that in all cases the data were heteroscedastic (Sokal & Rohlf, 1981).
9. After heteroscedasticity was demonstrated using a Barlett test, data were ranked

and a two factor non-parametric analysis of variance with equal sample size (Scheirer, Ray & Hare, 1976, in Zar, 1984) was used to investigate the effect of the two variables in each of tank trials 4 and 5 on shrimp growth, survival (arcsin transformed), FCR, FCE and production after 35 days.

10. The T-method for multiple comparisons was used to determine statistical differences between treatment means for the above ANOVAs (Sokal & Rohlf, 1981). Results were considered significant if $P < 0.05$.
11. A basic economic analysis was carried out using a computerised investment appraisal on the nursery systems to estimate the minimum price necessary for juvenile shrimp for the economic functioning of the secondary nursery systems.

3.2.2 Net cage nursery trial

As a comparison to the concrete nursery trials, one net cage nursery trial was conducted simultaneously to the first concrete nursery tank trial (trial 1). The net cage nursery trial utilised three replicate net cages of one treatment, *i.e.* stocking shrimp at 500 PL₁₅ m⁻². The trial was run for 35 days to PL₅₀, as for the concrete nursery tank trials.

The cages were 3 m in length, 3 m in width and 1.5 m in depth. The cages were constructed of 1 mm nylon mesh and were fixed at each corner, top and bottom, to 3 m long bamboo stakes, driven into the bottom of a 1.5 m deep pond in the fishery college, adjacent to the concrete nursery tank system (Figure 3.1). This pond was open to twice daily tidal water exchange so that the cages were supplied with no additional form of water exchange or aeration. The water depth in the cages varied from 0.8 to 1.2 m depending on the state of the tide and was a mean of 1 m in depth. The cages thus had a mean wetted surface area of 21 m² and bottom area of 6 m².

The cages contained no habitats or substrate and did not reach the pond bottom. Each cage however, contained one 1 mm mesh feeding tray of 0.25 m² in surface area as for nursery tank trial 5.

Feeding was carried out according to the schedule for the nursery tank trials at the normal feeding rate of reference diet 1 (see section 3.2.1.3). The feed was placed directly onto the feeding tray, rather than distributing it throughout the cages.

Water quality and shrimp performance was monitored according to the same schedule as for the nursery tank trials. Harvesting was carried out by untying the cages from their stakes and emptying all shrimp directly into bins full of aerated water.

Data analysis was conducted as for nursery tank trials 1 and 2 (see section 3.2.1.6) using one way ANOVA and the T-method for multiple comparisons. The results from the two nursery systems could thus be directly compared.

3.2.3 Economic analysis of nursery trials

An investment appraisal using a computer spreadsheet package (Lotus 123) was conducted on the concrete nursery tank system. This appraisal was carried out as a preliminary screening assessment. As such, it was used to give an indication of whether the nursery could be operated economically under the constraints experienced during this study, and which parameters most affect the viability of such a system. All costings were based on those recorded in southern Thailand during the nursery trials and were converted into pounds Sterling for the analysis.

A 'base case' was investigated for three alternative stocking densities which were most likely to be economically viable, *i.e.* 500, 1,000 and 2,000 shrimp m⁻². The base case was established using results generated from the trials conducted during this study at the relevant stocking densities. Since the selling price obtainable for shrimp juveniles is not accurately known, the base case used the lowest selling price for each stocking density which would yield a positive internal rate of return (IRR) on the capital investment. The net present value (NPV) of the system was also calculated. These calculations were based on a discount rate of 15 % and a tax rate on profits of 40 %. A sensitivity analysis was also conducted in order to assess the effects of variations in the cost or revenues for some of the most important parameters of the nursery system.

3.2.4 Net cage on-growing trial

One on-growing trial was conducted in order to assess the effects of secondary nursery rearing at variable stocking densities on the subsequent on-growing performance of juvenile *P. monodon*.

The juvenile shrimp stocked into the cages were those produced from the first concrete nursery tank trial (trial 1), and those produced from the net cage nursery trial. Samples of shrimp from these trials were stocked at a density of 1,000 PL₅₀ cage⁻¹ (125 shrimp m⁻²). The trial was run for a total of ten weeks. The trial utilized two replicate net cages per treatment for each of 5 treatments, with a total of ten cages. The treatments used were as follows:

Treatment 1: 0.887 g shrimp from nursery tank trial 1 nursed at 125 shrimp m⁻²,

Treatment 2: 0.550 g shrimp from nursery tank trial 1 nursed at 250 shrimp m⁻²,

Treatment 3: 0.413 g shrimp from nursery tank trial 1 nursed at 500 shrimp m⁻²,

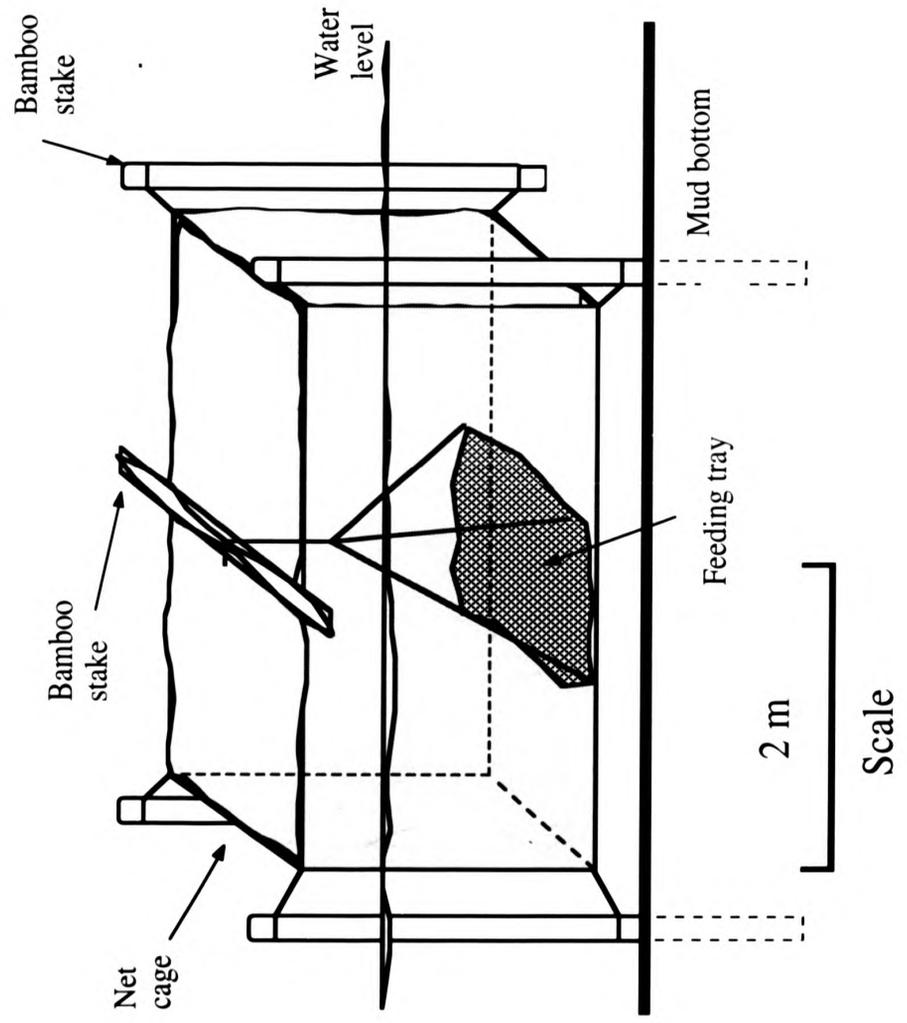
Treatment 4: 0.304 g shrimp from nursery tank trial 1 nursed at 1,000 shrimp m⁻², and

Treatment 5: 0.496 g shrimp from nursery cage trial 1 nursed at 500 shrimp m⁻².

The on-growing cages were 4 m in length, 2 m in width and 1.5 m in depth, had a mean wetted surface area of 32 m² and bottom area of 8 m². These cages were constructed of 5 mm nylon mesh, but otherwise were the same as the nursery cages described in section 3.2.2 (Figures 3.1 & 3.4).

Feeding was conducted using the larger sized pellets of reference diet 1 from nursery tank trial 1 (see section 3.2.1.3). A twice daily feeding regime was used in this trial (due to lack of time), feeding at 0800 and 1700 in equal portions for 13 days each fortnight (not feeding on the fortnightly sampling day). Feeding was according to the following schedule: week 0-2, 10 %, week 3-4, 9 %, week 5-6, 8 %, week 7-8, 7 % and week 9-10, 6 % of total wet body weight d⁻¹, allowing 5 % mortality per fortnight. The feed was placed directly onto the feeding tray as in the nursery cage trial.

Figure 3.4 Net cage on-growing system at Songkhla.



Water quality and shrimp growth was monitored according to the same schedule as for the nursery tank trials, except that sampling was conducted fortnightly instead of weekly. In addition, in this trial a total of 30 shrimp from each cage were sampled fortnightly for growth in length and weight. Harvesting was carried out as in section 3.2.2. On termination of the trial, all remaining shrimp were weighed and measured. The net cages were also cleaned every fortnight to remove any fouling organisms. Data analysis was conducted as for nursery tank trials 1 and 2 (section 3.2.1.6) using one way ANOVA and the T-method for multiple comparisons.

3.3 RESULTS

3.3.1 Concrete tank nursery trial 1: Stocking density

This trial investigated the effects of nursing shrimp at various stocking densities (125-1,000 shrimp m⁻²) on water quality and shrimp performance over 35 days in the concrete nursery tank system.

3.3.1.1 Water quality

There were few significant differences between treatments at differing stocking density with respect to water quality conditions during this trial. The main differences were a result of algal bloom 'crashes' on day 32-33 of all three replicates of the highest stocking density treatment (1,000 shrimp m⁻²). These crashes led to significantly ($P < 0.05$) lower transparency (figure 3.5) and higher suspended solids levels in the tanks stocked at the highest density (Table 3.3). Bloom crashes were seen to occur when the transparency decreased to < 0.4 m under the conditions of this trial.

Dissolved oxygen concentration (DO) generally remained high (Table 3.3, Figure 3.6) and higher than the minimal concentration of 4 mg l⁻¹ necessary for optimal shrimp growth (Liao & Murai, 1986). DO however, tended to decline with increasing stocking density and with time. DO also varied through the day, with concentrations decreasing to as low as 2 mg l⁻¹ briefly, in the morning following the bloom crashes in the three

Table 3.3 Mean water quality in weeks 1 to 5 of nursery tank trial 1.

Stocking density (shrimp m ⁻²)	Week	Temperature (°C)	Salinity (‰)	pH	Dissolved oxygen (% saturation)	Secchi depth (m)	TAN (mg l ⁻¹)	NO ₂ -N (µg l ⁻¹)	DRP (mg l ⁻¹)	Suspended solids (mg l ⁻¹)
125	1	29.7	29.8	8.62	110	0.98	0.00	2.0	0.05	74.8
	2	29.1	23.6	8.27	133	0.72	0.01	0.1	0.09	79.3
	3	30.1	21.7	8.31	130	0.87	0.04	0.8	0.00	46.8
	4	29.4	17.9	8.01	117	0.75	0.00	0.5	0.15	78.0
	5	28.8	22.0	8.13	111	0.57	0.03	0.7	0.21	98.1
250	1	29.7	29.8	8.53	109	0.95	0.00	2.1	0.07	86.6
	2	29.1	23.6	8.29	134	0.68	0.04	0.0	0.07	82.2
	3	29.9	21.7	8.29	126	0.78	0.22	0.8	0.01	51.1
	4	29.0	17.9	7.97	112	0.63	0.02	0.1	0.20	84.1
	5	28.5	22.0	8.16	113	0.51	0.05	0.6	0.30	105.8
500	1	29.7	29.8	8.54	109	0.93	0.00	1.5	0.13	85.2
	2	29.0	23.6	8.27	137	0.61	0.00	0.0	0.05	91.2
	3	29.9	21.7	8.35	131	0.68	0.04	0.5	0.02	49.8
	4	29.1	17.9	8.02	111	0.50	0.03	0.4	0.22	96.4
	5	28.6	22.0	8.13	112	0.44	0.10	0.9	0.35	102.0
1,000	1	29.7	29.8	8.56	110	0.90	0.00	1.7	0.04	88.8
	2	29.1	23.6	8.49	165	0.55	0.01	0.0	0.16	102.9
	3	30.0	21.7	8.35	128	0.56	0.15	0.5	0.02	59.1
	4	29.1	17.9	7.99	112	0.45	0.01	0.9	0.20	138.6
	5	28.5	22.0	7.97	82	0.50	0.20	1.2	0.55	124.9

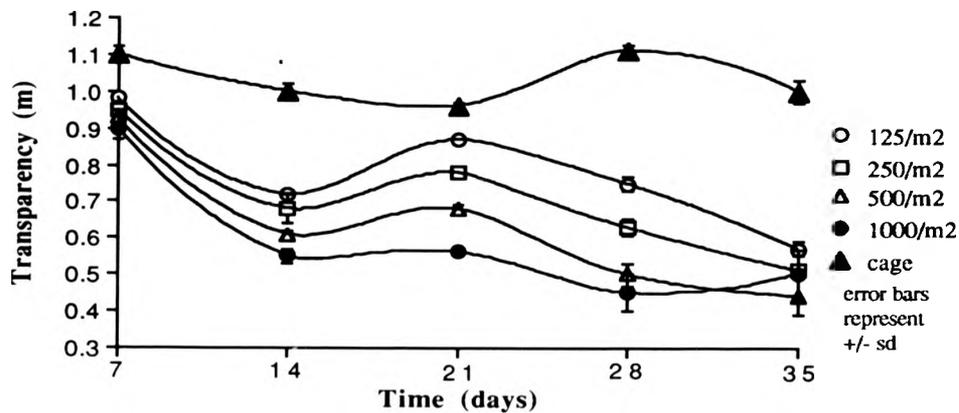


Figure 3.5 Change in mean weekly transparency with time for stocking density in nursery trial 1 and cage trial.

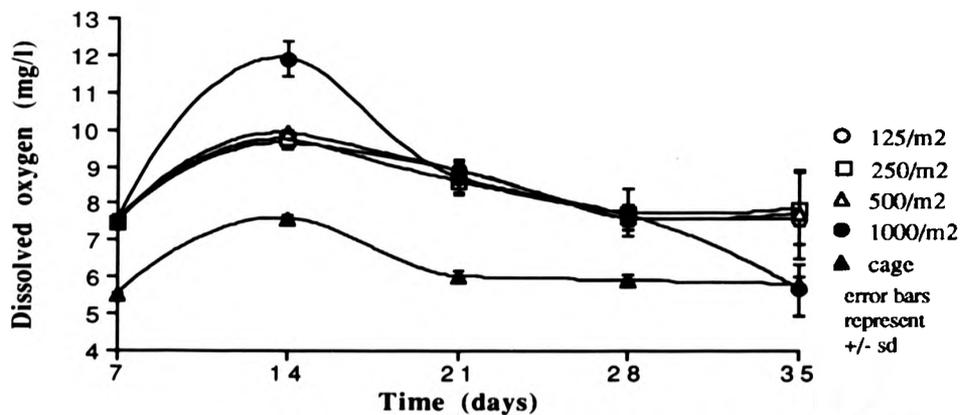


Figure 3.6 Change in mean weekly dissolved oxygen concentration with time for stocking density in nursery trial 1 and cage trial.

replicates of the highest density treatment. High DO (up to 16 mg l⁻¹, 250 % saturation) was also monitored in the highest density treatment during the afternoons. Particularly high DO was measured towards the end of the trial as the increasing algal biomass attained its peak photosynthetic rate.

Temperature, salinity and pH values recorded during the trial were stable and not significantly different between treatments (Table 3.3). They were all within the optimal ranges published for shrimp of 27-33 °C, 15-30 ‰ and pH 7.8-8.5 respectively (Valencia, 1976; Chen, 1985; Chien, 1992; Lester & Pante, 1992).

Dissolved nutrients, resulting from the breakdown of uneaten food and faeces and shrimp metabolism, were found at similar low concentrations for all treatments. No significant differences were found between treatments at any stage of the trial (Table 3.3). At no stage of the trial were toxic levels of TAN (> 0.6 mg l⁻¹ TAN, Tsai, 1989) or nitrite nitrogen (0.1-1.0 mg l⁻¹ NO₂-N, Chen, 1985; Chen & Chin, 1988; Chien, 1992) recorded.

3.3.1.2 Shrimp performance

Regression analysis of the four treatments of nursery trial 1 (not including the net cage trial data) revealed that post-larval shrimp growth was negatively density dependant. The weight attained after 35 days could be related to stocking density by the equation: $\text{Log}_e(\text{weight, g}) = 2.14 - 0.63 \text{Log}_e(\text{density, shrimp m}^{-2})$, $n = 4$, $r^2 = 0.951$, $P < 0.05$ (Figure 3.7). Similarly, the total length attained after 35 days could be related to stocking density by the equation: $\text{Log}_e(\text{total length, mm}) = 85.13 - 15.68 \text{Log}_e(\text{density, shrimp m}^{-2})$, $n = 4$, $r^2 = 0.984$, $P < 0.05$ (Figure 3.8).

One way ANOVA of shrimp growth also revealed that shrimp growth rate, measured as individual weight gain, specific growth rate (SGR) and growth in total length (Table 3.4), was inversely related to stocking density ($P < 0.05$). Juvenile shrimp at a mean weight of 0.887 g, mean length of 52.9 mm and mean SGR 12.1 % body weight d⁻¹ were produced after 35 days at 125 shrimp m⁻². Increasing the stocking density to 1,000 shrimp m⁻², reduced mean final weight to 0.304 g, mean length to 38.2 mm and mean

Table 3.4 Mean performance (weeks 1-5) of shrimp in nursery tank trial 1 and nursery cage trial.

Treatment	1		2		3		4		Net cage
	reference 1	normal	reference 1	normal	reference 1	normal	reference 1	normal	
Stocking density (shrimp m ⁻²)	125		250		500		1,000		500
Habitats (no. panels)	0		0		0		0		0
Substrates (cm depth)	0		0		0		0		0
Aeration (l sec ⁻¹ tank ⁻¹)	0		0		0		0		0
Diet	reference 1		reference 1		reference 1		reference 1		reference 1
Feeding rate	normal		normal		normal		normal		normal
Initial weight (g)	0.013 ± 0.000 ^a		0.013 ± 0.000 ^a		0.013 ± 0.000 ^a		0.013 ± 0.001 ^a		0.013 ± 0.001 ^a
Final weight (g)	0.887 ± 0.117 ^c		0.550 ± 0.098 ^b		0.413 ± 0.057 ^{ab}		0.304 ± 0.015 ^a		0.496 ± 0.010 ^b
SGR (% body wt d ⁻¹)	12.1 ± 0.4 ^c		10.7 ± 0.5 ^b		9.9 ± 0.4 ^b		9.0 ± 0.1 ^a		10.4 ± 0.2 ^b
Final length (mm)	52.9 ± 1.6 ^d		46.4 ± 1.1 ^c		43.3 ± 1.4 ^b		38.2 ± 0.8 ^a		42.2 ± 1.1 ^b
CV (length)	19.2 ± 2.3 ^a		21.6 ± 0.6 ^a		21.7 ± 2.9 ^a		25.5 ± 3.0 ^{ab}		29.6 ± 2.1 ^b
Total weight gain (g)	2625 ± 194 ^{ab}		3618 ± 259 ^{bc}		4612 ± 312 ^{cd}		6015 ± 1380 ^d		1616 ± 40 ^a
Food fed (g)	2446 ± 181 ^{ab}		3373 ± 206 ^b		5671 ± 259 ^c		8777 ± 1257 ^d		1536 ± 119 ^a
FCR	0.93 ± 0.06 ^a		0.93 ± 0.03 ^a		1.29 ± 0.08 ^{ab}		1.53 ± 0.52 ^b		0.95 ± 0.05 ^a
FCE	1.07 ± 0.07 ^b		1.07 ± 0.03 ^b		0.81 ± 0.03 ^{ab}		0.70 ± 0.25 ^a		1.06 ± 0.10 ^b
Survival (%)	93.3 ± 11.5 ^{ab}		98.5 ± 2.7 ^b		92.3 ± 6.8 ^{ab}		83.1 ± 15.9 ^{ab}		72.1 ± 4.0 ^a
Production (g m ⁻² d ⁻¹)	3.0 ± 0.2 ^a		4.1 ± 0.3 ^{ab}		5.3 ± 0.4 ^{bc}		6.9 ± 1.6 ^c		5.1 ± 0.1 ^{bc}

¹ For single criterion, mean values in the same row bearing different superscripts are significantly different (P < 0.05) by T-method.

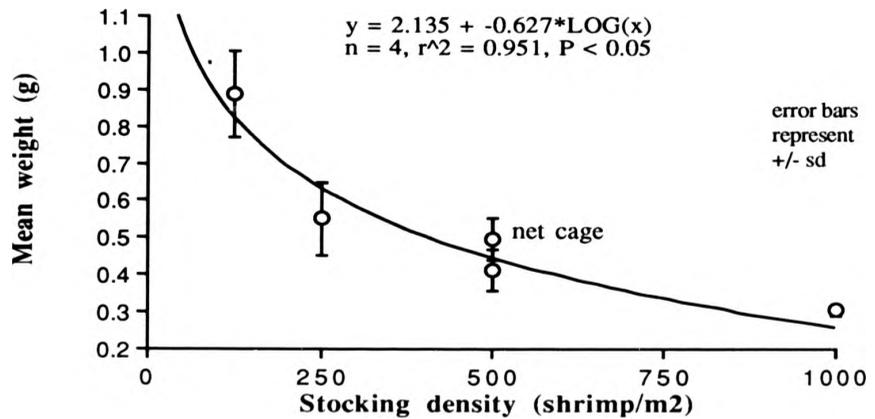


Figure 3.7 Relationship between mean weight and stocking density in nursery trial 1 and cage trial.

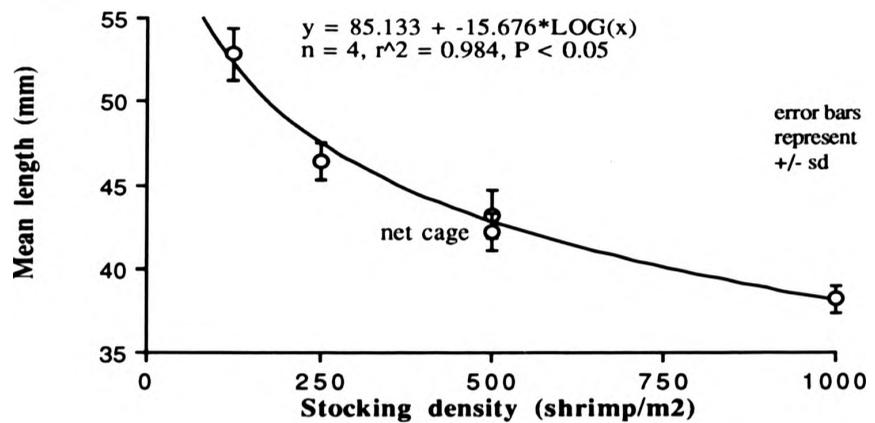


Figure 3.8 Relationship between mean length and stocking density in nursery trial 1 and cage trial.

SGR to 9.0 % body weight d^{-1} (Table 3.4, Figures 3.7 & 3.8).

Analysis of the coefficient of variation (CV) in total shrimp length following the 35 day secondary nursing period was conducted by non-parametric ANOVA (Kruskal-Wallis test) after a Bartlett test revealed heteroscedasticity (Table 3.4). This analysis revealed no significant difference in the CV in length between treatments, although nursing at higher density tended to increase the size range of the juvenile shrimp produced (Table 3.4, Figure 3.9).

Shrimp survival was very high at 83-99 % after 35 days and did not vary significantly with stocking density (Table 3.4)

The food conversion efficiency (FCE) of shrimp fed the formulated diet (reference diet 1, Table 3.2) was very high and significantly better at stocking densities of 125-250 shrimp m^{-2} , than at 1,000 shrimp m^{-2} (Table 3.4).

Overall shrimp production ($g\ m^{-2}\ d^{-1}$) was calculated from shrimp growth and survival rates. Although variable at the highest density tested, shrimp production was directly correlated with stocking density. The production attained after 35 days could be related to stocking density by the equation: $\text{Log}_e(\text{production, } g\ m^{-2}\ d^{-1}) = -5.98 + 4.24\ \text{Log}_e(\text{density, shrimp } m^{-2})$, $n = 4$, $r^2 = 0.992$, $P < 0.01$ (Figure 3.10). Production increased significantly ($P < 0.05$) from 3.0 to 6.9 $g\ m^{-2}\ d^{-1}$ as stocking density was increased from 125 to 1,000 shrimp m^{-2} (Table 3.4, Figure 3.10).

3.3.2 Net cage nursery trial

This trial investigated the effects of nursing shrimp at 500 shrimp m^{-2} in net cages for 35 days on water quality and shrimp performance. The results of this trial were compared to those for treatment 3 (at a stocking density of 500 shrimp m^{-2}) of tank trial 1.

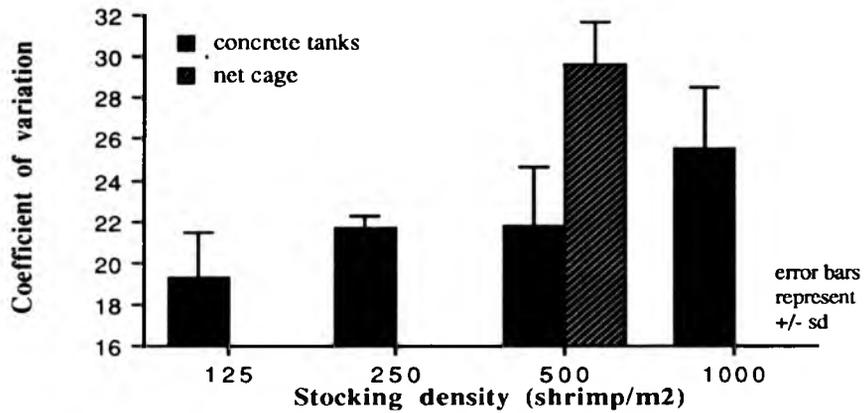


Figure 3.9 Relationship between coefficient of variation in length and stocking density in nursery trial 1 and cage trial.

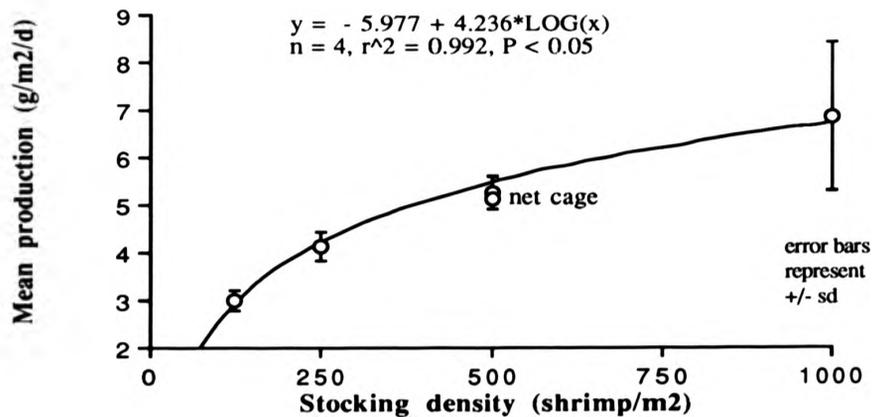


Figure 3.10 Relationship between mean production and stocking density in nursery trial 1 and cage trial.

3.3.2.1 Water quality

Water quality conditions in the net cage nursery trial were significantly ($P < 0.05$) different from those in concrete nursery tank trial 1, which was conducted simultaneously. This was largely a result of a difference in water supply, the cages being located in a pond on the fishery college site.

Temperature was an average 1°C higher, salinity was similar and pH was 0.7 pH units lower in the cages than the tanks over the trial period (Table 3.5). All of these parameters however, were within optimal ranges for shrimp.

The natural productivity (phytoplankton density) within the pond containing the cages was lower than in the tanks, due to dilution of the feed nutrients added into the large pond area. Thus, water transparency was higher and more stable (Table 3.5, Figure 3.5) and DO lower and more stable (Table 3.5, Figure 3.6) in the cages than the tanks.

Dissolved nutrient concentrations of TAN, $\text{NO}_2\text{-N}$ and DRP were low, fairly stable and not significantly different to those measured in tank trial 1 (Tables 3.3 and 3.5). Nutrient concentrations in the cages, as for the tanks, did not reach toxic concentrations at any stage of the trial, indicating good tidally-driven transmission of water through the cages.

3.3.2.2 Shrimp performance

One way ANOVA of shrimp growth was conducted for the net cage trial, together with the four treatments of tank trial 1. These analyses revealed that shrimp growth in terms of individual weight gain, SGR and growth in total length was not significantly different at a stocking density 500 shrimp m^2 in net cage or concrete tank nursery systems (Table 3.4, Figures 3.7 & 3.8). Juvenile shrimp at a mean weight of 0.496 g, mean length of 42.2 mm and mean SGR of 10.4 % body weight d^{-1} were produced from the net cage nursery after 35 days at 500 shrimp m^2 .

A non-parametric ANOVA (Kruskal-Wallis test) analysis was conducted on the

Table 3.5 Mean water quality in weeks 1 to 5 of nursery cage trial.

Cage trial	Week	Temperature (°C)	Salinity (‰)	pH	Dissolved oxygen (% saturation)	Dissolved oxygen (mg l ⁻¹)	Secchi depth (m)	TAN (mg l ⁻¹)	NO ₂ -N (µg l ⁻¹)	DRP (mg l ⁻¹)	Suspended solids (mg l ⁻¹)
1	1	30.4	29.9	7.78	77	5.5	1.10	0.00	1.0	1.00	100.5
	2	30.2	23.4	7.18	105	7.6	1.00	0.02	0.5	0.53	95.6
	3	30.8	22.0	7.68	92	6.0	0.96	0.05	0.2	0.26	78.2
	4	30.4	18.5	7.53	87	5.9	1.11	0.01	0.1	0.53	115.0
	5	30.0	21.8	7.50	86	5.8	1.00	0.02	0.2	0.27	55.0

coefficient of variation in length of shrimp following 35 days of cage nursing. This analysis revealed that nursing in net cages produced shrimp with a significantly ($P < 0.05$) larger size range than those nursed in concrete tanks at any density up to 500 shrimp m^{-2} (Table 3.4, Figure 3.9).

Shrimp survival was lower when nursed at 500 shrimp m^{-2} in net cages (72.1 %) compared to tanks (92.3 %), but the differences were not significant at the 5 % level (Table 3.4).

The FCR and FCE of shrimp fed the formulated diet (reference diet 1, Table 3.2) were slightly, but not significantly better for shrimp held in the cage as opposed to the tank nursery at a common density of 500 shrimp m^{-2} (Table 3.4).

Overall shrimp production was not significantly different for shrimp nursed at a common stocking density in net cages (5.1 $g\ m^{-2}\ d^{-1}$) or concrete tanks (5.3 $g\ m^{-2}\ d^{-1}$) (Table 3.4, Figure 3.10).

3.3.3 Concrete tank nursery trial 2: Habitat presence

This trial investigated the effects of nursing shrimp at a density of 500 shrimp m^{-2} in the presence of varying amounts of habitats (increasing the wetted surface area of the tanks by up to 160 %), on water quality and shrimp performance over 35 days in the concrete nursery tank system.

3.3.3.1 Water quality

In common with tank nursery trial 1, most of the water quality parameters monitored during this trial were unaffected by the treatments (different numbers of habitats) used.

DO was not significantly different between treatments, but decreased over the trial period to potentially stressful levels as low as 2 $mg\ l^{-1}$ in the mornings in the final week of the trial (Table 3.6, Figure 3.11). DO was generally lower than for the same stocking density (500 shrimp m^{-2}) in tank trial 1. This was due to the fact that the

Table 3.6 Mean water quality in weeks 1 to 5 of nursery tank trial 2.

Habitats (no. panels)	Week	Temperature (°C)	Salinity (‰)	pH	Dissolved oxygen (% saturation)	Secchi depth (m)	TAN (mg l ⁻¹)	NO ₂ -N (µg l ⁻¹)	DRP (mg l ⁻¹)	Suspended solids (mg l ⁻¹)
0	1	28.5	26.7	8.40	151	0.82	0.01	1.6	0.17	73.2
	2	29.2	26.8	8.44	127	0.97	0.00	0.3	0.14	104.9
	3	29.4	30.4	8.25	113	0.86	0.04	0.5	0.15	108.3
	4	29.4	31.0	8.04	78	0.84	0.09	0.5	0.28	83.6
	5	29.8	29.7	7.97	75	0.79	0.19	1.4	0.86	99.8
1	1	28.3	26.7	8.45	150	0.75	0.00	2.2	0.16	75.6
	2	29.1	26.8	8.46	127	0.96	0.00	2.5	0.04	106.4
	3	29.2	30.4	8.17	98	0.78	0.05	2.7	0.06	102.8
	4	29.3	31.0	7.93	70	0.80	0.03	0.0	0.38	81.7
	5	29.6	29.7	7.95	59	0.73	0.39	0.0	1.07	100.5
2	1	28.3	26.7	8.45	149	0.80	0.01	2.0	0.23	67.4
	2	29.1	26.8	8.39	128	0.95	0.00	1.6	0.13	103.1
	3	29.2	30.4	8.12	95	0.82	0.02	0.9	0.15	98.2
	4	29.2	31.0	7.95	73	0.84	0.05	0.0	0.13	89.9
	5	29.6	29.7	7.93	69	0.72	0.19	0.0	0.66	104.8
4	1	28.5	26.7	8.43	150	0.78	0.01	1.8	0.19	72.3
	2	29.1	26.8	8.41	124	0.96	0.00	1.6	0.10	99.8
	3	29.2	30.4	8.12	97	0.76	0.05	0.9	0.15	103.5
	4	29.2	31.0	7.99	71	0.77	0.06	0.3	0.51	82.2
	5	29.6	29.7	7.90	54	0.74	0.38	0.3	0.96	110.4

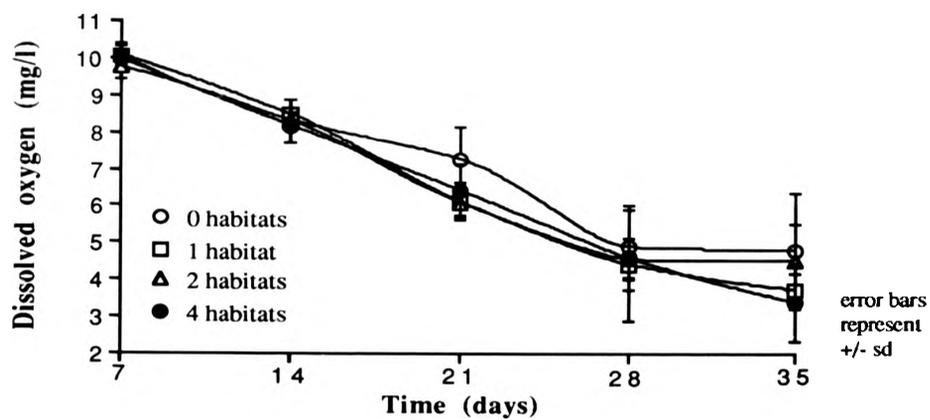


Figure 3.11 Change in mean weekly dissolved oxygen concentration with time for habitat number in nursery trial 2.

incoming water during this trial (3-6.7 mg l⁻¹) was lower in DO than in trial 1 (5.0-9.3 mg l⁻¹), and because the phytoplankton biomass in the tanks, as seen in the transparency levels, was lower for trial 2 than trial 1 (Tables 3.3 & 3.6).

Temperature and salinity were constant and within optimal ranges for shrimp growth for all treatments throughout the trial, although salinity was generally higher in this trial than in trial 1 (Table 3.6). pH was also within optimal limits, but tended to decline over the trial period from 8.4 to 7.9 for all treatments (Table 3.6).

Dissolved nutrient concentrations (TAN, NO₂-N and DRP) were also similar for all treatments during this trial. TAN and DRP concentrations increased slightly (but not to toxic levels) towards the end of the trial, and were maintained within optimal ranges for shrimp growth throughout the trial period (Table 3.6).

3.3.3.2 Shrimp performance

The presence of habitats increasing the wetted surface area (WSA) of the tanks in this trial by up to 160 %, had no significant effects on shrimp performance.

Shrimp growth rate was high, and improved over that achieved in tank trial 1 at a similar stocking density (500 shrimp m⁻²). Juvenile shrimp were produced at a mean weight of 0.492-0.591 g, mean total length of 43.6-46.7 mm at a mean SGR of 12.9-13.4 % body weight d⁻¹ after 35 days (Table 3.7, Figure 3.12). The highest growth rate was found in treatment 2 (1 habitat). This result may have been due to the decreased survival in this treatment (51.6 %) compared to the other treatments (56.6-58.3 %). These differences in survival however, were not significant at the 5 % level (Table 3.7, Figure 3.13).

Non-parametric ANOVA (Kruskal-Wallis test) of length data was conducted to examine the coefficient of variation in length of shrimp following 35 days of secondary nursing. This analysis revealed no significant differences between treatments (Table 3.7, Figure 3.14).

Table 3.7 Mean performance (weeks 1-5) of shrimp in nursery tank trial 2.

Treatment	1	2	3	4
Stocking density (shrimp m ⁻²)	500	500	500	500
Habitats (no. panels)	0	1	2	4
Substrates (cm depth)	0	0	0	0
Aeration (l sec ⁻¹ tank ⁻¹)	0	0	0	0
Diet	reference 1	reference 1	reference 1	reference 1
Feeding rate	normal	normal	normal	normal
Initial weight (g)	0.005 ± 0.000 ^a			
Final weight (g)	0.492 ± 0.063 ^a	0.591 ± 0.068 ^a	0.503 ± 0.048 ^a	0.505 ± 0.051 ^a
SGR (% body wt d ⁻¹)	12.9 ± 0.4 ^a	13.4 ± 0.3 ^a	13.0 ± 0.3 ^a	13.0 ± 0.3 ^a
Final length (mm)	44.0 ± 4.3 ^a	46.7 ± 4.4 ^a	45.5 ± 2.3 ^a	43.6 ± 3.3 ^a
CV (length)	27.3 ± 2.4 ^a	23.0 ± 5.7 ^a	24.4 ± 1.2 ^a	21.5 ± 1.4 ^a
Total weight gain (g)	3535 ± 691 ^a	3683 ± 60 ^a	3476 ± 490 ^a	3501 ± 151 ^a
Food fed (g)	3965 ± 95 ^a	4732 ± 153 ^a	4644 ± 610 ^a	4663 ± 629 ^a
FCR	1.15 ± 0.22 ^a	1.29 ± 0.07 ^a	1.37 ± 0.36 ^a	1.33 ± 0.13 ^a
FCE	0.89 ± 0.15 ^a	0.78 ± 0.04 ^a	0.76 ± 0.18 ^a	0.75 ± 0.07 ^a
Survival (%)	58.3 ± 4.3 ^a	51.6 ± 6.7 ^a	56.6 ± 8.5 ^a	56.8 ± 4.6 ^a
Production (g m ⁻² d ⁻¹)	4.0 ± 0.7 ^a	4.2 ± 0.1 ^a	4.0 ± 0.6 ^a	4.0 ± 0.2 ^a

¹ For single criterion, mean values in the same row bearing different superscripts are significantly different (P < 0.05) by T-method.

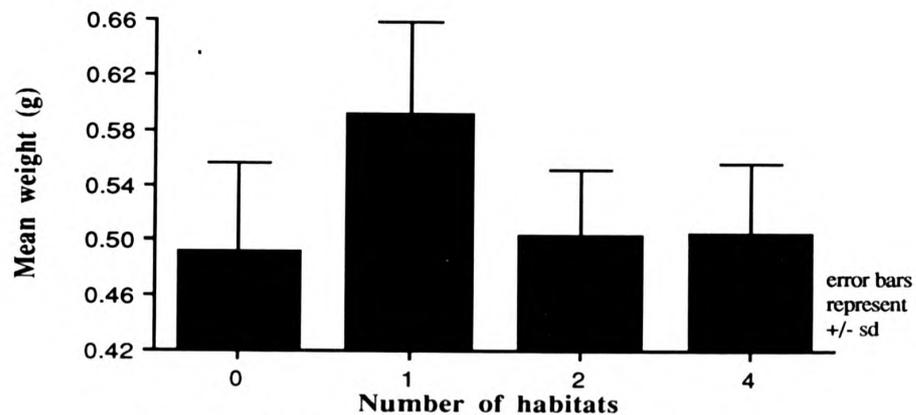


Figure 3.12 Relationship between mean weight and number of habitats in nursery trial 2.

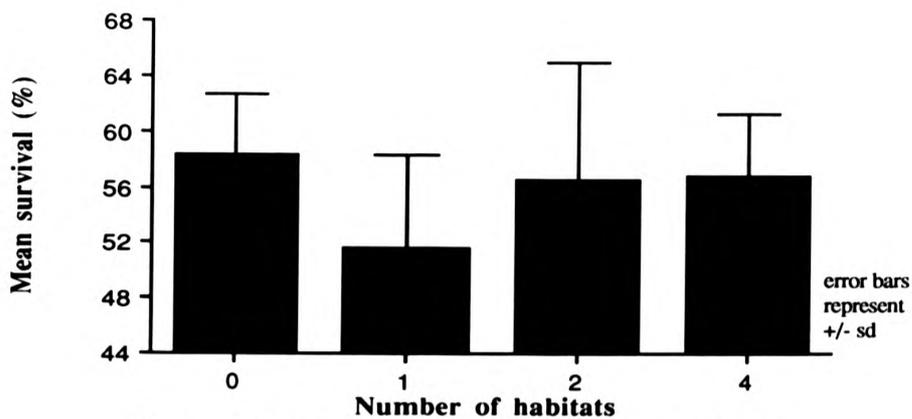


Figure 3.13 Relationship between mean survival and number of habitats in nursery trial 2.

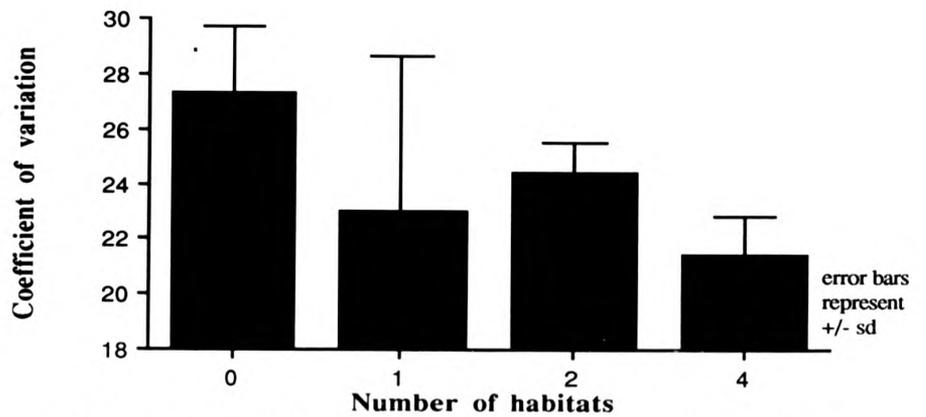


Figure 3.14 Relationship between coefficient of variation in length and number of habitats in nursery trial 2.

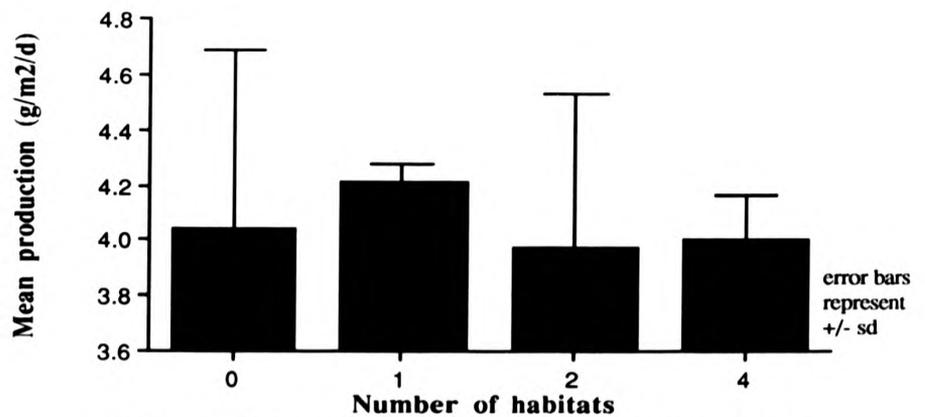


Figure 3.15 Relationship between mean production and number of habitats in nursery trial 2.

The FCR and FCE of shrimp fed the formulated diet (reference diet 1, Table 3.2) in this trial were not related to the presence of habitats, were generally good (Table 3.7) and were similar to those achieved in tank trial 1.

Shrimp production (at 4.0-4.2 g m⁻² d⁻¹) was not related to the presence of habitats, but was consistently poorer than that achieved at the same stocking density (500 shrimp m⁻²) in tank trial 1 (Table 3.7, Figure 3.15).

3.3.4 Concrete tank nursery trial 3: Stocking density/Dietary formulation with aeration and water filtration

Treatments 1, 3 and 4 examined the effects of stocking density (at 1,000, 2,000 and 3,000 shrimp m⁻²) on water quality and shrimp performance fed reference diet 1 (Table 3.2) over 35 days in the concrete nursery tank system. Treatment 2 was stocked at 1,000 shrimp m⁻² and was fed with a different dietary formulation (test diet 1), containing lower levels of protein and higher levels of lipid and energy (Table 3.2) for comparison to treatment 1 of this trial.

3.3.4.1 Water quality

There were few significant differences between treatments at differing stocking densities with respect to water quality conditions during this trial. However, due to unforeseen power cuts as a result of faulty wiring, the air blower failed twice on day 16 and day 30 of this trial. Figure 3.16 displays the change in mean morning DO concentrations over the 35 day trial period for the lowest (1,000 shrimp m⁻²) and highest (3,000 shrimp m⁻²) stocking densities (fed the reference diet) tested in this trial. The other two treatments were not included in this figure for purposes of clarity.

Despite increasing the water exchange rate, the first air blower failure resulted in a decrease in early morning (0700) DO to as low as 4 mg l⁻¹, the lowest levels being recorded in treatment 4 at the highest stocking density (3,000 shrimp m⁻²). The second air blower failure had similar results, with DO rapidly decreasing to as low as 2 mg l⁻¹ in the highest density treatment. This was due to the high oxygen demand of the large

biomass of shrimp present (Figure 3.16). In each case, DO had recovered to normal levels on the day following the power cuts as the air blower was restarted. The shrimp however, showed immediate signs of oxygen stress, coming up to the surface and hanging motionless until the air supply resumed. Shrimp mortality was also evident following both failures and feeding was suspended for two days after each failure in an attempt to reduce oxygen stress.

Apart from these two air blower failures, DO was stable at between 4 and 7 mg l⁻¹ (mean 6.5 mg l⁻¹) for all treatments throughout the trial due to the aeration provided by the air blower (Table 3.8). This was in contrast to the fluctuating and gradually declining DO measured in the high density treatments of tank trial 1 and in tank trial 2.

Water transparency was similar for all treatments and to results from tank trial 1. The Secchi depths recorded declined gradually over the 35 day trial period, but were never less than 0.4 m (Table 3.8). No phytoplankton crashes were recorded during this trial, despite the high stocking densities used and large quantities of feed applied, due to the maintenance of secchi depths at > 0.4 m throughout by water exchange.

Temperature and salinity values recorded were high, stable and within the optimal ranges for shrimp growth throughout the trial. pH was also within optimal levels throughout the trial, but tended to decline slightly over the trial period.

Dissolved nutrient levels of TAN, NO₂-N and DRP were low, increased only marginally over time and were within optimal levels for shrimp growth throughout the trial period.

3.3.4.2 Shrimp performance

3.3.4.2.1 Stocking density

Overall performance of shrimp was poor due to the two air blower failures occurring during this trial. The low DO concentrations monitored during the failures (particularly

Table 3.8 Mean water quality in weeks 1 to 5 of nursery tank trial 3.

Stocking density (shrimp m ⁻²)	Week	Temperature (°C)	Salinity (‰)	pH	Dissolved oxygen (% saturation)	Secchi depth (m)	TAN (mg l ⁻¹)	NO ₂ -N (µg l ⁻¹)	DRP (mg l ⁻¹)	Suspended solids (mg l ⁻¹)
1,000	1	28.7	28.9	8.30	102	0.66	0.00	0.3	0.01	80.3
	2	30.4	31.9	8.18	108	0.69	0.00	2.9	0.01	93.7
	3	29.4	29.2	7.94	108	0.52	0.00	10.3	0.01	80.4
	4	29.0	31.0	8.07	101	0.62	0.02	1.6	0.01	91.7
	5	28.8	32.5	7.78	105	0.57	0.02	1.5	0.00	99.7
1,000	1	28.4	28.9	8.27	99	0.63	0.00	2.2	0.00	87.9
	2	30.2	31.9	8.17	107	0.67	0.00	2.6	0.01	95.6
	3	29.5	29.2	7.94	106	0.53	0.01	9.4	0.01	85.2
	4	28.9	31.0	8.04	99	0.59	0.02	0.6	0.01	94.4
	5	28.7	32.5	7.79	103	0.61	0.02	1.3	0.01	106.4
2,000	1	28.6	28.9	8.27	101	0.67	0.00	0.5	0.00	88.4
	2	30.3	31.9	8.19	108	0.68	0.00	1.9	0.02	91.9
	3	29.5	29.2	7.93	105	0.52	0.00	9.8	0.00	99.2
	4	29.0	31.0	8.06	100	0.59	0.03	1.0	0.01	101.4
	5	28.8	32.5	7.71	102	0.52	0.04	2.2	0.02	106.2
3,000	1	28.5	28.9	8.28	98	0.64	0.00	1.2	0.01	89.8
	2	30.2	31.9	8.19	106	0.63	0.00	3.0	0.02	105.6
	3	29.4	29.2	7.93	104	0.50	0.02	9.8	0.01	91.3
	4	28.9	31.0	7.99	100	0.55	0.08	1.9	0.01	98.5
	5	28.8	32.5	7.60	100	0.48	0.16	2.9	0.06	101.4

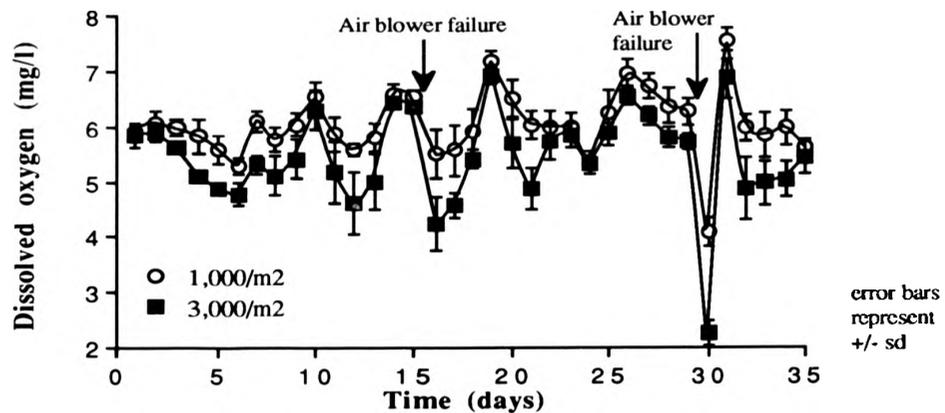


Figure 3.16 Change in mean am dissolved oxygen concentration with time for stocking density in nursery trial 3.

the first failure) were seen to result in high mortality. Shrimp mortality was directly related to stocking density, with shrimp stocked at the highest density (3,000 shrimp m⁻²) showing a significantly ($P < 0.05$) lower survival (14.1 %) than those stocked at 1,000 m⁻² fed the same diet (43.3 %) (Table 3.9, Figure 3.17).

In contrast to tank trial 1, higher stocking densities appeared to result in higher shrimp growth rates (Table 3.9, Figure 3.18). This result however, was anomalous since the higher mortality recorded in the treatments at high density resulted in final stocking densities of 433, 616 and 423 shrimp m⁻² for treatments 1, 3 and 4, stocked initially at 1,000, 2,000 and 3,000 shrimp m⁻².

The coefficient of variation in length of shrimp recorded after 35 days, as in tank trial 1 increased with stocking density, but the differences were not significant at the 5 % level (Table 3.9).

The FCR and FCE of shrimp fed reference diet 1 were good, comparable to those achieved in trials 1 and 2, but decreased significantly ($P < 0.05$) as stocking density increased (Table 3.9).

Overall shrimp production was low at 2.1-3.9 g m⁻² d⁻¹, but increased significantly with stocking density, despite the direct relationship between shrimp mortality and stocking density (Table 3.9, Figure 3.19).

3.3.4.2.2 Dietary formulation

No significant differences could be discerned between treatments 1 and 2, stocked at a common density of 1,000 shrimp m⁻², but fed different dietary formulations in terms of shrimp growth, survival or production (Table 3.9, Figures 3.17, 3.18 and 3.19). In each case however, shrimp performance tended to be lower for shrimp fed the test diet containing a lower level of protein and higher levels of lipid and energy.

The only significant differences recorded were in feeding efficiency, where the FCR and FCE of shrimp fed the test diet were significantly ($P < 0.05$) poorer than those

Table 3.9 Mean performance (weeks 1-5) of shrimp in nursery tank trial 3.

Treatment	1	2	3	4
Stocking density (shrimp m ⁻²)	1,000	1,000	2,000	3,000
Habitats (no. panels)	0	0	0	0
Substrates (cm depth)	0	0	0	0
Aeration (l sec ⁻¹ tank ⁻¹)	12	12	12	12
Diet	reference 1	test 1	reference 1	reference 1
Feeding rate	normal	normal	normal	normal
Initial weight (g)	0.013 ± 0.000 ^a			
Final weight (g)	0.200 ± 0.067 ^a	0.166 ± 0.019 ^a	0.214 ± 0.060 ^a	0.373 ± 0.077 ^b
SGR (% body wt d ⁻¹)	10.4 ± 0.9 ^a	9.9 ± 0.3 ^a	10.6 ± 0.8 ^a	12.2 ± 0.6 ^b
Final length (mm)	31.5 ± 2.5 ^a	29.2 ± 0.7 ^a	30.9 ± 2.8 ^a	37.3 ± 2.2 ^b
CV (length)	28.3 ± 3.8 ^a	29.8 ± 3.5 ^a	30.9 ± 3.4 ^a	32.5 ± 2.4 ^a
Total weight gain (g)	1830 ± 327 ^a	1320 ± 227 ^a	2812 ± 357 ^b	3418 ± 252 ^b
Food fed (g)	1960 ± 269 ^a	1900 ± 68 ^a	3913 ± 183 ^b	6854 ± 374 ^c
FCR	1.08 ± 0.06 ^a	1.47 ± 0.23 ^b	1.41 ± 0.17 ^b	2.01 ± 0.07 ^c
FCE	0.93 ± 0.05 ^c	0.69 ± 0.10 ^b	0.72 ± 0.08 ^b	0.50 ± 0.02 ^a
Survival (%)	43.3 ± 19.0 ^b	35.0 ± 5.5 ^{ab}	30.8 ± 11.3 ^{ab}	14.1 ± 3.8 ^a
Production (g m ⁻² d ⁻¹)	2.1 ± 0.4 ^a	1.5 ± 0.3 ^a	3.2 ± 0.4 ^b	3.9 ± 0.3 ^b

¹ For single criterion, mean values in the same row bearing different superscripts are significantly different ($P < 0.05$) by T-method.

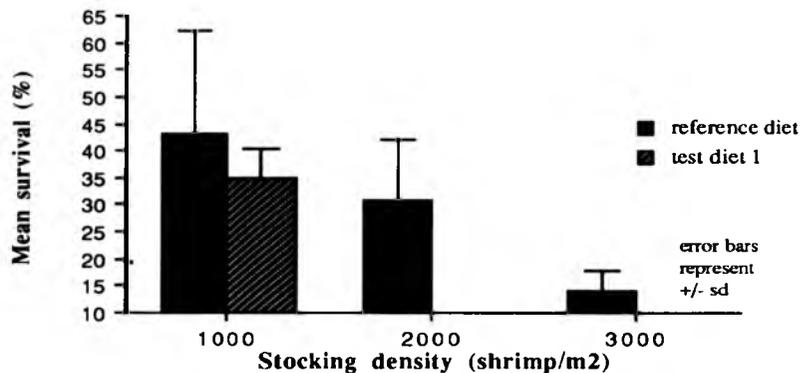


Figure 3.17 Relationship between mean survival and stocking density in nursery trial 3.

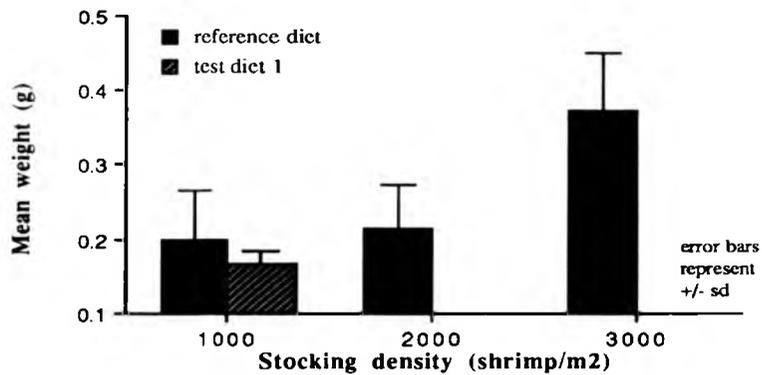


Figure 3.18 Relationship between mean weight and stocking density in nursery trial 3.

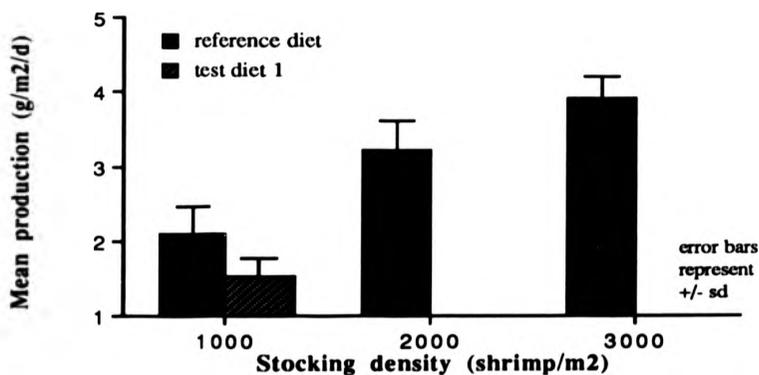


Figure 3.19 Relationship between mean production and stocking density in nursery trial 3.

nursed at the same density, but fed the reference diet (Table 3.9).

3.3.5 Concrete tank nursery trial 4: Habitat/Substrate presence with aeration and water filtration

This trial investigated the effects of the presence of habitats (increasing the WSA by 120 %) and/or substrates (10 cm of riverine sand) on the water quality and performance of shrimp stocked at 2,000 shrimp m² over 35 days in the concrete nursery tank system. This was done to investigate the advantage of habitat inclusion at high stocking density, after no advantage was found at low (500 shrimp m²) density (trial 3).

3.3.5.1 Water quality

Water quality conditions during this trial were mostly within the optimal range for shrimp growth, were not significantly different between treatments and were stable throughout the trial period. One of the exceptions included salinity which gradually increased from 31-34.6 ‰ over the trial period (Table 3.10). These levels were greater than the optimum (15-30 ‰, Chien, 1992), but not higher than the critical value of approximately 40 ‰ (Lester & Pante, 1992). Water transparency was similar between treatments, but tended to decrease during the trial from 0.87-0.53 m (Table 3.10), without reaching levels of < 0.4 m where phytoplankton bloom crashes may occur.

DO was high and stable at approximately 6.5 mg l⁻¹ (100 % saturation) throughout the trial. DO never decreased to < 4 mg l⁻¹ over the trial period due to the continual operation of the air blower.

The concentration of dissolved nutrients (TAN, NO₂-N and DRP) were also low and stable, apart from a slight increase in NO₂-N during week 3 due to high levels in the incoming water. The level of suspended solids also increased in all treatments towards the end of the trial period (Table 3.10).

On drainage of the tanks following this trial, it was evident that in the treatments

Table 3.10 Mean water quality in weeks 1 to 5 of nursery tank trial 4.

Habitats/ substrates	Week	Temperature (°C)	Salinity (‰)	pH	Dissolved oxygen (% saturation)	Secchi depth (m)	TAN (mg l ⁻¹)	NO ₂ -N (µg l ⁻¹)	DRP (mg l ⁻¹)	Suspended solids (mg l ⁻¹)
No habitats,	1	29.3	31.0	8.09	98	0.83	0.00	1.6	0.01	90.8
No substrates	2	28.6	32.2	8.23	101	0.69	0.00	2.1	0.01	94.5
	3	28.7	33.0	8.13	89	0.63	0.00	26.2	0.01	106.3
	4	28.9	34.5	7.90	100	0.57	0.00	3.2	0.00	118.7
	5	28.4	34.6	7.93	101	0.54	0.01	2.0	0.04	102.4
No habitats,	1	29.1	31.0	8.05	94	0.81	0.00	1.5	0.00	102.4
Substrates	2	28.8	32.2	8.18	103	0.68	0.00	1.1	0.01	94.3
	3	28.9	33.0	8.11	94	0.67	0.00	17.9	0.01	93.7
	4	29.1	34.5	7.92	106	0.62	0.00	1.7	0.00	111.6
	5	28.6	34.6	7.92	103	0.60	0.00	1.1	0.03	96.3
Habitats,	1	29.7	31.0	8.05	100	0.87	0.00	1.5	0.00	100.5
No substrates	2	28.8	32.2	8.20	102	0.69	0.00	1.4	0.01	94.2
	3	28.9	33.0	8.11	95	0.66	0.00	21.5	0.01	93.7
	4	29.0	34.5	7.86	104	0.62	0.00	3.1	0.00	120.3
	5	28.6	34.6	7.85	101	0.54	0.01	4.2	0.02	108.4
Habitats,	1	29.5	31.0	8.07	99	0.75	0.00	1.5	0.00	89.8
Substrates	2	28.8	32.2	8.17	101	0.72	0.00	0.2	0.01	94.1
	3	28.8	33.0	8.14	96	0.71	0.00	8.6	0.01	101.5
	4	28.9	34.5	7.89	104	0.61	0.00	2.9	0.01	116.2
	5	28.5	34.6	7.92	102	0.53	0.00	2.2	0.01	110.3

including sand substrates, a layer of fine, anoxic sediment completely covered the substrates, becoming trapped between the sand particles. In treatments without substrates however, these sediments were concentrated by the airstones into discrete areas of the tank floor, leaving most of the tank floor free from sediment.

3.3.5.2 Shrimp performance

Neither the presence of habitats (increasing the WSA of the tanks by 120 %) nor substrates (10 cm of riverine sand) were found to have any effects on shrimp growth rate, FCR or FCE during this trial. In addition, no interaction effects between habitats and substrates could be detected for any measure of shrimp performance.

Shrimp growth rate was relatively good for such a high stocking density (2,000 shrimp m⁻²) compared to the results from previous trials, although direct comparisons were difficult due to other variations between trials. Shrimp with mean weights of 0.257-0.309 g (Figure 3.20), mean total lengths of 33.7-36.3 mm at a mean SGR of 13.3-13.9 % body weight d⁻¹ were produced after 35 days. No significant differences were found between treatments for any of these parameters (Table 3.11).

The coefficient of variation in shrimp length was significantly ($P < 0.05$) higher in the presence of substrates, but was unrelated to the presence of habitats during this trial. It was however, relatively high at 23.3-28.1 (compared to previous trials) at the high stocking density used in this trial (Table 3.11).

The FCR and FCE of shrimp fed the formulated diet (reference diet 1, Table 3.2) were not significantly different between treatments and were relatively good at 1.23-1.3 and 0.78-0.82 for FCR and FCE respectively (Table 3.11).

In contrast to results from tank trial 2 (testing the presence of habitats at 500 shrimp m⁻²), shrimp survival increased significantly ($P < 0.05$) when habitats were included, particularly in the absence of sand substrates. Survival however, was low at 36.9-54.8 % at the high stocking density used during this trial, despite the good water quality conditions (Table 3.11, Figure 3.21).

Table 3.11 Mean performance (weeks 1-5) of shrimp in nursery tank trial 4.

Treatment	1	2	3	4
Stocking density (shrimp m ⁻²)	2,000	2,000	2,000	2,000
Habitats (no. panels)	0	0	3	3
Substrates (cm depth)	0	10	0	10
Aeration (l sec ⁻¹ tank ⁻¹)	12	12	12	12
Diet	reference 1	reference 1	reference 1	reference 1
Feeding rate	normal	normal	normal	normal
Initial weight (g)	0.002 ± 0.000 ^a			
Final weight (g)	0.273 ± 0.070 ^a	0.260 ± 0.048 ^a	0.257 ± 0.040 ^a	0.309 ± 0.027 ^a
SGR (% body wt d ⁻¹)	13.5 ± 0.8 ^a	13.4 ± 0.5 ^a	13.3 ± 0.4 ^a	13.9 ± 0.3 ^a
Final length (mm)	35.3 ± 3.5 ^a	33.7 ± 0.3 ^a	35.3 ± 1.0 ^a	36.3 ± 0.9 ^a
CV (length)	27.5 ± 5.8 ^a	28.1 ± 2.4 ^b	23.3 ± 2.7 ^a	28.1 ± 0.9 ^b
Total weight gain (g)	5323 ± 1045 ^a	4592 ± 337 ^a	6890 ± 952 ^b	6868 ± 304 ^b
Food fed (g)	7038 ± 2215 ^a	5838 ± 484 ^a	8758 ± 2234 ^a	8439 ± 753 ^b
FCR	1.30 ± 0.18 ^a	1.27 ± 0.08 ^a	1.26 ± 0.14 ^a	1.23 ± 0.06 ^a
FCE	0.78 ± 0.11 ^a	0.79 ± 0.06 ^a	0.80 ± 0.09 ^a	0.82 ± 0.04 ^a
Survival (%)	40.7 ± 5.3 ^b	36.9 ± 5.4 ^a	54.8 ± 3.5 ^d	45.5 ± 4.8 ^c
Production (g m ⁻² d ⁻¹)	6.1 ± 1.2 ^a	5.3 ± 0.4 ^a	7.9 ± 1.1 ^b	7.9 ± 0.4 ^b

¹ For single criterion, mean values in the same row bearing different superscripts are significantly different ($P < 0.05$) by T-method.

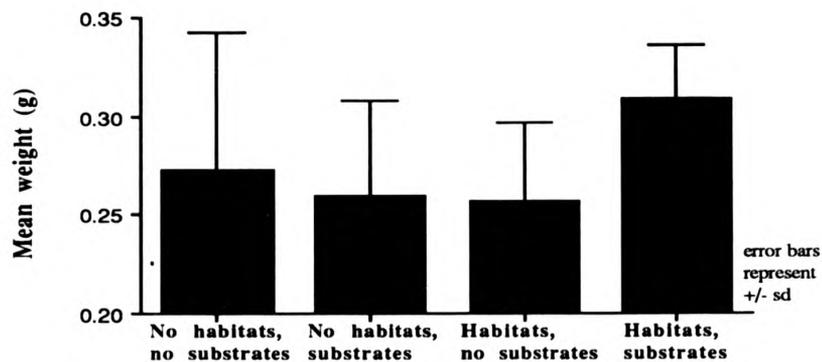


Figure 3.20 Relationship between mean weight and habitats/substrates in nursery trial 4.

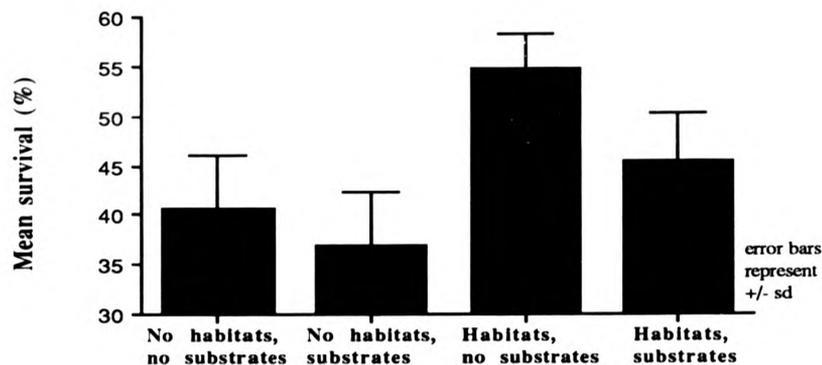


Figure 3.21 Relationship between mean survival and habitats/substrates in nursery trial 4.

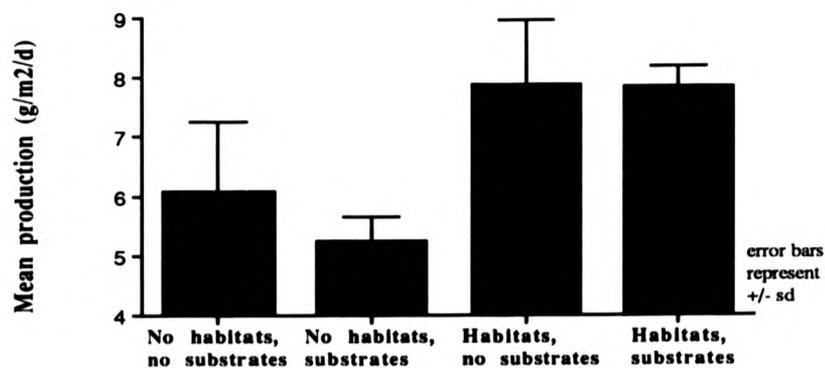


Figure 3.22 Relationship between mean production and habitats/substrates in nursery trial 4.

As with survival, shrimp production was significantly ($P < 0.05$) higher in the presence of habitats, but was unaffected by the presence of sand substrates. In the two treatments including habitats, the high mean shrimp production of $7.9 \text{ g m}^{-2} \text{ d}^{-1}$ was recorded, whilst production in tanks without habitats of 6.1 and $5.3 \text{ g m}^{-2} \text{ d}^{-1}$ was recorded in tanks without and with sand substrates respectively (Table 3.11, Figure 3.22).

3.3.6 Concrete tank nursery trial 5: Dietary formulation/Feeding rate with aeration and water filtration.

This trial investigated the effects of two dietary formulations (reference diet 2 and test diet 2, Table 3.2) and two feeding rates, low and *ad libitum*, on water quality and performance of shrimp stocked at $2,000 \text{ shrimp m}^{-2}$ over 35 days in the concrete nursery tank system. The low feeding rate was 30, 25, 20, 15 & 10 % wet body weight d^{-1} for weeks 1-5 respectively. The *ad libitum* feeding rate was 46, 60, 42, 42 & 30 % wet body weight d^{-1} for weeks 1-5 respectively (Table 3.1).

3.3.6.1 Water quality

In this trial, significant differences in DO were monitored between the treatments fed at the low feeding rate and those fed *ad libitum*. Additionally, a power cut due to faulty wiring resulted in a single failure of the air blower on day 15 of this trial. Figure 3.23 displays the change in mean morning DO concentrations over the 35 day trial period for those treatments fed at the low and *ad libitum* rates of the reference diet. The other two treatments were not included in this figure for purposes of clarity.

During the morning of the air blower failure, DO decreased rapidly to as low as 1 mg l^{-1} for the treatments fed *ad libitum* and 3.5 mg l^{-1} for the treatment fed the low feeding rate (Figure 3.23). In addition, for the two treatments fed *ad libitum*, early morning DO declined to $< 4 \text{ mg l}^{-1}$ (*i.e.* less than the optimal level for shrimp growth, Liao & Murai, 1986) for the final two weeks of the trial period (Figure 3.23). Mean DO during the day however, remained high and stable (approximately 6.5 mg l^{-1}) for treatments fed the low feed rate, but declined steadily to $3.6\text{-}4.2 \text{ mg l}^{-1}$ by the end of

Table 3.12 Mean water quality in weeks 1 to 5 of nursery tank trial 5.

Diet type/ feeding rate	Week	Temperature (°C)	Salinity (‰)	pH	Dissolved oxygen (% saturation)	Secchi depth (m)	TAN (mg l ⁻¹)	NO ₂ -N (µg l ⁻¹)	DRP (mg l ⁻¹)	Suspended solids (mg l ⁻¹)
Ref diet 2, low rate	1	28.4	34.9	8.07	85	0.87	0.00	0.6	0.00	82.8
	2	28.2	33.2	8.11	104	0.87	0.00	2.6	0.03	89.1
	3	28.7	29.4	8.23	104	0.65	0.00	3.6	0.11	96.1
	4	29.8	31.3	8.36	102	0.68	0.00	8.4	0.09	91.7
	5	30.4	31.3	8.29	103	0.62	0.00	14.7	0.23	93.1
Ref diet 2, <i>ad lib</i> rate	1	28.2	34.9	8.11	85	0.87	0.00	1.5	0.00	93.1
	2	28.2	33.2	8.10	105	0.83	0.00	4.9	0.02	107.9
	3	28.7	29.4	8.02	90	0.57	0.01	11.1	0.40	98.1
	4	29.8	31.3	7.95	74	0.58	0.06	16.3	0.34	96.7
	5	30.5	31.3	8.06	58	0.50	0.01	17.6	0.24	86.1
Test diet 2, low rate	1	28.3	34.9	8.11	85	0.89	0.00	2.5	0.00	88.3
	2	28.1	33.2	8.17	102	0.86	0.00	3.1	0.01	89.6
	3	28.7	29.4	8.19	101	0.62	0.00	6.8	0.08	92.8
	4	29.7	31.3	8.33	99	0.63	0.00	16.4	0.02	86.3
	5	30.4	31.3	8.36	104	0.63	0.00	13.0	0.23	98.3
Test diet 2, <i>ad lib</i> rate	1	28.4	34.9	8.10	85	0.89	0.00	1.8	0.00	86.5
	2	28.2	33.2	8.11	104	0.79	0.00	7.7	0.02	96.1
	3	28.8	29.4	7.97	86	0.58	0.01	11.4	0.33	97.3
	4	29.8	31.3	8.02	77	0.57	0.08	31.8	0.32	93.0
	5	30.6	31.3	8.04	68	0.63	0.01	50.2	0.32	87.9

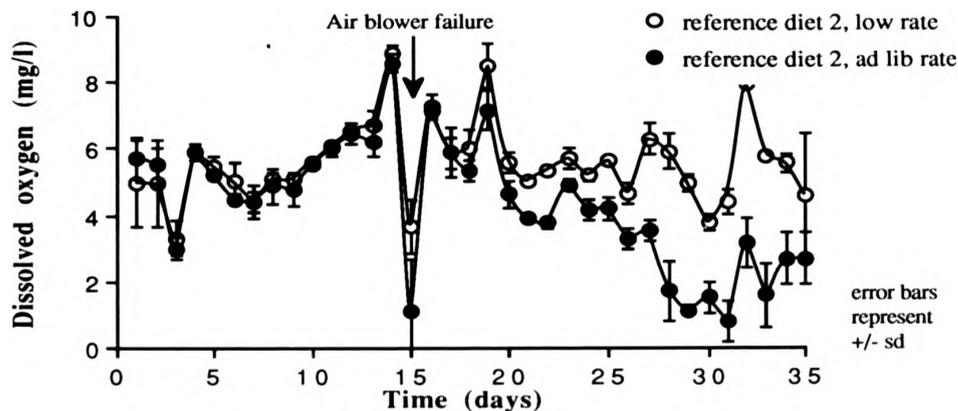


Figure 3.23 Change in mean am dissolved oxygen concentration with time for low and ad libitum feeding rates in nursery trial 5.

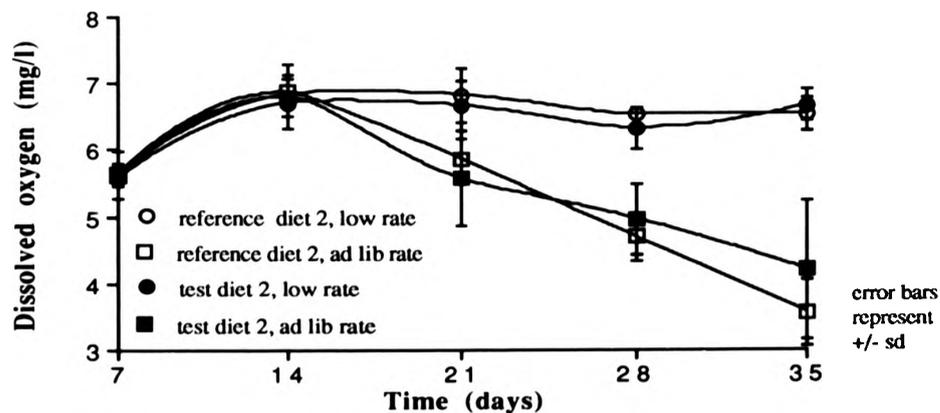


Figure 3.24 Change in mean weekly dissolved oxygen concentration with time for dietary formulation/feeding rate in nursery trial 5.

the trial in the treatments fed *ad libitum* (Table 3.12, Figure 3.24), despite the use of supplementary aeration.

Temperature and pH were stable and within the optimal range for shrimp growth during the trial period (Table 3.12), although high salinities of up to 35 ‰ were monitored during the first two weeks for all treatments.

Transparency was similar between treatments and did not decline to < 0.4 m over the trial period. Similarly, suspended solids levels were low, stable and were not significantly different between treatments.

Dissolved nutrient concentrations (TAN, NO₂-N and DRP) increased slightly during the trial period. However, they were within the tolerable limits for shrimp growth and did not vary significantly between treatments. The levels of these nutrients however, tended to be higher in the treatments fed *ad libitum* (Table 3.12).

3.3.6.2 Shrimp performance

Changing the dietary formulation to one containing higher levels of protein, lipid and energy (Test diet 2, Table 3.2) had significant ($P < 0.05$) negative effects on shrimp growth rate, survival and production, but decreased the coefficient of variation in shrimp length. Changing the feeding rate from low to *ad libitum* (Table 3.1) meanwhile, significantly ($P < 0.05$) increased the growth rate (weight increase and SGR), had no significant effects on shrimp survival or production, but led to a significant deterioration in the coefficient of variation in length, FCR and FCE (Table 3.13). Again, no interactive effects between these two variables were evident during this trial.

Growth rate was high for all treatments with shrimp produced after 35 days with mean weights of 0.509-0.577 g (Figure 3.25), mean total lengths of 42.9-45.3 mm, at a mean SGR of 15.0-15.3 % body weight d⁻¹ (Table 3.13).

The coefficient of variation in shrimp length was relatively low, but was significantly

Table 3.13 Mean performance (weeks 1-5) of shrimp in nursery tank trial 5.

Treatment	1	2	3	4
Stocking density (shrimp m ⁻²)	2,000	2,000	2,000	2,000
Habitats (no. panels)	3	3	3	3
Substrates (cm depth)	0	0	0	0
Aeration (l sec ⁻¹ tank ⁻¹)	24	24	24	24
Diet	reference 2	reference 2	test 2	test 2
Feeding rate	low	<i>ad libitum</i>	low	<i>ad libitum</i>
Initial weight (g)	0.003 ± 0.000 ^a			
Final weight (g)	0.511 ± 0.067 ^b	0.577 ± 0.001 ^c	0.509 ± 0.040 ^a	0.509 ± 0.021 ^a
SGR (% body wt d ⁻¹)	15.0 ± 0.4 ^a	15.3 ± 0.0 ^b	15.0 ± 0.02 ^a	15.0 ± 0.1 ^a
Final length (mm)	44.5 ± 2.2 ^b	44.2 ± 2.4 ^a	45.3 ± 0.8 ^b	42.9 ± 1.7 ^a
CV (length)	22.6 ± 1.8 ^c	25.0 ± 0.6 ^d	18.8 ± 0.3 ^a	21.2 ± 1.0 ^b
Total weight gain (g)	8024 ± 455 ^b	10025 ± 384 ^b	7262 ± 553 ^a	6534 ± 1372 ^a
Food fed (g)	9907 ± 936 ^a	27744 ± 2512 ^b	9196 ± 909 ^a	24880 ± 897 ^b
FCR	1.23 ± 0.09 ^a	2.87 ± 0.32 ^b	1.26 ± 0.06 ^a	3.92 ± 0.83 ^b
FCE	0.81 ± 0.07 ^a	0.35 ± 0.04 ^b	0.79 ± 0.04 ^a	0.26 ± 0.07 ^b
Survival (%)	32.4 ± 5.3 ^b	35.2 ± 1.4 ^b	29.1 ± 0.7 ^a	26.2 ± 4.9 ^a
Production (g m ⁻² d ⁻¹)	9.2 ± 0.5 ^b	11.5 ± 0.4 ^b	8.3 ± 0.6 ^a	7.5 ± 1.6 ^a

¹ For single criterion, mean values in the same row bearing different superscripts are significantly different ($P < 0.05$) by T-method.

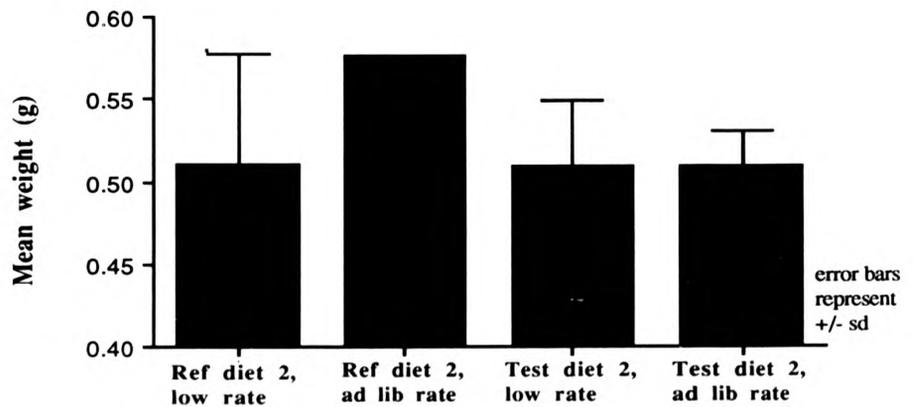


Figure 3.25 Relationship between mean weight and dietary formulation/feed rate in nursery trial 5.

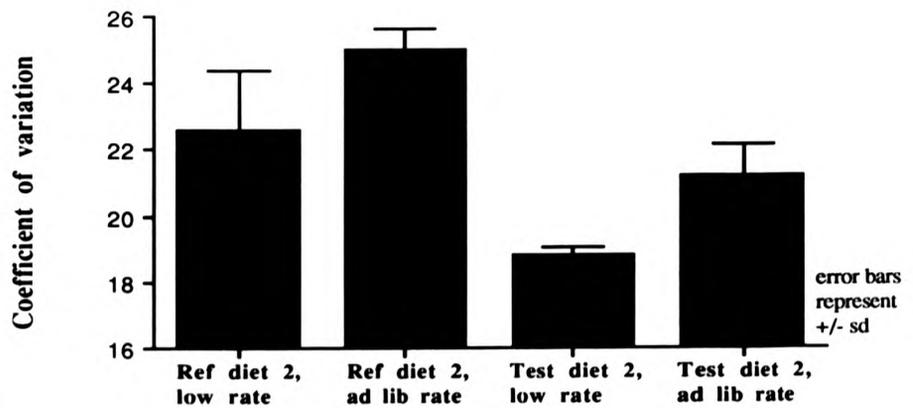


Figure 3.26 Relationship between coefficient of variation in length and dietary formulation/feed rate in nursery trial 5.

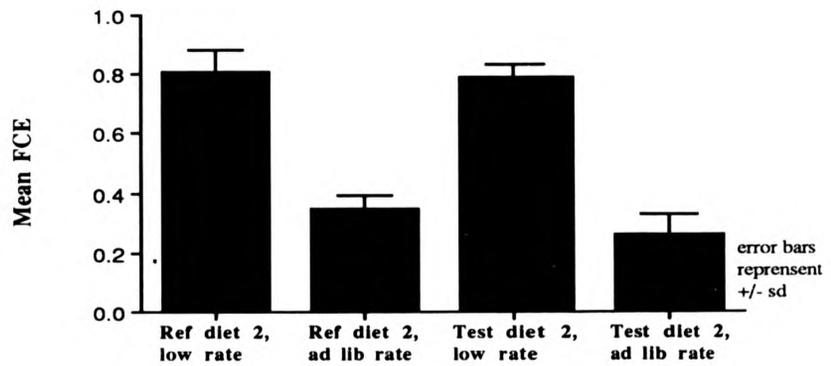


Figure 3.27 Relationship between mean FCE and dietary formulation/feed rate in nursery trial 5.

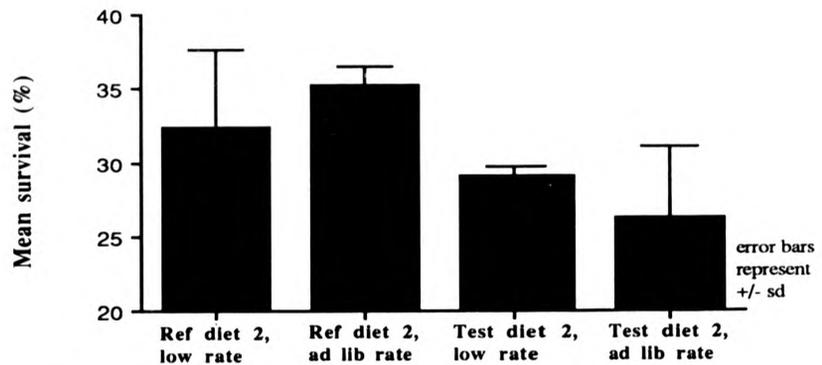


Figure 3.28 Relationship between mean survival and dietary formulation/feed rate in nursery trial 5.

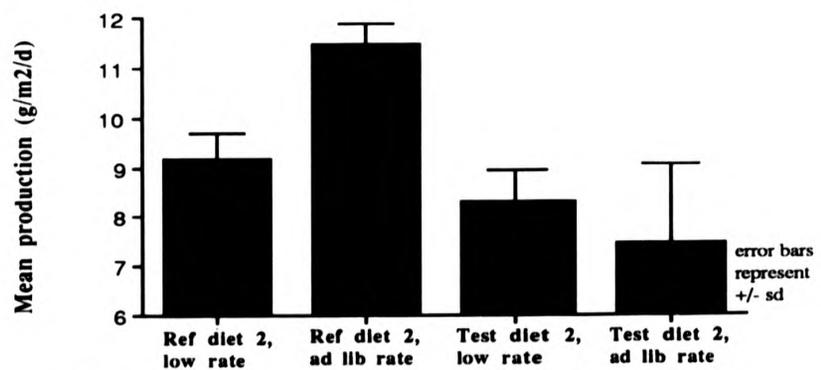


Figure 3.29 Relationship between mean production and dietary formulation/feed rate in nursery trial 5.

lower for shrimp fed the test diet (18.8-21.2), than those fed the reference diet (22.6-25.0). Changing the feeding rate from low to *ad libitum* also led to an increase in the size range of shrimp produced after 35 days (Table 3.13, Figure 3.26).

The FCR and FCE of shrimp fed the different diets at a common feeding rate were not significantly different. However, they were significantly better for shrimp fed at the low (FCR 1.23-1.26, FCE 0.79-0.81), compared to the *ad libitum* (FCR 2.87-3.92, FCE 0.26-0.35) feeding rate (Table 3.13, Figure 3.27).

Survival was poor, but was significantly higher for shrimp fed the reference diet (32.4-35.2 %), than those fed the test diet (26.2-29.1 %) (Table 3.13, Figure 3.28).

Mean shrimp production appeared to be largely independent of feeding rate, but was significantly higher for shrimp fed the reference diet. The highest production rate achieved in the nursery tanks of $11.5 \text{ g m}^{-2} \text{ d}^{-1}$ was obtained in this trial with shrimp fed the reference diet *ad libitum* (Table 3.13, Figure 3.29).

3.3.7 Synopsis of nursery trial results

3.3.7.1 Water quality

In the absence of supplementary aeration/water filtration, suitable water quality conditions could be maintained in nursery tank systems for up to one month (at 20 % water exchange d^{-1}) at stocking densities of up to 1,000 shrimp m^{-2} . Under these conditions, dissolved oxygen concentrations fluctuated widely due to daily cycles where photosynthesis and then respiration predominated, but rarely reached critical concentrations. Water transparency measurements of less than 0.4 m lead to phytoplankton bloom crashes (and hence stressed shrimp) when the nursing period exceeded one month or the stocking density was $> 1,000$ shrimp m^{-2} . The concentration of nutrients/metabolites in the water could be maintained below toxic levels under these conditions.

The water quality in net cage nursery systems (stocked at 500 shrimp m^{-2}) in tidally-

flushed ponds tended to be more stable than that achieved in un-aerated nursery tank systems stocked at a common density.

Increasing the stocking density of shrimp in nursery tank systems required supplementary aeration (and/or increased water exchange) for the maintenance of suitable water quality. Supplementary aeration tended to stabilise water quality, particularly dissolved oxygen concentrations, even at stocking densities of up to 3,000 shrimp m^{-2} . However, increasing the feeding rate to > 30 % wet shrimp body weight d^{-1} , increased the requirement for supplementary aeration (to > 0.07 $l\ sec^{-1}\ m^{-2}$) and water exchange (to > 40 % tank volume d^{-1}) at high stocking densities.

Failure of the air blower, providing aeration to high density nursery tanks, led to critically low concentrations of dissolved oxygen and high shrimp mortality, adding substantially to the risk of this method of nursing post-larvae.

The presence of sand substrates lead to siltation of the sand with anoxic mud, providing an inhospitable environment for benthic post-larvae. This situation occurred under the present conditions where there was inadequate filtration of incoming water containing high levels of suspended solids, or where over-feeding was practised. Other than increased siltation in the presence of substrates, neither the use of substrates nor habitats had any effects upon the water quality in the concrete nursery tank system.

3.3.7.2 Shrimp performance

Shrimp growth, size range, food conversion efficiency, survival and production were affected primarily by the density at which they were nursed. There were no clear relationships between shrimp performance and initial size despite variations from 0.002 to 0.013 g. The growth rate of shrimp was inversely related to stocking density. Figure 3.30 combines the results of all of the nursery tank trials in this series (except trial 3, due to very poor survival) to display the relationship between mean shrimp weight after 35 days of nursing and stocking density. In terms of the increase in mean length, the relationship to stocking density could be described by the equation: $\text{Log}_e(\text{length, mm}) = 77.17 - 12.27 \text{Log}_e(\text{density, shrimp } m^{-2})$, $n = 5$, $r^2 = 0.919$, $P < 0.05$ (Figure

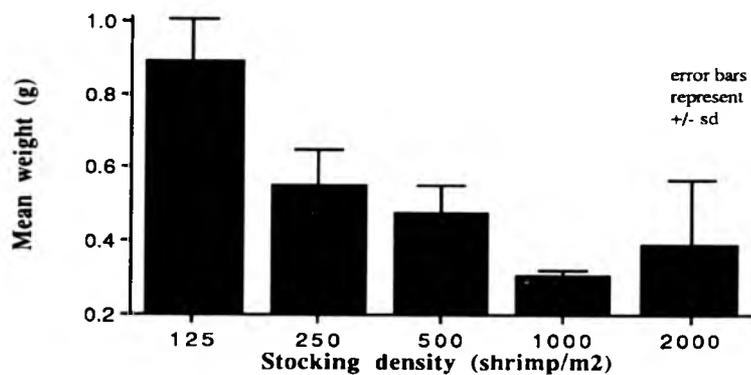


Figure 3.30 Relationship between mean weight and stocking density in nursery tank trials.

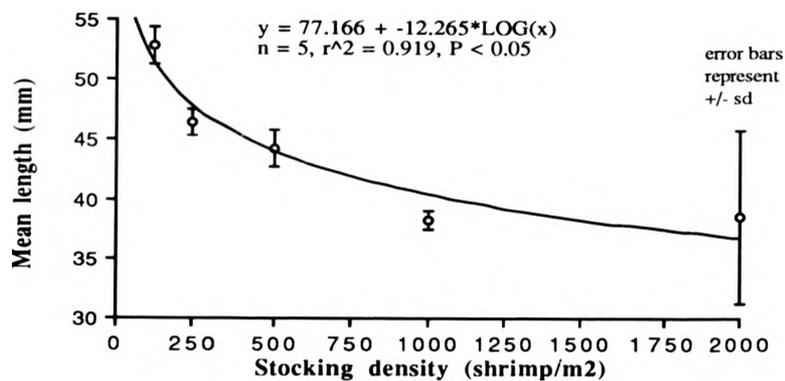


Figure 3.31 Relationship between mean length and stocking density in nursery tank trials.

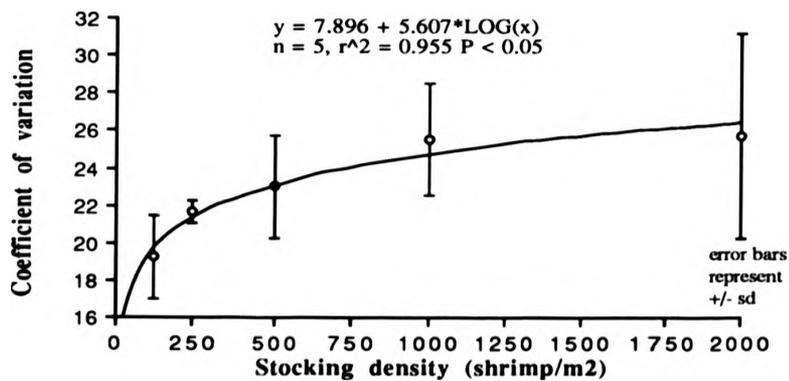


Figure 3.32 Relationship between mean coefficient of variation in length and stocking density in nursery tank trials.

3.31). Thus nursing at 125 shrimp m⁻² produced shrimp with a mean weight of 0.89 g and mean total length of 53 mm, while nursing at 1-2,000 shrimp m⁻² produced shrimp of 0.35-0.40 g and 38 mm after 35 days. As stocking density increases, not only did the shrimp grow more slowly, but their variation in size increased. Thus, the coefficient of variation in length of shrimp after 35 days could be related to stocking density by the equation: $\text{Log}_e(\text{length, mm}) = 7.90 + 5.61 \text{Log}_e(\text{density, shrimp m}^{-2})$, $n = 5$, $r^2 = 0.955$, $P < 0.05$ (Figure 3.32).

During secondary nursing, FCE was high at 0.6-1.1 at stocking densities from 125-2,000 shrimp m⁻². In common with growth rate, FCE was inversely related to stocking density. Figure 3.33 displays this relationship, which could be described by the equation: $\text{Log}_e(\text{FCE}) = 1.99 - 0.42 \text{Log}_e(\text{density, shrimp m}^{-2})$, $n = 5$, $r^2 = 0.923$, $P < 0.05$. Survival also tended to be inversely related to stocking density. Results from all the trials combined (except trial 3) showed that survival was high (> 90 %) at low density (125-250 shrimp m⁻²), intermediate at densities of 500-1,000 shrimp m⁻², but decreased to > 30 % as density was increased to 2,000 shrimp m⁻² (Figure 3.34).

Shrimp production was directly related to stocking density, reaching levels of 3 g m⁻² d⁻¹ at 125 shrimp m⁻² and > 6 g m⁻² d⁻¹ at densities of > 1,000 shrimp m⁻² (Figure 3.35). The effect of stocking density on shrimp production could be described by the equation: $\text{Log}_e(\text{production, g m}^{-2} \text{ d}^{-1}) = 1.99 - 0.42 \text{Log}_e(\text{density, shrimp m}^{-2})$, $n = 5$, $r^2 = 0.897$, $P < 0.05$ (Figure 3.35).

At a common stocking density of 500 shrimp m⁻², there was little difference between the performance of shrimp nursed in net cages or concrete tanks. Shrimp growth rate and FCE tended to be better, but survival and production were worse during net cage than during concrete tank secondary nursing.

The presence of habitats, used in order to increase the wetted surface area (WSA) of the concrete tanks, was seen to have variable effects on shrimp performance depending upon the stocking density used. When the stocking density was maintained at 500 shrimp m⁻², the use of habitats at an increase in WSA of up to 160 %, no significant effects on shrimp performance could be determined. When stocking densities were

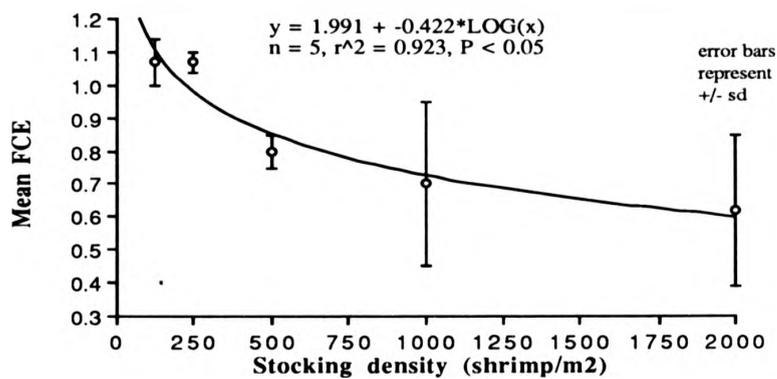


Figure 3.33 Relationship between mean FCE and stocking density in nursery tank trials.

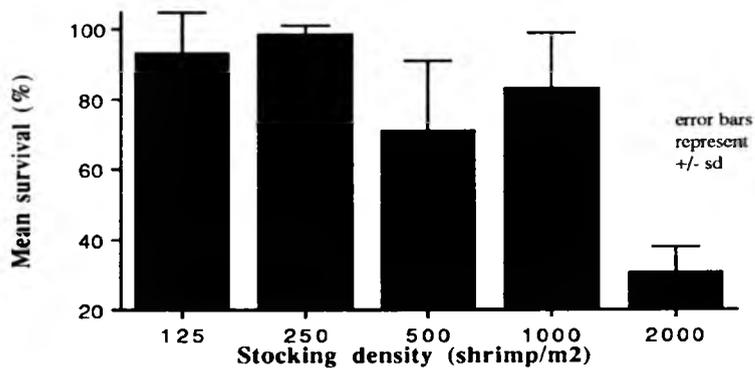


Figure 3.34 Relationship between mean survival and stocking density in nursery tank trials.

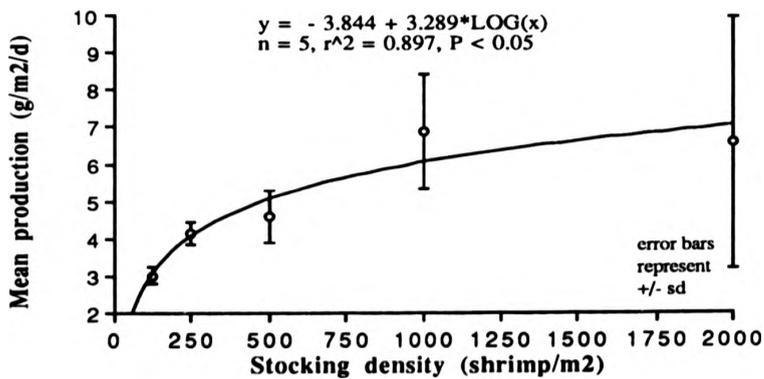


Figure 3.35 Relationship between mean production and stocking density in nursery tank trials.

increased to 2,000 shrimp m^{-2} however, the inclusion of habitats increasing the WSA of the tanks by 120 %, resulted in similar growth rates, but significantly enhanced survival (although survival was generally poor) and hence production rates.

The use of sand substrates was not found to have any significant effect on shrimp performance, although survival and production rates tended to decline in the presence of substrates.

The use of supplementary aeration and water exchange (at > 40 % tank volume d^{-1} for the fourth and fifth weeks of secondary nursing) was found to be necessary to support the production of shrimp nursed at densities $> 1,000$ shrimp m^{-2} .

Changing the diet from the reference to the test formulation resulted in significantly reduced shrimp growth rate, survival and production, but decreased the coefficient of variation in shrimp length. Changing the feeding rate from low to *ad libitum* (> 30 % wet body weight d^{-1}) also affected shrimp performance. Increasing the feeding rate in this way led to significantly increased shrimp growth rate, but produced shrimp with a higher size range. In addition, although having no significant effects on shrimp survival or production, increasing the feeding rate lead to a significant deterioration in FCR and FCE.

The growth rate, survival and performance of shrimp nursed at a common density varied considerably between the different nursery tank trials, as did the environmental conditions. The effects of shrimp quality (stress tolerance) on these results will be considered in chapter 4.

3.3.8 Economic analysis of nursery trials

For the purposes of economic analysis, the nursery tank system was costed for three stocking densities, *i.e.* 500, 1,000 and 2,000 shrimp m^{-2} . The nursery system comprised of twelve 25 m^2 tanks with a total surface area of 300 m^2 . The nursery had a 35 day cycle, rearing shrimp from PL_{15} to PL_{50} , with a total of ten crops possible per year.

3.3.8.1 Capital costs

According to the above specifications, the total capital cost for construction of the nursery system was £ 14,270 (Table 3.14).

3.3.8.2 Operational costs

Total annual operational costs for the nursery were £ 9,990 at 500 shrimp m⁻², £ 15,284 at 1,000 shrimp m⁻² and £ 25,993 at 2,000 shrimp m⁻² (Table 3.15). These costs were based on the assumptions that maintenance is set at 2 % of the capital costs and depreciation on capital is spread evenly over the first 6 years.

By far the most expensive item of the operational costs was seed cost, which increased with stocking density from £ 5,250 to £ 21,000 (53-81 % of operational costs). Depreciation on capital was the next highest cost at 9-24 % of operational costs, decreasing with stocking density. In contrast, variable costs of feed, labour and electricity were of relatively minor importance, each accounting for less than 10 % of the total operational costs (Table 3.15).

3.3.8.3 Juvenile production

The base case for producing juvenile shrimp was based on the results from four of the nursery tank trials conducted in this system (not including trial 3 due to air blower failure and mass mortality). The assumptions used for establishing the juvenile production costs are shown in Table 3.16. As stocking density during nursing increased, the growth, survival and FCR decreased. However, the production costs were lowest for a stocking density of 1,000 shrimp m⁻² due to high survival and production. In contrast, production costs at a stocking density of 2,000 shrimp m⁻² were very high, despite high production, due to very low survival (40 %).

3.3.8.4 Sensitivity analysis

The base case economic analysis used data derived from the nursery tank trials in order

Table 3.14 Capital costs of 300 m² concrete nursery tank system in Thailand¹.

Item	Units	Unit cost (£)	Total cost (£)
Concrete blocks	-	-	1270
Cement	-	-	750
Sand	-	-	500
Air blower (1.5 kW)	1	1350	1350
Pipework	-	-	5000
Water pump (2.2 kW)	2	1000	2000
Labour	4	-	1200
Control panels	2	550	1100
Miscellaneous equipment	-	-	1100
Total	-	-	14270

¹ 1994 prices in Baht equivalent (£ = 37 Bt).

Table 3.15 Operational costs of 300 m² concrete nursery tank system in Thailand^{1,2}.

Item	Unit cost (£)	Stocking density					
		500		1,000		2,000	
		Cost (£)	Cost (%)	Cost (£)	Cost (%)	Cost (£)	Cost (%)
Seed	0.0035 PL ₁₅ ⁻¹	5250	53.0	10500	68.7	21000	80.8
Feed	0.86 kg ⁻¹	452	4.6	586	3.8	795	3.1
Manager	270 year ⁻¹	270	2.7	270	1.8	270	1.0
Labourers (3)	108 year ⁻¹	325	3.2	325	2.1	325	1.3
Electricity	0.027 kWh ⁻¹	840	8.5	840	5.5	840	3.2
Maintenance ³	285 year ⁻¹	285	2.9	285	1.9	285	1.1
Miscellaneous	100 year ⁻¹	100	1.0	100	0.7	100	0.4
Depreciation ⁴	2378 year ⁻¹	2378	24.0	2378	15.6	2378	9.1
Total	-	9900	100.0	15284	100.0	25993	100.0

¹ 1994 prices in Baht equivalent (£ = 37 Bt).

² All figures are based on one year of operation at 10 cycles of 35 days year⁻¹.

³ Maintenance at 2 % of capital costs

⁴ Depreciation on capital spread evenly over first 6 years

Table 3.16 Base case assumptions for investment appraisal of 300 m² concrete nursery tank system in Thailand.

Stocking density (shrimp m ⁻²)	Mean survival (%)	Mean final weight (g)	Mean production (g m ⁻² d ⁻¹)	Mean FCR	Seed cost (£/1000)	Minimum selling price ¹ (£/1000)	Cost of production (£/1000)	Revenue (£)	Juvenile production ² (million yr ⁻¹)	Pond area stockable ³ (ha)
500	90	0.50	5	1.00	3.5	10.0	7.33	13,500	1.35	4.5
1,000	82	0.35	6	1.08	3.5	8.0	6.14	19,920	2.49	8.3
2,000	40	0.30	7	1.26	3.5	12.0	10.83	28,800	2.40	8.0

¹ Minimum selling price required to achieve positive internal rate of return (IRR)

² Juvenile production for one year of operation at 10 cycles of 35 days year⁻¹

³ On-growing pond area stockable at stocking density of 30 shrimp m⁻²

to establish the minimum selling price for juvenile shrimp to achieve a positive internal rate of return (IRR) on investment. The minimum price necessary varied with stocking density at £ 8, 10 and 12 per thousand juveniles at 1,000, 500 and 2,000 shrimp m⁻² respectively (Table 3.16).

The sensitivity analysis conducted showed that for departures of 10 and 20 % above and below the base values, the IRR was very sensitive to variations in selling price or survival, particularly at higher stocking densities (Table 3.17, Figures 3.36, 3.37, 3.38). If either selling price or survival decreased by > 10 %, break-even was not possible. If the selling price for shrimp produced at a nursing density of 2,000 shrimp m⁻², was reduced to £ 8 per thousand (likely, due to their smaller final size), a survival rate of > 60 % would be necessary to maintain a positive IRR.

The IRR was next most sensitive to fluctuations in seed cost, but break-even was possible if seed costs increased by 20 % at stocking densities of up to 1,000 shrimp m⁻². In contrast, capital and feed costs had very little effect on the profitability of the nursery system since they contributed little to the costs of production. By doubling the tank area of the nursery, the profitability of the nursery system was only slightly increased (Table 3.17).

3.3.9 Net cage on-growing trial

3.3.9.1 Water quality

Water quality parameters were not measured for individual treatments or replicates during the on-growing cage trial since the cages were located in a common pond with equal water exchange for all cages. The water quality conditions measured within a representative cage are recorded in Table 3.18.

Dissolved oxygen concentrations varied between 4.8 and 6.4 mg l⁻¹ and were never lower than 3.5 mg l⁻¹ during the early morning. Temperature and salinity were high and stable at 29.6-30.7 °C, and 31.2-33.0 ‰ respectively throughout the trial period. pH was also stable and slightly lower than optimum for shrimp growth at 6.92-7.66

Table 3.17 Sensitivity analysis of net present value (NPV) and internal rate of return (IRR) of 300 m² concrete nursery tank system.

Parameter	Change (%)	Stocking density (shrimp m ²)					
		500		1,000		2,000	
		NPV (£)	IRR (%)	NPV (£)	IRR (%)	NPV (£)	IRR (%)
Base case	0	-3429	8.35	-310	14.43	-5817	3.13
Selling price	-20	-11560	-14.64	-14024	-15.22	-20271	-90.26
	-10	-7495	-1.00	-7808	-1.83	-13044	-23.62
	+10	636	16.16	7188	27.28	1410	17.55
	+20	4701	23.20	14686	42.24	7401	45.22
Seed cost	-20	-268	14.51	6014	25.37	6830	26.70
	-10	-1849	11.51	2852	20.07	506	15.92
	+10	-5010	4.97	-3472	8.26	-12141	-17.58
	+20	-6591	1.29	-6633	1.18	-18465	-49.65
Capital cost	-20	-375	13.66	2544	20.63	-2963	7.78
	-10	-2002	10.77	1117	17.25	-4390	5.26
	+10	-4856	6.27	-1737	12.03	-7244	1.30
	+20	-6283	4.46	-3164	9.96	-8671	-0.30
Survival (%)	-20	-11560	-14.64	-12307	18.52	-23162	-103.15
	-10	-7495	-1.00	-6308	1.97	-14490	-25.36
	+10	636	16.16	5689	24.84	2855	20.07
	+20	-	-	11687	40.24	11527	53.47
Feed cost	-20	-3157	8.90	43	15.08	-5339	4.23
	-10	-3293	8.63	-133	14.75	-5578	3.69
	+10	-3566	8.06	-486	14.10	-6057	2.57
	+20	-3702	7.88	-663	13.77	-6296	2.00
Tank area	+100	4295	19.55	10534	25.78	-481	14.47

Notes:

Discount rate at 15 %

Tax on profits at 40 %

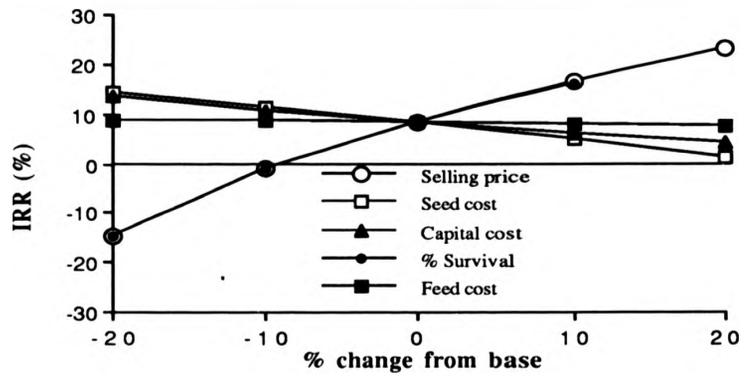


Figure 3.36 Sensitivity analysis of tank nursing at 500 shrimp/m2.

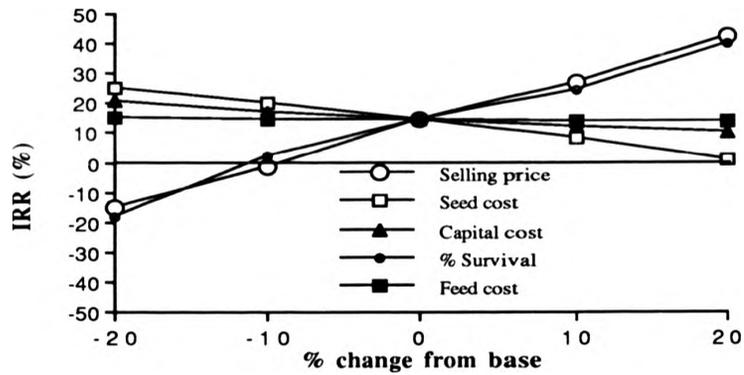


Figure 3.37 Sensitivity analysis of tank nursing at 1,000 shrimp/m2.

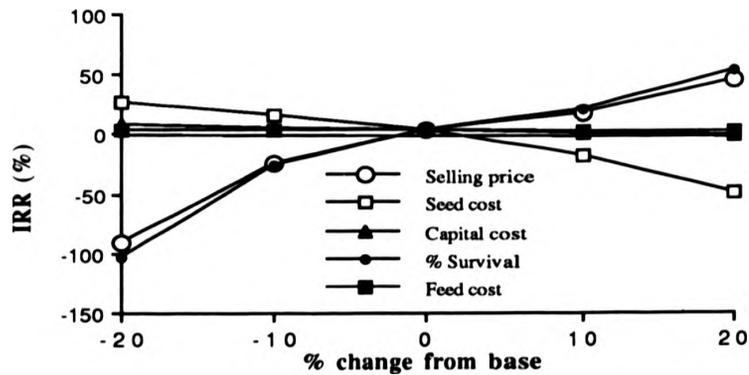


Figure 3.38 Sensitivity analysis of tank nursing at 2,000 shrimp/m2.

throughout the trial period. Water transparency was also stable and varied from 0.46 to 0.68 m over the trial period.

Suspended solids levels were 88.5-117.3 mg l⁻¹, and dissolved nutrient levels of TAN, NO₂-N and DRP, low or even undetectable throughout the trial period. At no stage were the water quality conditions monitored considered to be detrimental to shrimp growth or survival during this trial.

3.3.9.2 Shrimp performance

Shrimp stocked into the various treatments of the on-growing cage trial had significantly ($P < 0.05$) different initial weights since they came from the four treatments of nursery tank trial 1 (nursed at differing density) and the net cage nursery trial (Table 3.19).

Results from the trial revealed that the growth rate of the shrimp over the first six weeks was inversely proportional to their initial weight (Figure 3.39). In addition, from weeks 4-10, the mean weight (Figure 3.40) and length of the shrimp were not significantly different between treatments stocked with shrimp nursed at the different densities (Table 3.19).

Following ten weeks of on-growing, previous nursing density (and hence varying initial shrimp weight) had no significant effects on shrimp growth, CV in length, FCE, survival or production. Shrimp growth rate in terms of mean weight (Figure 3.41), mean total length and mean SGR (Figure 3.42) however, declined rapidly after 4-6 weeks. Overall growth tended to be directly related nursing density at up to 500 shrimp m⁻² (and hence decreasing initial weight) after 10 weeks of on-growing. The mean final weight of shrimp thus increased from 2.5-3.4 g and the SGR of the shrimp increased from 1.5 to 3 % body weight d⁻¹ as the nursing density increased from 125 to 500 shrimp m⁻² (Table 3.19 and Figures 3.41 & 3.42 respectively). Shrimp nursed in cages, rather than tanks, displayed the highest mean final weight of any treatment at 3.7 g (Table 3.19, Figure 3.41).

The coefficient of variation in length of shrimp following on-growing was highly

Table 3.18 Mean water quality in weeks 1 to 10 of on-growing cage trial.

On-growing Trial	Week	Temperature (°C)	Salinity (‰)	pH	Dissolved oxygen (% saturation)	Secchi depth (m)	TAN (mg l ⁻¹)	NO ₂ -N (µg l ⁻¹)	DRP (mg l ⁻¹)	Suspended solids (mg l ⁻¹)
1	1	29.6	31.7	7.34	84	0.49	0.03	1.7	0.01	114.3
	2	29.8	32.5	6.92	96	0.50	0.00	0.4	0.00	88.5
	3	30.2	31.5	7.65	79	0.53	0.07	0.7	0.01	100.7
	4	29.6	31.7	7.34	84	0.49	0.03	1.7	0.01	114.3
	5	29.8	32.5	6.92	96	0.50	0.00	0.4	0.00	88.5
	6	29.9	31.6	7.37	102	0.57	0.00	1.6	0.00	96.7
	7	29.7	31.2	7.61	74	0.46	0.00	0.5	0.00	93.0
	8	30.2	33.0	7.66	84	0.47	0.00	20.2	0.01	101.1
	9	30.1	31.4	7.62	88	0.58	0.00	5.4	0.01	117.3
	10	30.7	34.4	7.49	80	0.68	0.00	10.9	0.00	99.7

Table 3.19 Mean performance (weeks 1-10) of shrimp in on-growing cage trial.

Treatment	1	2	3	4	5
Stocking density (shrimp m ⁻²)	125	125	125	125	500
Nursing density (shrimp m ⁻²)	125	250	500	1,000	500
Nursery trial	tank 1	tank 1	tank 1	tank 1	cage
Diet	reference 1	reference 1	reference 1	reference 1	reference 1
Feeding rate	normal	normal	normal	normal	normal
Initial weight (g)	0.887 ± 0.117 ^c	0.550 ± 0.098 ^b	0.413 ± 0.057 ^{ab}	0.304 ± 0.015 ^a	0.496 ± 0.010 ^b
Final weight (g)	2.473 ± 0.375 ^a	2.671 ± 0.225 ^a	3.366 ± 0.184 ^a	2.423 ± 0.183 ^a	3.702 ± 0.109 ^a
SGR (% body wt d ⁻¹)	1.5 ± 0.2 ^a	2.3 ± 0.1 ^a	3.0 ± 0.1 ^a	3.0 ± 0.1 ^a	2.9 ± 0.0 ^a
Final length (mm)	75.1 ± 5.3 ^a	78.4 ± 4.9 ^a	82.9 ± 2.3 ^a	75.9 ± 2.0 ^a	84.6 ± 0.2 ^a
CV (length)	15.6 ± 2.2 ^a	13.9 ± 3.3 ^a	13.6 ± 4.6 ^a	13.2 ± 4.0 ^a	14.2 ± 4.6 ^a
Total weight gain (g)	1024 ± 29 ^a	1514 ± 56 ^a	2190 ± 142 ^a	1569 ± 74 ^a	2366 ± 84 ^a
Food fed (g)	7653 ± 122 ^a	7006 ± 172 ^a	7672 ± 1613 ^a	5533 ± 102 ^a	4602 ± 313 ^a
FCR	7.77 ± 2.08 ^a	4.63 ± 1.00 ^a	3.49 ± 0.51 ^a	3.48 ± 0.25 ^a	1.95 ± 0.06 ^a
FCE	0.14 ± 0.04 ^a	0.22 ± 0.03 ^a	0.29 ± 0.04 ^a	0.29 ± 0.03 ^a	0.52 ± 0.02 ^a
Survival (%)	72.2 ± 5.7 ^a	74.0 ± 7.2 ^a	75.1 ± 4.2 ^a	74.2 ± 8.2 ^a	71.8 ± 5.3 ^a
Production (g m ⁻² d ⁻¹)	1.8 ± 0.5 ^a	2.7 ± 0.4 ^a	3.9 ± 0.3 ^a	2.8 ± 0.2 ^a	4.2 ± 0.2 ^a

¹ For single criterion, mean values in the same row bearing different superscripts are significantly different (P < 0.05) by T-method.

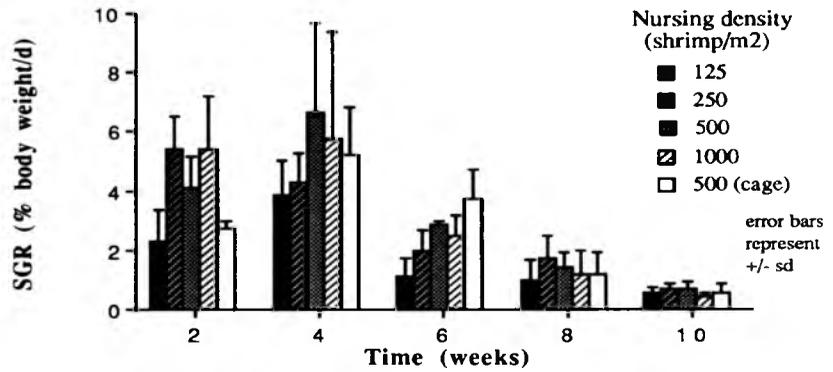


Figure 3.39 Relationship between mean SGR and nursing density over time in cage on-growing trial.

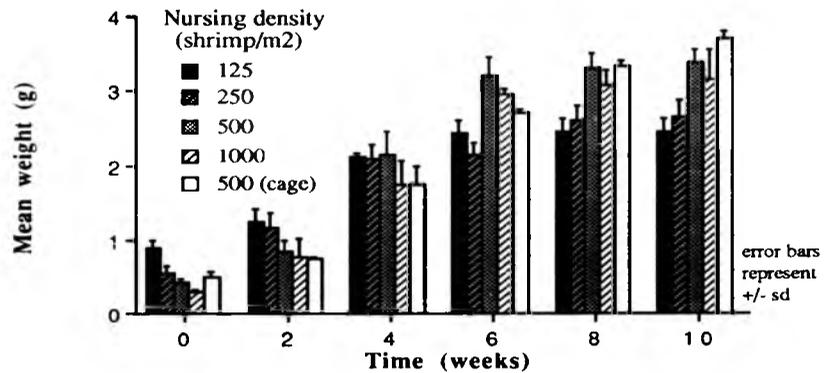


Figure 3.40 Relationship between mean weight and nursing density over time in cage on-growing trial.

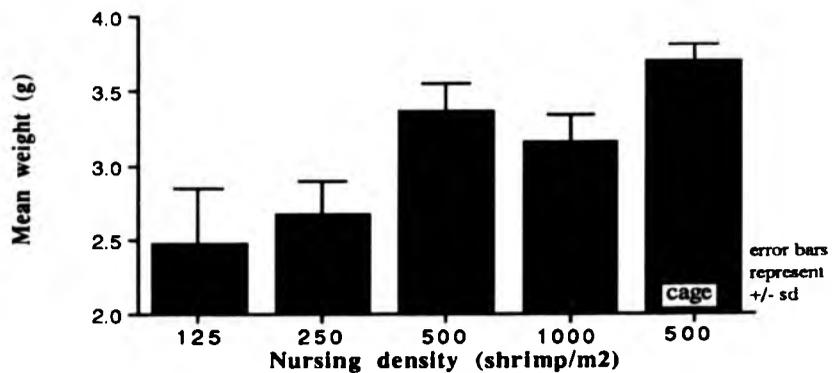


Figure 3.41 Relationship between mean weight and nursing density in cage on-growing trial.

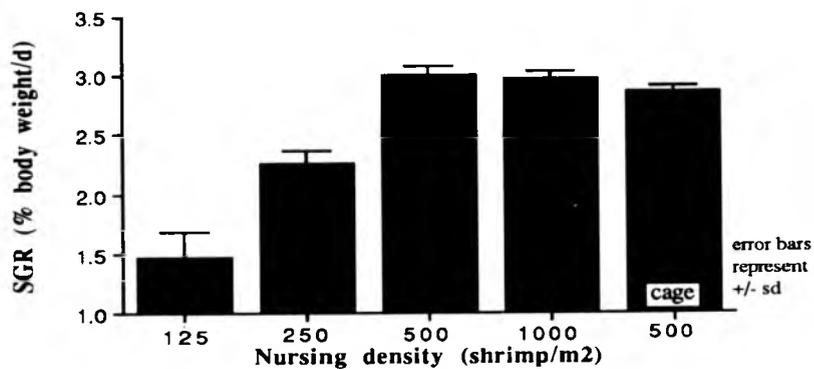


Figure 3.42 Relationship between mean SGR and nursing density in cage on-growing trial.

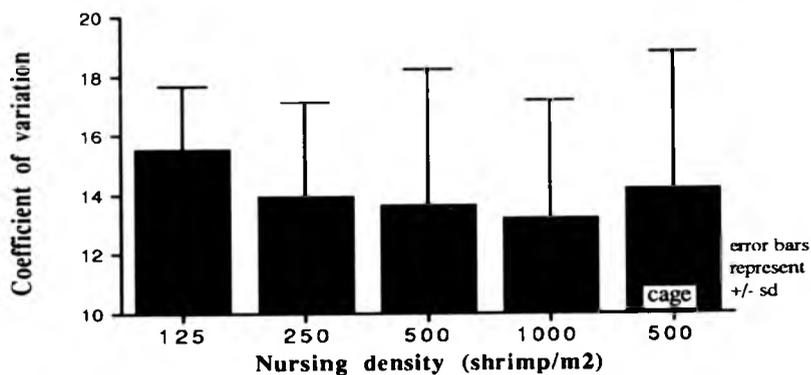


Figure 3.43 Relationship between coefficient of variation in length and nursing density in cage on-growing trial.

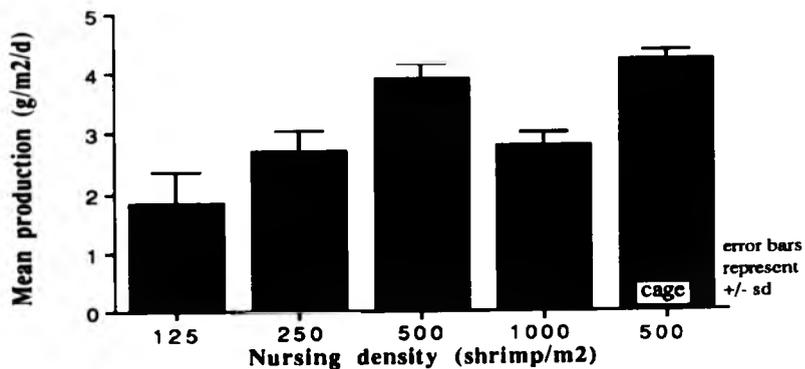


Figure 3.44 Relationship between mean production and nursing density in cage on-growing trial.

variable, but was low and tended to be inversely related to nursing density (Table 3.19, Figure 3.43). The initially large size range of shrimp from nursing at high density (see tank trial 1, section 3.3.1.2) therefore, did not translate into shrimp of highly variable size following the cage on-growing period.

Similarly to growth rate, the FCE of shrimp during on-growing tended to be inversely related to nursing density (Table 3.19) and was particularly high for cage-nursed post-larvae, although the differences were not significant at the 5 % level.

The survival of shrimp during the on-growing trial was high (mean 72-75 %) and was not significantly different between treatments (Table 3.19).

Overall shrimp production was low (1.8-4.2 g m⁻² d⁻¹), but tended to increase with increasing nursing density at up to 500 shrimp m⁻², and was highest for shrimp from the cage nursery trial (Table 3.19, Figure 3.44). These differences in production however, were not significant at the 5 % level.

3.4 DISCUSSION

3.4.1 Secondary nursery trials

3.4.1.1 Water quality

One of the potential benefits to intensive secondary nursing in tanks is the production of large numbers of juvenile shrimp seed under highly controlled conditions. Secondary nursing in ponds or cages involves placing the shrimp into conditions over which precise control of water quality, shrimp health and predation is difficult. It is partially because of these problems that increasing research (including the present study) is being conducted on high density secondary nursing in tanks. Secondary nursing in tanks facilitates maintenance of ideal water quality conditions during this important phase in the culture cycle. It also enables the culturist to inspect his stock regularly for signs of ill-health, and treat any problems as they arise, and to exclude predators which may

otherwise cause stock mortality.

The maintenance of water quality within nursery tanks permits optimal shrimp growth within the constraints of the system employed. In the concrete nursery tank trials conducted during the present study, maintenance of water quality was attempted using a number of techniques comprising aeration, water exchange and sand-filtration of inlet water. Although a number of parameters were monitored, the most critical ones were found to include DO, water transparency, temperature, salinity, pH and the concentration of ammonia.

The DO requirement for optimal growth and survival of shrimp is $> 4 \text{ mg l}^{-1}$ (Liao & Murai, 1986), whilst chronic DO concentrations $< 2 \text{ mg l}^{-1}$ are considered to be critical for post-larval shrimp (Chamberlain, 1988). During these trials, in the absence of supplementary aeration, a water exchange rate of 20 % tank volume d^{-1} was found to supply sufficient oxygen to tanks stocked at up to 1,000 shrimp m^{-2} for up to one month of nursing (Figures 3.6 & 3.11).

Under these conditions, the majority of the oxygen required by the shrimp is provided by the phytoplankton bloom. This bloom is stimulated to develop in the tanks through the fertilizing effects of applied feed and shrimp faeces. With further increases in either stocking density or rearing period however, overstimulation of the phytoplankton enhances the likelihood of its collapse ("bloom crash"). This was seen to occur on day 33 of trial 1 in this series when the water transparency decreased to $< 0.4 \text{ m}$ (Figure 3.5). In all subsequent trials, the transparency was maintained at $> 0.4 \text{ m}$ by water exchange at up to 40 % d^{-1} , and no further bloom crashes occurred. Dilution of the bloom by water exchange before transparency reaches this critical level was thus an effective technique for the maintenance of water quality.

If bloom crashes are permitted, the resulting high oxygen demand of the shrimp, the remaining live phytoplankton, the decaying dead phytoplankton and the uneaten food exceeds the oxygen supply from water exchange and the remaining live phytoplankton. Critical concentrations of DO and decreasing pH resulting from respiration of CO_2 lead to stressful conditions for the shrimp which can cause substantial mortality.

Even where bloom crashes are avoided, the lack of supplementary aeration (with water exchange rates of 20 % d⁻¹), particularly in tanks stocked at > 500 shrimp m⁻², tends to lead to large (and potentially stressful) daily DO fluctuations. This is due to the alternating dominance of algal photosynthesis during the day and respiration at night. In previous high density nursery tank trials with *P. vannamei*, AQUACOP (1985b) showed that water exchange rates of up to 400 % d⁻¹ were necessary to maintain water quality at stocking densities of 1-10,000 shrimp m⁻². They found that the exchange rate could be decreased to 5 % d⁻¹ if high levels of aeration were applied in combination with a phytoplankton and bacterial medium with both nutritive and purifying qualities. At low levels of water exchange, aeration may also be useful as a means of destratifying the water column to prevent critically low DO levels near the bottom of the tanks where most of the shrimp are located.

Unfortunately, there was not sufficient time available during the present study to conduct a thorough analysis of the benefits and disadvantages of aeration as opposed to water exchange for maintaining water quality of the concrete nursery tank system.

In the cage trial conducted during this study at 500 shrimp m⁻², water quality conditions were maintained within optimal limits by tidal water exchange of up to 0.5 m twice daily. Daily fluctuations in DO and other water quality parameters were less than in tanks stocked at the same density. This was a result of the less dense phytoplankton bloom in the cages since the fertilization effects of the shrimp faeces and applied feed were diluted in the large volume of water in the pond containing the cages. Increases in either the density of shrimp stocked in the cages or in the number of cages per pond however, may require supplemental aeration or water exchange, neither of which were tested during the present study. Results of previous research has shown that stocking densities can be increased if aeration (at 10 hp ha⁻¹) and water exchange (at 5-40 % pond volume d⁻¹) are employed during secondary nursing in ponds (Apud & Camacho, 1980; AQUACOP, 1985b; Sturmer *et al.*, 1992).

In order to increase stocking densities to > 500-1,000 shrimp m⁻² in secondary nursery tanks, ponds or cages, it is therefore vital to provide supplementary aeration and/or water exchange. During the tank trials conducted in this study, increasing the water

exchange rate (to 40 % d⁻¹ for weeks 4 and 5) and providing aeration (at 0.04-0.07 l sec⁻¹ m⁻²) via airstones supplied by an air blower, facilitated the maintenance of suitable water quality at stocking densities of at least 2,000 shrimp m⁻². Water quality conditions, particularly of DO and transparency were generally maintained at more constant levels, both daily and over the 35 day trial periods, with the use of supplementary aeration. However, air blower failure (during trials 3 and 5), due to a combination of faulty wiring and severe storms, resulted in critically low DO (Figures 3.16 & 3.23) and occasionally heavy shrimp mortality (57-86 %, Figures 3.17 & 3.28). DO stress was observed in the morning following the air blower failures and was characterised by the shrimp hanging motionless at the water surface and mortality within 6 h of the failure. These failures were evidence of the increased risk associated with such high-intensity nursery systems and the increased requirement for skilled management. The choice of nursery system employed (if any) will then depend upon decisions being made in each case taking account of the economic benefits and risks associated with each system.

The maintenance of water temperature, salinity and pH within the optimal range for shrimp production is also facilitated by nursing in tanks. If these parameters vary in the water source used for nursing the shrimp, they may be difficult to control in ponds, but can be manipulated relatively easily in tanks. If water is drawn from estuarine areas of the sea (a common occurrence for commercial shrimp farms), water of suitable quality with regard to these parameters may only be available at a particular stage of the tide. In tank nurseries, water exchange can be accomplished rapidly or even suspended, particularly where supplementary aeration can be accessed. Dilution of full strength seawater to salinities closer to the optimum for post-larval growth (15-30 ‰, Chien, 1992) is also easier in low-volume tanks than in pond or cage nurseries. Similarly, manipulation of pH to achieve the desired optimum of 7.5-8.5 (Chien, 1992) through the addition of lime, can be accomplished quickly and easily in tanks. In sub-tropical/temperate conditions, such as in north America, Taiwan or Israel, the enclosure of tank nurseries in greenhouses or the use of heated water has also permitted temperature manipulation in order to extend an otherwise limited growing season (Tseng, 1988; Samocha & Lawrence, 1992; Stern & Letellier, 1992; Sturmer *et al.*, 1992).

During the present study, water pumped from the brackish Songkhla Lake was found to be of nearly optimal quality in terms of temperature, salinity and pH for all the trials conducted. Water temperature was an average of 1 °C higher in the cage nursery trial than the tank trial conducted concurrently (Tables 3.3 & 3.5). This was probably due to the wind-chill effect on the water temperature in the tanks and may have been partially responsible for the slightly higher growth rate of the shrimp in the cages than the tanks at a common stocking density (Figure 3.7). However, this may also have been due to the increased availability of natural food in the cages. Salinity was usually within the optimal range, but did rise to $> 30 \text{ ‰}$ during some of the trials. These salinities however, were not found to result in stress as they are within the range of the euryhaline *P. monodon* (Chien, 1992; Lester & Pante, 1992). pH was generally within optimal ranges, but tended to decline during the trials due to the increased respiration (CO_2 production) of the greater biomass of shrimp towards the end of each trial.

Dissolved nutrient/metabolite (TAN, $\text{NO}_2\text{-N}$ and DRP) concentrations in the nursery tank trials were maintained below toxic levels throughout each of the trials. This was achieved with the use of water exchange rates of 20-40 % d^{-1} , in combination with aeration at stocking densities $> 1,000$ shrimp m^{-2} . Sand filtration of the inlet water was partially effective at reducing the concentration of suspended solids, but had no significant effects on nutrient levels in the tanks. This may have been because these nutrients were derived primarily from the metabolisation of the applied feed and were produced in the tanks in relation to the stocking density employed, rather than the concentration in the inlet water. High levels of these metabolites were only measured towards the end of tank trial 5 in the treatments fed at the high (*ad libitum*) feeding rate (Table 3.12). This was due to the high rate of feed wastage and hence leaching of nutrients in these treatments as evidenced by the poor FCE (0.2-0.3) obtained (Figure 3.27). The concentrations recorded however, were still less than those considered to be toxic (TAN 0.6 mg l^{-1} , $\text{NO}_2\text{-N}$ 1 mg l^{-1} , Chien, 1992). This result emphasises the importance of monitoring food consumption so that optimal growth is maintained without sacrificing water quality. Under the conditions of these trials, feeding pelleted diets *ad libitum* (at 30-60 % of wet shrimp body weight d^{-1}) resulted in high shrimp production and good water quality for up to 25 days, but low DO thereafter (Figure 3.23). Increasing the nursing period is thus not feasible without increasing either

aeration and/or water exchange rates. No significant differences were observed between the nutrient concentrations of tanks fed with diets varying in the level of protein, lipid and energy (Table 3.12). This was most likely a result of the relatively small quantities of food fed to the tanks ($< 0.05 \text{ kg m}^{-2} \text{ d}^{-1}$, Table 3.13), minimising the effects of increased leaching of nitrogen from the high protein diets or excretion of nitrogen by shrimp fed these diets.

During the cage nursery trial, low levels of dissolved nutrients were measured throughout due to the low stocking density employed, both in the cages themselves and in the pond as a whole. In other situations, dissolved nutrient concentrations may reach critical levels for a number of reasons. For example, if stocking densities (and hence feeding rates) are increased further, if poor quality inlet water is used (*i.e.* originating from canals used as both inlets and outlets to shrimp farms), if water exchange rates are low, if the nursing period is extended, or if fresh feeds rather than pelleted diets are used. These factors again illustrate the importance of proper site selection and management of intensive nursery culture systems.

Neither the presence of mesh habitats nor sand substrates was found to have any discernable effects on water quality in the nursery tanks. However, the sand substrates were observed to become covered in fine sediment derived from both uneaten and waste food and the inlet water supply. The sand substrate then provided an anoxic, inhospitable environment for the benthic post-larvae and may have been partially responsible for the decreased survival recorded for these treatments (Figure 3.21). This sediment tended to be either concentrated or resuspended and expelled from the tanks through the action of aeration and water exchange in tanks lacking substrates, thus providing a more hospitable environment. The concentration of suspended solids in the water supply to the tank nursery, even following sand filtration, was relatively high due to abstraction of water from Songkhla Lake, a shallow (mean depth 1.5 m) and silt-laden lagoon. This problem of sediment accumulation and the requirement for cleaning of the substrates between nursery cycles may limit any potential useful role of substrates to low-intensity systems where influent water carries very low levels of suspended solids. Sand substrates may be worthy of further investigation under these conditions as they have previously been suggested to result in increased productivity of tank

nurseries (Fegan, pers. comm.; Issar *et al.*, 1987; Sandifer *et al.*, 1987).

3.4.1.2 Shrimp performance

Perhaps the most important parameter in intensive nursery rearing of post-larval shrimp is that of the stocking density employed. *P. monodon* production is clearly directly related to stocking density (Figure 3.35), production increasing curvilinearly with stocking density. This increase in production however, is at the expense of individual growth rate, FCE and survival. A similar increase in production at higher stocking densities has been shown previously by other authors for *P. monodon* and other species nursing in ponds (Apud *et al.*, 1979; Tabbu, 1985; Sturmer & Lawrence, 1986), cages (Walford & Lam, 1987; Rodriguez *et al.*, 1993) and especially tanks at very high (3-8,000 shrimp m⁻²) densities (AQUACOP, 1985b; Sturmer & Lawrence, 1986, 1987b; 1988a; Samocha *et al.*, 1990; Sturmer *et al.*, 1992).

High density (2,000 shrimp m⁻²) secondary nursing in tanks during this study has facilitated shrimp production of up to 12 g m⁻² d⁻¹ (mean 5.5 g m⁻² d⁻¹). This maximum rate of production has exceeded previous results for this species of 1-10 g m⁻² d⁻¹ in tanks (Forster & Beard, 1974; AQUACOP, 1984; Juario & Benitez, 1988), 0.6-8.2 g m⁻² d⁻¹ in cages (Siddharaju *et al.*, 1982; Reyntjens, 1989; Rodriguez *et al.*, 1993), and 0.1-1.7 g m⁻² d⁻¹ ponds (Apud *et al.*, 1979; Apud & Camacho, 1980; Ravichandran *et al.*, 1982; Tabbu, 1985; Hamid, 1986). Recent research with *P. vannamei* in America has also confirmed the ability of high density tank nurseries to increase production rates. Stocking at densities of up to 6,000 shrimp m⁻², production rates of 14-65 g m⁻² d⁻¹ have been achieved over 28-45 days (Sturmer & Lawrence, 1987b; 1988a; Samocha *et al.*, 1990). The benefits of the higher productivity of such intensive tank nurseries must then be balanced against the drawbacks (including the lower value of the smaller shrimp produced and the increased costs and risks) associated with such systems compared with pond or cage nurseries. Economic analyses based on the productivity of these systems will then be able to identify which, if any, nursery system will prove optimal in each case (see section 3.4.2).

The production of shrimp in the net cage nursery trial was similar to that achieved at

a common stocking density in the tank trials ($5 \text{ g m}^{-2} \text{ d}^{-1}$, Figure 3.10). Growth and FCE tended to be higher, possibly due to increased natural food availability and higher temperatures and survival lower, due to the escape of small post-larvae on introduction to the cages. Similar productivity, up to a maximum of $7\text{-}8 \text{ g m}^{-2} \text{ d}^{-1}$, has previously been recorded nursing *P. monodon* in cages at densities of 400-600 shrimp m^{-2} (Siddharaju *et al.*, 1982; Reyntjens, 1989; Rodriguez *et al.*, 1993). Further increases in productivity using either pond or cage nurseries will probably be dependant upon the provision of supplementary aeration or water exchange in order to maintain water quality. This would then increase the costs and risks associated with these nurseries, reducing the advantages that they offer over tank-based nursery systems.

Concomitant to increasing production, shrimp growth rate in the nursery tanks has been shown to decrease curvilinearly in relation to increasing stocking density. In terms of mean shrimp length, this relationship can be expressed by the equation: $\text{Log}_e \text{ length (mm)} = 77.17 - 12.27 \text{ Log}_e \text{ density (shrimp m}^{-2}\text{)}$, $n = 5$, $r^2 = 0.919$, $P < 0.05$ (Figure 3.31). The reason for this inverse relationship between growth and stocking density is unclear, but may be due to a number of factors.

An increased metabolic rate, possibly related to stress, under high density has been suggested to account for reduced growth at high density in various finfish species (Yamagishi, 1962; Li & Brocksen, 1977; Haylor, 1992). This possibility was not investigated directly in the present study, but may have contributed to this phenomenon.

A decrease in water quality, particularly low DO and high levels of ammonia and nitrite, has also been associated with poor growth rates (Boyd & Musig, 1992). TAN concentrations of $> 0.6 \text{ mg l}^{-1}$ or nitrite concentrations of $> 1 \text{ mg l}^{-1}$ (particularly acting synergistically), have been suggested to produced stress and decrease shrimp growth rates (Wickins, 1976; Chen & Chin, 1988; Chen & Lin, 1992). DO concentrations $< 6 \text{ mg l}^{-1}$ have likewise been shown to depress growth rates of *P. chinensis* and feeding ceased at $< 1 \text{ mg l}^{-1}$ (Yang, 1990). Similarly, FCRs have been shown to increase if DO concentrations $< 2\text{-}3 \text{ mg l}^{-1}$ occur at night (Boyd, 1989). Such water quality stressors can thus lead to reduced shrimp growth and feeding efficiency, as has been shown for intensive shrimp on-growing ponds in Thailand (Funge-Smith &

Briggs, in press). In the present study, water quality with regard to the concentration of DO and dissolved nutrients tended to decrease with increasing density and may have contributed to decreased shrimp growth. The maintenance of adequate water quality is thus of importance for optimising production from intensive nursery systems. This has previously been recognised by Samocha & Lawrence (1992) and Sturmer *et al.* (1992), who, in reviewing intensive tank nursing of penaeids, emphasise the importance of water exchange, aeration and waste disposal under conditions of high stocking density. Research by their team has involved the development of nursery systems for *P. vannamei* stocked at densities of up to 6,000 shrimp m⁻². Results indicated that increasing water exchange rates up to 80 % d⁻¹, combined with continuous aeration via airlift pumps supplemented by compressed oxygen, were necessary in order to maintain water quality beyond 28 days at these densities (Samocha *et al.*, 1990, Samocha & Lawrence, 1992, Sturmer *et al.*, 1992).

A third possibility accounting for decreasing growth with increasing stocking density is an elevated number of aggressive interactions between shrimp in competition for food. In this case, the larger shrimp may out-compete smaller individuals for the available food, leading to reduced mean growth, feeding efficiency and possibly increased size variation in the shrimp produced. FCE was indeed seen to decline curvilinearly with increasing density (Figure 3.33). However, this may also have been partially due to a reduction in the amount of natural food, comprising plankton, macroalgae and benthic organisms available to the shrimp at high stocking density. Additionally, the size range of shrimp produced, as measured by mean coefficient of variation in shrimp length was seen to increase curvilinearly with density by the equation: $\text{Log}_e \text{ coefficient of variation} = 7.90 + 5.61 \text{ Log}_e \text{ density (shrimp m}^{-2}\text{)}$, $n = 5$, $r^2 = 0.955$, $P < 0.05$ (Figure 3.32). Nevertheless, the size range of shrimp produced has been shown during the present study and elsewhere (Sturmer & Lawrence, 1987a,b; Sturmer *et al.*, 1992) to be less from high density tank nurseries than from cage or pond nurseries. This is an important result in terms of the value of the juvenile shrimp produced following nursing since shrimp seed with a large size range are valued less highly by pond on-growers. This is because the shrimp are considered to express this variation throughout on-growing, reducing their value on harvesting (Clifford, 1992; Lee & Wickins, 1992; Stern & Letellier, 1992). The validity of this latter point

will be discussed as part of the on-growing study in section 3.4.3.2.

The survival rate of shrimp during nursing is an important factor in their economic efficiency. This is due to the high cost of post-larvae used for stocking the nurseries. If mortality is high, the economics of nursing can become compromised (Clifford, 1992). Survival rates achieved during pond, cage and tank secondary nursing typically vary from 45-90 %. Mortality in ponds and cages may often occur due to predation, lack of control over water quality and nutrition and sometimes losses due to the difficulty of harvesting the juveniles from ponds (Sturmer *et al.*, 1992). Mortality in tanks stocked at high density may also be high due to cannibalism or poor water quality resulting from inefficient management or equipment failure.

During the present study, survival was inversely related to stocking density (Figure 3.34). Survival rates were generally very high (> 90 %) at densities of < 250 shrimp m⁻², but these densities are unlikely to be economically attractive due to the high costs associated with tank nurseries (Sturmer *et al.*, 1992). As densities were increased to > 2,000 shrimp m⁻², survival decreased to < 40 % for a number of possible reasons. These included aeration failure, cannibalism and poor initial post-larval quality (as will be discussed in chapter 4). Recent research with intensive tank nursing of *P. vannamei* has also reported variable survival of 58-99 % at densities of 500-6,000 shrimp m⁻² (Sturmer & Lawrence, 1986, 1987b, 1988a; Samocha *et al.*, 1990). However, Sturmer *et al.* (1992) quote unpublished results suggesting that survival rates of > 92 % are possible at stocking densities of 7,800 *P. vannamei* m⁻² under optimal management. Thus, with advances in the technology for high density rearing of post-larval shrimp in tank nurseries, enhanced consistency and predictability of production may encourage the adoption of these systems.

As discussed in section 3.4.1.1, supplementary aeration and/or increased water exchange is necessary for sustained shrimp production in high density secondary nurseries. These techniques for water quality maintenance become increasingly necessary as stocking densities exceed 1,000 shrimp m⁻² and as the duration of secondary nursing extends beyond 25-30 days. Growth reduction in nursery ponds stocked at a maximum of only 500 shrimp m⁻² has been recorded after 35 days (Sturmer

& Lawrence, 1987a). In the present study, growth rates in tanks stocked at 2,000 shrimp m^{-2} declined after 25-30 days, but only in trials experiencing aeration failures. This suggests that under ideal management conditions, growth and production can be maintained beyond one month in tank nurseries. This contention is supported by results with tank nurseries stocked at up to 4,400 *P. vannamei* m^{-2} , where no growth reduction was evident during 42 days trials where high levels of aeration/oxygenation were maintained (Sturmer & Lawrence, 1987b).

The use of habitats in intensive nursery systems, although not yet widespread, increases the wetted surface area (WSA) of the tanks, providing more space and less competition for the shrimp. This may be particularly important during moulting where less encounters between the shrimp may help to reduce cannibalism during this vulnerable period (Sriraman & Sathiyamoorthy, 1988; Sandifer *et al.*, 1987). Indeed, Sandifer *et al.* (1987) found that increasing the WSA of nursery tanks stocked at 500 *P. vannamei* m^{-2} by 100 % increased survival from 58 to 82 %, but had no effects upon growth. In contrast, Parado-Esteva (1988) was unable to demonstrate the benefits of habitats in densely-stocked (5-30,000 shrimp m^{-2}) nursery tanks with *P. monodon*. Similarly, results from trial 2 in this series revealed no significant differences in growth, survival (52-59 %) or production when the WSA of tanks stocked at 500 *P. monodon* m^{-2} was increased by up to 160 %. It is possible that at these relatively low densities, habitat inclusion has no effect, but that when densities are increased, survival promotion may result. Samocha *et al.* (1990, 1992) however, found that increasing the WSA of tanks stocked at 3,100-3,300 *P. vannamei* m^{-2} by 160-265 % had no significant effects on shrimp growth, survival, FCE or production after 35 days. In contrast, results from trial 4 in this series (at 2,000 shrimp m^{-2}) revealed that survival rates were significantly increased (from 37-41 % to 46-55 %, Figure 3.21) in the presence of habitats increasing the WSA by 120 %. In addition, although growth was unaffected, production increased by 30-50 % as a result of the increased survival (Figure 3.22).

The reasons for these discrepancies are unclear, but may be related to the more cannibalistic nature of *P. monodon* (Tseng, 1988), enhancing the benefit of habitats during moulting. However, contradictory reports have been made on the effects of habitat inclusion during nursing of the cannibalistic freshwater prawn *Macrobrachium*

rosenbergii. Habitats have been shown to have minimal effects on growth and survival (Smith & Sandifer, 1979; Mulla & Rouse, 1985), no effects on survival, but slightly enhanced growth rates (Kneale & Wang, 1979), and better growth and survival (Sandifer & Smith, 1977) of prawns stocked at 1-2,000 post-larvae m². Further research in this area is therefore warranted.

A second hypothesis regarding the benefits of habitat inclusion involves an increase in FCE due to enhanced natural feeding as a result of the "fouling" communities associated with the habitats (Sturmer & Lawrence, 1988a). This theory was subsequently disputed in the trials mentioned previously (Sturmer *et al.*, 1990, 1992), where no increase in FCE was found in the presence of habitats. These results were confirmed in the present study where no differences in FCE could be detected at stocking densities of 500-2,000 shrimp m² for shrimp nursed in tanks with or without habitats. The contribution of habitats to enhanced natural feeding thus seems unimportant in high density nursery culture where high quality formulated feeds are provided.

The use of sand substrates, in addition to having no effects on water quality (section 3.4.1.1), were unable to enhance shrimp performance. In contrast, survival and production rates tended to decline as the substrates became fouled with fine sediments. Their use under the conditions of this study thus appears undesirable and uneconomic.

Little is currently understood regarding the effects of dietary formulation on the production of shrimp during secondary nursing. Various "natural" diets for *P. monodon* and other species during tank nursing have been tried including fresh/frozen feeds such as bivalve, fish or shrimp meal and live or frozen *Artemia* (Issar *et al.*, 1987; Parado-Estepa, 1988) and phytoplankton/bacterial cultures with both nutritive and purifying qualities (AQUACOP, 1985b). As stocking densities increase however, in order to supply the complete nutritional requirements of the shrimp and assist maintenance of water quality, formulated, pelleted diets become increasingly important (Apud *et al.*, 1979; Sturmer *et al.*, 1992). The nutritional requirements of post-larval shrimp during secondary nursing under field conditions are unknown and have not been investigated directly (Sturmer *et al.*, 1992).

Where the levels have been quoted, formulated diets for intensive nursing of shrimp have commonly contained 30-50 % protein, up to 8 % lipid and complete vitamin and mineral supplements (Samocho & Lawrence, 1992; Stern & Letellier, 1992; Sturmer *et al.*, 1992). During the present study, a commercially available post-larval diet was used as the reference formulation. The proximate analysis of this formulation altered slightly during the trials, but contained approximately 50 % protein, 4-7 % lipid, 25 % carbohydrate and 18-19 kJ g⁻¹ total energy (Table 3.2). Prior to a more thorough study of the nutritional requirements of post-larval shrimp (included in Chapters 5-8), these nursery trials examined the effects of changing the dietary formulation on shrimp performance. These trials were conducted in order to assess whether such manipulations of dietary profile would be expressed during the short duration (35 days) of the nursery trials. The proximate composition of the test formulations used are shown in Table 3.2 and involved modifications in dietary protein, lipid, carbohydrate and energy levels. Results were thus not attributable to a particular nutritional parameter, but to overall changes in dietary formulation.

Results revealed that significant differences in growth, survival, FCE and production could be detected after 35 days for shrimp fed different dietary formulations. Due to the many differences between the formulations used however, it was not possible to attribute these effects to particular nutritional parameters. The poor performance of the test diet during tank trial 5 may have been due to decreased consumption of this high energy diet. Although food consumption could not be monitored precisely, shrimp fed to satiation in the *ad libitum* treatments of trial 5 were seen to consume slightly less of the high energy test diet than the reference formulation. Although food consumption and FCE was lower for shrimp fed the high energy diet, these differences were not significant at the 5 % level (Table 3.13). Changing the dietary formulation has thus been shown to result in significant differences in shrimp performance. The effects of particular nutritional parameters on shrimp production will be investigated in more detail in chapters 5-8.

In addition to dietary formulation, the feeding regime (rate and frequency) utilised during secondary nursing is considered to affect shrimp performance and water quality. Investigations into the effects of feeding frequency on shrimp performance have yielded

conflicting results. It is generally considered however, that feeding 2-5 times daily can result in improved growth, FCE and production over that obtained feeding only once (Subramanian & Krishnamurthy, 1986; Issar *et al.*, 1987; Sampath & Srithar, 1987; Sturmer *et al.*, 1992). Frequent feeding is probably desirable since the shrimp are adapted to continuous feed availability during the hatchery phase (Stern & Letellier, 1992) and in order to reduce cannibalism during moulting. However, frequent feeding may increase the labour cost of nursing unless automated techniques are employed (Sturmer *et al.*, 1992). During this investigation, a feeding frequency of four times daily was used for all trials.

The effects of feeding rate on shrimp performance and water quality have received no direct attention during secondary nursing (Sturmer *et al.*, 1992). During this phase of the culture cycle, no clear guidelines exist, but rates of between 10 and 100 % wet body weight d^{-1} (usually decreasing with shrimp age) have been used with pelleted diets (Mock *et al.*, 1973; Apud *et al.*, 1979; AQUACOP, 1985b; Sandifer *et al.*, 1987; Sturmer & Lawrence, 1988a; Sturmer *et al.*, 1992).

Results from the present study revealed that good FCE (0.7-1.37), growth and survival of shrimp was obtained feeding pelleted diets at rates decreasing from 35 to 15 % body weight d^{-1} over the 35 day trial period. Decreasing the feeding rate to between 30 and 10 % body weight d^{-1} , resulted in good FCE (at approximately 0.8), growth (at a SGR of 15 % body weight d^{-1}) and production (at 8-9 $g\ m^{-2}\ d^{-1}$) (Trial 5, Table 3.13). The size range of shrimp produced at this feeding rate was also low (at a CV of 19-23) suggesting that this feeding rate is sufficient to maintain shrimp production at stocking densities of 2,000 shrimp m^{-2} . During the same trial, feeding shrimp to satiation (*ad libitum*), monitored by the use of feeding trays, revealed that shrimp would consume pelleted diets at between 30 and 60 % body weight d^{-1} . Feeding at these rates significantly improved shrimp growth rates, but significantly reduced FCE (from 0.8 to 0.3) and resulted in shrimp with a greater variation in final size. Water quality, particularly in terms of low DO also resulted from feeding at such high rates. Clearly, increasing the feeding rate leads to greater feed wastage, water quality problems and increased expenditure on feeds and the requirement for additional aeration/water exchange. Since the overall production of shrimp fed to satiation was not significantly

higher than those fed at low feeding rates, it appears that there is no benefit to increasing feeding rates to beyond 10-30 % body weight d^{-1} under the conditions of this study.

Particularly when shrimp nurseries are located at some distance from the on-growing ponds, stress on harvesting and transfer of the juveniles produced may limit the feasibility of incorporating a secondary nursery phase into the shrimp culture cycle (Licop, 1984; Olin & Fast, 1992; Stern & Letellier, 1992; Chanratchakool *et al.*, 1994). The harvesting and transfer techniques adopted in this investigation however, were found to be effective and successful. On transfer of the juveniles to cage on-growing, any stress accruing from these techniques did not appear to compromise subsequent growth or survival (see section 3.4.3.2). Similarly, Sturmer *et al.* (1992) managed to transfer 0.5-1 g juveniles from intensive tank nurseries to on-growing ponds without excessive mortality. Where long distances are involved however, research is still required into the best techniques for transportation of the shrimp juveniles. This is because traditional transport in plastic bags may prove ineffective due to the greater biomass of shrimp and their well developed rostral spines (capable of puncturing the bags), necessitating lower densities and hence increased expense during transportation.

Accurate estimation of shrimp numbers prior to on-growing is also important in order to optimise feeding management during on-growing. Where nursery systems (whether ponds, tanks or cages) are emptied directly into on-growing ponds, counting the shrimp can prove to be difficult (Wyban *et al.*, 1989; Lee & Wickins, 1992; Chanratchakool *et al.*, 1994). In addition, harvesting and counting all of the juveniles from tank or cage nurseries is considerably easier than from separate nursery ponds, not connected to the on-growing ponds. Scaled-up versions of the commercial counting procedures currently used following primary nursing (*i.e.* transfer of shrimp by volumes containing a known number of shrimp), as used in this tank study, seem to be feasible.

3.4.2 Economic analysis

It is generally accepted that the stocking of larger, older shrimp into the on-growing ponds will result in increased shrimp survival and hence production, probably due to

increased stress-tolerance (AQUACOP, 1985a; Lee & Wickins, 1992; Olin & Fast, 1992) and reduced predation (Malca, 1983; Clifford, 1985, 1992; Lee & Wickins, 1992). Stocking older shrimp also reduces the length of the grow-out period and facilitates the estimation of shrimp survival and feeding rates (AQUACOP, 1985b; Cordover, 1989; Samocha & Lawrence, 1992; Sturmer *et al.*, 1992). For these reasons, larger, older juvenile shrimp are more highly valued than ex-hatchery post-larvae (Bauman & Jamandre, 1990; Cao & Jiang, 1990; Fegan, 1992).

Currently in Southeast Asia, the majority of shrimp on-growers stock post-larval shrimp at PL₁₅ into their ponds. The current price for ex-hatchery PL₁₅ in Thailand is approximately £ 3.50 per thousand (Fegan, 1992, Fegan, pers. comm.). In Southern/Central America, the price tends to be lower, at £ 2.5-3.3 per thousand, with higher prices being paid for wild-caught seed. If older post-larvae are sold, the price in Thailand tends to increase by approximately £ 0.27 per thousand per day. Thus, depending upon the size, PL₅₀ would cost approximately £ 13 per thousand (Fegan, pers. comm.). In practice, few on-growers currently buy seed as old as PL₅₀ and this value is therefore only an estimation of their worth. Nevertheless, nursed juveniles have been reported to cost 2-3 times the price of post-larvae in India (Nielsen, 1991), PL₄₀ twice that of PL₂₀ in the Philippines (Primavera, 1983), PL₂₇₋₂₉ 80 % more than PL₁₃₋₁₅ in Taiwan (Mock, 1983) and juveniles 60 % more than post-larvae in South/Central America (Stern & Letellier, 1992). Thus, secondary-nursed PL₅₀ *P. monodon* may be expected to be worth approximately £ 7-10.5 per thousand without any further evidence regarding their enhanced on-growing potential. If it can be demonstrated that on-growing nursed juveniles results in increasing profits, *i.e.* through increased growth, survival and FCEs, then the value of nursed juveniles may increase further. No such work has yet been attempted.

The economic justification of the secondary nursing of shrimp thus depends upon whether the juveniles can be produced for less than their selling price. The cost of producing PL₅₀ from the concrete tank nursery system used in the present study varied depending on stocking density from £ 6.14-10.83 per thousand. The minimum selling price of £ 8-12 per thousand PL₅₀ necessary in order to break even thus seemed feasible. The size of the shrimp produced would naturally affect the selling price. Thus,

the lower stocking density systems producing larger juveniles seem more financially viable. However, at a selling price of £ 8 per thousand PL₅₀ at 0.35 g, a stocking density of 1,000 shrimp m⁻² was the most economically feasible under the conditions of this study.

The selling price of the juveniles produced was the most important variable affecting the economics of nursing. Survival however, was equally critical to profitability since the greatest operational expense is the initial cost of the post-larvae. At stocking densities of 2,000 shrimp m⁻², the low survival rate obtained (mean 40 %) was not sufficient to maintain profitability unless the selling price increased to £ 12 per thousand. Survival rates of > 60 % would be required for profitability if the selling price decreased to £ 8 per thousand. With advances in nursery rearing techniques, the cost of nursing juveniles may decline due to increased growth and survival rates and thus enhance the profitability of tank nursery systems.

Previous research has suggested that in America, production of 1 g juvenile *P. vannamei* in intensive (4,000 shrimp m⁻²) tank nurseries cost £ 5-6.8 per thousand (Juan *et al.*, 1988). Despite these relatively low production costs (due to very high stocking densities and survival in their system), these authors concluded that the on-growing of two crops per year using these juveniles was less profitable than the production of one crop using post-larval shrimp. However, their nursery system was more expensive than the system used during this study since it required the construction of greenhouses in order to nurse shrimp at higher temperatures during the North American spring. In addition, they point out that if shrimp growth rates during pond on-growing were 0.2 g shrimp⁻¹ week⁻¹ higher for juveniles than post-larvae, then the production of two crops using nursed juveniles would be more profitable than the production of one crop by direct stocking. That increased growth of shrimp nursed at high density is possible is discussed in section 3.4.3.2.

To commercial shrimp farmers, the cost of shrimp seed is not considered to be a major impediment to increasing shrimp production (Hopkins & Villalon, 1992). Instead, their major concerns are with seed quality and availability (AQUACOP, 1985a; Clifford, 1985; Hopkins & Villalon, 1992). If secondary nursing can be shown to improve the

quality of the seed (over that obtainable by direct stocking of PL₁₅s) for on-growing and the predictability of their availability, the value of nursed shrimp may increase over the long term. This would then increase the likelihood of cost-effective secondary nursing. The effects of secondary nursing on shrimp quality are explored further in chapter 4. It is already clear that in order to achieve maximum yields of higher value shrimp, nursery systems must be employed in conjunction with intensive grow-out (Sturmer *et al.*, 1992).

The relative financial advantages of cage or pond nurseries over tank nurseries were not explored during this work. Previous research however, has shown that both pond (Tabbu, 1985) and cage (Agbayani *et al.*, 1985; de la Pena *et al.*, 1985; Walford & Lam, 1987; Nielsen, 1991) nurseries can be run profitably under some conditions. Production costs in 1985 as low as £ 5 per thousand PL₃₅ have been recorded for cage nurseries (Walford & Lam, 1985). A more in-depth examination of the financial and managerial advantages and disadvantages of the three nursery system types is necessary before definitive conclusions can be drawn regarding the most suitable and cost-effective nursery system.

3.4.3 Net cage on-growing trial

3.4.3.1 Water quality

The water quality condition monitored in one representative cage during this trial were stable and within or close to the optimal range for all parameters throughout the ten week trial period. At no time was water quality therefore considered to be detrimental to shrimp health in any of the treatments of this trial. However, it was necessary to scrub clean the mesh sides of the cages weekly to remove fouling organisms and settled sediment in order to optimize transmission of water through the cages.

3.4.3.2 Shrimp performance

One of the commonly perceived problems with intensive secondary nursing of post-larval shrimp is that the stunting and increased size range of shrimp resulting from

being held at high density may be carried on into the on-growing phase of the culture cycle (Clifford, 1992; Lee & Wickins, 1992; Stern & Letellier, 1992; Chanratchakool *et al.*, 1994). This trial stocked juvenile shrimp nursed at a range of densities from 125 to 1,000 shrimp m⁻². The shrimp were stocked at a common density (1,000 juveniles m⁻²), but were smaller, with a larger size range in the treatments reared at higher densities.

Results from the trial revealed that the smaller shrimp nursed at higher density rapidly compensated for the stunting during nursing by growing more rapidly than those nursed at lower densities. This was probably a function of the higher growth rate of smaller shrimp, but shows that the stunting effect of holding shrimp at high density was not permanent. Juvenile shrimp were able to reach a common size, regardless of nursing density (between 125 and 1,000 shrimp m⁻²), when on-grown at a common density within 4-6 weeks. Results were similar for shrimp nursed in both tanks and cages at a common density. On-growing trials with *P. vannamei* following very high density (7,800 shrimp m⁻²) tank nursery culture have been conducted by Robertson *et al.*, (1992). These workers achieved good survival (81 %) and growth (0.12 g d⁻¹) over 7 weeks of on-growing in tanks stocked at 261 shrimp m⁻². Furthermore, a growth rate of 0.28 g d⁻¹ was achieved during subsequent cage on-growing at 40 juveniles m⁻². It therefore appears that the stunting of shrimp during high density nursing is not permanent, but is rapidly compensated for by increased growth rate during on-growing. Shrimp may therefore be stockpiled in nursery tanks until sufficient numbers are available to stock on-growing ponds (Samocha & Lawrence, 1992), on-growers require seed, the ponds are prepared and/or the conditions are suitable for on-growing. Indeed, there are reports that shrimp may be stockpiled in nursery tanks at high density for up to six months without affecting their future performance (Duenas *et al.*, 1983; Hirono, 1989).

Furthermore, the coefficient of variation in shrimp length, seen to be directly related to nursing density showed no relationship to nursing density following the on-growing trial. Indeed, the size range of shrimp following on-growing was reduced over that found after the 35 day nursery trials. This again, was probably due to the compensatory growth of the smaller shrimp stunted during nursing on-growing under less intensive

conditions. High density nursing of post-larval shrimp therefore, does not result in increased variability in stock size and hence reduced profits.

After 6 weeks of the on-growing trial, shrimp growth rates declined rapidly for all treatments. This was probably due to over-stocking of the cages. Thus, overall growth, FCR and production rates were low, although survival was high for all treatments. This suggests that high density nursing and the harvesting and transfer of shrimp to the on-growing ponds does not compromise subsequent on-growing performance provided stocking densities during on-growing are not excessive. The commercial on-growing of *P. monodon* in "intensive" systems in southeast Asia currently uses stocking densities of 30-100 shrimp m⁻². It is now considered that stocking densities in excess of 30-40 shrimp m⁻² will result in unsustainable production over the long term due to sediment and water quality deterioration (Csavas, 1990; Phillips *et al.*, 1993; Briggs & Funge-Smith, in press).

3.5 SUMMARY

Secondary nursing of shrimp in tanks for up to one month at up to 1,000 shrimp m⁻² can produce juvenile *P. monodon* at high growth and survival rates in the absence of supplementary aeration if water is exchanged at 20-40 % tank volume d⁻¹. Increasing the stocking density to 2,000 shrimp m⁻² requires supplementary aeration in order to maintain the concentration of dissolved oxygen within optimal limits for shrimp production. The requirements for aeration at high density adds to the risk associated with such nursery systems.

Shrimp growth, survival, FCE and size range is negatively density dependant, but production increases with stocking density. Increases in the size range and stunting of juvenile shrimp nursed at high density is not permanent, but is rapidly compensated for by high growth rates during on-growing at low density.

The addition of mesh habitats, but not sand substrates increases shrimp survival and production, but not growth or FCE at high stocking densities. This is probably due to

a reduction in cannibalism during moulting.

Changes in the dietary formulation and feeding regime (rate and frequency) of pelleted diets can significantly affect the production of shrimp during secondary nursing. This will be investigated further in chapter 8. During secondary nursing, feeding four times daily at between 10 and 30 % of wet body weight d^{-1} results in high production.

The high density culture, harvesting and transportation of juvenile shrimp over short distances following tank nursing does not appear to compromise subsequent on-growing performance.

The quality of the post-larvae stocked into the nursery systems may be an important factor in their ability to produce juvenile shrimp profitably and consistently. This will be investigated further in chapter 4.

The production of shrimp from net cage secondary nurseries is comparable to that achieved at a common density in tank nurseries. Increasing the density in net cages to > 500 shrimp m^{-2} may be possible, but will probably require supplementary aeration/water exchange. The use of tank nurseries however, may allow greater control and efficiency of production over that possible with the use of cages or ponds.

Secondary nursing of post-larval *P. monodon* in tanks may be profitable at a stocking density of 1,000 shrimp m^{-2} if a selling price for the juveniles of $> £ 8$ per thousand can be obtained. If the stocking density is increased to 2,000 shrimp m^{-2} , the selling price must increase to $£ 12$ per thousand or the survival rate must increase from a mean of 40 % to > 60 % to maintain profitability.

The stunting of shrimp during high density nursing is not permanent, but is compensated for by increased growth rate during on-growing. In addition, high density nursing of post-larval shrimp does not result in increased variability in stock size.

High density secondary nursing of *P. monodon* post-larvae has considerable potential for inclusion into intensive commercial culture systems. More research is necessary

regarding the economic benefits of nursing in tanks compared with ponds or cages, and the direct stocking of primary-nursed post-larvae. Further research into the effects of high density secondary nursing on subsequent on-growing performance is also warranted.

CHAPTER 4. Stress tests for determining vigour of post-larval/juvenile *Penaeus monodon*.

The weak can be terrible because they try furiously to appear strong.

Rabindranth Tagore, 1928

The information contained in Chapter 4 on the stress test has been summarised and published in *Aquaculture and Fisheries Management* 1992, 23, 633-637. Edited by D.H. Mills, R.J. Roberts & S.J. de Groot, Published by Blackwells.

4.1 INTRODUCTION

From a worldwide survey of shrimp farmers (Hopkins & Villalon, 1992), the variable quality of the seed available for stocking on-growing ponds was considered to be by far the most important impediment to increasing the production and/or profitability of intensive shrimp culture. Variable seed quality may present problems with regard to the reliability of hatchery supplies and the unpredictable survival rates achieved during on-growing.

Current on-growing procedure is to stock ex-hatchery post-larvae which have been reared in 'primary' nurseries from PL₄₋₇ to PL₁₂₋₂₀. Such hatchery reared post-larvae are cultured in intensive, artificial conditions often involving chemical disease treatments to give elevated growth and survival rates. They are thus often weaker and less resistant to environmental stress than their wild counterparts. When available, wild seed are therefore usually preferred to hatchery seed by on-growers.

Commonly, apparently similar ponds using the same on-growing techniques are seen to promote widely differing performance. In Thailand, currently the worlds largest producer of *P. monodon*, average survival rates in grow-out ponds range from 30 to 80 % (pers. observation). One of the reasons for this variability may be the quality of the post-larvae stocked (Bauman and Jamandre, 1990).

A technique utilised commercially which is thought to enhance the stress resistance or vigour of shrimp is that of rearing shrimp from PL₁₂₋₂₀ to PL₃₀₋₅₀ in tank, pond or cage 'secondary' nurseries. This allows ponds to be stocked with larger and presumably more stress resistant juvenile shrimp (see chapter 3). However, the effects of secondary nursing on stress resistance and the subsequent growth and survival rates of the shrimp during on-growing remain largely unstudied.

Previous work has suggested that stress tests, measuring shrimp seed survival after a period of environmental stress, may provide a relative measure of shrimp vigour (see section 2.4.3 and Tables 2.10 & 2.11). Bauman and Jamandre (1990) recommend stress tests over subjective quality control methods such as visual or microscopic examination or hatchery performance, or the determination of muscle-to-gut ratios (Bauman and Scurra, 1990) which have yet to be fully evaluated (see section 2.4.3).

A quick, simple, repeatable stress test method, suitable for use under commercial conditions, is required to provide a relative measure of shrimp vigour and to provide an objective determination of the effects of hatchery and nursery culture on shrimp vigour. Once this method is established, on-growing trials using stress tested shrimp can then be performed to determine whether shrimp vigour can be related to subsequent on-growing performance and thus indicate seedstock quality.

The purpose of these trials with *P. monodon* was thus to establish a suitable method of judging post-larval vigour and to conduct an initial investigation of the effects of high density secondary nursery rearing on the stress resistance of post-larval shrimp.

4.2 MATERIALS AND METHODS

The vigour of post-larval shrimp was tested by subjecting replicate groups of shrimp held in aquaria to a combination of low or high salinity and temperature stressors over a period of one hour. Preliminary trials were intended to establish a suitable combination of temperature and salinity which would result in approximately 50 % mortality after one hour. This combination of stressors was then used in a comparative

test of shrimp vigour for post-larvae both before and after secondary nursery rearing at PL₁₅ and PL₅₂₋₅₃ respectively.

4.2.1 Experimental animals

Post-larval *P. monodon* were obtained from two commercial hatcheries in Thailand, one east of Bangkok and one near Songkhla in the south. Both hatcheries used wild ablated broodstock from the Andaman Sea for spawning. Both also used the common algae/*Artemia* feeding regime, supplemented with flake and/or micro-capsules for feeding mysis and post-larval stage shrimp. Antibiotics were not used prophylactically in either hatchery. The post-larvae used had mean weights ranging from 0.002 to 0.005 g (mean weight of 15 batches 0.004 ± 0.001 g).

Post-larvae purchased from the hatcheries were delivered to a shrimp research facility at the Tinsulanonda Songkhla Fisheries College, Songkhla, southern Thailand in oxygenated seawater in double plastic bags stocked at 3-4,000 post-larvae per five litre bag. The post-larvae were acclimated to control conditions of 30 °C and 30 ‰ salinity for a minimum of 12 h prior to the trials. Temperature was measured by standard mercury thermometer (± 0.5 °C), and salinity with an Atago refractometer (± 1 ‰).

To investigate the effects of secondary nursery rearing on post-larval vigour, samples of PL₁₅ treated and tested identically to those in the first part of the investigation were used. These post-larvae were then on-grown at high density in concrete nursery tanks (see chapter 3) for 35 days and re-tested. The resulting PL₅₂₋₅₃ weighed between 0.170 and 0.580 g and were tested directly from the nursery tanks. Acclimation conditions for these shrimp were between 29.5-30 °C and 30-35 ‰ salinity.

4.2.2 Experimental system

Two or three replicate groups of between 200 and 500 shrimp (as available) were used in each of the stress tests. Shrimp were starved for at least 12 hours prior to each trial. Each replicate group of shrimp was transferred directly from the holding tank at approximately 30 °C and 30 ‰ salinity into an aerated aquarium containing 10 l of

water. This water was held at either an elevated or reduced combination of temperature and salinity with respect to the holding conditions. Increased temperature was achieved by placing the replicate aquaria into an outdoor covered tank which heated up to 32.5 °C. Reduced temperatures were achieved by carrying out the trials in an air-conditioned room at 20 °C.

Changes in salinity were achieved by mixing suitable quantities of fresh (0 ‰) and sea (35 ‰) water, and crude sea salt was dissolved in sea water and added to tanks where elevated (40 to 50 ‰) salinities were required. The water quality conditions in all test aquaria were checked and the aquaria left aerated overnight prior to the trials.

During each trial, the shrimp were left undisturbed and unfed in the aquaria for one hour. Extension of the trial time would have reduced the convenience of such a stress test for commercial operations. Mortality was assessed by prodding each shrimp (which was not obviously swimming) with a plastic pipette. If the shrimp moved, or showed the typical tail-flick escape response on being poked, they were counted as alive. If no movement resulted, they were considered dead.

A total of 15 combinations of temperature (varying from 20 to 32.5 °C) and salinity (from 0 to 50 ‰) were tested in the first part of the investigation. This resulted in the establishment of three potentially usable combinations of temperature and salinity. The second part of the investigation was then performed using these three combinations to determine the vigour of primary nursed shrimp to be used in the 35 day secondary nursery tank trials. The methodology for testing PL₁₅ prior to secondary nursing was identical to that used in the first part of this investigation. However, replicate groups of shrimp after secondary nursing were subjected to just one temperature/salinity combination of 20 °C and 10 ‰ to serve as a comparison between the same batch of shrimp before (PL₁₅) and after (PL₅₂₋₅₃) secondary nursing.

4.2.3 Data analysis

The following analyses were carried out:

1. 95 % Confidence limits were used to determine whether differences in shrimp survival were related to salinity and temperature shocks. 95 % confidence limits were used for all tests and were calculated as:

$$\text{C.L.} = \bar{x} \pm t_{0.05[n-1]} \frac{S}{\sqrt{n}}$$

where:

- \bar{x} = mean survival (%)
- $t_{0.05}$ = t distribution at $P < 0.05$
- S = standard deviation of mean survival
- n = number of replicates

2. For stress tests conducted simultaneously, a one way analysis of variance with equal sample size was used to analyse the effects of the stress tests on shrimp survival after one hour. The percentage survival data were arcsin transformed before ANOVA. Equality of variance was confirmed using a Bartlett test and normality could be demonstrated graphically (Sokol & Rohlf, 1981). ANOVAs were not used for comparison of data derived for shrimp before and following the secondary nursery trials since the data were derived from separate tests.
3. The T-method for multiple comparisons (Sokol & Rohlf, 1981) was used to determine statistical differences between treatment means for the ANOVAs completed. Results were considered significant if $P < 0.05$.

4.3 RESULTS

4.3.1 Stress testing of primary nursed post-larvae (PL₁₅)

Results from the stress tests showed that when ten batches of PL₁₅ were subjected to direct temperature decreases of 10 °C, from 30 to 20 °C (Table 4.1, Figure 4.1), a wide range of salinity tolerance from 10 to 40 ‰ resulted. Salinity had to be reduced to 7.5 ‰, or increased to 43 ‰ to cause 50 % mortality in one hour. Decreasing salinity to < 5 ‰, or increasing it to > 50 ‰ reduced survival to < 10 % after one hour.

A suitable stress test was thus derived by exposing PL₁₅ to a temperature/salinity combination representing a 10 °C temperature decrease and either a simultaneous decrease of 20-25 ‰, or an increase of 10-15 ‰ salinity.

When six batches of PL₁₅ were stressed by immersion into water heated by 2 °C, from 30 to 32-32.5 °C (Table 4.2, Figure 4.2), a wide range of salinity tolerance, from 10 to 40 ‰ again resulted. In this case, salinity had to be reduced to 3-4 ‰, or increased to 45-46 ‰ to cause 50 % mortality in one hour. At 32 °C, 100 % mortality was recorded when salinity was reduced to 0 ‰ or increased to > 50 ‰.

From the results of the above trials with PL₁₅, a standard stress test was developed. This entailed direct transfer of post-larvae from 30 °C and 30 ‰ salinity into water maintained at 20 °C and 7.5, 10 and 45 ‰ salinity for one hour. From the ten batches used during these trials, the precise salinity required to achieve a mean survival rate of 50 % after one hour would be 7.5 and 43 ‰ (Figure 4.1).

With the subsequent testing of three more batches of PL₁₅ from the same southern hatchery (used in the secondary nursery tank trials, see chapter 3), survival rates were seen to vary significantly between batches of shrimp. The standard stress tests, decreasing temperature from 30 °C to 20 °C and salinity from 30 ‰ to 7.5, 10 and 45 ‰ salinity were performed on these shrimp. Results showed survival rates of 20, 23 & 20 %; 77, 78 & 95 %; and 84, 91 & 95 % for the three salinity treatments of nursery trials 3, 4 and 5 respectively (Table 4.3, Figure 4.3).

Table 4.1 Stress tests from 30 °C, 30 ‰ to 20 °C over a range of salinities.

Treatment	Number of replicates	Temperature (°C)	Salinity (‰)	Initial number of shrimp	Mean survival		
					(%)	SE ¹	CL ²
1	3	20.5	10	500	91.6 ^a	2.1	9.2
2	3	20.5	30	500	98.5 ^a	0.5	2.0
3	3	20.0	40	500	87.8 ^a	1.8	7.6
4	3	20.0	0	500	0.0 ^{a3}	0.0	0.0
5	3	20.0	5	500	2.7 ^a	0.3	1.1
6	3	20.0	20	500	98.8 ^b	0.4	1.7
7	3	20.0	50	500	7.9 ^a	1.7	7.3
8	3	20.0	7.5	200	47.7 ^b	5.3	23.0
9	3	20.0	10	200	84.7 ^c	4.2	18.0
10	3	20.0	45	200	21.7 ^a	5.8	25.1

Initial conditions:

Treatments 1-3; Temperature 30°C, salinity 28 ‰, Age PL₁₄₋₁₈, post-larval source northern hatchery, mean weight 0.005 g

Treatment 4-7; Temperature 28.5°C, salinity 34 ‰, Age PL₁₄₋₁₅, post-larval source southern hatchery, mean weight 0.005 g

Treatment 8-10; Temperature 30°C, Salinity 32.5 ‰, Age PL₁₅, post-larval source southern hatchery, mean weight 0.005 g

¹ Standard Error of the mean

² 95 % confidence limits

³ For single criterion, mean value in the same block of columns bearing different superscripts are significantly different (P < 0.05) by T-method.

Table 4.2 Stress tests from 30 °C, 30 ‰ to 32 °C over a range of salinities.

Treatment	Number of replicates	Temperature (°C)	Salinity (‰)	Initial number of shrimp	Mean survival		
					(%)	SE ¹	CL ²
1	3	32.0	0	200	0.0 ^a	0.0	0.0
2	3	32.0	5	200	67.5 ^b	1.5	6.5
3	2	32.5	10	500	98.3 ^c	0.3	3.8
4	2	32.5	30	500	99.6 ^c	0.2	2.5
5	2	32.5	40	500	99.2 ^c	0.2	2.5
6	3	32.0	50	200	0.0 ^a	0.0	0.0

Initial conditions:

Temperature 30°C, salinity 28 ‰, Age PL₁₄₋₁₈, post-larval source northern hatchery, mean weight 0.005 g

¹ Standard Error of the mean

² 95 % confidence limits

³ For single criterion, mean value in the same column bearing different superscripts are significantly different ($P < 0.05$) by T-method.

Table 4.3 Stress tests on PL₁₅ prior to nursery tank trials 3, 4 & 5 from 30 °C, 30 ‰ to 20 °C at 7.5, 10 and 45 ‰ salinity.

Treatment	Number of replicates	Temperature (°C)	Salinity (‰)	Initial number of shrimp	Mean survival		
					(%)	SE ¹	CL ²
1	3	20.0	7.5	200	20.3	7.4	31.8
2	3	20.0	10	200	23.0	5.8	24.8
3	3	20.0	45	200	19.5	3.3	14.1
4	3	20.0	7.5	200	77.0	3.5	15.1
5	3	20.0	10	200	78.0	2.6	11.0
6	3	20.0	45	200	94.8	1.2	5.2
7	3	20.0	7.5	200	84.2	2.2	9.3
8	3	20.0	10	200	91.2	0.6	2.6
9	3	20.0	45	200	95.0	1.6	6.9

Initial conditions:

Treatments 1-3; Temperature 31°C, salinity 28 ‰, Age PL₁₅, post-larval source southern hatchery, mean weight 0.001 g. Used in nursery tank trial 3.

Treatments 4-6; Temperature 29°C, salinity 34 ‰, Age PL₁₅, post-larval source southern hatchery, mean weight 0.002 g. Used in nursery tank trial 4.

Treatments 7-9; Temperature 28°C, salinity 35 ‰, Age PL₁₅, post-larval source southern hatchery, mean weight 0.003 g. Used in nursery tank trial 5.

¹ Standard Error of the mean

² 95% confidence limits

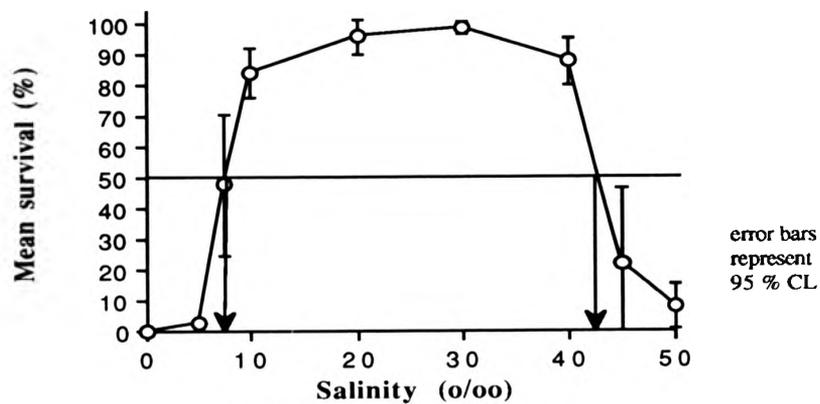


Figure 4.1 Mean survival of PL15 on direct transfer from water at 30 oC, 30 o/oo into 20 oC over a range of salinities after 1 h.

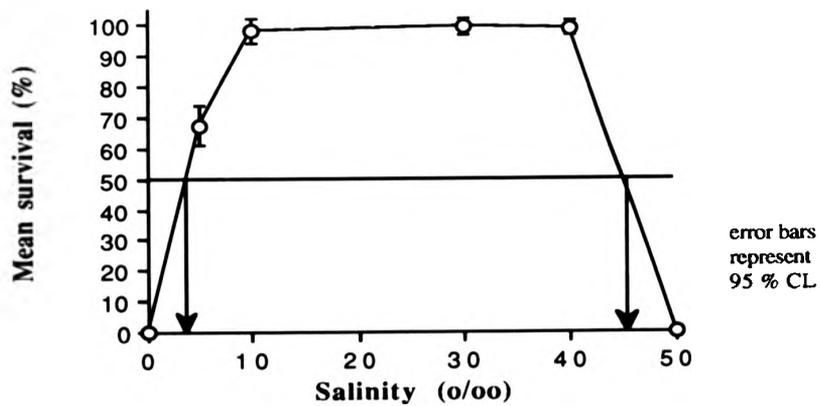


Figure 4.2 Mean survival of PL15 on direct transfer from water at 30 oC, 30 o/oo into 32 oC over a range of salinities after 1 h.

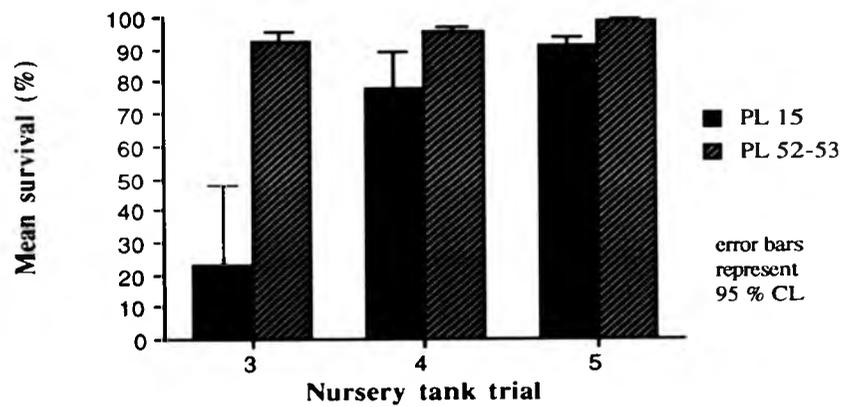


Figure 4.3 Mean survival of PL15 and PL52-53 on direct transfer from water at 30 oC, 30 o/oo salinity into 20 oC at 10 o/oo salinity after 1 h.

4.3.2 The effects of secondary tank nursery rearing on the vigour of post-larvae (PL₁₅₋₅₃).

4.3.2.1 Nursery tank trial 3

Results from the stress test carried out on PL₁₅ stocked in nursery tank trial 3 showed that poor survival of 20-23 % resulted from stress tests at 20 °C and 7.5, 10 and 45 ‰ salinity. These results were significantly ($P < 0.05$) lower than from the control test (Tables 4.1 & 4.3, Figures 4.1 & 4.3).

On conclusion of the 35 day nursery trial (investigating the effects of stocking density, see section 3.3.4) it was found that (taking into account an air blower failure), growth, survival and final production rates were very poor. On termination of the 35 day nursery trial, samples of shrimp from each treatment were subjected to a stress test at 20 °C and 10 ‰ salinity. Results from this test showed that elevated survivals of 85-96 % resulted (Table 4.4, Figure 4.3). No significant ($P < 0.05$) differences were found between the four treatments (stocking densities from 1,000-3,000 shrimp m²) on testing PL₅₂ from nursery trial 3. During this trial it was noticed that the smallest or currently moulting individuals were the ones most likely to die

4.3.2.2 Nursery tank trial 4

The PL₁₅ stocked in nursery tank trial 4 demonstrated high survivals of 77-95 % when stress tested at 20 °C and 7.5, 10 and 45 ‰ salinity. These results were significantly ($P < 0.05$) better than those from the control test and that of nursery trial 3 (Tables 4.1 & 4.3, Figures 4.1 & 4.3).

On conclusion of the 35 day nursery trial (investigating the effects of substrates and habitats, see section 3.3.5) it was found that growth, survival and final production rates were better than in nursery tank trial 3. During the first two weeks (prior to aeration failure in nursery trial 3), shrimp in nursery tank trial 4 increased in weight by 775 %, whilst the shrimp stocked at the same density in nursery tank trial 3 increased just 180 % under similar conditions, although these shrimp had a larger initial weight which

Table 4.4 Stress tests on PL₅₂₋₅₃ following nursery tank trials 3, 4 & 5 from 30 °C, 30 ‰ to 20 °C at 10 ‰ salinity.

Treatment	Number of replicates	Temperature (°C)	Salinity (‰)	Initial number of shrimp	Mean survival		
					(%)	SE ¹	CL ²
1	3	20.5	10	200	96.2	1.0	4.4
2	3	20.5	10	200	96.0	0.3	1.2
3	3	20.5	10	200	84.5	2.0	8.7
4	3	20.5	10	200	93.2	0.4	1.9
mean	12	20.5	10	200	92.5	1.5	3.3
5	3	20.0	10	200	93.3	0.9	3.8
6	3	20.0	10	200	94.8	0.6	2.6
7	3	20.0	10	200	97.2	0.4	1.9
8	3	20.0	10	200	97.2	0.6	2.6
mean	12	20.0	10	200	95.6	0.6	1.2
9	3	20.0	10	200	99.5	0.3	1.2
10	3	20.0	10	200	97.8	0.2	0.7
11	3	20.0	10	200	99.7	0.2	0.7
12	3	20.0	10	200	98.8	0.2	0.7
mean	12	20.0	10	200	99.0	0.2	0.5

Initial conditions:

Treatments 1-4; Temperature 30 °C, salinity 30 ‰, Age PL₅₂, post-larval source southern hatchery, mean weight 0.238 ± 0.090 g. From four treatments of nursery tank trial 3

Treatments 5-8; Temperature 30 °C, salinity 35 ‰, Age PL₅₃, post-larval source southern hatchery, mean weight 0.275 ± 0.024 g. From four treatments of nursery tank trial 4

Treatments 9-12; Temperature 29.5 °C, salinity 30 ‰, Age PL₅₂, post-larval source southern hatchery, mean weight 0.526 ± 0.033 g. From four treatments of nursery tank trial 5

¹ Standard Error of the mean

² 95 % confidence limits

may have been partially responsible for their slower growth rate. PL₅₃ from each treatment were again stress tested at 20 °C and 10 ‰ salinity. This test resulted in high survival rates of 93-97 % (Table 4.4, Figure 4.3). In this case, the presence of habitats, but not sand substrates was found to promote both significantly ($P < 0.05$) higher production (chapter 3) and higher vigour shrimp (Table 4.4, Figure 4.3) than PL₅₃ produced from nursery tank trial 3.

4.3.2.3 Nursery tank trial 5

The PL₁₅ stocked in nursery tank trial 5 showed high survival rates of 84-95 % when stress tested at 20 °C and 7.5, 10 and 45 ‰ salinity. These results were slightly higher than those obtained for nursery tank trial 3 (Table 4.3, Figures 4.1 & 4.3).

On conclusion of the 35 day nursery trial (investigating the effects of feeding rate and dietary formulation, see section 3.3.6) it was found that (again despite air blower failure), production rates were better than in the other nursery trials. The increase in weight of shrimp during nursery tank trial 5 for the first two weeks was 1,762 %, comparing favourably with nursery tank trials 3 and 4. The PL₅₂ from each treatment were again stress tested at 20 °C and 10 ‰ salinity, and resulting elevated survival rates of 98-100 % were recorded (Table 4.4, Figure 4.3). Both dietary formulation and feeding rate were found to affect shrimp production in this trial (chapter 3), but the vigour of shrimp produced from these treatments, although high, could not be differentiated using the standard stress test.

4.4 DISCUSSION

4.4.1 Stress testing of primary nursed post-larvae (PL₁₅)

Results from the stress testing of PL₁₅ confirmed the euryhaline nature of young *P. monodon*. From initial conditions of approximately 30 °C and 30 ‰ salinity, > 50 % survival after one hour resulted from tests either increasing the temperature to 32 °C or decreasing it to 20 °C at salinities of between 4-8 and 43-45 ‰. Similar results were

shown by Valencia (1976) who obtained > 50 % survival of *P. monodon* PL₁₅₋₃₄ acclimated to a temperature of 30 °C and salinity of 30 ‰ when shocked (at 30 °C) at salinities at either 4 or 46 ‰ over 24 hours.

During these trials, increasing the salinity from 30 ‰ to > 50 ‰, or reducing it to < 5 ‰, resulted in < 10 % survival after one hour. Similarly, Valencia (1976) demonstrated reduced survival when PL₁₅ were transferred from 35 to 45 ‰ and complete mortality at 55 ‰ after 24 hours. Mortality in these cases probably resulted from the reduced ability of penaeid post-larvae to osmoregulate and withstand extreme salinity variations, particularly in combination with temperature stress (Charmantier, 1987).

Post-larval *P. monodon* are clearly able to osmoregulate over a wide range of salinities. Outside of this range however, their osmoregulatory ability is rapidly reduced. Thus, it is difficult to define the precise decrease in salinity that is necessary to result in 50 % survival after one hour. Relative differences between two or more batches of shrimp however, may still be obtained if the tests are carried out simultaneously. From the results of the present study, 50 % survival was obtained using a temperature decrease of 10 °C from 30 to 20 °C, combined with a salinity increase of 13 ‰ from 30 to 43 ‰, or decrease of 22.5 ‰ from 30 to 7.5 ‰. These results were derived from tests done under the stated conditions. Variation in the results may be expected where acclimation temperatures are not controlled as salinity tolerance is dependant upon acclimation temperature (Valencia, 1976). Variation may also occur when post-larval age is not known precisely since salinity tolerance has been shown to be directly proportional to shrimp age (or size) (Durán Gómez *et al.*, 1991; Olin & Fast, 1992). This again, is probably due to the rapidly developing osmoregulatory capacity of post-larval shrimp (Charmantier *et al.*, 1988). Other departures from the standard curve derived during this study may be assumed to be due to shrimp vigour which itself may be related to a number of factors. These include the genetic history and source of the broodstock, the number of times the broodstock have spawned previously, the larval rearing conditions, and the food fed during larval rearing and primary nursing (Preston, 1985; Tackaert *et al.*, 1989; Arellano, 1990; Durán Gómez *et al.*, 1991).

During stress tests conducted at 20 °C, decreases in temperature were accomplished without ice in an air-conditioned room. Such rooms are normally available in commercial hatcheries, being used for the maintenance of algal stock cultures. In order to reduce temperatures lower than 20 °C the use of ice would be necessary. The use of ice also results in a dynamic change in water temperature and requires close monitoring in order to standardise the procedure. Such stress tests are thus inconvenient on a commercial scale.

If a means of cooling the water is unavailable, stress tests may be conducted successfully by increasing temperatures. In the present study, 50 % survival was obtained using a temperature increase of 2-2.5 °C from 30 to 32-32.5 °C, combined with a salinity increase of 15 ‰ from 30 to 45 ‰, or decrease of 26 ‰ from 30 to 4 ‰.

When the standard stress tests (initial conditions 30 °C, 30 ‰, test conditions 20 °C, 7.5, 10 or 45 ‰) were subsequently applied to three more batches of post-larvae from the same hatchery, significant differences between their survival rates suggested variations in the vigour of PL₁₅ produced from a single hatchery. Bauman and Jamandre (1990) stressed PL₁₅ *P. monodon* from various hatcheries by exposure to salinity decreases from 34 ‰ to 19 or 14 ‰ at 28 °C. They obtained 'moribund' fry rates of 0-33 % and 0-63 % respectively after one hour and suggested that the variation in survival was largely dependant on the source of post-larvae. Exposure for 2 hours had no significant additional effect, suggesting that extension of the stress test period (as suggested by Clifford, 1992) is not necessary. These authors also obtained similar results by exposure of PL₁₅ to 100-150 ppm of 37 % formalin in place of salinity stress. The use of formalin as a stressor may be easier than using salinity, but conformation of the formalin concentration is more difficult and may give rise to error. Salinity shocks may therefore present a more practical method of conducting stress tests.

4.4.2 The effects of secondary tank nursery rearing on the vigour of post-larvae (PL₁₅₋₅₅).

Results from the stress tests conducted on shrimp following 35 days of secondary

nursing (PL₅₂₋₅₃) clearly showed that older post-larvae are more resistant to temperature/salinity stress than are younger post-larvae (PL₁₅). These results confirm those of other authors investigating the effects of shrimp age/size on stress-resistance. Catedral *et al.* (1975, in Valencia, 1976) showed that whilst PL₁₀ *P. monodon* could tolerate temperatures from 24 to 35 °C and salinities from 5 to 39 ‰ when acclimated from 36 ‰, PL_{5,9} were less tolerant. Similarly, Ogle *et al.* (1988) showed that *P. vannamei* post-larvae were resistant (> 50 % survival) to salinity stress when transferred directly from 32 ‰ into 4-32 ‰ for 24 to 120 h, but that PL₉ were less resistant than PL₂₂. Increased resistance of post-larval shrimp to salinity shocks with increasing shrimp age has also been found by Tackaert *et al.* (1989), AQUACOP *et al.* (1991), Durán Gómez *et al.* (1991) and Olin & Fast (1992).

These results indicate that stress tolerance (vigour) is enhanced not by secondary nursing *per se*, but by the increasing developmental status in terms of gill development and hence osmoregulatory ability (Charmantier, 1987; Charmantier *et al.*, 1988, 1989) as the shrimp grow older. This result was confirmed by the observations during the stress tests that out of each batch of post-larvae tested, the smaller individuals were consistently less resistant to stress than larger shrimp. In addition, shrimp currently moulting were less resistant to stress than intermoult individuals. Any synchronisation of moulting during the testing of batches of shrimp may therefore predispose them towards low stress tolerance and confuse the results of the tests. This fact may partially account for the great differences between batches of shrimp tested from a single hatchery. During the stress tests, account should therefore be taken of the stage that the shrimp have reached in the moulting cycle. This may be particularly important for young post-larvae as moulting frequency declines with age (Lee & Wickins, 1992). The significance of these results in practical terms is that holding shrimp in nurseries allows the stocking of on-growing ponds with larger shrimp that are better able to withstand environmental stress. This therefore allows more accurate estimations of shrimp numbers in the pond, helps to optimise feeding management and may increase the final survival rate and hence profit on harvesting.

The standard stress test developed during the first part of this investigation revealed significant differences between the vigour of shrimp (PL₁₅) stocked into nursery trials

3, 4 and 5. Post-larval growth rate over the first two weeks of each nursery trial was significantly different between the three trials and tended to increase with the stress resistance of the PL₁₅ monitored on stocking the nursery tanks. Since the environmental conditions, diets and feeding rates during these trials were similar (although initial weights were slightly different), this result indicates that initial shrimp vigour (as detected by the stress tests) may have affected the subsequent growth rate of the shrimp during secondary nursing.

In Ecuador, many pond operators will only buy post-larvae of *Penaeus vannamei* which they have seen undergoing a stress test involving immersion of post-larvae into a container of cold water (D. Macintosh, pers. comm., 1992). Similarly, Bauman & Jamandre (1990) report from the Philippines that shrimp seed passing a stress test resulted in > 90 % survival during pond on-growing and hence were worth 30 % more than untested seed. Additionally, Nietes (1990) reports that farmers purchasing weak post-larvae in Thailand often experience survival rates of < 50 % during pond on-growing. In order to confirm these observations however, further work investigating the relationship between shrimp vigour and subsequent on-growing performance in both nurseries and ponds is required.

High stress tolerance or vigour (> 90 % survival) of juvenile shrimp (PL₅₂₋₅₃) was monitored following all of the secondary nursing trials. Differences between the vigour of shrimp between the separate nursery trials or between individual treatments of each nursery trial were therefore difficult to quantify. Results from stress testing PL₅₃ following nursery trial 4 however, indicated that the presence of habitats resulted in increases in both shrimp vigour and survival (see section 3.3.5.2). This indicates that stress tests may be able to assist in the determination of differences between treatments during nursery or on-growing trials. Previous research has already confirmed that differences in management practices during larval rearing may be detected through the use of stress tests on young post-larvae. The feeding of larvae with nutritionally-balanced diets rich in n-3 HUFAs in particular, has been shown to result in enhanced stress resistance of post-larval shrimp (Preston, 1985; Tackaert *et al.*, 1989; Arellano, 1990; Durán Gómez *et al.*, 1991). The development of diets for post-larval shrimp which are able to both increase performance (growth and survival) and enhance stress

resistance (vigour) may thus be able to improve the quality of hatchery-reared seed. The standard stress test developed here is clearly able to distinguish between the vigour of batches of young post-larvae (PL₁₅), but a more rigorous test will be required for effective evaluation of older post-larval/juvenile shrimp. Such tests may then be useful as a tool for evaluating differences between nursery rearing practices.

With the increasing expense and intensity of pond on-growing, it may be possible to show by such stress tests whether it would be more profitable to discard a weak batch of post-larvae and stock more vigorous shrimp assuming they are available. The use of stress tests may serve to enhance the reputation of hatcheries as well as the reliability of pond on-growing operations.

4.5 SUMMARY

A stress test designed to indicate the relative vigour of post-larval *Penaeus monodon* Fabricius aged 15 days post-metamorphosis (PL₁₅) was developed. This entailed direct transfer of batches of hatchery-reared and primary-nursed post-larvae from water at 30 °C and 30 ‰ salinity into waters at reduced or increased temperature and salinity for one hour. The conditions chosen for subsequent stress tests to compare the vigour of other batches of post-larvae were those causing 50 % mortality after one hour. Those conditions were found to be 20 °C and either 7.5 or 43 ‰ salinity.

With the subsequent testing of three more batches of PL₁₅ from the same source, survival rates were seen to vary significantly ($P < 0.05$, from 20-90 %) between shrimp batches. Despite slight differences in mean initial weight, these results may indicate variations in the vigour of batches of PL₁₅ produced by a single hatchery.

These tests were able to quantify differences in the stress tolerance (vigour) of various batches of post-larval (PL₁₅) shrimp. Alternative techniques for determining shrimp quality, including behavioural, visual or histological analysis may be used in combination with stress tests, but alone have, as yet, been unable to quantify differences between the vigour of different batches of post-larvae.

The stress resistance of PL₅₂₋₅₃, following secondary nursing was significantly ($P < 0.05$) better than that of PL₁₅ prior to the secondary nursery period. Older or secondary nursed shrimp are thus more likely to survive transfer from the hatchery to the on-growing ponds and subsequent on-growing than are only primary-nursed PL₁₅.

Results suggested that stocking low-vigour PL₁₅ may result in poor shrimp growth rates during secondary nursing. However, further work is necessary to confirm whether shrimp vigour, as measured by the stress tests, can be related to subsequent nursing or on-growing performance and hence seed quality.

Relative to the enhanced quality of older post-larvae, the treatments tested during the secondary nursery period appeared to have little effect on shrimp vigour. A more rigorous stress test is necessary to enable the determination of differences in vigour between batches of secondary-nursed PL₅₀.

Further work on stress testing should consider the state of gill, gut and digestive gland development and the stage in the moulting cycle of the shrimp in order to enhance the standardisation of the stress test and further explore the effects of developmental stage on stress resistance.

CHAPTER 5. The effect of dietary lipid and lecithin levels on production and carcass composition of post-larval *Penaeus monodon*.

I find no sweeter fat than sticks to my own bones.

Walt Whitman, 1855-92

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5.1 INTRODUCTION

Dietary lipids play a number of important roles in the nutrition of crustaceans. Their main functions are as highly digestible and rich sources of dietary energy and as essential fatty acids necessary for the maintenance and functional integrity of biomembranes (D'Abramo, 1990; Tacon, 1990; Zhou, 1990; Cuzon, 1993).

There is good evidence to suggest that penaeid shrimp do not have the ability to synthesise long chain polyunsaturated (PUFA) or highly unsaturated (HUFA) fatty acids of the n-6, or particularly n-3 series rapidly enough to satisfy their nutritional requirements (Kanazawa *et al.*, 1979d; Kayama *et al.*, 1980). A supply of linolenic acid (18:3(n-3)) or preferably eicosapentaenoic acid (20:5(n-3)) or docosahexaenoic acid (22:6(n-3)) must therefore be supplied pre-formed in the diet (AQUACOP, 1978; Kanazawa *et al.*, 1978, 1979a-e, 1985; Jones *et al.*, 1979b; Teshima & Kanazawa, 1984; Kanazawa, 1985; Castell *et al.*, 1986). Although the above authors have shown that the addition of 1 % of n-3 series PUFAs or HUFAs to the diet has increased shrimp production, the precise quantitative requirements for these fatty acids have yet to be determined for any species (D'Abramo, 1990).

Because marine animal lipids such as cod or pollack liver oil or clam oil are rich in PUFAs and HUFAs, these lipids have usually promoted better growth and survival than plant seed oils when included in shrimp diets (Guary *et al.*, 1976; Kanazawa *et al.*, 1977; AQUACOP, 1978; Catacutan & Kanazawa, 1985; Dominy & Lin, 1989; Ali,

1990; Catacutan, 1991a). Marine animal oils however, are expensive and some authors have achieved good shrimp production feeding diets containing mixtures of fish or clam and plant (*i.e.* soybean and sunflower) oils (Deshimaru *et al.*, 1979; Read, 1981; Briggs, unpublished data) or plant oils such as peanut or crude degummed soybean oil alone (Colvin, 1976b; Pascual, 1986).

Once the requirements for fatty acids and cholesterol are met, it is probable that most of the dietary lipid is used as an energy substrate. Although some fish species are capable of utilising up to 30 % dietary lipid, there is evidence that crustaceans cannot and are thus less able to utilise lipids as energy sources for sparing dietary protein from catabolism for energy (Halver, 1976; New, 1976a,b, 1980; Hanson & Goodwin, 1977; Castell, 1979, 1982; Capuzzo, 1982; Millikin, 1982; D'Abramo, 1990; Cuzon, 1993). This is thought to be a reflection of the low lipid levels present in their natural diet (1-6 %) (Dall *et al.*, 1991) and in their tissues (3.3-9.3 %) (Colvin, 1976b; Teshima *et al.*, 1977; Clarke & Wickins, 1980; Chanmugan *et al.*, 1983). In addition, dietary lipid levels of between 10 and 17 % are generally believed to have adverse effects upon the growth and survival of crustaceans (Forster & Beard, 1973; Deshimaru *et al.*, 1979, 1985; Teshima & Kanazawa, 1984; Bautista, 1986). Excess dietary lipid may also result in fatty carcasses which can reduce storage times and consumer acceptability (Sedgwick, 1979; Chanmugan *et al.*, 1983).

Previous work on the dietary lipid requirements of post-larval/juvenile *Penaeus monodon* has suggested optimum levels of 5-10 % when fed either purified or practical style diets in the laboratory (Alava & Lim, 1983; Deshimaru *et al.*, 1985; Bautista, 1986; Pascual, 1986; Hajra *et al.*, 1988; Shiau & Chou, 1991; Briggs, unpublished data). However, little account has been taken of the influence of diet type, lipid quality, or the roles of phospholipids and dietary energy density on the total lipid requirements. Recently however, up to 12 % lipid has been used successfully in low-protein, energy-balanced diets fed to juvenile *P. monodon* (Sheen & Chen, 1992).

Phospholipids are known to play an important role in the growth and survival of marine crustaceans including penaeid shrimp. Kanazawa *et al.* (1979f) showed that juvenile *Penaeus japonicus* Bate grew faster when fed a diet containing phospholipid-rich clam

lipids than one containing pollack liver oil as the lipid source. These authors then succeeded in isolating lecithin, and specifically phosphatidylcholine (PC), as the specific fraction of the phospholipid responsible for this effect.

The first studies on the phospholipid requirements of *P. monodon* were by Pascual (1986, 1988). She found enhanced growth and FCR as soy lecithin levels were increased from 0 to 2 % (equivalent to 0-0.4 % PC) in practical style diets containing 10 % of various lipid sources. Chen (1993) later went on to suggest that 1.25 % of 80 % pure soy PC (equivalent to 1 % PC) was required in purified diets fed to *P. monodon*. However, neither the precise requirement for lecithin, nor the influence of dietary lecithin on total lipid requirements have been established for this species. In contrast, the growth-promoting effects of increased levels of soy lecithin have been demonstrated for both *P. japonicus* at 3-6 % (0.7-1.4 % PC) (Teshima *et al.*, 1982, 1986b,c; Kanazawa *et al.*, 1985) and homarid lobsters at 6-8 % (1.4-1.9 % PC) (Conklin *et al.*, 1980, 1981; Bowser & Rosemark, 1981) of casein-based diets.

A preliminary, unpublished study by this author investigated the effects of a range of lipid sources on the production of post-larval *P. monodon*. The isonitrogenous, isoenergetic semi-purified diets contained 6 % of a range of basal lipid sources in combination with 3 % soy lecithin and 0.5 % cholesterol at a total dietary lipid level of 9.5 %. Results showed that a diet containing basal lipid sources of cod liver oil:soybean oil at a ratio of 3:1 was able to support shrimp production equal to a diet containing cod liver oil alone. Both of these diets supported better shrimp production than those containing basal lipid sources of capelin oil, rape seed oil or soybean oil alone. In addition, a diet containing 6 % soy lecithin and 3 % cod liver oil, resulted in significantly better production than any of those containing 3 % soy lecithin. These results confirmed the growth-promoting effects of combinations of basal lipid sources and soy lecithin in diets for *P. monodon*.

In the present study, the effects of varying the dietary levels of basal lipid (cod liver oil:soybean oil at a ratio of 3:1), from 0 to 6 % and soybean lecithin, from 0 to 9 %, on the production and carcass composition of post-larval *P. monodon* were investigated using semi-purified diets. This study was conducted in order to establish the total

dietary lipid, fatty acid and lecithin (PC) requirements of this species at a constant dietary energy level.

5.2 MATERIALS AND METHODS

5.2.1 Experimental animals

Post-larvae used in this trial were on-grown (in the shrimp larval rearing facility of the Institute of Aquaculture, University of Stirling) from nauplii obtained from the Frippak Feeds hatchery in Aberdeen, Scotland. Larvae were fed algae (*Isocrysis galbana* Parke 1949, *Tetraselmis suecica* (Kylin) Butcher 1959) and newly-hatched *Artemia* nauplii in static seawater (30 °C, 35 ‰ salinity) black conical bins (75 l) until metamorphosis into post-larvae. After reaching the post-larval stage, they were weaned to a commercial flake diet (Waterlife Research Industries Ltd., West Drayton, Middlesex) and the salinity of the water gradually reduced to 22.5 ‰ over a period of ten days. Post-larvae were transferred from the larval rearing bins to the recirculated brackish-water (22.5 ‰) tank system (Appendix 1) at ten days post-metamorphosis (PL₁₀) to acclimate them to the experimental conditions. At this stage they were weaned to the basal experimental pelleted diet for a period of 7 days.

5.2.2 Experimental design

Before starting the trial, the post-larvae (PL₁₇) were starved for 24 h, blotted dry with tissue paper and weighed (± 0.001 g) using a top pan balance (Mettler AJ100). Experimental shrimp were selected with mean weights of 22 ± 3 mg. Twenty shrimp of this size were randomly allocated to each of 24 fibreglass tanks of dimensions 0.75 X 0.7 X 0.2 m deep (0.53 m² area, 105 l volume) at a stocking density of 38 shrimp m⁻². Eight dietary treatments were tested, each in triplicate, in the brackish-water recirculating system. This system incorporated biological and mechanical filtration, protein stripping, buffering, temperature control and aeration. The tanks were covered with opaque lids and supplied with brackish-water at the rate of 6 l min⁻¹ per tank.

All shrimp were group-weighted (as above) at the beginning of the trial and every ten days thereafter for the 50 day duration of the experiment. At these times, the tanks were scrubbed clean to prevent bacterial build-up. In addition, prior to the first feeding of each day, any dead shrimp were removed from the tanks to prevent cannibalism, the mortalities were recorded and uneaten food and faeces (but not exuviae) removed by siphon.

5.2.3 Water quality

Measurements of dissolved oxygen, temperature, salinity, pH, total ammonia nitrogen (TAN) and nitrite nitrogen ($\text{NO}_2\text{-N}$) were made daily in randomly selected tanks. Dissolved oxygen was measured with a YSI model SI B oxygen meter, pH with a WTW pH 95 electronic pH meter, salinity with a hand held Atago refractometer, temperature with a thermometer and TAN and $\text{NO}_2\text{-N}$ with Tetra water test kits (Tetra Werke, Melle, Germany). External lighting was on a 12:12 h light:dark cycle. Water (from a preheated and salinity adjusted supply) was added to the biofilter to replace losses from cleaning and evaporation as required.

Water quality was maintained relatively constant throughout the duration of the trial. Dissolved oxygen remained above 6.7 mg l^{-1} ($> 100 \%$ saturation), temperature was maintained at $29.4 \pm 0.6 \text{ }^\circ\text{C}$, pH at 8.1 ± 0.05 and salinity at $22.5 \pm 1.5 \text{ }^\circ\text{‰}$. TAN remained below 0.01 mg l^{-1} and $\text{NO}_2\text{-N}$ below 0.001 mg l^{-1} throughout the trial period. Water quality was not considered to be detrimental to the growth of the shrimp during the trial.

5.2.4 Diet formulation, preparation and feeding

Eight isonitrogenous ($43.3 \pm 1.3 \%$ protein), isoenergetic ($20.2 \pm 0.6 \text{ kJ g}^{-1}$) semi-purified diets (Table 5.1) were formulated using casein and gelatin (3:1) as the protein sources. The optimal dietary lipid source (from a previous unpublished trial) of cod liver oil and soybean oil at a 3:1 ratio was chosen as the basal lipid source for this trial. Basal lipid levels of 0 to 6 % were combined with various levels of powdered soybean lecithin (18 % PC, Nutripur, Lucus Meyer, Hamburg, Germany) from 0 to 9 % and

Table 5.1 Composition and proximate analysis (% dry weight) of the diets used in the lipid/lecithin trial.

Ingredient	Diet							
	1	2	3	4	5	6	7	8
Casein	31.0	31.0	31.0	31.0	31.0	31.0	31.0	31.0
Gelatin	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2
Yellow dextrin	49.2	42.4	42.4	35.7	28.9	42.4	35.7	28.9
α cellulose	0.0	3.8	3.8	7.5	11.3	3.8	7.5	11.3
Vitamin premix ¹	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix ²	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Astaxanthin ³	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cod liver oil	0.0	0.0	2.3	2.3	2.3	4.5	4.5	4.5
Soybean oil	0.0	0.0	0.7	0.7	0.7	1.5	1.5	1.5
Soybean lecithin	3.0	6.0	3.0	6.0	9.0	0.0	3.0	6.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
+ water (20-30 %)	+	+	+	+	+	+	+	+
Proximate analysis ⁴								
Protein	41.2	45.2	44.1	44.0	42.1	44.0	42.6	43.1
\pm SD	0.2	0.3	0.4	0.5	0.4	0.3	0.5	0.6
Ether extract ⁵	2.8	3.7	4.1	6.0	8.2	4.1	6.8	7.0
\pm SD	0.1	0.1	0.2	0.2	0.4	0.3	0.4	0.4
Total lipid ⁶	3.0	4.1	4.5	6.6	8.9	4.7	8.0	8.6
\pm SD	0.2	0.4	0.5	0.4	0.5	0.3	0.5	0.7
Carbohydrate ⁷	51.0	42.9	44.1	38.6	35.6	44.0	38.5	33.4
Fibre	0.8	3.2	3.3	6.6	8.9	3.6	6.9	10.0
\pm SD	0.2	0.3	0.4	0.5	0.6	0.5	0.6	1.0
Ash	4.0	4.6	4.0	4.2	4.5	3.7	4.0	4.9
\pm SD	0.3	0.2	0.2	0.4	0.2	0.3	0.4	0.4
Moisture	15.4	10.7	14.7	10.1	8.8	10.5	9.1	10.3
\pm SD	0.3	0.3	0.3	0.1	0.2	0.2	0.2	0.1
Total energy (kJ g ⁻¹) ⁸	19.7	19.7	19.8	19.6	19.6	19.8	19.8	19.3
Total energy (kJ g ⁻¹) ⁹	20.3	19.5	19.5	20.5	20.6	19.8	20.2	21.1
Pr:E ratio (mg pr. kJ ⁻¹ TE) ¹⁰	20.9	23.0	22.3	22.4	21.5	22.2	21.5	22.3
Carbohydrate:lipid ratio	17.0	10.5	9.8	5.9	4.0	9.4	4.8	3.9

- continued over

Table 5.1 - continued

¹ Vitamin Premix (from Deshimaru & Kuroki, 1974) contributes the following per 100 g of finished diet: Choline Chloride 800 mg, inositol 400 mg, cholecalciferol 0.6 mg, ascorbic acid (sodium salt) 100 mg, A tocopherol acetate 40 mg, niacinamide 80 mg, pantothenic acid 30 mg, thiamine hydrochloride 6 mg, riboflavin 20 mg, para-aminobenzoic acid 40 mg, menadione 4 mg, pyridoxine hydrochloride 6 mg, folic acid 1.5 mg, biotin 0.6 mg, cyan-ocobalamin 0.04 mg, Beta carotene 4 mg, cellulose powder 1564 mg

² Mineral Premix (modified from Briggs *et al.*, 1988) contributes the following per 100 g of finished diet: CaHPO₄.2H₂O 3001.3 mg, MgSO₄.7H₂O 510 mg, NaCl 240 mg, KCl 200 mg, FeSO₄.7H₂O 10 mg, ZnSO₄.7H₂O 22 mg, MnSO₄.4H₂O 10.1 mg, CuSO₄.5H₂O 3.1 mg, CoSO₄.7H₂O 1.9 mg, CaIO₃.6H₂O 1.2 mg, CrCl₃.6H₂O 0.5 mg

³ 50 % Astaxanthin in cod liver oil from Chr. Hansen's Laboratorium A/S, Copenhagen, Denmark

⁴ All results based on triplicate analyses

⁵ Analysed By Soxhlet method

⁶ Analysed by method of Folch-Lees (Folch *et al.*, 1957)

⁷ By difference in dry weight

⁸ By calculation

⁹ By bomb calorimetry

¹⁰ Using calculated TE figures

a constant level of 0.5 % cholesterol (96 % 5-cholesten-3B-ol, Sigma Chemical Company Ltd.) to provide final total dietary lipid levels of between 3.5 and 12.5 % at 3 % increments.

Dextrin and cellulose levels were varied to maintain isoenergetic diets. Since neither digestible nor metabolisable energy levels are known for *P. monodon*, dietary energy levels were calculated using mean total energy values of 23.6 kJ g⁻¹ for protein, 39.5 kJ g⁻¹ for lipid (using total lipid levels) and 17.2 kJ g⁻¹ for carbohydrate (Cho *et al.*, 1982; Jobling, 1983; Henken *et al.*, 1986). The total energy level of each diet was also measured on duplicate samples using an adiabatic autobomb calorimeter (Gallenkamp model CBA-350-K). Total energy (TE) levels of 20.2 ± 0.6 kJ g⁻¹ (by bomb calorimetry) and protein to energy (Pr:E) ratios of 22.0 ± 0.7 mg protein kJ⁻¹ TE (using calculated energy values for comparison with other studies) were maintained for all diets (Table 5.1).

Diets were prepared by mixing the finely (< 0.125 mm) ground ingredients in a Hobart bowl mixer. The lipid components were then added prior to further blending. 20-30 % of warm water was then added until the mix bonded together under light hand pressure. The dough was cold extruded in a Hobart pellet mill through a 3 mm die. The resulting pellets were dried in a convection oven at 38 °C for 6 h to < 10 % moisture (except diet 1 at 15.4 % moisture). The pellets were subsequently ground and sieved. Particles between 1 and 2 mm diameter were retained and stored at -20 °C until required.

Feeding was carried out twice daily at 0900 and 1700 for nine days out of every ten (not feeding on sampling day). The shrimp were fed at 20 % of body weight daily for days 1-29 and 10 % of body weight daily for days 31-50 on a wet shrimp/dry feed basis. The amount of food fed was adjusted at every ten day sampling period and resulted in practically constant food availability and hence approximated *ad-libitum* feeding with minimal wastage. All diets remained stable in water for > 12 h as a result of the binding properties of gelatin and the finely-ground nature of the ingredients.

5.2.5 Sample collection and analysis

Diets and whole shrimp (on termination of the trial) were ground and frozen at -15°C for subsequent proximate and fatty acid analyses. All analyses were based upon the mean of three replicates. Determinations of crude protein for diets were by macro-Kjeldahl analysis (AOAC, 1980) and for carcasses by the method of Lowry (Lowry *et al.*, 1951), total lipid by the Soxhlet and Folch-Lees (Folch *et al.*, 1957) methods, total ash by combustion at 450°C for 12 h, total crude fibre by Fibretek and total carbohydrate by difference in dry weight and moisture by drying at 105°C for 24 h.

Lipids from shrimp carcasses were extracted using chloroform-methanol (2:1 v/v) following the method of Folch *et al.* (1957). Lipid class composition was determined by high performance thin-layer chromatography using the double development system of Olsen & Henderson (1989). Lipid classes were identified by comparison with commercial standards (Sigma Chemical Co. Ltd.) after spraying and cleaning with cupric sulphate (Christie, 1982). Quantification was made by flying spot densitometry using a Shimadzu CS-930 scanner. Methyl esters of total lipid were prepared by acid catalysed transmethylation (Christie, 1982) after the addition of 10 % by weight of 23:0 as internal standard. Following purification, fatty acid methyl esters were separated using a Packard 436 gas chromatograph equipped with a CP wax 51 column (50 m by 0.32 mm) (Chrompack U.K., Ltd.). A column temperature of 235°C and a 0.2 microlitre sample with a methyl ester concentration of approximately 3 mg ml^{-1} were used. Peaks were identified by comparison with a marine oil standard. The true percentage concentration of each identified fatty acid was calculated in relation to the integral peak area of the internal standard.

5.2.6 Data analysis

Data obtained from this trial was analysed as described below:-

1. Specific growth rate (SGR), survival, production and food conversion efficiency (FCE) were analysed as described in section 3.2.1.6.

2. Apparent protein efficiency ratio (PER)

$$\text{PER} = \frac{\text{Wet protein fed (g)}}{\text{Dry protein fed (g)}}$$

3. The effects of dietary total lipid and lecithin levels on shrimp growth, survival (arcsin transformed), production, FCE, PER and carcass composition after 50 days were analysed using a non-parametric analysis of variance (Kruskal-Wallis) test after Bartlett tests revealed that in all cases the data were heteroscedastic (Sokal & Rohlf, 1981).
4. Where appropriate, the T-method for multiple comparisons was used to determine statistical differences between treatment means for the above ANOVAs (Sokal & Rohlf, 1981). Results were considered significant if $P < 0.05$.
5. Regression analyses were used to identify possible relationships between the dietary total lipid and lecithin levels and shrimp growth, survival, production, FCE, PER and carcass composition (Sokal & Rohlf, 1981).

5.3 RESULTS

5.3.1 Dietary lipid and fatty acid analysis

The lipid extraction techniques used in this study, *i.e.* the Soxhlet and the Folch-Lees procedures, were only able to extract 56-80 % and 63-86 % respectively of the total lipid added to the test diets (Table 5.1). The remaining lipid was probably either bound to the protein or carbohydrate portions of the diet or was lost selectively in the pelleting process as lipid tended to be squeezed from the diet during extrusion.

From the class analysis of the lipid sources used in the test diets (Table 5.2),

Table 5.2 Lipid fraction analysis of the dietary lipids used in the lipid/lecithin trial.

Component	Lipid type			
	Soybean oil	Cod liver oil	Lecithin	Cholesterol
Crude lipid (% of dry diet)	100.0	100.0	98.1	100.0
Lipid classes (% of total lipid in sample by TLC densitometry)				
SE (sterol esters)	-	-	-	-
TAG (triacylglycerol)	78.0	87.6	5.3	-
FFA (free fatty acids)	8.4	Trace	1.3	-
Cholesterol	13.6	12.4	1.2	100.0
MGDG ¹	-	-	5.0	-
Glycolipids	-	-	7.4	-
DGDG ²	-	-	1.6	-
PE ³	-	-	6.4	-
PG/PI ⁴	-	-	53.7	-
PC ⁵	-	-	18.1	-
LPC ⁶	-	-	-	-
Unknown	-	-	Trace	-

¹ Monogalactyldiacylglycerols

² Digalactyldiacylglycerols

³ Phosphatidylethanolamine

⁴ PG, phosphatidylglycerol, PI, phosphatidylinositol

⁵ Phosphatidylcholine

⁶ Lysophosphatidylcholne

endogenous cholesterol levels were expected to be enhanced due to the high levels of cholesterol (12-14 %) in the basal lipid sources used. The low level of cholesterol in the lecithin (1.2 %, Table 5.2) contributed little to overall dietary levels. Analysis of the cholesterol present in the diets showed dietary levels of 0.6-1.0 % (Table 5.3). Analysis of phosphatidylcholine (PC) levels in the lipid sources used (Table 5.2) revealed that PC was present at 18 % of the lecithin fraction and not in any other lipid source. Analysis of the PC present in the diets (Table 5.3) indicated levels of 0.4 % for diet 1, 0.5-0.6 % for diets 2 and 3, 0.8 % for diets 4 and 7, and 1.2 % for diets 5 and 8. Diet 6 lacking soy lecithin however, contained only 0.03 % PC.

Analysis of the fatty acid profile of the lipid sources (Table 5.4) and the diets (Table 5.5) showed that diets containing cod liver oil (diets 3-8) had a high level of n-3 series fatty acids (0.5-0.9 % of the diet) and a relatively high ratio of n-3:n-6 PUFAs and HUFAs (0.3-0.9). This was due to the high proportion of eicosapentaenoic (20:5(n-3)) and docosahexaenoic (22:6(n-3)) acids present in cod liver oil. These HUFAs were either lacking or found in negligible quantities in soybean oil and lecithin, which were instead dominated by n-6 series fatty acids, particularly linoleic acid (18:2(n-6)) (Table 5.4).

5.3.2 Shrimp production

Despite poor overall performance, the absence of basal lipids (containing n-3 series fatty acids) in diets 1 and 2 resulted in relatively high shrimp growth rates but poor FCE, PER and production due to survival rates which were significantly lower than for any diets containing a source of basal lipid. Diet 6, lacking lecithin, however, resulted in relatively good survival, but very poor growth, FCE, PER and hence production (Table 5.6, Figures 5.1-5.3).

In the presence of lecithin at a constant dietary level and at least 3 % basal lipids, increases in growth rate, FCE, PER, survival and production were directly related to basal and hence total lipid levels until the lecithin fraction constituted 46-63 % of the total lipid added, before decreasing again at higher levels (Table 5.6, Figures 5.1-5.3). Thus, at 6.6-8.0 % total lipid, 6 % lecithin was optimum (diet 4), whereas at 4.1-4.7

Table 5.3 Lipid fraction analysis of the diets used in the lipid/lecithin trial.

Component	Diet							
	1	2	3	4	5	6	7	8
Crude lipid (% of dry diet)	3.0	4.1	4.5	6.6	8.9	4.7	8.0	8.6
Lipid classes (% of total lipid)								
SE	-	-	-	-	-	-	-	-
TAG	12.4	11.7	42.1	32.7	31.5	74.8	55.2	36.5
FFA	3.3	3.0	2.1	1.9	1.9	1.9	1.3	0.2
Cholesterol	21.6	15.2	15.9	12.7	11.2	22.7	12.3	8.5
MGDG ¹	4.4	5.8	5.6	4.5	8.5	-	2.7	4.7
Glycolipids	5.6	6.4	}	}	}	}	}	}
DGDG ²	1.7	2.0	0.6	2.1	1.1	-	0.6	1.4
PE ³	19.7	15.0	12.0	12.6	10.3	-	7.7	13.8
PG/PI ⁴	16.5	24.7	10.6	21.4	21.8	-	8.3	20.5
PC ⁵	14.8	15.8	10.7	11.8	13.4	0.6	9.7	13.9
LPC ⁶	-	0.4	0.4	0.3	0.3	-	0.5	0.5
Unknown	Trace	Trace	Trace	Trace	Trace	Trace	1.7	Trace

¹ Monogalactyldiacylglycerols

² Digalactyldiacylglycerols

³ Phosphatidylethanolamine

⁴ PG, phosphatidylglycerol, PI, phosphatidylinositol

⁵ Phosphatidylcholine

⁶ Lysophosphatidylcholne

Table 5.4 Fatty acid analysis of the dietary lipids used in the lipid/lecithin trial.

Component	Dietary component			
	Soybean oil	Cod liver oil	Lecithin	Cholesterol
Crude lipid (% of dry diet)	100.0	100.0	100.0	100.0
Fatty acids (% of total lipid)				
Fatty acids	78.6	78.9	39.4	0.0
Non FAs	21.4	21.1	60.6	100.0
14:0	0.1	3.9	-	-
15:0	-	0.4	-	-
16:0	8.1	11.2	8.5	-
16:1	0.1	6.4	0.1	-
16:2	0.2	0.4	-	-
16:3	-	0.8	-	-
16:4	-	0.8	-	-
18:0	3.1	2.0	1.5	-
18:1(n-9)	19.1	11.9	4.3	-
18:1(n-7)		3.3	0.9	-
18:2(n-6)	41.0	2.0	21.3	-
18:3(n-6)	-	1.1	-	-
18:3(n-3)	5.4	-	2.1	-
18:4(n-3)	-	2.2	-	-
20:0	0.3	0.2	0.1	-
20:1	0.2	4.7	-	-
20:4(n-6)	-	0.5	-	-
20:4(n-3)	-	0.6	-	-
20:5(n-3)	-	8.9	-	-
22:1	0.3	3.2	0.2	-
22:5(n-3)	-	1.6	-	-
22:6(n-3)	0.1	8.5	0.4	-
24:1	-	0.4	-	-
Unidentified	0.6	4.1	0.4	-
Total saturated FAs	11.6	17.6	10.0	-
Total monoenes	19.7	29.8	5.4	-
Total n-3	5.5	21.8	2.5	-
Total n-6	41.0	3.6	21.3	-
n-3:n-6 ratio	0.1	6.1	0.1	-

Table 5.5 Fatty acid analysis of the diets used in the lipid/lecithin trial.

Component	Diet							
	1	2	3	4	5	6	7	8
Total lipid (% of dry diet)	3.0	4.1	4.5	6.6	8.9	4.7	8.0	8.6
Fatty acids (% of total lipid)								
Fatty acids	44.4	36.6	59.3	55.9	54.2	61.4	68.9	66.3
Non FAs	55.6	63.4	40.7	44.1	45.8	36.8	33.1	33.7
14:0	0.1	0.1	1.3	1.0	0.8	2.2	1.9	1.5
15:0	-	-	0.1	0.1	0.1	0.2	0.2	0.1
16:0	8.9	7.7	9.0	9.7	9.8	8.2	9.4	9.9
16:1	0.1	-	2.3	1.7	1.4	4.1	4.5	1.6
16:2	-	-	0.2	0.1	0.1	2.0	0.3	-
16:3	0.1	0.1	0.3	0.2	0.2		0.2	-
16:4	-	-	0.3	0.2	0.2	0.3	0.4	0.2
18:0	1.6	1.4	1.8	1.8	1.8	2.0	2.1	2.2
18:1(n-9)	5.3	5.1	9.2	7.9	7.3	13.6	14.3	13.2
18:1(n-7)	0.9		1.7	1.6	1.5			
18:2(n-6)	24.2	20.0	18.6	20.8	22.2	10.3	17.7	19.4
18:3(n-3)	2.4	1.6	2.3	2.4	2.4	1.7	2.6	2.5
18:4(n-3)	-	-	0.9	0.6	0.5	1.1	1.2	0.9
20:0	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
20:1	0.1	0.1	1.7	1.2	0.9	2.8	2.1	1.9
20:4(n-6)	-	-	0.2	0.1	0.1	0.3	0.3	0.2
20:4(n-3)	0.1	-	0.2	0.2	0.1	0.3	0.3	0.2
20:5(n-3)	-	-	3.3	2.3	1.7	3.7	4.1	2.9
22:1	0.1	0.3	1.1	0.8	0.7	2.2	1.3	1.2
22:5(n-3)	-	-	0.6	0.4	0.3	0.6	0.7	0.4
22:6(n-3)	0.1	-	2.8	1.9	1.5	2.3	2.7	1.8
24:1	-	-	0.2	0.1	0.1	-	-	-
Unidentified	0.3	-	1.3	0.4	0.4	3.4	2.5	5.9
Total saturates	10.7	9.3	12.3	12.8	12.6	12.9	13.7	14.0
Total monoenes	6.5	5.6	16.1	13.4	11.9	22.7	22.2	17.9
Total n-3	2.5	1.7	10.1	7.9	6.5	9.6	11.7	8.8
Total n-6	24.2	20.0	18.8	20.9	22.2	10.6	18.0	19.6
n-3:n-6 ratio	0.1	0.1	0.5	0.4	0.3	0.9	0.6	0.4

Table 5.6 Performance of post-larval *P. monodon* fed diets varying in lipid and lecithin level over the 50 day lipid/lecithin trial period.¹

Diet	Basal lipid level (%)	Lecithin level (%)	Total lipid level (%)	Survival (%)	Mean body weight (g)		SGR (% body wt d ⁻¹)	Production (g m ⁻² d ⁻¹)	FCE	PER
					Initial	Final				
1	0.0	3.0	3.0	18.3 ²	0.020 ^a	0.486 ^{ab}	6.32 ^a	0.050 ^{ab}	0.18 ^{ab}	0.44 ^{ab}
± SD	-	-	-	7.6	0.001	0.165	0.84	0.026	0.05	0.11
2	0.0	6.0	4.1	25.0 ^{ab}	0.023 ^a	0.501 ^{ab}	6.00 ^{ab}	0.063 ^{ab}	0.20 ^{ab}	0.43 ^{ab}
± SD	-	-	-	13.2	0.001	0.289	1.38	0.029	0.10	0.22
3	3.0	3.0	4.5	43.3 ^{bc}	0.024 ^a	1.009 ^b	7.19 ^a	0.270 ^d	0.48 ^c	1.09 ^c
± SD	-	-	-	16.1	0.001	0.636	1.55	0.128	0.16	0.37
4	3.0	6.0	6.6	56.7 ^c	0.023 ^a	0.498 ^{ab}	6.14 ^a	0.193 ^c	0.38 ^{bc}	0.86 ^{bc}
± SD	-	-	-	5.8	0.001	0.124	0.41	0.031	0.07	0.16
5	3.0	9.0	8.9	43.3 ^{bc}	0.024 ^a	0.352 ^{ab}	5.38 ^{ab}	0.107 ^b	0.22 ^{ab}	0.52 ^{ab}
± SD	-	-	-	12.6	0.001	0.064	0.55	0.055	0.08	0.20
6	6.0	0.0	4.7	43.3 ^{bc}	0.021 ^a	0.132 ^a	3.69 ^b	0.030 ^a	0.12 ^a	0.27 ^a
± SD	-	-	-	2.9	0.001	0.020	0.29	0.010	0.03	0.07
7	6.0	3.0	8.0	45.0 ^{bc}	0.023 ^a	0.373 ^{ab}	5.59 ^{ab}	0.110 ^b	0.25 ^{ab}	0.58 ^{ab}
± SD	-	-	-	5.0	0.001	0.076	0.71	0.017	0.05	0.12
8	6.0	6.0	8.6	45.0 ^{bc}	0.021 ^a	0.320 ^{ab}	5.38 ^{ab}	0.090 ^{ab}	0.21 ^{ab}	0.49 ^{ab}
± SD	-	-	-	5.0	0.001	0.071	0.47	0.012	0.01	0.02

¹ Based on the mean of three replicates

² For single criterion, mean values in the same column bearing different superscripts are significantly different ($P < 0.05$) by T-method

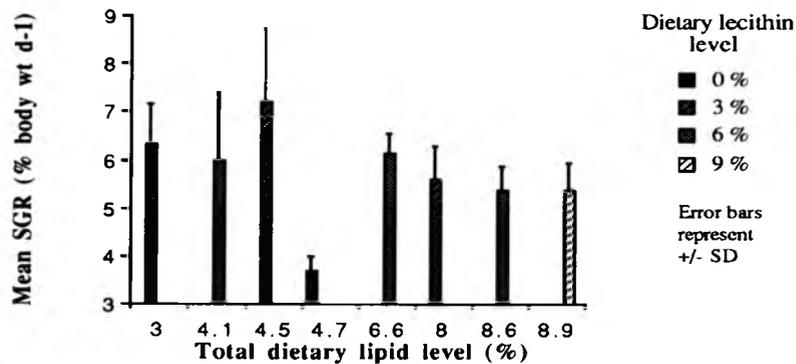


Figure 5.1. Relationship between total dietary lipid and lecithin levels and mean SGR of shrimp over 50 days.

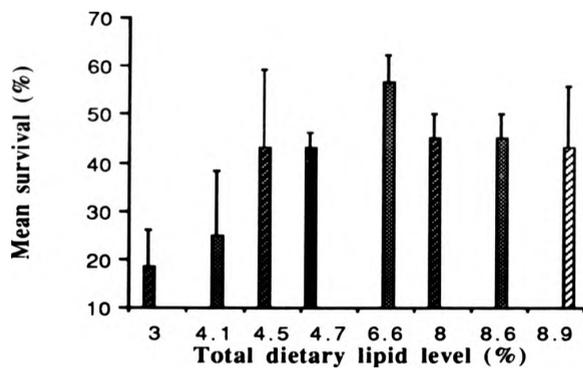


Figure 5.2. Relationship between total dietary lipid and lecithin levels and mean survival of shrimp after 50 days.

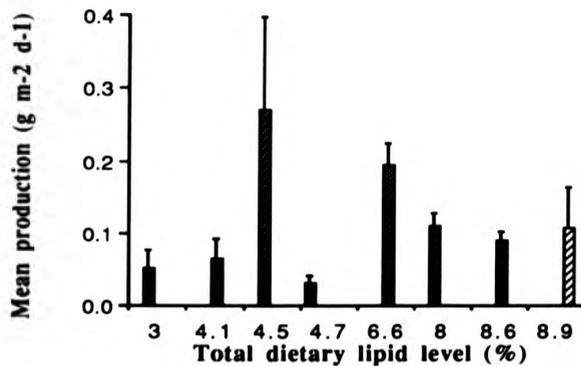


Figure 5.3. Relationship between total dietary lipid and lecithin levels and mean production of shrimp after 50 days.

% total lipid, the optimum lecithin level was 3 % (diet 3). At a constant level of total dietary lipid, shrimp performance increased until lecithin constituted 46-63 % of the total lipid added, before decreasing as the proportion of lecithin increased further.

At a constant level of 3 % of added basal lipids, as dietary lecithin levels were increased from 3 to 9 % (and hence total lipid levels from 4.5-8.9 %), the specific growth rate decreased linearly from 7.2 to 5.4 % body weight d^{-1} ($A = 8.971$, $b = -0.410$, $r^2 = 0.993$, $P < 0.05$). Similarly, with increasing dietary lecithin (and total lipid levels), production decreased linearly from 0.27 to 0.11 $g\ m^{-2}\ d^{-1}$ ($A = 0.437$, $b = -0.037$, $r^2 = 0.999$, $P < 0.05$). However, at high levels of total dietary lipid (8-8.9 %), increasing the level of dietary lecithin from 3 to 9 % did not significantly affect growth, survival, FCE, PER or production (Table 5.6, Figures 5.1-5.3)

The growth, production, FCE and PER of shrimp were highest when fed diets containing 43.3 ± 1.3 % protein and $19.7 \pm 0.2\ kJ\ g^{-1}$ total energy (by calculation), with the addition of 3 % basal lipids and 3 % lecithin (at 46 % of the total lipid added) at a total lipid level of 4.5 % (Table 5.6). This diet (diet 3) promoted significantly higher production than any other diet and better FCE and PER than any diet other than diet 4 containing 3 % basal lipid and 6 % lecithin (6.6 % total lipid). However, a total dietary lipid level of 4.5-8.9 %, consisting of any combination of 0.5 % cholesterol, 3-6 % basal lipid and 3-6 % lecithin (0.7-1.0 % dietary cholesterol and 0.5-1.2 % dietary PC) resulted in relatively good shrimp performance (Table 5.6, Figures 5.1-5.3). These diets also contained a high levels (0.7-1.8 %) of energy-supplying monoenes and a high ratio (0.3-0.6) of n-3:n-6 fatty acids derived from relatively high levels of n-3 and relatively low levels of n-6 series fatty acids (Table 5.5).

Survival rates tended to increase as lecithin additions increased from 0 to 6 % of diets containing up to 6.6 % lipid (particularly at 46-63 % of the total lipid), but did not increase as lecithin and total lipid levels were raised further. The most noticeable effect on survival was that of basal lipids which, when omitted, resulted in the significantly ($P < 0.05$) lowest survival rates of 18-25 % (Table 5.6, Figure 5.2).

5.3.3 Carcass composition

On termination of the trial on day 50, proximate analysis of the shrimp fed the various diets revealed that the dietary formulations had no significant ($P < 0.05$) effect on carcass composition (Table 5.7). Carcass lipid levels were similar for shrimp fed all diets at 4.3-6.2 % of dry weight.

5.4 DISCUSSION

5.4.1 Dietary lipid and fatty acid analysis

Results from the fatty acid profile analysis of the test diets confirmed that diets containing no cod liver oil possessed low levels of n-3 series fatty acids (0.07-0.08 % of the diet) and in particular, negligible amounts of the HUFAs 20:5(n-3) and 22:6(n-3). This deficiency may have accounted for the poor performance of shrimp fed these diets, particularly in terms of survival, underlining the importance of the dietary fatty acid profile as previously suggested for *P. monodon* (Pascual, 1983; Deshimaru *et al.*, 1985; Kanazawa, 1985a,b; Tacon, 1990; Catacutan, 1991a).

Diet 6 however, containing relatively high levels of n-3 HUFAs (due to the inclusion of 6 % basal lipids), but lacking lecithin, resulted in low levels of dietary n-6 series fatty acids. Although survival was acceptable with this diet, growth rate, FCE, PER and hence final production were very poor, suggesting that high levels of n-3 HUFAs may be responsible for enhanced survival, but that high levels of both n-3 and n-6 series fatty acids may be required for optimum growth and FCE.

Similar results have been found with juvenile *P. monodon* over a limited range of lecithin levels (Pascual, 1985, 1986, 1988) and both larval and juvenile *P. japonicus* fed phospholipid-rich diets (Kanazawa *et al.*, 1979a, 1985; Teshima *et al.*, 1986b,c). Teshima *et al.* (1982, 1986b,c) and Kanazawa (1985a,b) have concluded that it is either the choline and/or inositol and unsaturated fatty acid components of lecithin that are the effective portions for shrimp production. This may have been reflected in the results of

Table 5.7 Carcass composition (% dry weight) of shrimp on termination of the lipid/lecithin trial.

Proximate analysis ¹	Diet								
	1	2	3	4	5	6	7	8	PL15 (day 0)
Protein ²	54.0 ^a	41.5 ^a	52.0 ^a	42.5 ^a	41.5 ^a	41.0 ^a	50.5 ^a	50.1 ^a	53.1 ^{a3}
± SD	11.3	0.5	5.7	0.5	0.5	0.1	2.1	9.5	4.0
Lipid ⁴	4.3 ^a	5.0 ^a	4.5 ^a	5.0 ^a	6.2 ^a	6.0 ^a	5.8 ^a	4.4 ^a	4.8 ^a
± SD	0.1	0.2	0.7	0.1	0.5	0.1	0.5	0.1	0.3
Ash	19.5 ^a	17.5 ^a	18.1 ^a	15.9 ^a	18.6 ^a	19.8 ^a	17.3 ^a	19.3 ^a	20.9 ^a
± SD	2.9	0.7	0.9	4.9	2.1	2.0	2.4	0.8	1.0
Moisture	77.9 ^a	76.9 ^a	77.8 ^a	77.2 ^a	77.3 ^a	77.7 ^a	77.4 ^a	77.4 ^a	79.8 ^a
± SD	0.8	0.1	0.1	0.6	0.2	1.0	0.1	1.1	0.8
Carbohydrate and fibre ⁵	22.2	36.0	25.4	36.6	33.7	33.2	26.4	26.2	21.2

¹ Analyses (except for carbohydrate) based on mean of three replicates from carcasses pooled by treatment

² Analysed by method of Lowry (Lowry *et al.*, 1951)

³ For single criterion, mean values in the same row bearing different superscripts are significantly different ($P < 0.05$) by T-method

⁴ Analysed by method of Folch-Lees (Folch *et al.*, 1957)

⁵ By difference in dry weight

this trial, which showed enhanced performance of *P. monodon* fed diets including lecithin which contained high quantities of PC (18 %), PI/PG (54 %) and linoleic acid (21 %), and particularly in the presence of cod liver oil supplying relatively high levels of n-3 series HUFAs. None of the diets used during this trial however, contained quantities of n-3 series fatty acids or n-3:n-6 ratios comparable to the levels found in the shrimp carcass. Typically, shrimp body lipids comprise 23-34 % n-3 and 5-18 % n-6 series fatty acids at n-3:n-6 ratios of 1.5-6.8 (Shewbart *et al.*, 1973; Guary *et al.*, 1974, 1976; Joseph & Meyers, 1975; Colvin, 1976b; Teshima *et al.*, 1977; Clarke & Wickins, 1980; Chanmugan *et al.*, 1983).

5.4.2 Shrimp production

Overall shrimp performance during this trial was poor due probably to the purified nature of most of the ingredients used leading to diets which were unpalatable and had poor amino acid profiles. This phenomenon has been noticed previously with other species (New, 1980; Boghen & Castell, 1981; Maugele *et al.*, 1983b), but is partially compensated for by the more easily defined nature of semi-purified diets.

In diets containing 43.3 ± 1.3 % protein and 19.7 ± 0.2 kJ g⁻¹ total energy (by calculation), the best ($P < 0.05$) growth, FCE, PER and production was obtained with the inclusion of 3 % basal lipid and 3 % lecithin (at 46 % of the total lipid added) at a total lipid level of 4.5 %. This diet contained 0.15 % 20:5(n-3) and 0.13 % 22:6(n-3) and had a n-3:n-6 series fatty acid ratio of 0.5. In the absence of information on the quantitative requirements of shrimp for essential fatty acids, D'Abramo (1990) has suggested that dietary levels of 0.075 % may satisfy the requirement for any particular fatty acid. However, n-3 series HUFAs have previously been shown to act as feeding attractants in shrimp diets, suggesting that higher levels may increase feed ingestion (Bryant *et al.*, 1989). The results of this study showed that increasing dietary lipid levels to 8-8.6 %, and hence the level of the HUFAs 20:5(n-3) and 22:6(n-3) to 0.23-0.33 % and 0.15-0.22 % respectively, did not enhance shrimp performance. These diets however, did not have elevated ratios of n-3:n-6 series fatty acids. Successfully increasing the level of dietary lipid may thus require addition of n-3 series fatty acids (by increasing the inclusion of cod liver oil for example) to raise the n-3:n-6 ratio to

a level approaching that found in the shrimp carcass (1.5-6.8). Diets lacking a source of basal lipids, and hence n-3 series HUFAs resulted in very poor survival and production, confirming the essentiality of n-3 series HUFAs for *P. monodon*.

Pascual (1985) showed that *P. monodon* exhibited improved growth and FCR when lecithin levels were increased from 0 to 2 % in practical style diets containing 10 % total lipid. Her trials, however, were not designed to demonstrate the effects of higher levels of dietary lecithin. In the present study, it was found that although growth rate, FCE, PER and production were decreased by levels of dietary lecithin levels above 3 %, additions of up to 6 % dietary lecithin tended to enhance survival at total lipid levels of up to 6.6 %. Shrimp growth, FCE, PER and production rates were probably reduced at higher levels of dietary lecithin due to the increased levels of total lipid, n-6 series fatty acids and hence the reduced ratio of n-3:n-6 series fatty acids in these diets. Increased dietary levels of 18:2(n-6) (reducing n-3:n-6 ratios), resulting from inclusion of high levels of plant oils, have previously been shown to reduce growth of juvenile *P. monodon* (Catacutan, 1991a) and *P. japonicus* (Deshimaru *et al.*, 1979).

In common with the results of this study, larval *P. japonicus* have been shown to require 3 % lecithin in terms of growth and 6 % in terms of survival (0.7-1.8 % PC) in diets containing, optimally, 0.5-1 % cholesterol and 6-8 % pollack liver oil as the basal lipid source (Teshima *et al.*, 1982; Kanazawa *et al.*, 1985). These requirements, they suggested, may have been due to the role of lecithin in lipid (especially cholesterol) intestinal absorption and transport in the shrimp body, and may thus be modified by the overall dietary fatty acid composition as shown in the results of this trial.

Teshima *et al.* (1986a,b) also demonstrated that a deficiency of phospholipids in casein-based diets significantly reduced weight gain, FCR and lipid retention rates of *P. japonicus*. In contrast, Tackaert *et al.* (1991) were only able to demonstrate improved growth of post-larval *P. japonicus* for 17 days, after which time phospholipid-free diets stimulated better performance. The reason for this discrepancy is unclear.

A source of basal lipids (> 3 %) is therefore necessary to supply high levels of the

essential n-3 series fatty acids at relatively high (0.4-0.6) n-3:n-6 ratios, while lecithin at 46-63 % of the added lipid (providing 0.5-0.8 % dietary PC) has been shown able to enhance survival and growth at up to 6 % and 3 % respectively of diets containing 4.1-8.0 % total lipid. Previous work has shown that diets containing up to 1 % PC resulted in enhanced growth but not survival of juvenile *P. monodon* (Chen, 1993). Additionally, semi-purified diets containing total dietary lipid levels of between 5 and 10 % (with little attention to phospholipid levels) have usually been shown to result in better growth, survival and FCR than diets either lacking in, or containing more than 10 % dietary lipid when fed to *P. monodon* (Alava & Lim, 1983; Deshimaru *et al.*, 1985; Bautista, 1986; Sheen & Chen, 1992), *P. japonicus* (Shudo *et al.*, 1971; Deshimaru & Shigeno, 1972; Kanazawa *et al.*, 1977, 1979a-d, 1985; Deshimaru *et al.*, 1979; Teshima & Kanazawa, 1984), *P. indicus* (Colvin, 1976; Read, 1981; Ali, 1990) and *P. merguensis* (Sedgwick, 1979).

5.4.3 Carcass composition

The fact that no significant differences were found in the carcass lipid levels of shrimp during the trial period suggested that feeding diets containing up to 8.9 % lipid does not necessarily result in fatty carcasses. The levels of lipid measured in shrimp carcasses following this trial (4.3-6.2 % dry weight) were similar to the levels measured in other penaeid species (3.3-9.3 %) (Guary *et al.*, 1974; Colvin, 1976b; Teshima *et al.*, 1977; Clarke & Wickins, 1980; Chanmugan *et al.*, 1983). High lipid diets may thus have potential in protein sparing without adverse effects on shrimp quality. However, there were signs that the carcass lipid levels tended to increase with dietary lipid level, as was shown by Catacutan (1991a). It is possible that fatty carcasses may occur after an entire rearing cycle of feeding high-lipid diets. These aspects of lipid nutrition should therefore be the subject of further investigation.

5.5 SUMMARY

Despite poor overall performance due to the purified dietary ingredients used, leading to amino acid imbalances, diets lacking basal lipids resulted in poor shrimp survival and

hence production, whilst an absence of dietary lecithin resulted in poor growth, production, FCE and PER. Although not significantly better than the other diets, the highest production was obtained with a diet containing 3 % basal lipid and 3 % lecithin at a total lipid level of 4.5 %, protein level of 44.1 % and total energy level of 19.8 kJ g⁻¹ (by calculation). This diet contained 0.7 % cholesterol, 0.5 % PC, 0.15 % 20:5(n-3) and 0.13 % 22:6(n-3) at a n-3:n-6 series fatty acid ratio of 0.5.

The addition of dietary lecithin (particularly at 46-63 % of the total lipid added) at up to 3 % significantly ($P < 0.05$) increased shrimp production, largely as a result of increased growth rate. At low levels of dietary lipid (< 6.6 %), inclusion of 6 % dietary lecithin also tended to increase survival.

Increasing the total dietary lipid level from 4.5 to 8.9 % had no significant effect on growth, survival, FCE, PER or carcass lipid levels, although the highest growth rate was achieved at 4.5 % (3 % lecithin) and survival at 6.6 % (6 % lecithin). The total lipid requirements are consistent with those reported by other workers for penaeid shrimp, and are probably a result of the requirement for essential fatty acids, the stimulant properties of lecithin and the calorogenic effects of dietary lipid.

CHAPTER 6. The effect of dietary carbohydrate and lipid levels and their ratio on production, carcass composition, midgut gland amylase activity, diet digestibility and histology of post-larval *Penaeus monodon*.

The information contained in Chapter 6 has been submitted for publication in *Aquaculture and Fisheries Management*. Edited by D.H. Mills, R.J. Roberts & S.J. De Groot, Published by Blackwells.

6.1 INTRODUCTION

Dietary lipid is required by shrimp and other animals as a source of essential fatty acids and sterols for the maintenance and functional integrity of bio-membranes, to aid transport of fat-soluble vitamins, cholesterol and phospholipids and as a highly digestible (80-93 %, Teshima & Kanazawa, 1983; Catacutan, 1991a) source of energy (Tacon, 1990; Akiyama *et al.*, 1992). Unlike fish however, shrimp are not thought to be able to tolerate high levels of dietary lipid (Capuzzo, 1982; Millikin, 1982; D'Abramo, 1990; Cuzon, 1993).

The majority of work conducted on penaeid shrimp has suggested that dietary levels of between 5 and 10 % result in optimum growth and survival (Deshimaru & Shigeno, 1972; Colvin, 1976; Kanazawa *et al.*, 1977, 1985; Deshimaru *et al.*, 1979, 1985; Read, 1981; Alava & Lim, 1983; Teshima & Kanazawa, 1984; Bautista, 1986; Briggs *et al.*, 1994, chapter 5). The variation in these requirements is thought to depend largely upon species, age, lipid source or quality and the level of other dietary nutrients, particularly the alternative energy substrates of protein and carbohydrate (D'Abramo, 1990; Tacon, 1990; Akiyama, 1992; Akiyama *et al.*, 1992; Cuzon, 1993).

More recent work however, has suggested that dietary lipid levels of up to 12 % may be acceptable in nutrient-balanced diets fed to juvenile *P. monodon* (Catacutan, 1991a; Sheen & Chen, 1992). These results, if confirmed, may lead to the increasing use of lipid in shrimp diets as an energy source, sparing dietary protein for growth. This

situation has occurred in trout diets where lipid levels have been increased to as much as 24 % of the diet (Akiyama, 1992). In unbalanced diets however, dietary lipid levels of 10-17 % have been shown to result in decreased growth, survival and FCE in *P. monodon* (Deshimaru *et al.*, 1985; Bautista, 1986) and *P. japonicus* (Deshimaru *et al.*, 1979; Teshima & Kanazawa, 1984). High levels of dietary lipid have also been associated with fat deposition in the carcass, leading to reductions in dress-out yield, storage times and consumer acceptability (Clifford & Brick, 1979; Sedgwick, 1979; Chanmugan *et al.*, 1983).

In shrimp it has been supposed that carbohydrate may offer a promising alternative to lipid as a non-protein energy source (Colvin, 1976a; Bages & Sloane, 1981, Akiyama *et al.*, 1992). Although no specific dietary requirement for carbohydrate has been established, carbohydrates provide the cheapest source of dietary energy (Tacon, 1990). They are also important metabolic intermediates (Matty, 1989; Tacon, 1990; Akiyama *et al.*, 1992) and reduced growth, survival and protein deposition rates have resulted from feeding diets lacking carbohydrate (Teshima & Kanazawa, 1984; Alava & Pascual, 1987). Complex carbohydrates such as gelatinised starch also have an important role in binding formulated diets (Millikin, 1982; Tacon, 1990). The utilization of carbohydrates by crustaceans appears to be species specific and varies depending upon the complexity of the carbohydrate source, its dietary level and digestibility (Andrews *et al.*, 1972; Sick & Andrews, 1973; Deshimaru & Yone, 1978; Abdel-Rahman *et al.*, 1979; Capuzzo, 1982; Pascual, 1983; Pascual *et al.*, 1983; Alava & Pascual, 1987).

Crustaceans are able to metabolize complex starches and disaccharides well (at optimum dietary levels of 20-35 %), but simple sugars, such as glucose perform poorly as dietary energy sources (New, 1976a,b, 1980; Deshimaru & Yone, 1978; Abdel-Rahman *et al.*, 1979; Ali, 1982; Alava & Pascual, 1987; Gómez Díaz & Nakagawa, 1990; Shiau & Peng, 1992; Ali, 1993; Briggs, 1991). Moreover, metabolic studies have indicated that crustaceans utilise protein and lipid preferentially as energy sources with carbohydrate being of relatively minor importance except during moulting (Shewbart *et al.*, 1973; Tacon, 1990; Zhou, 1990). However, a protein sparing effect at high levels of dietary carbohydrate has been suggested for penaeid shrimp (Colvin, 1976a; Sedgwick, 1979; Bages & Sloane, 1981; Ali, 1982; Teshima & Kanazawa, 1984; Bautista, 1986; Shiau

& Chou, 1991; Shiau *et al.*, 1991a; Shiau & Peng, 1992), homarid lobsters (Capuzzo & Lancaster, 1979) and the freshwater prawn, *Macrobrachium rosenbergii* (Clifford & Brick, 1978, 1979). In addition, a suitable source of carbohydrate for use in chitin synthesis may substitute for carbon chains from amino acids and have some nutritional benefit and a protein sparing role in this way (New, 1976a,b). Dietary levels of over 30-40 % carbohydrate however, have been shown to reduce growth and survival (Andrews *et al.*, 1972; Bages & Sloane, 1981; Pascual *et al.*, 1983; Alava & Pascual, 1987; Catacutan, 1991b), and result in degenerative histological changes to the midgut gland of *P. monodon* (Pascual *et al.*, 1983).

Although many carbohydrase enzymes (including amylase, maltase, sucrase, chitinase and cellulase) have been detected in penaeid shrimp (Yokoe & Yasumasu, 1964; Van Wormhoudt *et al.*, 1972; Hood & Meyers, 1977; Lee & Lawrence, 1982, 1985; Maugle *et al.*, 1982a,b, 1983a,b; Lovett & Felder, 1990b), the apparent digestibility of carbohydrates is known to be low (usually 57-75 %, Condrey *et al.*, 1972; Fenucci *et al.*, 1982; Maugle *et al.*, 1983b; Akiyama *et al.*, 1989). Because the natural diet of shrimp is low in carbohydrate, it is thought that carbohydrate digestion may be poor due to the low activity of carbohydrase enzymes such as amylase, particularly in young shrimp (Elliot *et al.*, 1989). In support of this, supplementation of diets with microencapsulated amylase has been shown to increase starch digestion, growth, moulting and protein sparing in *P. japonicus* (Maugle *et al.*, 1983a,b). Chen & Lin (1990) were able to increase the growth rate of post-larval *P. monodon* by including acetone extracts from shrimp midgut glands in the diet, but showed that this was unrelated to midgut gland enzyme activity.

The hypothesis that suitable dietary formulations may be predicted from the relative digestive enzyme spectra of shrimp (Fair *et al.*, 1980; Lee *et al.*, 1980) has yet to be confirmed. Although it has been shown that *P. setiferus* can increase the activity of their digestive enzymes in response to a diet containing low levels of digestible protein (Lee & Lawrence, 1985), it is unclear whether shrimp can increase amylase activity and diet digestibility in response to high levels of dietary carbohydrate. This ability has been indicated to a small degree in *P. stylirostris* (Fenucci *et al.*, 1982), *P. japonicus* (Maugle *et al.*, 1983b) and *Homarus americanus* (Hoyle, 1973), but was not observed

in *Palaemon serratus* (Van Wormhoudt *et al.*, 1980). In addition, although post-larval *P. monodon* were observed to have altered digestive enzyme activities depending upon the diet (live or compounded) fed, growth rate could not be related directly to these activities (Chen & Lin, 1992).

It has been suggested that the best utilised diets for some crustacean species are those containing relatively high levels of carbohydrate and low lipid. Carbohydrate:lipid (C:L) ratios as high as 3-4:1 (including 8-10 % lipid) have been found to promote good growth and protein sparing in *P. monodon* (Bautista, 1986; Shiau & Chou, 1991; Shiau *et al.*, 1991a; Shiau & Peng, 1992), other species of penaeid shrimp (Andrews *et al.*, 1972; Colvin, 1976; Sedgwick, 1979; Cuzon *et al.*, 1993), *M. rosenbergii* (Clifford & Brick, 1978, 1979; Millikin *et al.*, 1980) and the crayfish *Procambarus acutus acutus* (Davis & Robinson, 1986). In contrast, some studies have shown that diets containing high levels of lipid (10-13 %) at C:L ratios of as little as 1.6-3.0:1 may result in protein sparing (Bages & Sloane, 1981; Alava & Pascual, 1987; Catacutan, 1991a,b; Sheen & Chen, 1992). These results suggest that the C:L ratio and the contribution that these non-protein energy sources make to the energy density of the diet may be as important as the dietary level of either nutrient.

For *P. monodon*, the few studies completed have suggested a dietary requirement for 5-10 % total lipid and 20-30 % carbohydrate. However, no study to date has analysed the effects of a wide range of carbohydrate and lipid levels and their ratio on the growth and survival of this species. The aims of this trial were therefore to study the effects of a range of lipid (4.5-12.8 %) and carbohydrate (20.8-45.4 %) levels resulting in a range of C:L ratios (1.6-10.1:1) in isonitrogenous, isoenergetic, semi-purified diets on the production, carcass composition, midgut amylase activity, diet digestibility and histology of post-larval *P. monodon*.

6.2 MATERIALS AND METHODS

6.2.1 Experimental animals

Post-larvae used in this trial were obtained and treated as in section 5.2.1 except that the post-larvae were transferred from the larval rearing bins to the recirculated brackish-water tank system (Appendix 1) at 10 days post-metamorphosis (PL₁₀) to acclimate them to the experimental conditions. At this stage they were weaned to the basal experimental pelleted diet for a period of 14 days.

6.2.2 Experimental design

Experimental design was as in section 5.2.2 except that 24 day old post-larvae were selected with mean weights of 27 ± 2 mg. Fifty shrimp of this size were randomly allocated to each of 24 fibreglass tanks of dimensions 0.75 X 0.7 X 0.2 m deep (0.53 m² area, 105 l volume) at a stocking density of 95 shrimp m⁻². Eight dietary treatments were tested, each in triplicate, in the brackish-water recirculating system. The tanks were supplied with brackish-water at the rate of 6 l min⁻¹ per tank.

Every ten days the shrimp were group-weighed to determine growth, survival and feeding rates, and individually weighed on termination of the trial on day 50 to quantify within-tank size variation.

6.2.3 Water quality

Water quality was monitored and maintained as in section 5.2.3. Water quality remained relatively constant throughout the duration of the trial. Dissolved oxygen remained above 6.6 mg l⁻¹ (> 100 % saturation), temperature was maintained at 30.0 ± 1.0 °C, pH at 8.4 ± 0.2 and salinity at 25 ± 2 ‰. Total ammonia nitrogen remained below 0.01 mg l⁻¹ and nitrite nitrogen below 0.01 mg l⁻¹ throughout the trial period. Water quality was not considered to be detrimental to the growth of the shrimp during the trial.

6.2.4 Diet formulation and feeding

Diets were formulated and prepared as in section 5.3.4, except that seven isonitrogenous (44.6 ± 0.7 % protein), isoenergetic (20.4 ± 0.4 kJ g⁻¹) semi-purified diets (Table 6.1) were formulated using casein and gelatin (3.6:1) as the protein sources. The best dietary carbohydrate source (from a previous trial, Briggs, 1991) of raw corn starch was included at levels from 21 to 45 %. The optimal dietary basal lipid source (from a previous unpublished trial) of cod liver oil and soybean oil at a 3:1 ratio was included at levels from 1.7 to 9 % at approximately 1.3 % increments. This equated to carbohydrate:lipid (C:L) ratios of between 1.6 and 10.1. In addition, constant levels of 0.5 % cholesterol (96 % 5-cholesten-3B-ol, Sigma Chemical Company Ltd.) and 3 % of powdered soybean lecithin (18 % PC, Nutripur, Lucus Meyer, Hamburg, Germany) were included (except for diet 1 at 1.5 % and diet 2 at 2 % lecithin) to give total dietary lipid levels ranging between 4.5 and 12.8 % (Table 6.1).

The dietary energy level was maintained within a narrow range by the manipulation of dietary α cellulose levels. α cellulose was considered as non-digestible filler (Tacon, 1990) and the dietary carbohydrate levels were based on those of starch only. Total dietary energy levels were measured by bomb calorimetry and calculated using mean total energy values of 23.6 kJ g⁻¹ for protein, 39.5 kJ g⁻¹ for lipid (using total lipid levels) and 17.2 kJ g⁻¹ for carbohydrate, as in section 5.2.4. Total energy (TE) levels of 20.4 ± 0.4 kJ g⁻¹ (by bomb calorimetry) and protein:energy (Pr:E) ratios of 22.7 ± 0.7 mg protein kJ⁻¹ TE (using calculated energy values for comparison with other studies) were thus maintained for all the formulated diets (Table 6.1).

All formulated diets contained 1 % trimethylamine hydrochloride (TMAH) as a feeding attractant (Costa-Pierce & Laws, 1985; Hartati & Briggs, 1993) which was found to counter the poor palatability of the semi-purified diets noted in previous studies at this laboratory (section 5.4.2) and elsewhere. The formulated diets were also supplemented with 1.7 % L-Arginine due to the deficiency of this amino acid in the protein components of the diets (Tacon & Cowey, 1984; Teshima *et al.*, 1986a). All formulated diets also included 1 % chromic oxide marker for use in determining the digestibility

Table 6.1 Composition and proximate analysis (% dry weight) of the diets used in the C:L ratio trial.

Ingredient	Diet							
	1	2	3	4	5	6	7	8
Casein	31.0	31.0	31.0	31.0	31.0	31.0	31.0	-
Gelatin	8.5	8.5	8.5	8.5	8.5	8.5	8.5	-
Raw corn starch	45.0	41.0	37.0	33.0	29.0	25.0	21.0	-
α Cellulose	0.0	2.3	4.7	7.1	9.8	12.5	15.2	-
Vitamin premix ¹	4.0	4.0	4.0	4.0	4.0	4.0	4.0	-
Mineral premix ²	4.0	4.0	4.0	4.0	4.0	4.0	4.0	-
Astaxanthin ³	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-
Cod liver oil	1.3	2.2	2.6	3.8	4.8	5.8	6.8	-
Soybean oil	0.4	0.7	0.9	1.3	1.6	1.9	2.2	-
Soy lecithin	1.5	2.0	3.0	3.0	3.0	3.0	3.0	-
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	-
Trimethylamine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	-
L-Arginine	1.7	1.7	1.7	1.7	1.7	1.7	1.7	-
Fresh <i>Mytilus</i>	-	-	-	-	-	-	-	100.0
+ water (20-30 %)	+	+	+	+	+	+	+	-

- continued over

Table 6.1 - continued

Proximate analysis ⁴	1	2	3	4	5	6	7	8
Protein	43.4	44.8	44.0	44.1	45.3	45.1	45.3	64.2
± SD	0.7	1.0	1.7	2.6	1.3	2.0	2.4	2.6
Ether extract ⁵	3.0	4.9	5.8	8.1	8.7	8.9	9.8	5.7
± SD	0.2	0.3	0.3	0.4	0.5	0.4	0.3	0.4
Total lipid ⁶	4.5	6.2	8.1	8.6	9.1	10.7	12.8	8.5
± SD	0.2	0.5	0.5	0.2	0.2	0.3	0.4	0.2
Carbohydrate ⁷	45.4	40.2	37.0	34.1	29.3	25.8	20.8	12.5
Fibre	1.5	3.7	6.1	8.3	11.5	13.8	16.5	5.1
± SD	0.1	0.1	0.4	0.3	0.5	0.7	0.9	0.3
Ash	5.2	5.1	4.8	4.9	4.8	4.6	4.6	9.7
± SD	0.2	0.6	0.1	0.1	0.1	0.1	0.1	0.2
Moisture	4.2	4.3	4.7	3.6	3.1	3.3	3.5	84.7
± SD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	2.1
Total energy (KJ g ⁻¹) ⁸	19.8	19.9	20.0	19.7	19.3	19.3	19.3	20.7
Total energy (kJ g ⁻¹) ⁹	19.8	20.0	20.4	20.7	20.7	20.3	20.6	22.2
Pr:E ratio (mg pr. kJ ⁻¹ TE) ¹⁰	21.9	22.5	22.1	22.4	23.4	23.4	23.4	31.1
Carbohydrate:lipid ratio	10.1	6.5	4.6	4.0	3.2	2.4	1.6	1.5

¹ Vitamin Premix as Table 5.1

² Mineral Premix as in Table 5.1

³ Astaxanthin as in Table 5.1

⁴ All results based on triplicate analyses

⁵ Analysed by Soxhlet method

⁶ Analysed by method of Folch-Lees (Folch *et al.*, 1957)

⁷ By difference in dry weight

⁸ By calculation

⁹ By bomb calorimetry

¹⁰ Using calculated TE figures

of the diets during the trial (NRC, 1983).

Based on previous experience (chapter 5), feeding was carried out twice daily at 0900 and 1700 for nine days out of every ten (not feeding on sampling day). The shrimp were fed at 35 % of body weight daily for days 1-9, 30 % for days 11-19, 25 % for days 21-29, 20 % for days 31-39 and 15 % for days 41-49 on a wet shrimp/dry feed basis. This feeding rate was higher than that used in chapter 5 because all of the food was consumed at the lower rate fed during that trial.

The seven formulated diets were compared to a control diet of chopped fresh mussel (*Mytilus edulis*) fed at the rate of 230, 200, 160, 130 and 100 % of wet weight (consistent with the weight-specific ration of pelleted diets on a dry weight basis) for the five nine day periods respectively.

6.2.5 Midgut gland amylase level determination

Midgut glands were excised on termination of the trial from frozen (-20 °C) shrimp. Individual glands were homogenized in 2 ml of chilled, 0.1 M phosphate buffer (pH 7) then centrifuged at 20,000 rpm for 30 minutes at 2 °C and assayed using 200 μ l subsamples. The assays were run using the Phadebas amylase kit (Pharmacia Diagnostics AB, Uppsala, Sweden). Each assay was performed in duplicate from extracts prepared from each of three replicate shrimp from each treatment. The protein contents of the supernatant solutions used in the amylase assay were determined using a Bradford assay standardised against bovine gamma globulin (Bradford, 1976). Amylase activity was expressed as $u\ mg^{-1}$ soluble protein where one unit (u) is defined as the amount of enzyme catalysing the hydrolysis of 1 μ mol glucosidic linkage per minute at 37 °C.

6.2.6 Diet digestibility determination

In order to determine the dry matter digestibility of the diets, the technique developed in this laboratory (Hartati & Briggs, 1993) was used. During the final 10 days of the trial, all tanks were siphoned clean prior to the first feeding of the day. The diets were

then fed and three hours later (before excessive leaching was presumed to have occurred) all faecal material was siphoned from the tanks, sieved to separate faeces from uneaten food and exuviae, pooled daily for each replicate of each treatment and stored at -15°C for subsequent analysis. 50-100 mg triplicate samples of moisture-free diets and faeces were analysed for chromic oxide content after the method of Furukawa & Tsukahara (1966).

The apparent dry matter digestibility co-efficient (ADC) for each diet was computed according to the formula:

$$\text{ADC} = 100 - \left[100 \times \frac{\% \text{ indicator in food}}{\% \text{ indicator in faeces}} \right]$$

6.2.7 Sample collection, analysis and histology

Shrimp and diet samples were collected and analysed as in section 5.2.5 except that lipid class and fatty acid analyses of diets and shrimp were not performed. In addition, six shrimp from each treatment were fixed for histological examination. Shrimp carapaces were slit and tissues injected for rapid penetration of the Davidson's fixative (Bell & Lightner, 1988, Appendix 2). The tissues were then dehydrated in ascending grades of alcohol, cleared in chloroform, embedded in wax and sectioned on a rotary microtome at 5 µm. The tissues were then stained with haematoxylin and eosin (H & E) and the periodic acid-Schiff (PAS) reaction for carbohydrate (Drury & Wallington, 1980). The sections were then examined by light microscope (Olympus CHB) for histopathological changes in the midgut gland as compared to shrimp fed the live food control and normal histology as detailed in Bell & Lightner (1988).

6.2.8 Data analysis

Data obtained from this trial was analysed as described below:-

1. Specific growth rate (SGR), survival, production and food conversion efficiency (FCE) were analysed as described in section 3.2.1.6. Protein efficiency ratio (PER) was analysed as described in section 5.2.6.
2. The effects of dietary carbohydrate:lipid ratio on shrimp growth, survival (arcsin transformed), production, FCE, PER and carcass composition after 50 days were analysed using a non-parametric analysis of variance (Kruskal-Wallis) test after Bartlett tests revealed that in all cases the data were heteroscedastic (Sokal & Rohlf, 1981).
3. Where appropriate, the T-method for multiple comparisons was used to determine statistical differences between treatment means for the above ANOVAs (Sokal & Rohlf, 1981). Results were considered significant if $P < 0.05$.
4. Regression analyses were used to identify possible relationships between the dietary carbohydrate:lipid ratio and shrimp growth, survival, production, FCE, PER and carcass composition (Sokal & Rohlf, 1981).

6.3 RESULTS

6.3.1 Shrimp production

Production and growth of shrimp was significantly ($P < 0.05$) better when fed the control diet of fresh mussel (*M. edulis*) than when fed any of the test diets (Table 6.2). This was probably due to the low acceptance and sub-optimal amino acid balance of the semi-purified dietary ingredients used.

Shrimp growth rate did not vary significantly between the formulated diets tested (Table 6.2, Figure 6.1). However, as the C:L ratio was decreased (*i.e.* increased lipid and decreased carbohydrate) shrimp survival and hence production significantly ($P < 0.05$) increased (Table 6.2, Figures 6.2 & 6.3). The production of post-larval *P. monodon*

Table 6.2 Performance of post-larval *P. monodon* fed diets varying in carbohydrate and lipid levels over the 50 day C:L ratio trial period.¹

Diet	Carbohy- drate level (%)	Lipid level (%)	Cho: lipid ratio	Mean survival (%)	Mean body weight (g)		SGR (% body wt d ⁻¹)	Production (g m ⁻² d ⁻¹)	FCE	PER	Digestibility (%)
					Initial	Final					
1	45.4	4.5	10.1	32.7 ^{ab2}	0.026 ^a	0.734 ^a	6.70 ^a	0.41 ^a	0.22 ^a	0.51 ^a	42.19 ^c
± SD	-	-	-	5.0	0.001	0.022	0.20	0.05	0.02	0.05	0.58
2	40.2	6.2	6.5	27.3 ^a	0.027 ^a	0.948 ^a	7.06 ^a	0.42 ^a	0.23 ^a	0.51 ^a	36.43 ^d
± SD	-	-	-	8.3	0.001	0.267	0.44	0.06	0.02	0.05	0.85
3	37.0	8.1	4.6	33.3 ^{ab}	0.026 ^a	0.906 ^a	7.06 ^a	0.51 ^{ab}	0.25 ^a	0.56 ^{ab}	39.13 ^{de}
± SD	-	-	-	6.4	0.001	0.165	0.15	0.04	0.01	0.02	1.18
4	34.1	8.6	4.0	46.0 ^b	0.028 ^a	0.711 ^a	6.49 ^a	0.57 ^{ab}	0.24 ^a	0.55 ^{ab}	20.20 ^b
± SD	-	-	-	2.0	0.001	0.026	0.12	0.04	0.01	0.01	1.33
5	29.3	9.1	3.2	51.3 ^{bc}	0.028 ^a	0.736 ^a	6.54 ^a	0.67 ^{bc}	0.27 ^a	0.60 ^{ab}	27.12 ^b
± SD	-	-	-	3.1	0.001	0.064	0.12	0.05	0.02	0.05	2.69
6	25.8	10.7	2.4	48.0 ^b	0.025 ^a	0.820 ^a	6.95 ^a	0.69 ^{bc}	0.30 ^a	0.67 ^b	37.50 ^d
± SD	-	-	-	8.0	0.001	0.096	0.13	0.04	0.03	0.06	0.67
7	20.8	12.8	1.6	53.3 ^{bc}	0.027 ^a	0.806 ^a	6.78 ^a	0.76 ^c	0.27 ^a	0.60 ^{ab}	32.56 ^c
± SD	-	-	-	10.1	0.001	0.081	0.23	0.07	0.03	0.07	1.44
8	12.5	8.5	1.5	47.3 ^b	0.026 ^a	1.554 ^b	8.17 ^b	1.35 ^d	0.29 ^a	0.46 ^a	-
± SD	-	-	-	3.1	0.001	0.092	0.34	0.14	0.06	0.10	-

¹ Based on the mean of three replicates

² For single criterion, mean values in the same column bearing different superscripts are significantly different ($P < 0.05$) by T-method

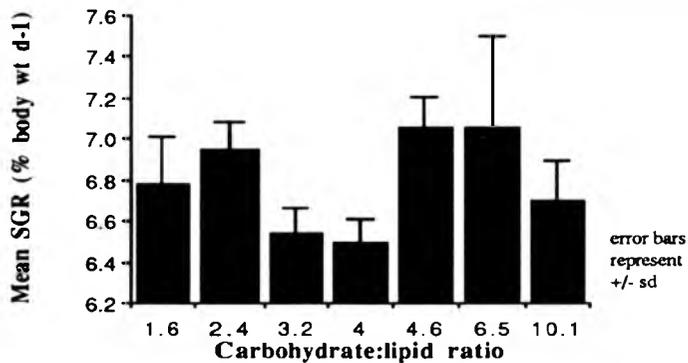


Figure 6.1 Relationship between dietary carbohydrate to lipid ratio and mean SGR of shrimp over 50 days.

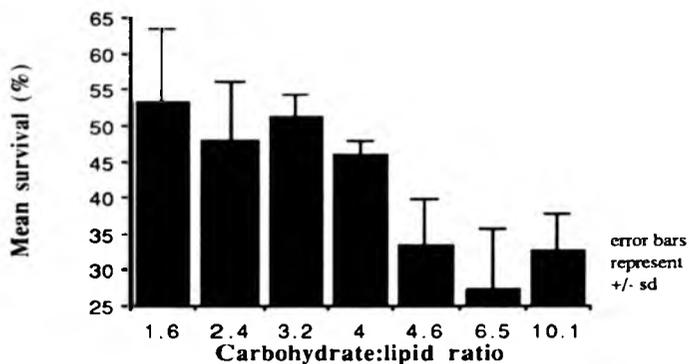


Figure 6.2 Relationship between dietary carbohydrate to lipid ratio and mean shrimp survival after 50 days.

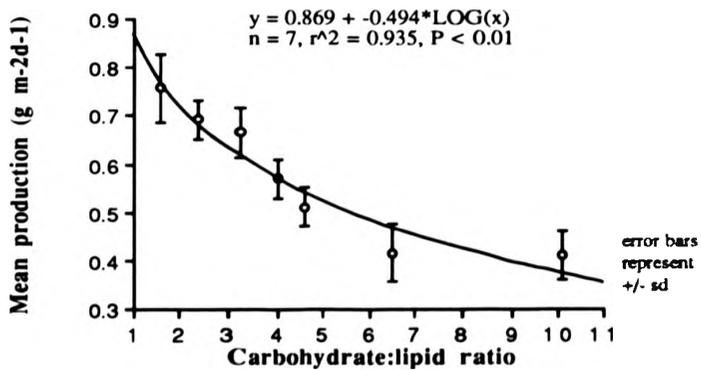


Figure 6.3 Relationship between dietary carbohydrate to lipid ratio and mean shrimp production over 50 days.

over the 50 day trial was negatively related to the dietary C:L ratio (Figure 6.3). Production decreased curvilinearly in relation to increasing dietary C:L ratio whereby Log_e production ($\text{g m}^{-2} \text{d}^{-1}$) = $0.869 - 0.494 \text{ Log}_e$ C:L ratio, $r^2 = 0.935$ and $P < 0.01$. Thus, diets containing > 9.1 % lipid and < 30 % carbohydrate at C:L ratios of $< 3.2:1$ promoted the best shrimp survival and production. The highest rates of shrimp survival and production were achieved on a diet containing 55 % of the TE from protein, 26 % from lipid and 19 % from carbohydrate.

Shrimp FCE did not vary significantly, although the trend was for increasing FCE as the C:L ratio decreased, probably due to the greater overall production rates achieved on these high lipid diets (Table 6.2). In contrast, PER increased significantly with decreasing C:L ratio, with the best PER of 0.67 being achieved on a diet containing 25.8 % carbohydrate and 10.7 % lipid, at a C:L ratio of 2.4 (Table 6.2, Figure 6.4).

6.3.2 Carcass composition

The different dietary C:L ratios tested in this trial had no significant effect upon carcass protein, ash, moisture or carbohydrate levels of shrimp fed formulated diets or fresh *Mytilus* (Table 6.3). In contrast, at a constant level of dietary protein (44.6 ± 0.7 %), total energy ($20.4 \pm 0.4 \text{ kJ g}^{-1}$) and hence protein:energy ratio ($22.7 \pm 0.7 \text{ mg protein kJ}^{-1} \text{ TE}$), as the dietary lipid level increased (*i.e.* at progressively lower C:L ratios) the level of lipid in the shrimp carcasses increased linearly from 5.4 to 7.4 % ($A = 4.095$, $b = 0.252$, $r^2 = 0.909$, $P < 0.01$) (Table 6.3, Figure 6.5). However, only diet 7, containing the highest dietary lipid level (12.8 %) promoted a significantly ($P < 0.05$) higher level of carcass lipid than diets containing < 5.7 % lipid (Table 6.3).

6.3.3 Midgut gland amylase level

Analysis of the amylase levels detectable in the shrimp midgut glands on termination of the trial revealed no significant difference between amylase activity in shrimp fed the various dietary formulations or between the formulated diets and fresh *Mytilus* (Table 6.4). Higher levels of dietary carbohydrate however, tended to increase the levels of midgut gland amylase, while shrimp fed fresh *Mytilus* displayed the lowest level of

Table 6.3 Carcass composition (% dry weight) of shrimp on termination of the C:L ratio trial.

Proximate analysis ¹	Diet								
	1	2	3	4	5	6	7	8	PL ₁₅ (d 0)
Protein ²	52.3 ^a	49.2 ^a	50.4 ^a	50.4 ^a	48.6 ^a	50.4 ^a	50.0 ^a	48.0 ^a	53.1 ^{a3}
± SD	1.9	0.6	3.5	2.0	2.5	3.3	1.4	2.4	3.5
Lipid ⁴	5.4 ^a	5.6 ^a	5.7 ^a	6.5 ^{ab}	6.4 ^{ab}	6.8 ^{ab}	7.4 ^b	6.9 ^{ab}	4.8 ^a
± SD	0.7	0.3	0.7	1.0	0.7	0.4	1.0	0.4	0.5
Ash	19.3 ^a	18.5 ^a	17.8 ^a	19.2 ^a	19.6 ^a	18.0 ^a	18.0 ^a	17.5 ^a	20.9 ^a
± SD	1.3	1.2	0.9	0.6	0.3	0.5	0.6	0.4	1.0
Fibre and Carbohydrate ⁵	23.0	26.7	26.1	23.9	25.4	24.8	24.6	27.6	21.2
Moisture	76.3 ^a	76.3 ^a	75.8 ^a	76.2 ^a	76.3 ^a	76.0 ^a	75.8 ^a	76.4 ^a	78.6 ^a
± SD	1.2	1.0	1.0	0.9	0.1	0.6	0.5	0.8	1.0

¹ Analyses (except for carbohydrate) based on mean of three replicates from carcasses for each replicate of each treatment

² Analysed by method of Lowry (Lowry *et al.*, 1951)

³ For single criterion, mean values in the same row bearing different superscripts are significantly different (P < 0.05) by T-method

⁴ Analysed by method of Folch-Lees (Folch *et al.* 1957)

⁵ By difference in dry weight

Table 6.4 Midgut gland amylase levels of shrimp on termination of the C:L ratio trial.

Parameter	Diet							
	1	2	3	4	5	6	7	8
Dietary carbohydrate level (%)	45.4	40.2	37.0	34.1	29.3	25.8	20.8	12.5
Midgut amylase (μ mg ⁻¹ sol. pr) ¹	2.1 ^a	3.1 ^a	3.5 ^a	3.0 ^a	2.8 ^a	2.8 ^a	2.2 ^a	1.7 ^{a2}
\pm SD	0.2	0.7	0.3	0.6	0.8	0.9	1.0	0.8

¹ Assays based on duplicate measurements on extracts prepared from triplicate tanks of shrimp per diet (n = 6)

² For single criterion, mean values in the same row bearing different superscripts are significantly different (P < 0.05) by T-method

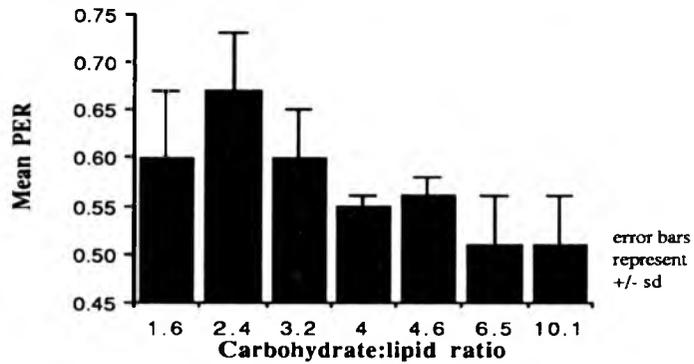


Figure 6.4 Relationship between dietary carbohydrate to lipid ratio and mean PER of shrimp over 50 days.

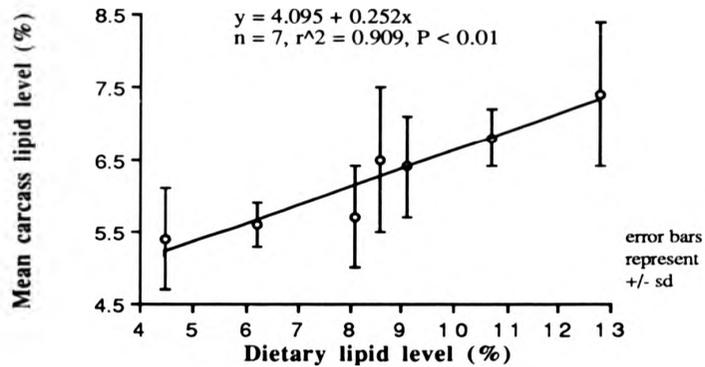


Figure 6.5 Relationship between lipid level in the diet and in the shrimp carcass after 50 days.

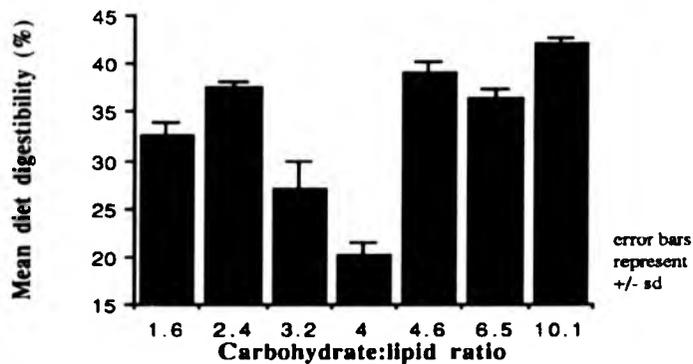


Figure 6.6 Relationship between dietary carbohydrate to lipid ratio and mean diet digestibility by shrimp over days 40-50.

amylase activity.

6.3.4 Diet digestibility

During this trial very low digestibility values were recorded for all of the formulated diets tested (Table 6.2, Figure 6.6). However, from the results obtained, significant ($P < 0.01$) differences were found between the formulated diets tested. The highest digestibility (36-42 %) was achieved by shrimp fed diets 1-3 *i.e.* at high carbohydrate (37-46 %) and low lipid (4.5-8.1 %) levels (C:L ratios of $> 4.6:1$). However, high lipid (> 10.7 %) and low carbohydrate (< 26 %) diets at C:L ratios of $< 2.4:1$ (diets 6 & 7) were also relatively well (33-38 %) digested.

6.3.5 Midgut gland histology

In general, no pathological changes were noticed in the tissues of shrimp fed any of the trial diets. However, there were differences between midgut gland tissue of shrimp fed the various diets. In shrimp fed diets with high C:L ratios (diets 1-3), compared to those fed the control diet, midgut gland tissue showed few large secretory B-cells with many vacuoles and low levels of lipid and carbohydrate storage. In contrast, in shrimp fed diets with low C:L ratios (diets 5-7), the midgut gland tissues displayed large numbers of vacuoles and lipid storage in the small absorptive and secretory R-cells. High levels of dietary lipid therefore seemed to increase lipid storage in the midgut gland, as reflected in the higher levels of carcass lipid (Table 6.3, Figure 6.5). However, the changes did not seem pathological, but rather a physiological adaption to these diets, as reflected in the higher survival rate shown by shrimp fed these diets (Table 6.2, Figure 6.2).

6.4 DISCUSSION

6.4.1 Shrimp production

No significant differences were found between the growth rate of shrimp fed any of the

formulated diets during this trial. Similarly, Deshimaru *et al.*, (1985) reported that there was no relationship between total dietary lipid level (from 4.3 to 10.5 %) and growth in *P. monodon*. However, their diets were formulated using different nutrient sources so direct comparisons may not have been valid. In the present study, diets containing the highest level of lipid and lowest level of carbohydrate (lowest C:L ratios) resulted in the best shrimp survival and hence production rates.

Results suggest that when carbohydrate levels are maintained at < 30 % and lecithin levels at 3 %, inclusion of basal lipids (containing PUFAs and HUFAs) at up to 9 % (12.8 % total lipid) in energy-balanced diets can lead to enhanced shrimp survival and production. The generally low rate of survival obtained during this trial was mostly due to cannibalistic mortalities following moulting induced by handling stress on weighing. This stress however, was relatively constant for all diets tested.

In common with the results of this trial, Tacon (1990) recommends 10-12 % lipid in diets for omnivorous shrimp species at a 5:1 ratio of animal (marine fish):plant oils. Catacutan (1991a) found optimum growth and survival of *P. monodon* at dietary lipid (cod liver oil) levels of 12.8 %. Sheen & Chen (1992) found that isonitrogenous, isoenergetic diets resulted in optimum growth rate of juvenile *P. monodon* when between 8 and 12 % of lipid derived from cod liver oil and corn oil (2:1) were added. Similarly, Bautista (1986) found that total lipid levels (cod liver oil:soybean oil at a ratio of 1:1) of up to 10 % promoted better growth and FCE than diets containing 5 % lipid, but that 15 % dietary lipid resulted in high carcass lipid levels and presumably deleterious histopathological changes in the midgut gland.

In contrast, Colvin (1976b) suggested that there was no advantage to be gained from supplementing compound diets fed to *P. indicus* with 5 % of various seed oils (to give total dietary lipid levels of 10 %). However, the oils used did not supply long-chain polyunsaturated fatty acids (PUFAs) which may be required for optimal lipid retention efficiency (Kanazawa *et al.*, 1979d). D'Abramo (1990), in a review of lipid requirements of shrimp, reported that high levels of lipid are usually associated with significant growth retardation and lipid deposition in the carcass. He points out however, that the requirements are influenced by the overall balance of energy sources

in the diets.

Results from a previous trial (Briggs *et al.*, 1994, chapter 5) revealed that shrimp growth and production was optimum at 4.5-6.6 % total lipid, in the presence of 3 % lecithin. This was probably due to the presence of lecithin enhancing lipid transport and absorption (Cuzon, 1993) and possibly increasing feed attractivity and ingestion (Bryant *et al.*, 1989). Increases in dietary lipid up to 8.9 %, although not affecting survival, resulted in decreased growth and hence production rates. These diets however, included carbohydrate levels of > 33 % which may have led to protein:energy imbalances.

In the present study, dietary carbohydrate levels of > 30 % (*i.e.* when contributing > 60 % of the non-protein energy (NPE)), resulted in depressed shrimp survival and production. Thus, as the dietary lipid level was increased until it contributed > 40 % NPE (*i.e.* at C:L ratios of < 3.2:1), shrimp production was increased. The poor survival and production rates obtained with diets highest in carbohydrate was probably due to the fact that diets 1 and 2 contained < 3 % basal lipid and only 1.5 and 2 % lecithin respectively, less than the 3 % basal lipid and 3-6 % lecithin found to be optimal for growth and survival of *P. monodon* (Briggs *et al.*, 1994, chapter 5). However, reduced shrimp growth and survival have also frequently been associated with dietary carbohydrate levels exceeding 30-40 %, probably due to poor diet digestibility (Shiau & Peng, 1992), deleterious histopathological changes (Pascual *et al.*, 1983) and possibly nutrient imbalances (Clifford & Brick, 1978; Akiyama, 1992).

The lowest levels of carbohydrate tested (21-29 %), in combination with high levels of lipid (9-13 %) at C:L ratios of 1.6-3.2:1 were found to result in optimum survival and production in this trial. Similarly, Catacutan (1991b) found that growth, FCE and survival of juvenile *P. monodon* tended to decrease with increasing carbohydrate inclusion levels (up to 35 %) in diets containing 40 % protein and 9-12 % lipid. Better survival and moulting of juvenile penaeid shrimp has previously been noted with semi-purified diets containing 10-20 % carbohydrate than with those containing 40 % (Abdel-Rahman *et al.*, 1979; Pascual *et al.*, 1983). Optimum growth of juvenile penaeid shrimp has also been noted to result from diets containing carbohydrate levels of up to 20 % (Bautista, 1986; Alava & Pascual, 1987) or 30 % (Andrews *et al.*, 1972; Shiau

& Peng, 1992).

In contrast, optimum growth of juvenile *P. monodon* (Pascual *et al.*, 1983) and *P. indicus* (Ali, 1982) has sometimes been observed at dietary carbohydrate levels of 40 %. In both of these studies however, the total energy level of their diets increased from 12.5 to 17 kJ g⁻¹ (by calculation) with increasing carbohydrate levels of 10 to 40 %, leading to difficulties with the interpretation of their results, particularly with regard to feed intake levels. The inverse relationship noted in this study (and that of Pascual *et al.*, 1983) between growth and survival may also partially explain these discrepancies. Moreover, Bages & Sloane (1981) found that growth of post-larval *P. monodon* was proportional to protein content between 25 and 55 %, but independent of carbohydrate (starch) levels between 10 and 40 % at 10 % dietary lipid. There therefore seems to be no benefit in adding dietary carbohydrate at over 30 % in diets for juvenile penaeid shrimp.

6.4.2 Carcass composition

Carcass lipid tended to increase with increasing dietary lipid levels. Although carcass lipid was only significantly increased at the highest dietary lipid level tested, shrimp fed this diet did not accumulate significantly more lipid than those fed the fresh *Mytilus* diet. This increase in carcass lipid was due to the greater deposition rates of lipid by shrimp fed high lipid diets, but may have also resulted from increased (although unquantifiable) feed ingestion.

In common with the results of this study, levels of dietary lipid in excess of 10 % have previously been recognised as having adverse effects upon carcasses lipid levels, which can reduce storage times and consumer acceptability (Andrews *et al.*, 1972; Forster & Beard, 1973; Deshimaru & Kuroki, 1974; Clifford & Brick, 1979; Sedgwick, 1979, NRC, 1983; Cho *et al.*, 1985; Bautista, 1986; D'Abramo, 1990; Catacutan, 1991a). In contrast, but also in common with this study, relatively high carbohydrate levels of 30-35 % have been shown to depress lipid deposition rates in juvenile *P. monodon* (Alava & Pascual, 1987; Catacutan, 1991b). This may be because stored lipid can be rapidly withdrawn from the abdominal muscle and the midgut gland of shrimp when required

(Barclay *et al.*, 1983). This lipid can then be used for energy production in high carbohydrate, low digestibility diets where the animal has a poor ability to convert the excess carbohydrate energy into lipids or non-essential amino acids (Tacon, 1990). Alternatively, low carcass lipid levels may result from feeding diets high in carbohydrate through increases in the rate of lipid excretion as shown by Catacutan (1991b). High rates of lipid deposition or mobilisation may thus be due to nutrient imbalances or deficiencies in relation to energy levels and/or lipid quality. Optimal balancing of energy sources is thus an area requiring further research (D'Abramo, 1990; Tacon, 1990; Akiyama *et al.*, 1992).

6.4.3 Midgut gland amylase level

Analysis of the amylase levels in the midgut glands of shrimp in this study suggested that during the 50 day rearing period, the shrimp were not able to significantly increase the level of amylase activity in response to high carbohydrate diets over that measured in shrimp fed diets low in carbohydrate. This suggests that the shrimp may have been unable to utilise high levels of dietary carbohydrate. However, due to the poor growth rates monitored and the fact that the digestive system of shrimp may not be complete until PL₃₅ (Motoh, 1984), it is difficult to draw any firm conclusions from this result.

Similar results have been observed with *Palaemon serratus*, where maximal specific amylase activity was achieved with only 2.8 %, and optimum growth at 3.5 % carbohydrate (Van Wormhoudt *et al.*, 1980). It is not clear from these results however, whether the amylase activity detected was sufficient to digest high levels of carbohydrate or whether high dietary carbohydrate levels result in poor shrimp growth for other reasons such as excessive levels of dietary energy, limiting feed and protein intake (Maugle *et al.*, 1983b), or histopathological changes in the tissues (Pascual *et al.*, 1983).

Although these latter reasons may have contributed to the results, some studies have shown that shrimp may be able to alter the activity of some digestive enzymes in response to dietary composition (Lee & Lawrence, 1985; Lee *et al.*, 1985; Chen & Lin, 1992). Interpretation of these results however, is complicated by the fact that the

proximate composition of the midgut gland may vary with dietary formulation (Lee *et al.*, 1985), and because shrimp age and the stage in the moult cycle also affect enzyme activity (Van Wormhoudt, 1983; Lee *et al.*, 1984; Elliot *et al.*, 1989). In addition, digestive enzyme activity does not seem to be related to either growth rate (Lee *et al.*, 1984; Van Wormhoudt *et al.*, 1980; Galgani *et al.*, 1988; Chen & Lin, 1992) or diet digestibility (Fenucci *et al.*, 1982; Lee *et al.*, 1984; Lee & Lawrence, 1985; Smith *et al.*, 1985; Eusebio, 1991), although there was some evidence for this in larval *P. monodon* (Jones *et al.*, 1979a,b).

Despite these problems with analysis of digestive enzyme activity, Hoyle (1973) suggested that although amylase activity in *H. americanus* was not significantly affected by increasing dietary carbohydrate levels, a slight increase occurred over the trial period, suggesting some level of adaption. A trend towards increasing amylase activity as dietary carbohydrate levels were increased to 37 % was also noted in this study. Similar results were also found with *P. japonicus* (Maugle *et al.*, 1983b) and *P. stylirostris* (Fenucci *et al.*, 1982).

In contrast, research supplementing shrimp diets with microencapsulated α amylase (Maugle *et al.*, 1983a,b) or acetone extracts from shrimp midgut glands (Chen & Lin, 1990) has shown that this exogenous enzyme could enhance shrimp growth. It was suggested that this may occur through a protein sparing action since the digestibility of the carbohydrate source (starch) included at 19 % of the diet containing an amylase supplement increased over that achieved by shrimp fed diets lacking supplementary amylase (Maugle *et al.*, 1983b). These results suggest that endogenous amylase activity may indeed limit growth on diets high in carbohydrate. The technique of supplementing microencapsulated amylase to shrimp diets would thus seem to offer a better means of enhancing the utilisation of dietary carbohydrate and possibly promote protein sparing and the development of cheaper diets.

6.4.4 Diet digestibility

The low and fluctuating digestibility values obtained during this trial were probably due to contamination of the faeces with uneaten food and exuviae, and possibly differential

estion of chromic oxide and faeces. The data may therefore have little value in interpreting the results of this study. Catacutan (1991b) used adult (30-40 g) rather than juvenile *P. monodon* in order to estimate the apparent digestibility of practical diets. Her use of adult shrimp facilitated faecal collection and hence digestibility determination. She found digestibilities of 93-94 % for protein and 90-93 % for lipid, but only 76-87 % for dry matter, possibly due to low carbohydrate and/or α cellulose digestibility. In her study, lipid at 9.5-12 % inclusion, was thus well digested (as was protein), and dry matter digestion increased significantly with the C:L ratio. This result was obtained despite the fact that carbohydrate digestibility has previously been shown to be low (36-76 %) in shrimp (Fenucci *et al.*, 1982; Akiyama *et al.*, 1988; Akiyama, 1991). The direct relationship between diet dry matter digestibility and C:L ratio noticed for diets 1-4 in this study and for the diets used by Catacutan (1991b), may thus reflect the strong correlation between dry matter digestibility and dietary α cellulose (fibre) levels in both of these studies (see chapter 7). Diets 5-7 in this study however, despite containing high levels of α cellulose, contained low (< 30 %) levels of carbohydrate and were digested relatively well. This suggests that dry matter digestibility may depend on the dietary levels of both carbohydrate and α cellulose. In addition, the use of α cellulose as a binder can cause problems with differential binding of ingredients such as lipid.

6.4.5 Midgut gland histology

Physiological changes in the midgut gland (increased numbers of vacuoles and lipid storage in the R-cells), in conjunction with high carcass lipid levels, were recorded in shrimp fed the high lipid diets. Over the trial period, these changes did not seem pathological, but require evaluation over a complete rearing cycle to ascertain possible deleterious long-term effects. Similar histopathological changes have been noticed by previous authors when the levels of various carbohydrate sources were raised from 10 to 30-40 % (Alava & Pascual, 1987; Storch *et al.*, 1984), when lipid levels were raised over 15 % (Bautista, 1986) or under starvation (Vogt *et al.*, 1985). However, Pascual *et al.* (1983) found histopathological changes (degeneration of the midgut gland and hyperplasia of the gill filaments' cuticle) in *P. monodon* fed all of their semi-purified diets suggesting that the purified ingredients themselves rather than lipid or

carbohydrate levels *per se* may be partially responsible.

6.5 SUMMARY

Shrimp fed diets with high lipid and low carbohydrate levels (low C:L ratios) achieved significantly ($P < 0.01$) higher survival and production rates, but also higher carcass lipid levels than those fed diets with high C:L ratios. Results indicated that total dietary lipid levels of 9.1-12.8 % were optimum in nutrient-balanced diets fed to post-larval *P. monodon*. When carbohydrate levels were maintained at < 30 % and lecithin levels at 3 %, inclusion of basal lipids at up to 9 % of the diet (C:L ratios of $< 3:1$) improved shrimp production. During this trial, the best partitioning of dietary energy at a total energy level of 19.6 ± 0.3 kJ g⁻¹ (20.4 ± 0.4 kJ g⁻¹ by calorimetry) and protein:energy ratio of 22.7 ± 0.7 mg protein kJ⁻¹ TE (by calculation) was obtained in a diet containing protein accounting for 55 %, lipid 26 % and carbohydrate 19 % of the total energy.

These results indicate that post-larval *P. monodon* appear to be able, not only to tolerate higher levels of dietary lipid than is generally acknowledged, but to achieve higher production when fed semi-purified diets containing between 9.1 and 12.8 % total dietary lipid at low C:L ratios of $< 3.2:1$. It is probable that such increases in dietary lipid from the 7-10 % level usually regarded as optimum (Alava & Lim, 1983; Deshimaru *et al.*, 1985; Bautista, 1986; Hajra *et al.*, 1988) up to 12 % can stimulate shrimp production only in diets containing balanced quantities of protein and non-protein energy.

The use of higher lipid levels however, may be constrained by increasing carcass lipid levels (ideally this should be studied over a complete production cycle) and limits imposed by diet pelletising, shrimp processing and diet storage (due to increased lipid oxidation). Further trials (chapters 7 & 8) will investigate whether, by decreasing the protein content to below 40 %, increased levels of the non-protein energy sources of carbohydrate and particularly lipid can maintain shrimp production.

CHAPTER 7. Protein-sparing effects of dietary lipid and carbohydrate on production, carcass composition and diet digestibility of juvenile *Penaeus monodon*.

People who are greedy have extraordinary capacities for waste - they must, they take in too much.

Norman Mailer, 1968

7.1 INTRODUCTION

Determination of the maximum sparing role of non-protein energy sources is necessary in order to optimise protein levels and reduce diet cost. The total energy level and protein:energy ratio of shrimp diets is important because on the one hand, excessive dietary energy can limit intake of amino acids and other essential nutrients to levels below that required for optimum growth (NRC, 1983). On the other hand, an insufficient dietary energy density can reduce growth rate due to utilisation of nutrients for energy rather than growth (Robinson & Wilson, 1985; Tacon, 1990).

Protein is the most expensive macro-component of shrimp diets. It is also used preferentially for catabolism for energy by shrimp and its oxidation is therefore economically wasteful (Alava & Pascual, 1987; Tacon, 1990). The optimal protein content of diets for post-larval/juvenile *Penaeus monodon* Fabricius is generally indicated to be between 40 and 46 % (Lee, 1971; AQUACOP, 1977; Khannapa, 1977; Alava & Lim, 1983; Bautista, 1986; Hajra *et al.*, 1988; Liao & Liu, 1990; Shiau *et al.*, 1991a; Akiyama, 1992; Akiyama *et al.*, 1992). It is thought however, that a reduction in the expensive protein fraction may be achieved by the manipulation of the protein (amino acid) content to the optimum level required for growth, while maximising the protein-sparing effects of the non-protein energy sources of lipid and carbohydrate (Sedgwick, 1979; Cho & Kaushik, 1985; Cho *et al.*, 1985; Bautista, 1986; Alava & Pascual, 1987; D'Abramo, 1990; Shiau & Chou, 1991; Akiyama *et al.*, 1992).

Reductions in dietary protein level from 39-45 % to 35-36 % have previously been achieved without compromising growth in tank trials with *P. monodon* by increasing

dietary carbohydrate levels from 20-27 % to 30-35 % (Bages & Sloane, 1981; Shiau & Chou, 1991; Shiau & Peng, 1992). Protein levels have also been successfully reduced to as little as 30 % by further increasing carbohydrate levels to 36 % (Shiau & Peng, 1992). Similar results have also been achieved with other omnivorous penaeid species including *P. setiferus* (Andrews *et al.*, 1973), *P. indicus* (Colvin, 1976a), *P. merguensis* (Sedgwick, 1979), and *P. vannamei* (Cuzon *et al.*, 1993), confirming the protein sparing role of dietary carbohydrate.

The optimum protein level for any species is probably a function of the energy density of the diet (Tacon, 1990). To date only three trials have attempted to determine the protein:energy requirements of *P. monodon*. Recalculating the results of these trials has suggested that protein requirements are directly related to dietary energy density. Thus, protein requirements were 52 % at a protein:energy ratio of 29 mg protein kJ⁻¹ total energy (TE), 39 % at 23-24 mg protein kJ⁻¹ TE and only 36 % at 20 mg protein kJ⁻¹ TE (Bautista, 1986; Shiau & Chou, 1991). These trials however, did not investigate the protein-sparing effects of lipid at > 10 % of the diet. In contrast, Hajra *et al.* (1988) found that 46 % protein was optimum at a relatively low protein:energy ratio of 22 mg protein kJ⁻¹ TE. This latter trial however, used diets formulated using a range of different ingredients, leading to some confusion with interpretation of their results.

Whilst carbohydrates are required primarily as dietary energy sources, lipids (or more precisely, fatty acids, phospholipids and sterols) have various metabolic functions to perform before any surplus is available for catabolism for energy (Tacon, 1990; Akiyama *et al.*, 1992). Excessively high levels of both lipids and carbohydrates have however, been reported to result in poor production and deleterious changes in shrimp tissues, including high carcass lipid levels for lipid-rich diets (Andrews *et al.*, 1972; Forster & Beard, 1973; Deshimaru & Kuroki, 1974; Clifford & Brick, 1979; Deshimaru *et al.*, 1979, 1985; Sedgwick, 1979, Pascual *et al.*, 1983; Cho *et al.*, 1985; Bautista, 1986; D'Abramo, 1990).

Contrary to previous results, some research has shown that juvenile *P. monodon* demonstrate better growth and survival on diets with high levels of lipid (10-13 %) and low levels of carbohydrate (19-33 %) *i.e.* at low C:L ratios (Bages & Sloane, 1981;

Alava & Pascual, 1987; Catacutan, 1991a,b; Sheen & Chen, 1992; chapter 6). This suggests that high dietary lipid levels can be utilised and may have potential in protein-sparing, although this has yet to be confirmed experimentally.

The aims of this trial were therefore to determine 1) the maximal sparing action of lipid and carbohydrate on dietary protein, 2) the optimum proportions of energy provided by protein, lipid and carbohydrate and 3) the optimum energy density and protein:energy ratio of diets containing balanced nutrient profiles fed to juvenile *P. monodon*.

7.2 MATERIALS AND METHODS

7.2.1 Experimental animals

Shrimp post-larvae used in this trial were not reared from larvae on site, but were air-freighted from a hatchery in Singapore. Otherwise, the post-larvae were treated as in section 5.2.1, except that the shrimp were transferred from the freight cartons to the recirculated brackish-water tank system (Appendix 1) at 16 days post-metamorphosis (PL₁₆) to acclimate them to the experimental conditions. At this stage they were weaned from *Artemia salina* nauplii to the basal experimental diet for a period of 33 days.

7.2.2 Experimental design

Experimental design was as in section 5.2.2, except that 49 day old juveniles were selected with mean weights of 205 ± 3 mg. Fifty shrimp of this size were randomly allocated to each of 24 fibreglass tanks of dimensions 0.75 X 0.7 X 0.2 m deep (0.53 m² area, 105 l volume) at a stocking density of 95 shrimp m². Eight dietary treatments were tested, each in triplicate, in the brackish-water recirculating system. The tanks were supplied with brackish-water at the rate of 6 l min⁻¹ per tank.

Every ten days the shrimp were group-weighed to determine growth, survival and feeding rates, and individually weighed on termination of the trial on day 50 to quantify within-tank size variation.

7.2.3 Water quality

Water quality was monitored and maintained as in section 5.2.3. Water quality remained relatively constant throughout the duration of the trial. Dissolved oxygen remained above 6.6 mg l^{-1} ($> 100 \%$ saturation), temperature was maintained at $30.0 \pm 1.0 \text{ }^\circ\text{C}$, pH at 8.5 ± 0.4 and salinity at $25 \pm 2 \text{ }^\circ\text{‰}$. Total ammonia nitrogen remained below 0.01 mg l^{-1} and nitrite nitrogen below 0.01 mg l^{-1} throughout the trial period. Water quality was not considered to be detrimental to the growth of the shrimp during the trial.

7.2.4 Diet formulation and feeding

Diets were formulated and prepared as in section 5.2.4, except that eight semi-purified diets were formulated for this trial (Table 7.1). The first series of four diets (diets 1-4) included protein levels from 38 to 44 %, using casein and gelatin (3:1) as the protein sources. The best dietary carbohydrate source (from a previous trial, Briggs, 1991) of raw corn starch was included at relatively constant levels of 27-33 %. Final dietary carbohydrate levels were 34-38 %. The best dietary lipid sources (from a previous unpublished trial and chapter 5), of cod liver oil and soybean oil at a 3:1 ratio, 1.5-3 % soybean lecithin (18 % PC, Nutripur, Lucus Meyer, Hamburg, Germany) and 0.5 % cholesterol (96 % 5-cholesten-3B-ol, Sigma Chemical Company Ltd.) were used. These lipid sources replaced protein at final total lipid levels of 6.6 to 12.0 % of the diet. Diets were maintained isoenergetic at $21.1 \pm 0.2 \text{ kJ g}^{-1}$ total energy (TE) (by bomb calorimetry) by manipulation of dietary α cellulose levels. α cellulose was considered as non-digestible filler (Tacon, 1990) (although it may preferentially bind lipids) and the dietary carbohydrate levels were based on those of starch only. These diets thus contained protein:lipid (Pr:L) ratios of 3.2-6.7:1, carbohydrate:lipid (C:L) ratios of 2.8-5.7:1 and protein:energy (Pr:E) ratios (using calculated energy values for comparison with other studies) of 19.6-22.6 mg protein kJ g^{-1} TE (Table 7.1).

The second series of five diets (diets 5-8 plus diet 4 from the first series), were formulated from the same ingredients to contain carbohydrate levels from 26 to 42 % in isonitrogenous ($38.3 \pm 0.1 \%$ protein), isolipidic ($12.1 \pm 0.1 \%$) diets. These diets

Table 7.1 Composition and proximate analysis (% dry weight) of the diets used in the protein-sparing trial.

Ingredient	Diet							
	1	2	3	4	5	6	7	8
Casein	32.1	30.6	29.1	27.5	27.5	27.5	27.5	27.5
Gelatin	10.7	10.2	9.7	9.2	9.2	9.2	9.2	9.2
Raw corn starch	33.0	31.0	29.0	27.0	15.0	21.0	33.0	39.0
α Cellulose	8.0	9.5	10.5	12.6	24.6	18.6	6.6	0.6
Vitamin premix ¹	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Mineral premix ²	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Astaxanthin ³	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cod liver oil	1.8	3.3	4.8	6.3	6.3	6.3	6.3	6.3
Soybean oil	0.6	1.1	1.6	2.1	2.1	2.1	2.1	2.1
Soy lecithin	1.5	2.0	3.0	3.0	3.0	3.0	3.0	3.0
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Taurine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
L-Arginine	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
+ water (20-30 %)	+	+	+	+	+	+	+	+

- continued over

Table 7.1 - continued

Proximate analysis ⁴	1	2	3	4	5	6	7	8
Protein	44.0	42.1	40.3	38.3	38.3	38.3	38.2	38.2
± SD	0.7	0.5	0.3	0.4	0.6	0.4	0.5	0.6
Ether extract ⁵	5.6	6.9	7.9	10.0	10.1	9.8	10.3	11.1
± SD	0.1	0.0	0.0	0.2	0.2	0.3	0.1	0.3
Total lipid ⁶	6.6	8.9	10.9	12.0	12.1	12.2	12.3	12.1
± SD	0.1	0.0	0.0	0.2	0.2	0.3	0.1	0.3
Carbohydrate ⁷	37.7	35.1	34.2	33.7	25.7	27.6	36.8	41.7
Fibre	6.5	9.0	10.0	11.1	19.1	17.1	7.8	2.8
± SD	0.7	0.1	0.3	0.9	0.1	0.3	0.2	0.1
Ash	5.2	4.9	4.6	4.9	4.8	4.8	4.9	5.2
± SD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Moisture	7.0	5.8	7.3	4.3	5.1	6.8	6.1	6.5
± SD	0.1	0.2	0.2	0.0	0.1	0.2	0.1	0.1
Total energy (kJ g ⁻¹) ⁸	19.5	19.5	19.7	19.6	18.2	18.6	20.2	21.0
Total energy (kJ g ⁻¹) ⁹	20.9	20.9	21.4	21.2	20.2	20.4	21.5	22.0
Pr:E ratio (mg pr. kJ ⁻¹ TE) ¹⁰	22.6	21.6	20.5	19.6	21.0	20.6	18.9	18.2
Protein:lipid ratio	6.7	4.7	3.7	3.2	3.2	3.1	3.1	3.2
Carbohydrate:lipid ratio	5.7	3.9	3.1	2.8	2.1	2.3	3.0	3.4

¹ Vitamin Premix as in Table 5.1

² Mineral Premix as in Table 5.1

³ Astaxanthin as in Table 5.1

⁴ All results based on triplicate analyses

⁵ Analysed by Soxhlet method

⁶ Analysed by method of Folch-Lees (Folch *et al.*, 1957)

⁷ By difference in dry weight

⁸ By calculation

⁹ By bomb calorimetry

¹⁰ Using calculated TE figures

thus contained TE levels from 20.2 to 22.0 kJ g⁻¹ (by calorimetry), C:L ratios of 2.1-3.4:1 and Pr:E ratios (using calculated energy values for comparison with other studies) of 18.2 to 21.0 mg protein kJ⁻¹ TE (Table 7.1).

Total dietary energy levels were measured by bomb calorimetry and calculated using mean total energy values of 23.6 kJ g⁻¹ for protein, 39.8 kJ g⁻¹ for lipid (using total lipid levels) and 17.2 kJ g⁻¹ for carbohydrate, as in section 5.2.4 .

All formulated diets contained 1 % taurine as a feeding attractant due to the poor acceptance of semi-purified diets in previous unpublished studies in this laboratory and the efficacy of taurine as a feed attractant (Hartati & Briggs, 1993). The diets were also supplemented with 1.7 % L-Arginine due to the deficiency of this amino acid in the protein components of the diets (Tacon & Cowey, 1984; Teshima *et al.*, 1986a). All diets also included 1 % chromic oxide marker for use in determining the digestibility of the diets during the trial (NRC, 1983).

Based on previous experience (chapters 5 & 6), feeding was carried out twice daily at 0900 and 1700 for nine days out of every ten (not feeding on sampling day). the shrimp were fed at 40 % of body weight daily for days 1-9, 30 % for days 11-19, 20 % for days 21-29 and 15 % for days 31-49 on a wet shrimp/dry food basis. This feeding rate was adapted from those used in chapter 6 due to possible underfeeding noted during the first ten days of that trial.

7.2.5 Diet digestibility determination

The apparent dry matter digestibility co-efficient (ADC) of the test diets was determined by the method of Furukawa & Tsukahara (1966), as described in section 6.2.6. and according to the formula:

$$\text{ADC} = 100 - \left[100 \times \frac{\% \text{ indicator in food}}{\% \text{ indicator in faeces}} \right]$$

7.2.6 Sample collection and analysis

Shrimp and diet samples were collected and analysed as in section 6.2.7, except that histological analysis was not done.

7.2.7 Data analysis

Data obtained from this trial was analysed as described below:-

1. Specific growth rate (SGR), survival, production and food conversion efficiency (FCE) were analysed as described in section 3.2.1.6. Protein efficiency ratio (PER) was analysed as described in section 5.2.6.
2. Diets 1-4, varying only in protein:lipid ratio, and diets 4-8, varying only in carbohydrate (and hence total energy) level were separated for statistical analysis. The effects of dietary protein:lipid ratio (for diets 1-4) and dietary carbohydrate level (for diets 4-8) on shrimp growth, survival (arcsin transformed), production, FCE, PER and carcass composition after 50 days were analysed using a non-parametric analysis of variance (Kruskal-Wallis) test after Bartlett tests revealed that in all cases the data were heteroscedastic (Sokal & Rohlf, 1981).
3. Where appropriate, the T-method for multiple comparisons was used to determine statistical differences between treatment means for the above ANOVAs (Sokal & Rohlf, 1981). Results were considered significant if $P < 0.05$.
4. Regression analyses were used to identify possible relationships between the dietary protein:lipid ratio and the dietary carbohydrate level and shrimp growth, survival, production, FCE, PER and carcass composition (Sokal & Rohlf, 1981).

7.3 RESULTS

7.3.1 Shrimp production

The SGR of the shrimp during the present study was lower than that achieved in previous trials in this thesis. This was probably due to the larger initial size of the shrimp used in this trial, since older shrimp have more fully developed digestive systems and since the growth rate of shrimp tends to decrease with size. However, the survival rate of shrimp in the present study was higher than that achieved previously. This was probably due to the use of shrimp imported as post-larvae in this trial, compared to the use of shrimp reared from larvae on site in the previous trials. It seems likely therefore, that there were differences in the vigour (quality) of shrimp between the trials which affected the results. Overall shrimp production in the present study was thus considerably higher than that achieved in previous trials in this thesis due to the larger initial size and higher survival of shrimp in this trial.

Shrimp growth, survival, FCE, PER and production were not significantly different when the level of dietary protein was decreased from 44.0 to 38.3 % and protein:lipid ratios from 6.7 to 3.2 by increasing lipid levels from 6.6 to 12.0 % at constant total energy (TE) ($21.1 \pm 0.2 \text{ kJ g}^{-1}$, by calorimetry) and carbohydrate ($35.2 \pm 1.8 \%$) levels (Table 7.2, Figures 7.1-7.4). However, as protein levels were decreased and lipid levels increased, PER tended to increase as the limited supply of protein was being spared for growth by lipid (Table 7.2, Figure 7.4).

Whilst maintaining a constant level of dietary protein ($38.3 \pm 0.1 \%$) and lipid ($12.1 \pm 0.1 \%$), increasing the level of carbohydrate from 26 to 42 % and hence total energy from 20.2 to 22.0 kJ g^{-1} significantly ($P < 0.05$) reduced growth rate (Table 7.2, Figure 7.5), but increased ($P < 0.05$) survival (Table 7.2, Figure 7.6). This resulted in production which, although not significantly different, tended to be enhanced at low levels of carbohydrate (26-34 %) and total energy (20.2-21.2 kJ g^{-1}) and at low C:L ratios (2.1-2.8:1) (Table 7.2, Figure 7.7). None of these diets however, had any significant effect upon FCE or PER (Table 7.2).

Table 7.2 Performance of juvenile *P. monodon* fed diets varying in carbohydrate and lipid level over the 50 day protein-sparing trial period.¹

Diet	Protein level (%)	Carbohydrate level (%)	Lipid level (%)	Mean survival (%)	Mean body weight (g)		SGR (% body wt d ⁻¹)	Production (g m ⁻² d ⁻¹)	FCE	PER	Digestibility (%)
					Initial	Final					
1	44.0	37.7	6.6	80.7 ^a	0.205 ^a	2.300 ^a	4.82 ^a	3.14 ^a	0.26 ^a	0.60 ^a	59.24 ^{e2}
± SD	-	-	-	4.2	0.005	0.346	0.31	0.55	0.02	0.03	0.38
2	42.1	35.1	8.9	86.0 ^a	0.208 ^a	2.095 ^a	4.61 ^a	3.03 ^a	0.26 ^a	0.63 ^a	67.53 ^d
± SD	-	-	-	3.5	0.005	0.210	0.16	0.20	0.01	0.03	0.36
3	40.3	34.2	10.9	86.0 ^a	0.205 ^a	1.997 ^a	4.53 ^a	2.88 ^a	0.26 ^a	0.65 ^a	56.58 ^b
± SD	-	-	-	3.5	0.010	0.374	0.37	0.59	0.02	0.04	0.22
4	38.3	33.7	12.0	86.7 ^{a,xy}	0.207 ^{a,x}	2.068 ^{a,xy}	4.60 ^{a,xy}	3.02 ^{a,x}	0.25 ^{a,x}	0.65 ^{a,x}	45.33 ^{a,y}
± SD	-	-	-	4.2	0.004	0.131	0.09	0.19	0.02	0.05	1.38
5	38.3	25.7	12.1	78.0 ^x	0.200 ^x	2.331 ^y	4.91 ^y	3.08 ^x	0.24 ^x	0.63 ^x	39.02 ^x
± SD	-	-	-	4.0	0.008	0.155	0.07	0.12	0.01	0.02	0.65
6	38.3	27.6	12.2	86.7 ^{xy}	0.206 ^x	2.069 ^{xy}	4.62 ^{xy}	3.03 ^x	0.25 ^x	0.65 ^x	39.38 ^x
± SD	-	-	-	5.0	0.006	0.166	0.22	0.37	0.01	0.03	1.11
7	38.2	36.8	12.3	82.0 ^{xy}	0.207 ^x	1.743 ^x	4.26 ^x	2.33 ^x	0.23 ^x	0.59 ^x	68.35 ^z
± SD	-	-	-	7.2	0.010	0.106	0.13	0.32	0.03	0.07	0.17
8	38.2	41.7	12.1	90.7 ^y	0.202 ^x	1.752 ^x	4.32 ^x	2.67 ^x	0.25 ^x	0.65 ^x	69.78 ^z
± SD	-	-	-	1.2	0.014	0.102	0.17	0.24	0.01	0.03	0.51

¹ Based on the mean of three replicates

² For single criterion, mean values in the same column bearing different superscripts are significantly different (P < 0.05) by T-method

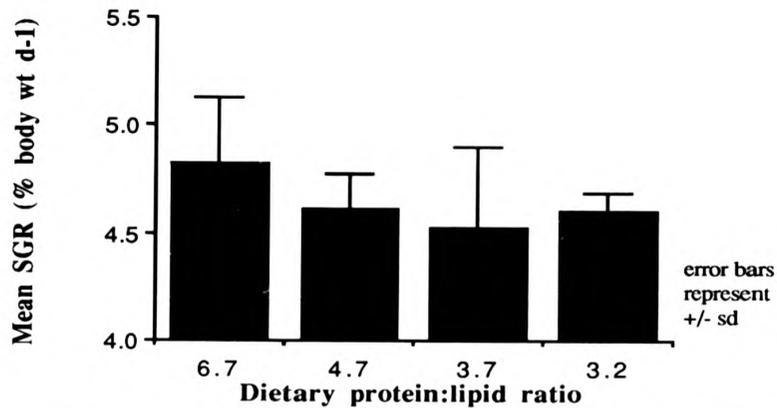


Figure 7.1 Relationship between dietary protein:lipid ratio and mean SGR of shrimp over 50 days.



Figure 7.2 Relationship between dietary protein:lipid ratio and mean shrimp survival after 50 days.

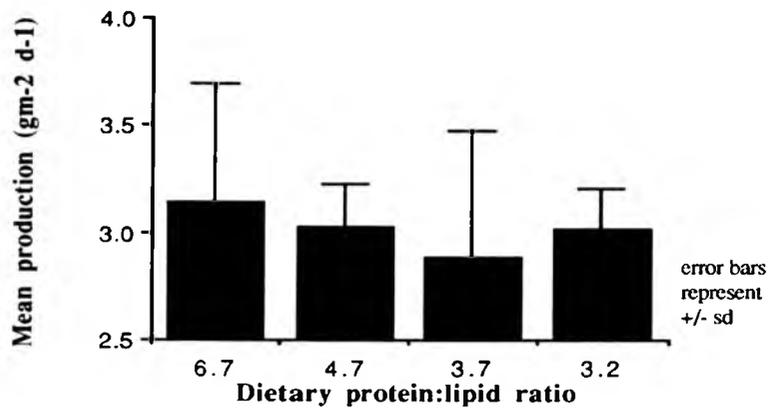


Figure 7.3 Relationship between dietary protein:lipid ratio and mean shrimp production over 50 days.



Figure 7.4 Relationship between dietary protein:lipid ratio and mean PER of shrimp over 50 days.

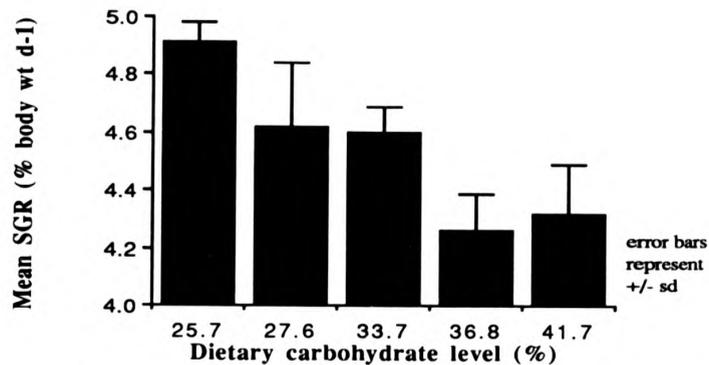


Figure 7.5 Relationship between dietary carbohydrate level and mean SGR of shrimp over 50 days.

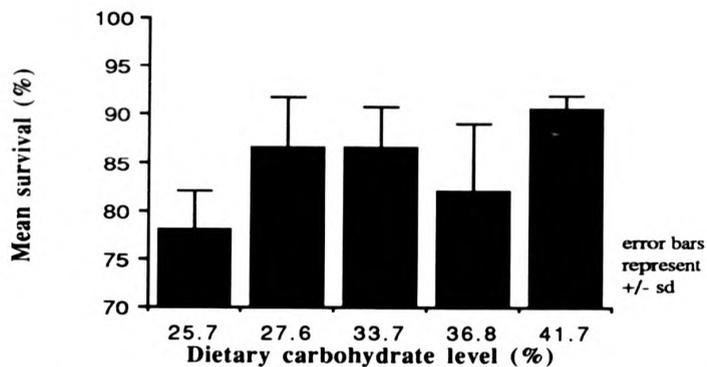


Figure 7.6 Relationship between dietary carbohydrate level and mean shrimp survival after 50 days.

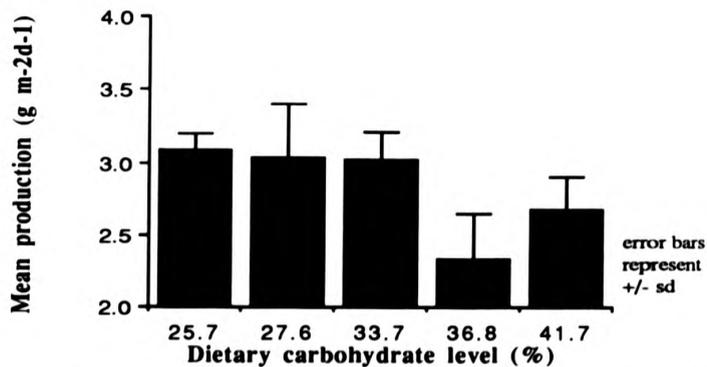


Figure 7.7 Relationship between dietary carbohydrate level and mean shrimp production over 50 days.

Shrimp growth, survival, FCE, PER and production were not significantly affected when fed isoenergetic diets in which protein was progressively replaced by lipid at Pr:E ratios of between 19.6 and 22.6 mg protein kJ^{-1} TE. At constant levels of dietary protein (38 %) and lipid (12 %), the optimum Pr:E ratio in terms of shrimp growth and production was 21 mg protein kJ^{-1} TE at a total energy level of 20.2 kJ g^{-1} (by calorimetry), or 18.2 kJ g^{-1} (by calculation).

In terms of energy partitioning, at a constant dietary protein level, optimum shrimp growth and production was achieved on diets containing 38 % protein (46-48 % of the TE), 12 % lipid (24-26 % TE), and 28-34 % carbohydrate (25-30 % TE). Decreasing the amount of energy derived from protein and lipid by increasing dietary carbohydrate levels tended to decrease shrimp growth and production, but increase survival.

7.3.2 Carcass composition

Differences between the diets tested in this trial had no significant effects upon the carcass composition of the shrimp (Table 7.3). However, decreasing the protein:lipid ratio of the diets by replacing protein with up to 12 % lipid tended to produce shrimp with higher levels of carcass lipid. Changing the levels of carbohydrate and hence total energy in isonitrogenous (38 % protein), isolipidic (12 %) diets had no significant effects upon carcass composition (Table 7.3).

7.3.3 Diet digestibility

Relatively low dry matter digestibility values (39-70 %) were recorded for all of the diets tested in the present study (Table 7.2). These values however, were similar to those reported (34-73 %) from previous research with juvenile penaeid shrimp (Fenucci *et al.*, 1982; Lee & Lawrence, 1985; Smith *et al.*, 1985; Akiyama, 1991; Shiao & Peng, 1992). From the results obtained, significant ($P < 0.01$) differences were found between the diets tested. At relatively constant levels of dietary energy and carbohydrate (diets 1-4), the digestibility of diets was inversely related to the dietary protein:lipid ratio, with the significantly ($P < 0.01$) highest level of digestibility (67.5 %) occurring at 20.9 kJ g^{-1} total energy, 42 % protein and 9 % lipid (Figure 7.8).

Table 7.3 Carcass composition (% dry weight) of shrimp on termination of the protein-sparing trial.

Proximate analysis ¹	Diet								
	1	2	3	4	5	6	7	8	PL ₁₅ (d 0)
Protein ²	49.5 ^a	49.4 ^a	49.6 ^a	48.9 ^{a,x}	49.3 ^x	49.3 ^x	49.7 ^x	49.7 ^x	52.1 ^{a,x,3}
± SD	0.1	0.5	0.3	0.7	0.5	0.3	0.7	0.2	1.5
Lipid ⁴	4.7 ^a	5.1 ^a	4.7 ^a	5.5 ^{a,x}	6.4 ^x	6.5 ^x	6.2 ^x	6.5 ^x	4.8 ^{a,x}
± SD	0.2	0.6	0.3	0.5	0.5	1.0	1.0	0.3	0.5
Ash	18.8 ^a	18.4 ^a	18.4 ^a	18.2 ^{a,x}	17.4 ^x	17.9 ^x	18.0 ^x	18.9 ^x	20.9 ^{a,x}
± SD	0.9	1.0	0.8	0.2	1.1	0.7	0.3	0.8	1.0
Fibre	7.8 ^a	7.7 ^a	7.8 ^a	7.5 ^{a,x}	8.3 ^x	8.4 ^x	8.3 ^x	8.1 ^x	8.5 ^{a,x}
± SD	0.1	0.1	0.4	1.2	0.4	0.2	0.1	0.4	0.5
Carbohydrate ⁵	19.2	19.0	19.5	20.5	18.6	17.9	17.8	16.8	13.7
Moisture	69.5 ^a	69.3 ^a	69.9 ^a	69.6 ^{a,x}	67.4 ^x	68.9 ^x	67.8 ^x	68.7 ^x	74.6 ^{a,x}
± SD	0.5	1.6	0.2	0.8	1.6	1.2	0.2	3.0	1.2

¹ Analyses (except for carbohydrate) based on mean of three replicates from carcasses for each replicate of each treatment

² Analysed by method of Lowry (Lowry *et al.*, 1951)

³ For single criterion, mean values in the same row bearing different superscripts are significantly different ($P < 0.05$) by T-method

⁴ Analysed by method of Folch-Lees (Folch *et al.* 1957)

⁵ By difference in dry weight

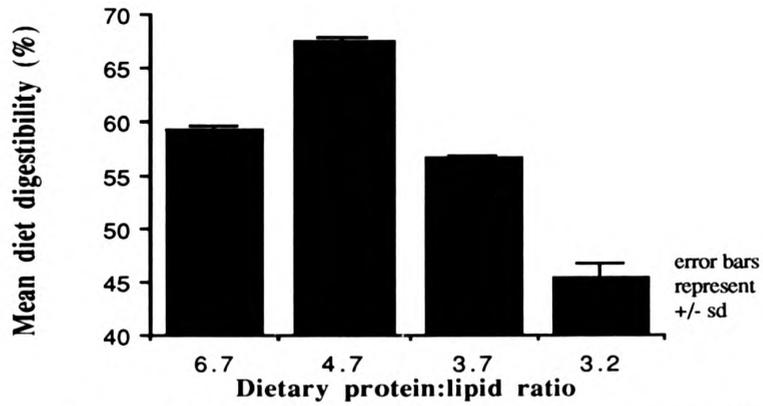


Figure 7.8 Relationship between dietary protein:lipid ratio and mean diet digestibility over days 40-50.

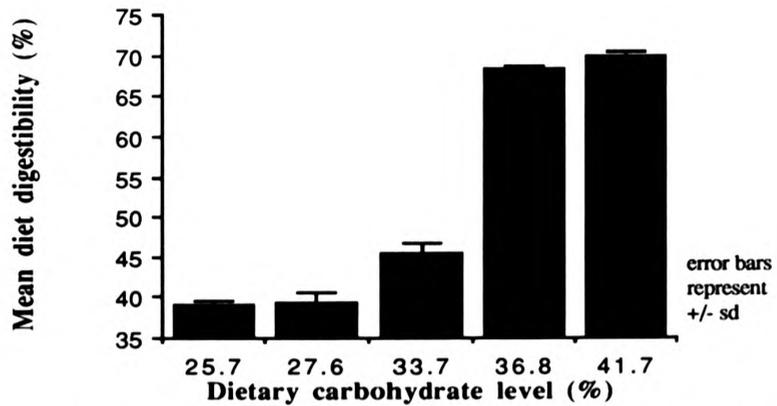


Figure 7.9 Relationship between dietary carbohydrate level and mean diet digestibility over days 40-50.

In addition, as the dietary carbohydrate level was increased from 26 to 42 % (and total energy from 20.2 to 22.0 kJ g⁻¹), diet dry matter digestibility increased significantly ($P < 0.01$) (Figure 7.9). In contrast, diets with low levels of total energy and protein and high levels of lipid (and cellulose) were not well digested. The digestibility of these isonitrogenous, isolipidic diets however, tended to be inversely related to shrimp growth rates (Table 7.2).

7.4 DISCUSSION

7.4.1 Shrimp production

The optimal inclusion level of protein in juvenile shrimp diets will depend upon a number of factors. Primary among these are the cost of the ingredients, the amino acid profile of the protein source(s) used, the growth rate and the food and protein conversion efficiencies of the shrimp fed diets varying in protein content. Reducing the dietary protein content and increasing (or at least maintaining) FCE and PER without compromising growth will assist reduction in feed costs due to the over-riding expense of the protein component (Liao & Liu, 1990; Akiyama *et al.*, 1992). Feeding diets low in protein will also reduce waste production through a decrease in ammonia excretion (Forster & Gabbott, 1971; Shiau & Chou, 1991; Boyd & Musig, 1992; Phillips *et al.*, 1993) and promote the efficiency of protein usage (Tacon, 1990).

Because feed intake by shrimp is regulated by dietary energy density (Sedgwick, 1979; Adolph, 1981; Church & Pond, 1982), the protein requirement will partially be a function of the energy level (Tacon, 1990). Maximum growth may therefore be achieved by the low consumption of diets high in both protein and energy or high consumption of diets low in both of these components. The levels of non-protein energy sources (lipid and carbohydrate) in the diet must be such that they are able to supply as much of the energy requirements as possible, sparing dietary protein for growth alone. The quantity of lipid and carbohydrate, and hence total energy, required will then depend upon the protein content of the diet and the maximum amount of lipid and carbohydrate that can be tolerated in the diet without adversely affecting shrimp growth,

survival or body composition.

Carbohydrates are generally believed to have a minor role as energy stores in crustaceans (Jungreis, 1968; Heath & Barnes, 1979; Regnault, 1981; Barclay *et al.*, 1983; Zhou, 1990). This however, is due to the low level of carbohydrates in the natural diet of shrimp, their low ability to digest and store carbohydrates and hence low body levels. Despite this, the majority of the trials conducted to date investigating protein sparing have attempted to substitute carbohydrate for protein in shrimp diets. This is largely because carbohydrates provide the cheapest source of energy in shrimp diets (Akiyama *et al.*, 1992).

Colvin (1976a) reduced the protein content of isoenergetic (17.5 kJ g⁻¹ TE, by calculation), isolipidic (approximately 5 %) diets fed to juvenile *P. indicus* from 53 to 43 % by increasing the level of starch from 13 to 29 % without compromising growth, survival or FCR. He also found that the PER was inversely related to protein content in these diets, confirming the protein sparing role of carbohydrate. Similarly, Sedgwick (1979) successfully reduced protein levels from 51 to 34-42 % in diets fed to juvenile *P. merguensis* containing 16-18 kJ g⁻¹ TE (by calculation) and 2-6 % lipid by the addition of up to 30 % starch.

Similar results have been obtained with *P. monodon*. Bages & Sloane (1981) found that the protein content of isoenergetic (17-19 kJ g⁻¹ TE, by calculation), isolipidic (10 %) diets fed to post-larvae could be reduced from 55 to 35 % without a significant decrease in production when corn starch inclusion was increased from 10 to 30 %. Bautista (1986) found a non-significant decrease in the growth rate of juveniles when the protein level of isoenergetic (15-18 kJ g⁻¹ TE, by calculation), isolipidic (10 %) diets was decreased from 50 to 40 % and the carbohydrate level increased from 0 to 10 % or 20 to 30 %. Shiau & Chou (1991a) achieved similar results, successfully reducing the protein content of isolipidic (9 %) diets from 40 to 36 % by increasing dietary carbohydrate levels from 27 to 35 %. Shiau *et al.* (1991) promoted maximum growth of juveniles when fed isolipidic (10 %) diets where protein was reduced from 52 to 40-44 % and carbohydrate increased from 21 to 31-35 %. Finally, weight gain of juveniles was not significantly reduced and PER was significantly increased when the protein

content of isolipidic (8-9 %) diets was reduced from 41 to 31 % and carbohydrate (starch) levels increased from 27 to 36 % (Shiau & Peng, 1992).

These results suggest that complex carbohydrates such as starch may have a protein sparing role when included at 29-36 % of diets containing low levels of protein (31-44 %), total energy (15-19 kJ g⁻¹, by calculation) and lipid (2-10 %). The results of the present study also show that the growth rate of juvenile *P. monodon* does not decline significantly when the protein level is decreased from 44 to 38 % of diets where lipid and carbohydrate levels are adjusted to maintain energy density. In contrast to the results of the above authors however, this study shows that when juvenile *P. monodon* are fed 38 % protein diets containing higher levels of lipid (12 %), shrimp growth is inversely related to the dietary carbohydrate level over the range 26 to 42 % and hence total energy (18 to 21 kJ g⁻¹, by calculation).

The discrepancy between these results is probably due to the differences in dietary energy density. The high lipid levels tested in this trial (at low levels of carbohydrate) may thus have provided sufficient energy to meet the requirements of the shrimp, so that increases in carbohydrate resulted in excessive energy levels (> 18.2 kJ g⁻¹, by calculation) and possibly reduced diet consumption (although this could not be quantified). If this were true, the shrimp may therefore have not been able to ingest sufficient protein to maintain maximum growth rate. In confirmation, isonitrogenous (40-45 % protein) and isolipidic (8-10 %) diets containing high levels of carbohydrate (30-40 %) and hence total energy (18-22 kJ g⁻¹, by calculation), have previously been shown to result in decreased growth and survival (Bautista, 1986; Alava & Pascual, 1987; Catacutan, 1991b; Shiau & Chou, 1991) and deleterious changes to the mid-gut gland (Pascual *et al.*, 1983) of *P. monodon*.

Previous research has shown that lipid is second in importance to protein as an energy reserve in starved crustaceans (Schafer, 1968; Speck & Urich, 1969; Regnault, 1981; Barclay *et al.*, 1983; Zhou, 1990). These authors have suggested however, that lipid may be catabolised more rapidly than protein, but that because it is normally at a low level in the shrimp carcass, its contribution to the energy requirements are lower than that of protein. Because it is possible to include relatively high levels of highly

digestible lipid into shrimp diets, it is surprising that the ability of lipid to spare protein for growth in has received little direct attention from shrimp nutritionists.

Preliminary research in this area suggested that dietary lipid levels in excess of 10 % result in decreased growth and survival, fatty carcasses and processing difficulties (D'Abramo, 1991, chapters 5 & 6). However, this again is probably due to excess energy in relation to the protein content of the diets used (*i.e.* Pr:E ratios which are too low to support maximum shrimp production). Catacutan (1991b) demonstrated that maximum growth of juvenile *P. monodon* was obtained when fed high protein (51 %), high energy (20 kJ g⁻¹, by calculation) diets containing 19 % carbohydrate when lipid (cod liver oil) levels were increased from 0.8 to 12.8 % (C:L ratios of 1.9-4.2). In this case, the high level of lipid may have been acceptable since reduced intake of the high energy diets may not have prevented the intake of sufficient protein due to its high dietary level. Sheen and Chen (1992) also showed that lipid levels of up to 8-12 % promoted optimal growth of juvenile *P. monodon* fed isoenergetic, isonitrogenous diets. The results of a previous study in this thesis (chapter 6) also found that 9.1-12.8 % lipid at carbohydrate:lipid (C:L) ratios of < 3:1 (13-26 % carbohydrate) promoted maximum survival and production of post-larval *P. monodon* when fed high protein (45 %), high energy diets (19.3 kJ g⁻¹, by calculation).

The results of the present study confirm that under these conditions high levels of dietary lipid may be used for protein sparing in high energy diets. Thus, protein levels could be reduced from 44 to 38 % in isoenergetic diets (19.6 ± 0.1 kJ g⁻¹, by calculation) when carbohydrate levels were maintained at 34-38 % and lipid levels increased from 6.6 to 12.0 % (C:L ratio of 2.8:1), including 8.4 % basal lipids, 3 % lecithin and 0.5 % cholesterol. This diet promoted a slight increase in survival and PER with no significant reduction in growth, FCE or production. At dietary levels of 38 % protein and 12 % lipid however, optimum growth and production were achieved when the dietary carbohydrate level was reduced to 26 % (C:L ratio of 2.1:1) and the level of total energy thereby reduced to 18.2 kJ g⁻¹, at a high Pr:E ratio of 21 mg protein kJ⁻¹ TE (by calculation). These results underline the protein-sparing ability of balanced C:L and P:E ratios.

It therefore seems that protein levels may be reduced to less than 40 % in diets containing appropriate quantities of both lipids (8-12 %) and carbohydrates (< 30-35 %) at low levels of total energy. In such low protein diets, the total energy level must be maintained at a low level (< 19 kJ g⁻¹, by calculation) by manipulation of the C:L ratio to 3-4:1 at 8-10 % lipid and approximately 2:1 at 12 % lipid. Although such low-protein, low energy diets may not significantly increase the growth and survival rates over that of shrimp fed diets high in protein and energy, they will be cheaper. In addition, since this trial demonstrates that although poor, the FCE of shrimp fed such diets was not significantly lower than that of shrimp fed high protein diets, waste production will decrease due to the lower ammonia production rate resulting from the feeding of low-protein diets.

7.4.2 Carcass composition

As discussed in chapter 6, most of the studies investigating the effects of high dietary lipid levels (> 10 %) on shrimp have suggested that such diets result in increased deposition of lipid in the shrimp carcass. For example, Catacutan (1991b), feeding isonitrogenous diets to juvenile *P. monodon*, found there was a direct relationship between dietary (9.5-11.9 %) and carcass (3.3-4.9 %) lipid levels. Her high lipid diets however, also contained lower levels of carbohydrate and hence total energy, suggesting that energy from lipids excess to requirements was being stored in the carcass. Davis & Robinson (1986) however, showed that the carcass lipid content of crayfish (*Procambarus acutus acutus*) fed isonitrogenous, isoenergetic diets was maximum at 6 % dietary lipid, but declined as dietary lipid levels were increased further. They suggested that this was due either to reduced food consumption or impaired absorption of high dietary lipid levels. In confirmation of this, they reported that the growth rate of crayfish was significantly reduced at the highest lipid levels tested (12-15 %). In the present study however, such reductions in growth rate were not seen when diets contained up to 12 % lipid, sparing dietary protein in isoenergetic diets. This suggests that in diets which have balanced nutrient profiles, high levels of dietary lipid may be utilised efficiently. In this case lipid and carbohydrate rather than protein may be catabolised for shrimp metabolism, so that high levels of dietary lipid or energy need not result in significantly increased rates of lipid deposition.

7.4.3 Diet digestibility

Results from the first part of this study showed that at constant levels of dietary energy and carbohydrate, the dry matter digestibility of diets tended to increase with dietary protein level and decrease with lipid level, *i.e.* at the highest Pr:E ratio. The highest digestibility was achieved in diets containing 19.5 kJ g⁻¹ total energy (by calculation), 42 % protein and 9 % lipid at a Pr:E ratio of 22.6 mg protein kJ⁻¹ TE.

Penaeid shrimp are known to be able to digest protein efficiently. Using the chromic oxide marker technique, apparent protein digestibility has been shown to range from 73 to 94 % for a range of penaeid species (Fenucci *et al.*, 1982; Teshima & Kanazawa, 1983; Lee & Lawrence, 1985; Smith *et al.*, 1985; Akiyama, 1991; Catacutan, 1991b; Shiau & Peng, 1992). In addition, these authors have shown that protein digestibility is largely independent of shrimp species, size (0.5-40 g) or dietary protein level (22-40 %) over the ranges tested.

Lipid digestibility, although less well researched, has also been reported to be high in penaeid shrimp, with apparent digestibility values of 90-93 % (at 9-12 % of the diet) for 30-40 g *P. monodon* (Catacutan, 1991b). Lower apparent lipid digestibility values however, have been reported for other shrimp species. Teshima & Kanazawa (1983) reported 75-88 % digestibility for a range of lipid sources at 8 % of the diet fed to 20-30 g *P. japonicus*. Akiyama (1991) report lipid digestibilities of 70-75 % for large *P. monodon* and *P. vannamei* fed diets containing 94 % soybean meal. However, Fenucci *et al.* (1992) quoted 51-69 % lipid digestibility feeding diets containing 6-7 % lipid to juvenile *Pleoticus muelleri* (Bate), and Smith *et al.* (1985) report only 45-65 % lipid digestibility for 4 g *P. vannamei* fed 6 % lipid diets. These differences may be species specific, but may also indicate that younger shrimp are less able to digest dietary lipid. If the latter is true, this may explain the poor digestibility of high lipid, low protein diets recorded for juvenile *P. monodon* during this study. More likely however, the decrease in dry matter digestibility of diets low in protein and high in lipid was probably related to the increased level of indigestible α cellulose (up to 13 %) in these diets.

Results from the second part of the present study, feeding diets at constant levels of protein (38 %) and lipid (12 %), showed that dry matter digestibility was directly related to carbohydrate and hence energy levels, but inversely related to growth. Catacutan (1991b) reported the same relationship with adult (30-40 g) *P. monodon* fed a 40 % protein diet. A previous trial in this series (chapter 6) also showed that dry matter digestibility was directly related to dietary carbohydrate level and C:L ratios. A similar result was found with juvenile *P. muelleri* by Fenucci *et al.* (1992), where carbohydrate digestibility (from 47 to 84 %) was directly related to increasing levels of dietary carbohydrate from 2.5-17.9 %.

This data appears to suggest that despite the low digestibility values reported for carbohydrates (36-76 %, see chapter 6), diet digestibility increases with carbohydrate inclusion level. However, in each of the above examples (including the present study), diets which contained low levels of carbohydrate also contained high levels of the indigestible "filler" α cellulose. Thus in this study, diet dry matter digestibility decreased linearly with increasing α cellulose level ($A = 73.41$, $b = -1.56$, $r^2 = 0.80$, $P < 0.05$), whilst the relationship for the data of Catacutan (1991b) could be described as $A = 86.99$, $b = -0.59$, $r^2 = 0.95$, $P < 0.05$. The high digestibility of diets rich in carbohydrate may thus reflect the effects of low α cellulose rather than high carbohydrate levels. This may also explain the lack of correlation noted in this study, and by most other authors, between diet digestibility and shrimp growth rate. Diet digestibility may thus be a poor indicator of the nutritional value of diets unless the levels of indigestible fillers such as α cellulose are maintained constant.

7.5 SUMMARY

Both lipid and carbohydrate are capable of sparing dietary protein for growth in shrimp diets. The level of protein in diets fed to juvenile *P. monodon* can be reduced from 44 to 38 % without a significant decrease in growth, survival, FCE, PER or production if the dietary energy level is maintained by the isoenergetic substitution of lipid for protein.

With the inclusion of 12 % lipid to diets containing 38 % protein, the best growth and production of shrimp was obtained on a diet containing 26 % carbohydrate at a low C:L ratio of 2.1:1 and a high Pr:E ratio of 21 mg protein kJ^{-1} TE. This diet had a calculated total energy density of 18.2 kJ g^{-1} (20.2 kJ g^{-1} by calorimetry), where 49 % of the total energy was derived from protein, 26 % from lipid and 24 % from carbohydrate.

Carcass lipid levels were not significantly increased by feeding diets containing lipid levels of up to 12 %, probably indicating the enhanced catabolism of lipid for energy in low protein, nutrient balanced diets.

Diet dry matter digestibility tended to be directly related to dietary protein and carbohydrate level and C:L ratio. However, diet digestibility was unrelated to growth rate or overall shrimp production. These results may have been due to the strong inverse relationship between the dietary levels of indigestible α cellulose and diet digestibility.

CHAPTER 8. The protein-sparing effect of carbohydrate on the production and carcass composition of post-larval *Penaeus monodon* fed low energy, practical-style diets under laboratory and field conditions.

8.1 INTRODUCTION

The ability of lipid and carbohydrate to spare dietary protein from catabolism for energy was reported in chapter 7. It was shown that the protein content of semi-purified diets could be reduced from 44 to 38 % by the isoenergetic substitution of lipid for protein in high energy (19.6 kJ g⁻¹, by calculation) diets, without compromising shrimp production. In addition, it was shown that at high levels of dietary lipid (12 %), carbohydrate levels > 26 % reduced the growth of shrimp fed low protein (38 %), high energy semi-purified diets (18-21 kJ g⁻¹, by calculation).

The present study was designed to investigate the ability of high levels of carbohydrate to spare dietary protein in low lipid (6-7 %), low energy, isoenergetic (18.4 kJ g⁻¹, by calculation) practical-style diets fed to post-larval *P. monodon*. The use of high lipid levels (10-12 %) was not attempted in this trial since, in order to maintain a low dietary energy density but include high levels of protein, it was not possible to include > 7 % lipid without the inclusion of a non-nutrient filler. This was avoided since it would be neither practical nor desirable commercially. In addition, the use of carbohydrate rather than lipid to spare protein is advantageous since carbohydrates represent the cheapest source of dietary energy (Tacon, 1990).

Previous work with penaeid shrimp has shown that protein can be spared (reduced from 40-50 % to 28-40 %, without significant growth impairment) by the approximately isoenergetic substitution of carbohydrate for protein in isolipidic, low energy (16-18 kJ g⁻¹ TE, by calculation) diets. This has been shown for juvenile *P. indicus* (Colvin, 1976a) and *P. merguensis* (Sedgwick, 1979) when fed practical-style diets, and recently (during the completion of this thesis) for juvenile *P. monodon* when fed casein-based diets (Shiau & Chou, 1991; Shiau & Peng, 1992). Similar work however, has yet to be conducted on post-larval *P. monodon* fed practical-style diets. It is possible that younger, post-larval shrimp may not be able to assimilate high levels of dietary

carbohydrate due to the poor development of the enzymatic system during their early life history (Fenucci *et al.*, 1982; Elliot *et al.*, 1989; Macdonald *et al.*, 1989; Lovett & Felder, 1990a,b) and/or to the low levels of carbohydrate present in the natural prey of young shrimp (Lee & Lawrence, 1985).

The majority of nutritional trials with shrimp (and fish) which have been published in the scientific press have used semi-purified or purified ingredients. This is because their use permits precise control over dietary constituents and the final proximate composition of the diets, enhancing replicability. The purified ingredients are also usually well assimilated. In addition, their use is thought to reduce interference (antagonism) between dietary constituents. Problems are encountered however, including the usually poor palatability of purified diets unless supplemented with feeding attractants, possible amino acid deficiencies, the unknown applicability of results generated with such diets to the nutritional requirements of shrimp fed on practical-style diets, and the more rapid growth rate noted by most authors on practical, fresh or live diets (Biddle *et al.*, 1977; New, 1980; Boghen & Castell, 1981; Maugle *et al.*, 1983b; Akiyama *et al.*, 1988).

In order to reduce variation in diet quality with the various practical-style formulations used in the present study, the animal protein content was kept constant. The only changes made were a progressive replacement of soybean meal with broken rice in the lower protein diets. This was necessary since previous research has shown that shrimp production is affected not only by the quantity of the ingredients (particularly protein), but also by their quality (Fenucci *et al.*, 1982; Smith *et al.*, 1985; Tacon & Jackson, 1985; Hajra *et al.*, 1988). Thus, protein sources containing amino acid patterns similar to that of the shrimp body (usually animal proteins) have been shown to have the highest nutritive value to *P. monodon* (Pascual, 1983; Deshimaru *et al.*, 1985; Dy Penaflores, 1989), as well as to other shrimp and fish species (Phillips & Brockway, 1956; Deshimaru & Shigeno, 1972; Arai, 1981; Kanazawa, 1985; Tacon & Cowey, 1985; Wilson, 1985).

One of the major problems encountered with laboratory-based nutrition trials is the lack of knowledge regarding their applicability to the requirements of the shrimp under commercial culture conditions. It has often been noted that growth of shrimp under

laboratory conditions is poorer than that obtained in ponds. The reasons for this include the lack of natural productivity in the laboratory, the presence of growth-promoting factors in live feeds and pond water, stress under captive conditions, and the poor palatability of semi-purified diets (Biddle *et al.*, 1977; Stahl & Ahearn, 1978; Maguire & Bell, 1981; Anderson *et al.*, 1987; Hunter *et al.*, 1987; Leber & Pruder, 1988; Burgett, 1989; Castille & Lawrence, 1989; Moss, 1992; Moss *et al.*, 1989, 1992). It is therefore important to investigate the effects of diets under both laboratory and commercial culture conditions.

The aims of the present study using practical-style dietary formulations were therefore to determine 1) the sparing action of carbohydrate on protein in low energy diets, 2) the optimum energy density and protein:energy ratio of diets containing balanced nutrient profiles, 3) whether the nutritional requirements of post-larval *P. monodon* established with the use of semi-purified diets are applicable to practical-style dietary formulations, and 4) whether the nutritional requirements are affected by the use of laboratory facilities as apposed to outside nursery tank rearing systems.

8.2 MATERIALS AND METHODS

8.2.1 Experimental animals

Shrimp post-larvae/juveniles used in the laboratory trial were air freighted from a hatchery in Singapore. Otherwise, the post-larvae were treated as in section 5.2.1, except that the shrimp were transferred from the freight cartons to the recirculated brackish-water tank system (Appendix 1) at 18 days post-metamorphosis (PL₁₈) to acclimate them to the experimental conditions. At this stage they were weaned to the basal experimental diet for a period of 16 days.

Shrimp post-larvae used in the field trial were delivered from the Aquastar hatchery at PL₁₅ as described for the nursery trials in section 3.2.1.2. On arrival, the shrimp were acclimated directly to the experimental tanks (Figures 3.1-3.5).

8.2.2 Experimental design

Experimental design for the laboratory trial was as in section 5.2.2, except that 34 day old post-larvae/juveniles were selected with mean weights of 69 ± 3 mg. Thirty shrimp of this size were randomly allocated to each of 12 fibreglass tanks of dimensions 0.75 X 0.7 X 0.2 m deep (0.53 m² area, 105 l volume), at a stocking density of 57 shrimp m⁻². Four dietary treatments were tested, each in triplicate, in the brackish-water recirculating system. The tanks were supplied with brackish-water at the rate of 6 l min⁻¹ per tank.

Every ten days over the 50 day laboratory trial period, the shrimp were group-weighed to determine growth, survival and feeding rates. In addition, all of the shrimp were individually weighed on termination of the trial to quantify within-tank size variation.

Experimental design for the field trial was as in section 3.2.1. 15 day post-larvae (PL₁₅) with mean weights of 4 mg were used. 50,000 shrimp of this size were randomly allocated to each of 12 concrete nursery tanks of dimensions 25 X 2.5 X 1 m deep (25 m² area, 25 m³ volume), at a stocking density of 2,000 shrimp m⁻². Four dietary treatments were tested, each in triplicate, in the brackish-water flow-through system. The tanks were supplied with sand-filtered brackish-water (20-40 % water exchange d⁻¹) and aerated using 12 airstones tank⁻¹, providing an air volume of 0.07 l sec⁻¹ m⁻². Three mesh habitats were positioned in each tank increasing the wetted surface area of each tank to 110 m². Substrates were not used.

Every seven days over the intended 35 day field trial period, the shrimp were measured for total length and group-weighed to determine growth, survival and feeding rates (see section 3.2.1.5). Unfortunately, due to air blower failure, the field trial was terminated on day 21 due to low DO conditions in all treatments on day 22. Prior to the expected mass-mortality, the tanks were drained on day 22 and the remaining shrimp counted. Samples of 100 shrimp tank⁻¹, as measured for total length on day 21, were used to quantify within-tank size variation.

On the sampling days, the tanks in the laboratory trial were scrubbed clean to prevent

bacterial build-up. In addition, prior to the first feeding of each day, any dead shrimp were removed from the tanks to prevent cannibalism, mortalities were recorded and uneaten food and faeces (but not exuvia) removed by siphon. It was not possible to perform any of these tasks during the field trial.

8.2.3 Water quality

Water quality during the laboratory trial was monitored and maintained as in section 5.2.3. Water quality remained relatively constant throughout the duration of the trial. The dissolved oxygen concentration (DO) remained above 6.6 mg l^{-1} (100 % saturation), temperature was maintained at $30.0 \pm 1.0 \text{ }^\circ\text{C}$, pH at 8.4 ± 0.5 and salinity at $25 \pm 2 \text{ }^\circ\text{‰}$. Total ammonia nitrogen (TAN) remained below 0.01 mg l^{-1} and nitrite nitrogen ($\text{NO}_2\text{-N}$) below 0.01 mg l^{-1} throughout the trial period.

Water quality during the field trial was monitored and maintained as in section 3.2.1.4. Water quality remained relatively constant throughout the duration of the trial. The mean values for the water quality parameters measured are shown in Table 8.1. Water quality was different between the laboratory and the field trials. Whilst water quality was not considered to be detrimental to the growth of the shrimp in either trial, the higher temperature (mean $30 \text{ }^\circ\text{C}$, compared to $28 \text{ }^\circ\text{C}$) and the higher salinity (mean $25 \text{ }^\circ\text{‰}$, compared to $12 \text{ }^\circ\text{‰}$) in the laboratory as compared to field trials may have enhanced the growth of shrimp in the laboratory trial.

8.2.4 Diet formulation, preparation and feeding

The same four practical-style diets were used in both the laboratory and field trials conducted during this study. The ingredients used in the diets were obtained from the Aquastar feed mill in Ranod, southern Thailand. Protein was supplied by a combination of shrimp head, soybean, squid and fish meals. Lipid was derived from these meals and from the inclusion of cod liver oil, 2 % soy lecithin and 0.2 % cholesterol. Carbohydrate was included in the form of broken rice and wheat flour. The diets also included 6 % wheat gluten meal as a binder, 1.5 % of a commercial vitamin premix (including 0.02 % vitamin C phosphate), and 2.5 % of a commercial mineral premix.

Table 8.1 Mean water quality parameters recorded during the 21 day field trial period.

Parameter ¹	Diet				mean
	1	2	3	4	
Temperature (°C)	27.7	27.9	28.0	28.0	27.9
Salinity (‰)	11.8	11.8	11.8	11.8	11.8
pH	7.8	7.9	7.8	7.8	7.8
DO (mg l ⁻¹)	7.3	7.2	7.1	7.1	7.2
DO (% saturation)	97.6	97.1	96.5	97.0	97.1
Secchi depth (m)	0.85	0.86	0.87	0.85	0.86
Suspended solids (mg l ⁻¹)	41.2	41.2	43.9	42.8	42.3
TAN (mg l ⁻¹) ²	0.4	0.4	0.4	0.4	0.4
Nitrite nitrogen (mg l ⁻¹)	0.1	0.1	0.1	0.0	0.1
Nitrate nitrogen (mg l ⁻¹)	0.2	0.2	0.2	0.1	0.2
DRP (mg l ⁻¹) ³	0.1	0.1	0.1	0.0	0.1

¹ All parameters measured twice daily and the mean over the period calculated

² TAN = total ammonia nitrogen

³ DRP = dissolved reactive phosphorus

The proximate analysis of these ingredients is shown in Table 8.2.

Four isoenergetic $19.6 \pm 0.3 \text{ kJ g}^{-1}$ ($18.4 \pm 0.2 \text{ kJ g}^{-1}$, by calculation), isolipidic ($6.7 \pm 0.5 \%$) diets were formulated for this study (Table 8.3). The isoenergetic diets were formulated to contain different levels of protein (35-44 %) at different protein:energy ratios (Pr:E), from 19-24 mg protein kJ^{-1} TE (using calculated energy values for comparison with other studies). The inclusion levels of the major protein-supplying ingredients (shrimp head, squid and fish meals) were maintained constant whilst manipulating dietary protein ratios. This was accomplished by altering the inclusion levels of soybean meal and rice bran in order to maintain the attractivity of the diets to the shrimp. Total energy levels were maintained constant by varying the level of dietary carbohydrate from 32 to 43 %. This resulted in C:L ratios of 4.8-6.7:1 for all diets.

Total dietary energy levels were measured by bomb calorimetry and calculated using mean total energy values of 23.6 kJ g^{-1} for protein, 39.5 kJ g^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate, as in section 2.5.4.

Diets were prepared at the small research-scale feed mill of Aquastar shrimp farm in Ranod. The finely ($< 0.125 \text{ mm}$) ground ingredients were first mixed in a Hobart bowl mixer. The lipid components were then added prior to further blending. 20-30 % warm water was then added until the mix bonded together under light hand pressure. The dough was cold extruded in a home made pellet mill through a 3 mm die. The resulting pellets were sun-dried on canvas sacking for 1-4 h to $< 10 \%$ moisture. The pellets were subsequently ground and sieved to two sizes. Size 1 was a crumble of 0.5-1 mm particle size and was used in the field trial. Size 2 was a pellet of 1-2 mm particle size and was used in the laboratory trial.

Based on previous experience (chapters 5-7), feeding during the laboratory trial was carried out twice daily at 0900 and 1700 for 9 days out of every 10 (not feeding on sampling day). The shrimp were fed at 40 % of body weight daily for days 1-9, 35 % for days 11-19, 25 % for days 21-29, 20 % for days 31-39 and 15 % for days 41-49 on a wet shrimp/dry food basis (as in chapter 7).

Table 8.2 Proximate analysis (% dry weight) of the feedstuffs used in the practical diet trial.

Ingredient	Protein	Lipid	Fibre	Ash	Carbohydrate	Moisture
Fish oil	0.0	100.0	0.0	0.0	0.0	0.0
Shrimp head meal	35.5	1.7	3.1	33.1	26.6	7.8
Soybean meal	47.1	3.4	6.7	5.7	37.1	11.1
Squid meal	61.1	6.5	0.2	16.0	16.2	12.5
Danish fish meal	69.0	13.6	0.6	13.8	3.0	6.0
Broken rice	9.2	1.4	1.2	0.7	87.5	12.3
Wheat flour	17.2	2.5	0.8	0.9	78.6	10.5
Wheat gluten meal	78.6	2.5	0.8	0.8	17.3	5.6
Soy lecithin	0.0	100.0	0.0	0.0	0.0	0.0
Cholesterol	0.0	100.0	0.0	0.0	0.0	0.0
Vitamin premix ¹	0.0	0.0	0.0	100.0	0.0	0.0
Vitamin C ²	0.0	0.0	0.0	100.0	0.0	0.0
Mineral premix ³	0.0	0.0	0.0	100.0	0.0	0.0

¹ Vitamin premix as used by Aquastar feed mill, Ranod, Thailand

² Vitamin C was used as the stabilised phosphate

³ Mineral premix as used by Aquastar feed mill, Ranod, Thailand

Table 8.3 Composition and proximate analysis (% dry weight) of the diets used in the practical diet trial.

Ingredient	Diet			
	1	2	3	4
Fish oil	1.3	1.5	1.6	1.8
Shrimp head meal	12.0	12.0	12.0	12.0
Soybean meal	27.6	21.5	14.3	7.3
Squid meal	10.0	10.0	10.0	10.0
Danish fish meal	12.0	12.0	12.0	12.0
Broken rice	12.5	18.5	25.8	32.7
Wheat flour	12.4	12.3	12.1	12.0
Wheat gluten meal	6.0	6.0	6.0	6.0
Soy lecithin	2.0	2.0	2.0	2.0
Cholesterol	0.2	0.2	0.2	0.2
Vitamin premix	1.48	1.48	1.48	1.48
Vitamin C	0.02	0.02	0.02	0.02
Mineral premix	2.5	2.5	2.5	2.5
+ Water (20-30 %)	+	+	+	+

- continued over

Table 8.3 - continued

Proximate analysis ¹	1	2	3	4
Protein	44.5	42.0	38.3	35.3
± SD	0.5	0.3	0.4	0.6
Ether extract ²	4.0	5.2	5.5	6.0
± SD	0.2	0.3	0.3	0.2
Total lipid ³	6.0	7.1	7.1	6.4
± SD	0.2	0.4	0.4	0.2
Carbohydrate ⁴	31.7	34.1	38.4	43.1
Fibre	4.7	4.4	4.3	3.6
± SD	0.1	0.1	0.2	0.2
Ash	13.1	12.4	11.9	11.6
± SD	0.5	0.4	0.2	0.3
Moisture	7.6	8.8	7.2	8.2
± SD	0.1	0.1	0.1	0.1
Total energy (kJ g ⁻¹) ⁵	18.3	18.6	18.5	18.3
Total energy (kJ g ⁻¹) ⁶	19.6	19.2	19.6	19.5
Pr:E ratio (mg pr. kJ ⁻¹ TE) ⁷	24.3	22.6	20.8	19.3
Protein:carbohydrate ratio	1.4	1.2	1.0	0.8
Carbohydrate:lipid ratio	5.3	4.8	5.4	6.7

¹ All results based on triplicate analyses

² Analysed By Soxhlet method

³ Analysed by method of Folch-Lees (Folch *et al.*, 1957)

⁴ By difference in dry weight

⁵ By calculation

⁶ By bomb calorimetry

⁷ Using calculated TE figures

Based on previous experience (chapter 3), feeding during the field trial was carried out four times daily (25 % of the daily ration per feeding) at 0700, 1100, 1500 and 1900 for 6 days out of every 7 (not feeding on the sampling day). The shrimp were fed at 35, 30 and 25 % of body weight daily for weeks 1-3 respectively on a wet shrimp/dry feed basis, allowing for 5 % mortality week¹ (as in trials 1-4 in chapter 3).

All diets remained stable in water for > 10 h as a result of the binding properties of wheat gluten meal and the finely-ground nature of the ingredients.

8.2.5 Sample collection and analysis

Shrimp and diet samples from the laboratory trial were collected and analysed as in section 7.2.6, and those from the field trial as in section 3.2.1.5.

8.2.6 Data analysis

Data obtained from the laboratory and field trials were analysed as described below:-

1. Shrimp performance (SGR, survival, production, FCE and carcass composition) in both trials were measured using the techniques described in section 3.2.1.6. PER was measured using the technique described in section 5.2.6.
2. The results from the laboratory and field trials were separated for statistical analysis. The effects of dietary protein:carbohydrate ratio (Pr:C) on shrimp growth, survival (arcsin transformed), production, FCE, PER and carcass composition after 50 days (laboratory trial) and 21 days (field trial) were analysed using a non-parametric analysis of variance (Kruskal-Wallis) after Bartlett tests revealed that in all cases the data were heteroscedastic (Sokal & Rohlf, 1981).
3. Where appropriate, the T-method for multiple comparisons was used to determine statistical differences between treatment means for the above ANOVAs (Sokal & Rohlf, 1981). Results were considered significant if

P < 0.05.

8.3 RESULTS

8.3.1 Shrimp production

This trial, unlike previous trials in this thesis used practical-style dietary formulations. Despite the larger initial weight of the shrimp used in the present laboratory trial, the SGR of the shrimp was similar to that achieved in the trials in chapters 5 & 6. Due to this relatively rapid growth rate (for older post-larvae/juveniles), and the higher survival of shrimp in this trial, shrimp production (yield per unit area) was considerably enhanced. Shrimp production in the present trial however, was not improved over that achieved in the trial in chapter 7, although shrimp growth rate was higher due to the lower stocking density and smaller shrimp used.

The overall results of this study investigating the effects of the same four diets on shrimp performance were similar between the laboratory and field trials. Survival rates were high at 78-86 %, FCE was 0.3-0.5 and PER 0.9-1.2 (Tables 8.4 & 8.5, Figures 8.1-8.3). Although the trends between the dietary formulations and shrimp growth rate and production were similar between the two trials, the values were different due to the different initial weight (age) of the shrimp stocked and their stocking densities. Thus, the SGR of the young PL₁₅ stocked during the field trial ranged from 13-15 % body weight d⁻¹, whilst that of the larger PL₃₄ stocked in the laboratory trial ranged from 7.6-7.8 % body weight d⁻¹ (Tables 8.4 & 8.5, Figure 8.4). Similarly, the production of the shrimp from the high density (2,000 shrimp m⁻²) field trial ranged between 4.5 and 6.6 g m⁻² d⁻¹, whilst that of the shrimp from the low density (57 shrimp m⁻²) laboratory trial ranged between 2.5 and 3.1 g m⁻² d⁻¹ (Tables 8.4 & 8.5, Figure 8.5).

In both trials, diet 4, containing the lowest protein level (35.3 %) (at the lowest Pr:C (0.8) and Pr:E (19.3) ratios), resulted in the poorest shrimp performance. This was demonstrated in terms of growth rate (P < 0.05 in the field trial), survival, production (P < 0.05 in both trials), and FCE (P < 0.05 in the field trial). PER however, although not significantly different between diets in the field trial, tended to be

Table 8.4 Performance of post-larval *P. monodon* fed practical diets varying in protein and carbohydrate level over the 50 day laboratory trial period.¹

Diet	Protein level (%)	Carbohydrate level (%)	Lipid level (%)	Mean survival (%)	Mean body weight (g)		SGR (% body wt d ⁻¹)	Production (g m ⁻² d ⁻¹)	FCE	PER
					Initial	Final				
1	44.5	31.7	6.0	80.0 ^a	0.07 ^a	3.50 ^b	7.84 ^a	3.08 ^b	0.44 ^a	0.99 ^{a2}
± SD	-	-	-	14.5	0.00	0.36	0.21	0.23	0.04	0.09
2	42.0	34.1	7.1	83.3 ^a	0.07 ^a	3.13 ^{ab}	7.62 ^a	2.89 ^{ab}	0.44 ^a	1.04 ^{ab}
± SD	-	-	-	8.8	0.00	0.15	0.10	0.19	0.01	0.03
3	38.3	38.4	7.1	85.6 ^a	0.07 ^a	3.21 ^{ab}	7.72 ^a	3.06 ^b	0.42 ^a	1.11 ^b
± SD	-	-	-	1.9	0.00	0.08	0.03	0.09	0.01	0.03
4	35.3	43.1	6.4	77.8 ^a	0.07 ^a	2.90 ^a	7.55 ^a	2.50 ^a	0.40 ^a	1.15 ^b
± SD	-	-	-	5.1	0.00	0.12	0.09	0.26	0.01	0.03

¹ Based on the mean of three replicates

² For single criterion, mean values in the same column bearing different superscripts are significantly different ($P < 0.05$) by T-method

Table 8.5 Performance of post-larval *P. monodon* fed practical diets varying in protein and carbohydrate level over the 21 day field trial period.¹

Diet	Protein level (%)	Carbohydrate level (%)	Lipid level (%)	Mean survival (%)	Mean body weight (g)		Mean total length (mm)		SGR (% body wt d ⁻¹)	Production (g m ⁻² d ⁻¹)	FCE	PER
					Initial	Final	initial	final				
1	44.5	31.7	6.0	85.1 ^a	0.004 ^a	0.079 ^b	24.27 ^b	1.48	14.44 ^b	6.03 ^b	0.42 ^b	0.95 ^{a3}
± SD	-	-	-	0.3	0.001	0.005	0.36	-	0.28	0.37	0.01	0.02
2	42.0	34.1	7.1	84.8 ^a	0.004 ^a	0.086 ^b	24.09 ^b	1.48	14.84 ^b	6.57 ^b	0.45 ^b	1.07 ^a
± SD	-	-	-	0.2	0.001	0.007	0.76	-	0.36	0.51	0.05	0.12
3	38.3	38.4	7.1	85.2 ^a	0.004 ^a	0.080 ^b	23.53 ^b	3.27	14.51 ^b	6.16 ^b	0.42 ^b	1.09 ^a
± SD	-	-	-	0.8	0.001	0.009	0.77	-	0.51	1.14	0.03	0.07
4	35.3	43.1	6.4	83.8 ^a	0.004 ^a	0.061 ^a	22.64 ^a	0.13	13.22 ^a	4.51 ^a	0.32 ^a	0.91 ^a
± SD	-	-	-	1.0	0.001	0.001	0.03	-	0.09	0.03	0.03	0.07

¹ Based on the mean of three replicates

² CV = coefficient of variation in final length = SD/mean * 100

³ For single criterion, mean values in the same column bearing different superscripts are significantly different (P < 0.05) by T-method

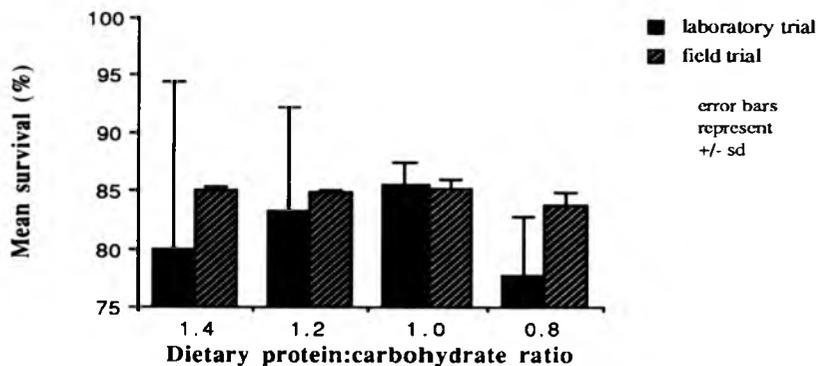


Figure 8.1 Relationship between dietary protein:energy ratio and mean shrimp survival after laboratory and field trials.

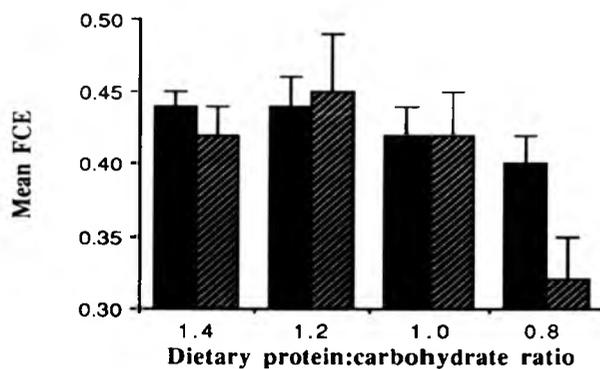


Figure 8.2 Relationship between dietary protein:energy ratio and mean FCE of shrimp over laboratory and field trial trials.

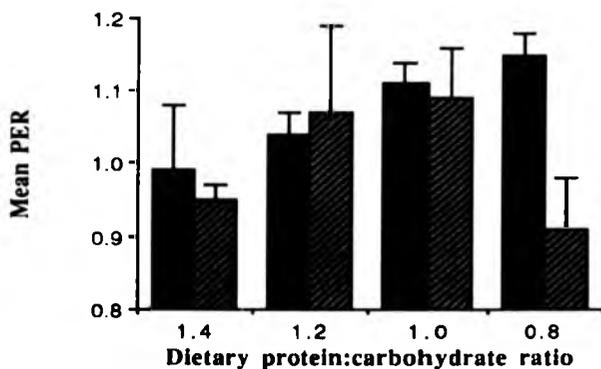


Figure 8.3 Relationship between dietary protein:energy ratio and mean PER of shrimp over laboratory and field trials.

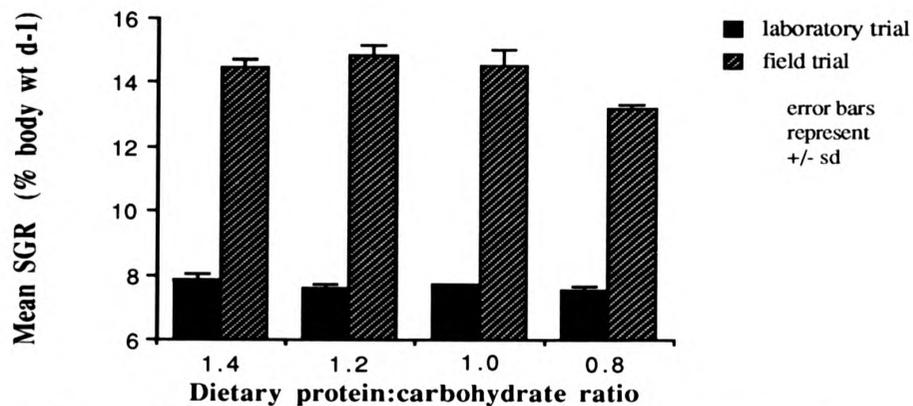


Figure 8.4 Relationship between dietary protein:energy ratio and mean SGR of shrimp over laboratory and field trials.

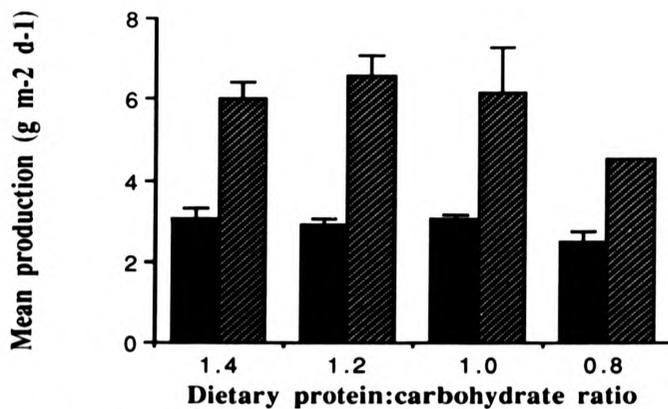


Figure 8.5 Relationship between dietary protein:energy ratio and mean shrimp production after laboratory and field trials.

enhanced at lower dietary protein levels (and Pr:C and Pr:E ratios). This relationship was significant ($P < 0.05$) in the laboratory trial (Tables 8.4 & 8.5, Figures 8.1-8.5).

For both trials however, shrimp growth, survival, production and FCE were unaffected by a reduction in dietary protein content from 44.5 to 38.3 % when the diets were maintained isoenergetic by the supplementation of carbohydrate. These diets also had Pr:C ratios of 1.0-1.4 and Pr:E ratios of 20.8-24.3 mg protein kJ^{-1} TE (Tables 8.4 & 8.5, Figures 8.1-8.5).

8.3.2 Carcass composition

Following the 50 day laboratory trial, the shrimp fed diet 2 contained significantly lower levels of lipid and ash, and higher levels of carbohydrate in their carcasses than shrimp fed the other diets. However, the variation in carcass lipid levels of shrimp fed the various diets was small at 5.5 to 6.1 % of dry body weight (Table 8.6). Otherwise, there were no significant differences between the carcass composition of shrimp fed the different diets. The carcass composition of shrimp from the field trial was not determined due to loss of the samples during transportation to Scotland.

8.4 DISCUSSION

8.4.1 Shrimp production

The requirement of post-larval/juvenile *P. monodon* for dietary protein is currently thought to be approximately 40 % (Khannapa, 1977; AQUACOP, 1977; Alava & Lim, 1983; Bautista, 1986; Shiau *et al.*, 1991a; Table 2.13), with the majority of commercially-produced diets containing 45-50 % protein (Table 2.12). Reducing the level of dietary protein without compromising shrimp growth or FCE is perhaps the most effective means of reducing the cost of and nitrogenous waste production from formulated shrimp diets.

Work has been conducted on reducing the dietary protein content through the sparing

Table 8.6 Carcass composition (% dry weight) of shrimp on termination of the 50 day laboratory trial.

Proximate analysis ¹	Diet			
	1	2	3	4
Protein ²	48.1 ^a	48.4 ^a	48.2 ^a	48.8 ^{a3}
± SD	0.3	0.9	0.4	0.2
Lipid ⁴	6.0 ^b	5.5 ^a	6.0 ^b	6.1 ^b
± SD	0.1	0.0	0.1	0.1
Ash	17.2 ^c	15.3 ^a	16.8 ^b	16.7 ^b
± SD	0.2	0.2	0.1	0.0
Carbohydrate ⁵	21.5	23.6	21.7	21.4
Fibre	7.2 ^a	7.2 ^a	7.3 ^a	7.1 ^a
± SD	0.5	0.4	0.1	0.5
Moisture	74.7 ^a	74.8 ^a	75.1 ^a	74.5 ^a
± SD	0.1	0.1	0.1	0.3

¹ Analyses (except for carbohydrate) based on mean of three replicates from carcasses for each replicate of each treatment

² Analysed by method of Lowry (Lowry *et al.*, 1951)

³ For single criterion, mean values in the same row bearing different superscripts are significantly different ($P < 0.05$) by T-method

⁴ Analysed by method of Folch-Lees (Folch *et al.*, 1957)

⁵ By difference in dry weight

action of both lipid and carbohydrate. A previous trial in this thesis (chapter 7) has shown that the isoenergetic substitution of lipid for protein can reduce the protein requirement of juvenile *P. monodon* from 44 to 38 % at a dietary energy density of 19.6 kJ g⁻¹ (by calculation). At these protein (38 %) and lipid (12 %) levels, chapter 7 also showed that increasing the carbohydrate levels from 26 to 42 % lead to a decrease in shrimp growth and production. This was probably due to excessive increases in dietary energy density (up to 21 kJ g⁻¹, by calculation), reducing the protein intake to sub-optimal levels. Although these results indicate the protein-sparing ability of dietary lipid, lipid itself is expensive, may have long-term effects on the shrimp carcass and the incorporation of high levels of it may require the adoption of specialised pelleting techniques. High energy diets using high levels of lipid to replace protein may not therefore result in more cost-effective diets. Low energy diets, substituting carbohydrates and lipid (at a low level) for protein may have a greater potential for reducing diet cost and waste discharge.

The results of both the laboratory and field trials show that at low levels of dietary energy (18.4 ± 0.2 kJ g⁻¹, by calculation), the protein content of diets fed to post-larval *P. monodon* can be reduced from 44.5 to 38.3 %. Such reductions in protein levels were achieved without a significant decrease in growth, survival, FCE or production by the isoenergetic substitution of carbohydrate for protein (*i.e.* at low Pr:C and Pr:E ratios). The 38 % protein diet also contained 38 % carbohydrate at a Pr:C ratio of 1:1. Similar results have been achieved previously by Bages & Sloane (1981) who showed that a Pr:C ratio of 1.2:1 could promote high survival and production of post-larval *P. monodon* in diets containing 35 % protein and 30 % carbohydrate. It therefore seems that even young post-larval *P. monodon* are able to utilise high levels (30-38 %) of dietary carbohydrate for energy production.

In the present study, the lower protein diets also resulted in increased PER as all available protein was being used anabolically for growth and there was less available for wasteful catabolism for energy. However, further reductions in dietary protein content to 35.3 % significantly reduced shrimp production. This result may have been due to the shrimp being unable to ingest sufficient protein at these dietary Pr:E ratios. Further reductions in energy density may thus be necessary in such low protein diets

in order to permit sufficient ingestion of dietary protein. In support of this, Shiau & Peng (1992) were able to reduce the protein content of diets fed to juvenile *P. monodon* to 34.5 % and 30.8 %, at dietary energy densities of 17.2 and 16.6 kJ g⁻¹ (by calculation) respectively without a significant decrease in growth, FCE or survival. Alternatively, the 35 % protein diet also contained > 40 % carbohydrate which may have compromised growth and/or had deleterious effects on the shrimp carcass as noted at such high levels by previous authors (Pascual *et al.*, 1983; Catacutan, 1991b). Future research may be directed at using a lower C:L ratio in such low protein/low energy diets in order to reduce the potentially damaging effects of such high levels of carbohydrate.

The results of the present study are in agreement with those of previous authors who have demonstrated the protein-sparing effects of isoenergetic substitution of protein by carbohydrate in practical-style, low energy diets fed to juvenile shrimp (Table 2.14). Colvin (1976a) reduced the protein level from 53 to 43 % of diets fed to *P. indicus* by increasing carbohydrate to 29 %. Sedgwick (1979) showed that growth rate of *P. merguensis* did not decline when the dietary protein level was decreased from 51 % to approximately 34 % by increasing carbohydrate to 20 %. Recent work with juvenile *P. monodon* has yielded similar results with semi-purified diets. Shiau & Chou (1991) showed that dietary protein could be reduced from 39 to 36 % by increasing the level of carbohydrate from 27 to 35 % at energy densities of 18 and 17.3 kJ g⁻¹ (by calculation) respectively. Subsequently, Shiau & Peng (1992) showed that it was possible to reduce the protein content further from 41 to 31 % without a significant decrease in growth rate when carbohydrate levels were increased from 27 to 36 % and the energy density decreased further from 17.7 to 16.6 kJ g⁻¹ (by calculation) respectively.

These results confirm that it is not possible to determine the protein requirements of shrimp in isolation and that in terms of growth rate, a range of protein (and Pr:E ratio) optima will exist depending upon the energy density of the diet. The results also confirm that both lipid and carbohydrate can be used by the shrimp for protein-sparing, but that there are limits, primarily concerning the energy density of the diet and the assimilation efficiency of these nutrients, within which protein-sparing can be

optimized.

In both this trial (diet 3) and the trial conducted in chapter 7 (diet 5) optimum protein-sparing without significantly decreasing growth or production was achieved by feeding shrimp a diet containing 38.3 % protein at a dietary energy density of 18.2-18.5 kJ g⁻¹ (by calculation) and a Pr:E ratio of 21 mg protein kJ⁻¹ TE. In both of these diets protein accounted for 49 % of the total dietary energy. A range of C:L ratios responsible for protein-sparing in such diets was demonstrated at 2.1-5.4:1. Thus, lipid can be included at 7-12 % (15-26 % TE) and carbohydrate at 26-38 % (24-36 % TE) of these diets. The final choice of carbohydrate and lipid levels will depend upon striking a balance between the growth-promoting and diet processing restriction effects of high lipid diets, the relative cost of the energy substrates, and possible deleterious effects of high levels of lipid or carbohydrate on the shrimp carcass.

This study indicates that nutritional trials may yield similar results with the use of semi-purified and practical-style diets. Although the more easily defined nature of semi-purified diets may thus remain preferable, their applicability to the formulation of commercial diets remains suspect. Differences between the efficiency of assimilation of various practical ingredients (particularly lower cost plant-based protein sources), and their possibly toxic properties, necessitates the testing of practical dietary formulations prior to commercial use.

Similar results were achieved with shrimp fed the same diets under laboratory and outdoor concrete nursery tank conditions. This was despite differences between the trials including shrimp age, size and stocking density, trial length and water quality. Previous work has suggested that shrimp (at least under semi-intensive conditions) are able to obtain a significant proportion of their nutrition from the natural productivity of earthen ponds (Maguire & Bell, 1981; Anderson *et al.*, 1987; Hunter *et al.*, 1987; Burgett, 1989; Castille & Lawrence, 1989). This includes foods such as bacteria, microalgae, detritus, faeces and larger prey items, which are largely derived from the fertilization of the pond from applied feeds and fertilizers. Such food items have been proved capable of promoting shrimp growth by up to 89 % when shrimp pond water (not filtered to < 5 microns) is used in tank-based trials (Leber & Pruder, 1988; Moss,

1992; Moss *et al.*, 1989, 1992).

During the present field trial stocking densities were high and, although shrimp pond water was not used, the water was only crudely sand-filtered and originated from a highly eutrophic brackish-water lagoon. This lagoon contained fish and shrimp cages and also received the effluent from numerous intensive shrimp ponds. Under such intensive nursery tank conditions, any natural productivity available to the shrimp did not seem capable of reducing the protein requirements of shrimp to below that achieved in the laboratory trial. The results of laboratory-based nutrition trials may therefore be applicable to intensive nursery tank rearing trials, whilst differences might still be expected under lower density, pond-based trials. However, no firm conclusions could be drawn due to the differences between the two trials conducted during this study. In addition, the ability of natural productivity to supply essential nutrients and permit a decrease in dietary nutritional quality without sacrificing shrimp production requires further study. This should ideally be conducted under pond conditions over a range of stocking densities and for shrimp of different ages.

8.4.2 Carcass composition

The carcass composition of shrimp following the laboratory trial was relatively constant for all diets tested. Carcass protein levels (48-49 %) were not significantly different for shrimp fed diets containing 35 to 45 % protein at an energy density of 18.4 kJ g⁻¹ (by calculation). These results are similar to those in chapter 7 (49-50 % carcass protein) using casein-based diets containing 38-44 % protein at an energy density of 19.6 kJ g⁻¹ (by calculation).

Since there were no significant differences between either the FCE or growth rate of shrimp fed diets containing 38-45 % protein, these results suggest that dietary protein levels > 38 % are not utilized solely for growth, but that the excess is instead catabolised for energy production.

It has been shown previously that dietary and carcass protein levels are unrelated in fish species fed low energy diets (Cowey & Sargent, 1972; Page & Andrews, 1973; Poston,

1975; Millikin, 1982; Daniels & Robinson, 1986). Although little work has been conducted with shrimp, Alava & Lim (1983) showed that there was a direct relationship between dietary and carcass protein levels for juvenile *P. monodon*. However, the growth rate was not significantly reduced by a decrease in protein from 51-40 % of high energy semi-purified diets (19-20 kJ g⁻¹, by calculation). This suggests that high levels of protein may be utilised for growth in high energy diets, although such diets resulted in significantly decreased survival, leading to difficulties with the interpretation of their results.

The levels of carcass lipid were similar for all diets tested in the present study (5.5-6.1 %) and similar to those recorded in chapter 7 (4.7-6.5 %), utilising high levels of dietary lipid (up to 12 %) in semi-purified diets. In neither study were carcass lipid levels related to either dietary protein or lipid levels. In contrast, previous work has shown that increasing the levels of dietary lipid and/or protein (and/or energy) leads to an increase in carcass lipid level in both shrimp (Catacutan, 1991b) and fish (Watanabe, 1982; Daniels & Robinson, 1986). This is due to the storage of excess energy from protein and/or lipid as body lipid or carbohydrate (Cowey & Sargent, 1979).

Increasing the level of non-protein energy from lipids or carbohydrates without affecting the production or carcass composition of shrimp may thus only be possible when diets contain the correct balance of energy substrates. Alava & Lim (1983) showed that carcass lipid levels were unaffected when carbohydrate levels were increased to replace protein as the major energy source in diets fed to juvenile *P. monodon*. In addition, the carcass lipid and protein levels of the crayfish, *Procambarus acutus acutus* were shown to be unaffected by the isoenergetic substitution of lipid for carbohydrate in isonitrogenous diets (Davis & Robinson, 1986).

8.5 SUMMARY

The isoenergetic substitution of carbohydrate for protein is able to maintain the growth rate of post-larval *P. monodon* fed low energy, practical-style diets. Good growth may be achieved with the use of low protein (38.3 %), low energy diets where dietary

carbohydrate levels are increased to 38 % at a C:L ratio of 5.4:1 and a Pr:E ratio of 21 mg protein kJ^{-1} TE. This diet had a calculated total energy density of 18.5 kJ g^{-1} (19.6 kJ g^{-1} by calorimetry), where 49 % of the total energy was derived from protein, 15 % from lipid and 36 % from carbohydrate.

The results from laboratory-based trials appear to be applicable to high-density nursery tank trials. These results have potentially beneficial implications for the formulation of low cost, low pollution post-larval/juvenile shrimp diets.

CHAPTER 9. General discussion of nutrition trials.

The nutrition trials conducted during this study were designed to provide data for optimising the protein:energy ratio and maximising the contribution of non-protein energy sources in post-larval/juvenile *P. monodon* diets. This information is necessary for the formulation of least-cost, low pollution diets resulting in optimum shrimp production. To accomplish these objectives, a series of trials were conducted in order to establish the relative requirements for the building blocks (protein, lipid and carbohydrate) and energy of *P. monodon*. These trials were first aimed at elucidating the qualitative and quantitative requirements for lipids and carbohydrates, and the optimal ratio of these nutrients in isonitrogenous, isoenergetic diets. The protein-sparing ability and optimum energy provision from lipids and carbohydrates were then investigated, compared to the results of previous authors working with young shrimp (and fish) of various species and models for shrimp generated.

Both the quality and quantity of dietary lipid were demonstrated to affect the production of *P. monodon*. A preliminary trial conducted during this study showed that from a range of dietary lipid sources, fish (cod liver) oil was able to support maximum growth and survival of *P. monodon*. Similar results have been obtained previously with *P. monodon* (Catacutan, 1991a), *P. japonicus* (Guary *et al.*, 1976; Kanazawa *et al.*, 1977), *P. vannamei* (Dominy & Lim, 1989), and *P. merguensis* (AQUACOP, 1978). However, a 3:1 mixture of cod liver and soybean oil was shown to be as effective as cod liver oil alone, and result in cost savings. This result confirms the ability of mixtures of marine (fish and bivalve) and plant oils to promote good growth at reduced cost as suggested previously for other species of shrimp (Deshimaru *et al.*, 1979; Read, 1981; Ali, 1990).

The inclusion of soy lecithin (especially at 46-63 % of the total dietary lipid) was shown to slightly enhance shrimp growth at 3 %, and survival at 6 % of the diet (chapter 5). This requirement for lecithin is higher than the 2 % previously reported for *P. monodon* (Pascual, 1986, 1988, who did not test higher levels), but consistent with the reported requirements of *P. japonicus* (Teshima *et al.*, 1982, 1986b,c; Kanazawa *et al.*, 1985) and homarid lobsters (Conklin *et al.*, 1980, 1981; Bowser & Rosemark, 1981) fed

casein-based diets. Inclusion of 3-6 % lecithin resulted in dietary levels of 0.5-0.8 % phosphatidylcholine (PC), thought to be the active fraction of lecithin (chapter 5). This is consistent with recent work which has shown that up to 1 % dietary PC improved the growth of *P. monodon* (Chen, 1993). Reductions in the requirement for supplementary lecithin may be expected in practical-style diets containing endogenous PC.

This study demonstrated that basal lipids (including n-3 series HUFA), lecithin and cholesterol were required for optimising the growth and survival of *P. monodon*. Dietary levels of n-3 HUFAs of 0.15 % 20:5(n-3) and 0.13 % 22:6(n-3) at a n-3:n-6 series fatty acid ratio of 0.5 were sufficient to maximise shrimp growth and survival. Further increases in n-3 HUFAs did not enhance shrimp production (chapter 5). This is in contrast to the results of previous authors working with larval and post-larval *P. japonicus* (Kanazawa *et al.*, 1978, 1979a,c,e,d, 1985; Jones *et al.*, 1979b; Teshima & Kanazawa, 1984) who suggested that there was a requirement for 0.5-1.0 % of these HUFAs. This result may be due to species-specific differences. More consistent with the results of this trial however, D'Abramo (1990) maintains that dietary levels of 0.075 % may satisfy the requirement for any particular fatty acid. Further increases in n-3 HUFA levels may instead enhance shrimp production due to their attractant qualities (Bryant *et al.*, 1989), or possibly to an increase in the n-3:n-6 series fatty acid ratio to that approaching the ratio found in shrimp tissues.

Penaeid shrimp, unlike some fish species, are not thought to be able to tolerate high levels of dietary lipid. Most authors have reported that lipid levels of > 10 % result in decreased growth and survival and increased deposition of lipid in the carcass (Deshimaru *et al.*, 1979, 1985; Teshima & Kanazawa, 1984; Bautista, 1986). However, it is likely that these effects are more associated with energy imbalances than lipid levels *per se*. In support of this, the present study has shown that in diets containing high levels of protein (43 %), carbohydrate (> 33 %) at a high energy level (19.7 kJ g⁻¹, by calculation), increasing the lipid level above 5-7 % (and C:L ratios over 4) decreased shrimp growth and production (chapter 5). However, in diets containing 44 % protein and 19.6 kJ g⁻¹ energy (by calculation), reducing the carbohydrate level to < 30 % (and the C:L ratio to < 3.2) and increasing lipid levels to 9.1-12.8 %, resulted in similar growth, but significantly higher survival and production than that

achieved on low (< 6.2 %) lipid diets (chapter 6). This result indicated that *P. monodon* are not only able to tolerate higher levels of lipid than has generally been acknowledged, but that higher production can result from high lipid diets containing balanced quantities of protein and non-protein energy. The inclusion of sufficient quantities of antioxidants to prevent lipid rancidity may also be important in high lipid diets. High shrimp production on diets with low C:L ratios may be due to the attractant qualities or calorogenic effects of marine lipids, or possibly to the poor assimilation/histopathological effects of high levels of carbohydrate. Whilst this study was in progress, similar results were obtained by other authors feeding diets containing 12-12.8 % lipid at a C:L ratio of 1.5-1.8 (Catacutan, 1991a,b; Sheen & Chen, 1992).

Further work showed that lipid could spare protein for growth in juvenile *P. monodon* fed isoenergetic diets. Dietary protein levels could be reduced from 44 to 38 % without affecting shrimp production by the isoenergetic substitution of lipid for protein (chapter 7). At these levels of protein (38 %) and lipid (12 %), optimum growth and production was achieved by shrimp fed diets containing the lowest levels of carbohydrate (26 %) and energy (18.2 kJ g⁻¹, by calculation) tested, at a low C:L ratio of 2.1:1 and a high Pr:E ratio of 21 mg protein kJ⁻¹. Such protein-sparing at low C:L ratios has previously been documented for various fish species (Shimeno *et al.*, 1979, 1980, 1985; Berger & Halver, 1987; Cho, 1987; Alstead & Jokumsen, 1989), but until now has not been demonstrated for shrimp. Furthermore, in such nutrient balanced diets, increases in dietary lipid levels did not result in significantly higher levels of carcass lipid, suggesting that this phenomenon may occur only where diets contain an excessive ratio of lipid energy:total energy. However, due to the short duration of the trials (50 days), it may be prudent to examine lipid deposition rates over a longer period before definitive conclusions can be drawn.

During this study (not included here, but published in Briggs, 1991), it was shown that as with lipids, both the quality and quantity of dietary carbohydrate affect shrimp production. In common with the results of previous trials with various shrimp species (Deshimaru & Yone, 1978; Abdel-Rahman *et al.*, 1979; Pascual *et al.*, 1983; Kanazawa, 1985a; Alava & Pascual, 1987; Briggs, 1991; Ali, 1993), disaccharides such as sucrose and polysaccharides including corn and wheat starch were found to be

preferable to monosaccharides such as glucose. More complex carbohydrates are thought to be utilised more efficiently by shrimp due to the slower absorption of glucose units cleaved from polysaccharides (Abdel-Rahman *et al.*, 1979; Furuchi & Yone, 1982; Shiao & Peng, 1992), their greater use as energy substrates (Capuzzo & Lancaster, unpublished data in Capuzzo, 1981; Alava & Pascual, 1987) and also to their greater binding qualities, particularly when cooked (New, 1976a,b; Tacon, 1990).

Although no specific dietary requirement for carbohydrates has been established, the inclusion of 10-35 % in diets for *P. monodon* has led to improved growth and survival (Bages & Sloane, 1981; Alava & Pascual, 1987; Catacutan, 1991b; Sheen & Chen, 1991; Shiao & Chou, 1991). However, inclusion of higher levels (35-40 %) has led to poor growth, survival, digestibility and histopathological changes in shrimp tissues (Pascual *et al.*, 1983; Alava & Pascual, 1987; Catacutan, 1991b). The results from this study showed that increasing the level of dietary carbohydrate from 21 to 45 % and C:L ratios from 1.6 to 10.1 in isoenergetic diets significantly reduced shrimp survival, PER and hence production, but had no effect on growth (chapter 6).

The reason for poor shrimp performance on diets containing high C:L ratios is unclear but may have been related to differences in dietary lipid levels. Catacutan (1991b) however, did not find this and showed that growth, survival and FCE were reduced in shrimp fed diets containing relatively constant levels of lipid (9.5-11.9 %) when carbohydrate levels were increased from 22 to 39 %. Additionally, good growth and survival has previously been recorded for *P. monodon* fed diets containing lipid levels as low as 5-8 % (Alava & Lim, 1983; Deshimaru *et al.* 1985; Bautista, 1986; chapter 5). In contrast, recent studies have suggested that high lipid levels (12-13 %) may indeed be responsible for increased shrimp growth, survival and production (Catacutan, 1991a; Sheen & Chen, 1992).

Low digestibility of high carbohydrate diets has also been suggested to account for their poor performance (Fenucci *et al.*, 1982; Akiyama *et al.*, 1988; Akiyama, 1991; Shiao & Peng, 1992), but digestibility was relatively high for high carbohydrate diets during this trial. However, the digestibility measurements may have been inaccurate due to the difficulty of faeces collection and because of differences between the α cellulose content

of the diets (see later). The possibility that poor digestibility of high carbohydrate diets is due to low levels of amylase was investigated during this study (chapter 6). It was shown that amylase levels in the midgut gland were not significantly increased in response to increasing levels of dietary carbohydrate. Similar results have been achieved previously by some authors working with penaeid shrimp (Van Wormhoudt *et al.*, 1980; Elliot *et al.*, 1989), whilst others have suggested that some degree of adaption is possible (Hoyle, 1973; Fenucci *et al.*, 1982; Maugle *et al.*, 1983b). Chen & Lin (1992) however, found that feeding fresh and compounded diets to post-larval *P. monodon* succeeded in altering their digestive enzyme profiles, but not their growth rates. Further research has shown that dietary supplementation of microencapsulated amylase lead to increased starch digestion, growth, moulting and protein-sparing in *P. japonicus* (Maugle *et al.*, 1983a,b), suggesting that endogenous amylase production may indeed be insufficient to support maximum shrimp production. Supplementation of diets with digestive enzymes may therefore offer more potential to enhance the utilisation of dietary carbohydrate and possibly promote protein-sparing and the production of cheaper diets.

Deleterious changes in the midgut gland of shrimp were not observed in shrimp fed the high carbohydrate diets in chapter 6, suggesting that this was not the reason for their poor performance.

Despite the poor performance of shrimp fed high carbohydrate diets, limited amounts of carbohydrates do have potential for protein-sparing in diets fed to young shrimp. The present study showed that protein levels could be reduced from 44 to 38 % without a significant decrease in shrimp production by the isoenergetic substitution of carbohydrate for protein (chapter 8). In this trial, the 38 % protein diet contained only 7 % lipid, 38 % carbohydrate (at a C:L ratio of 5.4:1), 18.5 kJ g⁻¹ TE (by calculation) and a Pr:E ratio of 21 mg protein kJ⁻¹. A further reduction in the protein level to 35 % lead to decreased growth and production. This was probably due either to an imbalance in the Pr:E ratio, preventing sufficient intake of protein for maximal growth, or to the poor assimilation of the high levels of carbohydrate (43 %) present in this diet.

The use of carbohydrate to spare dietary protein has previously been shown for various

shrimp species (Colvin, 1976a; Sedgwick, 1979). Recent work with juvenile *P. monodon* has shown that growth is not significantly impaired when protein levels are reduced from 39-41 % to 36 % (Shiau & Chou, 1991) or even 31 % (Shiau & Peng, 1992). These results were achieved by increasing carbohydrate levels to 35-36 % (at 8-9 % lipid), and decreasing the total energy density to 17.3 or 16.6 kJ g⁻¹ and the Pr:E ratio to 20 or 18.5 mg protein kJ⁻¹ (by calculation) respectively. Increasing the level of carbohydrate in low protein diets may thus only be possible when dietary energy levels are reduced.

As mentioned above, during two of the nutrition trials (chapters 6 & 7) conducted in this study, measurements of apparent dry matter digestibility were taken using the chromic oxide marker method of Furukawa & Tsukahara (1966). These analyses suggested poor dry matter digestibilities of 20-42 % and 39-70 % for the trials in chapters 6 and 7 respectively. The poor overall results appeared to be largely due to contamination of the faeces with uneaten food and partially digested exuviae. This contamination was due to the feeding habits of the shrimp which include mastication of the pelleted diets outside their bodies, regurgitation of indigestible food particles, the shredding and partial consumption of cast exuviae and coprophagy. In the large, dark tanks used for the nutritional trials in the present study, it proved to be very difficult to separate true faeces from these other wastes, leading to underestimations of diet digestibility. Faecal collection was particularly difficult during these trials due to the small size of the faeces produced by post-larval/juvenile shrimp.

Most of the investigations conducted to date on diet digestibility in shrimp have used large (4-40 g) shrimp held in specially designed digestibility tanks with false floors in an effort to simplify faecal separation and collection (Fenucci *et al.*, 1982; Teshima & Kanazawa, 1983; Lee & Lawrence, 1985; Smith *et al.*, 1985; Akiyama, 1991; Akiyama *et al.*, 1988; Catacutan, 1991b). These techniques would therefore seem necessary for the collection of useful data on diet digestibility. The results of these authors have generally provided high digestibility values for protein (73-94 %) and lipid (70-93 %). However, there have been indications that lipid digestibility is lower (46-69 %) in young shrimp (Smith *et al.*, 1985; Fenucci *et al.*, 1992). In addition, the digestibility of carbohydrate is generally low at 36-76 % (Fenucci *et al.*, 1982; Akiyama *et al.*,

1988; Akiyama, 1991), leading to dry matter digestibilities of only 34-73 % (Lee & Lawrence, 1985; Smith *et al.*, 1985; Akiyama *et al.*, 1988; Shiao & Peng, 1992).

Although it might be expected that diets which are highly digestible (high in proteins and lipids) may also promote good growth rates, neither the present study nor any other trial conducted to date has shown a direct relationship between diet digestibility and shrimp growth. In chapter 6 it was shown that although relatively good dry matter digestibility was measured for shrimp fed diets containing the lowest levels of carbohydrate (possibly due to high lipid digestibility), there were indications of increasing digestibility at high levels of carbohydrate which were unrelated to growth. This direct relationship between dietary carbohydrate level and diet digestibility was also observed in chapter 7, but diet digestibility in this trial was inversely related to growth. Similar relationships have previously been demonstrated with adult *P. monodon* (Catacutan, 1991b) and juvenile *Pleoticus muelleri* (Fenucci *et al.*, 1992). Although these relationships appear to refute claims of poor carbohydrate digestibility, in each of these studies, the poorly digested, low carbohydrate diets also contained high levels of the indigestible filler α cellulose (to maintain dietary energy density). In these trials, diet digestibility was inversely related to α cellulose level. The level of dietary α cellulose may thus have more influence on digestibility than the level of carbohydrate and may partially explain the poor correlation between digestibility and growth. Further studies, using large shrimp in digestibility chambers are required using diets containing constant levels of α cellulose or other fillers in order to understand the effects of increasing levels of carbohydrate and lipid on digestibility and growth in shrimp.

The results of the present study and those of previous authors suggest that shrimp do not have fixed requirements for protein (or lipid or carbohydrate), but that a range of protein (and Pr:E) optima will exist depending upon the energy density of the diet. The generally high protein requirements of shrimp and fish reported in scientific publications (and used in commercial diets) may thus be an artifact of the sub-optimal amino acid profiles of the protein sources used and the energy density of the diets used, as has been suggested previously for fish (Bowen, 1987; Yakupitiyage, 1989). These optima will in turn be influenced by the choice of criteria used to determine diet performance, *i.e.* whether maximum growth (or production) or maximum growth per unit cost is the

primary consideration. In addition, both lipid and carbohydrate may be used for sparing protein in shrimp diets. However, their ratio may be as important as the percentage inclusion of either nutrient within the limits of the requirements for specific nutrients (such as fatty acids, PC and hormone precursors), the ability of the shrimp to assimilate those nutrients, the possibly deleterious effects on shrimp tissue of excessively high levels, and the feasibility of processing diets containing the various levels of these nutrients.

A consistent pattern emerges when the relative contribution of energy from proteins, lipids and carbohydrates included in the most successful diets of this study and other published data (Tables 2.13, 2.14, 2.16 & 2.20) is recalculated using standard fuel values (23.6, 39.5 and 17.2 kJ g⁻¹ for protein, lipid and carbohydrate respectively). This analysis confirms that the protein requirements of shrimp depend upon the energy density of the diet. Figure 9.1 shows the linear relationship between dietary protein level and the Pr:E ratio of diets producing maximum growth of various species of omnivorous penaeid shrimp. From this relationship it is possible to estimate the Pr:E ratio and hence total energy level (Figure 9.2) required for optimum growth of shrimp at any protein level. Thus, at 30 % dietary protein, the optimal Pr:E ratio will be 18.5 mg protein kJ⁻¹ TE and the optimal TE level will be 16 kJ g⁻¹. At 50 % protein however, these requirements will increase to 27.5 mg protein kJ⁻¹ and 18 kJ g⁻¹ respectively. Further, previous authors have reported similar optimum Pr:E ratios (as mg protein kJ⁻¹ TE) for various fish species *i.e.* 23-30 for tilapia, *Oreochromis niloticus* (Yakupitiyage, 1989), 21-28 for catfish, *Ictalurus punctatus* (Garling & Wilson, 1976), 23-24 for sea bream, *Sparus aurata* (Vergara Martin, 1992), and 21-29 for red drum, *Sciaenops ocellatus* (Daniels & Robinson, 1986). This data suggests that reported differences between the protein requirements of omnivorous shrimp and fish species may be related more to the dietary energy content than any specific requirements for protein (amino acids).

Further analysing the results of the present study and previous trials with young shrimp, the relative requirements for lipids and carbohydrates at various protein levels can be established (Figure 9.3). In this instance, only information from trials (in this study and from the literature) with *P. monodon* was used due to the paucity of data on other

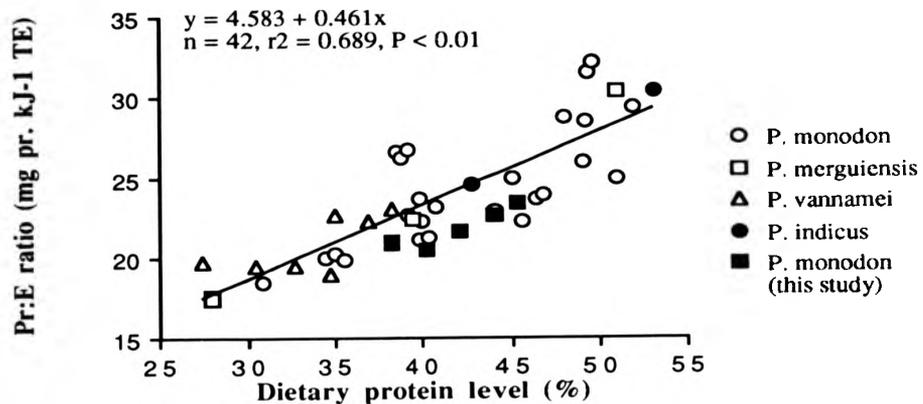


Figure 9.1 Relationship between optimum dietary protein level and protein:energy ratio in shrimp diets.

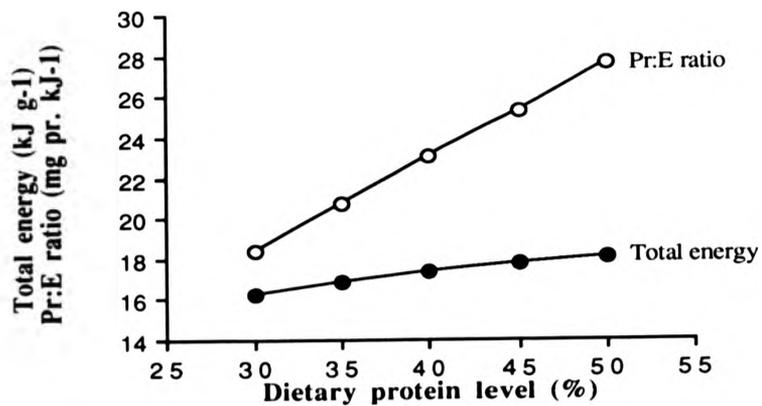


Figure 9.2 Relationship between dietary protein level, protein:energy ratio and total energy in diets promoting optimum growth of post-larval *P. monodon*.

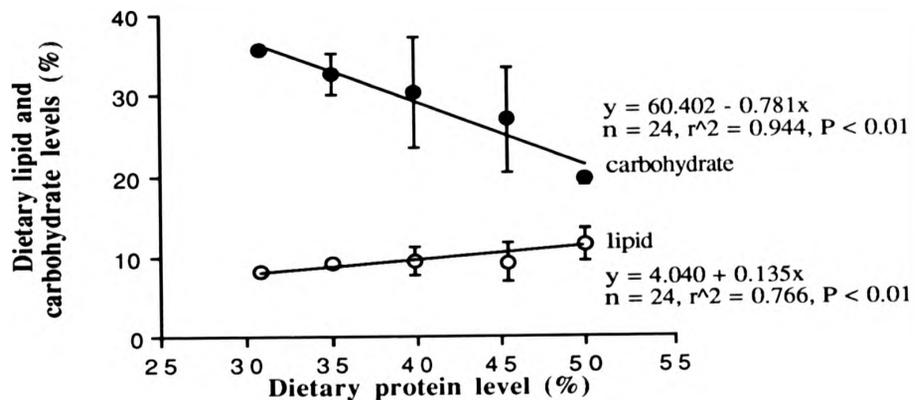


Figure 9.3 Relationship between protein, lipid and carbohydrate in diets promoting optimum growth of post-larval *P. monodon*.

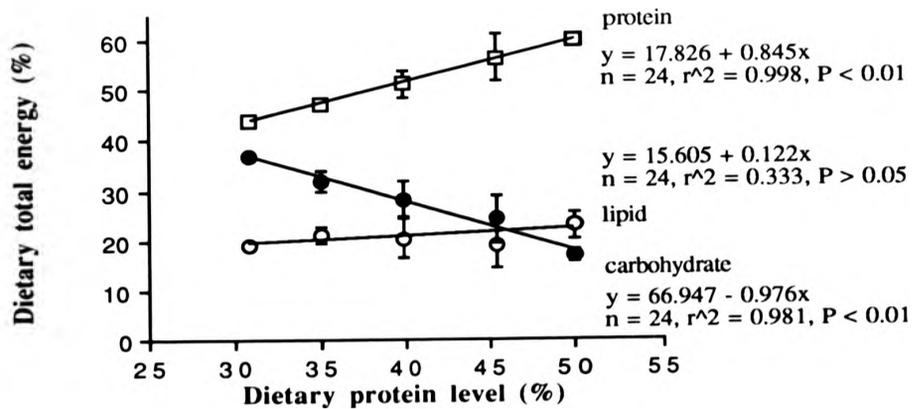


Figure 9.4 Relationship between energy provision from protein, lipid and carbohydrate in diets promoting optimum growth of post-larval *P. monodon*.

omnivorous shrimp species. This analysis reveals that at low levels of dietary protein (30 %), high levels of carbohydrate (up to 36 %), at high C:L ratios of 4.5:1 are required, whilst much lower levels of carbohydrate (21 %), at low C:L ratios (1.8:1) are necessary in high (50 %) protein diets. This data includes only a narrow range of lipid levels (7-13 %) and reflects the predominant use of carbohydrate to spare dietary protein in shrimp diets. Although attempts to further increase lipid levels in low (< 38 %) protein diets have not been made, such increases may be unfeasible due to increased lipid deposition in the shrimp carcass. Nevertheless, this is an area requiring further research.

At the levels of protein, lipid and carbohydrate suggested as optimal from this analysis, the relative contribution of these substrates to the energy requirements of *P. monodon* can be assessed (Figure 9.4). This analysis reveals that protein optimally provides approximately 50 % (44-60 %) of the total energy requirements, *i.e.* at a protein energy:total energy (PE:TE) ratio of approximately 1:2. This ratio however, is influenced by the dietary protein level and varies from 1:1.6 at 30 % protein to 1:2.3 at 50 % protein. This ratio is a useful way of presenting the protein and energy requirements of a species since it indicates the amount of non-protein energy which can be added to the diet without any detrimental effects on growth. Interestingly, the optimum PE:TE ratio reported here for shrimp is similar to that reported for a range of fish species. For example, the optimum ratio for rainbow trout, *Salmo gairdnerii* has been reported as 1:2.0 (Lee & Putnam, 1973), for sea bream, *S. aurata* as 1:1.8 (Vergara Martin, 1992), for tilapia, *O. niloticus* as 1:2.3 (Wee & Ng, 1986) or 1:1.6 (Yakupitiyage, 1989), and for tilapia, *Sarotherodon mossambicus* as 1:1.9 (Jauncey, 1982). These similarities may be due to the ectothermic nature of all fish and shrimp species.

Figure 9.4 also shows that non-protein energy (NPE) may be further broken down into lipid (LE) and carbohydrate (CE) energy. Once again, the relative proportions of energy derived from lipids and carbohydrates is influenced by the dietary protein level. Thus at 30 % dietary protein, LE and CE account for 20 and 38 % of TE respectively at a LE:CE ratio of 0.5:1, whilst at 50 % protein, this ratio increases to 1.3:1 at 22 % and 17 % respectively. These LE:CE ratios are lower than that found to be optimum for

fish *i.e.* 2.3:1 for *O. niloticus* (Yakupitiyage, 1989), due possibly to the higher tolerance for lipid and lower tolerance for carbohydrate by fish (New, 1980).

These ratios of protein, lipid, carbohydrate and the amount of energy that they provide in shrimp diets are those that result in the best growth and production. However, of at least equal importance, particularly in terms of the formulation of commercial diets, is the cost of the diet in relation to the growth it promotes. Throughout the present study emphasis has been placed on reducing the dietary protein level without decreasing growth. This is partially in an attempt to reduce diet cost since protein is the most expensive of the energy substrates. In order to formulate least-cost diets, the amount of protein included must supply the minimum quantity of amino acids necessary for tissue synthesis, with lipids and carbohydrates being used to satisfy the energy requirements.

Table 9.1 shows the proximate composition, energy provision and cost of some representative ingredients used in commercial shrimp diets. The cost of providing energy from a high quality Danish fish meal can be seen to be 2.7 p mJ⁻¹ TE. Of this energy, 73 % is derived from protein, 24 % from lipid and 2 % from carbohydrate. In contrast, although fish oil is more expensive per kilo, the cost of providing energy (100 % lipid energy) is lower than providing it from fish meal at 2.1 p mJ⁻¹. Carbohydrate energy from ingredients such as wheat flour or broken rice is the cheapest at 1.6 p mJ⁻¹, with 73-85 % of the energy being derived from carbohydrate, 12-22 % from protein and 3-5 % from lipid. Maximising the use of carbohydrates and to a lesser extent lipids for energy provision in shrimp diets can thus result in significant cost savings in shrimp diets.

The use of high quality proteins such as squid meal have been shown to improve shrimp growth (Cruz-Rique *et al.*, 1987; AQUACOP & Cuzon, 1989), but their use may be restricted to low level inclusion as feeding attractants due to their high cost (7.2 p mJ⁻¹) as sources of energy. The use of plant proteins such as soybean meal however, have shown potential to partially replace fish meal as a source of protein and energy in shrimp diets without affecting growth (Akiyama, 1988, 1992; Akiyama & FSGP, 1989; Tacon, 1993, 1994). Soybean meal is a very cheap source of energy costing 1.5 p mJ⁻¹,

Table 9.1 The provision and cost of energy from protein, lipid and carbohydrate sources in shrimp diets.

Major nutrient	Ingredient	Protein		Lipid		Carbohydrate		Ingredient cost ¹	
		% of ingredient	% of TE ingredient	% of ingredient	% of TE ingredient	% of ingredient	% of TE ingredient	£ kg ⁻¹	p MJ ⁻¹
Protein	Danish fish meal	69.0	73	13.6	24	3.0	2	0.60	2.7
	squid meal	61.1	73	6.5	13	16.2	14	1.43	7.2
	soybean meal	47.1	59	3.4	7	37.1	34	0.29	1.5
Lipid	fish oil	0.0	0	100.0	100	0.0	0	0.83	2.1
Carbohydrate	wheat flour	17.2	22	2.5	5	78.6	73	0.29	1.6
	broken rice	9.2	12	1.4	3	87.5	85	0.29	1.6

¹ Ingredient cost based on current wholesale price in Thailand

with 59 % of the energy being derived from protein. The use of such protein sources, combined with the protein-sparing effects of optimal levels of lipids and carbohydrates therefore has considerable potential to reduce diet cost. Investigations into the use of alternative protein sources were not conducted during this study due to lack of time, but have been researched elsewhere (*i.e.* Tacon & Jackson, 1985; Cruz-Rique *et al.*, 1986, 1987, 1989; Akiyama, 1988; Cruz-Suarez *et al.*, 1987; AQUACOP & Cuzon, 1989; Akiyama *et al.*, 1992; Tacon, 1993, 1994).

Using the optimal ratios of protein, lipid, carbohydrate and energy derived from data from this study and in the literature, a series of diets at various protein levels have been formulated to demonstrate the cost savings possible through a reduction in dietary protein level (Table 9.2). In this case, protein from fish meal has been progressively replaced by carbohydrate from broken rice and wheat flour. The cost of such diets decreases by 22 % (from £ 0.69-0.54 kg⁻¹) as the protein level is reduced from 50 to 30 %. Provided that the reductions in ingredient cost are passed on to the shrimp producers, the use of such 30 % protein diets could reduce feed costs from 48-60 % to 37-47 % of the production costs. However, the economic advantage of using such low protein diets depends upon the FCE and growth rate possible on such diets. It also requires that the dietary formulation contains sufficient high quality protein (squid, shrimp head and soybean meals in this case) and lipid (cod liver oil), and levels of carbohydrate that will not result in reduced growth or histopathological changes in the shrimp tissues.

Although these diets have not been tested, the results of this study have shown that reductions in dietary protein levels to as low as 38 % can result in growth, survival and FCE of *P. monodon* equal to that on higher (44-45 %) protein diets (chapters 7 & 8). Other research has suggested that further reductions to 35 % (Shiau & Chou, 1991; Shiau & Peng, 1992) or even 31 % (Shiau & Peng, 1992) protein may be possible in nutrient-balanced diets, emphasising the cost savings possible. Further reductions in dietary protein levels may also be expected under pond on-growing conditions with access to natural productivity. In support of this, previous research has suggested that shrimp under semi-intensive pond culture (17-20 shrimp m⁻²) can derive up to 43-77 % of their nutrition from natural food sources (Maguire & Bell, 1981; Anderson *et al.*,

Table 9.2 The calculated composition and proximate analysis of diets formulated at optimum protein, lipid and carbohydrate levels for production of post-larval *P. monodon*.

Ingredient	Ingredient cost (£ kg ⁻¹) ¹	Dietary formulation (% of diet)				
		1	2	3	4	5
fish oil	0.83	1.5	2.1	2.6	3.3	3.6
shrimp head meal	0.46	12.0	12.0	12.0	12.0	12.0
soybean meal	0.29	12.0	12.0	12.0	12.0	12.0
squid meal	1.43	10.0	10.0	10.0	10.0	10.0
Danish fish meal	0.60	34.0	24.5	16.4	7.0	0.0
broken rice	0.29	9.0	13.2	17.3	21.2	26.2
wheat flour	0.29	9.3	14.0	17.5	22.3	24.0
wheat gluten meal	1.54	6.0	6.0	6.0	6.0	6.0
lecithin	0.63	2.0	2.0	2.0	2.0	2.0
cholesterol	7.14	0.2	0.2	0.2	0.2	0.2
vitamin/mineral premix	2.29	4.0	4.0	4.0	4.0	4.0
Proximate analysis						
protein (%)	-	50.7	45.0	40.2	34.6	30.3
lipid (%)	-	10.6	10.0	9.5	9.1	8.5
carbohydrate (%)	-	21.5	25.4	29.2	32.8	37.6
total energy (kJ g ⁻¹) ²	-	19.9	19.0	18.4	17.5	17.1
protein:energy ratio (mg pr. kJ ⁻¹ TE) ²	-	25.5	23.7	21.9	19.8	17.8
carbohydrate:lipid ratio	-	2.0	2.5	3.1	3.6	4.4
Diet cost (£ kg ⁻¹) ³	-	0.69	0.65	0.61	0.57	0.54

¹ Ingredient cost based on current wholesale price in Thailand

² By calculation

³ Diet cost accounts for cost of ingredients only

1987; Hunter *et al.*, 1987). Unfortunately, such research was not possible during the present study, but should prove to be a profitable area for future research. Specifically, attention should be focused on the development of diets which supply only what is unavailable from the pond and on feeds which stimulate the microbial/detrital biomass to enhance natural food availability. The nutritional profile of these diets will again be dependent upon the intensity of the culture operation.

A second important reason for reducing the protein content of shrimp diets is in the reduction in waste loadings that this facilitates. Reducing the pollution from shrimp farms has received attention recently due to increasing concern over the environmental impact of intensive shrimp farming (Akiyama, 1992; Phillips *et al.*, 1993; Briggs & Funge-Smith, 1994, in press). Minimising feed losses presents the most effective way of reducing pollution from fish/shrimp farms (Seymour & Bergheim, 1991; Boyd & Musig, 1992). This can be achieved through manipulation of the nutritional profile or processing of the feeds or through optimisation of the feeding regimes used. Reducing the dietary protein level and optimising the Pr:E ratio through protein-sparing is necessary since ammonia nitrogen excretion in shrimp and fish is directly related to protein level (Rychly, 1980; Kaushik & Oliva-Teles, 1986; Watanabe *et al.*, 1987; Koshio *et al.*, 1992; Li & Lovell, 1992). Maintenance or improvement of growth rate and FCR is essential in order to reduce the waste production of low protein diets. Use of poor quality proteins must therefore be controlled to maintain feed digestibility and assimilation (Tacon & Jackson, 1985; Cho & Kaushik, 1985; Kaushik & Cowey, 1991).

In the present study using practical-style diets (chapter 8), reduction in the dietary protein level from 44 to 38 % was achieved by the substitution of soybean meal with broken rice. These low-protein diets resulted in non-significant changes in shrimp growth, survival and FCE and a significant increase in PER as protein was being spared for growth by carbohydrate. Although ammonia production levels were not monitored, this result indicates the waste reduction potential of such low-protein diets. Once again, further reductions in dietary protein and fishmeal inclusion levels, and hence pollution loadings may be expected under pond culture conditions, where natural productivity contributes to shrimp nutrition.

The processing technology used in diet manufacture may also help to reduce pollution from diets. For example, commercial extrusion of diets (necessary for the production of high lipid diets) also results in the expansion and fusion of dietary ingredients (particularly starchy carbohydrates), promoting binding (Seymour & Bergheim, 1991). This is particularly important for shrimp which tend to be slow feeders and break up feed pellets outside their bodies, increasing the wastage of poorly-bound diets. Optimised feeding management through monitoring and maximisation of shrimp feeding with the use of feed nets and regular feed applications provides a final means of reducing feed wastage under commercial conditions (Kaushik & Gomes, 1985; Kaushik & Cowey, 1991; Chanratchakool *et al.*, 1994; Briggs & Funge-Smith, 1994, in press).

This thesis has highlighted some of the problems with estimating the dietary energy requirements of shrimp. The majority of the nutritional trials conducted on shrimp have failed to measure dietary energy density. Of the authors who have quoted energy levels, some have used calculated figures for total (gross) or digestible energy, using a variety of conversion factors (and proximate analysis techniques) whilst others have measured total energy density using calorimetry. It is necessary to adopt a single technique in order to standardise the results of nutrition trials from various authors.

As discussed in the introduction (section 2.5.3.9), metabolisable or digestible energy values have the most relevance to the requirements of the shrimp. However, accurate measurement of these values is difficult for shrimp and, with the lack of standard reference diets for which these values are known, they should be determined for each diet of each trial. Because of the difficulty of doing these measurements, total energy values are most frequently used. For estimating total energy, bomb calorimetry is the method of choice (Jobling, 1983; Henken *et al.*, 1986), but is rarely used. Calculation of total energy values through proximate analysis followed by the use of energy conversion terms is the most commonly used method but suffers from a number of drawbacks. These include under-estimation of total energy content, the use of different methods for determining sample proximate composition (and compounding of errors if carbohydrate values are determined "by difference"), the use of different conversion terms (and even units), and the inapplicability of standard conversion terms for all biological materials (Craig *et al.*, 1978; Osborne & Voogt, 1978; Cho *et al.*, 1982;

Jobling, 1983; From & Rasmussen, 1984; Henken *et al.*, 1986).

Despite the drawbacks of using calculated total energy values, this method has been used in this thesis to assist comparison between this study and previous trials. This is because where authors have not measured or quoted energy values, it is only possible to estimate energy density through calculation using standard values. The total energy values estimated using such calculations were on average 3-6 % less than those measured by bomb calorimetry. This level of underestimation is similar to that noted by Henken *et al.* (1986) when comparing the various methods for determining energy levels. The relationships derived in this thesis for the partitioning of dietary energy from proteins, lipids and carbohydrates are therefore not strictly accurate, but provide an estimate based on the data available. Further research should use bomb calorimetry for more accurate identification of energy requirements, but there will still be problems with this approach due to the unknown digestibility of the ingredients used. This will be a particular problem in practical diets using novel protein sources. Accurate determination of energy levels thus requires the simultaneous measurement of nutrient and energy digestibility and accurate quantification of food consumption.

Attempts to study the effects of common diets on the performance of shrimp under laboratory and nursery tank conditions were inconclusive due to a range of differences between the two trials conducted (chapter 8). Nevertheless, the results indicated similar requirements under these conditions and suggested that laboratory-based research may be applicable to intensive nursery culture. Further work in this area however, is justified.

This thesis has sought to gain a better understanding of the interrelationships between proteins, lipids, carbohydrates and energy in the diet of young *P. monodon*. General models have been proposed whereby the optimum level of these factors in terms of shrimp production, diet cost and waste production can be estimated. It is hoped that this information will be of use for the formulation of cheap, low pollution diets for intensive rearing of young shrimp and as a baseline for further research into optimal diets for older shrimp during pond on-growing.

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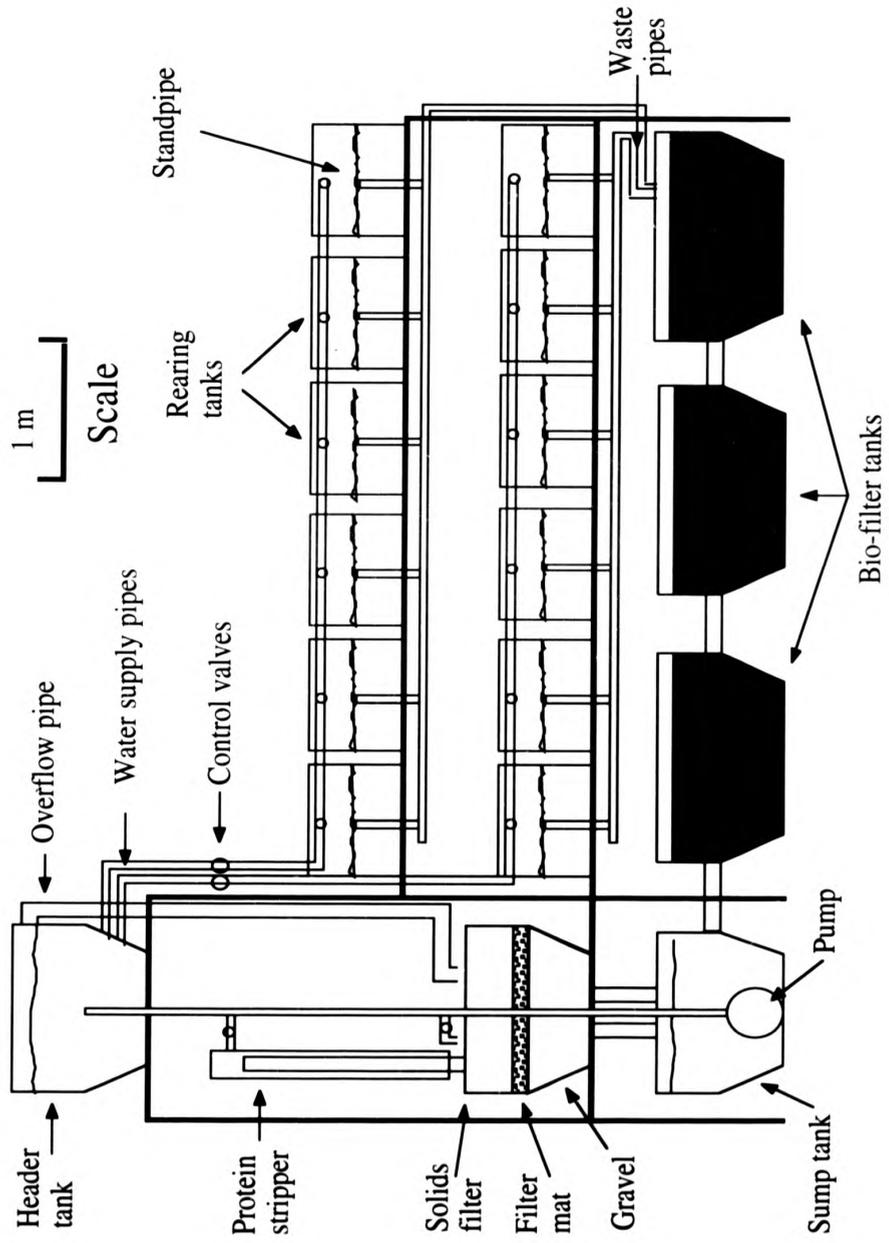
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Appendix 1 The experimental post-larval shrimp rearing system at Stirling.



Appendix 2. Formula for Davidson's fixative (after Bell & Lightner, 1988).

1. 330 ml 95 % ethyl alcohol
2. 220 ml 100 % formalin (saturated aqueous solution of formaldehyde gas, 37-39 % solution)
3. 115 ml glacial acetic acid
4. 335 ml distilled water

Stored at room temperature