THE EFFECT OF ORGANIC FERTILISER AND FORMULA FEED IN POND CULTURE OF THE FRESHWATER PRAWN, *Macrobrachium rosenbergii* (de Man).

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The research presented in this thesis is the result of investigations conducted by myself. The thesis is entirely my own composition, and has not been, nor will be, submitted for any other degree.

M.H. MacLean

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THIS THESIS IS DEDICATED TO OOMIE, MY MOTHER AND FRIEND,
FOR HER CONSTANT INTEREST, ADVICE AND SUPPORT.

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ABSTRACT

The supplementation of pelleted prawn feed with chicken manure significantly increased the mean growth rate, weight at harvest and marketable percent of the freshwater prawn *Macrobrachium rosenbergii* (de Man) under the conditions of complete pond trials described. Improved prawn production, when both feed and manure were applied to ponds, may be attributed to manure. The application of manure only resulted in significantly reduced yield, but did not produce significantly different mean growth rate, weight at harvest, marketable percent and marketable yield as compared to when feed only was applied. There was a definite requirement for pelleted feed but the application of both feed and manure was beneficial. At the levels employed, simultaneously decreased pellet input and increased manure load actually increased prawn mean growth rate, weight at harvest, yield, marketable percent and marketable yield. There were no significant differences between treatments for the measured parameters of water and sediment chemistry, or benthic macroinvertebrates. Mineralisation of organic matter and assimilation of inorganic nutrients appeared efficient and the water was eutrophic in all treatments.

A second pond experiment evaluated the frequency of supplemental manure application, as every 3.5, 7, or 14 days. Prawn mean growth rate, weight at harvest, yield and marketable yield were superior when manure was applied every 14 days. Decreasing application frequency resulted in increased autotrophic biomass and improved efficiency of algal production but higher oxygen requirement. The manure may have induced short term shifts between autotrophy and heterotrophy. Phytoplankton appeared to be generally nitrogen dependent. There was an overall decrease in sediment nutrient concentrations with time, and rapid organic decomposition and mineralisation were indicated.

In the final experiment, which employed enclosures, water and sediment total bacterial biomass were determined. After two months of the trial, water total bacterial biomass in the treatment which partially replaced feed with manure was 3.7 times that in the feed only treatment, 2.0 times that in the manure only treatment, and 1.7 times that of the commercial feed plus manure treatment. In all treatments, water bacterial biomass showed a general increase over time in all treatments, whereas the sediment bacterial biomass was more erratic. The occurrence of high sediment bacterial levels in the feed dominated treatments at an early stage in the experiment indicated overfeeding. High total nutrient concentrations in the water indicated a large capacity to support algae and the low inorganic nutrient concentrations indicated their rapid utilisation. Prawns suffered a space effect when enclosed, and although survival was high in all treatments, mean weight at harvest was small.

Both the feed conversion and financial ratios favoured the treatment wherein feed was partially replaced by applications of manure every 14 days. Correlations between prawn production and feed, for data combined from the three experiments, indicated that each of growth rate, mean weight at harvest and yield were strong but less than those when both feed and manure were added. Manure was strongly correlated with marketable yield, the index of economic concern.

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CHAPTER ONE

INTRODUCTION

1.1 PRAWN AQUACULTURE SYSTEMS

Aquaculture of the freshwater prawn, *Macrobrachium rosenbergii* (de Man), is a very young science. Culture truly started after 1962 (New, 1988) and the fundamental lifecycle research by Ling and Merican in Malaysia (Ling and Merican, 1961; Ling, 1962). Widespread interest in the commercial culture of the species developed globally, most successfully in Hawaii (Fujimura and Okamoto, 1970), Thailand (New *et al.*, 1982) and Taiwan (Chen, 1976; Liao and Chao, 1982). Worldwide aquaculture production of the species has risen in approximately twenty-five years to 21,000 metric tons in 1989 (FAO, 1991). The most recent production records reveal that Thailand produced 50-78% of the world tonnage during 1984-1989 (FAO, 1989, 1991), with other significant contributions from Taiwan (New, 1988) and Brazil (FAO, 1989, 1991). Despite this development, production of the species is small relative to that of other aquaculture species both within countries where the prawn is cultured and on a global scale (New, 1988; FAO, 1989, 1991).

Further commercial expansion has been limited by constraints including a poorly developed global commodity market (pers. comm. Professor Ang Kok Jee, Universiti Pertanian Malaysia, Serdang; Lee and Wickins, 1992; New, 1988), the cost of feedstuffs or diets (Ang *et al.*, 1989; Roberts and Bauer, 1978; Samples and Leung, 1985; Shang and Fujimura, 1977), of labour (Shang and Fujimura, 1977), and of seed[1] (Ang *et al.*, 1989), poor pond management (Ang *et al.*, 1989), and biological restrictions or difficulties such as the warm climatic requirement (Sandifer and Smith, 1985), heterogeneous growth (Lee and Wickins, 1992) and associated territoriality (New, 1988), long larval life (New, 1988), and low proportion of tail meat (Lee and Wickins, 1992).

[1]In this content "seed" refers to postlarvae or juveniles of *Macrobrachium rosenbergii*. More generally, the term is used additionally for larvae, fry, fingerling or any small form of a cultured aquatic species.

Aquaculture, at the on-growing stage from juveniles to marketable adults, is associated with high investment costs, especially for land purchase and pond construction (Lee and Wickins, 1992; Shang and Fujimura, 1978). With respect to operating costs, feed may account for 13-27% (Shang and Fujimura, 1977), 30% (Liao and Chao, 1982), or even 42% (Roberts and Bauer, 1978), and when combined with seed costs, the two running expenditures may total 50-70% of production costs (Lee and Wickins, 1992).

However, costs are dependent upon the country of production, particularly in terms of land and labour expenses, and the mode of production especially as regards feed, seed and electricity demands. Three systems of on-growing monoculture, summarised by Lee and Wickins (1992), are roughly defined on the basis of stocking density and attention to pond management. *Macrobrachium* density in extensive ponds is usually less than 5 m^{-2}, with yields of 200-300 kg ha^{-1} $year^{-1}$ as entirely derived from natural pond productivity, although the addition of fertilisers may be employed to stimulate productivity. Semi-intensive culture is characterised by stocking densities of 5-20 m^{-2}, yields of 1-5 tonnes $year^{-1}$ and achieved through the application of supplemental feeds. Other husbandry practices may include the addition of fertilisers, water exchange through pumping, mechanical aeration, predator control, pond bund maintenance and greening[1], and prawn sampling to determine feeding requirements and animal health. Intensive or superintensive grow-out[2] culture with high prawn densities is considered unfeasible due to the aggressive territorial nature of the species (Lee and Wickins, 1992). However, Ang *et al.* (1989) suggested that successful grow-out could be achieved at densities of 40 m^{-2}, and Sandifer *et al.* (1982) reported that

[1]the planting, and possibly cropping, of terrestrial plants in order to stabilise the pond banks; typically used plants include grasses such as elephant grass *Pennisetum* spp., Sudan grass *Sorghum sudanense*, lalang *Imperata rundinacea*, and star grass *Cynodon plectostachyus*, and legumes such as centro *Centrosema pubescens* and stylo *Stylosanthes humilis* or vines such as puero *Pueraria phaseoloides* (Little and Muir, 1987).

[2]the culture of a species to market size, from postlarvae or juveniles in the case of *Macrobrachium*

prawns stocked at 32 m^3 (1.3 g for 138 days) attained a mean size of 16.2 g and survival of 73.2% although those stocked at 83 m^3 (1.0 g for 130 days) attained 8.5 g and 66.5% survival. *M. rosenbergii* has been shown to be a successful component in polyculture systems with finfish such as tilapia, silver carp, bighead carp, grass carp, milkfish, grey mullet, and catfish (Martinez-Silva *et al.*, 1976; Buck *et al.*, 1981; Malecha *et al.*, 1981; Liao and Chao, 1982; Behrends *et al.*, 1985; Wohlfarth *et al.*, 1985; D'Abramo *et al.*, 1986; Lilyestrom *et al.*, 1987.)

Although most farm operations may be classified as earthen pond semi-intensive monocultures (Lee and Wickins, 1992), an incredible variation in management practices exists. Table 1.1 summarizes the mechanisms and production of some semi-intensive systems, based mostly on research rather than commercial reports as necessitated by public literature availability. Management techniques are dictated by such factors as financial limitations, economic aims, market availability, infrastructural support systems, and conditions on the farm itself. For example, water exchange may be operated as flow-through or topping-up and accomplished by gravity or pump, and mechanical aeration may be managed typically by paddlewheel aerators, airblowers or jetblowers. Feed types include waste fisheries products, other fresh feeds of plant and animal matter, prepared diets, or commercially produced and specifically formulated pellets. The utilisation of each management technique is dependent upon existing husbandry practices. Management strategies are influenced by biological factors including the size, activity and productivity of the biotic biomass particularly as prawns, plankton, bacteria and benthic invertebrates. Chemical factors including the quality of the water source and pond internal elemental dynamics, physical factors such as the level of suspended or dissolved inorganic matter and the depth of the benthic sediment layer, and meterological factors including temperature, rainfall and wind all influence the selection and effect of management strategies. The great multitude of practices, each specific to the degree of

Table 1.1. Management and production in semi-intensive culture of *Macrobrachium rosenbergii* (de Man).

Reference	Location and stock	Management	Production
Shang and Fujimura, 1977	Hawaii; earthen ponds 4000-8000m^2; juvenile prawns at 16.14-21.52m^{-2}	210-240 days; fed cbsp to demand (roughly results in FCR 3.3:1); selective harvest	marketable weight = 45g 50% survival 3364-3925$kgha^{-1}$
Willis and Berrigan, 1977	Florida; earthen ponds 250m^2; prawns of 0.05-0.06g at 5 & 10m^{-2}	168 days; fed 25% protein pp at mean 15%$bwday^{-1}$ (2-30%) water change 400$lha^{-1}min^{-1}$; aeration if oxygen problems	17-34g weight 38-92% survival 643-1633$kgha^{-1}$
Stahl, 1979	Hawaii; outdoor pools of 23.69m^2 with earth substrate; prawns of 0.24g at 16.15m^{-2}	50 days; fed 26.4% protein pp at 12%[b] $bwday^{-1}$ with or without phytoplankton; water change 18% per day; continuous air	2.51 ±[a] 0.37g weight 92.6 ±[a] 3.25% survival 375$kgha^{-1}$
Smith *et al.*, 1981	South Carolina; earthen ponds 2500m^2; prawns of 0.32 & 0.42g stocked at 8.61 & 6.46m^{-2}	167 & 154 days; fed 25% protein pp at mean 5.8%$bwday^{-1}$ (1-20%)	18.3 & 22.4g 77.9 & 79.1% 1314 & 1208$kgha^{-1}$
Adisukresno *et al.*, 1982.	Indonesia; earthen pond 2000m^2; prawns of 30 days age stocked at 5.8m^{-2}	150 days duration; fed cbsp to demand; free-flowing H_2O to replace losses	30-40g weight 49-65% survival[b] 2688 $kgha^{-1}$

cbsp = chicken broiler starter pellet; pp = prawn pellet; cd = compound diet; fp = fish pellet; $bwday^{-1}$= body weight per day; a = standard error; b = calculated; c = standard deviation; d = 95% confidence interval.

Table 1.1 cont'd. Management and production in semi-intensive culture of Macrobrachium rosenbergii (de Man).

Reference	Location and stock	Management	Production
Boonyaratpalin and New, 1982	Thailand; cement ponds 50m^2; prawns of 0.12g at 5m^{-2}	119 days; fed 25% protein cd to demand; phytoplankton present; water change every 21 days	12.46 ±* 1.59g 101 ±* 16% survival 642 ±* 178kgha^{-1}
Menasveta and Piyatiratitivokul, 1982	Thailand; earthen pond 2400m^{-2}; prawns of 0.86g at 5m^{-2}	180 days; fed 40% protein cd at 5%bwday^{-1}	47.8 ±* 29.13g weight 48% survival 1312 kgha^{-1}
New and Singholka, 1982	Thailand; earthen pond 2000-6000m^2; prawns of 7-30 days age at 5m^{-2}	180-240 days; fed cbsp initially at 6.25kgha^{-1} day^{-1} and then to demand of about 37.5kgha^{-1}day^{-1}; ideally 140-280lha^{-1}min^{-1} to cover losses or greater	Ideally 70g + 50% survival 1250kgha^{-1}
Smith *et al.*, 1982	South Carolina; earthen ponds 700m^2;prawns of 0.04g at 10.8m^{-2}	137 days; fed 25% protein pp at 4-10%bwday^{-1}; water to replace losses	7.4g weight 51.3% survival 415kgha^{-1}
Ra'anan *et al.*, 1984	Israel; earthen ponds 200m^2; prawns of 0.5g at 15m^{-2}	184 days; fed 25% protein fp to give fcr of 3:1; overnight aeration; 5 selective + final harvest	Marketable prawns = 30.60 ±* 0.75g and 2631 ±* 158kg ha^{-1}; unmarketable prawns = 8.67 ±* 0.46g and 125 ±* 31kgha^{-1}; 69% survival
Costa-Pierce *et al.*, 1987	Hawaii; earthen ponds 200m^2; prawns of 1.2g at 11.5m^{-2}	286 days; fed 27.2% protein pp 15%bwday^{-1} for 42 days and then 40kgha^{-1}day^{-1}; water change 50-100l ha^{-1}min^{-1}	21.9 ±^{d} 4.9g weight 72 ±^{d} 11% survival 1735 ±^{d} 180 kgha^{-1}

realisable ambition as limited by an inordinate number and relative importance of factors, has not clarified our knowledge of the dynamics of prawn culture. Given the nature of the animal and its intimate association with the sediment, our understanding of the species and environment need to be thorough. The practice of *M. rosenbergii* aquaculture has preceded a mature scientific basis.

1.2 PRAWN BEHAVIOUR, DIGESTIVE PHYSIOLOGY AND NUTRITION

1.2.1 Behaviour and Digestive Physiology

The freshwater prawn locates feed mainly by smell and touch employing the antennae and antennules, and transports feed toward the mouth using the first, and perhaps second, pair of thoracic legs (Ling, 1969a). Feeding behaviour includes antennular flicking, a sweeping scanning movement by the first pair of walking legs, and movement of the mouthparts (Heinen, 1980; Harpaz and Steiner, 1987; Harpaz *et al.*, 1987). Chemoreceptors associated with feeding include those sited at the antennules and involved in the initial arousal and search for food, the pereiopod receptors which serve to assist seize and convey the food particle, those of the mouthparts which ultimately accept or reject the item, and those of the foregut whose role remains undefined (Heinen, 1980). Strong stimuli to most decapods include low molecular weight amino acids, rather than compounds such as fatty acids, sugars, alcohols, and starches (Heinen, 1980). Extracts of various foods and pure compounds elicited responses from perfused *M. rosenbergii* pereiopod chemoreceptor cells (Derby and Harpaz, 1988), but in general, artificial mixtures simulating natural materials are less stimulating than the natural feeds (Carr in Heinen, 1980; Johnson in Heinen, 1980; Mackie in Heinen, 1980; McLeese in Heinen, 1980). The species generally feeds and is more active at night as illustrated by increased movement (Peebles, 1979), a 12% increase in metabolic rate during the dark as opposed to the light cycle (Nelson *et al.*, 1977c), and stimulated growth and enhanced survival under conditions of continuous darkness (Withyachumnarnkul *et al.*, 1981). However, Peebles

(1979) noted maximal activity of movement at sunset and dawn, there being a reduction in activity between 2400 and 0500.

The crustacean digestive tract may be divided into a fore-gut, ectodermal in origin and chitin lined, a mid-gut, endodermal in origin and mostly devoid of chitin, and a hind-gut, of similar origin and lining as the fore-gut (Vonk, 1960). The fore-gut consists of the mouth, the oesophagus, and the stomach which is divided into cardiac and pyloric sections. In most Malacostracans the cardiac stomach is specialised into a gastric mill for mastication. However, the Caridea display a secondary reduction or absence of this apparatus and in *Macrobrachium* the exterior mandibular appendage is used to break up and triturate food particles prior to ingestion (Patwardhan, 1935; Vonk, 1960). The pyloric stomach acts as a filter, sieving items before entry into the mid-gut. The intestine, hepatopancreas, and two caeca make up the mid-gut. The hepatopancreas is the site of enzyme secretion and nutrient absorption. However, normal peristalsis and particularly strong antiperistalsis in the midgut transport digestive juices to the hindgut and stomach (Vonk, 1960), thus allowing enzyme activity in all three tissues (Tsai *et al.*, 1986).

Digestive enzyme analysis (Lee *et al.*, 1980) of *M. rosenbergii* hepatopancreas extracts indicated significant activity for the proteases trypsin, chymotrypsin, pepsin, carboxypeptidase A and B and leucine aminopeptidases, and for the carbohydrase amylase. Lipolytic enzymes demonstrated high esterase activity, particularly of acetate chains, but negligible lipase activity of stearate chains. Comparable activities of proteases and amylases classified the prawn as an omnivore and indicated that complex proteins and starch can be readily hydrolysed (Lee *et al.*, 1980). Given that the reported specific activity of the pepsin was low, and that pepsin-like enzymes had not been previously reported in crustacea (Vonk, 1960; New, 1976), it is possible that there was contamination with bacterial enzyme. New (1976) considered that bacteria may be the source

of gut cellulase and chitinase, although Fair *et al.* (1980) believed that cellulase activity in the hepatopancreas tissue suggested an endogenous source, as did Nip *et al.* (1985) concerning a midgut collagenolytic enzyme fraction. The quantity and activity of digestive enzymes are seemingly not limiting factors to prawn nutrition (Newman *et al.*, 1982), probably unlike the residence time in, and the absorption capacity of, the hepatopancreas (Lee *et al.*, 1980). In the generalised crustacean, the intestine functions in water and ion, particularly Na^+, Cl^- and K^+, regulation and has a very limited role in nutrient absorption (Ahearn, 1982). The functions of the anterior and posterior midgut caeca and of the hindgut remain in dispute (Ahearn, 1982). Strong peristalsis and antiperistalsis in the hindgut of crustacea (Fox, 1952; Vonk, 1960), particularly the phenomena of rhythmic rectal contractions resulting in the anal uptake of water, have been attributed to initiation of intestinal antiperistalsis followed by peristalsis and hydrostatic pressure induced defecation (Fox, 1952). Prawns *Leander adspersus* and *Palaemonetes varians* exhibited this phenomenon to a size of 4.5 cm, larger individuals being too opaque for observation, while in very small specimens of *Lepidurus*, freshly evacuated faeces were frequently anally reingested (Fox, 1952). Certain authors (Dall in Ahearn, 1982; Malley in Ahearn, 1977) support the theory that solutes from anal egested water are transported across the hindgut epithelium.

Regurgitation is a consistent habit in *M.rosenbergii*, with feed orally egested from four to twenty hours after a meal and accounting for an average of 76.8% of total egesta (Newman *et al.*, 1982). Regurgitated material is composed of large particles of plant matter, primarily cellulose and lignin, indicating a sorting mechanism of the digesta. The phenomena of regurgitation may be of energetic consequence to the prawn, obviating the need to break down and digest large and resistant particles and allowing for the ingestion of new food following evacuation of the stomach (Newman *et al*, 1982). Regurgitation has been noted in other

decapods (Vonk, 1960) and particularly carideans, occurring within 2.5 to 16 hours after a meal and similarly composed of large particles of refractory materials (Forster and Gabbott, 1971).

1.2.2 Natural Diet

In its natural environment, the adult prawn is a voracious omnivore. It will consume a wide range of presented feeds including animal matter such as pieces of aquatic worms, various insect developmental stages (larvae, pupae and adult), molluscs, crustacea, and fish flesh or offal, and plant matter such as rice, wheat, peas, beans, groundnuts, corn, coconut, fruit, and aquatic plants (Ling and Merican, 1961; Bardach *et al.*, 1972; Ling and Costello, 1976). The diet of juveniles is similar to that of the adult (Ling, 1962; Ling 1969b; Bardach, 1972; Ling and Costello, 1976).

Cannibalism in this species is well documented, particularly amongst adult males (Ling and Merican, 1961; Bardach *et al.*, 1972, Adisukresno, 1977). Populations of *M. rosenbergii* have been identified as hierarchial (Lee and Wickins, 1992; Ra'anan and Cohen, 1984) in which large adult males are most territorial and aggressive (Kuris *et al.*, 1987). Although females are homogenously size distributed, male populations are skewed due to the presence of several morphotypes (Fujimura and Okamoto, 1970; Cohen *et al.*, 1981; Malecha, 1986; Kuris *et al.*, 1987; Lin and Boonyaratpalin, 1988). Size variation has been attributed to differences in individual relative growth rates (Ra'anan and Cohen, 1984), and the removal of large prawns from culture systems recommended in order to decrease competition between prawns and allow compensatory growth of smaller prawns (Fujimura and Okamoto, 1970; Malecha, 1986; Lin and Boonyaratpalin, 1988). However, the "leapfrog" growth pattern whereby males attain the top class in the hierarchy at a size greater than existing males in that class (Ra'anan and Cohen, 1985; Ra'anan *et al.*, 1991), has lead to the suggestion that in aquaculture production a few large top class individuals be maintained in order to act as "targets" for the transforming males from the lower class

(Ra'anan et *al.*, 1991).

Specific studies on coprophagy in *Macrobrachium* have not been conducted. However, according to various authors (Newell 1965; Johannes and Satomi, 1966; Frankenberg and Smith, 1967) many benthic omnivores are partially coprophagic, and New (1988) claims coprophagy is common in crustacea. In aquaria studies exposing a variety of marine animals to a wide range of faeces, Frankenberg and Smith (1967) recorded faecal pellet ingestion as a percent of bodyweight 48-hours^{-1} (%bw). Faecal ingestion by the Penaeidea *Penaeus setiferus* ranged from 0.1-2.4%bw and by the Caridea *Palaemonetes pugio* from 8.2-83.0%bw. Other crustacea indicated rates of 1.1-70.0%bw. Ingestion rate estimates of same species' faeces are hampered by an inability to separate the initial pellet from that produced by the experimental animal. The authors calculated that *Pagurus longicarpus* (120 mg) ingesting *Callinassa major* faeces and *Palaemonetes pugio* (460 mg) ingesting *Penaeus setiferus* could satisfy 9% and 333% respectively of their metabolic rate from the ingested faeces. Ingestion rates were highly correlated to faecal content of carbon and nitrogen, suggesting that high levels of organic matter and protein encourage reingestion. This would indicate that such factors as the nutritional value of the initial diet, the physiological status of the animal, and the environmental conditions would influence not only the content of the faeces, but its potential reingestion. Johannes and Satomi (1966) reported that there was approximately a 50% reduction in the content of carbon, protein and phosphorus following reingestion of own faeces by *Palaemonetes pugio*. Newell (1965) reported a 96% decrease in nitrogen content but relatively unchanged carbon content in resvacuated faeces of the prosobranch *Hydrobia ulvae*. The paper concluded that the nutritional value of faecal pellets was the associated microbial populations rather than the pellet itself.

M. rosenbergii is known to consume its own shed exuviae (Nelson et *al.*, 1977c). However, the amount of the moult which is ingested and assimilated

is unknown (Nelson *et al.*, 1977c) and probably influenced by such factors as prawn condition, prawn density and food availability.

Gut content analysis, as a quantitative indicator of the diet is at least hampered if not invalidated by the biological phenomena of antiperistalsis and regurgitation. The mechanisms of reversing the normal direction or non-linearity of the digesta and of sorting to prevent larger particles from passing into the hepatopancreas have the consequence that different feeds will remain in the stomach for different durations (Newman *et al.*, 1982). Further inaccuracies associated with gut content analysis involve the chemical complexity of the particles given that the very wide range of items consumed will be digested at different rates. Naturally recalcitrant items, for instance chitin or cellulose as opposed to protein, will remain in the hepatopancreas longer. Other potential sources of error associated with quantitative gut content analysis include the difficulty in identification of feedstuffs, particularly given the prawn feeding habits of preingestion grinding and broad tastes, and the nature of the digesta to clump despite dilution and mixing. In a quantitative description of fish diets, Bowen (1985) stated that for some species stomach contents may not accurately reflect diet due to the rapid and complete digestion of certain feed items thereby resulting in underestimate or exclusion from scoring, and the differential rates of digestion for various prey resulting in over-representation of the more unmanageable items. He further elaborated upon the factors which influence diet, including the effects of the diel cycle, seasonality, fish size and territoriality. Bowen (1985) reported that only stomach contents as quantified on the basis of percent composition by weight "begin" to identify the nutritional importance of a food item. However a major source of error is the requirement to estimate the prey weight, as necessitated when only parts of the prey remain, when dietary items such as certain invertebrates or algae are too small to weigh, or when detritus constitutes a notable portion of the stomach content. Beyond the problem

of accurate identification and quantification of the food items, and preferably their relation to stomach capacity, the problem of quantifying the energetic costs, under standard conditions, of acquisition, digestion, and absorption remains.

Under extensive culture conditions, Ling (1969b) recommended that in a properly managed pond, natural foods were the main source of prawn feed. Nonetheless a daily supplement of 5 percent body weight could be added comprising a variety of feeds such that animal and plant material were supplied in a ratio of approximately 3:1. However, as culture systems become more intensive by increasing stocking density, natural foods are insufficient to satisfy dietary requirements. As described by Hepher (1979) in relation to warmwater fish pond culture, an increase in fish stock and a subsequent decrease in the ratio of available natural food in relation to feed requirement, results in insufficient energy and protein relative to demand. Various authors declared that with increasing prawn stock, additional feeds must be made available by the application of commercial formula feed pellets (Biddle *et al.*, 1977; Sandifer and Smith, 1979), and the fledgling industry looked to complete feeds to increase marketable yields. Consequently, a variety of pelleted feeds have been used including feeds formulated for pigs, chicken broilers, gamebirds, catfish, trout, and marine shrimp (Sandifer and Smith, 1985). Application rates to earthen ponds have varied from 2% to 50% body weight per day. Application of broiler starter has resulted in feed conversion ratios (FCRs) of, on average, 3.3 (Malecha *et al.*, 1984). The FCRs of penaeid marine ration have been reported as 2.62-4.92 (Smith *et al.*, 1976) and 1.96-7.40 (Smith *et al.*, 1982).

With the aim of improving the economics of feeding, research has been devoted to designing the ideal formulation of a diet specific to the requirements of *M.rosenbergii*. Controlled laboratory experimentation has defined specific nutrient requirements for some nutrients. However, the

extension of laboratory results to pond conditions is presumptuous given that the earthen substrate, natural biota, and very possibly differing prawn physiology will affect nutrition and growth. Nonetheless, laboratory studies are necessary and valuable, up to a point, in forming a set of possible baseline criteria. Most experimental diets have employed reasonably high quality meals[4], however, the wide variety of feedstuffs and other variables confuse interpretation of results. The lack of internationally accepted reference diets and of controlled standardised test conditions prevent direct comparison between experiments. Dietary variables such as protein quality, energy content, diet stability, attractability, and digestibility, prawn variables such as age or size, and moult and sexual stage, culture system variables such as water quality, sediment exposure, and natural feed availability, and management variables such as stocking density, feeding rate, frequency and mode of application will affect prawn production parameters. Overall, certain aspects of *Macrobrachium* ecology and biology will specify the prawns nutrient requirements as distinct from those of other aquatic species.

1.2.3 Proteins

Protein constitutes a large portion (65-75%) of the total dry weight of animal tissue (NRC, 1983; Walton, 1985), and reportedly 63-67% of whole *M.rosenbergii* (Choo, 1973; Nelson *et al.*, 1977c; Poh, 1985). Proteins are wide ranging in their functions, acting in structure (eg. collagen, elastin, glyco- and mucoproteins), storage (eg.casein, ferritin), transport (eg. haemo- and myoglobin, haemocyanin, serum albumin, ß-lipoprotein), contraction (eg. myosin and actin), protection (eg. fibrinogen, thrombin and antibodies), and as enzymes, hormones and toxins. Regardless of function or origin, all proteins are composed of one or more polypeptide chains consisting of various, yet specific, sequences of

[4]an ingredient which has been dried and ground or otherwise reduced to a particle size slightly larger than flour; prepared from animal and plant matter such as prawn/shrimp, fish, meat, bone, blood, alfalfa, coconut, corn, cottonseed, peanut, rapeseed and soybean.

protein α-amino acids, of which there are twenty commonly occuring. The pathways of amino acid synthesis are specific for each amino acid and are complex multienzyme sequences regulated by feedback control. The ability to synthesize amino acids and the form of nitrogen employed to that end varies between organisms. Animals, including fish, typically cannot produce ten amino acids, arginine (ARG), histidine (HIS), isoleucine (ILE), leucine (LEU), lysine (LYS), methionine (MET), phenylalanine (PHE), tryptophan (TRP), threonine (THR), and valine (VAL), which are thus said to be essential. (NRC, 1983).

Dietary protein is therefore required by crustacea in both a quantitative and a qualitative sense, with an obvious interdependence such that a high quality protein composed of a balanced array of amino acids, particularly including those essential, will be required in lower quantity than a low quality protein lacking in the indispensable amino acids.

The protein requirement for *M. rosenbergii* probably lies within the range of 15-40% with an average near 30% (Table 1.2). As might be expected, there is some evidence that protein requirement decreases with age (Goodwin and Hanson in Clifford and Brick, 1978), but as tabulated and mentioned, long experimental trials and variable conditions limit data interpretation. The optimal dietary protein requirements for Penaeids are reported over a similarly wide range, 28-60% (NRC, 1983). As a basic assumption, the qualitative essential amino acid requirement of *M.rosenbergii* may be similar to most other species. However Stahl and Ahearn (1978) found that in purified diets presented to juvenile *M.rosenbergii*, inclusion of ARG, LYS, MET, and TRP had no benefit on growth. Nonetheless, further studies on juveniles fed either a standard commercial pellet based on fish and soy meals or that same feed repelleted after the addition of 1% (dry weight of feed) of eight individual amino acids, indicated that improved growth rate was achieved with supplementation of ARG, LEU, ILE, and PHE (Farmanfarmaian and Lauterio,

Table 1.2. Estimated protein requirement as a percent of diet for *Macrobrachium rosenbergii*; protein values in brackets refer to the levels tested.

Reference	Protein requirement	Protein source	Prawn age or size and duration of trial	Culture system
Boonyaratpalin and New, 1982	15 (15,25,35)	Meals of shrimp, fish, soy and peanut	Post-larvae (0.12g) stocked at $5m^{-2}$ for 119 days	Concrete ponds
Farmanfarmaian and Lauterio,1979	20-25 (20,25)	Fish meal and soy meal	Juvenile (0.5g) individually stocked at 81^{-1} for 14 weeks	Plexiglass tanks compartmentalised by plastic netting
Perry and Tarver, 1984	25 (25,30)	Fish meal and soy meal	Postlarvae (8-12mm TL) stocked at $4.4m^{-2}$ for 141 days	Earthen ponds
Stanley and Moore, 1983	27 (27,28,41,44)	Meals of corn, soy, crab and meat and bone	Juvenile (1.9g) stocked at $10m^{-2}$ for 10 weeks	Cages placed on mud pond bottom
Fruechtenicht, 1988	30 (14,30,51)	Unavailable	Juvenile (3.1g) and adult (13.8g) individually stocked for 12 weeks	Compartmentalised aquaria
Balazs and Ross, 1976	35% (15,25,35)	Fish meal and soy meal	Juvenile (16mm OL; 0.1g) stocked at $17m^{-2}$ for 244 days	Outdoor fibreglass tanks
Millikin *et al.*, 1980	40 (23,32,40,49)	Fish meal and soy proteinate	Juvenile (0.15g) stocked at $53m^{-2}$ (excl substrates) for 14 weeks	Fibreglass tanks with artificial hides

1979). The EAA pattern in body tissue may be used as an indicator of dietary requirement, and tail muscle analysis of *M.rosenbergii* (Farmanfarmian and Lauterio, 1980) suggested a particularly high requirement for ARG as compared to various species of fish and marine shrimps (see Tacon, 1987a). In adult *Macrobrachium ohione*, Miyajima *et al.* (1977) inferred from radioactive assay that the species had a dietary requirement for ALA, ARG, HIS, ILE, LEU, LYS, MET, PHE, THR, TYR, and VAL (TRP was not assayed). Hence, no definite essentiality composite is as yet available for the freshwater prawn, but as suggested by Tacon (1987a) high intra-specific variation in the quantitative EAA requirement of fish species possibly may be attributed to methodological artifact rather than a real difference. Furthermore, in a benthic omnivore such as *Macrobrachium* the incorporation of optimum dietary amino acid levels into commercial diets is perhaps not critical given that gut and environmental bacteria may be important amino acid contributors, most particularly in pond systems (Stahl and Ahearn, 1978).

1.2.4 Energy

Aquatic organisms require much less energy than terrestrial animals for several reasons;

- the losses of digestible energy in urine and gill excretions are lower because approximately 85% of the nitrogenous waste is as ammonia rather than urea or uric acid
- the specific dynamic action (SDA), or the energy expenditure due to the assimilation of ingested feed, is lower
- the maintenance requirements are lower as there is less energy demand to maintain position in the water and to regulate body temperature (NRC, 1983).

Clifford and Brick (1978) suggested that SDA in *M.rosenbergii* was a threshold phenomenon related to the percentage of protein in the diet and not to the quantity of food ingested. These authors found that SDA

increased over the protein range 15-25%, above which there was no effect. Nelson and Kropp (1985) reported that the ammonia excretion rate was not determined by either dietary nitrogen content or assimilation. The utilisation of stored energy substrates in crustacea varies although carbohydrate mobilisation predominates in freshwater species. Based on the principles of respiratory thermochemistry and indirect calorimetry, Clifford and Brick (1983) reported that after four days of starvation juvenile *M.rosenbergii* utilised carbohydrate predominantly, as indicated by a metabolic substrate ratio (MSR) for %protein : %lipid : %carbohydrate of 8:18:74. After eight days of starvation there was a decrease in metabolism and a shift toward lipid and protein oxidation although carbohydrate remained predominant giving an MSR of 15:34:51. The authors suggested that the carbohydrate source was initially blood glucose, followed by glycogen and other storage polysaccharides. Fair and Sick (1982) observed a rapid decrease in serum levels of certain amino acids (PRO, GLU, MET, and PHE) after five days of starvation indicating a critical role for these compounds in tissue metabolism. A review of the literature suggested that substrate hierarchy in crustacea was variable, and possibly a function of animal weight or methodological technique (Clifford and Brick, 1983).

1.2.5 Lipids

Lipids are water-insoluble organic biomolecules with varied biological functions acting as structural components of membranes, as a source of energy, and in the production and transport of fat-soluble vitamins, hormones and prostaglandins. Lipids may also combine with other biomolecules to yield hybrids such as glycolipids and lipoproteins. Lipids are said to be complex or simple if they respectively are composed of or bereft of fatty acids. Complex lipids include the various glycerides such as triacylglycerol and phosphoglycerol, the sphingolipids, and the waxes. The simple lipids, which are very active but occur in smaller quantity, include the terpenes, such as the fat-soluble vitamins, carotenoids and

ubiquinone, and the steroids including cholesterol which is the precursor of bile acids and sex hormones. The prostaglandins function in a variety of hormonal and regulatory mechanisms (Lehninger, 1979).

Certain finfish are able to utilise up to 20-30% of dietary total lipid (Halver, 1976; Cho *et al.*, 1985). However, it is unlikely that such high levels are suitable for crustaceans (Biddle *et al.*,1977, New, 1980), and the requirement for penaeids is probably 6-8.8% (Deshimaru *et al.*, 1979; Kanazawa *et al.*, 1981; Deshimaru and Shigeno, 1972). The lack of specifically designed and well controlled experiments on lipid requirements in *M.rosenbergii* prevents tabulation of specific references. Based on reviews of more general studies, various authors have advised using less than 10% total lipid (Biddle *et al.*, 1977; Forster, 1975; New 1980), and that 7.5-10.0 may be quite appropriate (Lovell, 1978; Corbin *et al.*, 1983). Higher levels, even up to 14.7%, have been used in research trials (Farmanfarmaian and Lauterio, 1979; Hilton *et al.*, 1984; Millikin *et al.*, 1980), but excessive quantities are known to have an adverse effect upon prawn production (Andrews *et al.*, 1972; New, 1980).

Over one hundred fatty acids have been isolated, each possessing a long hydrocarbon chain and a terminal carboxyl group, but differing in chain length and the number and position of unsaturated bonds. Animals contain predominantly C_{16} and C_{18} but have a dietary essentiality for certain fatty acids (EFA) (Lehninger, 1979). Many terrestrial animals require linoleic acid (18:2n-6) (Aaes-Jorgensen, 1961) and this requirement can be satisfied by longer acids of the same series (20:2n-6, 22:4n-6). *Onchorhynchus mykiss* requires linolenic acid (18:3n-3) (Castell *et al.* in NRC, 1983), probably due primarily to increased unsaturation and consequent membrane fluidity, while *Tilapia sillii* requires n-6 fatty acids only (Kanazawa *et al.* in NRC, 1983). Bell *et al.* (1986) concluded that probably both 18:2n-6 and 18:3n-3 are essential for freshwater fish. In a study with juvenile *M.rosenbergii* employing a commercial diet low in

n-3 fatty acids and the same supplemented with 3% shrimp head oil rich in n-3 fatty acids, and hence not isocaloric, Sandifer and Joseph (1976) concluded on the basis of growth response that the n-3 polyunsaturated acids (PUFA) are nutritionally important. Analyses indicated that the prawn tissue n-3 fatty acid levels reflected the diets while those of n-6 were lower suggesting the utilization of 18:2n-6 as an energy source. There was evidence of chain elongation and desaturation for both series. The ratio of n-6:n-3 decreased by 25-30% in both treatments between the diet and the prawn tissue such that the tissue ratios were 2.7 and 1.5 from the control ration and from the augmented ration. Chanmugam *et al.* (1983) also reported that n-6 PUFA predominate in adult *M.rosenbergii* tissue lipids. Analysis of whole animal pond reared (but without feed application) *M.rosenbergii* revealed total lipid as 3.18% and a predominance of n-6 PUFA in each of total lipid, phospholipid and triglyceride classes. Alternately, these authors reported a total lipid level of 1.32% and a predominance of n-3 acids in each lipid class of wild *Penaeus aztecus*. Further research on penaeids has suggested that both linoleic and linolenic are essential (Kanazawa *et al.*, 1977) but that dietary n-3 are more effective than n-6 PUFA (Kanazawa *et al.*, 1977), and that there is limited capacity for conversion of the EFA to the more elongated and desaturated acids (Kanazawa *et al.*, 1979, Colvin, 1976; Kayama *et al.*, 1980). Various factors such as the water salinity and temperature and the fish diet, sex, and reproductive state will affect tissue lipid composition, although triacylglycerides tend to reflect the diet, and phospholipids both the culture conditions and the EFA requirement of the fish (NRC, 1983).

Marine crustaceans are unable to synthesise sterols *de novo* (Zandee, 1967; Teshima and Kanazawa, 1971) and hence require a form of dietary sterol, such as 0.5% cholesterol of the dry diet (Castell *et al.*, 1975; Kanazawa *et al.*, 1971). In the single study on *M.rosenbergii*, Briggs *et al.* (1988) reported that supplementation to semi-purified diets of cholesterol above

0.12% (level of endogenous cholesterol in the basal diet) had no advantage, suggesting that the prawn has a very low cholesterol requirement and/or is capable of sterol synthesis from acetate or mevalonic acid. Supplementation of the phospholipid lecithin from 1% to 8% also improved growth in marine crustacea (Kanazawa *et al.*, 1979; Conklin *et al.*, 1980). Again, Briggs *et al.* (1988) found no growth or survival advantage with supplementation of lecithin (above the basal dietary level of 0.048% phospholipid), and concluded, as did Milton *et al.* (1984), that dietary lecithin was non-essential.

1.2.6 Carbohydrates

Carbohydrates are polyhydroxy aldehydes, ketones or their derivatives, and serve as fuel (glucose, sucrose), as a storage form of energy (starch, glycogen), in cell structure (cellulose, peptidoglycan), and as glycoproteins in association with proteins involved in lubrication (mucopolysaccharides), blood plasma (fibrinogen, blood-group proteins), connective tissue (collagen), in the formation of chitin, and various hormones and enzymes. Monosaccharides are the simplest sugars, of which glucose is the most abundant and the most common compound upon which more complex carbohydrates are structured. Oligosaccharides contain from two to ten monosaccharide units, and polysaccharides more than ten units. Starch, the plant fuel storage polysaccharide including α-amylose , amylopectin and the derivative dextrin, and cellulose, the plant structural polysaccharide, contribute to the high biospheric carbohydrate level. Glycogen is the main storage carbohydrate in animals, typically located in the liver (Lehninger, 1979).

The utilisation of carbohydrates in finfish decreases with increasing complexity from monosaccharides to disaccharides, simple polysaccharides, dextrins, cooked starches, and lastly raw starches (Halver, 1976). Conversely, crustaceans are able to use complex polysaccharides more efficiently than simple sugars (Biddle *et al.*, 1977; Gomez and Nakagawa,

1990; New, 1980), although there seem to be considerable species specific differences (Gomez and Nakagawa, 1990; New, 1976). Studying *M.rosenbergii* and using isonitrogenous and isocalorific diets based on a semi-purified preparation employing either glucose, sucrose, glycogen, soluble starch, potato starch or dextrin at 40% inclusion, Gomez and Nakagawa (1990) concluded that soluble starch and potato starch were used most efficiently as evaluated by growth, feed efficiency, and body composition. Glucose supplied prawns had the lowest weight gain. Carbohydrate source did not affect survival, but did influence the lipid composition of the hepatopancreas in that soluble and potato starches resulted in highest levels of free fatty acids and phospholipids, thus possibly indicating active lipid metabolism (Gomez and Nakagawa, 1990). Fair *et al.* (1980) replaced up to 30% (0, 5, 15, 30%) precooked starch with fibre cellulose in *M. rosenbergii* rations without detriment to growth, and indeed stimulated growth up to 20% fibre in a second trial (0, 5, 20%). Good prawn growth was achieved as protein was replaced employing high inclusion levels of dietary carbohydrate-rich barley (Ashmore *et al.*, 1985) and corn silage (Moore and Stanley, 1982). Similarly, protein was spared when a high fat:carbohydrate ratio of 1:3-1:4.5 was utilised (Clifford and Brick, 1978; Millikin *et al.*, 1980).

1.2.7 Ash, Minerals, and Vitamins

Inorganic matter may be required by *M. rosenbergii* in order to supply minerals and salts (Newman *et al.*, 1982), although the uptake of ash also exhibited by marine crustaceans which are hypotonic to the external medium, suggests that salt absorption may be involuntary (Forster and Gabbott, 1971). Forster and Gabbott (1971) determined that 32.2% of the inorganic fraction of the diet, which was 15.5% ash, was assimilated by *Palaemon serratus* while Newman *et al.* (1982) reported 47-58% of the inorganic component of the diet, which was 13.7% ash, was absorbed by *M.rosenbergii*. Inclusion of a mineral premix in semi-purified diets has proved beneficial for growth of various penaeids and a Ca:P ratio near 1:1

optimal (Sick *et al.*, 1972; Kanazawa *et al.*, 1984). Studying *P.japonicus*, Kanazawa *et al.* (1984) observed best growth, survival, and feed conversion given 1-2% of each Ca and P, 0.1-0.5% Mg, and 0.09-1.8% K, but not Fe, Cu or Mn. Such specific nutritional studies with *Macrobrachium* are lacking, but in both marine and freshwater crustaceans the water medium will act as a source of certain ions (Shewbart *et al.*, 1973; Fieber and Lutz, 1985) and thus dietary requirement will to an extent be dependent on environmental conditions. However dietary phosphorus will probably be necessary as it is neither typically abundant in the aquatic ecosystems nor readily absorbed across epithelial surfaces, and because it is involved in many important biochemical compounds and processes.

Vitamin supplementation, as mineral, is a routine though somewhat blind practice in the preparation of crustacean diets. For the generalised crustacea, vitamins C, E, and most of the B group are nutritionally required, vitamins A and D may be synthesised from nutritional precursors, and dietary vitamin K may be detrimental (Fisher, 1960). Further research indicated a requirement for thiamine and pyroxidine (Deshimaru and Kuroki, 1979), but no effect was evident on growth by varying the form of carotenoid pigment supplementation (Yamada *et al.*, 1990) in *P japonicus*. Postlarval *M. rosenbergii* cultured in individual cages were not affected by the deletion from purified diets of fat-soluble vitamins, but three water-soluble vitamins influenced health, growth and survival (Heinen, 1988). Vitamin C deficiency resulted in subcuticular coloration lesions, incomplete moulting, and mortality. Deletion of pyridoxine and riboflavin significantly decreased and increased growth rate respectively.

1.2.8 Nutrient Assimilation

Assimilation efficiencies of the dietary components by *M. rosenbergii*, calculated on the basis of ingested feed and egested material as both regurgitate and faeces, are reportedly high. Dry matter, lipid, carbohydrate, and inorganic matter were assimilated with 77-80%, 94-97%,

83-87%, and 47-56% efficiency over, yet unaffected by, the temperature range of 22.4°C to 34.7°C (Newman *et al.*, 1982). Other Carideans have indicated similar levels, and a mean nitrogen assimilation efficiency of 89% (Forster and Gabbott, 1971). Several nutrient assimilation or digestibility studies, such as those employing chromic oxide, are invalidated by negation of the assumption of consistent homogeneity of the food material and the marker (Ashmore *et al.*, 1985; Fair *et al.*, 1980), and those employing the natural marker ash are invalidated by the negation of the assumption that there is no change in ash weight as food passes through the gut (Fair *et al.*, 1980).

1.3 ROLE OF FEED AS FEED OR FERTILISER

Various authors (New, 1980; Weidenbach, 1982) have commented on the lack of evidence concerning the role of formula pelleted feeds in pond culture of prawns. In semi-controlled outdoor fibreglass tank studies, Balazs and Ross (1976) concluded that algae were of nutritional benefit to *M. rosenbergii*, while Boonyaratpalin and New (1982) claimed that natural food in concrete ponds receiving formulated feed and infrequent water exchange significantly affected growth. In an earthen pond study conducted by Fujimura and Okamoto (1970) insignificant differences for both growth and survival between prawns raised on water stable trout chow pellets and those raised on either pig or poultry starters or gamebird feed, suggested that the prawn is able to locate and use small particles of broken feed. Similarly, Stanley and Moore (1983) found no growth difference between prawns fed bound or unbound diets in pond cages. Referring to earthen ponds, New (1980) reported that much of the applied pelleted feed acts as a fertiliser to increase pond productivity and thus lead only indirectly to production of the cultured prawn. New and Singholka (1982) stated that their recommended initial levels of feeding were well above prawn requirements and that the food acts as an expensive yet effective fertiliser.

Stomach content analysis by Waidenbach (1982) indicated that *M.rosenbergii* consumed both feed pellet and natural pond biota. The study quantified the individual components of the stomach contents and hence, due to the previously defined reservations concerning such analyses, the relevant data is excluded. However, another measurement based on the population of the prawns and not on the population of each stomach individually, identified the frequency with which feed items occurred between different individual prawns. This measure of frequency is of no value for quantitatively assessing stomach content nor nutritional value, but merely reflects the uniformity with which the predators select the diet (Bowen, 1985). Of the stomachs examined 95-100% contained formula feed pellets, 100% contained fine particulate matter (material less than 250 µm including sediments, detritus, plant and animal remains, benthic and planktonic algae, sand, and commercial pellets), 21-41% contained pond macroflora, and 88-92% contained pond macrofauna. The macrofauna reportedly included prawn pieces, mosquito fish, chironomids and ants, ostracods, copepods, cladocerans and miscellaneous fauna. The study, conducted under standard Hawaiian conditions in earthen ponds supplied with pelleted feeds at application rates of 45 kg ha^{-1} day^{-1} but without manure application and with stocking densities of 16.14-21.52 postlarvae m^{-2} (Fujimoto *et al.*, 1977), further supported the argument for a role of natural feeds even in the presence of pelleted diets.

Food wastage is not a phenomenon specific to crustacea, although the external manipulation of diet particles by Macrobrachium does suggest that losses may be especially high. Beveridge *et al.* (1991) reported that 1% to 30% of applied feed may remain uneaten by rainbow trout and Atlantic salmon cultured intensively. These authors, in summarising results from various publications, suggested that losses are greater in instances where trash fish is applied or where the fish are cultured in cages as opposed to ponds. Estimates of feed unconsumed by rainbow trout in earth ponds and supplied with pelleted diet ranged from 1-10%. Beveridge *et al.* (1991)

indicated that no similar data are available for crustacea held in ponds, although from laboratory studies with P. monodon Wickins (1985) reported 11% of feed remained uneaten, and from literature data on feed conversion ratios Phillips et al. (1992) considered that shrimp feed wastage may be higher than 11%.

1.4 POND PRODUCTIVITY

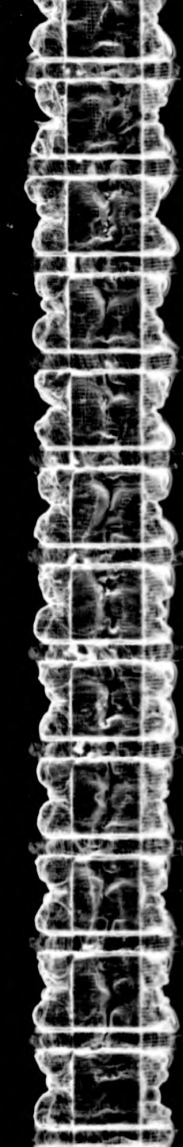
1.4.1 The Process of Decomposition

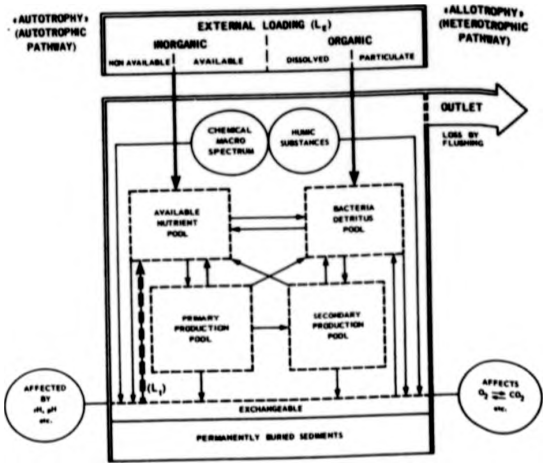
Organic matter, as a potential source of carbon and nutrients, perpetuates pond productivity. The synthesis of new cell biomass is accomplished principally by photosynthetic primary producers but also by autotrophic cyanobacteria and anaerobic bacteria. Organic matter which is tied up in living biomass is unavailable for immediate recycling. However upon death, organic matter becomes the object of deterioration and decomposition with the resultant release of carbon and nutrients to fuel production and growth. A simple trophic model of the interlinked aquatic foodchains is presented in Figure 1.1. The origin of the available organic matter may be autochthonous as dead plant or animal material or the excreta and exudate of living organisms present either in the water column or within the sediment layers. Alternately, the organic matter may be allochthonous as applied feed, foliage, or manure, or as organic matter which enters the system through water exchange, water runoff, or miscellaneous diffuse sources such as windborne pollen or grasses, or point sources such as bird excreta. Organic matter may be particulate and of extremely variable composition, or dissolved as either low molecular weight compounds including amino and volatile fatty acids, or more complex and refractory compounds of higher molecular weight including, for example, extracellular bacterial products or humic and phenolic compounds. Dissolved organic matter will be converted into the particulate form by a variety of chemical, physical and biological reactions (Wooton, 1984), although the concentration of dissolved organic matter, at least in natural waterbodies, remains approximately an order of magnitude greater than that

Figure 1.1.
Internal dynamic pathways in the aquatic environment (OECD, 1982).

Figure 1.1.

Internal dynamic pathways in the aquatic environment (OECD, 1982).




«AUTOTROPHY»
(AUTOTROPHIC
PATHWAY)
EXTERNAL LOADING (L_e)
INORGANIC
ORGANIC
NON AVAILABLE
AVAILABLE
DISSOLVED
PARTICULATE
«ALLOTROPHY»
(HETEROTROPHIC
PATHWAY)
OUTLET
LOSS BY
FLUSHING
CHEMICAL
MACRO
SPECTRUM
HUMIC
SUBSTANCES
AVAILABLE
NUTRIENT
POOL
BACTERIA
DETRITUS
POOL
PRIMARY
PRODUCTION
POOL
SECONDARY
PRODUCTION
POOL
(L_i)
AFFECTED
BY
eH, pH
etc.
AFFECTS
$O_2 \rightleftharpoons CO_2$
etc.
EXCHANGEABLE
PERMANENTLY BURIED SEDIMENTS

of particulate forms (Saunders, 1976).

Bacteria play the major role in converting dissolved organic matter to particles (Bowen, 1987), both in order to produce new cells and to produce the extracellular organic matrix (Paerl 1974, 1978; Hobbie and Lee, 1980; Costerton *et al.*, 1981) whose mass may exceed by many fold that of the bacteria themselves (Paerl, 1978; Hobbie and Lee, 1980). The terms "substrate" or "resource" refer to an organic compound (Anderson, 1987) and imply one which has the potential to host enzymatic degradation. Detritus may be defined as non-living particulate organic matter (see Moriarty and Pullin, 1987), however, in association with the detritus will develop viable microbial populations such that detritus without attached or embedded microorganisms is rare (Bowen, 1987).

The process of decomposition in aquatic systems, as outlined below, was developed by Anderson (1987) as based on that for terrestrial systems (Swift *et al.* in Anderson, 1987). The variables of the environment, including the physical parameters, the organisms and the quality of substrate, regulate the three interactive processes of decomposition; catabolism, comminution, and leaching. Catabolism is a chemical process of enzymatic degradation of a substrate, and is mostly microbially mediated. Comminution is predominately a physical process leading to the reduction of the resource conducted biotically by animal feeding activities and abiotically by factors such as water currents. Leaching is an entirely physical process describing the removal of soluble or labile materials from the resource by the water itself.

The quality of a substrate is determined by its chemical composition, specifically the type of carbon compounds present, the concentration of nutrients present, and the presence of modifiers or chelating agents which deter degradation. Compounds such as simple sugars or starch are readily catabolised while others such as lignin and chitin are more recalcitrant.

Nutrient concentration may limit decomposition by directly affecting the catabolic microbes. Furthermore nutrients may be inversely related to the concentration of other components, specifically carbon, and thus potentially influenced by substrate carbon availability. Certain compounds such as tannins have been implicated as modifiers in determining feeding and in inhibiting enzymes, although their role in actually controlling decomposition rates is unclear (Anderson, 1987).

Substrate quality is also determined by physical characteristics such as particle size, surface to volume ratio and surface texture. These features will influence the degree of leaching possible and the quantity and quality of organisms which are attracted to and successfully able to degrade the substrate through either comminution or catabolism. Hence, the organismal population act as an environmental determinant of decomposition (Anderson, 1987).

In general bacterial decomposition is more important than fungal decomposition in aquatic systems (Moriarty, 1987). Nonetheless, the physical attributes of the substrate influence the relative importance of the two groups in that the unicellular thallus of many aquatic bacteria is more effective at colonising particulate resources of less than 1 mm, whereas the hypomycetes of fungi are better adapted to larger surfaces (Anderson, 1987; Bowen, 1987). Obviously, not only the initial size of the particle, but the rapid process of leaching and continuing process of comminution will influence the character of the microbial invasion (Anderson, 1987). The interplay between organisms within and across trophic levels represents complex ecological phenomena associated with commensalism, synergism, mutualism, competition, amensalism, predation, and parasitism (see Anderson and Macfadyen, 1976; see Tenore and Coull, 1980; see Atlas and Bartha, 1987; see Sommer, 1989).

The main physical factors affecting decomposition are temperature and

oxygen (Anderson, 1987). Temperature affects the rate of breakdown very roughly as a 50% increase for every 5°C increment (Little and Muir, 1987), probably mediated through the effect of increasing temperature on accelerating autolysis and increasing bacterial activity while decreasing bacterial generation time (Brock, 1966; Rheinheimer, 1985). In all micro-organisms, temperature affects the life-processes (Rheinheimer, 1985) most particularly as growth rate, nutritional requirements, and fatty acid composition (Ingraham, 1962, 1987; Frecht *et al.*, 1973). Oxygen may be considered an important factor limiting heterotrophic productivity (Colman and Edwards, 1987). However the majority of aquatic microbes are facultative anaerobes, and even obligate aerobes (eg: Nitrosomonas europaea and *Nitrobacter winogradskyi* which function in nitrification) are impaired only at very low (2 mg l^{-1} or less) oxygen concentrations (Rheinheimer, 1985), whist obligate anaerobes (eg: *Pseudomonas stutzeri* which functions in denitrification) may be inhibited by the presence of oxygen (Brock, 1966).

The rate of decomposition of a substrate, theoretically consistently decreasing as the most to least catabolisable fractions are mineralised, is complicated by the association of new products due to such processes as flocculation of previously dissolved organic matter and the formation of new particulate matter, or microbial synthesis (Anderson, 1987). In practice, the components which cannot be degraded further have extremely limited nutritional value (Bowen, 1987) and become incorporated into the sediment (Anderson, 1987; Bowen, 1987).

1.4.2 The Stimulation of Productivity

The intentional addition of organic matter to fuel production in finfish aquaculture is a centuries old management technique. Particularly in Asia and the Far East, manure and foliage have been applied to pond polycultures of carps. Dependent upon the availability of resources,

especially as waste from agriculture, or household produce, an incredible variety of organic material has been applied to ponds (Ling, 1967; Prowse, 1967). However, certain resources have been used frequently and thus directed research to focus on sewage or human waste (Edwards *et al.*, 1980; Edwards, 1984; Piedrahita and Tchobanoglous, 1987), and animal manures (Schroeder, 1978; see Wohlfarth and Schroeder, 1979; Buck *et al.*, 1981; Fair and Fortner, 1981; Malecha *et al.*, 1981; Wohlfarth *et al.*, 1985; see Little and Muir, 1987; see Wohlfarth and Hulata,1987; Zhu *et al.*,1990). Application rates cited in the literature have varied enormously, for fish culture, over the range 12.5-200 kg ha^{-1} day^{-1} as dry matter of poultry, cattle or pig manure (Moav *et al.*, 1977; Burns and Stickney, 1980; Malecha *et al.*, 1981; Barash *et al.*, 1982; Cohen *et al.*, 1983; Wohlfarth *et al.*, 1985; Zhang *et al.*, 1987; Zhu *et al.*, 1990; Green, 1992). The nutrient composition of manures varies greatly between and within animal species (Table 1.3) and will thus obviously affect the value and amount required of the fertiliser.

A review on the use of manure in aquaculture (Wohlfarth and Schroeder, 1979) reported that certain studies indicated a superiority of cattle manure while others indicated chicken manure was more effective. Similar inconclusive results on the effects of the combined addition of feeds and manures resulted from an apparent synergism in some experiments yet sub-additive action in other studies. However a more recent review indicated that superiority of a manure type or a fish species is not apparent, and increasing stock density results in increased yields only to a certain level (10,000 fish ha^{-1}) of density (Wohlfarth and Hulata, 1987). A compilation of conversion ratios for both feed and manure in controlled experimental ponds in Israel indicated that FCRs for manure, either as cattle or poultry, were 0.6-3.8, and for pellets (25% protein) were 0.5-1.8 when added to manured ponds, or up to 2.9 when applied without manure but in parallel ponds (Wohlfarth and Hulata, 1987). Fish yields from these experimental systems were highest with high protein pelleted feeds at a

Table 1.3. Animal manure nutrient content (% dry matter) for nitrogen (N), phosphorus (P), and potassium (K), and their carbon:nitrogen ratios (C:N).

Manure	N	P	K	C:N
Poultry	3.8[d]	1.9[d]	1.8[d]	9[d]
Chicken broiler	3.5[b]	2.0[a]	1.7[a]	-
Duck	2.3[b]	1.4[a]	1.2[a]	-
	4.4[c]	1.1[c]	-	10[c]
	2.2[d]	1.1[d]	1.2[d]	10[a]
Swine	2.3[d]	0.8[d]	1.3[d]	24[d]
Sheep	1.9[d]	0.8[d]	0.9[d]	29[d]
Cattle	1.9[d]	0.6[d]	1.4[d]	19[d]
Buffalo	1.4[c]	0.2[c]	-	26[c]
	1.2[d]	0.6[d]	0.7[d]	19[d]
Human	7.2[d]	1.7[d]	2.4[d]	8[d]
Septage	6.9[c]	1.9[c]	-	6[c]

[a] = Taiganides in Hopkins and Cruz, 1982 (P calculated from P_2O_5 phosphate with multiplication by 0.4364 and K calculated from K_2O potash with multiplication by 0.8302 according to Tacon (1987b)); [b] = Hopkins and Cruz, 1982; [c] = Edwards, 1986; [d] = Tacon, 1987b.

mean of 42 kg ha^{-1} day^{-1}, and yields from intensive manuring with supplemental pellet feeding were greater at a mean of 35 kg ha^{-1} day^{-1} than those for manure alone at a mean of 32 kg ha^{-1} day^{-1}.

Similarly a survey of the literature reflecting a wider range of environmental conditions and management strategies indicated that fish grown in mono- or poly-culture systems produced yields of 1-35 kg ha^{-1} day^{-1} in ponds supplied with chicken, cattle, pig, or duck manure alone (Moav *et al.*, 1977; Collis and Smitherman, 1978; Wohlfarth *et al.* in Wohlfarth and Hulata, 1987; Bok and Jongblood, 1984; Zhu *et al.*, 1990). Higher maximum yields to 43-52 kg ha^{-1} day^{-1} have been recorded when both manure and feeds are applied (Barash *et al.*, 1982; Cohen *et al.*, 1983; Wohlfarth *et al.*, 1985).

Considered as the principal nutrient input, Wohlfarth and Hulata (1987), summarised the following advantages of manure;

- the low cost of manures can dramatically reduce input expenses
- the need to acquire feedstuffs from elsewhere is reduced or avoided
- the use of manure relieves problems associated with its disposal, including environmental pollution resulting from its accumulation
- fish raised on the natural feeds produced from manure degradation tend to have a lower fat content than fish raised on formula pelleted feeds

However, Wohlfarth and Hulata (1987) also noted the following disadvantages associated with the use of manures;

- the proper management of manured systems is more difficult than systems to which feeds are applied
- determining the appropriate amount of manure to be applied is more difficult than estimating feed requirements due to the variability in manure quality (particularly moisture and

nutrient content)

- manure decomposition and use of the degradation products are influenced by factors beyond control
- manure availability may be a problem unless livestock are situated locally in feedlot units
- fish raised on manure may meet consumer resistance
- the economics of manure verses feedstuffs most favours the use of manure when feeds are expensive and fish are inexpensive
- manure usage is not well suited to colder water systems due to temperature dependent decomposition
- manure usage is not suited to super-intensive systems

It is accepted that organic matter may theoretically provide nutrition to pond fishes by three mechanisms; direct consumption, or either autotrophic or heterotrophic stimulation of pond productivity (Tang, 1970; Wohlfarth and Schroeder, 1979). The relative importance of the mechanisms has been disputed, but is likely to be a function of the conditions of culture including the system, the species and the climate. However, manures are considered generally as an indirect source of feed (Schroeder, 1978; Boyd, 1982; Wohlfarth and Hulata, 1987). To consider the potential effect upon the autotrophic and heterotrophic populations, and to monitor the development of any potentially deleterious conditions, certain water quality parameters must be regularly determined.

1.4.3 The Assessment of the Pond Status

In aquaculture water serves the overall purpose of medium, while its physical, chemical and biological characteristics are important in determining the oxygen supply and energetics of excretion, nutrition and growth of the cultured species (Phillips *et al.*, 1991).

Water temperature affects all life processes but most importantly in aquaculture growth rate, as mediated through feeding rate and digestion

rate, and flesh quality. Optimal temperature is species specific and an important factor in the culture of the target species and, in extensive and semi-intensive systems, the organisms which comprise its biotic diet.

Dissolved oxygen supply may be typically attributed to internal processes and dynamics in extensive or semi-intensive culture when the water residence time is long, but almost entirely on the water supply in most intensive aquaculture systems (Phillips *et al.*, 1991), although generally ponds tend to have a relatively low water exchange even in intensive systems (Beveridge *et al.*, 1991). According to Colman and Edwards (1987), the goal of pond water chemical balance is the generation of sufficient oxygen for the target species and their food items, and the prevention of the buildup of toxic metabolites. Aerobic decomposition, which proceeds most rapidly when dissolved oxygen levels are near saturation, confers carbon dioxide as the primary product, while anaerobic decomposition, which is a slower process, leads to organic primary end products such as alcohols and organic acids (Boyd, 1979). Dissolved oxygen (DO) is an important water quality parameter and its depletion is often the main cause of sudden and massive fish mortalities (Fast, 1986). DO concentration is determined by the processes of photosynthesis, respiration and diffusion (Boyd, 1979; Fast, 1986) and the solubility is affected by the physical factors of temperature, pressure and salinity (Boyd, 1979; Fast, 1986).

Oxygen production is determined by the abundance and species of photosynthesing organisms, directly as oxygen generation and indirectly as the effects of shading through turbidity. Stratification (thermal, chemical or biological) and the physical attenuation of light will influence the DO profile of a water body, and both seasonal and diel fluctuations may be wide ranging. The daily range of saturation may extend from 25% to 200% (Fast, 1986) or even to extreme supersaturation at 250% (Boyd, 1979). The effect of such fluctuation is poorly documented (Boyd,

1979), but DO below 25% saturation (Rappaport *et al.* in Boyd, 1979) or 1-2 mg l^{-1} (Fast, 1986) is accepted as potentially deleterious although these observations are somewhat species specific. Hence, interest has been directed towards the prediction of low DO typically occurring at dawn, as based upon factors such as temperature, secchi disc reading, fish biomass, phytoplankton biomass, pond biological oxygen demand and wind speed and DO concentrations at dusk or during the night (Romaire *et al.*, 1978; Boyd, 1979; Meyer and Brune, 1982; Madenjian *et al.*, 1987a, 1987b).

The hydrogen ion concentration is also important due to its influence upon the toxicity of substances such as ammonia and free-CO_2. The pH usually exhibits a diel pattern as determined by the intensity of photosynthesis and the concentration of free-CO_2 (Rimon and Shilo, 1982). Values of pH between 7-8 (Fast, 1986) or 6.5-9.0 at daybreak (Ellis in Boyd, 1979) are desirable for most species, but a wide daily variability in pH may be damaging to fish (Boyd, 1979; Fast, 1986).

Waters with low alkalinity are typically variable in pH, low in nutrients, unproductive, and low in fish yields (Hickling, 1971; Boyd, 1979; Schaeperclaus in Fast, 1986). As modified by Fast (1986), Schaeperclaus classified pond productivity over the range of 0-5 mequivl l^{-1} (0-250 mg l^{-1}) as waters from strongly acid and unsuitable for aquaculture purposes to those which exhibit optimal productivity. The predominant bases bicarbonate, carbonate and hydroxide are the major factors which influence alkalinity, and which vary in proportion with the acids carbon dioxide and carbonic acid in relation to photosynthetic activity (Boyd, 1979).

Turbidity is caused by both organic matter including phytoplankton and inorganic material such as silt or clay. It may limit light penetration thereby controlling photosynthesis and oxygen production, and determining the health of the ecosystem. Inorganic turbidity can reduce the effects of manuring as phosphorus particles tend to be absorbed or adsorbed on to

the particles (Fast, 1986), although manure may also be used to decrease inorganic turbidity caused by negatively charged colloidal clay particles (Swingle and Smith in Fast, 1986). A load of 2440 kg ha^{-1} barnyard manure applied twice or thrice will cause precipitation of some clay particles (Swingle and Smith in Fast, 1986), as will appropriate applications of hay or aluminum sulphate which, though nontoxic to aquatic organisms, can cause a reduction in pH (Irwin and Stevenson in Fast, 1986; Boyd, 1979).

Various reports have indicated that either phosphorus, nitrogen, or both are key or limiting nutrients required by fertilisation programmes (Hickling, 1971; Woynarovich, 1975; Boyd and Musig, 1981; Yusoff, 1987). The absolute concentrations and ratios of the inorganic phosphorus and nitrogen compounds are the best indicators of potential nutrient limitation. Ratios of each of total phosphorus and total nitrogen to chlorophyll are also used as indicators of the chlorophyll production per unit of nutrient. Orthophosphate present in the water may be taken up by phytoplankton, bacteria, or sediments (Rigler, 1956, 1964; Hepher, 1958a, 1958b; Fitzgerald, 1970; Kimmel and Lind, 1970). Indeed orthophosphate present in the water the day after fertilisation declines by 90% within 1-2 weeks (Zeller, 1952; Hepher, 1958; Boyd and Musig, 1981). Accumulation of sediment phosphorus decreases the ability to remove further phosphorus from water (Eren *et al.*, 1977). However, pond sediments are variously reported as sinks or sources of phosphorus (Hepher, 1966; Metzger and Boyd, 1980; Boyd and Musig, 1981) probably as dependent on such factors as history of enrichment, trophic state, level of external loading, flushing rate, basin morphometry and sediment type (Marsden, 1989). The extremely leached soil typical of the wet tropics (Deshmukh, 1986; Yaacob and Shamsuddin in Yusoff, 1987), may limit the amount of nutrients entering the pond system during filling or water exchange. Nitrogen compounds also may accumulate in sediment particularly as ammonium salts, or form gaseous N, and either remain in the water or be lost to the atmosphere (Goldman and Horne, 1983). Ammonia which is not precipitated,

may be nitrified, or assimilated by cyanobacteria (Shilo and Rimon, 1982; van Rijn *et al.*, 1984).

The original sources of ammonia include diet, fertiliser, or water supply, and once in the system ammonia may be regenerated through excretion or mortality. Ammonia is an important phytoplankton nutrient, but also a powerful fish toxin particularly at high temperature and pH when the unionised fraction increases (Shilo and Rimon, 1982; Stirling, 1985). Excessively applied protein rich diets have been implicated in the production of high ammonia levels and fish growth depression (Kausik, 1980; Colt and Armstrong, 1979; Shilo and Rimon, 1982), and have lead to a call for further research into optimal feeding and fertilising programmes (Shilo and Rimon, 1982).

Organic matter exerts a biochemical oxygen demand (BOD) which is a measure of the respiration of the whole respiring community and therefore an important parameter which can be used to indicate potential oxygen stress. Oxygen uptake is a function of the respiratory demands of the bacterial, phytoplanktonic and zooplanktonic communities, and of the quantity of organic matter (Beveridge in Stirling, 1985). Historically BOD refers to the oxygen consumed after a five day dark incubation period and is a measure employed in the waste industry. For application to aquaculture a shorter incubation is more suitable (Boyd, 1979; Little and Muir, 1987). The BOD measured over 12-dark-hours at pond temperature, $BOD_{5,5}$, is appropriate as a reflection of the overnight oxygen demand associated with pond respiration and substrate specific decomposition (Little and Muir, 1987). The chemical oxygen demand (COD) is a measure of the oxidisable organic matter but determined chemically (Beveridge in Stirling, 1985) and hence measures more complete oxidation which may be beyond the capacity of the existing microbial community. Although COD does not indicate the rate of oxidation or oxygen demand as does $BOD_{5,5}$, the ratio of COD:BOD can reflect longer term oxygen requirements (Little and Muir, 1987).

Chlorophyll concentration is typically used as a measure of phytoplankton biomass, although variations in the ratios of chlorophyll to biomass on the basis of species, physiological state, water trophic state and light are known to exist (Hunter and Laws, 1981; White *et al.*, 1988). Nonetheless, absolute chlorophyll concentrations and the ratios of chlorophyll to nutrients are useful, albeit imperfect, indicators of the degree of trophy and the efficiency of the system.

Fish production and gross primary productivity have been found to be strongly correlated (Melack, 1976; McConnell *et al.*, 1977; Liang *et al.*, 1981). The usual method of measuring algal productivity by dark and light bottle incubation is actually a measure of water column productivity because it is the summation of water column net productivity and respiration. Net productivity in the water column (NP_{wc}) measures net algal productivity (NP_a) minus both algal and nonalgal respiration (R_a and R_{na}) and is thus an underestimate of NP_a. In ponds receiving a large loading of organic inputs, the difference between NP_{wc} and NP_a may be very large. Although NP_a is the appropriate measure for research in aquatic feeding pathways (McConnell, 1963), it cannot be measured directly by the oxygen method, and hence gross productivity is usually quoted (Colman and Edwards, 1987). Even then, the "glass-effect" on eliminating the flow of water and nutrients and promoting the sedimentation of the plankton, and the "dark-effect" on decreasing the rate of respiration may introduce inaccuracies to the method of dark-light bottle incubation and primary productivity determinations (Olah *et al.*, 1978).

A valuable predictor of potential fish harvest has been identified as the morphoedaphic index (MEI). This index is a function of the concentration of total dissolved solids and the mean water depths, and has proven an accurate predictor in fisheries management (Ryder, 1965; Ryder *et al.*, 1974; Allan in McConnell *et al.*, 1977; Melack, 1976). Unfortunately, however, MEI is not recommended for pond aquaculture being inappropriate

for small shallow ponds (McConnell *et al.*, 1977).

The above water quality parameters (with the exception of MEI) constitute some of the most appropriate abiotic and biotic tools to assess the status of the dynamic pond environment. Alternately, or preferably additionally, the macroinvertebrate and microbial communities may be examined.

Benthic invertebrates are "animals living in or on the sediments and dependent upon the decomposition cycle for most if not all of (their) basic food supply" (Brinkhurst, 1974). Certain populations of benthic invertebrates, or indeed the balance of various populations, have been characterised as indicative of the trophic status of the water body and many indices have been developed to summarise benthic data (Shannon, 1948; Brinkhurst, 1966; Chandler, 1970; see Hellawell, 1977, 1986). Some indices suffer from certain limitations such as sample-size dependency (Gray, 1974) and the tendency of benthic communities to aggregate rather than be randomly distributed in the sediment (Pielou, 1969). However they may be useful tools in drawing general conclusions from complex ecological data (Pearson and Rosenberg, 1978), especially if several types are employed simultaneously (Hellawell, 1986). Unfortunately, it would appear that no indices have been developed specifically for tropical benthos, and the application of indices from temperate regions to the tropics has not been fully assessed. Furthermore few studies have been undertaken to relate benthic ecology to aquaculture, although it is understood that the macrobiotic benthos may play an important role in pond ecology. Perhaps the most important effect of the benthos is in terms of its oxygen demand, although certain populations, such as many species of chironomids, are tolerant of very low dissolved oxygen (Pinder, 1986). Nonetheless growth of chironomids is inhibited when DO is below 4% saturation (Jónasson and Kristiansen, 1967) and most benthic organisms require 1 mg l^{-1} in order to maintain positive production (Martien and Benke, 1977). An important role of the macroinvertebrate population is that of sediment mixing and

aeration, and subsequent nutrient release (Holdren and Armstrong, 1980; White, 1986). Alternately, competition for feed between cultured benthic feeders and macroinvertebrates may, at least temporarily, shift nutrients away from a cultured target species (Bardach *et al.*, 1972; White, 1986). The degree of intensification of aquaculture systems is considered a major factor influencing the importance of natural macrobenthos to the target species (White, 1986; Stirling and Wahab, 1990).

Similarly, microbes including bacteria, fungi and protozoa, both reflect and influence the pond ecological status. As discussed above bacterial decomposition is of primary importance in aquatic ecosystems. Although the methods for studying both fungal and protozoal biomass and growth in mixed assemblages are not well developed (Moriarty, 1987), the methods for estimating total bacterial numbers, volumes and biomass in both natural waters and sediments have developed remarkably in recent years due to advances in staining techniques, epifluorescent microscopy and image analysis (van Es and Meyer-Reil, 1983; Getliff and Fry, 1989; Fry, 1990). However, the methods have not been applied frequently to aquaculture systems yet and may serve to clarify the role of bacteria in pond productivity.

1.4.4 Aquaculture and Productivity

In an earthen pond environment the complexities of the abiotic processes and biotic interactions, which to an extent will be site and management specific, have granted the systems the status of "black-boxes" (Samples and Leung, 1985; Anderson, 1987) of which the dynamic metabolism is poorly understood. This is particularly surprising as most of the world's yield of farmed fish is raised in manured ponds (Wohlfarth in Colman and Edwards, 1987). However, research in aquaculture has tended to focus upon the cultured organism and their specific requirements, rather than addressing, as particularly suitable in the tropics, the complete resource system upon which much aquaculture in the developing world depends

(Edwards, 1992). The ancient practice of stocking with multiple species each with a generally specific and non-competing feeding niche, has perhaps volumetrically optimised production, but lacks a detailed basis of appreciation for the complex operational processes. Species selection, historically sensible, has been transplanted across the continents and centuries. However as "new" (different) species are scientifically investigated for their biological suitability for aquaculture, and further encouraged by market interests, it is necessary to consider appropriate production mechanisms. Extensive aquaculture is not recommended for ponds, and most tropical inland aquaculture is semi-intensive (Beveridge and Phillips, 1992). In general, system intensification associated with higher stocking densities and dependence upon applied feeds has characterised much of finfish aquaculture in recent years. Intensification has increased fish yields, although sometimes only temporarily (Csavas, 1992), but not without considerable effects upon the environment (Beveridge *et al.*, 1991; Weston, 1991) and the indigenous biota (Weston, 1991) including the natural fish stocks (Maitland, 1989; Weston, 1991; Cataudella and Crosetti, 1992). Furthermore, overproduction as often associated with industrialisation or intensification may lead to market saturation and a fall in unit price (Csavas, 1992; Ferdouse, 1990). Semi-intensive culture is less environmentally harmful (Csavas, 1992) and a beneficial system (Primavera, 1989; Edwards, 1992). Armed with the anatomical, physiological and behavioral adaptations to feed on either plankton, benthos or detritus, a species (Beveridge, 1984), such as M. rosenbergii, may be especially well suited to semi-intensive systems stimulated by organic fertilisation.

Tropical aquaculture has a weak research base (Edwards, 1992) and systematic studies on water quality dynamics are needed (Boyd, 1979; see Lannan *et al.*, 1986). Furthermore, a major experimental effort is required in order to consider the effect of pond fertilisation on the production of natural food and its utilisation by cultured aquatic species (see

Moriarty and Pullin, 1987). Research is required on the ecological efficiency of the pond as inputs of organic matter and yield of cultured biomass (Moriarty, 1987). Studies on nutrient dynamics are lacking in prawn culture, specifically as mass balance equations (Beveridge *et al.*, 1991), and nutrient dynamics as related to the role of the sediments must also be addressed (Edwards, 1992)

In view of the foregoing introduction concerning both the biology of *Macrobrachium rosenbergii* and general pond productivity, the present research project was designed to study the effect of applications of formula feed and manure on pond culture of the freshwater prawn. It was anticipated that research into the ecology of the managed pond ecosystem would extend the current limitations of our knowledge on *Macrobrachium* pond culture dynamics.

1.5 THE AIMS OF THE PRESENT STUDY

The aims of the present project were outlined as follows;

1) To investigate whether formula feed, presented as pellet, may be partially replaced by organic manure in pond culture of *Macrobrachium rosenbergii* (Experiment 1),
2) To investigate the effect of manuring frequency (Experiment 2),
3) To investigate if juvenile prawns respond to feed and manure over a short period of exposure (Experiment 3),
4) To identify the effects of inputs on water quality (particularly as nutrient and chlorophyll concentrations, and primary productivity), sediment chemistry (as nutrient concentrations) and benthic macrofauna (as density) (Experiments 1, 2, and 4),
5) To identify the effects of inputs on water and sediment bacteriology (as total biomass) (Experiment 4),
6) To consider the value of employing enclosures (mesocosms) in such studies (Experiments 3 and 4).

CHAPTER TWO

THE STUDY SITE

2.1 Malaysia

Peninsular Malaysia lies between the latitudes 1° 15' N to 6°45' N and the longitudes 99° 35' E and 104° 20' E. It has a maximum length of 740 km, a maximum width of 322 km, and covers an area of 134,680 km^2. A central mountain range runs from the southeast to the northwest, reaching an elevation of about 2400 m above sea level (Yusoff, 1987; Ang, 1990). Limestone deposits are restricted to the northwest and central regions, so most peninsular waters are typically soft, acidic and lacking in buffering capacity (Yaacob and Shamsuddin in Yusoff, 1987). Overall the country experiences a northeast monsoon during November to March and a southwest monsoon during May to August. Annual rainfall is usually within the range of 1800-3500 mm, but like other climatic factors, exhibits geographic variation.

2.2 Universiti Pertanian Malaysia

Universiti Pertanian Malaysia (UPM; Agricultural University of Malaysia) is located at Serdang 23 km from the capital Kuala Lumpur. The university was previously operated as a plantation for both palm oil and rubber. The land is gently sloping and the aquaculture unit is situated at 31 m above sea level. The soils are predominantly yellow or reddish-yellow latosols derived from quartzite sedimentary rock and of variable fertility (Panton, 1964). The water supply to the aquaculture unit originates from three ground sources on campus which join to form a stream and reach the site within 1 km from the farthest source. The drainage basin consists of plantation, crop, and grazing lands. Stream water is collected in an impoundment from where it is pumped either to the hatchery unit or the pond complex.

2.2.1 Hatchery

The Fakulti Perikanan dan Sains Samudera (Faculty of Fisheries and Marine Science), established in 1979, constructed a small hatchery on site at UPM. The hatchery was expanded with technical and financial assistance

from the Government of Japan through the Japanese International Cooperation Agency (JICA). The JICA project commenced in 1984, and a new bio-physico-chemical hatchery system was operational by mid 1986 (Plate 2.1).

Stream water is pumped from the impoundment into an underground 14 ton concrete reservoir, passed through a silica-sand-gravel filter capable of filtering 240 l min^{-1} and stored in one 14 ton tank and two 7 ton elevated tanks. The bio-physico-chemical hatchery system (bpc) consists of five 16 ton tanks, three 32 ton tanks, and eight biostream units. The bpc is open to the air but is roofed with opaque sheeting which passes light and concentrates heat. The culture method may employ either "green" or "clear" water[5], but the former is used most frequently in order to improve both prawn/fish growth and water quality. Water and wastes from each tank are recirculated from a central drain to its complimentary biostream unit via an airlift pump. The biostream unit is a maze of concrete paths through which tank water flows, and wastes collect and undergo decomposition. Wastes may be either settled algae, fish/prawn faeces, or food organisms/diets. Some bpc tanks and biostreams are employed for the culture of food organisms such as algae including *Chlorella* and rotifers *Brachionus*. Other bpc tanks are used for larval rearing of fish such as lampam java *Puntius goniontus* and sea bass *Lates calcarifer*, or freshwater prawns *M. rosenbergii*. Each bpc unit of tank and biostream operates independently, allowing for different combinations of water conditions specifically in terms of algal density and saline concentration. Broodstock are maintained in separate tanks in the hatchery or in ponds in the neighbouring pond complex.

[5]referring to whether algae have or have not been intentionally added to the water; recommended algae are unicellular greens, usually *Chlorella* spp.

Plate 2.1

Facilities at Universiti Pertanian Malaysia;

a) the hatchery,

b) the pond complex.

2.2.2 Pond Complex

The ponds are situated on level ground and the perimeter of the complex fenced. Water is pumped from the impoundment through an underground system of channels to each pond, so no ponds are operated in series. The channels run under the bunds which are roughly 1 m wide. A simple screw valve controls the water flow into each pond through a 10.16 cm diameter inlet pipe to which are clipped two mesh bags (250 µm) for filtering.

Rectangular ponds of 110 m^2 (10 m x 11 m), 220 m^2 (20 m x 11 m) and 440 m^2 (20 m x 22 m) were constructed in 1985. The prawn ponds used for the following experiments (Plate 2.1) had similar histories of management and stocking practices. Pond outlets are situated diagonally opposite to the inlets and consist of a cement sluice adapted to house two 10.16 cm standpipes to control pond water height. A standpipe inside the pond at a distance of roughly 50 cm from the sluice maintains the pond water depth at 1 m. An elbow joint and short horizontal pipe direct water through the sluice gate and out of the drainage system. A second standpipe located inside the cement sluice itself functions to hasten draining and was capped on the pond side throughout each experiment. Poles and overhead power cables provide electricity for light and equipment such as aerators or pumps. An armed security guard is detailed to overlook the hatchery and pond complex outside of normal working hours. Despite the security guard and fencing, predators or competitors such as the monitor lizard *Varanus salvator*, white-collared kingfisher *Halycon chloris*, cinnamon bittern *Ixobrychus cinnamomeus*, and little egret *Egretta garzetta*, were occasionally viewed or at least their recent presence in evidence. Prior to the commencement of the second trial, an internal perimeter fence of 1 m height was erected around the prawn ponds to further reduce the risk of entry by the monitor lizards. Snakes including the King cobra *Naja hannah*, the regal python *Python reticulatus* and a pit viper (unconfirmed identity but possibly *Ancistrodon rhodostoma*) were not uncommon on site due to the proximity of the supply river and pockets of natural forest.

2.2.3 Weather Station

The UPM weather station is located approximately 1 km from the pond site. Data for the five year period 1981-1985 indicated average monthly values for rainfall of 21.10 - 418.10 mm, relative humidity 92-97%, maximum daily temperature 31.0-34.7°C, minimum daily temperature 20.7-23.5°C, daily sunshine duration 4.05 - 7.92 hours, evaporation 82.70 - 178.30 mm, and wind speed 0.00 - 1.18 m s^{-1}. Annual precipitation (AP) at UPM classifies the site as Type 2 Humid wherein AP is usually > 2000 mm and some months have less than 100 mm (Jackson, 1977). Specific climatic data for each experiment will be presented in an appendix for reference.

2.2.4 Laboratories

The hatchery buildings are equipped with facilities for diet preparation, freezers for the storage of feedstuffs, diet or cultured animals, and a basic laboratory where feed and animals may be weighed or simple analyses such as oxygen titration, may be conducted. The Faculty building at a distance of 2 km from the hatchery/pond complex is furnished with both wet and dry laboratories, computing facilities and a library.

CHAPTER THREE

EXPERIMENT ONE:
THE EFFECT OF FEED AND FERTILISER ON PRODUCTIVITY IN POND TRIALS

3.1 INTRODUCTION

Natural pond productivity is neither optimally employed nor promoted in freshwater prawn culture. The common practice in the intensive and semi-intensive commercial sector is to feed a protein-rich formulated diet at moderate to high levels. However, an alternative approach which is characterised by less costly production, is the stimulation of the natural pond environment. Several researchers have addressed the subject of fertilisation in freshwater prawn systems (Stahl, 1979; Buck *et al.*, 1981; Wohlfarth *et al.*, 1985), but there is a lack of research on the effects on the pond chemistry and biota of variable loadings of manure and feed under conditions typical of tropical semi-intensive commercial culture.

Commercial culture of *M.rosenbergii* is not well developed in Malaysia despite initial research on the species having been conducted in the country (Ling and Merican, 1961; Ling, 1962, 1967, 19691, 1969b). Indeed there are no extension manuals published by the Government of Malaysia on recommended methods of prawn pond culture. However a Department of Fisheries textbook describes general aquaculture techniques in natural waters and recommends the application of 3-4 tons per acre (7.41-9.88 tons per hectare) of animal manure (MDF, 1985). If inorganic fertiliser is applied, the Government advise that nitrogen fertiliser is not required, but that fertiliser containing phosphorus alone will suffice. Hence, feeding and fertilising procedures appropriate for the culture of *M. rosenbergii* under Malaysian conditions are not readily identifiable.

Neighbouring Thailand has to some extent similar climate and environmental conditions as Malaysia. The Thai Fisheries Department does not specifically recommend the addition of manure to *Macrobrachium* ponds. However in freshwater fish culture, the application of 1250 - 1562 kg ha^{-1} $month^{-1}$ of dry buffalo, cow or chicken manure is recommended by the Government of Thailand (Pongsuwan and Sithimung, 1989). Fishes which can survive low dissolved oxygen concentrations, may be grown in ponds

receiving as much as 100 kg wet chicken manure ha^{-1} day^{-1} (Edwards and Kaewpaitoon, 1984), although lower levels such as 500 kg wet chicken manure ha^{-1} $fortnight^{-1}$ are also recommended for fish culture (Ali, 1986). Species criteria and water management practices will determine the appropriate level of manure. The Network of Aquaculture Centres in Asia recommends that 200 kg wet chicken manure per hectare per spring tide water exchange is appropriate for the culture of marine Penaeids (NACA, 1986). A review of international extension manuals does not reveal any specific pond manuring recommendations for *N. rosenbergii* culture. New and Singholka (1982) stated that fertilisation in prawn ponds is unneccessary during the first two months of culture as excess feed acts as a fertiliser, but that in general, the use of fertilisers in later stages could be encouraged to reduce costs.

In order to achieve better production of the freshwater prawn it is necessary that appropriate techniques for their culture be identified, and thereafter extended to the farm sites. The Malaysian population is rapidly increasing and fish, including finfish and shellfish, provides roughly 60% of the national protein intake. Based upon the estimated future requirement for fish and the present fish landings and proportions of fisheries trash fish, supply will be 250,000 tons short of demand, or 36% short of the total requirement, in the year 1995 (Labon in Ang, 1990). The development of good finfish and shellfish husbandry practices suitable for widespread application within the country is required, but is dependent upon a thorough understanding of the underlying principles of the culture environment. The earthen pond environment must be understood in order to boost production by Malaysian fish farmers, the majority of whom integrate their culture with animal husbandry and agriculture (Ang, 1990).

In the complex pond system a host of parameters must be analysed in order to understand the dynamics of the system. Factors which require assessment are prawn survival and production, water quality and sediment chemistry,

benthic macrofauna, and water and sediment microbiology. By way of an initial study, Experiment 1 considered prawn production and certain aspects of both the water and sediment chemical and biotic status.

3.2 MATERIALS AND METHODS

3.2.1 Pond Preparation

Eight ponds of 220 m^2 were cleared of plants, and dried over several days until deep cracks had developed in the bottom. Standard pond preparation techniques were followed. Quick lime, CaO, and seasoned semi-dry chicken manure were added at rates of 700 kg ha^{-1} and 250 kg ha^{-1} respectively. Water was added to a depth of 10 cm. After five days, a further 90 cm of water was added. Diesel emulsion (50 l diesel fuel + 50 ml soap detergent + 5 l water) was added at a rate of 55.05 l ha^{-1} (pers. comm. Professor Ang Kok Jee) to eliminate air-breathing insects. The emulsion was allowed to clear for six days before the prawns were introduced.

3.2.2 Stocking, Management and Harvesting

M. rosenbergii (de Man) post-larvae (PL) were obtained from the hatchery unit on site. Ponds were stocked with 52-day-old (from hatching) prawns at 10 m^{-2}. Arithmetic mean and standard error at stocking were 0.05 ± 0.01 g (1.50 ± 0.04 cm orbital length). Silver carp, *Hypophthalmichthys molitrix* (Val.), of 5.74 g were stocked at 0.1 m^{-2} two weeks later. After a further three weeks, grass carp *Ctenopharyngodon idella* (Val.), were introduced at one fish of mean weight 1.8 kg per pond. Scheduled stocking of the grass carp was delayed by three weeks because the initial supply was diseased and a second had to be obtained. These stocking rates were insufficient to classify the system as a typical polyculture (Tapiador *et al.*, 1977), and the fish were considered as mechanisms for biological control over the extreme development of plant life.

Four treatments, randomly allocated to eight ponds, employed a high

benthic macrofauna, and water and sediment microbiology. By way of an initial study, Experiment 1 considered prawn production and certain aspects of both the water and sediment chemical and biotic status.

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Four treatments, randomly allocated to eight ponds, employed a high

quality 30% protein diet and/or a seasoned chicken manure. The diet formulation (Table 3.1) was designed at UPM by least-cost linear programming (Poh, 1985) and the feed was prepared onsite. Dry ingredients were ground to 0.5 mm, accurately weighed, and mixed thoroughly together. Oil was added to produce a dough which was passed through a low pressure pelleter with a ring die of 1.5 mm. Resulting dough strings were dried at 35°C, and if required, crumbled and sieved. The pellet sizes employed were 1 mm diameter from day 1 to day 49 and 1.5 mm from day 50 onwards.

The treatment feed-plus-manure (FPM) was supplied with feed application rates (Table 3.2) representing local commercial levels and manure loads suitable for Malaysian fish culture conditions (pers. comm. Professor Ang Kok Jee, UPM, and Mr. Lim Hai San, Lion Aquafarms Sdn. Bhd., Puchong). The feed-only (FO) treatment was fed as above but not fertilised with manure. The decreased-feed-increased-manure (DFIM) regime was given feed at reduced rates and sufficient manure such that the total phosphorus load was equivalent to FPM. The manure-only (MO) treatment received the same absolute amount of manure as FPM, but no feed. Treatments were conducted in duplicate with the exception of MO which was unreplicated due to an unexpected shortage of PL.

Feed was applied twice daily at 0700 and 1900 hours. The feeding requirement was defined based on the prawn stocking biomass for the first five weeks, and on an assumed average prawn weight of 1 g for the subsequent two weeks due to the difficulty in sampling such a small size. Commencing on day 49 and continuing fortnightly, the absolute feed requirement was calculated based on the mean weight of prawns, the feeding rate as a percent of the body weight (Table 3.2), and an assumed mortality. Prawn weight was determined from sampling thirty individual prawns per pond (day 49, 63, 77, 91, and 105). Sampling was conducted with a cast net of 370 cm diameter and 1 cm mesh size across the stretched diagonal. Prawn standing biomass was calculated assuming mortalities based

Table 3.1. Prawn feed formulation.

Feed Ingredient	% Inclusion
Fish meal (Malaysian by-catch; whole animal machine dried)	11.21
Shrimp meal (Malaysian by-catch; whole animal sun dried)	21.56
Soy bean meal (extracted)	9.63
Copra meal (expelled)	10.95
Wheat flour	33.31
Palm oil	4.82
Fine sand	4.82
Mineral mix[1]	3.00
Vitamin mix[2]	0.20
Basfin[3]	0.50

[1] Sick and Beaty (1975)

[2] NRC (1977)

[3] polymethylcarbamide crosslinking carbohydrate and protein

Table 3.2. Pond inputs (feed as a percent of body weight per day).

Schedule	FPM[1]	FO[2]	DFIM[3]	MO[1]
Day 1 - 49	15.0	15.0	10.0	-
Day 50 - 63	10.0	10.0	7.5	-
Day 64 - 125	5.0	5.0	2.5	-

[1] 250 kg manure ha^{-1} 14 $days^{-1}$

[2] no manure

[3] sufficient manure such that pond input was isophosphoric with FPM

on previous grow-out trials at UPM of 15%, 20%, 30% and 40% of the initial stock commencing on days 38, 50, 64 and 92 respectively. Chicken manure from battery reared layers was applied fortnightly (day 13, 25, 38, 50, 64, 78, 92, and 106), during weeks alternate to water sampling. Periodic analysis of the manure for *Salmonella* spp. using routine culture techniques, consistently proved negative. Water exchange was maintained at 5% day^{-1} by pumping water into each pond through an inlet pipe covered with two mesh (250 µm) bags. Decreasing dawn dissolved oxygen levels at the pond bottoms (to 2.49 mg l^{-1} in DFIM and 2.95 mg l^{-1} in FPM) led to the introduction of aeration on day 117 and thereafter paddlewheel aerators were employed nightly in every pond from 1900 to 0700 hours.

Harvesting took place on day 126. The water level was lowered to 10 cm on the afternoon before harvest. The prawn biomass of each pond was measured and the prawns divided into four morphotypic sex-types as berried females, unberried females, small males (including juveniles), and adult males. This classification was based on the sex-types described by Cohen *et al.* (1981) and Kuris *et al.* (1987). Sex-type groups were weighed and the number of prawns in each group counted in order to determine morphotype proportions. From each sex-type, twenty five individuals were randomly selected and weighed. Mean prawn weight at harvest (irrespective of morphotype) was calculated as a function of total biomass and total number. Marketable percent was determined based on the ratio within each sex-type of the randomly sampled individuals which were > 20.00 g. This ratio was weighted by the appropriate morphotype proportion and the contributions for each sex-type summed for each pond. Annual marketable yield was calculated given a possible 2.6 batch cycles per year under prevailing conditions.

3.2.3 Water Chemistry

Water sampling and measurements were conducted approximately every two weeks (days 3, 17, 29, 44, 56, 70, 84, 98, and 113) between 0745 and 0915

hours. A small paddle boat was used to gain access to the centre of the pond without disturbing the sediment, and the sampling schedule was completed prior to the introduction of the paddlewheel aerators.

Samples for dissolved oxygen (DO) were collected in biological oxygen demand (BOD) bottles temporarily strapped to a stick and immersed to depth. A system using two glass tubes of 3 mm internal diameter mounted in a rubber bung inserted into the neck of the bottle allowed for gentle air displacement. Samples at 20 cm and 70 cm were taken to indicate both surface and bottom DO conditions. Given the height of the BOD bottle and the tubing, 70 cm was decided as the maximum depth possible to ensure the integrity of the sediment. DO samples were fixed immediately with manganous sulphate and alkaline iodide solutions. Unscheduled DO samplings were made closer to harvest and on occasions of extreme climatic conditions.

Temperature was measured *in situ* with a mercury thermometer at a depth of 20 cm after allowing for a three minute stabilization period. An integrated water sample was obtained using a PVC pipe of 4 cm internal diameter. Four tube samples were sufficient to fill a 4 l polypropylene storage bottle. Water samples were transferred to the laboratory for immediate analysis or preservation. Samples for filtration were passed through glass fibre filter papers, and spectrophotometric analyses were performed using a double beam 1 cm cell path spectrophotometer (Shimadzu UV-210A).

Determinations of pH were made with a standardised Metrohm 620 pH meter. Chlorophyll-a (CHL) was determined according to the technique of Holm-Hansen and Riemann (1978) by vacuum filtering a known volume of water (250-500 ml), extracting the pigment in 90% methanol, and measuring absorbance at 750 nm and 665 nm. The concentration of CHL was calculated using the equation of Jones (1979a);

$CHL = Ve/Vs \times f/g \times A$

where CHL = chlorophyll-a ($\mu g\ l^{-1}$)

Ve = volume (ml) of the extract

Vs = volume (l) of the filtered sample

f = factor equivalent to the reciprocal of the specific absorption coefficient (SAC) for CHL, multiplied by 1000; assuming a SAC for CHL in absolute methanol of 77.9 $l\ g^{-1}\ cm^{-1}$ (Riemann, 1978).

g = path length (cm)

A = absorbance at 665 nm corrected for that at 750 nm

Dissolved oxygen (DO) was determined iodometrically by the Winkler method using starch as an indicator and modified with sodium azide to eliminate nitrite interferences (APHA, 1985).

Total nitrogen (TN) was digested with sulphuric acid and potassium persulphate into ammonia which was analysed spectrophotometrically at 635 nm by the phenol-hypochlorite method (Adamski, 1976).

Total phosphorus (TP) was digested with sulphuric acid and potassium persulphate into soluble inorganic phosphorus which was analysed spectrophotmetrically at 882 nm using the molybdate reaction method (Eisenrich *et al.*, 1975).

3.2.4 Sediment Chemistry

Sampling for sediment chemistry was conducted once monthly (days 5, 34, 59, 88 and 117) between 0800 and 1100 hours. From a paddle boat, an Ekman grab (15 cm x 15 cm) was used to collect three samples per pond. These samples were pooled and returned to the laboratory. A subsample was taken from each well mixed sample and analysed for total nitrogen by the Kjeldahl method, and total phosphorus using the perchloric-sulphuric acid method and measurement at 660 nm (Black, 1965). Organic carbon was determined by the Walkely-Black method using potassium dichromate as the

oxidant and ferrous sulfate as the titrant (Black, 1965). Although carbon occurs in soil in both organic and inorganic forms, it is known to be predominantly or entirely in organic form in humid regions under conditions of extreme leaching (Black, 1965).

3.2.5 Sediment Macroinvertebrates

Benthic macroinvertebrates were separated from the sediment samples once monthly (days 34, 59, 88 and 117). Pooled samples were passed through a 250 µm (Wentworth) sieve, and separated out onto a white enamel tray (45 cm x 21 cm). To ensure no invertebrates had been missed, the sieved sample was quickly scanned under a low power dissecting microscope. Organisms were preserved in 70% alcohol, and enumerated and identified by eye or by microscope as required. In order to identify the oligochaetes and chironomids to species, it was necessary to view certain morphological characteristics and so individuals had to be mounted on glass slides and viewed under a high power Olympus microscope. Prior to mounting, the specimens were transferred from 70% alcohol, to 30% alcohol, and then to water (Brinkhurst, 1971). The excess water was removed by blotting with tissue, the specimen placed on a glass slide, and several drops of polyvinyl lactophenol (PVLP) and a coverslip added. Correct orientation of the head of the chironomid was vital for proper viewing and identification. Therefore head capsules had to be separated from the body, and mounted such that the ventral side faced up. Slides were labelled, and placed on a hot-plate at 50 - 60°C for about 24 hours, or until dry. The drying process was monitored and more PVLP added if the initial supply evaporated and the development of airspaces threatened. Identification required the aid of several keys as indicated below.

Invertebrates in General	Macan,TT. (1964) Quigley,M. (1977)
Mollusca	Berry,A.J. (1963)
Oligochaeta	Brinkhurst,R.O. and Jamieson,B.G.M. (1971)

Chironomidae larvae	Wiederholm,T. (1983)
Chironomidae pupae	Wilson,R.S. and McGill, J.D.(1982)
Ceratopogonidae larvae	Fitter,R. and Manuel,R. (1986)
Coleoptera larvae	Fitter,R. and Manuel,R. (1986)
Trichoptera larvae	Edington,J.M. and Hildrew,A.G. (1981)
Ephemeroptera pupae	Macan,T.T. (1970)

The identifications of two groups were confirmed by experts as follows; chironomids by Dr. Peter Cranston of Division of Entomology, Commonwealth Scientific and Industrial Research Organisation, Clevland, Australia, and, oligochaetes by Dr. Mike Ladle of the River Laboratory, Freshwater Biological Association, East Stoke, England.

The number of organisms per species, and the species diversity index as measured by the Shannon-Wiener function and described by Lloyd and Ghelardi (1964), were analysed.

3.2.6 Nutritional Analyses

Triplicate samples of prawn diet and chicken manure were analysed for moisture, total nitrogen, total phosphorus, and organic carbon. Moisture was defined as the weight change in samples after drying at 105°C for twelve hours. Subsequent determinations were performed on dried, ground samples and the results expressed as a percent of the dry weight.

Total nitrogen was determined by the Kjeldahl nitrogen method. Results were expressed as percent nitrogen, and as percent protein following multiplication by a factor of 6.25. Total phosphorus and organic carbon were analysed as described for sediment samples.

3.2.7 Input:Output Conversions

Input:output conversion ratios were calculated from the quantity of dry feed and manure load in relation to wet prawn yield as both total and

marketable yields. A partial economic assessment was made based on the cost to produce one kilogram of marketable prawns given existing costs for feed and manure at the equivalent of 1991 prices. Feed costs were unusually low due to their formulation by least-cost linear programming and preparation within the university. Prices in 1991 were the equivalent of £0.31 kg^{-1} dry prawn diet and £28.80 $tonne^{-1}$ dry chicken manure.

3.2.8 Climatic Analysis

Climatic data was obtained daily throughout the experimental period from the UPM weather station as described in 2.2.3. Relative humidity was measured with wet and dry thermometers and temperature with minimum and maximum thermometers, all of which were housed in a Stevenson screen. Rainfall was measured employing a Dines tilting-siphon system, and sunshine duration using a Campbell-Stokes recorder. Wind speed was determined by a cup anemometer, and evaporation by an American Class A evaporation pan. Water balance was calculated as the daily difference between rainfall and evaporation. Climatic data are presented in Appendices 3.1 and 3.2.

3.2.9 Statistical Analysis

The method oneway analysis of variance (ANOVA) with a single factor of treatment was adopted such that a series of ANOVAs were constructed corresponding to each sampling day. Data for prawn mean weight were transformed by log to the base ten. The assumptions of normal distribution for each population and equal variances for all populations at each sampling time were checked and confirmed. Scheffe's multiple comparison test was employed to isolate the source of any statistical differences as indicated by the ANOVA results. It was not possible, due to small number of replicates, to thoroughly check for normality of the data for each of prawn harvest and both water and sediment chemistry on each sampling day. However, because there was no reason to assume that the logged data were non-normal and since the analysis of variance is very robust to non-

normality (Zar, 1984), ANOVAs were conducted and the results accepted given that homoscedascity was proven. Throughout the thesis, the letter "F" is used to indicate the F statistic of probability, also known as the "p" value. In order to gain further insight into the physicochemical relationships, water data from each sampling period were stacked together. Tests for normality and homoscedascity proved positive, and analyses were conducted for correlation, regression and ratio values. Standard Pearson product-moment correlations, and also partial correlations were calculated between TN, TP and CHL in order to account for any interdependency of TN and TP. All statistics were performed using the package SPSSx (SPSS Inc.) on the university mainframe.

3.3 RESULTS

3.3.1 Pond Inputs

Pond inputs over the experimental period are presented in Table 3.3. Despite the consistent feeding rate as a percent of estimated prawn biomass in the treatments FPM and FO (Table 3.1), the absolute amounts of feed added to these two treatments were quite different due to the difference in mean size of prawns at sampling. In absolute terms, the amount of feed added to the FO and DFIM treatments were very similar. Nutrient loads into DFIM and FPM were isophosphoric as defined in the experimental protocol. The amount of manure loaded to each of FPM and MO was 11.50 dry g m^{-2} 14 day^{-1} as defined, and that to DFIM was 14.08 dry g m^{-2} 14 day^{-1} as calculated. The feed and manure content and load of TN, TP, and OC are given in Table 3.3, and the cumulative loads of each nutrient from both inputs are presented in Appendices 3.3 - 3.5.

3.3.2 Prawn Sampling Weights

Weight data from fortnightly sampling are presented in Table 3.4. Analyses of variance at each sampling period indicated differences between treatments commencing from the first sampling day. Throughout the trial,

Table 3.3. Load of prawn feed, chicken manure, total phosphorus (TP), total nitrogen (TN) and organic carbon (OC) over the experimental cycle; absolute load (dry g m^{-2} $cycle^{-1}$) and percent load of each of total input, total phosphorus, total nitrogen and organic carbon (%).

Component	FPM	FO	DFIM	MO
Feed	255.11 (73.50)	172.26 (100.00)	174.80 (60.82)	- (0.00)
Manure	92.00 (26.50)	- (0.00)	112.60 (39.18)	92.00 (100.00)
TP as feed[1]	1.33 (42.63)	0.90 (100.00)	0.91 (29.26)	- (0.00)
TP as manure[1]	1.79 (57.37)	- (0.00)	2.20 (70.74)	1.79 (100.00)
TN as feed[2]	11.92 (83.94)	8.04 (100.00)	8.17 (74.54)	- (0.00)
TN as manure[2]	2.28 (16.06)	- (0.00)	2.79 (25.46)	2.28 (100.00)
OC as feed[3]	77.04 (81.16)	52.02 (100.00)	52.79 (70.70)	- (0.00)
OC as manure[3]	17.88 (18.84)	- (0.00)	21.88 (29.30)	17.88 (100.00)

[1] based on percent TP values of 0.52 for feed and 1.95 for manure.
[2] based on percent TN values of 4.67 for feed and 2.48 for manure.
[3] based on percent OC values of 30.20 for feed and 19.43 for manure.

Table 3.4. Prawn weight (g) at sampling; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
0	0.05^{a} (0.01)	0.05^{a} (0.01)	0.05^{a} (0.01)	0.05^{a} (0.01)
49	3.00^{a} (0.45)	1.61^{a} (0.26)	3.57^{b} (0.47)	2.80^{a} (0.37)
63	5.80^{b} (0.50)	3.75^{a} (0.34)	8.13^{b} (0.74)	5.51^{ab} (0.72)
77	8.43^{b} (0.45)	6.10^{a} (0.33)	12.31^{c} (0.60)	6.41^{ab} (0.59)
91	13.04^{b} (0.81)	8.62^{a} (0.41)	16.73^{c} (0.73)	7.79^{a} (0.87)
105	17.55^{b} (0.93)	12.40^{a} (0.46)	23.36^{c} (1.09)	11.18^{a} (0.74)
126[1]	23.19^{b} (0.18)	13.44^{a} (1.29)	28.89^{b} (0.35)	13.28^{a} (na)

[1] = as calculated from harvest data; total biomass/total number.

the FO or the MO prawns consistently weighed less than the FPM prawns which in turn weighed less than the DFIM prawns. FO and MO prawn weights were never significantly different from one another, although MO prawns weighed more than FO prawns on the first three sampling days, while the reverse was true for the last two sampling days. FPM sampled prawns were significantly heavier than FO and MO prawns from days 63 and 91 onwards respectively. DFIM prawns were significantly heavier than FO, MO, and FPM prawns from days 49, 77, and 77 onwards respectively. Mean weights at harvest, calculated as total biomass/total number, were statistically similar for FO and MO regimes, which were statistically different from the similarly grouped FPM and DFIM treatments.

The trends of the sampling data and the clear superiority of the DFIM mean weight are illustrated in Figure 3.1.

3.3.3 Prawn Growth Rate and Harvest Data

The mean growth rate for each treatment is expressed in Table 3.5. Analyses indicated that FO and MO growth rates were not significantly different from one another, but were statistically different from FPM and DFIM which also were not statistically different from one another.

Harvest characteristics were most extreme in MO and DFIM treatments, with the exception of percent survival. Biomass and yield were significantly lower in MO treatment as compared to the other treatments, which were grouped statistically together. Differences between treatments for the parameter percent survival indicated two statistical groupings as MO and DFIM lower than FO, and FPM compatible to both. Statistical differences were also evident for percent marketable wherein MO and FO were similar to one another, and different from FPM and DFIM, which were also similar to one another. Marketable yield indicated two statistical groupings as MO and FO lower than DFIM, and FPM again comparable to both. Disregarding the extreme MO treatment, highest percent survival corresponded to lowest

Figure 3.1.
Prawn mean weight (g) at sampling.

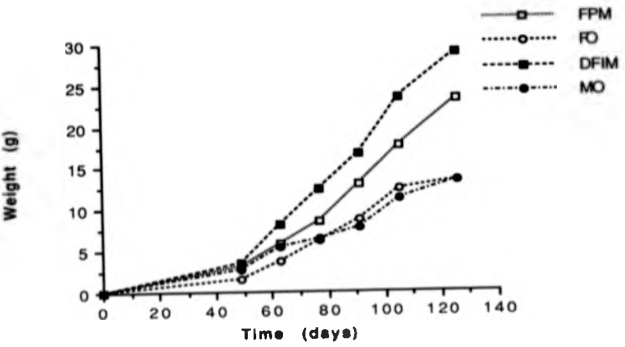

FPM
FO
DFIM
MO
Weight (g)
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
Time (days)

Table 3.5. Prawn growth rate and harvest data; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Parameter	FPM	FO	DFIM	MO
Growth rate ($g\ day^{-1}$)	0.18^{b} (0.00)	0.11^{a} (0.01)	0.23^{b} (0.00)	0.11^{a} (na)
Weight (g)	23.19^{b} (0.18)	13.44^{a} (1.29)	28.89^{b} (0.35)	13.28^{a} (na)
Biomass ($kg\ pond^{-1}\ cycle^{-1}$)	20.69^{b} (1.89)	21.35^{b} (2.18)	23.07^{b} (8.78)	7.82^{a} (na)
Yield ($kg\ ha^{-1}\ cycle^{-1}$)	940.46^{b} (85.46)	970.44^{b} (10.01)	1048.00^{b} (40.00)	355.44^{a} (na)
Percent survival (%)	40.59^{ab} (4.00)	72.82^{b} (6.23)	36.32^{a} (1.82)	26.77^{a} (na)
Percent marketable (%)	52.21^{b} (3.26)	13.28^{a} (4.07)	74.56^{b} (2.00)	11.12^{a} (na)
Marketable yield ($kg\ ha^{-1}\ year^{-1}$)	1283.88^{ab} (195.72)	336.14^{a} (106.15)	2029.40^{b} (23.18)	103.00^{a} (na)

na =not applicable

percent marketable and lowest marketable yield, and lowest percent survival corresponded to highest percent marketable and highest marketable yield.

3.3.4 Prawn Sex-Type Data

Adult males were heaviest and small males lightest in all treatments (Table 3.6). Females were intermediate, although the presence of eggs did not consistently rate the berried females as either heavier or lighter than the unberried females. The mean weights of each sex-type revealed treatment trends similar to those of sampling weights and overall harvest weights. Each sex-type of FO and MO was significantly lighter than those of FPM. The exception was in the small male morphotype, which was not evident in the DFIM treatment. DFIM sex-types were heaviest, significantly so over all FO and MO types and over berried females in FPM, but not so over unberried females and adult males in FPM.

Sex-types were also considered on the basis of their percent representation of the total harvest number, yield and marketable yield. Adult males made up from 39% to 56% of the number, 48% to 68% of the yield, and 67% to 100% of the marketable yield depending on treatment. Small males contributed little to yield and nothing to marketable yield in all treatments. However, males as a whole constituted a larger proportion of number, yield and particularly marketable yield than females as a whole in all treatments. Again the balance between berried and unberried females was inconsistent. Differences between treatments were the lack of small males and high percent of adult males in DFIM and the high percent of small males in FO, especially since the latter was not accompanied by an increase in mean weight of small males. Of further particular note was the relation between the adult male percent of yield and the overall marketable yield of Table 3.5. Both the numeric and statistical trend of these two parameters were in parallel with one another. Alternately, the adult male contribution to the percent of

Table 3.6. Prawn harvest data for each sex-type; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference ($P<0.05$) and superscripts in uncommon rows are unrelated).

Parameter	FPM	FO	DFIM	MO
Weight (g)				
Type 1[1]	18.32^{b} (0.46)	12.84^{a} (0.35)	21.89^{c} (0.40)	13.16^{a} (0.43)
Type 2	18.51^{b} (0.45)	13.20^{a} (0.29)	19.73^{b} (0.39)	12.66^{a} (0.49)
Type 3	6.73^{ab} (0.39)	5.81^{a} (0.33)		6.91^{b} (0.25)
Type 4	32.50^{b} (1.67)	17.42^{a} (0.78)	35.00^{b} (2.30)	17.64^{a} (1.04)
Percent of number(%)				
Type 1	19.58^{b} (0.71)	22.18^{bc}(0.60)	24.72^{c} (0.08)	11.72^{a} (na)
Type 2	29.30^{a} (2.29)	16.60^{a} (2.12)	19.10^{a} (1.46)	34.47^{a} (na)
Type 3	6.81^{a} (0.11)	16.50^{c} (0.18)	0.00 (0.00)	14.08^{b} (na)
Type 4	44.31^{a} (1.46)	44.72^{a} (1.35)	56.18^{b} (1.38)	39.73^{a} (na)
Percent of yield (%)				
Type 1	16.16^{a} (0.76)	23.94^{a} (2.74)	18.72^{a} (0.18)	12.60^{a} (na)
Type 2	22.68^{ab}(1.28)	16.58^{ab}(0.98)	13.03^{a} (0.78)	32.03^{c} (na)
Type 3	1.88^{a} (0.09)	7.47^{b} (0.29)	0.00 (0.00)	7.10^{b} (na)
Type 4	59.28^{ab}(0.44)	52.01^{a} (2.05)	68.25^{b} (0.60)	48.27^{a} (na)
Percent of marketable yield (%)				
Type 1	11.21 (1.96)	0.00 (0.00)	24.54 (0.07)	0.00 (na)
Type 2	17.80 (0.28)	0.00 (0.00)	8.13 (0.62)	0.00 (na)
Type 4	70.99 (1.68)	100.00 (0.00)	67.33 (0.69)	100.00 (na)

[1] Where types 1, 2, 3, and 4 refer to berried females, unberried females, small males, and adult males respectively.
na = not applicable

marketable yield was a reverse function to the marketable yield, high adult male percent related to small marketable yield and low adult male percent related to high marketable yield. The trends expressing the relation between treatments of the contributions of sex-types to each of harvest number, yield, and marketable yield are presented graphically in Figure 3.2.

3.3.5 Water Quality Concentrations and Ratios

Mean values for the water parameters are presented in Table 3.7. Mean dawn DO concentrations were higher in the FO and MO treatments than in the FPM and DFIM treatments. There were no significant differences between treatments for any of the measured parameters, although the DFIM levels of TN, TP, and CHL were always greatest followed by those of FPM. Concentrations of TN and CHL were very similar in FO and MO, but the mean TP value of the FO treatment slightly above that of MO. Figure 3.3 plots the concentrations of TN, TP and CHL over time. The mean CHL:TN and CHL:TP ratios (Table 3.7) were very alike between treatments with the exception of the FO CHL:TP ratio which was low. The mean TN:TP ratio was lowest in the FO treatment and highest in the DFIM treatment.

3.3.6. Water Quality Correlations and Regressions

Pearson correlations between the parameters TN, TP and CHL were highly significant with $F<0.01$ (Table 3.8). In each treatment, the correlation coefficient for TN-CHL were highest, followed by that for TN-TP, and lastly TP-CHL. Partial correlations between TN and CHL while controlling TP were also significant at $F<0.01$ or $F<0.05$. However, apparent correlation between TP and CHL vanished in all treatments while controlling TN. Regression equations (Table 3.9) for CHL against TN and TP singly or in combination were acceptable at $F<0.01$. Chlorophyll was predicted most accurately by TN alone. TN and TP together illustrated a slight reduction in the prediction value R^2 adjusted for each of FPM, FO, and MO. Total phosphorus alone was a poorer, although valid, predictor of

Figure 3.2.

Prawn sex-type as a percent of;

a) harvest number,

b) yield, and

c) marketable yield.

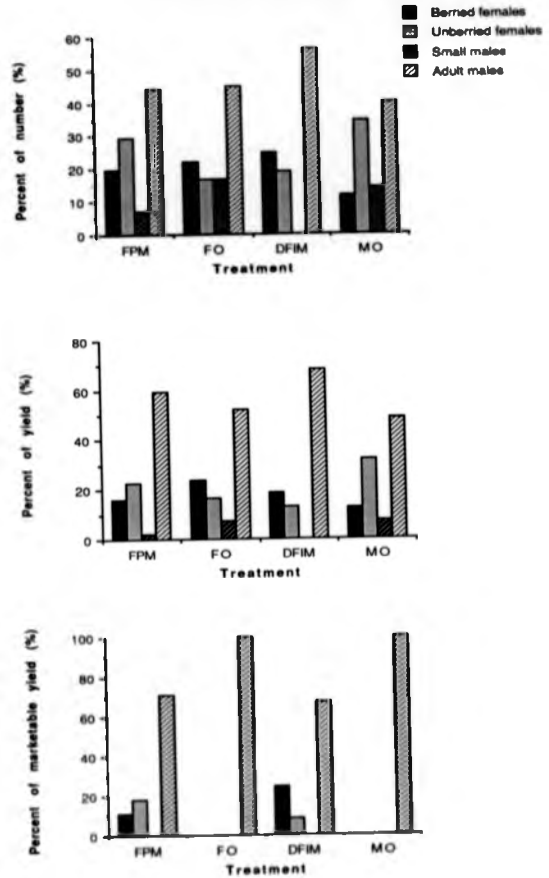

Berried females
Unberried females
Small males
Adult males
Percent of number (%)
60
50
40
30
20
10
0
FPM
FO
DFIM
MO
Treatment
Percent of yield (%)
80
60
40
20
0
FPM
FO
DFIM
MO
Treatment
Percent of marketable yield (%)
100
80
60
40
20
0
FPM
FO
DFIM
MO
Treatment

Table 3.7. Water quality data and ratios between the parameters total nitrogen (TN), total phosphorus (TP), and chlorophyll (CHL); arithmetic mean and standard error except for pH which is presented as the range of values measured over the experimental period.

Parameter	FPM	FO	DFIM	MO
Temperature (°C)	29.1 (0.2)	29.2 (0.2)	29.0 (0.2)	29.1 (0.2)
Dissolved oxygen (mg l^{-1})	4.66 (0.44)	5.21 (0.40)	4.45 (0.38)	5.18 (0.45)
pH	6.65 - 8.57	6.71 - 8.15	6.72 - 8.80	6.69 - 8.06
Total nitrogen (mg l^{-1})	2.079 (0.358)	1.868 (0.316)	2.708 (0.456)	1.869 (0.343)
Total phosphorus (µg l^{-1})	100.4 (14.8)	94.5 (9.5)	110.0 (15.8)	84.5 (8.6)
Chlorophyll (µg l^{-1})	87.2 (15.8)	71.2 (11.7)	99.4 (17.0)	73.7 (14.1)
CHL:TN	0.040 (0.003)	0.037 (0.003)	0.036 (0.002)	0.038 (0.005)
CHL:TP	0.84 (0.10)	0.69 (0.08)	0.86 (0.09)	0.81 (0.13)
TN:TP	20.18 (1.69)	18.33 (1.66)	23.40 (1.67)	20.79 (2.55)

Figure 3.3.

Water concentrations of;

a) total nitrogen (TN),

b) total phosphorus (TP), and

c) chlorophyll (CHL).

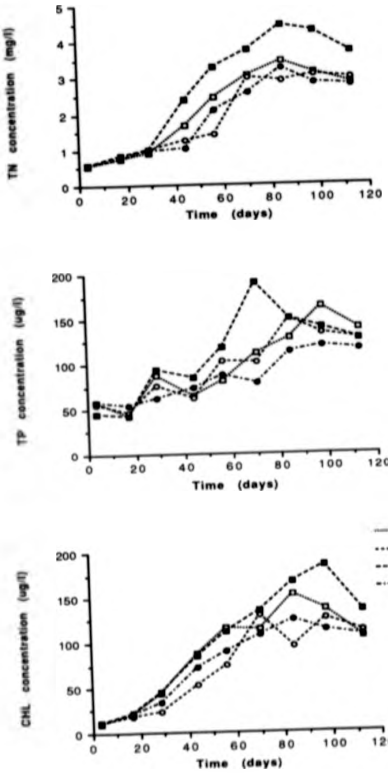

TN concentration (mg/l)
0
1
2
3
4
5
0
20
40
60
80
100
120
Time (days)
TP concentration (ug/l)
0
50
100
150
200
0
20
40
60
80
100
120
Time (days)
CHL concentration (ug/l)
0
50
100
150
200
0
20
40
60
80
100
120
Time (days)
FPM
FO
DFM
MO

Table 3.8. Pearson correlation coefficients between the water parameters total nitrogen (TN), total phosphorus (TP), and chlorophyll (CHL), and partial correlation coefficients between TN and CHL controlling TP and between TP and CHL controlling TN.

Treatment	Pearson correlation coefficient			Partial correlation coefficient		
		TN	TP		TN	TP
FPM	TP	+0.849[a1]				
	CHL	+0.946[a]	+0.759[a]	CHL	+0.877[a]	-0.260
FO	TP	+0.884[a]				
	CHL	+0.920[a]	+0.845[a]	CHL	+0.691[a]	+0.176
DFIM	TP	+0.919[a]				
	CHL	+0.957[a]	+0.852[a]	CHL	+0.845[a]	-0.239
MO	TP	+0.908[a]				
	CHL	+0.930[a]	+0.837[a]	CHL	+0.740[b]	-0.042

[1] = a and b refer to F levels of probability of 0.01 and 0.05 respectively.

Table 3.9. Regression equations between the log of the water parameters total nitrogen (TN), total phosphorus (TP), and chlorophyll (CHL).

Treatment	Regression equation	R^2	F
FPM	CHL = + 1.54 + 1.19 TN	+0.89	0.000
	CHL = + 1.25 - 0.63 TP	+0.55	0.000
	CHL = + 2.02 + 1.35 TN - 0.26 TP	+0.88	0.000
FO	CHL = + 1.50 + 1.22 TN	+0.84	0.000
	CHL = - 1.66 + 1.74 TP	+0.70	0.000
	CHL = + 0.94 + 1.04 TN + 0.30 TP	+0.83	0.000
DFIM	CHL = + 1.48 + 1.17 TN	+0.91	0.000
	CHL = - 0.83 + 1.36 TP	+0.71	0.000
	CHL = + 1.97 + 1.36 TN - 0.28 TP	+0.91	0.000
MO	CHL = + 1.51 + 1.26 TN	+0.85	0.000
	CHL = - 2.90 + 2.44 TP	+0.66	0.005
	CHL = + 1.70 + 1.31 TN - 0.11 TP	+0.82	0.003

R^2 = R^2 adjusted

F = F statistic of probability

chlorophyll.

3.3.7. Sediment Quality Concentrations and Ratios

There were no significant differences between treatments for any of sediment TN, TP, or OC. However mean concentrations of each parameter were highest in the FO treatment, and TP in MO was very low (Table 3.10). The MO treatment was most stable over time for all parameters, and FPM showed little variation over time for TN and OC, while FO indicated extreme OC variation (Figure 3.4).

The ratios TN:TP and OC:TN were low in all treatments, but somewhat higher in the ponds receiving a single input (FO or MO) rather than both feed and manure (Table 3.11). The OC:TP ratio was particularly low in FPM and high in the MO treatment.

3.3.8. Sediment Macroinvertebrates

There were no significant differences between treatments regarding the densities of benthic macroinvertebrates (Table 3.12). The densities were relatively constant over time in the FO and MO treatments, but peaks in density occurred on days 59 and 88 in the DFIM and FPM treatments respectively, and the mean density in DFIM was greater than under any other regime. The number of species ranged from 4-8 m^{-1}, but no differences between treatments were evident. Species diversity was highest in FO and lowest in FPM.

Tables 3.13 - 3.16 characterise the species profiles on each of the sampling days. The presence of *Chironomus* spp. ranged from 2.73 - 68.15% of the total sample, but as means the genus was most evident in the treatment DFIM (44.42%), followed closely by FO (40.15%) and FPM (39.54%), and lastly MO (31.11%). Similarly the percent of *Limnodrilus hoffmeisteri* ranged from 0.00-47.35%, but was greatest in FO (23.83%), followed by FPM (15.56%) and DFIM (12.88%), and MO (3.82%).

Table 3.10. Sediment chemistry parameters; arithmetic mean and standard error.

Parameter	FPM	FO	DFIM	MO
Total nitrogen (mg g^{-1})	0.84 (0.08)	1.13 (0.21)	0.92 (0.19)	0.84 (0.02)
Total phosphorus (mg g^{-1})	0.38 (0.06)	0.40 (0.05)	0.36 (0.04)	0.24 (0.02)
Organic carbon (mg g^{-1})	7.57 (0.81)	11.89 (2.20)	10.44 (1.41)	8.48 (0.82)

Table 3.11. Ratios between the sediment parameters total nitrogen (TN), total phosphorus (TP), and organic carbon (OC); arithmetic mean and standard error.

Ratio	FPM	FO	DFIM	MO
TN:TP	2.14 (0.27)	3.12 (0.31)	2.47 (0.43)	3.56 (0.30)
OC:TN	9.06 (0.75)	13.99 (1.81)	9.54 (1.05)	10.09 (0.72)
OC:TP	19.46 (3.44)	26.62 (2.15)	24.13 (3.33)	35.76 (3.35)

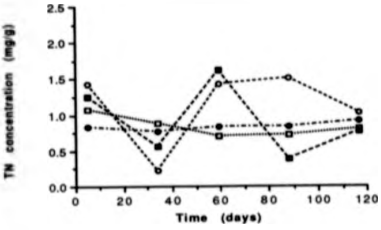
Figure 3.4.

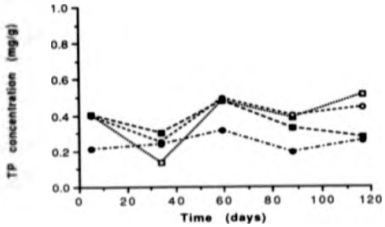
Sediment concentrations of;

a) total nitrogen (TN),

b) total phosphorus (TP), and

c) organic carbon (OC).


TN concentration (mg/g)
2.5
2.0
1.5
1.0
0.5
0.0
0
20
40
60
80
100
120
Time (days)


TP concentration (mg/g)
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
Time (days)

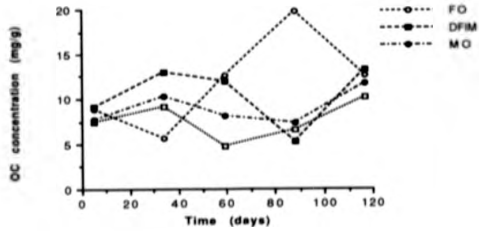

OC concentration (mg/g)
20
15
10
5
0
0
20
40
60
80
100
120
Time (days)
FPM
FO
DFIM
MO

Table 3.12. Benthic macroinvertebrate density (number organisms m^{-2}) and diversity (number species m^{-2}), and species diversity index according to the Shannon-Weiner function; arithmetic means.

Sampling day	FPM	FO	DFIM	MO
34	181 : 5	473 : 5	684 : 6	500 : 4
59	256 : 5	715 : 5	1166 : 5	222 : 6
88	1255 : 8	528 : 6	763 : 5	278 : 4
117	361 : 5	666 : 7	493 : 6	819 : 5
Mean	513 : 6	595 : 6	776 : 6	454 : 5
Species diversity index	1.64	1.96	1.89	1.80

Table 3.13. Species density (number organisms m^{-2}) of benthic macroinvertebrates and their percent contribution of the total on day 34; arithmetic mean with the exception of MO.

Species	FPM	FO	DFIM	MO
Oligochaeta				
Limnodrilus hoffmeisteri (Claparéde)	-	21 (4.44)	14 (2.05)	-
Aulodrilus piguetti (Kowalewski)	-	-	-	-
Dero (dero) obtusa (Udekem)	-	-	-	-
Conchostracae				
Cyclestheria hislopi (Baird)	-	-	-	-
Mollusca				
Melanoides tuberculata (Müller)	42 (23.20)	160 (33.83)	101 (14.77)	56 (11.20)
Indoplanorbis exustus (Deshayes)	7 (3.87)	-	-	-
Coleoptera				
Berosus sp.	-	-	-	-
Trichoptera	-	-	7 (1.02)	-
Diptera				
Chironomidae				
Clinotanypus sp.	14 (7.74)	56 (11.84)	104 (15.21)	83 (16.60)
Chironomus sp.	90 (49.72)	201 (42.50)	257 (37.57)	111 (22.20)
Ceratopogonidae	28 (15.47)	35 (7.39)	201 (29.38)	250 (50.00)

Table 3.14. Species density (number organisms m^{-2}) of benthic macroinvertebrates and their percent contribution of the total on day 59; arithmetic mean with the exception of MO.

Species	FPM	FO	DFIM	MO
Oligochaeta				
Limnodrilus hoffmeisteri (Claparéde)	-	118 (16.50)	146 (12.52)	-
Aulodrilus piguetii (Kowalewski)	-	-	-	-
Dero (dero) obtusa (Udekem)	-	-	-	-
Conchostracae				
Cyclestheria hislopi (Baird)	-	-	-	-
Mollusca				
Melanoides tuberculata (Müller)	7 (2.73)	111 (15.53)	194 (16.64)	97 (43.69)
Indoplanorbis exustus (Deshayes)	7 (2.73)	-	-	-
Coleoptera				
Berosus sp.	-	-	-	28 (12.61)
Trichoptera	-	-	-	14 (6.31)
Diptera				
Chironomidae				
Clinotanypus sp.	173 (67.58)	76 (10.63)	111 (9.52)	41 (18.47)
Chironomus sp.	7 (2.73)	361 (50.49)	347 (29.76)	14 (6.31)
Ceratopogonidae	62 (24.23)	49 (6.85)	368 (31.56)	28 (12.61)

Table 3.15. Species density (number organisms m^{-2}) of benthic macroinvertebrates and their percent contribution of the total on day 88; arithmetic mean with the exception of MO.

Species	FPM	FO	DFIM	MO
Oligochaeta				
Limnodrilus hoffmeisteri (Claparéde)	444 (35.38)	250 (47.35)	14 (1.84)	-
Aulodrilus piguetti (Kowalewski)	-	21 (3.98)	-	-
Dero (dero) obtusa (Udekem)	-	-	-	-
Conchostracae				
Cyclestheria hislopi (Baird)	7 (0.56)	-	-	-
Mollusca				
Melanoides tuberculata (Müller)	69 (5.50)	104 (19.70)	83 (10.88)	14 (5.04)
Indoplanorbis exustus (Deshayes)	7 (0.56)	-	-	-
Coleoptera				
Berosus sp.	-	-	-	-
Trichoptera	7 (0.56)	-	-	-
Diptera				
Chironomidae				
Clinotanypus sp.	62 (4.94)	35 (6.63)	14 (1.84)	56 (20.14)
Chironomus sp.	555 (44.22)	104 (19.70)	520 (68.15)	83 (29.86)
Ceratopogonidae	104 (8.28)	14 (2.64)	132 (17.29)	125 (44.96)

Table 3.16. Species density (number organisms m^{-2}) of benthic macroinvertebrates and their percent contribution of the total on day 117; arithmetic mean with the exception of MO.

Species	FPM	FO	DFIM	MO
Oligochaeta				
Limnodrilus hoffmeisteri (Claparéde)	97 (26.87)	180 (27.03)	173 (35.09)	125 (15.26)
Aulodrilus piguetii (Kowalewski)	-	28 (4.20)	28 (5.68)	-
Dero (dero) obtusa (Udekem)	-	-	28 (5.68)	-
Conchostracae				
Cyclestheria hislopi (Baird)	-	-	-	-
Mollusca				
Melanoides tuberculata (Müller)	21 (5.82)	90 (13.51)	7 (1.42)	28 (3.42)
Indoplanorbis exustus (Deshayes)	7 (1.94)	-	-	-
Coleoptera				
Berosus sp.	-	-	-	-
Trichoptera	-	7(1.05)	-	-
Diptera				
Chironomidae				
Clinotanypus sp.	-	14 (2.10)	-	111 (13.55)
Chironomus sp.	222 (61.49)	319 (47.91)	208 (42.19)	541 (66.06)
Ceratopogonidae	14 (3.88)	28 (4.20)	49 (9.94)	14 (1.71)

3.3.9. Input:Output Conversions

The conversion ratio data (Table 3.17) indicated that in terms of dry weight of inputs, least material had to be added to FO in order to achieve one kilogram of prawns. However, taking into consideration the costs of the diet and manure, and the marketable proportion of prawns in each treatment, the MO and DFIM treatments were most financially successful. The FO treatment had a very unfavourable conversion ratio on the basis of cost to produce marketable *M rosenbergii*.

3.4 DISCUSSION

The larger sampling weights and faster growth rate evident in DFIM supported the hypothesis that the feeding rate may be reduced given additional organic fertilisation is provided. Indeed although the feeding rate for FPM and FO were similar, the superior growth of the FPM prawns must be attributed to the contribution of the manure. Furthermore the equal absolute loads of feed into the FO and DFIM ponds suggested that the application of manure was responsible for the significantly heavier prawn mean weight and faster growth rate in the DFIM treatment. Manure not only replaced, but improved upon, the growth characteristics of the prawns receiving formulated pellets.

The range of growth rates, 0.11 - 0.23 g day^{-1}, of the present trial were not dissimilar to those previously reported for *M. rosenbergii*: 0.18 - 0.29 g day^{-1} (Willis and Berrigan, 1977), 0.21 g day^{-1} (Brick and Stickney, 1979), and 0.10 g day^{-1} (Malecha *et al.*, 1981), 0.05 g day^{-1} (Smith *et al.*, 1982). Mean weights at harvest consolidated the trends established at sampling. However mean size was balanced by survival in each of FPM, FO and DFIM such that the yields between these treatments did not indicate significant differences. The highest survival occurred wherein prawn mean weight was low, and with the exception of MO, intermediate survival was accompanied by high individual mean weight. Survival and yield of the

Table 3.17. Input:output conversion ratios based on dry weight of feed and manure inputs and on wet weight of prawn output, and cost (£) to produce one kilogram of total and marketable prawns (M.Prawn).

Conversion Ratio	FPM	FO	DFIM	MO
Feed:Prawn	2.71:1	1.78:1	1.67:1	-
Manure:Prawn	0.98:1	-	1.07:1	2.59:1
Input:Prawn	3.69:1	1.78:1	2.74:1	2.59:1
Cost:Prawn	0.87:1	0.55:1	0.55:1	0.08:1
Cost:M.Prawn	1.66:1	4.13:1	0.74:1	0.67:1

present trial may be compared to the literature data presented in Table 1.1. The most similar trials in terms of prawn stocking details, those of Willis and Berrigan (1977) and Smith *et al.*, (1982), had comparable production figures to the present experiment. The MO treatment compared well to the trial of Malecha *et al.* (1981) in which cow manure alone was added to ponds stocked with prawns of 0.08 g at 6.7 m^{-2} and production resulted in 13 g mean weight, 48% survival and 353 kg ha^{-1} after 131 days.

Given the nature of the social hierarchy of a mixed *M. rosenbergii* population, the ratio of the sexual morphotypes influences production characteristics. The dominant (51.1 - 61.2%) proportion of males within the total population of the present study was consistent with that reported by Wang *et al.* (1975). However Smith *et al.* (1981) observed more females (52.8 - 57.9%) in all treatments (six) which were stocked with either juveniles alone or with a combination of post-larvae and juveniles at densities from 2.15 m^{-2} to 8.61 m^{-2}, yet more males (55.3 - 56.1%) in 66% of the treatments (two of three) stocked with postlarvae. Cohen *et al.* (1981) observed a 1:1 abundance ratio between males and females in ponds stocked with PL at densities of 3 - 15 m^{-2} and Hulata *et al.* (1988) reported male composition from 45.4 - 54.3% for prawns stocked at 1.25 m^{-2} in a sex ratio of 1:1 male:female. However, Karplus *et al.* (1986) recorded that the population structure of prawns stocked at densities of 1-4 m^{-2} and batch harvested after 16 weeks displayed female proportions of 56.9 - 63.3%. Higher female abundance has also been reported by Sandifer *et al.* (1982) and Smith *et al.* (1978).

Three possible mechanisms to explain the frequent occurrence of female abundance dominance were suggested by Smith *et al.* (1978) based on possible differences in sex ratio at stocking, pond environmental conditions which favour the development of females and, potential selective mortality of males. Prawn behaviour and ecology would suggest that given the vulnerability during moulting (Peebles, 1978) and the habit

of females to seek shelter between the claws of an adult male during the moult process (Peebles in Peebles, 1979), males are more exposed to aggression possibly leading to death. Theoretically this explanation would seem most valid, however, overall management strategies which preclude or promote aggression are of primary importance. An extreme case of unequal sex ratio was reported by Lin and Boonyaratpalin (1988) as 4 females : 1 male. Unfortunately given that a full 28% of the total number of prawns were not sexed by the authors because the prawns were either soft shelled or below market size and immediately restocked into different ponds, the reported ratio may be questioned. Furthermore the practice of selective harvesting during the fourth to seventh months of culture resulted in the female contribution of each of the first and second harvests as being greater than 80%, presumably due to the unimodal growth of the female population (Cohen *et al.*, 1981; Karplus *et al.*, 1986; Lin and Boonyaratpalin, 1988) and the growth suppression (Cohen *et al.*, 1981; Karplus *et al.*, 1986) of smaller males by the dominant males. Hence the early removal of the females (Lin and Boonyaratpalin, 1988) may have caused increased sexually induced aggression between males intent upon sequestering the remaining females for mating and thus overall low male survival.

Percent yield also was biased toward the males (55.4 - 68.3%) in the present trial. Smith *et al.* (1981) reported that the male contribution to the pond biomass production after a single batch harvest was greater than the female contribution in 66% of the treatments (six of nine), whereas Sandifer *et al.* (1982), observed female biomass dominance in one trial and male biomass dominance in a second trial using the same system and batch harvesting. Under the selective harvesting system employed by Lin and Boonyaratpalin (1988) the marketable biomass was 36% male and 59% female, with the remaining 5% as unsexed soft shelled prawns.

In the present trial adult male mean weight was significantly greater than

that of the female, a finding consistent with that of Karplus *et al.* (1986). However male mean weight is very dependent upon the proportion of small males in the population, which in the present trial averaged 22%. Brody *et al.* (1980) and Cohen *et al.* (1981) reported that small males constituted 50% of the total male population, but Sagi *et al.* (1986) recorded only 13% small males in a mixed population. Smith *et al.* (1981) observed that the small males proportion relative to other males was roughly 1:1 (43.7 - 50.8%), and that unlike the adult males, the mean weight of the small males was unaffected by stocking density. In males other than those classed as small, increasing density was associated with decreasing mean weight and decreasing population size skew. The small male proportion in the study by Karplus *et al.* (1986) increased with stocking density from 32.8 - 56.5% as the superior blue-claw male percentage decreased from 20 - 10%. This response in male morphotype proportionality with stocking density was not consistent with the work of Cohen *et al.* (1981) or Cohen and Ra'anan (1983), and was attributed to differences in experimental design including treatment replication and stocking density.

Analysis of the female sex-types in the present study revealed no consistent dominance of either berried or unberried females for any of number, yield or marketable yield. Smith *et al.* (1981) recorded that berried females were consistently heavier than unberried females but that the former constituted less than half of the female population, the frequency increasing from 30.0%, to 40.2%, and 44.2% as the stocking density decreased from 15, 7, 3 m^{-2}. The authors attributed higher density with a smaller size of mature females due to a lower growth rate and earlier maturation. Similarly Mulata *et al.* (1988) observed 45% of the females were berried, but alternatively Lin and Boonyaratpalin (1988) reported that 64% of the females bore eggs.

As a percent of marketable yield based upon a market size of 20.00 g, adult males were most significant contributors at 67 - 100%. Given the

present culture and harvest conditions, adult males were most likely to attain the marketable weight. However, the variation to be expected between studies under different management strategies and market criteria is high as exemplified by Lin and Boonyaratpalin (1988) who, in contrast to the present trial, identified the marketable yield as 36% male and 59% female, with the remaining 5% as sexually unidentifiable.

Prawn production was probably not affected deleteriously by the water quality parameters measured. Water temperature was within the 18-34°C range considered acceptable by New and Singholka (1982), but may have fluctuated over the daily cycle to beyond the ideal range of 29-31°C (New, 1988). The measured values for pH were occasionally outwith the optimal range of 7.0-8.5 (New and Singholka, 1982; New, 1988), but within the chronic critical range of 6.5-9.0 (Sandifer and Smith, 1985). The possibility of dangerously low concentrations of dissolved oxygen was minimised by the introduction of the aerators when dawn DO fell below 3 mg l^{-1}. New and Singholka (1982) identified the ideal DO concentration for *M rosenbergii* as > 75% saturation, which based on the mean dawn temperature equates to roughly 5.80 mg l^{-1}, but over the entire daily cycle would be in the range of approximately 5.40 - 5.85 mg l^{-1}. However, such an open-ended criterion is subject to question as neither the effect of very high DO concentrations nor very wide ranges in concentration have been studied. Avault (1987) reported that the species could tolerate DO as low as 1 mg l^{-1}, but Sharp (in Sandifer and Smith, 1985) suggested oxygen became limiting at 2.9 mg l^{-1} at 28°C or 4.7 mg l^{-1} at 33°C. Malecha (1983) considered that stress was caused at levels below 25-30% saturation, or at the current temperatures about 1.80 - 2.34 mg l^{-1}.

The concentrations of the water quality parameters TN, TP, and CHL were high and could be classified as eutrophic according to the OECD trophic state classification (OECD, 1982). Limnological studies of tropical waters have reported total nitrogen concentrations of 0.6 - 12.3 mg l^{-1} in a

shallow reservoir (Sreenivasan, 1974), 1.03 - 7.56 mg l^{-1} in highly productive temple ponds (Sreenivasan, 1976), and 1.3 - 2.5 mg l^{-1} in a shallow eutrophic lake (Kalff, 1983). Total phosphorus values have been determined as 28 - 79 µg l^{-1} (Kalff, 1983) and 20 - 60 µg l^{-1} in a shallow and heavy silted lake (Fatima and Sharr, 1987). Chlorophyll concentrations have been reported as 9 - 48 µg l^{-1} (Kalff, 1983), 13 - 237 µg l^{-1} in a tropical flood-plain lake (Schmidt, 1973), and 15 - 300 µg l^{-1} in a shallow eutrophic lake (Ganf, 1974). Hence, by comparison the data of the present experiment was similar to literature reports of the tropics and indicated a probable eutrophic status in all ponds.

The extensive literature on nutrient limitation and eutrophication has identified phosphorus and nitrogen as the most usual limiting nutrients to phytoplankton growth (OECD, 1982; Reynolds, 1984). There exists a strong research and publication bias toward temperate conditions and caution with extrapolation to tropical conditions is urged. However, individual phytoplankton species have generally similar nutrient requirements (Moss, 1980; Reynolds, 1984) and the internal nutrient ratios have been identified as 42:7:1 (by weight) as C:N:P (Redfield in Moss, 1980). Hence the mean TN:TP ratios of the present trial, which were of the order of 18-23:1, suggested phosphorus limitation. Various authors have suggested the critical TN:TP ratio to define phosphorus limitation as TN:TP > 17 (Sakamoto, 1966), > 12 (Dillon and Rigler, 1974), and > 10 (Chiadani and Vighi, 1974). Furthermore, Schindler (1977) reported phosphorus proportional phytoplankton development in fertilised lakes with an TN:TP ratio as low as 5:1 due to nitrogen fixation. In tropical African lakes, Kalff (1983) concluded the presence of phosphorus limitation, partially on the basis of Sakamoto's ratio classification. However, threshold criteria based on total nutrients should only be applied, when either phosphorus or nitrogen may be proven as limiting phytoplankton growth on the basis of the levels of the dissolved inorganic nutrients. Furthermore, the high concentrations of TN and TP in the prawn ponds

indicated great capacity to support chlorophyll and the ratios for both CHL:TN and CHL:TP suggested efficient algal production. Nonetheless, partial correlations revealed more significance between TN and CHL than TP and CHL.

Water trophic status is, however, only an indication of the nutrient state at a point in time. The sediments of lakes and ponds are capable of both taking up and releasing nutrients and thereby altering conditions in the water column. Phosphorus is bound or adsorbed onto iron hydroxides and/or onto clay particles, or is bound in apatite or in organic matter (Golterman, 1988). Fixation into the sediment is encouraged by calcium carbonate content and by total colloids but discouraged by high organic colloids (Hepher, 1958a). The release of phosphorus from lakes during anaerobic conditions was documented many years ago (Mortimer, 1941, 1942) and has continued to be the subject of research (Sakata, 1987; Nürnberg, 1988). However, phosphorus may also be released under aerobic conditions (Andersen, 1975; Ryding and Forsberg, 1977; Gardner *et al.*, 1981; Andersen, 1982; Boström, 1984), the rate of release being influenced by many factors. Phosphate liberation may be increased by an increase in temperature (Andersen, 1974; Kamp-Nielsen, 1975; Sinke and Cappenberg, 1988), by a decrease in sediment redox potential (Hallberg in Andersen, 1975; Tessenow in Andersen, 1975), by pH although various factors including the balance between acidity, iron, aluminum and hardness will determine the liberation (Andersen, 1974, 1975; Boström, 1984; Golterman, 1988), by the dissolution of ferro-manganese oxides and hydroxides (Sakata, 1987), by overlying water nitrate concentrations (Andersen, 1982), by wind induced resuspension (Ryding and Fosberg, 1977; Kettunen and Stenmark, 1982), by bioturbation associated with benthic invertebrates primarily due to their excretion (Gardner *et al.*, 1981) or to the transport of interstitial water (Misniewski and Planter, 1985), and, by microbial processes (Sinke and Cappenberg, 1988). In shallow pond ecosystems to which organic nutrients have been added, the most important

factors concerning the dynamics of TP and TN would probably be wind, bioturbation, redox potential and microbial activity.

Sediment TN is found in both organic and inorganic phases, the latter mainly as ammonium and nitrate ions. Under appropriate conditions organic nitrogen undergoes ammonification to ammonia and nitrification to nitrite and nitrate. The rate of nitrification is dependent upon the nature of the sediment organic matter, and the sediment temperature, carbon and oxygen levels (OECD, 1986). The rate of denitrification leading to the production of elemental nitrogen and nitrous oxide depends on the supplies of nitrate, carbon, and ferrous material and on temperature (OECD, 1986). Release of sediment ammonium occurs when the overlying water is either oxic or anoxic (Tanaka and Fukuhara, 1981) and is facilitated by the rapid remineralisation of labile organic materials (Sakata, 1987), and the activity of benthic animals principally via mechanical bioturbation but also by ammonium excretion (Fukuhara and Sakamoto, 1988). Water temperature may influence microbial decomposition of organic matter and the ammonium pool, but appears to have no direct effect on nitrogen release from sediments (Fukuhara and Sakamoto, 1988). Jones (1979b) indicated that sediments in contact with oxygenated water were oxidizing to a depth of 5-10 mm and that nitrate reductase activity was often at a maximum at a depth of 10 - 15 mm. In contrast, under reducing conditions the nitrate reductase activity was greatest at the water-sediment interface. Ryding and Forsberg (1977) found heavy internal loading of nitrogen from lake sediments to the water body, but also a substantial loss of nitrogen to the atmosphere through denitrification.

In fish ponds, Shilo and Rimon (1982) indicated that the ammonium uptake by the sediment in combination with that by the cyanobacteria (and presumably the phytoplankton in general as dependent upon the plankton profile) maintained a steady state low ammonia concentration in the water column thereby minimising the toxicity of ammonia to fish. Furthermore,

van Rijn *et al.* (1984) reported that emptying and drying the pond after harvest aerated the sediment causing nitrification of the ammonium. Upon pond refilling, the nitrate was lost to the atmosphere through denitrification, thus increasing the capacity of the sediment to take up ammonium ion derived from fish excretion, feed, manure and water input sources of nitrogen.

The sediment TP concentrations of the present study were low as compared to some data in the literature. However, published data is strongly biased toward temperate waterbodies which are the subject of concern related to eutrophication. Pettersson and Istvánovics (1988) reported concentrations of 578-781 µg g^{-1} for a freshwater lake, Gunatilaka (1988) reported 900-1300 µg g^{-1} for a reed swamp, Mawson *et al.* (1983) identified the "heavy organic muck" of a freshwater lake as having a mean TP concentration of 1994 µg g^{-1}, and in a review Nürnberg (1988) presented concentrations ranging from 0.5 - 10.3 mg g^{-1}. The sediment TN concentrations of the present study were again low compared to the data in the literature. Pettersson and Istvánovics (1988) reported 2.4 - 3.9 mg g^{-1} in a lake with a strong trophic gradient and Granéli (1979) determined concentrations of 6.5 - 14.8 mg g^{-1} from temperate eutrophic lakes. Present organic carbon values also were low compared to 30 -150 mg g^{-1} in West Bengal fish pond sediments (Mandal and Moitra, 1975), and to total carbon (TC) of 25 ±9.6 mg g^{-1} in oligotrophic Lake Taupo (Viner, 1989), and 38-46 mg g^{-1} in other New Zealand lakes (M'Coull in Viner, 1989).

Concentrations of water TN and TP in the present study showed some fluctuation over time particularly for the treatments FO and DFIM. Such fluctuations suggest that the sediments played temporary roles as both sources and sinks of nutrients. Given that the mobility of phosphate and ammonium ions are affected by water temperature influences on microbial processes and pH changes which may be induced by photosynthesis, directional changes in the flux of nutrients even over short time periods

may be supported. The stability of the nutrient concentrations under the MO regime suggested a most balanced system in which organic matter was rapidly decomposed, and nutrients mineralised and reutilised. The relatively constant level of TN and TP in the MO treatment may have been attributed directly to the relatively small pond input, particularly as OC, and the presumably much reduced extent of bioturbation by the small biomass of prawns. Given organic loading in all treatments as feed, manure and plankton, the lack of an overall and consistent increase in the sediment nutrient concentrations indicated that both the mineralisation of organic matter and the assimilation of inorganic nutrients were efficient processes. Unfortunately, based on Figures 3.3 and 3.4, it is difficult to associate water and sediment nutrient concentration peaks and troughs.

TC:TN ratios of 7.2-18.7 have been reported for Israeli fertilised fish ponds (Hepher, 1965), of 7.4-14.3 for fibreglass pools provided with an earthen substrate and phytoplankton and/or feed for the stocked *M. rosenbergii* (Stahl, 1979) and 12 ± 3 (Viner, 1989). According to Lityński (reported in Trojanowski *et al.*, 1982) the OC:TN ratio of sediment containing organic matter reflects the mineralisation of organic matter. The degradation of organic matter with a ratio of OC:TN < 17 results in rapid mineralisation and the release of inorganic nitrogen. Alternately, a ratio of OC:TN > 33 causes the uptake of inorganic nitrogen from the environment. Values between 17 and 33 indicate mineralisation without the release of ammonia due to the complete nitrogen usage by the bacteria. Similarly, Stahl (1979) claimed that at TC:TN ratios < 30 ammonia will be released due to decomposition, and at TC:TN > 30 microorganisms will extract mineral nitrogen from the sediment to satisfy nutrient demands in order to utilise all the carbon of the organic matter. The ratios OC:TN of the present experiment were < 30 and indeed < 17 and hence suggestive of rapid organic decomposition and mineralisation. Furthermore, Vollenweider (1968) indicated that the TN:TP ratio may be indicative of

denitrification. The present low TN:TP ratios were similar to those reported by Viner (1989) of 3.7 ± 1.0 and suggested denitrification and possibly inadequate supplies of nitrogen.

The density and variety of benthic macroinvertebrates may, in some circumstances, be used as indicators of trophic state. Two interrelated trends accompany an increase in organic enrichment. The first is a reduction from the condition of few individuals of many species to a state of many individuals representative of a small number of species. The second is the disappearance of particular indicator species and their replacement with different species (Hellawell, 1986). Hence, the small number of species represented in the present trial may have indicated that all ponds were organically enriched. However, such a condition is also typical of a temporary or young water body, including fish culture ponds.

The large peaks in benthic macroinvertebrate density in FPM and DFIM on days 88 and 59 respectively, and their greater overall means, may have been associated with greater input of feed and manure relative to the FO and MO treatments. A comparison between the equally manured FPM and MO treatments, suggested that the variable macroinvertebrate density over time and the higher mean density in the FPM treatment may be attributed to the feed input. However the role of feed as a direct nutrient source is supported by the decrease in macroinvertebrate density between days 34 and 59 in MO as suggestive that in the treatment without feed, prawn grazing on the benthic population caused a reduction in the standing density at a time when the densities in the ponds receiving feed were increasing. Hence, the prawns in the MO treatment may have been limited by natural feeds only after day 34, given that before that sampling date the standing density of macroinvertebrates was not less than that in other regimes. The later upturn in the density in the MO treatment may reflect decreased grazing resultant from the much reduced prawn population and the allowed resurgence of the macrobenthos. Indeed the effects of grazing are

impossible to separate from the effects of the treatments, although the consistency of the FO macrobenthos suggested that any grazing was well balanced with recruitment, possibly due to the reliance of the prawns on the pellets.

The presence of specific benthic macroinvertebrates as indicators of trophic status has lead to the development of several macrobenthos classification systems. A high percent of either *Chironomus* spp. or *Limnodrilus hoffmeisteri* is often indicative of a highly organic system (Saether, 1979; Hellawell, 1986). In the present study the percent contribution of each of these groups suggested that the MO treatment was the least organically laden, which was the case for TN and OC but not TP loads. However, the two indicators were not consistent in their classification order of the other three treatments and the value of such indices in the present circumstances must be questioned. Indeed, the Shannon-Weiner diversity index suggested that the cleanest water was to be found in the FO treatment, followed by DFIM, MO, and FPM. Comparison with the mean chlorophyll concentrations indicated that although the *Chironomus* spp. percent was most similar to CHL levels, none of the three commonly used indicators of trophic status were appropriate to the present experimental conditions. This was probably a function of the temporary nature of the ponds, and suggests that such indices are most appropriate for use in permanent water bodies.

Competitive interaction between chironomids and oligochaetes due to their generally similar environmental preferences and requirements (Lellack, 1969), warrant the summation of their contributions to the whole population as an indication of pond conditions. The combined percent of tubificid oligochaetes and chironomids in all treatments increased from 38.80 - 58.78% on day 34 to 81.24 - 94.87% on day 117, thus implying an increasing eutrophication with time. The patterns of increase were very similar in FO and FPM and the mean combined contributions were 73.83% and

75.04% as opposed to 65.36% for DFIM and 52.11% for MO. These figures again indicated that the MO treatment was least organically rich.

However, a better criterion to indicate increasing eutrophication, is a shift from a chironomid dominated to an oligochaete dominated community (Saether, 1979), as the tubificid worms *Limnodrilus* spp. and *Tubifex* spp. are most tolerant of the low DO associated with extremely enriched sediment (Hellawell, 1986). *Clinotanypus* spp. prefer soft sediments of shallow warm water of variable quality (Fittakau and Roback, 1983) and larvae of the genus *Chironomus* either filter feed or graze on detritus and live predominantly in soft standing waters (Pinder and Reiss, 1983). *Chironomus* spp. are often associated with organically enriched waters (Hawkes, 1979), but are less resistant to low DO than tubificids (Hellawell, 1986). *Limnodrilus* spp. can live in accumulated organic deposits and feed on bacteria enriched detritus (Brinkhurst and Cook, 1974), and tolerate conditions of low oxygen for prolonged periods (Hellawell, 1986). However none of the benthic communities of the present trial indicated a consistent shift from chironomids to *Limnodrilus* spp., although the percent *L. hoffmeisteri* did increase over time in each treatment from 0-4% on day 34 to 15-35% on day 117. This increase in the oligochaeta population was at the expense of families other than the chironomidae.

Prawn mean biomass and yield increased with macrobenthos mean density, suggesting a relation between the factors. However, the inability to quantitatively analyse accurately for *M. rosenbergii* stomach contents, as discussed in the introduction, would prevent firm conclusions on the extent of dietary contribution afforded by the benthic macroinvertebrate population to the prawns. The lack in the literature of macrobenthic trophic indices appropriate to tropical waterbodies also prevents definitive classification of the ponds on the basis of their benthic fauna.

However species density data and descriptive accounts of tropical benthic fauna are available. In an Indian fish pond of 1.45 m depth and a bottom sediment of soft dark clay, Ali *et al.* (1978) reported that oligochaetes and chironomids constituted 9% and 91% respectively of the macroinvertebrate population on the pond bottom. Of the seven genera of Chironomidae reported only *Chironomus* spp. was abundant, and of the eleven genera of Oligochaeta, abundance was noted for three species of which one was *L. hoffmeisteri*. Michael (1968) recorded 158 - 1660 macrobenthos m^{-2} in unstocked fish ponds of which oligochaetes dominated the bottom sediment and, Raman *et al.* (1975) reported total densities ranging from 376 - 3389 m^{-2} in stocked fish ponds, in which the dominant groups were oligochaetes and dipteran larvae. In shallow equatorial Lake George, the mid-lake benthic sediments were characterised as soft and unstable and was poor in macroinvertebrate diversity, being dominated by oligochaetes and chironomids (Burgis *et al.*, 1973). Hence the macrobenthos densities of the present trial were not dissimilar to those previously reported for the tropics, and most of the identified species have been previously noted in the descriptive and systematic literature of such areas (Costa, 1967; Brandt, 1974; Ali and Issaque, 1975; Hashimoto *et al.*, 1981).

To summarize, best prawn production and marketable yield were achieved under the regime DFIM indicating that feed levels can be reduced from higher levels provided manure supplementation at 14.08 dry g m^{-2} 14 $days^{-1}$ is applied. The benefit of manure was evident in a comparison of prawn production of the FO treatment with either FPM or DFIM. However, feed was a necessary input as indicated by the poor prawn growth in MO compared with the equally manured FPM.

The isophosphoric nature of the treatments FPM and DFIM did not appear to determine the water phosphorus levels. High variation between replicates prevented any statistical differences in water quality from being

observed. Chlorophyll concentrations appeared to mirror total nitrogen levels, and the Pearson correlations and the regression equations between the two parameters were very strong. Partial correlation analyses revealed that CHL values were strongly correlated to TN but not to TP, suggesting that nitrogen determined algal biomass. Sediment chemistry data fluctuated greatly with time, with the exception of MO for all parameters and FPM for TN and OC. Variations in sediment nutrients, especially TP, suggested temporal shifts from nutrient sink to source. Macrobenthos suggested that all treatments were eutrophic. The mean density of sediment macroinvertebrates was greatest in the DFIM treatment and lowest in the MO treatment mirroring prawn yield trends. However, the effects of prawn grazing on the benthos prevents definitive conclusion on the value of this relationship.

Conversion ratios indicated that the cost of production of marketable prawns under the FO regime was most unattractive. Economically, the DFIM treatment was inexpensive, which, coupled with the high absolute production in that treatment, identified it as the most favourable and recommended management system.

Appendix 3.1. Climatic conditions during the experimental period day 1 - day 126 (20th October 1987 - 22nd February 1988).

Parameter	Mean and standard error	Minimum	Maximum
Relative humidity (%)	97 (1)	88	100
Maximum air temperature (°C)	32.0 (0.2)	26.0	34.3
Minimum air temperature (°C)	22.3 (0.1)	20.4	24.1
Rainfall (mm day^{-1})	7.91 (1.29)	0.00	92.4
Rainfall duration (hr day^{-1})	1.01 (0.16)	0.00	10.50
Sunshine duration (hr day^{-1})	5.37 (0.25)	0.00	10.65
Wind speed (m s^{-1})	0.79 (0.02)	0.22	1.65
Evaporation (mm day^{-1})	3.86 (0.14)	0.15	9.56
Water balance[1] (mm day^{-1})	+4.04 (1.25)	-5.67	+88.52

[1] water balance (mm day^{-1}) = rainfall (mm day^{-1}) - evaporation (mm day^{-1})

Appendix 3.2. Selected climatic conditions during each time period between water sampling days; arithmetic mean and standard error.

Time period	Sunshine duration (hr day^{-1})	Water balance[1] (mm day^{-1})
Day 3 - 16	4.20 (0.57)	+7.22 (3.21)
Day 17 - 28	3.95 (0.78)	+6.36 (4.75)
Day 29 - 43	6.37 (0.56)	-2.85 (0.71)
Day 44 - 55	2.23 (0.71)	+5.34 (3.19)
Day 56 - 69	6.05 (0.68)	+4.15 (3.34)
Day 84 - 97	5.61 (0.60)	+8.23 (7.04)
Day 98 - 112	4.04 (0.75)	+6.19 (0.78)

[1] water balance (mm day^{-1}) = rainfall (mm day^{-1}) - evaporation (mm day^{-1})

Appendix 3.3. Cumulative TN[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FPM	FO	DFIM	MO
Day 1 - 12	0.05	0.05	0.03	-
Day 1 - 24	0.38	0.09	0.35	0.29
Day 1 - 37	0.71	0.14	0.67	0.57
Day 1 - 49	1.71	0.85	1.47	0.89
Day 1 - 63	3.85	1.73	3.21	1.14
Day 1 - 77	5.37	2.53	4.42	1.43
Day 1 - 91	7.58	3.94	6.19	1.71
Day 1 - 105	10.47	5.61	8.21	2.00
Day 1 - 125	14.20	8.04	10.96	2.28

[1] based on percent TN values of 4.67 and 2.48 for feed and manure respectively.

Appendix 3.4. Cumulative TP[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FPM	FO	DFIM	MO
Day 1 - 12	0.005	0.005	0.003	-
Day 1 - 24	0.23	0.01	0.24	0.22
Day 1 - 37	0.46	0.02	0.47	0.45
Day 1 - 49	0.77	0.10	0.78	0.67
Day 1 - 63	1.20	0.19	1.21	0.90
Day 1 - 77	1.56	0.28	1.57	1.12
Day 1 - 91	2.00	0.44	2.01	1.35
Day 1 - 105	2.51	0.63	2.50	1.57
Day 1 - 125	3.12	0.90	3.11	1.79

[1] based on percent TP values of 0.52 and 1.95 for feed and manure respectively.

Appendix 3.5. Cumulative OC[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FPM	FO	DFIM	MO
Day 1 - 12	0.29	0.29	0.19	-
Day 1 - 24	2.82	0.58	2.66	2.23
Day 1 - 37	5.37	0.90	5.13	4.47
Day 1 - 49	12.22	5.52	10.77	6.70
Day 1 - 63	26.43	11.17	22.51	8.94
Day 1 - 77	36.65	16.33	30.77	11.17
Day 1 - 91	51.36	25.46	42.69	13.41
Day 1 - 105	70.41	36.29	56.32	15.64
Day 1 - 125	94.92	52.02	74.67	17.88

[1] based on percent OC values of 30.20 and 19.43 for feed and manure respectively.

CHAPTER FOUR

EXPERIMENT TWO;
THE EFFECT OF MANURE APPLICATION FREQUENCY
ON PRODUCTIVITY IN POND TRIALS

4.1 INTRODUCTION

The overall benefits of pond fertilisation in finfish culture have been well documented (Chapter 1). Reported research has focused on phytoplanktivorous species such as silver carp and the filter-feeding tilapias, omnivorous species such as common carp, and extensive polycultures in which a balanced array of fish species optimise usage of the autotrophic and heterotrophic chains of natural productivity. In a review on manure usage in fish farming, Wohlfarth and Schroeder (1979) reported application frequencies ranging from daily to yearly. However, it is generally recommended that fertilisers are applied on a frequent basis (Little and Muir, 1987; Wohlfarth and Schroeder, 1979), either daily (FAO, 1983; Zhu *et al.*, 1991), biweekly (Hepher, 1975), weekly (Horvath *et al.*, 1984), or fortnightly (Bard *et al.*, 1976). The discussion concerning optimal methodology of application prevails, primarily due to the many variables of culture including species selection and existing management and environmental conditions (Colman and Edwards, 1987).

The beneficial utilisation of organic fertilisers in pond culture of the freshwater prawn has been reported (Buck *et al.*, 1981; Wohlfarth *et al.*, 1985) and further was determined in Experiment 1. However, studies concerning the optimal application frequency of manures to such cultures are lacking. The present experiment was designed to evaluate the effect of manuring frequency on prawn production and pond dynamics in a semi-intensive grow-out trial. Three frequencies were employed within the range typical of fish culture as noted above, and manure was applied every 3.5, 7, and 14 days.

4.2 MATERIALS AND METHODS

4.2.1 Pond Preparation

Six ponds of 440 m² were cleared of plants and dried until deep cracks had developed. However the condition of the ponds in the region of the cement

sluices appeared poor so the ponds were immediately filled and tested for water retention. The percent water loss over several periods of twenty four hours ranged from 4.08% to 14.70% of standing volume. Pond repairs were therefore conducted. Following draining, polyvinyl chloride (pvc) plastic sheeting (EPC Nylex) of 0.25 mm thickness was draped over the cement sluice. The pvc extended to roughly 1 m on either side of the sluice, 1 m inward from the standpipes and up to the top of the cement wall thus above the water line. The edges of the plastic were secured about 15 cm below the pond bottom and across the top of the sluice with bricks. Additional clay was added to weigh down the pvc on the pond bottom. Ensuring that ponds were plant-free and well dried, agricultural lime $CaMg(CO_3)_2$, and seasoned chicken manure were added at 1159 kg ha^{-1} and 250 kg ha^{-1} respectively. Unfortunately, quick lime as used in the Experiment 1 was unavailable, but the equivalent liming rate was achieved based upon calculations of neutralising values and efficiency ratings according to the method outlined by Boyd (1982). The remainder of the procedure for pond preparation was similar to the previous experiment. Three days were then allowed in order to test the water retention of the ponds, and the percent water loss in twenty four hours determined as 1.60% to 4.68% of standing volume. The repairs were considered successful, and the experiment begun.

4.2.2 Stocking, Management and Harvesting

Post-larval prawns of the same age as stocked in Experiment 1 were unavailable from either the hatchery on site or nearby commercial hatcheries, due in the main to the delay caused by the necessary pond repairs. Primarily because of financial restrictions, it was decided not to wait for another batch of PL. Juvenile *M. rosenbergii* were obtained from the Lion's Group commercial farm sited nearby at Puchong. According to the farm Management, the stock were 78-day-old (from hatching) and had been transferred from the hatchery to nursery cages after thirty days. In the cages the prawns had been fed the same UPM 30% protein diet (Table

3.1) at 15% body weight day^{-1}. Arithmetic mean and standard error at stocking were 0.55 ± 0.04 g (3.32 ± 0.04 cm orbital length). Two weeks later silver carp, *H. molitrix* (Val.), of 1.67 ± 0.09 g (5.30 ± 0.09 cm standard length) were stocked at 0.1 m^{-2}. Grass carp, *C. idella* (Val.), were also stocked at this stage although over several days as the commercial supply suffered a 58% mortality following transportation to UPM. Immediate replacement of the dead fish was possible because of excess initial ordering, and the final stock in each pond consisted of two individuals of 1.6 kg and 1.2 kg

Treatments tested the effect of fertilising frequency on pond productivity and prawn production. As identified from Experiment 1, the DFIM regime with manure fertilisation of 14.08 dry g m^{-2} 14 days^{-1} produced greatest total and marketable prawn yields and thus was chosen to serve as the treatment control in Experiment 2. The schedule of absolute manure and percent feed application employed in the DFIM treatment of the previous experiment (Table 3.2) was again employed, but with a forward adjustment of 26 days to account for the difference in age (78-52 days) of the prawn stock. The same percent feeding and absolute manuring rates were applied to two other treatments which employed fertilisation every 7 and 3.5 days. Treatments were referred to as M14 (manure every 14 days at 13.51 kg 440 m^2), M7 (manure every 7 days at 6.75 kg 440 m^2) and M3.5 (manure every 3.5 days at 3.38 kg 440 m^2), and were conducted in duplicate. The same 30% protein diet formulation (Table 3.1) was applied twice daily at 0700 and 1900 hours. The initial pellet size of 1 mm was applied until day 20, after which 1.5 mm was used. Feeding requirement was based on prawn stocking biomass until day 11 inclusive and on an assumed average prawn weight of 1 g for the subsequent two weeks in parallel to Experiment 1. Commencing on day 23, the absolute feed requirement was calculated based on the mean weight of prawns, feeding rate as a percent of body weight, and an assumed mortality. Prawn weight was determined from sampling 50 prawns per pond according to the schedule established in Experiment 1 (day

23, 37, 51, 65, and 79). Pond standing biomass was calculated assuming the same mortality rate as previously described (day 12, 24, 38, 66). Seasoned chicken manure was applied as required at 0800 hours. Periodic analyses of the manure for *Salmonella* spp. consistently proved negative. Water exchange was maintained at 5% day^{-1} by pumping water into the ponds through mesh (250 µm) covered inlet pipes. Decreasing dawn dissolved oxygen levels (to 2.70 mg l^{-1} in M14 instigated the nightly operation of a paddlewheel aerator in each pond from day 90 to harvest.

Harvesting took place 100 days after stocking the prawns. The harvest procedure followed that employed in the previous experiment. Annual prawn marketable yield was calculated given a possible 3.2 batch cycles per year under prevailing conditions. Due to the two sizes of grass carp stocked into each pond, weight gain was calculated based on the difference between stocked and harvested weights. Results for silver carp were reported as mean weight for each treatment.

4.2.3 Water Chemistry

Water sampling and measurements were conducted from a small paddle boat every two weeks (days 3, 16, 28, 44, 58, 72 and 86) timed to occur immediately prior to the application of manure in M3.5 and M7. The sampling was achieved between 0745 and 0915 hours. Samples for DO were collected and analysed as in Experiment 1. Measurements *in situ* included temperature, secchi disk transparency, conductivity, and dissolved solids. Transparency was determined using a standard 20 cm diameter secchi disk of black and white quadrants. Readings at the disappearance and reappearance of the disk were averaged to give a mean transparency depth. Conductivity (COND) and dissolved solids (DS) were also measured in the field using a Hach Portable Conductivity/DS meter suspended at 20 cm below the water surface.

An integrated sample was obtained from each pond as previously described

and taken to the laboratory for analysis. Samples from the water supply at the dam were taken on days 3, 44, and 86. Laboratory determinations of DO, pH, CHL, TN, and TP were analysed as described in Experiment 1. Further analyses included alkalinity, chemical oxygen demand, ammonia, nitrite, nitrate, dissolved reactive phosphorus, total solids, suspended solids, and turbidity. As in Experiment 1, and where required by the procedural analyses, glass fibre filter papers and the double beam spectrophotometer with 1 cm cells (Shimadzu UV-210A) were employed.

Total alkalinity (ALK) was determined by the titrimetric method using hydrochloric acid and BDH 4.5 indicator solution (Stirling, 1985).

Chemical oxygen demand (COD) was determined using the heat of dilution procedure (Boyd, 1979) based on potassium dichromate and sulphuric acid. Correction to the standard method (APHA, 1985) was achieved using the equation (Boyd, 1979);

$Y = 3.02 + 1.505\ X$

where Y = standard COD (mg l^{-1})

X = heat of dilution COD (mg l^{-1})

Total solids (TS) were determined in pre-ignited pre-weighed crucibles by evaporating to dryness 50.00 ml of water sample at 105°C. Suspended solids (SS) were measured by filtering 500 ml samples through pre-weighed glass fibre filter papers and drying to constant weight at 105°C (APHA,1985).

Total ammonia ($NH_4+NH_4^+$) was determined by the phenol-hypochlorite method (Adamski, 1976) and spectrophotometric measurement at 635 nm. The unionised ammonia fraction (NH_4) was calculated based on the pH and temperature of the water at the time of sampling (Stirling, 1985).

Nitrite (NO_2) was measured at 540 nm following reaction with sulphanilamide and then N-(1-naphthyl)-ethylenediamine dihydrochloride

(Strickland and Parsons, 1972).

Nitrate (NO_3) was reduced to nitrite by cadmium-copper coupling and measured as above (Golterman *et al.*, 1978; Strickland and Parsons, 1972).

Dissolved reactive phosphorus (DRP) was analysed spectrophotometrically at 882 nm by the molybdate reaction technique (Eisenrich *et al.*,1975).

Turbidity was measured using a computerised Hach spectrophotometer (DR/2000) standardised with formazan.

4.2.4 Biochemical Oxygen Demand ($BOD_{0.5}$)

The $BOD_{0.5}$ was assessed as the change in DO concentration in a sample of water incubated for twelve hours in the dark. In each pond, two samples of water from 70 cm were sampled using 300 ml BOD bottles. One sample was immediately fixed (as for DO in Experiment 1) and the second was wrapped in aluminium foil and incubated overnight in the pond by suspending the sample at 70 cm from a float. Initial samples were taken a 1900 hours, and the incubated sample recovered and fixed the next morning at 0700 hours. Both samples were analysed using the Winkler titration modified with sodium azide, and the change in DO concentration over twelve hours calculated. The procedure was followed on three occasions (days 39-40, 52-53, and 80-81).

4.2.5 Diel Cycle

The daily fluctuations of DO, pH and temperature were analysed on two occasions (days 41-42 and 54-55). All measurements were made in the field. DO was analysed using a YSI oxygen meter, pH with a Schott-Gerate CG819 pH meter, and temperature with a mercury thermometer. All readings were made at 20 cm and 70 cm following a three minute stabilisation period, at 0700, 1300, 1900, and 0700 hours.

4.2.6 Primary Productivity

Estimates of primary productivity were made on three occasions (days 40, 53, and 81) by incubation of two light and dark BOD bottles at each depth of 20 cm and 70 cm for exactly three hours between 1100 and 1500 hours. The change in DO concentration per unit volume was measured employing the dark and light bottle technique (Vollenweider, 1969). The initial sample and each incubated sample were fixed in the field and quickly transferred to the laboratory for analysis by the modified Winkler method.

Calculations of primary productivity in g carbon m^{-3} day^{-1} were made as suggested by Westlake (1969) and Cole (1983). The dark and light bottle data for each incubation period was extrapolated to daily gross (GPP) and net (NPP) primary production on the basis of a twelve hour day and a respiratory quotient of 1.2. Thus, g of C fixed m^{-3} day^{-1} = mg O_2 released l^{-1} $hour^{-1}$ x 12 x 12/(32 x 1.2).

In order to assess the ratio of production:respiration, gross productivity values as oxygen produced per twelve hours were compared to respiration values as oxygen consumed per twenty-four hours.

4.2.7 Sediment Chemistry

Samples of sediment were collected on days 7, 31, 65 and 89, and analysed for TN, TP and OC as in Experiment 1.

4.2.8 Prawn Stomach Contents

Despite the previously mentioned reservations on stomach content analysis, and due to the possible usefulness of quantifying the data on the basis of percent composition by weight (Bowen, 1985) (Chapter 1), an assessment of the stomach contents of a small number of prawns was attempted. However, preliminary tests on non-experimental prawns were not

sufficiently successful to confidently judge the method reliable and indicative of the prawn diet. Thus the stomach analysis on the harvested prawns was not conducted.

The method employed in the preliminary tests involved catching twenty prawns of approximately 5 - 15 g with a cast net from a nearby pond which was receiving both feed and manure inputs. Pelleted feed was applied to a small area of the pond twenty minutes prior to netting in order to attempt to lure prawns and thus increase the catch efficiency. Indeed, pellet was evident in the oesophagus of some of the prawns. All of the netted animals were immediately killed and transferred to the laboratory. The cardiac stomach was gently excised from the thorax, split open, and flushed with distilled/deionized water. The contents were then examined under both dissecting and binocular microscopes.

Upon dissection, many of the stomachs were found to be empty, yet conversely, some stomachs were so full that their intact removal was not possible. Under the microscope, items such as sand granules, phytoplankton, and assorted invertebrate appendages were evident. So too was tissue most likely to have been some of the stomach wall. It was evident that the definite identification of the stomach contents would have been difficult given the degree of mastication and digestion, and any attempt at quantification misleading. The full reasoning for not employing the procedure involved these practical difficulties, together with the questionable theoretical validity of stomach contents in a species which is a benthic omnivore ingesting material including silica grains, soft-bodied oligochaetes and chitinous prawns, and practising regurgitation.

4.2.9 Sediment Macroinvertebrates

The analysis of benthic macroinvertebrates was cancelled due to the unsatisfactory results of the preliminary tests on the prawn stomachs, and the limited interpretation possible on sediment macroinvertebrate data in

the absence of gut analysis.

4.2.10 Input:Output Conversions, Climatic and Statistical Analyses

Conversion, climatic and statistical analyses were conducted as described for Experiment 1, and climatic data is presented in Appendices 4.1 and 4.2. The diel measurements were plotted and primary productivity and respiration data tabulated as arithmetic means. The data for each of sediment macroinvertebrate population and nutritional and climatic analyses are presented as mean values.

4.3 RESULTS

4.3.1 Pond Inputs

Pond inputs over the experiment are presented in Table 4.1. Feed inputs were very similar because the mean weights of prawns at each sampling also were similar. Manure loads were identical between all treatments as defined by the experimental protocol.

4.3.2 Prawn Sampling Weights

Prawn weights at each sampling period were very similar between treatments (Table 4.2). Only on days 23 and 79 were any statistical differences noted, wherein M7 and M14 were superior respectively. However, mean weight was consistently heaviest for M14 from sampling day 65 onwards and including overall harvest. Figure 4.1 illustrates weight increase over time.

4.3.3 Prawn Growth Rate and Harvest Data

The mean growth rate, Table 4.3, for the prawns of M14 was higher, but not significantly, compared with the other two treatments. Similarly, all harvest characteristics with the exception of percent survival were highest, but not significantly so, in M14. The values for percent survival were very similar between treatments.

Table 4.1. Load of prawn feed and chicken manure over the experimental cycle; absolute load (dry g m^{-2} $cycle^{-1}$) and percent of total input (%).

Component	M3.5	M7	M14
Feed	110.20 (56.61)	110.90 (56.76)	114.26 (57.49)
Manure	84.48 (43.39)	84.48 (43.24)	84.48 (42.51)

Table 4.2. Prawn weight (g) at sampling; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	M3.5	M7	M14
0	0.55^{a} (0.04)	0.55^{a} (0.04)	0.55^{a} (0.04)
23	2.16^{a} (0.15)	2.68^{b} (0.13)	2.15^{a} (0.15)
37	5.90^{a} (0.23)	5.33^{a} (0.16)	5.75^{a} (0.20)
51	7.93^{a} (0.29)	7.44^{a} (0.23)	7.51^{a} (0.31)
65	8.28^{a} (0.26)	8.67^{a} (0.25)	9.25^{a} (0.35)
79	12.02^{a} (0.36)	11.06^{a} (0.20)	13.73^{b} (0.40)
100[1]	15.13^{a} (0.56)	14.94^{a} (0.29)	17.32^{a} (1.64)

[1] = as calculated from harvest data; total biomass/total number.

Figure 4.1
Prawn mean weight (g) at sampling.

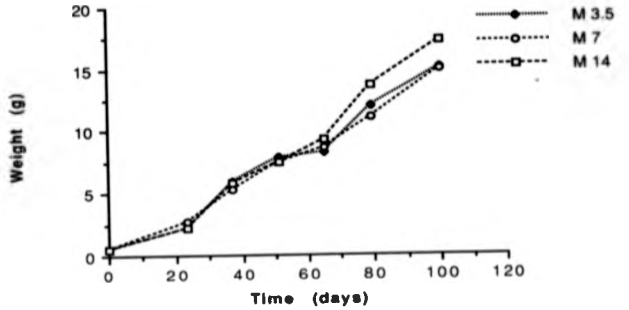

20
15
10
5
0
Weight (g)
0
20
40
60
80
100
120
Time (days)
M 3.5
M 7
M 14

Table 4.3. Prawn growth rate and harvest data; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Parameter	M3.5	M7	M14
Growth rate ($g\ day^{-1}$)	0.15^a (0.01)	0.15^a (0.01)	0.17^a (0.02)
Weight (g)	15.13^a (0.56)	14.94^a (0.29)	17.32^a (1.64)
Biomass ($kg\ pond^{-1}\ cycle^{-1}$)	50.32^a (0.45)	52.36^a (0.59)	55.85^a (0.75)
Yield ($kg\ ha^{-1}\ cycle^{-1}$)	1143.70^a (10.29)	1189.93^a (135.04)	1268.84^a (17.41)
Percent survival (%)	75.69^a (2.08)	79.50^a (7.50)	73.89^a (6.00)
Percent marketable (%)	29.03^a (6.55)	22.57^a (0.78)	43.35^a (14.73)
Marketable yield ($kg\ ha^{-1}\ year^{-1}$)	1064.42^a (249.09)	862.78^a (127.23)	1768.14^a (622.03)

4.3.4 Prawn Sex-Type Data

Adult males were heaviest and small males lightest in all treatments (Table 4.4). Berried females were consistently heavier than unberried females. Each sex-type of M14 was heavier than it's counterpart of the other two treatments, significantly so for berried females and adult males.

As a percent, the balance of sex-types was quite similar between treatments, with the exception of more berried and fewer unberried females in M14. The same trend is evident for the percent of yield. Adult males made up a substantial percent of the number, the yield, and the marketable yield in each treatment. Small males contributed little to the yield and nothing to the marketable yield in all treatments. As a whole, males made up a slightly larger proportion of the percent yield, and a much larger proportion of the percent of marketable yield, than females as a whole in all treatments. There were no significant differences for percent of number or percent of yield. An inverse relationship seemed apparent between the contribution of adult males to the percent of marketable yield (Table 4.4) and the overall marketable yield (Table 4.3). The contributions of each sex-type in each treatment to the harvest parameters are graphically presented in Figure 4.2.

4.3.5 Fish Survival and Production

All grass carp survived and both the growth rate and weight gain increased with decreasing frequency of manure application (Table 4.5). Survival of silver carp ranged from 70-100% but indicated no evident trends between treatments for survival. Due to high variation within treatments there were no significant differences for any of the fish production parameters.

4.3.6 Water Quality Concentrations

Water quality data summarised over the entire sampling period are presented in Table 4.6. For all treatments, early morning mean temperature

Table 4.4. Prawn harvest data for each sex-type; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference ($F<0.05$) and superscripts in uncommon rows are unrelated).

Parameter	M3.5	M7	M14
Weight (g)			
Type 1[1]	15.15^a (0.25)	14.48^a (0.22)	18.67^b (0.39)
Type 2	14.21^a (0.34)	14.05^a (0.29)	15.35^a (0.46)
Type 3	3.64^a (0.12)	3.75^a (0.11)	3.78^a (0.12)
Type 4	22.67^a (0.63)	21.01^a (0.43)	26.89^b (0.68)
Percent of number (%)			
Type 1	16.75^a (5.64)	18.24^a (7.54)	24.70^a (0.33)
Type 2	33.02^a (6.69)	33.06^a (8.77)	27.92^a (1.52)
Type 3	13.07^a (1.13)	10.18^a (1.12)	11.30^a (0.06)
Type 4	37.16^a (2.18)	38.52^a (2.35)	36.08^a (1.24)
Percent of yield (%)			
Type 1	16.61^a (4.04)	18.01^a (6.67)	24.44^a (1.22)
Type 2	31.22^a (5.90)	30.41^a (8.53)	23.23^a (0.73)
Type 3	3.49^a (0.31)	3.00^a (0.52)	2.84^a (0.47)
Type 4	48.68^a (2.16)	48.58^a (2.37)	49.49^a (0.03)
Percent of marketable yield (%)			
Type 1	3.59 (2.33)	0.00 (0.00)	14.76 (11.27)
Type 2	7.30 (5.86)	2.91 (0.81)	8.17 (0.00)
Type 4	89.11 (8.19)	97.09 (0.81)	77.07 (19.45)

[1] Where types 1, 2, 3, and 4 refer to berried females, unberried females, small males and adult males respectively.
na = not applicable

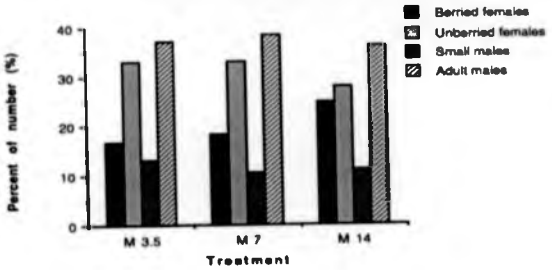
Figure 4.2

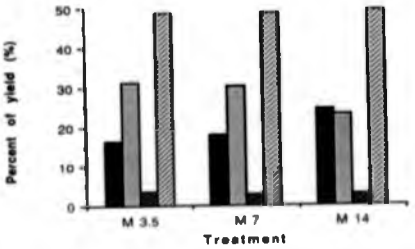
Prawn sex-type as a percent of;

a) harvest number,

b) yield, and

c) marketable yield.


Berried females
Unberried females
Small males
Adult males
Percent of number (%)
40
30
20
10
0
M 3.5
M 7
M 14
Treatment


Percent of yield (%)
50
40
30
20
10
0
M 3.5
M 7
M 14
Treatment

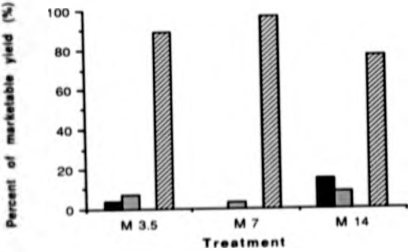

Percent of marketable yield (%)
100
80
60
40
20
0
M 3.5
M 7
M 14
Treatment

Table 4.5. Fish growth rate and harvest data; arithmetic mean and standard error.

Fish species	M3.5	M7	M14
Grass carp			
Growth rate (g day^{-1})	11.8 (2.7)	14.0 (1.1)	15.1 (3.5)
Weight gain (g)	1017.5 (227.7)	1200.0 (98.2)	1298.3 (304.1)
Survival (%)	100.00	100.00	100.00
Silver carp			
Growth rate (g day^{-1})	2.24 (0.27)	3.02 (0.00)	2.62 (1.06)
Weight (g)	193.92 (22.88)	260.90 (0.10)	227.00 (91.40)
Survival (%)	85.23 (12.50)	98.87 (1.14)	82.96 (12.50)
Yield (kg ha^{-1} $cycle^{-1}$)	7.15 (0.21)	11.35 (0.14)	8.79 (4.59)

Table 4.6. Water quality parameters; arithmetic mean and standard error except pH which is presented as the range of values measured.

Parameter	M3.5	M7	M14
Temperature (°C)	29.0 (0.2)	29.1 (0.2)	28.9 (0.2)
Dissolved oxygen (mg l^{-1})	4.67 (0.35)	5.26 (0.53)	4.45 (0.45)
pH	6.69 - 8.77	6.73 - 8.65	6.70 - 9.16
Conductivity (µS cm^{-1})	108.6 (6.4)	106.6 (5.2)	117.0 (7.1)
Total alkalinity (mequival l^{-1})	1.0 (0.1)	1.0 (0.1)	1.1 (0.1)
Total nitrogen (mg l^{-1})	1.982 (0.372)	2.128 (0.244)	2.791 (0.441)
Total ammonia (µg l^{-1})	<7.8 (na)	<23.0 (na)	<32.1 (na)
Unionized ammonia (µg l^{-1})	<7.8 (na)	<8.4 (na)	<12.4 (na)
Nitrite (µg l^{-1})	<2.0 (na)	<2.0 (na)	<2.0 (na)
Nitrate (µg l^{-1})	3.0 (0.7)	2.3 (0.6)	4.0 (0.8)
Total phosphorus (µg l^{-1})	75.0 (11.5)	100.4 (15.2)	135.2 (15.3)
Dissolved reactive phosphorus (µg l^{-1})	11.1 (1.5)	8.9 (1.0)	10.9 (1.1)
Total solids (mg l^{-1})	130.6 (13.4)	116.0 (8.5)	136.5 (13.9)
Suspended solids (mg l^{-1})	32.1 (8.9)	26.3 (6.0)	32.2 (7.8)
Dissolved solids (mg l^{-1})	56.6 (3.0)	56.1 (2.0)	59.8 (2.2)
Chemical oxygen demand (mg l^{-1})	36.9 (5.1)	36.7 (2.0)	52.4 (7.9)
Chlorophyll (µg l^{-1})	91.7 (28.6)	97.7 (19.0)	137.4 (29.6)
Secchi depth (cm)	49.0 (7.0)	42.1 (5.1)	37.1 (5.7)
Turbidity (FTU)	44 (18)	31 (8)	62 (21)

na = not available

was approximately 29°C and DO 4.5 - 5.3 mg l^{-1}, and pH was measured over the range 6.69 - 9.16. Mean DO was higher in M7 rather than with either less or more frequent applications. The parameters TN, TP, and CHL increased on average with decreasing frequency of manure application. The values of TN, TP, and CHL were quite high at 1.9 - 2.8 mg l^{-1}, 75 - 135 µg l^{-1} and 92 - 137 µg l^{-1} respectively. The parameters of cond, NO_3, TS, SS, DS, COD and turbidity were lowest in M7 and highest in M14, being intermediate in M3.5. Secchi depth was inversely related to CHL, but not related to solids or turbidity.

The levels of NH_3+NH_4^+ were frequently below detectable levels, and for that reason it was not possible to calculate accurate means. However, as a rough guide, if samples below the detectable limit are considered to have concentrations equal to the detectable limit (7.8 µg l^{-1}), values representing the maximum possible means may be presented. By this approach, a small increase in concentration was evident with decreasing frequency of manure (Table 4.6). Levels of nitrite were consistently below the detectable limit, and neither NO, nor NH_3 were deleterious to the prawns (Armstrong *et al.*, 1976, 1978).

Analyses of variance for the major nutrients TN, NO_3, TP, and DRP, and for CHL, indicated that there were no significant differences between treatments at any sampling time. Figure 4.3 indicates the concentrations of these nutrients and CHL in order to visualize any possible relationships. For all regimes trends in the CHL concentration seemed most similar to those of TN, and to a lesser extent TP. A possible inverse relation existed with DRP.

The parameters measured in the early morning at the water supply dam had the following values for arithmetic mean and standard error; temperature 29.2 (0.8)°C, TN 0.538 (0.004) mg l^{-1}, NH_3+NH_4^+ <7.8 (na) µg l^{-1}, NO_3 <2.0 (na) µg l^{-1}, NO_2 517.2 (259.8) µg l^{-1}, TP 47.5 (1.7) µg l^{-1}, DRP 7.8 (0.4)

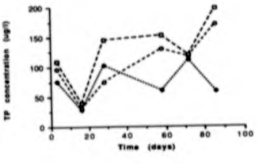
Figure 4.3

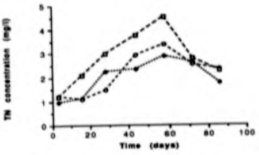
Water concentrations of;

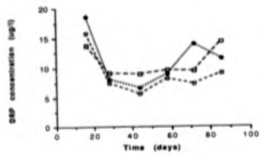
a) total phosphorus (TP), b) total nitrogen (TN),

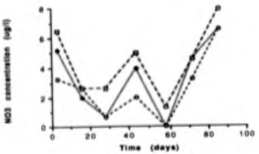
c) dissolved reactive phosphorus (DRP) d) nitrate (NO_3), and

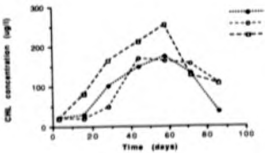
e) chlorophyll (CHL).


TP concentration (ug/l)
Time (days)


TN concentration (mg/l)
Time (days)


DRP concentration (ug/l)
Time (days)


NO3 concentration (ug/l)
Time (days)


CHL concentration (ug/l)
Time (days)
M3.5
M7
M14

$\mu g\ l^{-1}$, COD 14.9 (4.5) $mg\ l^{-1}$, and CHL 7.0 (1.8) $\mu g\ l^{-1}$. The range of pH was 6.75 - 6.98.

4.3.7 Water Quality Ratios, Correlations and Regressions

Table 4.7 presents the ratios between the same five parameters as above. An increasing mean ratio was evident with decreasing frequency of manure application for each of the ratios CHL:TN, CHL:NO_3, CHL:TP, and CHL:DRP. Although this trend was very consistent, attention should be paid to the relatively high standard errors. On average for all treatments, the ratios were approximately 0.04, 39.0, 0.8, and 14.0 for CHL:TN, CHL:NO_3, CHL:TP, and CHL:DRP respectively. The ratio TN:TP was high at about 21.0, but no obvious trend related to manure frequency was suggested.

Pearson correlation coefficients (Table 4.8) indicated for each treatment that the highest correlation of any nutrient with CHL was that of TN ($F<0.01$). TP was also well correlated with CHL, at 0.05 in M3.5 and M14, and at 0.01 in M7. However, TP and TN were themselves highly correlated ($F<0.05$ for each treatment). DRP was also correlated ($F<0.01$) with CHL in M7, but unlike the other treatments, it too was correlated with TP and TN (both $F<0.05$). CHL and COD were highly correlated ($F<0.01$) in all treatments. Partial correlation coefficients between TN and CHL were significant ($F<0.01$) in all treatments, whereas between TP and CHL there apparent correlations were lost.

A series of regression equations were generated for CHL determination from the various major nutrients (Table 4.9). TN was the best single predictor of CHL, and high values for R^2 adjusted were achieved for each treatment (0.79, 0.86, and 0.91 for M3.5, M7 and M14 respectively). The addition of TP to the equation made very slight alterations to the fit of the data (0.77, 0.83, and 0.92). Singly, TP predicted CHL with a poorer fit (0.32, 0.44, and 0.32) but the procedure of generating the equation was still valid ($F<0.05$ in all cases). The available nutrients, NO_3 and DRP, were

Table 4.7. Ratios between the water parameters total nitrogen (TN), total phosphorus (TP), nitrate (NO_3), dissolved reactive phosphorus (DRP), and chlorophyll (CHL); arithmetic mean and standard error.

Ratio	M3.5	M7	M14
CHL:TN	0.037 (0.005)	0.040 (0.005)	0.043 (0.005)
CHL:TP	0.74 (0.16)	0.81 (0.16)	0.86 (0.18)
CHL:NO_3	35.16 (14.88)	36.75 (12.65)	44.46 (12.77)
CHL:DRP	10.09 (2.99)	15.43 (3.37)	15.67 (2.86)
TN:TP	22.11 (3.70)	22.34 (3.01)	19.79 (2.85)

Table 4.8. Pearson correlation coefficients between the water parameters total nitrogen (TN), nitrate (NO_3), total phosphorus (TP), dissolved reactive phosphorus (DRP), chemical oxygen demand (COD), secchi disc transparency (SCH) and chlorophyll (CHL), and partial correlation coefficients between TN and CHL controlling TP and between TP and CHL controlling TN.

Treatment	Pearson correlation coefficient						Partial correlation coefficient		
M3.5		TN	NO_3	TP	DRP	COD	SCH		TN	TP
	NO_3	-0.360								
	TP	+0.609[b]	-0.158							
	DRP	+0.272	-0.004	+0.251						
	COD	+0.846[a]	-0.168	+0.633[b]	+0.163					
	SCH	-0.906[a]	+0.155	-0.505	+0.170	-0.881[a]				
	CHL	+0.899[a]	-0.212	+0.631[b]	+0.203	+0.920[a]	-0.922[a]	CHL	+0.835[a]	+0.242
M7	NO_3	-0.153								
	TP	+0.662[b]	+0.049							
	DRP	-0.653[b]	+0.090	-0.693[b]						
	COD	+0.795[a]	-0.128	+0.534[b]	-0.408					
	SCH	-0.841[a]	+0.268	-0.838[a]	+0.640[b]	-0.896[a]				
	CHL	+0.933[a]	-0.120	+0.700[a]	-0.720[a]	+0.814[a]	-0.888[a]	CHL	+0.858[a]	+0.309
M14	NO_3	+0.047								
	TP	+0.564[b]	+0.256							
	DRP	+0.314	+0.312	+0.375						
	COD	+0.939[a]	+0.253	+0.467	+0.301					
	SCH	-0.934[a]	-0.212	-0.819[b]	-0.394	-0.933[a]				
	CHL	+0.957[a]	-0.002	+0.624[b]	+0.185	+0.854[a]	-0.933[a]	CHL	+0.947[a]	+0.366

[1] = a and b refer to F levels of probability of 0.01 and 0.05 respectively.

Table 4.9. Regression equations between the log of the water parameters total nitrogen (TN), nitrate (NO_3), total phosphorus (TP), dissolved reactive phosphorus (DRP), secchi disk (SCH), and chlorophyll (CHL).

Treatment	Regression equation	R^2	F
M3.5	CHL = + 1.43 + 1.43 TN	+0.79	0.000
	CHL = - 0.19 + 0.98 TP	+0.32	0.050
	CHL = + 1.07 + 1.28 TN + 0.21 TP	+0.77	0.003
	CHL = + 1.88 - 0.30 NO_3	-0.04	0.487
	CHL = + 1.38 + 0.41 DRP	-0.05	0.526
	CHL = + 1.36 - 0.14 NO_3 + 0.51 DRP	-0.16	0.749
	CHL = + 4.93 - 1.98 SCH	+0.83	0.000
M7	CHL = + 1.25 + 2.03 TN	+0.86	0.000
	CHL = - 0.47 + 1.15 TP	+0.44	0.016
	CHL = + 0.81 + 1.80 TN + 0.26 TP	+0.83	0.000
	CHL = + 1.91 - 0.18 NO_3	-0.08	0.697
	CHL = + 3.52 - 1.72 DRP	+0.47	0.008
	CHL = + 3.60 - 0.02 NO_3 - 1.83 DRP	+0.44	0.039
	CHL = + 5.81 - 2.51 SCH	+0.76	0.001
M14	CHL = + 1.34 + 1.66 TN	+0.91	0.000
	CHL = - 1.18 + 1.47 TP	+0.32	0.040
	CHL = + 0.75 + 1.63 TN + 0.28 TP	+0.92	0.000
	CHL = + 2.07 - 0.01 NO_3	-0.11	0.995
	CHL = + 1.71 + 0.37 DRP	-0.06	0.565
	CHL = + 1.69 + 0.22 NO_3 + 0.33 DRP	-0.19	0.754
	CHL = + 5.27 - 2.26 SCH	+0.86	0.000

R^2 = R^2 adjusted
F = F statistic of probability

generally very poor predictors of CHL. However in M7, the DRP fraction both singly and in combination with NO_3, resulted in acceptable regression equations ($F<0.05$ in both cases).

4.3.8 Biochemical Oxygen Demand

$BOD_{5.5}$ values, presented in Table 4.10, suggested increasing oxygen demand with decreasing frequency of manure application during two of three determinations and on average. On the nights studied, $BOD_{5.5}$ ranged from 1.6 - 2.4 mg O_2 l^{-1} 12-hour-day^{-1}.

4.3.9 Diel data

The diel data are presented in Figures 4.4 and 4.5. As expected DO increased from 0700 hours to a peak usually at 1900 hours but occasionally at 1300. The daily range in DO was greatest in surface as opposed to bottom waters. The parameter pH also showed more diel variation in the surface waters where it always peaked at 1900 hours.

Dawn DO values in both surface and bottom waters were lowest M3.5. The parameters pH and temperature showed little variation between treatments, except that the peak to a temperature maximum was slightly more acute in M3.5 than in the other regimes.

4.3.10 Primary Productivity

Primary productivity data is presented in Table 4.11. With only one exception, values for both GPP and NPP bottom data were consistently at least half those of surface data. GPP values ranged from 0.22-7.25 and NPP values from 0.00-3.69 g C m^{-3} 12-hour-day. On average, net productivity increased with increasing frequency of manuring. On three occasions, no net carbon was fixed at the pond bottom, possibly due to shading. Certainly, CHL levels were quite high and near their peak for two (day 53 in M7 and M14) of those values. Data for both GPP and NPP on sampling day 81 in M14 were unusual. Surface GPP was less than bottom GPP both because

Table 4.10. Biochemical oxygen demand (mg O_2 l^{-1} 12-hour-day^{-1}) as measured using incubated bottles suspended at 70 cm water depth; arithmetic mean and standard error.

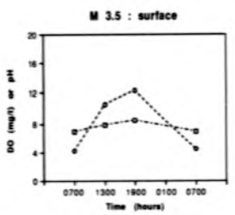
Sampling day	M3.5	M7	M14
Day 39-40	1.8 (0.1)	2.4 (0.2)	2.0 (0.5)
Day 52-53	2.0 (0.6)	2.0 (0.4)	2.2 (0.3)
Day 80-81	1.6 (0.3)	1.6 (0.8)	1.9 (1.1)
Mean and standard error	1.8 (0.2)	2.0 (0.3)	2.0 (0.3)

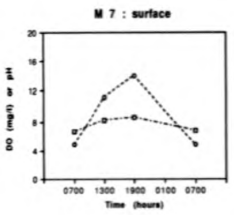
Figure 4.4

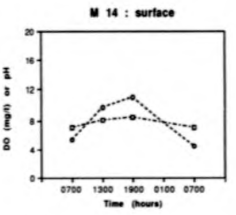
Diel measurements of dissolved oxygen (DO), pH and temperature on days 41-42; surface and bottom refer to sampling depths of 20 cm and 70 cm respectively.

a) M3.5 : surface b) M7 : surface c) M14 : surface

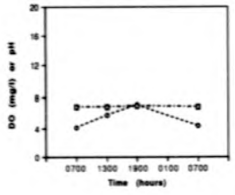
d) M3.5 : bottom e) M7 : bottom f) M14 : bottom

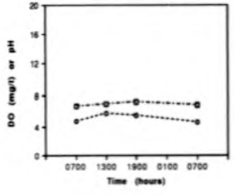

M 3.5 : surface
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)

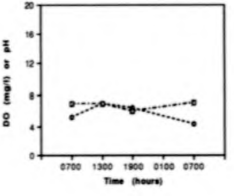

M 7 : surface
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)


M 14 : surface
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)

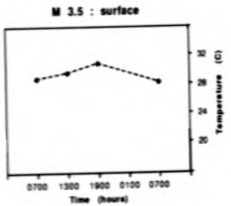
126

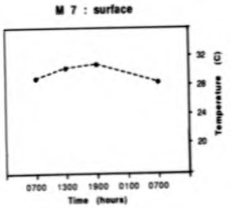

M 3.5 : bottom
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)

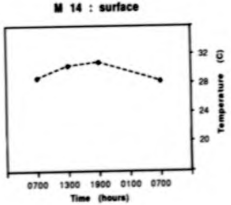

M 7 : bottom
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)


M 14 : bottom
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)

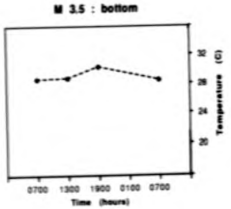

DO
pH

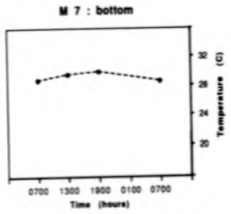

M 3.5 : surface
32
28
24
20
Temperature (C)
0700 1300 1900 0100 0700
Time (hours)


M 7 : surface
32
28
24
20
Temperature (C)
0700 1300 1900 0100 0700
Time (hours)


M 14 : surface
32
28
24
20
Temperature (C)
0700 1300 1900 0100 0700
Time (hours)

1264


M 3.5 : bottom
32
28
24
20
Temperature (C)
0700 1300 1900 0100 0700
Time (hours)


M 7 : bottom
32
28
24
20
Temperature (C)
0700 1300 1900 0100 0700
Time (hours)

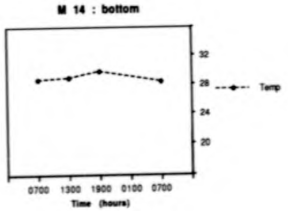
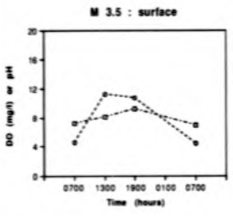

M 14 : bottom
32
28
24
20
Temp
0700 1300 1900 0100 0700
Time (hours)

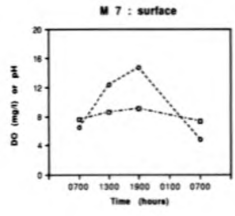
Figure 4.5

Diel measurements of dissolved oxygen (DO), pH and temperature on days 54-55; surface and bottom refer to sampling depths of 20 cm and 70 cm respectively.

a) M3.5 : surface b) M7 : surface c) M14 : surface

d) M3.5 : bottom e) M7 : bottom f) M14 : bottom


M 3.5 : surface
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)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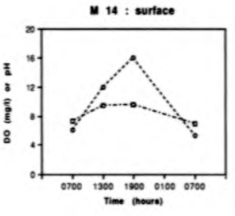
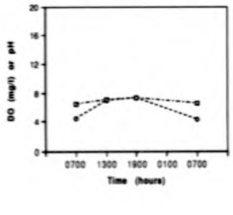
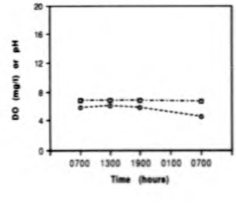
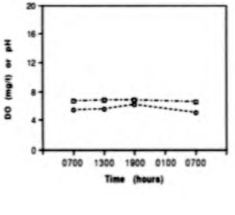
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)


M 14 : surface
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)


M 3.5 : bottom
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)


M 7 : bottom
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)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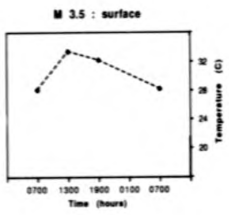
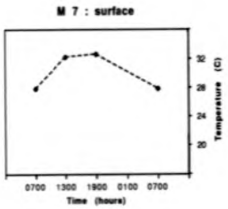
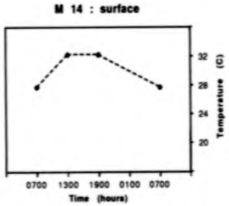
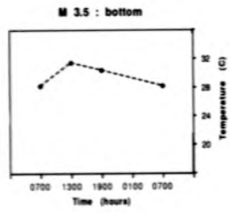
DO (mg/l) or pH
20
16
12
8
4
0
0700 1300 1900 0100 0700
Time (hours)

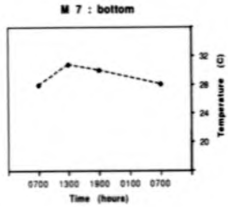

DO
pH


M 3.5 : surface
Temperature (C)
32
28
24
20
0700 1300 1900 0100 0700
Time (hours)


M 7 : surface
Temperature (C)
32
28
24
20
0700 1300 1900 0100 0700
Time (hours)


M 14 : surface
Temperature (C)
32
28
24
20
0700 1300 1900 0100 0700
Time (hours)


M 3.5 : bottom
Temperature (C)
32
28
24
20
0700 1300 1900 0100 0700
Time (hours)


M 7 : bottom
Temperature (C)
32
28
24
20
0700 1300 1900 0100 0700
Time (hours)

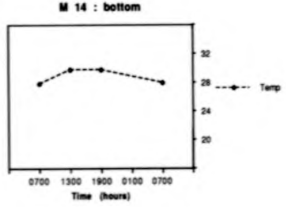

M 14 : bottom
Temperature (C)
32
28
24
20
0700 1300 1900 0100 0700
Time (hours)
Temp

Table 4.11. Carbon fixed by gross (GPP) and net (NPP) primary production (gC m^{-2} 12-hour-day^{-1}), as measured using incubated bottles suspended at the surface (20 cm) and bottom (70 cm) of the pond.

Sampling day		M3.5		M7		M14	
		Surface	Bottom	Surface	Bottom	Surface	Bottom
Day 40	GPP	4.52	2.19	3.68	1.77	3.31	1.58
	NPP	3.54	0.93	2.44	0.65	1.07	0.28
Day 53	GPP	3.95	1.76	6.40	2.29	7.25	1.88
	NPP	2.54	0.91	3.48	0.00	4.32	0.00
Day 81	GPP	2.46	1.11	5.30	1.00	1.39	2.19
	NPP	1.77	0.50	3.69	0.14	0.27	0.00
Mean and standard error	GPP	2.67 (0.45)		3.41 (0.64)		2.93 (0.63)	
	NPP	1.70 (0.39)		1.65 (0.47)		0.99 (0.55)	

surface NPP was very low and bottom respiration very high. The low value for NPP at the surface may have been due to initial and incubation conditions in one of the two pond replicates; specifically, the initial bottle DO was 15.64 mg l^{-1} and as such 220% saturation and higher than in any other pond at any other time; furthermore, pond temperatures ranged from 33.7°C to 33.5°C during the incubation period, figures also greater than at any other occasion, and probably still an underestimate of the water temperature within the light bottle. The high respiration at the pond bottom was also unusual, as typically surface respiration was greater than bottom. Possibly a decline in phytoplankton after day 56 encouraged high pond bottom respiration.

Calculations relating GPP to respiration (Table 4.12), supported the finding that respiration was high relative to primary productivity on Day 81 in the M14. In general, the GPP:R ratio increased with decreasing frequency of manuring. Values ranged from 0.54:1 to 1.50:1.

4.3.11 Sediment Quality Concentrations and Ratios

The mean values for each of the sediment chemistry parameters were similar between treatments (Table 4.13). Analyses of variance revealed only two cases of statistical difference, both of which occurred on Day 31 and concerned M7. The TN concentration of this treatment was significantly smaller than both the other treatments and the OC concentration was significantly less than in M14. The variation in nutrient concentrations over the experimental period are graphically presented in Figure 4.6. TN values ranged from 1.11 mg g^{-1} to 3.13 mg g^{-1} (0.11-0.31%), and illustrated a general decrease with time in each treatment but with most fluctuations in M7. TP concentrations ranged from 0.35 mg g^{-1} to 0.75 mg g^{-1} (0.04-0.08%). There was an initial downward trend in both M7 and M14, and all treatments showed an increase on the final sampling day so that final concentrations were very similar to initial concentrations. Initial values for organic carbon were low (although not statistically different) in M3.5

Table 4.12. The ratio between gross primary production ($gO_2\ m^{-2}$ 12-hour-day^{-1}) and respiration ($gO_2\ m^{-2}$ 24-hour-day^{-1}) on a whole pond basis (arithmetic mean of surface and bottom samples).

Sampling day	M3.5	M7	M14
Day 40	1.50:1	1.16:1	0.69:1
Day 53	1.26:1	0.76:1	0.95:1
Day 81	1.37:1	1.27:1	0.54:1
Mean and standard error	1.38 (0.07) : 1	1.06 (0.16) : 1	0.73 (0.12) : 1

Table 4.13. Sediment chemistry parameters; arithmetic mean and standard error.

Parameter	M3.5	M7	M14
Total nitrogen (mg g^{-1})	2.46 (0.14)	1.93 (0.33)	2.33 (0.26)
Total phosphorus (mg g^{-1})	0.58 (0.03)	0.48 (0.06)	0.61 (0.06)
Organic carbon (mg g^{-1})	11.35 (0.92)	13.91 (2.17)	14.64 (1.27)

Table 4.14. Ratios between the sediment parameters total nitrogen (TN), total phosphorus (TP), and organic carbon (OC); arithmetic mean and standard error.

Ratio	M3.5	M7	M14
TN:TP	4.41 (0.34)	4.10 (0.45)	3.69 (0.12)
OC:TN	4.82 (0.58)	7.64 (0.89)	6.49 (0.25)
OC:TP	19.96 (1.77)	31.63 (6.11)	23.79 (0.68)

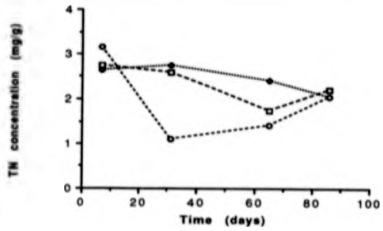
Figure 4.6

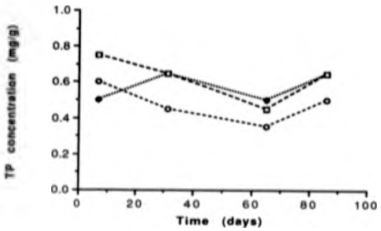
Sediment concentrations of;

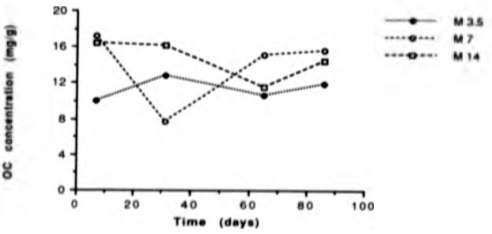
a) total nitrogen (TN),

b) total phosphorus (TP), and

c) organic carbon (OC).


TN concentration (mg/g)
0
1
2
3
4
0
20
40
60
80
100
Time (days)


TP concentration (mg/g)
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Time (days)


OC concentration (mg/g)
0
4
8
12
16
20
0
20
40
60
80
100
Time (days)
M 3.5
M 7
M 14

compared to the other treatments. In all regimes the range in concentrations were 7.65 mg g^{-1} - 17.2 mg g^{-1} (0.77-1.72%). Ponds receiving M14 were most variable over time for sediment OC values, but again each treatment attained a final value similar to the initial concentration. For each sediment parameter considered, the concentrations in the treatment receiving most frequent additions of manure were most stable over time. The mean ratios between sediment TN, TP, and OC are presented in Table 4.14. Values for TN:TP and for OC:TN were very low.

4.3.12 Input:Output Conversions

The conversion analysis, summarised in Table 4.15, indicated very good conversion ratios overall, and optimal cost for marketable prawns under the treatment receiving manure application every 14 days.

4.4 DISCUSSION

Prawn mean growth rate, weight, yield, percent marketable, and marketable yield were superior, on average, in M14 ponds. The lack of statistical significance for fish and prawn production is not atypical of pond studies due to frequent high variation between replicates (Buck *et al.*, 1970; Malecha, 1989).

In a single publication concerning the effect of manure application frequency on *M. rosenbergii*, Wohlfarth *et al.* (1985) concluded that prawn production was unaffected by manuring, as daily or weekly, or by feeding. However prawn stocking density was very low, 0.5 - 1.5 m^{-2}, and fish, in a four species polyculture stocked at 7450-11950 ha^{-1} (644-700 kg ha^{-1}), would have contributed considerable faeces to the pond benthos and thus masked possible effects of allochthonous fertiliser application frequency on prawn production.

Table 4.15. Input:output conversion ratios, based on dry weight of feed and manure inputs and on wet weight of outputs as prawns alone or in combination with fish, and cost (£) to produce one kilogram of total and marketable prawns (M.Prawn).

Conversion Ratio	M3.5	M7	M14
Feed:Prawn	0.96:1	0.93:1	0.90:1
Manure:Prawn	0.74:1	0.71:1	0.67:1
Input:Prawn	1.70:1	1.64:1	1.57:1
Input:Output	1.66:1	1.60:1	1.52:1
Cost:Prawn	0.32:1	0.31:1	0.30:1
Cost:M.Prawn	1.10:1	1.37:1	0.69:1

In a polyculture system of Chinese carps, Zhu *et al.* (1990) reported that the net yield of silver carp significantly increased when the frequency of manure application was increased, while bighead carp showed little response. Silver carp net yield increased with manure application every 30, 10, 7 and 5 days as 0.7, 0.8 4.5 and 5.2 kg ha^{-1} day^{-1}. In a second experiment employing four carp species and more frequent manure applications, the authors observed that increasing the frequency from 7 to 5 days had no consistent effect on total fish yield. However with daily manuring, the net yield for each of silver carp and common carp indicated a significant statistical increase compared with manuring every 5 and 7 days. Bighead carp, a zooplanktivore, and crucian carp, a benthic detritivore, did not respond to manure application frequency, and grass carp were not included in the polyculture system. The silver carp results of Zhu *et al.* (1990) do not support the present findings for that species. This difference may have resulted from differences between the experimental designs, particularly as stocking density which was 4.5 m^{-2} in the research of Zhu *et al.* (1990) and 0.1 m^{-2} in the present work, and the absolute load of manure added which was 13.7-47.6 dry kg pig manure ha^{-1} day^{-1} in the experiments reported by Zhu *et al.* (1990) and 8.5 dry kg chicken manure ha^{-1} day^{-1} in the present research.

The balance of prawn sex-types indicated that the population was approximately equally distributed between males and females, a ratio also observed by Cohen *et al.* (1981). These results reflected the use of management practices which did not encourage aggression between individuals, most importantly as moderate stocking density, the availability of sufficient feed, a relatively short grow-out period, and batch harvest. Total yield was slightly male dominated, and marketable yield strongly biased toward the males as in Experiment 1.

Unberried females consistently outnumbered berried females, similar to the findings of Smith *et al.* (1971) and Hulata *et al.* (1988), and showed an

increasing trend with decreasing manuring frequency. This trend could not be explained by a density factor as proposed by Smith *et al.* (1971) given, not only the equal initial stocking densities, but also the lack of a trend between manuring frequency and survival. Due to characterisation of the species into three distinct adult males morphotypes displaying three particular mating habits and capacities (Kuris *et al.*, 1987), it is possible that the trend in berried females may have reflected the proportions of adult male sex-types had the experiment considered further morphometric characterisation. As a unit, the adult male was the most numerable single sex-type (36.1-38.5%), which contributed nearly half of the yield (48.6-49.5%) and most of the marketable yield (77.1-97.1%).

As in the previous experiment, prawn production probably was not affected by unsuitable temperatures. The concentration of DO was sufficient until the end of the experiment, after which DO was maintained above critical levels by artificial aeration. A single high pH value (>9.0 in M14) during fortnightly morning sampling, and maximal pH values (>9.0 in M3.5, M7 and M14) during the diel sampling on day 54-55, were above recommended levels for long-term exposure (Sandifer and Smith, 1985). The effect on prawns of brief exposure to high pH is not documented, although chronic exposure is reported to cause the formation of calcium carbonate on gill surfaces (Smith and Sandifer in Sandifer and Smith, 1985). Alkalinity values as measured fortnightly were acceptable as *M. rosenbergii* is susceptible to total alkalinity >180 mg l^{-1}, or 3.60 mequival l^{-1} (Sandifer and Smith, 1985). The present levels of both ammonia and nitrite were below levels deemed deleterious to the freshwater prawn. Armstrong *et al.* (1978) reported that the toxicity of ammonia to *M. rosenbergii* is greatly influenced by water pH, but that both NH_3 and NH_4^+ are toxic. The proportions of the two forms found to be toxic were related to pH with toxicity determined by NH_3 at high pH and by NH_4^+ at low pH. At pH 7.60, Armstrong *et al.* (1978) indicated reduced growth in sublethal concentrations of 32 mg l^{-1} total ammonia, and LC_{50} values of 115 mg l^{-1}

total ammonia at 24-hours and of 44 mg l^{-1} at 144-hours. At this moderate pH level, only about 2% of the total ammonia would be in the form NH_3 and hence the ammonium ion probably would have been responsible for most of the toxicity. The authors predicted safe levels of NH_3+NH_4^+ as 1.0 mg l^{-1} at pH 8.34 and 3.2 mg l^{-1} at pH 6.83. *N.rosenbergii* is less sensitive to nitrite than are fish, possibly due to differences in respiratory pigments, and reduced growth occurred at 1.8 mg l^{-1} and LC_{50} at 130 for 24-hours and 4.5 mg l^{-1} for 192-hours (Armstrong *et al.*, 1976).

The present study indicated that despite nearly equal additions of nutrients to each treatment, water concentrations of TN, TP, and CHL, and, ratios of CHL:TN and CHL:TP, increased with decreasing manuring frequency. The slightly greater feed inputs which were associated with decreasing manuring frequency (Table 4.1) as calculated from prawn biomass estimates, provided insufficient TN or TP to directly account for the increases in water nutrient levels. Table 4.16 compares input load and water concentration values for each of phosphorus and nitrogen, utilising M3.5 as the baseline. The results indicated that the excess (relative to the baseline) TP loaded into M7 and M14 was respectively 7 times and 3 times less than the excess water TP concentration. Similarly, the excess TN loaded into M7 and M14 were, in both cases, 4 times less than the excess TN concentrations. Thus, the slightly different feed inputs were not responsible for the different water nutrient concentrations. Therefore, the differences noted for water quality parameters between treatments must have been associated with the frequency of manure application.

The increase in concentrations of CHL with decreasing manuring frequency indicated increased autotrophic biomass, while efficient algal production was suggested by the increased ratios of CHL:TN and CHL:TP. The relationship of increasing ratios for CHL:TP with increasing TP was similar, with the work of Sakamoto (1966) and Dillon and Rigler (1974).

Table 4.16. A comparison of the feed input and associated nutrient load[a] and the water concentration values[b] for total phosphorus (TP) and total nitrogen (TN), utilising M3.5 as the baseline.

Determinant	M3.5	M7	M14
Absolute Feed ($g\ m^{-2}\ cycle^{-1}$)	110.20	110.90	114.26
Excess Feed ($mg\ l^{-1}$)[c]	0.00	0.70	4.06
Excess TP Load ($\mu g\ l^{-1}$)	0.00	3.64	21.1
Excess TP Water ($\mu g\ l^{-1}$)	0.00	25.39	60.15
TP Factor	na	6.98	2.85
Excess TN Load ($mg\ l^{-1}$)	0.00	0.033	0.190
Excess TN Water ($mg\ l^{-1}$)	0.00	0.146	0.809
TN Factor	na	4.42	4.26

[a] assuming the TP and TN concentrations were 0.52% and 4.67% (Experiment 1)

[b] using mean values (Table 4.6)

[c] assuming water depth of 1m

na = not applicable

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[a] assuming the TP and TN concentrations were 0.52% and 4.67% (Experiment 1)

[b] using mean values (Table 4.6)

[c] assuming water depth of 1m

na = not applicable

Increasing mean $BOD_{0.5}$, decreasing mean NPP and decreasing mean GPP:R ratios with decreasing application frequency suggested higher oxygen requirement and utilisation, due to either algal respiration, and/or stimulated organic decomposition and bacterial respiration. Linearly increasing CHL mean concentrations with decreasing manuring frequency suggested that algal respiration was responsible for the trends in $BOD_{0.5}$, NPP and GPP:R. Furthermore the time plot of CHL concentration, which indicated that treatment peaks occurred at day 58 in M3.5 and M14, and on day 44 in M7, may be related to the oxygen demand. Particularly, peaks in the $BOD_{0.5}$ values occurred on days 52-53 in M3.5 and M14, and on day 39-40 in M7. Hence, given the limitations of the sampling timetable, high $BOD_{0.5}$ values seemed to immediately precede or accompany high CHL concentrations. Boyd (1974) reported that highest COD and BOD (5 days at 20°C) coincided with highest CHL concentrations, thus verifying that phytoplankton constituted the major oxygen demand in catfish ponds supplied with pelleted fomula feed and occasional inorganic fertiliser applications. In the present experiment the high correlation coefficients between CHL and COD also suggested that oxygen consumption was strongly oriented toward the algae.

However, the slight increasing trend in $NH_3+NH_4^+$ with decreasing manure application frequency may have indicated greater mineralization of organic matter by bacteria, implying greater bacterial respiration. Furthermore, the absolute levels of all inorganic compounds, and particularly the nitrogenous inorganic compounds, were very low, indicating tight control over phytoplankton growth and efficient turnover of organic matter. Hence, bacterial respiration must have contributed considerably to pond oxygen dynamics, and the generally low mean GPP:R, reflected high CHL levels and presumed high bacterial levels. A further factor in support of the argument for a considerable bacterial respiratory component may be found by comparing the time plot for CHL concentration (Figure 4.3) and the data for GPP:R ratios (Table 4.12). Near equal CHL levels in M3.5 and M7 days

on both of the primary productivity sampling days 40 and 53 indicated that the very different GPP:R ratios between these treatments on each of the two occasions probably may be attributed to non-algal respiration. Hence, again given the confines of the sampling schedule, heterotrophic respiration was greater with weekly manuring than with more frequent application. The same comparison may be made for M7 and M14 on sampling day 81. The CHL values are not as similar to each other as in the previous case, yet the vastly different GPP:R ratios indicated that there was greater bacterial respiration in M14.

The decrease in GPP from day 53 to day 81 in most instances (excepting only the bottom sample in M14) reflected the decrease in CHL levels in all treatments (Figure 3.3), both as oxygen production and consumption. Assuming a decline in chlorophyll concentration reflects a decline in algal density in the water column and an increase in the detritus on the pond bottom, conditions at that time favoured an increase in the heterotrophic community and high benthic respiratory demand. Therefore it might have been expected that the values for BOD_{5} on day 80-81 were higher than recorded, however these samples were taken at 70 cm depth and not at the water-sediment interface. Measurement of true sediment respiration requires the use of an *in situ* respiratory dome situated on the benthos (Costa-Pierce, 1984; Garza in Madenjian, 1990).

Values for both GPP and NPP bottom data were at least half of those of surface data, with a single exception. This relationship was undoubtedly a function of the light absorbing components of the aquatic system: the water itself, dissolved yellow pigments, particulate matter, and photosynthetic biota (Kirk, 1986). Pure water absorbs very weakly in the blue and green regions of the spectrum but more strongly above 550 nm, whereas gilvin, the yellow coloured humic acids formed during the decomposition of plant matter, has a high absorption coefficient at lower wavelengths. Particulate matter scatters light readily and thus presents

special problems to the measurement of its absorption capacity, although highly turbid waters containing, for instance, suspended sediment will result in considerable particle induced light absorption. Lastly, the absorption of light by the photosynthetic pigments contributes to the attenuation of photosynthetically available radiation with depth (Kirk, 1986). In the present experiment the three occurrences of no net carbon fixed possibly indicated reduced bottom photosynthesis associated primarily with algal shading given that the CHL levels were quite high and even at their peak for two of the values (M3.5 and M14 on sampling day 58). The presence of little inorganic material in the water column was suggested by the quite deep secchi readings given the CHL values.

The present values for gross productivity, particularly in the surface waters, were moderately high. Tropical and subtropical waters receiving organic manures tend to have the highest GPP values (Colman and Edwards, 1987). The gross primary productivity of an Amazonian flood-plain lake had an average of 1.1 gC m^{-2} day^{-1} (Schmidt, 1973), but the highly productive Philippine Lake Lanao had an average GPP of 2.6 gC m^{-2} day^{-1} (Lewis, 1974) and the sewage fed Indian Ooty Lakes an average GPP of 3.16 gC m^{-2} day^{-1} (Sreenivasan, 1980). Gross productivities of 6.0-11.0 gC m^{-2} day^{-1} (Sreenivasan, 1964) and 0.06-9.14 gC m^{-2} day^{-1} (Sreenivasan, 1976) were reported in Southern Indian temple ponds used for bathing and washing clothes. In Indian hypereutrophic waters used for fish culture, Sreenivasan (1980) reported mean GPP values of 5.26 gC m^{-2} day^{-1} for a moat receiving town sewage, and 13.51 gC m^{-2} day-1 for a sewage stabilisation pond. Under Malaysian conditions, GPP values for a shallow lake were 0.08-1.31 gC m^{-2} day^{-1} (Fatimah *et al.*, 1984), for unenriched waters 0.3-1.6 gC m^{-2} day^{-1} with a mean of 0.8 gC m^{-2} day^{-1} and for manure enriched fish ponds 1.3-10.2 gC m^{-2} day^{-1} with a mean of 5.1 gC m^{-2} day^{-1} (Richardson and Lim, 1975), and for chemically fertilised fish ponds 0.72-12.52 gC m^{-2} day^{-1} with a mean of 3.87 gC m^{-2} day^{-1} (Prowse, 1972).

Certain values of the GPP and NPP data sampled from the bottom of the ponds did not suggest sufficient oxygen production to justify the mean dawn DO concentrations. However, higher oxygen production in the surface waters likely provided oxygen to the pond bottom during nightly water cooling and sinking. The diel data indicated an average daily change in temperature of the surface waters of 3°C on day 41-42 and 4°C on day 54-55. The common temperature peak at 1900 on day 41-42 yet frequent peak at 1300 on day 54-55 was most likely to be explained by climatic conditions. In general cloud and rain occurred in the afternoon, and specifically days 41 and 54 gauged 1.45 and 7.75 hours of sunshine respectively explaining the different temperature maxima on the two diel sampling days. The duration of sunshine, rather than any internal pond dynamics, will also have been the most important factor in the difference in both DO and pH values between the two sampling dates, and hence to compare different days would be inappropriate.

On each diel sampling occasion, the patterns of oxygen and pH increase were quite typical of eutrophic water bodies. Particularly in the surface waters, DO and pH increased during sunlight hours due to the process of photosynthesis which produced oxygen and removed carbon dioxide thus shifting the pH up. The same, albeit smaller, trends in the bottom waters also indicated photosynthesis.

Zhu *et al.* (1990) concluded that increasing manure (fermented pig) application from every five or seven days to daily, resulted in a higher rate of organic decomposition and mineralisation due to increased bacterial activity. More frequent application of manure from every 5 days to daily, increased BOD by 47%, COD by 15%, NH_4^+ by 60%, and the rate of bacterial decomposition by 43% (derived from Zhu *et al.*, 1990). Although the authors concluded that daily applications resulted in higher fish yield (as compared to applications every 5 or 7 days) through stimulation and nutritional contribution of the heterotrophic chain, the trends in

many (BOD, COD, NH_4^+, turbidity, bacterial decomposition) of the water parameters which supported this conclusion had stabilised or even reverted as manure application was altered from 5 to 7 days. Indeed, as application frequency decreased from every 5 days to every 7 days, COD increased by 5%, NH_4^+ by 21%, and bacterial decomposition by 10% (derived from Zhu *et al.*, 1990). Translated to daily alterations, COD increased by 3.75% when manure was applied every day as opposed to every 5 days and by 2.5% when manure was applied every 7 days as opposed to every 5 days. Similarly, NH_4^+ increased daily by 15% and 10% and bacterial decomposition by 10.75% and 5% for each of manure 5 days to daily and manure 5 to 7 days respectively. Hence it is evident that the heterotrophic chain was more active when manuring both every day and every 7 days as opposed to every 5 days. Thus a shift in the emphasis of the trophic pathways occurred between days 1 and 7, and although the daily rate of change may not have been linear over the period, the calculation above for the daily increments in each of COD, NH_4^+ and bacterial decomposition, suggests that the shift may have occurred near a manuring schedule of every 3-4 days.

A temporal shift from autotrophy to heterotrophy has been described over the grow-out cycle in shrimp aquaculture ponds (Madenjian, 1990). This shift, identified on the basis of changes in pond net production as estimated from DO concentrations measured every ten minutes, was attributed to the constant addition of organic matter as feed. The present experiment suggested that trophic reactions to the addition of manure were dynamic and experienced a temporary stabilisation or recovery phase. Detailed work by Madenjian (1990) did not report short term trophic changes possibly as a function of the consistent daily loading of nutrients as feed, no manure having been applied.

In the present experiment, the Pearson correlation coefficients suggested that CHL values in each treatment were most strongly related to water TN. The partial correlation coefficients and the regression equations

indicated that CHL values in each treatment were indeed most strongly related to water TN, and only due to an intercorrelation between TN and TP, were CHL and TP related. The strength of all three indices increased with decreasing frequency of manure application. As defined previously the mean TN:TP ratios of 20-22 suggested phosphorus limitation. However, employing the OECD (1982) criteria as defined for temperate lakes, that inorganic-N:ortho-P ratios of greater than 15 indicate non-nitrogen limitation, the present three treatments would be classified as nitrogen limited. Furthermore, based on critical concentrations of 5 $\mu g\ l^{-1}$ for available phosphorus and 80 $\mu g\ l^{-1}$ for available nitrogen (pers. comm. Dr.C.S.Reynolds, Freshwater Biological Association, Ambleside), present levels of inorganic P were not likely to have been limiting while those of inorganic N were possibly limiting. Thus, ratios of available N : available P, which are more powerful indicators of phytoplankton growth control than the total nutrient ratios and concentrations, support nitrogen limitation. This is in keeping with the strong CHL to TN partial correlations and regressions. As identified by the OECD report (1982), there is a tendency for waters to shift from phosphorus to nitrogen dependency with increasing trophic status and according to their own criteria, the waters have been shown to be eutrophic. The low levels of available N and P illustrated that most of the nutrients were tied up in organic matter and that the processes of decomposition and nutrient reutilisation were efficient.

The present low levels of the inorganic nutrients may be compared to data from geographically proximal Malaysian reservoirs. Ho (1976) recorded values for NH_4 of 0.07 - 0.39 mg l^{-1}, NO_2 <0.05 mg l^{-1}, NO_3 2.15 - 3.11 mg l^{-1} and DRP 0.03- 0.15 mg l^{-1}, and Arumugam and Furtado (1980) reported greater maximum concentrations of inorganic nutrients as NH_4 0 - 0.9 mg l^{-1}, NO_2 0.5 - 7.2 mg l^{-1}, NO_3 0-0.3 mg l^{-1}, and PO_4 0- 0.8 mg l^{-1}. Similarly in simulated pond studies on the use of feeds and fertilisers in prawn studies, Stahl (1979) reported NH_4^+ 0 - 1.58 mg l^{-1}, NO_3 0 - 1.86 mg l^{-1},

and DRP 0 - 0.55 mg l^{-1}.

The possibility that the phytoplankton were light limited in the present experiment requires consideration. The approximate incident solar radiation in Malaysia is 156,000 gcal cm^{-2} $year^{-1}$ (Prowse, 1972), or 818 µEinst m^{-2} s^{-1} as based upon the conversion ratio given by Bannister (1974) that 1 Einst m^{-2} 12-hours^{-1} of visible (400 - 700 nm) solar light corresponds to 0.0084 gcal cm^{-2} min^{-1} of total solar energy. Harris (1980) reported that phytoplankton growth rates are saturated at light intensities of 50-120 µEinst m^{-2} s^{-1}. Hence in order for the photosynthetically available radiation (PAR) of 818 µEinst m^{-2} s^{-1} to be reduced to the maximum growth saturation point of 120 µEinst m^{-2} s^{-1} the extinction coefficient of light would have to be 6.82. Given the typical chlorophyll specific extinction coefficient of 0.0016 m^2 mg CHL^{-1} (Bannister, 1974), and assuming that the other factors of light absorption were minimal, the CHL concentration would have to be 426 µg l^{-1} in order to lower the PAR to limiting levels. In the present experiment there were no instances at which the critical CHL concentration was reached and it may be concluded that the phytoplankton probably were not light limited. In support of the assumption that light absorption by non-algal elements was low, the secchi disk readings were seen to decrease as CHL concentration increased and solids concentrations fluctuated. Indeed 76-86% of the variation in the secchi disk data may be attributed to CHL as indicated by the log-log regression equations fitted for CHL determination based on secchi reading. Hence, even if assuming 25% of the secchi variation was associated with non-algal absorption, the CHL concentration would have had to have been 320 µg l^{-1} in order that the PAR was lowered to limiting levels.

The water quality at the dam was notable for the high NO_3 concentrations recorded as compared to the ponds. The levels suggested contamination from the surrounding drainage basin with inorganic fertilisers as synthetic

nitrogenous fertilisers, in which the nitrogen is predominantly in the form of nitrate, readily affect both aquifer and river waters (OECD, 1986). An alternate explanation, that the nitrate may have been formed from slow conversion from organic nitrogen, seems less likely given the low dam levels of both phosphate and ammonia which usually indicate contamination from animal faeces. However, even at 500 $\mu g\ l^{-1}$, the UPM dam water NO_3 level were moderate. Mean concentrations for rivers draining the Scottish highlands are generally less than 500 $\mu g\ l^{-1}$, but certain rivers in south-east England may have 10 $mg\ l^{-1}$ and some surface waters in The Netherlands 4-18 $mg\ l^{-1}$ (OECD, 1986).

Although it would seem most likely that the present systems were on balance nitrogen limited, the lack of a definitive conclusion suggests that general temporal means may be insufficient to classify certain water bodies. Temporal shifts in limitation existed and indeed Reynolds (1984) reported that continuous limitation by any given nutrient is rare. Furthermore, analysis of water data may indicate the nutrient status at any given point in time, but does not consider the possible influence of nutrient sources within the pond environment.

As discussed previously, the sediments of lakes and ponds influence the nutrient status of the water column. As in Experiment 1, the sediment concentrations of both TN and TP were low. Despite fluctuations of the total nutrient values over time, there was a general decrease in sediment concentrations of TN and TP in the present study, with the exception of TP in M3.5. This suggested that the sediments were not acting as sinks for the retention of DRP, NH_4^+ and NO_3^-, but possibly rather as sources for water column phytoplankton. Again however it is difficult to associate water and sediment nutrient concentration peaks and troughs (Figures 4.3 and 4.6). The present low sediment OC:TN ratios again suggested rapid organic decomposition and mineralisation. Equally, the low TN:TP ratios suggested denitrification and possibly inadequate supplies of nitrogen.

To summarize, best prawn production was achieved in M14. Increasing the application frequency of fertiliser decreased the mean CHL concentration and hence presumably algal biomass, and resulted in less efficient CHL production per unit of TN or TP. CHL was significantly correlated to TN, increasingly so with decreasing manure application frequency, and was related to TP only through intercorrelation to TN. Light limitation was unlikely. More frequent manuring increased the GPP:R ratio, relieving potential oxygen depletion in M14. Although levels of total ammonia were very low, there was an increase in concentration with decreasing frequency of manure application, suggesting greater decomposition of organic matter. Both autotrophic (CHL) and heterotrophic (NH_3+NH_4^+) communities appeared to be stimulated most by manure application every 14 days. The balanced GPP:R ratio and high mean dawn DO levels of M7 did not guarantee best prawn production. Indeed, M7 had the lowest yield of marketable prawns and smallest input:output conversion ratio for cost of production:marketable prawn. Economic analysis favoured the application of manure fortnightly.

Appendix 4.1. Climatic conditions during the experimental period day 1 - day 100 (25th July 1989 - 1st November 1989).

Parameter	Mean and standard error	Minimum	Maximum
Relative humidity (%)	95 (1)	86	100
Maximum air temperature (°C)	32.1 (0.1)	26.7	34.3
Minimum air temperature (°C)	22.4 (0.1)	20.9	23.7
Rainfall (mm day^{-1})	8.07 (1.43)	0.00	64.00
Rainfall duration (hr day^{-1})	0.92 (0.15)	0.00	6.30
Sunshine duration (hr day^{-1})	4.91 (0.25)	0.00	9.90
Wind speed (m s^{-1})	1.00 (0.05)	0.45	5.30
Evaporation (mm day^{-1})	4.14 (0.11)	0.98	8.32
Water balance[1] (mm day^{-1})	+4.02 (1.43)	-5.73	+59.5

[1] water balance (mm day^{-1}) = rainfall (mm day^{-1}) - evaporation (mm day^{-1})

Appendix 4.2. Selected climatic conditions during each period between water sampling days; arithmetic mean and standard error.

Time period	Sunshine duration (hr day^{-1})	Water balance[1] (mm day^{-1})
Day 3 - 15	5.74 (0.53)	-3.02 (0.55)
Day 16 - 27	5.40 (0.79)	+0.19 (2.65)
Day 28 - 43	4.67 (0.65)	+5.92 (4.91)
Day 44 - 57	4.67 (0.69)	+3.52 (2.65)
Day 58 - 71	4.55 (0.55)	+9.90 (3.99)
Day 72 - 85	5.25 (0.80)	+6.93 (5.18)

[1] water balance (mm day^{-1}) = rainfall (mm day^{-1}) - evaporation (mm day^{-1})

CHAPTER FIVE

EXPERIMENT THREE;
THE DESIGN AND USE OF ENCLOSURES FOR EXPERIMENTATION IN FEEDING AND FERTILISING TRIALS - A PILOT NURSERY STUDY

5.1 INTRODUCTION

Frequent high variation in fish and prawn production has been reported between different ponds in close proximity and undergoing similar treatment (Tacket, 1968; Buck *et al.*, 1970; Costa-Pierce, 1990), and was identified in Experiments 1 and 2. Under research conditions this situation is adjusted to, whenever possible, by the use of many replicate ponds. However, the economic and practical availability and utilisation of this approach may be preemptive. Furthermore it is a reaction rather than a solution to the problem of seemingly random inexplicable differences between ponds. Consequently an alternative means to potentially overcome such differences is deserving of attention.

Enclosures, or mesocosms, have been employed for many years by limnologists and ecologists as effective tools to study the dynamics of aquatic systems. Various authors have successfully utilised mesocosms to examine water-sediment interactions (Mazumder *et al.*, 1989; Sakamoto *et al.*, 1989), microbes (Newhook and Briand, 1987; Sondergaard *et al.*, 1988; Pace and Funke, 1991), phytoplankton (McAllister *et al.*, 1961; Lund, 1972; Reynolds *et al.*, 1985; Sondergaard *et al.*, 1988), zooplankton (Arumugam and Geddes, 1986; Pace and Funke, 1991), and fish (Arumugam and Geddes, 1986; Mazumder *et al.*, 1989). The great advantages afforded by enclosures is their ability to allow semi-controlled experimentation representative of "near-natural" situations yet remain relatively free of the artificial conditions associated with laboratory studies. The degree of "naturalness" depends upon the design of the enclosure, particularly in terms of the volume of water enclosed, the continuity with the interfaces of both water-air and water-sediment, and the time length of the experimentation. Smaller enclosures generally have been utilised for shorter periods such as those used by Pace and Funke (1991) which contained 45 l for 4 days or those of Arumugam and Geddes (1986) which enclosed 1000 l for 26 days. Larger enclosures, with internal volumes of 16000 m^3 - 18500 m^3, have been operated for 11 months (Lund, 1972; Reynolds *et al.*, 1985).

Although most mesocosm studies to date have been situated in lakes, the utilisation of enclosures in earthen ponds, including those of aquacultural significance, is applicable. The use of a set of experimental units within a single earthen pond may lend initial equality to each unit in terms of both water and sediment character and nutrient content. Furthermore enclosures permit manipulation of specific controllable factors in an active *in situ* manner determined in general by the husbandry practices of aquaculture, but also specific to the target species.

An enclosure allowing for experimentation of feed and fertilisation trials with the freshwater prawn *M. rosenbergii* was designed. A trial of 30 days duration employed two treatments previously used in the grow-out trials to assess both the appropriateness of the mesocosm design and the effect of feed and manure on the nursery[4] stage of the prawn. The unavoidable discrepancy between Experiments 1 and 2 in terms of prawn stocking size, also justified a study on the nursery stage. The experiment acted as a pilot trial to consider the suitability of the enclosures for studying system dynamics and prawn production for longer experimental periods.

5.2 MATERIALS AND METHODS

5.2.1 Enclosure Design and Pond Preparation

Four octagonal enclosures each with an internal area of 10 m^2 (Figure 5.1) were constructed in a single pond of 440 m^2. The required length of the enclosure sides was calculated based on the area of a regular polygon (Greer and Hancox, 1989);

$$\text{Area} = 1/4\ N\ L^2\ \text{cotangent}\ 180/N$$

where N = the number of sides and L = the length of sides.

Eight vertical wood supports (200 cm x 5.08 cm x 5.08 cm) were set 162 cm

[4] a brief period of culture during which PL (or occasionally juveniles), are added to ponds or to nets located in ponds (or occasionally to other facilities), with a view to acclimatising the prawns to the pond environment; after a period of some weeks, the juveniles are harvested and restocked into grow-out ponds.

Plate 5.1.

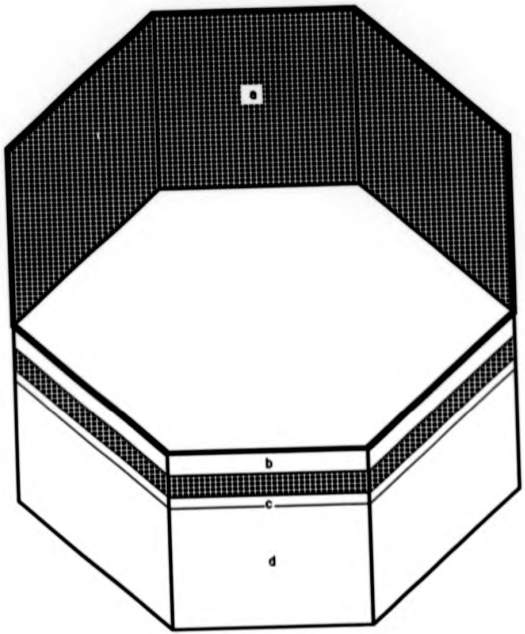
Enclosure design indicating;

a) internal sleeve of netting (1 mm mesh; 150 cm high),

b) pvc plastic slide panel (0.25 mm thick; 16 cm and 8 cm high respectively on the inside and outside of the enclosure),

c) styrofoam collar (2 cm wide; 5 cm high), and

d) external sleeve of pvc plastic (0.25 mm thick; 105 cm high).


a
b
c
d

apart and firmly set (50 cm) into the pond bottom and a trough of sediment cleared between them. Eight horizontal wood crossbars (167 cm x 2.54 cm x 5.08 cm) were added to join the upper edge of the posts. A double sleeve design was employed during the trial; an external wall of plastic sheeting to isolate the water and biotic communities, and an internal wall of netting to prevent damage to the plastic sheet by prawn claws. Plastic netting of 1 mm mesh size was first wrapped around and stapled onto the outside of the vertical supports, thus achieving a functional side length of 167 cm. The standard width of the netting was insufficient to attain the total height of the enclosure so two sheets had to be stitched together. The bottom of the netting occupied the trough, the lower edge at 8 cm below the usual pond bottom. The upper margin was wrapped over the horizontal bars and stapled to their undersides. A single sheet of polyvinyl chloride (pvc) plastic lining (EPC Nylex) of 0.25 mm thickness was wrapped around the outside of the netting. The lower edge of the plastic lining was also positioned at 8 cm below pond bottom, and then the sediment replaced and firmly packed down. At the position of the horizontal crossbars, a pvc plastic strip of 24 cm was stapled over the vertical wood supports such that 16 cm and 8 cm of plastic hung over the netting on the inside and outside of the enclosure respectively. This panel was intended to act as a slide panel, if needed, to prevent prawns from climbing out of the mesocosm. Throughout the trial, weekly checks around the exterior of each enclosure ensured that the mud and plastic netting were continuous and that no holes had developed.

A primary platform of wood (11 m x 48 cm x 150 cm) was constructed down the length of the pond and two mesocosms situated on either side. Three shorter secondary platforms (5 m x 48 cm x 150 cm) crossed the primary so that each enclosure was easily accessible.

Prior to construction, the pond was cleared of plants and dried. Inevitably during the period of construction, more plants grew and rain

water accumulated. Upon completion of the building, further clearing, draining and drying were undertaken. The enclosures and the pond proper were each prepared individually. Agricultural lime, $CaMg(CO_3)_2$, and seasoned chicken manure were added to each enclosure (1.16 kg and 128.9 g respectively) and to the pond proper (46.36 kg and 5.16 kg respectively). During pond filling the plastic sleeves were left lowered near the pond bottom in order to ensure similar initial conditions between enclosures. Water was added to a depth of 10 cm and after five days a further 90 cm of water was added. The plastic sheeting was raised upward on the following day. To the upper 5 cm of the plastic were sewn eight strips of styrofoam (164 cm x 2 cm x 5 cm) to act as a suspension collar. The plastic, secured only at the pond bottom, was therefore free to move with changing water currents or depths. The plastic reached 105 cm above pond bottom, but the active height was approximately 100 cm after allowing for bowing of the plastic during water exchange or rain.

After pond filling the water inlet was modified to allow water addition to each individual mesocosm. Attached to the pond inlet pipe was a reducer (10.16 cm to 5.08 cm internal diameter) and flexible hose (5.08 cm i.d.) which was supported by a series of "Y" shaped wooden stakes to the wooden platform to which it was secured with occasional clips. The hose with mesh (250 µm) bag attached permitted water addition directly into each enclosure by temporarily locating the hose over each enclosure. A simple diffuser structure consisting of netting stretched between and stapled to two beams of wood (260 cm x 5.08 cm x 5.08 cm and 230 cm x 5.08 cm x 5.08 cm) and held 20 cm apart by cross bars (30 cm x 2.54 cm x 2.54 cm) spread the direction of the flow of water. Each enclosure had a diffuser which remained in position bridging two sides of the mesocosm. During the experiment, a constant 5% day^{-1} water exchange was achieved by the addition of a known volume of water, the volume being calculated based on flow rate. The pond proper was also allotted a timed water input of 5% day^{-1}.

5.2.2 Stocking, Management and Harvesting

Post-larval *M. rosenbergii* (de Man) were obtained from the hatchery unit on site. Enclosures were stocked with 52-day-old (from hatching) prawns at 10 m^{-2}. Arithmetic mean and standard error at stocking were 0.05 ± 0.01 g (1.61 ± 0.02 cm orbital length). Neither silver nor grass carp were stocked due to the brief nature of the trial, the emphasis on studying prawn growth rather than pond productivity, and the non-availability of appropriate stock.

Two treatments considered as the extremes of feeding and manuring application in the previous trials, namely feed-only (FO) and decreased-feed-increased-manure (DFIM) with manure application every 14 days, were employed in duplicate. The same 30% protein diet (Table 3.1) was employed and manure was applied as scheduled in Experiment 1 (day 13 and 25). The DFIM treatment received manure at an application rate which was isophosphoric with an imagined feed plus manure treatment in which 11.5 dry g m^{-2} 14-$days^{-1}$ was received, and fed at 15% body weight day^{-1} as opposed to 10% in DFIM. As in the first five weeks of Experiment 1 the feeding rate was based on prawn stocking biomass and prawns were not sampled nor any mortality assumed.

Harvesting was accomplished on Day 38 although on the previous afternoon the styrofoam collars were removed and the pond water level slowly drained down to 10 cm. Early on the morning of harvest further draining and pumping of individual enclosures ensured that each mesocosm was fully dried and harvested. Enclosures were checked for continuity with the sediment throughout the harvesting process. The total biomass of each enclosure was measured, the total number of prawns counted, and the individual weights of twenty five randomly sampled individuals taken.

5.2.3 Water Chemistry

Dawn readings for dissolved oxygen were made at least weekly and more

frequently when the weather was overcast using a YSI oxygen meter. Readings for DO were always greater than 6.10 mg l^{-1}.

5.2.4 Climatic and Statistical Analyses

Climatic measurements were obtained as described in Experiment 1, and data are presented in Appendix 5.1. Prawn data was analysed statistically as described previously.

5.3 RESULTS

5.3.1 Pond Inputs

Pond inputs over the experimental period are presented in Table 5.1. Given the briefness of the experiment and the small size of the prawns and hence the small amount of feed required, the nutrient loads into FO were very much lower than those into DFIM. The TN load into FO was approximately 6x less than that into DFIM, the TP load 28x less, and the OC load 7x less. The accumulative loads of each nutrient are given in Appendices 5.2 - 5.4.

5.3.2 Prawn Growth Rate and Harvest Data

Mean growth rates, expressed in Table 5.2, did not indicate any difference between the two treatments. The mean weight at harvest was significantly greater in DFIM than FO. Both biomass and survival in DFIM were also greater than in FO although not significantly so.

5.4 DISCUSSION

A density-independent "space effect" on some species of crustacea has been identified. Aiken and Waddy (1978) reported that both space and density were important in limiting the growth of juvenile lobsters *Homarus americanus*, and suggested that the space required for unrestricted growth was equivalent to 75(carapace length)^2 over the range 50-80 mm carapace length. Similarly Juarez *et al.* (1987) indicated a space effect on the

Table 5.1. Load of prawn feed, chicken manure, total phosphorus (TP), total nitrogen (TN) and organic carbon (OC) over the experimental cycle; absolute load (dry g m^{-2} $cycle^{-1}$) and percent load of each of total input, TP, TN and OC (%).

Component	FO	DFIM
Feed	2.78 (100.00)	1.85 (7.37)
Manure	- (0.00)	23.26 (92.63)
TP as feed[1]	0.015 (100.00)	0.010 (2.36)
TP as manure[1]	- (0.00)	0.414 (97.64)
TN as feed[2]	0.140 (100.00)	0.093 (11.58)
TN as manure[2]	- (0.00)	0.710 (88.42)
OC as feed[3]	0.911 (100.00)	0.606 (9.34)
OC as manure[3]	- (0.00)	5.884 (90.66)

[1] based on percent TP values of 0.55 for feed and 1.78 for manure
[2] based on percent TN values of 5.04 for feed and 3.05 for manure
[3] based on percent OC values of 32.75 for feed and 25.30 for manure.

Table 5.2. Prawn growth rate and harvest data; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference at $F<0.05$, and superscripts in uncommon rows are unrelated).

Parameter	FO	DFIM
Growth rate ($g\ day^{-1}$)	0.06^{a} (0.01)	0.06^{a} (0.01)
Weight (g)	1.75^{a} (0.08)	2.08^{b} (0.06)
Biomass ($g\ enclosure^{-1}$)	119.71^{a} (5.50)	135.35^{a} (0.79)
Percent survival (%)	63.00^{a} (4.00)	66.00^{a} (6.00)

growth of *M. rosenbergii* when prawns of initial weight of 0.021 g (12.4 mm) were reared for 6 weeks in individual compartments of 0.001 - 0.01 m^2. The authors suggested that there was a critical area requirement for unrestricted growth regardless of chemical and/or visual communication between individual prawns.

In the present study the initial unit area per 0.05 g (16.1 mm) prawn was 0.1 m^2. Experiment 1 which employed the same treatments and similar prawn size at stocking (0.05 g) in whole pond experiments, reported mean sizes of approximately 1.5 g for FO prawns and 3.0 g for DFIM prawns on day 38 (by interpolation as first sampling was on day 49). As the harvest weights of the present trial were larger for FO but smaller for DFIM prawns as compared to the whole pond study, it would seem that the mesocosms were not causing a consistent space effect. Translating the results to standing biomass, prawns in the FO treatment represented 11.0 g m^{-2} (1.75 g mean weight with 63% survival from initial stock of 10 m^{-2}) whereas those in DFIM were harvested at 13.7 g m^{-2} (2.08 g m^{-2} mean weight with 66% survival). A completely accurate comparison between Experiments 1 and 3 is not possible because the survival on day 38 of Experiment 1 could only be estimated given the length of the trial. However, based on roughly calculated biomass figures in the present experiment, no consistent space effect was evident over the range 11.0 - 13.7 g m^{-2}.

The early appearance of an advantage afforded by the DFIM treatment was evident in this trial, and supported the weight and biomass results of Experiment 1. Apparently the higher feeding levels of the FO treatment in Experiments 1 and 3 were not required. Possibly, manure was advantageous either as a better catalyst for system productivity, or a better feed which offered either a nutrient profile more balanced to the prawn requirements than the formulated pellets, or contained a specific growth enhancing factor. The combination of both feed and manure may have improved the management strategy and facilitated prawn growth.

Overfeeding is a possibility but it would seem likely that if there were any excess pellet it would act as a fertiliser similar to manure. Certainly the manure may have been a better catalyst to system productivity leading to the production of natural feeds, but more likely the combination of feed and manure was ideal considering the poor performance of the manure-only regime in Experiment 1. The effects of feed and manure upon system productivity including the microbial community and the possibility of direct manure consumption will be addressed further in Experiment 4.

The lack of significance between treatments for the parameter survival may have been due to a true reflection of treatments, the short duration of the trial, and/or the variation within treatments. In order to assess the enclosure design as a means to decrease variation between replicates, coefficients of variation were calculated for the harvest variables mean weight, yield and survival in the present experiment and the FO and DFIM treatments of Experiment 1. The results, presented in Table 5.3, indicated that the mean variation for each parameter was higher in the enclosure study than in the pond trial. Given the difference in the length of the trials and the nature of young populations (as in Experiment 3) to be very dynamic and hence subject to more variation than older more stable populations, it would be premature to conclude that the enclosures were a source of variation themselves. However, despite the limitations of the comparison, the mesocosms did not seem to afford protection against high variation of some prawn population characteristics. Similarly, based on a comparison of golden perch (*Macquaria ambigua*) populations in a pond and in 1000 l enclosures, Arumugam and Geddes (1986) reported that the range and variation of survival in the enclosures (15.5 - 47.0%) was similar to that in the pond.

Practically, the enclosures described were effective in retaining organisms, particularly due to the continuous seal between the walls and

Table 5.3. Values for the coefficient of variation[1] for the harvest parameters mean weight, yield and survival in the FO and DFIM treatments of Experiment 1 and Experiment 3.

Parameter	Experiment and Treatment	Coefficient of Variation (C.V.)	Mean and standard error of C.V.
Weight	Exp1 FO	13.54	
	Exp1 DFIM	1.70	7.62 (5.92)
	Exp3 FO	15.63	
	Exp3 DFIM	13.53	14.58 (1.05)
Yield	Exp1 FO	1.46	
	Exp1 DFIM	5.40	3.43 (1.97)
	Exp3 FO	6.50	
	Exp3 DFIM	0.84	3.67 (2.83)
Survival	Exp1 FO	12.10	
	Exp1 DFIM	7.08	9.59 (2.51)
	Exp3 FO	8.98	
	Exp3 DFIM	12.86	10.92 (1.94)

[1] coefficient of variation = standard error/arithmetic mean

the sediment, the height of the walls, and the internal netting sleeve which prevented damage of the plastic sheeting by the prawns. Furthermore the external sheeting material remained intact and successfully isolated the enclosed water from the pond proper. Management practices were accomplished with ease as the platforms and flexible inlet hoses gave ready access to each individual enclosure and water quality problems were not in evidence. Dawn dissolved oxygen levels remained high throughout the experimental period, although if required the design would readily lend itself to aeration of individual enclosures.

The published design for enclosure use in shallow water ponds by Arumugam and Geddes (1986) situated the enclosures along the pond bank for ease of management and to grant a shallow water refuge to the motile biotic organisms. Although the design was acceptable for studies concerning the interaction between zooplankton and zooplanktivorous fish, the situation along the bank would render the design inappropriate for research concerned with nutrient dynamics due to the effects of runoff from the banks into the enclosures. Even if pond banks are equally sloped, the possibility for unequal runoff exists particularly in areas receiving very directional rain and/or tropical monsoons. A further disadvantage of enclosure-bank continuity is ease of access by predators or competitors such as birds, lizards, snakes, frogs, insects, or macrophytes.

Over the extent of the experiment the enclosure design proved to be robust and stable, and easily constructed and managed. Post-larval prawn growth did not appear to be consistently restricted in the present trial by an enclosure induced space effect, but variation commonly associated with earthen ponds did not appear to be reduced. On the basis of both prawn production and mesocosm stability, trials employing enclosures as tools for research into system productivity and dynamics appeared justified. The advantage of space allocation and therefore the potential for greater treatment replication is both practicable and appealing.

Appendix 5.1. Climatic conditions during the experimental period day 1 - day 38 (16th June 1990 - 23rd July 1990).

Parameter	Mean and standard error	Minimum	Maximum
Relative humidity (%)	96 (1)	91	100
Maximum air temperature (°C)	31.9 (0.1)	29.5	35.1
Minimum air temperature (°C)	22.9 (0.1)	21.0	24.0
Rainfall (mm day^{-1})	7.83 (2.55)	0.00	88.40
Rainfall duration (hr day^{-1})	0.97 (0.21)	0.00	5.10
Sunshine duration (hr day^{-1})	5.24 (0.47)	0.00	9.45
Wind speed (m s^{-1})	0.88 (0.04)	0.37	1.71
Evaporation (mm day^{-1})	3.92 (0.23)	1.16	8.11
Water balance[1] (mm day^{-1})	+3.92 (2.52)	-5.54	+84.40

[1] water balance (mm day^{-1}) = rainfall (mm day^{-1}) - evaporation (mm day^{-1})

Appendix 5.2. Cumulative TN[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FO	DFIM
Day 1 - 12	0.045	0.030
Day 1 - 23	0.090	0.415
Day 1 - 37	0.140	0.803

[1] based on percent TN values of 5.04 and 3.05 for feed and manure respectively.

Appendix 5.3. Cumulative TP[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FO	DFIM
Day 1 - 12	0.005	0.003
Day 1 - 23	0.010	0.213
Day 1 - 37	0.015	0.424

[1] based on percent TP values of 0.55 and 1.78 for feed and manure respectively.

Appendix 5.4. Cumulative OC[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FO	DFIM
Day 1 - 12	0.295	0.197
Day 1 - 23	0.590	3.335
Day 1 - 37	0.911	6.490

[1] based on percent OC values of 32.75 and 25.30 for feed and manure respectively.

CHAPTER SIX

EXPERIMENT FOUR:
THE EFFECT OF FEED AND FERTILISER
ON WATER AND SEDIMENT BACTERIA IN ENCLOSURE TRIALS

6.1 INTRODUCTION

The natural consumption of fresh and decaying organic matter by the freshwater prawn lends credence to the application of organic matter as agricultural wastes or animal manures to semi-intensive prawn culture. Indeed the beneficial use of organic manures in *M. rosenbergii* culture has been reported (Buck *et al.*, 1981; Wohlfarth *et al.*, 1985), but the dietary pathway from the fertiliser to the prawn has not been experimentally investigated.

The addition of organic matter to natural waters stimulates microbial biomass and productivity (Moriarty, 1986; Schroeder, 1978). The bacteria, occupy a key position in the aquatic heterotrophic foodweb and play a vital role in environmental ecology. The bacteria affect abiotic factors including inorganic nutrients and dead organic matter or detritus, and viable communities including algae, zooplankton, protozoa and meiofauna (Fenchel and Jorgensen, 1977; Moriarty, 1986; Güde, 1989).

The aims of the present study were to determine if the bacterial population was stimulated by the addition of feed or manure. The study was designed as in the previous experiments to evaluate the application of chicken manure and formula feed pellets. Due to the high replicate variation commonly associated with pond trials, the enclosure design employed in Experiment 3 was used in the present trial in an attempt to isolate the true treatment effects. This was deemed especially necessary in microbial studies in which unequal conditions, particularly dilution, would have a dramatic effect on bacterial density and hence total productivity.

6.2 MATERIALS AND METHODS

6.2.1 Enclosure Design and Pond Preparation

Eight octagonal enclosures of area 10 m^2 were constructed in a single pond of 440 m^2. The design as outlined in Experiment 3 was employed with the following alterations in the platform construction and water distribution system (Plate 6.1). A primary platform of wood (21 m x 40 cm x 150 cm) was constructed down the entire length of the pond and four mesocosms situated on either side. Five shorter secondary platforms (5 m x 40 cm x 150 cm) crossed the primary so that each enclosure was easily accessible. The water inlet was modified as described previously with a flexible hose which was supported by a series of "Y" shaped wooden stakes to the platform, in this case the middle secondary platform. A "T" joint divided the hose into two similar hoses which ran down the length of the primary platform, being held in place by occasional clips. These hoses permitted water addition directly into each enclosure, one hose being used for four enclosures. Mesh bags and diffusers were used as in Experiment 3 and a constant 5% day^{-1} water exchange was achieved for each enclosure and the pond proper.

Decreasing dawn bottom dissolved oxygen (DO) levels (to 2.55 mg l^{-1} in DFIM and 2.70 mg l^{-1} in FPM) instigated the installation of an aeration system after 87 days of culture. A Hitachi Bibicon air compressor with a 0.75 kw motor capable of delivering air at a rate of 80 l min^{-1} was installed on the pond bund. A single pvc pipe running along the primary platform and fitted with eight stopcocks, delivered air to each enclosure via eight flexible air hoses and eight air stones. The flexible hoses were directed horizontally to the centre of each mesocosm and then dropped vertically by simple use of a string taut to the far side of each enclosure. By this means, stones were kept in suspension several centimeters above the pond bottom and in the very centre of the mesocosm. The compressor was used nightly from 1900 hours to 0700 hours for the final two weeks of the trial and maintained dawn DO levels near saturation.

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Eight octagonal enclosures of area 10 m^2 were constructed in a single pond of 440 m^2. The design as outlined in Experiment 3 was employed with the following alterations in the platform construction and water distribution system (Plate 6.1). A primary platform of wood (21 m x 48 cm x 150 cm) was constructed down the entire length of the pond and four mesocosms situated on either side. Five shorter secondary platforms (5 m x 48 cm x 150 cm) crossed the primary so that each enclosure was easily accessible. The water inlet was modified as described previously with a flexible hose which was supported by a series of "Y" shaped wooden stakes to the platform, in this case the middle secondary platform. A "T" joint divided the hose into two similar hoses which ran down the length of the primary platform, being held in place by occasional clips. These hoses permitted water addition directly into each enclosure, one hose being used for four enclosures. Mesh bags and diffusers were used as in Experiment 3 and a constant 5% day^{-1} water exchange was achieved for each enclosure and the pond proper.

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Plate 6.1.

The enclosures;

a) layout in the pond showing platforms, enclosures and inlet hoses, and,

b) air delivery from the Hitachi Bibicon compressor.

Pond preparation was similar to that of Experiment 3 except for the absolute level of agricultural lime and chicken manure added to the pond proper, respectively 41.72 kg and 4.64 kg, because there were four more mesocosms than in the previous experiment each of which required separate additions of inputs and thus reduced the area of the pond proper.

6.2.2 Stocking, Management and Harvesting

Juvenile prawns, hatched at the unit on site and reared in nursery cages in a nearby pond until they were 78 days old (from hatching) were stocked into the enclosures at 10 m^{-2}. Arithmetic mean and standard error at stocking were 0.67 ± 0.08 g (3.52 ± 0.05 cm orbital length). Two weeks later, silver carp, *H. molitrix* (Val.), of 1.32 ± 0.03 g (4.28 ± 0.04 cm standard length) were stocked at 0.1 m^{-2}, or one individual per enclosure. One silver carp of 150 g and two grass carp, *C. idella* (Val.), of 2.9 kg and 0.7 kg were stocked into the main pond.

Treatments in this trial were the same as those in Experiment 1. The same 30% protein diet formulation (Table 3.1) and seasoned chicken manure were applied as scheduled (Table 3.2) and as required to the four treatments in duplicate; feed-plus-manure (FPM), feed-only (FO), decreased-feed-increased-manure (DFIM), and manure-only (MO). As in Experiment 2 feed application rates were based on prawn stocking biomass until day 11, on an assumed mean weight of 1 g for the subsequent two weeks, and then on prawn mean weight, in this case of ten individuals per enclosure. Dates for prawn sampling, feed application rate, pellet size, and mortality rate followed the same scheduling of days as defined in Experiment 2.

Feed was applied twice daily and the sizes of pellet used followed the same schedule in Experiment 2. Manure was applied at 0800 hours (day 12, 24, 38, 52, 66 and 80). Throughout the study manure was periodically analysed for *Salmonella* spp., but all tests proved negative.

Harvesting took place on day 100, and the enclosure draining followed the procedure as outlined in Experiment 3 (Plate 6.1). Enclosures were checked for continuity with the sediment throughout the harvesting process. Prawn biomass was measured and prawns divided into four sex-types as in Experiments 1 and 2. These morphotype groups were weighed and their number counted. A random sample of ten individuals from each type, or if there were not as many as ten then all individuals, were separately weighed. Mean prawn weight and growth rate were calculated as before.

6.2.3 Water Chemistry

All water sampling occurred prior to the installation of the air compressor, and therefore all data reported (including DO) are for that period. Dissolved oxygen was measured at least weekly using a YSI oxygen meter. An integrated water sample was obtained from each enclosure every two weeks (day 3, 16, 28, 44, 58, 72 and 86) using a pvc pipe of 4 cm internal diameter. Samples were taken between 0800 and 0815 hours, stored in polypropylene 1 l bottles and transferred immediately to the laboratory. Upon arrival at the laboratory, subsamples were taken for total bacterial determinations. Every sixth week, viable bacterial analyses were also conducted and at those times subsamples for viability were taken before any other. Details of the procedures for subsampling and subsequent bacterial analyses follow.

The remainder of the water sample was available for chemical analysis. Determinations for CHL, COD, and TP were made as previously described. On the final two sampling days, TN, NH_3+NH_4^+, NO_2, and NO_3 were also analysed. Non-availability of sufficiently pure potassium persulphate had previously prevented the determination of TN and NH_3+NH_4^+.

6.2.4 Total Bacteria:Water

Within forty minutes of pond sampling, the sample was gently inverted several times, and subsamples taken as follows. Using sterile pipettes,

20.00 ml of water was transferred to sterile polystyrene/polyethylene Sterilin universal vials. As a preservative, 400 µl of formaldehyde (prefiltered through Whatman 0.2 µm cellulose nitrate filters) was added. The subsamples were tightly capped, inverted several times to mix, and stored in a dark cool cupboard until microscopic analysis, usually within two weeks.

The method for fluorescent staining and direct enumeration of bacteria was based on those by Francisco *et al.* (1973), Jones and Simon (1975) and Hobbie *et al.* (1977). The procedure was most similar to that recommended by Fry (1988). A pond water volume of between 500 µl to 2000 µl (depending on the apparent eutrophic status) was transferred from a well mixed subsample to a second sterile vial which contained between 8300 µl to 6800 µl of dilution water (distilled deionized water prefiltered through Whatman 0.2 µm cellulose nitrate filters and autoclaved, and to which was added prefiltered formalin to a final concentration of 2%). The vial was capped, mixed for five seconds on a vortex mixer, and 1200 µl acridine orange (AO) added. Preliminary tests (Appendix 6.1) employing a range of final AO concentrations on water samples from a nearby pond indicated that a final AO concentration of 60 mg l^{-1} was optimal (prepared using dilution water and refiltered through glass fibre and 0.2 µm cellulose nitrate filters). Therefore 1200 µl of AO was used consistently in the preparations. After capping and further vortex mixing for five seconds, the preparation was placed in the dark for a precise three minutes.

Following incubation, the preparation was remixed for five seconds on the vortex and added to the tower of a Sartorius 25 mm filter. A few millilitres of dilution water had already wetted the filter paper to ease the distribution of bacteria. The filter paper, a Nucleopore 0.2 µm polycarbonate membrane, had previously been stained with 2000 mg l^{-1} irgalan black (Ciba-Geigy Dyestuffs: prepared using dilution water containing a 2% final concentration of acetic acid and refiltered through

glass fibre and 0.2 µm cellulose nitrate filters) and quickly rinsed in dilution water. Two volumes of approximately 10 ml each were used to rinse the filter tower. The filter paper was transferred to a glass slide on which a drop of paraffin (prefiltered through 0.2 µm cellulose nitrate filters and autoclaved) had been placed. A second drop of paraffin was placed on top of the paper and a coverslip positioned. Glycerin was required for viewing the slide under the glycerin-immersion microscope lens.

A Nikon Fluophot Microscope set for incident light epifluorescence with AO staining (employing a high-pressure 200 W mercury lamp; B excitation filter IF420-490; dichroic mirror DM505; absorption filter 515 W; UV-F 100x objective set to a maximum numerical aperture of 1.3, and 10X eyepiece objective) was used for viewing the slide mounts. Systematically moving over a large area of the filter paper, bacteria were counted until at least 500 individual bacteria and 50 fields of vision had been viewed. Bacteria had distinct outlines and were typically white or pale green, although occasionally orange. A field of 2x2 grids on a 10x10 eyepiece graticule was adopted as standard throughout the procedure. Calibrated against a stage micrometer, this size grid equated to 400 μm^2. To enumerate filamentous bacteria, exactly 100 fields of vision were examined. Calculations for the number of total bacteria per µl followed the formula;

$$\frac{\text{average count per 2x2 grid}}{\text{grid area (400 } \mu m^2\text{)}} \times \frac{\text{effective filtration area of membrane filter (3.4636 x10}^6\text{ µm2)}}{\text{volume of subsample filtered (µl)}}$$

The resultant value was multiplied by a constant (1.02) to account for the dilution of the sample by the addition of formalin and by 1000 to express the concentration in bacteria ml^{-1}.

As an indication of precision, counts on three separate filter preparations from the same subsample resulted in a coefficient of

variation or precision of 0.61% at 8.22 x 10^6 bacteria ml^{-1} of pond water.

6.2.5 Respiring Bacteria:Water

An active electron transport system, indicative of respiration, may be illustrated in bacteria by the formation within the cells of dark red formazan crystals following incubation of a bacterial sample with the tetrazolium salt 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT). In theory actively respiring bacteria may be counted by bright field and epifluorescence microscopy by the presence of one or more formazan dots per bacterial cell. The method was first described by Zimmerman *et al.* (1978), although the adaptions suggested by Fry (1990) were employed in the present experiment.

Within thirty minutes of sampling, water samples were stained with INT to give a final concentration of 200 mg l^{-1}, mixed and incubated in the dark for twenty minutes. The reaction was stopped with 37% formalin by adding a volume equivalent to 1% of that in the universal and the procedure for acridine orange staining and mounting completed. The presence of formazan dots was noted in large organisms such as phytoplankton and cyanobacteria, but not in the heterotrophic bacteria. Trials with a pure culture of *Aeromonas sobria* grown in the laboratory under nutrient rich conditions resulted in easy identification of formazan dots in these large cells and suggested that the problem with the sample may be due to the small size of the bacteria. An alternative explanation may have been a detection limitation associated with the ocular mechanics of the microscope. However, this would not seem to have been the case as the surface of the 0.2 µm polycarbonate filter paper could be seen to be pitted. Indeed these surface variations appeared as dots and were initially confused with formazan dots, but given that they appeared under bright field conditions with filtered (0.2 µm) and sterilised water samples prepared under strict conditions in which the filter papers were sterilised and all reagent preparation and dilution waters were both filtered and sterilised, they

were attributed to the paper surface.

Two possible solutions to the problem were considered. The addition of substrates such as NADPH, NADH, and sodium succinate will increase the size of the small viable cells so that the formazan dots would be more readily identifiable (Fry, 1990). However, given that the experiment considered the effect of nutrients (feed and manure) on the bacterial population and based on the comments of Quinn (1984), this method was not adopted due to the possibility of inducing activity in barely viable bacteria which lacked the cellular reserves of substrates. Further support for this decision was found more generally in Tabor and Neihof (1982), van Es and Meyer-Reil (1983), and O'Carroll (1988). A second solution was the adaptation of the INT method as proposed by Tabor and Neihof (1982, 1984) in which bacteria are transferred from a filter paper to a gelatin film on a coverslip, the paper removed, and a gelatin film sprayed over the bacteria so embedding them. However this approach would have been terribly time consuming and on that basis could not be adopted.

6.2.6 Total Bacteria:Sediment

A sediment sample was obtained every four weeks (days 4, 30, 59 and 87) using a modified syringe of 3 cm internal diameter. Samples were taken at 0800 hours, stored in the syringe inside a plastic bag and transferred to the laboratory. Within twenty minutes of sampling, the sample was gently extruded and a subsample of 1.00 g wet weight taken from the uppermost 0.5 cm centre section of the syringe. The subsample was placed in a sterile universal vial containing 8600 µl dilution water and 400 µl formalin. The vial was tightly capped, inverted several times, and stored in a dark cool cupboard until microscopic analysis within ten weeks. A second subsample of approximately 1 - 2 g was accurately weighed to two decimal places on preweighed aluminum foil and transferred to an oven for drying at 60°C for twenty-four hours.

Prior to microscopic analysis, the subsample was inverted several times by hand, mixed for twenty seconds on the vortex, allowed to sit for five seconds and a 20 µl volume was extracted. This volume was found to cover 40-70% of the background area with particulate matter as recommended (Clarke and Joint, 1986; Getliff and Fry, 1990). All volume transfers were done under sterile conditions using autoclaved pipette tips and autopipettes. The sediment was added to a second sterile vial which contained 8780 µl dilution water and mixed on the vortex for five seconds. After the addition of 1200 µl AO and similar mixing, the preparation was placed in the dark for a precise three minutes. As with pond water, preliminary tests employing a range of AO concentrations on a sediment sample from a nearby pond indicated that a final AO concentration of 60 mg l^{-1} was optimal (Appendix 6.1). The procedure for sediment analysis proceeded as described above for pond water with the exception that bacterial counts were separated to those located either "on" or "off" the background particulate matter. Once both minimum criteria of 50 fields and 500 bacteria were achieved, the number of bacteria located "on" the detritus were multiplied by two, based on the assumption that for every visible bacterium on the particulate matter another bacterium was hidden beneath it as proposed by Goulder (1977). Hence the total number of bacteria per field (N) could be calculated based on the equation;

$$N = F + 2R$$

where F and R represent the number of bacteria counted respectively "off" and "on" the background detritus. Thereafter, calculations for the number of bacteria per µl followed the formula given for pond water as above. The resultant value was multiplied by a constant (10.00) to account for the dilution of the sample, multiplied by 1000, and divided by the percent dry matter of the sediment to express the concentration in bacteria g^{-1} dry sediment. Counts on three separate filter preparations from the same subsample resulted in a coefficient of variation or precision of 0.62% at 12.41×10^{9} bacteria g^{-1} dry pond sediment.

6.2.7 Total Bacteria:Prawn Alimentary Tract

An assessment of the total bacterial loads within the alimentary tract of the prawns at harvest was intended. Unfortunately two preliminary tests on non-experimental prawns did not reveal the presence of bacteria and thus the procedure was cancelled. The method employed in the preliminary tests was based on modifications of the procedures recommended in APHA (1970) and those described above for the total bacterial assessment of the pond water and sediment. At each preliminary test six prawns of approximately 10 g were sampled with a cast net from a pond located near the experimental pond and receiving both feed and chicken manure inputs. The prawns were immediately transferred live to the laboratory in a container of pond water. At the lab the prawns were treated sequentially as follows. The animal was quickly killed and the carapace lightly scrubbed with a sterile brush and distilled/deionized water. With the aid of a sterile knife, the carapace was removed and discarded. The alimentary tract was exposed and the intestine from the anus to, and including, the pyloric stomach was teased out. The tissue was added to a preweighed vial containing 6.00 ml of dilution water and 240 µl of formalin. The cardiac stomach was also removed, snipped open, and flushed with 1.00 ml of dilution water into a second preweighed sterile vial. This process was repeated for the remaining five prawns. Upon completion of the flushing of the stomachs, 240 µl of formalin was added to the second vial. Each vial was then reweighed and placed onto the dispersing tool of a tissue grinder (T25 Janke fitted with a S2510G dispersing tool) for 3 minutes at 8000 rpm in the case of the intestines and 1 minute at 8000 rpm for the stomachs. A container of ice held around the vial helped to keep the sample cool. The grinder was rinsed with 4.00 ml of dilution water collected into the vial. The grinder was sterilised by swabbing with alcohol between vials.

For microscopic viewing, several subsamples of the preparations above were processed. A sample of 4.00 ml was analysed without any dilution, and both

1.00 ml and 20.00 µl were diluted to 10.00 ml. Appropriate volumes of acridine orange were employed, and the procedure for staining and viewing under epifluorescence conditions followed.

6.2.8 Total Bacteria: Photography and Image Analysis

Photographs of bacteria were taken upon completion of the counting procedure. A Nikon FX-35 WA camera, Nikon UFXII exposure meter set to 400ASA and -2 exposure, and Kodak TMY 400ASA black and white film were employed. Films were developed to negative strips for assessment of bacterial size.

To estimate cell dimensions, a video camera and image analyser were employed. The system was similar to that utilized by Getliff and Fry (1989). A Panasonic Newvicon electron tube camera (Model WV-80) produced a monochrome video image. The image analyser, a Solitaire Plus, consisted of a main Z80B processor running a Task Programming Language (TPL) and equipped with a 3.5" disk drive, a Thompson high resolution colour monitor, an Ampex terminal, and an Epson FX1000 printer. The video image was segmented into 512x256 pixels and each pixel was assigned a grey level value from a range of one hundred and twenty seven values denoting shade depth. A series of previously selected enhancement features were applied to reduce noise and clarify the image. The enhanced grey image was thresholded to produce a binary image, which was then edited by a further series of previously selected editing features designed to remove debris, or bacteria which were either poorly thresholded or focused. Objects were then measured for area and perimeter, from which central length and width were estimated, and volume was calculated. The constants and equations for these parameters were given by Fry and Davies (1985). The data was temporarily stored on floppy disks and printed to hardcopy for security, but also was downloaded to the university VAX mainframe system for future statistical analysis. Bacterial biovolume was calculated as the product of count and volume. Bacterial biomass was estimated based on the

recommended conversion factor of 310 fgC μm^{-3} (Fry, 1990).

Initial video images were captured by separate methods for each of the water and sediment samples. In the case of pond water, the image was obtained by directly mounting the photographic negatives in a light box. The Newvicon camera was fitted with a Tiffen 48 mm to 59 mm lens and a Cannon 58 mm closeup lens. Calibration was achieved employing photographic negatives of the stage micrometer. The list of commands employed in the image analysis of water samples is presented in Appendix 6.2. Due to the presence of much debris in the photographs of pond sediment, the negatives were not used directly for image analysis due to interference and low contrast of bacteria against the background. An intermediate step of drawing the outline of projected bacteria was introduced. Negatives were mounted in standard glass slides and projected a constant distance to a frame size of 70 cm x 46 cm as recommended by Fry and Davies (1985). The bacterial outlines were drawn on paper using a brown felt-tip pen, as were several micrometer distances to establish calibration during image analysis. The hand drawings were then captured by the Newvicon camera using a Tarcus 12.5 mm lens. The list of commands employed in the image analysis of sediment samples is presented in Appendix 6.3.

As an indication of the precision of the image analysis for mean volume determination, duplicate sets of measurements were taken for each of a water and a sediment sample. The method exhibited a coefficient of variation of 1.08% at 0.2035 μm^3 for water and 0.30% at 0.1528 μm^3 for sediment.

6.2.9 Viable Bacteria:Water

Water sampled according to the procedure above was also analysed for viable counts every six weeks. The technique of Patrick (1978) using membrane filters was adapted. Phosphate buffered saline was employed in the preparation of serial dilutions and a Gelman Science manifold system

was used for rapid filtration. Millipore membrane HA grided 0.45 µm sterile filter papers were incubated on tryptone glucose extract agar plates held at 30°C for 24 hours. Triplicate counts were made at two or three serial dilutions.

6.2.10 Nutritional, Climatic and Statistical Analyses

Triplicate samples of the prawn diet and chicken manure, and of the fish meal and shrimp meal used in the diet formulation, were analysed for moisture, total nitrogen, total phosphorus and organic carbon as described in Experiment 1.

Crude protein was determined as Kjeldahl total nitrogen multiplied by a factor of 6.25 (AOAC, 1975). Crude lipid was analysed by ether extraction using the Soxhlet method (AOAC, 1975). Ash was defined as the weight change in samples after igniting in a muffle furnace at 550°C for four hours (AOAC, 1975). Crude fibre was analysed on lipid extracted samples by digestion with both acid and base, and following ashing (AOAC, 1975). The crude carbohydrate percent was calculated as nitrogen free extractives (NFE) by the subtraction from 100% of the dry percents of crude protein, crude lipid and ash as follows;

NFE = 100 - (% crude protein + % crude lipid + % ash).

Gross energy was calculated from assumed physiological fuel values for each of crude protein, crude lipid and crude carbohydrate as follows;

Gross energy (kcal g^{-1}) = (X + Y + Z) / 100

where X = % crude protein x 5.4 kcal g^{-1}

Y = % crude lipid x 9.5 kcal g^{-1}

Z = % crude carbohydrate x 4.1 kcal g^{-1}

Climatic measurements were obtained as described in Experiment 1, and the data are summarised in Appendices 6.4 and 6.5. Statistical analyses were analysed as before with the exception that nitrogen compounds were not

included in ANOVAs, correlations or regressions, because of the infrequency of their analysis.

6.3 RESULTS

6.3.1 Pond Inputs

Pond inputs over the experimental period are presented in Table 6.1. Feed inputs into FPM and FO were quite similar as based on equal feeding rates and only slightly differential growth. The nutrient loads into FPM and DFIM were isophosphoric as defined in the experimental protocol. The feed and manure levels of TN, TP and OC are given in Table 6.1 and the accumulative loads of each nutrient from both sources are presented in Appendices 6.6-6.8.

6.3.2 Prawn Sampling Weights

Weight data from bimonthly sampling are presented in Table 6.2. At each and every sampling period, analysis of variance revealed that MO prawns were significantly lighter than any other prawns. There were no statistical differences between prawns of FPM, FO and DFIM treatments. The mean weight at harvest, as calculated by total biomass/total number, indicated the same statistical pattern as the sampling data. FPM prawns were heaviest, followed closely by FO and then DFIM prawns. The clear weight advantage of the prawns receiving feed is evident in Figure 6.1.

6.3.3 Prawn Growth Rate and Harvest Data

The mean growth rate for each treatment is expressed in Table 6.3. As in the sampling data there are significant differences between MO and all other treatments. Similarly, harvest weight, biomass and yield were each grouped as one for FPM, FO and DFIM, and placed above MO. Biomass and yield were highest in FPM, followed by DFIM, and then FO. Percent survival was very high, ranging from 75% to 98% between treatments. Two significant groupings for percent survival were assigned with FO and FPM lower than

Table 6.1. Load of prawn feed, chicken manure, total phosphorus (TP), total nitrogen (TN) and organic carbon (OC) over the experimental cycle; absolute load (dry g m^{-2} $cycle^{-1}$) and percent load of each of total input, TP, TN and OC (%).

Component	FPM	FO	DFIM	MO
Feed	155.51 (69.27)	149.82 (100.00)	91.70 (50.83)	- (0.00)
Manure	69.00 (30.73)	- (0.00)	88.72 (49.17)	69.00 (100.00)
TP as feed[1]	0.86 (41.15)	0.82 (100.00)	0.50 (23.92)	- (0.00)
TP as manure[1]	1.23 (58.85)	- (0.00)	1.59 (76.08)	1.23 (100.00)
TN as feed[2]	7.84 (78.87)	7.55 (100.00)	4.62 (63.03)	- (0.00)
TN as manure[2]	2.10 (21.13)	- (0.00)	2.71 (36.97)	2.10 (100.00)
OC as feed[3]	50.93 (74.47)	49.07 (100.00)	30.03 (57.22)	- (0.00)
OC as manure[3]	17.46 (25.53)	- (0.00)	22.45 (42.78)	17.46 (100.00)

[1] based on percent TP values of 0.55 for feed and 1.78 for manure
[2] based on percent TN values of 5.04 for feed and 3.05 for manure
[3] based on percent OC values of 32.75 for feed and 25.30 for manure.

Table 6.2. Prawn weight (g) at sampling; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
0	0.67^{a} (0.08)	0.67^{a} (0.08)	0.67^{a} (0.08)	0.67^{a} (0.08)
23	2.33^{b} (0.12)	2.36^{b} (0.08)	2.28^{b} (0.08)	1.48^{a} (0.04)
37	3.29^{b} (0.20)	3.37^{b} (0.19)	3.88^{b} (0.13)	1.70^{a} (0.06)
51	4.51^{b} (0.28)	5.16^{b} (0.43)	6.17^{b} (0.32)	1.92^{a} (0.07)
65	6.93^{b} (0.57)	7.37^{b} (0.48)	7.12^{b} (0.49)	2.22^{a} (0.15)
79	7.07^{b} (0.39)	7.50^{b} (0.39)	8.02^{b} (0.28)	2.81^{a} (0.17)
100[1]	10.89^{b} (1.06)	9.50^{b} (0.68)	9.34^{b} (0.42)	3.29^{a} (0.36)

[1] = as calculated from harvest data; total biomass/total number.

Figure 6.1.
Prawn mean weight (g) at sampling.

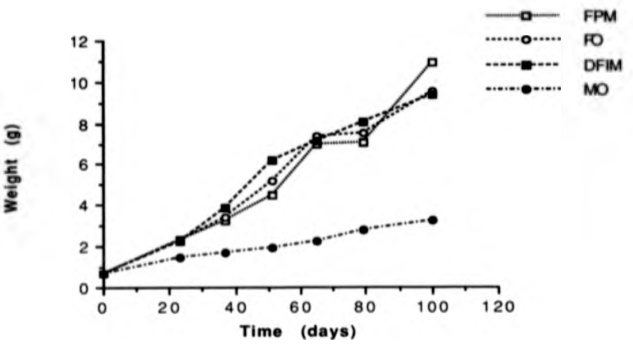

FPM
FO
DFIM
MO
Weight (g)
0
2
4
6
8
10
12
0
20
40
60
80
100
120
Time (days)

Table 6.3. Prawn growth rate and harvest data; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Parameter	FPM	FO	DFIM	MO
Growth rate ($g\ day^{-1}$)	0.10^{b} (0.01)	0.09^{b} (0.01)	0.09^{b} (0.01)	0.03^{a} (0.01)
Weight (g)	10.89^{b} (1.06)	9.50^{b} (0.68)	9.34^{b} (0.42)	3.29^{a} (0.36)
Biomass ($g\ enclosure^{-1}\ cycle^{-1}$)	854.79^{b} (28.77)	718.99^{b} (75.21)	799.36^{b} (50.13)	323.27^{a} (30.82)
Yield ($kg\ ha^{-1}\ cycle^{-1}$)	854.79^{b} (28.77)	718.99^{b} (75.21)	799.36^{b} (50.13)	323.27^{a} (30.82)
Percent survival (%)	79.00^{a} (5.00)	75.50^{a} (2.50)	85.50^{ab} (1.50)	98.50^{b} (1.50)

MO, and DFIM common to both.

No prawns in any treatment attained the 20.00 g lower limit assigned for marketable potential. The heaviest weight achieved was 18.07 g (FPM - berried female). Hence no data for marketable percent or marketable yield can be presented.

6.3.4 Prawn Sex-Type Data

As indicated in Table 6.4 small males were lightest in all treatments, but adult males were not necessarily heaviest. Berried females were the heaviest sex-type in both FPM and FO, and adult males were the heaviest morphotype in DFIM. Berried females were heavier than unberried females in each and every treatment. Significant differences between treatments were restricted to the weight of unberried females in MO verses the other three regimes.

The contributions of the various sex-types to the percent of harvest number were very similar for FPM, FO and DFIM treatments. Adult males contributed 43% to 46% of the number, and 46% to 51% of the yield depending on treatment. With the exception of MO, small males contributed little to percent yield. As a whole, males in each treatment constituted a larger percent of harvest number than females in each treatment. Other than DFIM, the same was true for percent of yield. Excepting MO wherein there were no females with eggs, berried females formed a much higher percent of both number and yield as compared to unberried females. Significant differences between treatments were limited to that between MO and all other regimes for the contribution of small males to both percent number and percent yield. The contributions of each sex-type in each treatment to the harvest parameters are graphically presented in Figure 6.2.

Table 6.4. Prawn harvest data for each sex-type; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference and superscripts in uncommon rows are unrelated).

Parameter	FPM	FO	DFIM	MO
Weight (g)				
Type 1[1]	11.98[a] (0.63)	10.25[a] (0.53)	10.53[a] (0.51)	
Type 2	10.07[b] (0.66)	8.75[b] (0.46)	9.37[b] (0.59)	3.52[a] (0.18)
Type 3	2.90[a] (0.30)	3.09[a] (0.20)	3.16[a] (0.29)	3.04[a] (0.15)
Type 4	10.55[a] (0.79)	9.53[a] (0.60)	11.33[a] (0.61)	
Percent of number (%)				
Type 1	11.36[a] (0.55)	14.72[a] (4.46)	12.74[a] (6.79)	
Type 2	32.45[a] (2.70)	32.89[a] (6.86)	36.98[a] (7.09)	39.64[a] (2.64)
Type 3	9.34[a] (2.58)	6.11[a] (4.84)	6.40[a] (1.65)	60.36[b] (2.64)
Type 4	46.85[a] (0.43)	46.28[a] (2.44)	43.88[a] (1.35)	
Percent of yield (%)				
Type 1	13.25[a] (0.23)	18.34[a] (7.18)	14.94[a] (7.46)	
Type 2	32.71[a] (2.88)	31.28[a] (6.39)	36.28[a] (7.18)	42.26[a] (2.42)
Type 3	2.71[a] (0.71)	2.17[a] (1.76)	2.22[a] (0.97)	57.74[b] (2.42)
Type 4	51.33[a] (1.95)	48.21[a] (2.55)	46.56[a] (2.12)	

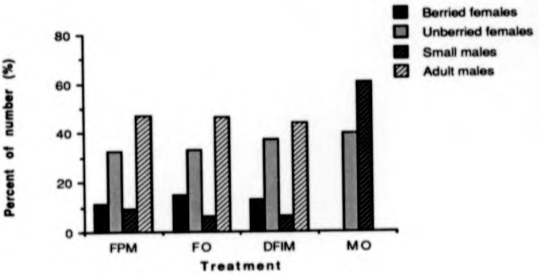
[1] Where types 1, 2, 3, and 4 refer to berried females, unberried females, small males, and adult males respectively.

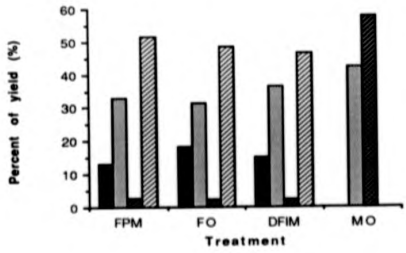
Figure 6.2.

Prawn sex-type as a percent of;

a) number, and

b) yield.


Berried females
Unberried females
Small males
Adult males
Percent of number (%)
80
60
40
20
0
FPM
FO
DFIM
MO
Treatment


Percent of yield (%)
60
50
40
30
20
10
0
FPM
FO
DFIM
MO
Treatment

6.3.5 Fish Survival and Production

Silver carp survival was 100% in all treatments. Growth rate and mean weight at harvest were highest in the FO ponds and lowest in the MO ponds (Table 6.5).

The fish stocked into the pond proper to control excess plant growth were also weighed at harvest. The silver carp doubled its stocking weight to reach 300 g and the two grass carp attained weights of 1.9 kg (from 0.7 kg) and 3.4 kg (from 2.9 kg).

6.3.6 Water Nutrient Concentrations and Ratios

Table 6.6 presents the concentration of each parameter. It is evident that the DO was similar in all treatments but that of MO in which the level was higher, at 7.6 rather than approximately 5.4 mg l^{-1}. Levels of TN, COD and CHL were highest in MO and lowest in FO regimes. TN ranged from 1.589 - 2.233 mg l^{-1}. The CHL concentration in MO was moderate to high, and more than double that in either FPM or FO which were quite low, whilst in DFIM the photosynthetic pigment level was moderate. TP values varied little between treatments, averaging approximately 103.0 µg l^{-1}. The levels of NH_3 and of NO_2 were below critical levels for prawns (Armstrong *et al.*, 1976, 1978). Values of NO_3 were very low in DFIM and MO, but higher in FO and FPM treatments. Because of the infrequency of sampling the nitrogenous compounds, only limited statistical analyses were performed on TN. There were no significant differences noted at any time for any parameter due to similar trends between treatments and high variance within treatments due at least in part to the low number of replicates. The parameter ratio CHL:TP was highest in MO, and higher in DFIM over FPM and FO. Figure 6.3 illustrates the concentration of TP and CHL at each sampling period, and suggests a positive relation between the parameters.

Table 6.5. Fish growth rate and harvest data; arithmetic mean and standard error.

Silver carp	FPM	FO	DFIM	MO
Growth rate ($g\ day^{-1}$)	0.68 (0.23)	0.86 (0.04)	0.69 (0.09)	0.34 (0.19)
Weight (g)	59.77 (19.92)	75.05 (3.68)	60.26 (6.97)	30.52 (16.72)
Survival (%)	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)	100.00 (0.00)

Table 6.6. Water quality parameters and CHL:TP ratio; arithmetic mean and standard error.

Parameter	FPM	FO	DFIM	MO
Dissolved oxygen ($mg\ l^{-1}$)	5.02 (0.24)	5.40 (0.31)	5.63 (0.44)	7.64 (0.40)
Total nitrogen ($mg\ l^{-1}$)[1]	1.743 (0.232)	1.589 (0.211)	2.101 (0.440)	2.233 (0.488)
Total ammonia ($\mu g\ l^{-1}$)[1]	97.2 (na)	84.2 (na)	25.2 (na)	62.5 (na)
Nitrite ($\mu g\ l^{-1}$)[1]	12.1 (12.1)	13.0 (11.2)	2.8 (0.0)	2.8 (2.8)
Nitrate ($\mu g\ l^{-1}$)[1]	327.6 (236.8)	221.8 (97.1)	48.0 (6.9)	64.9 (2.3)
Total phosphorus ($\mu g\ l^{-1}$)	100.1 (14.2)	102.1 (13.1)	116.0 (13.6)	94.3 (8.4)
Chemical oxygen demand ($mg\ l^{-1}$)	28.4 (2.8)	27.4 (2.7)	29.8 (2.9)	30.1 (2.7)
Chlorophyll ($\mu g\ l^{-1}$)	46.2 (10.4)	45.0 (10.5)	64.8 (15.5)	93.9 (24.3)
CHL:TP	0.41 (0.05)	0.38 (0.06)	0.48 (0.07)	0.88 (0.21)

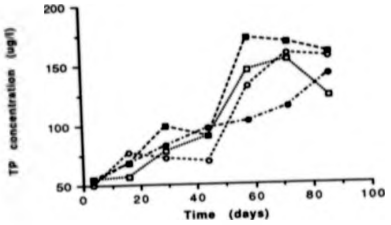
[1] = measured only on the last two sampling days

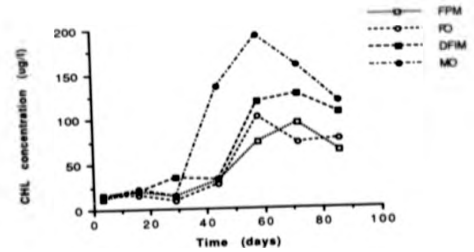
Figure 6.3.

Water concentrations of;

a) total phosphorus (TP), and

b) chlorophyll (CHL).


TP concentration (ug/l)
200
150
100
50
0
20
40
60
80
100
Time (days)


CHL concentration (ug/l)
200
150
100
50
0
20
40
60
80
100
Time (days)
FPM
FD
DFIM
MD

6.3.7 Water Quality Correlations and Regressions

Pearson correlations between TP, CHL and COD were constantly high (Table 6.7). Regression lines were quite similar between treatments, especially between FO and DFIM (Table 6.8). The quality of the fit of the data to the line, indicated by R^2, and the worth of the procedure of fitting that line, indicated by F, confirmed the value of the equations.

6.3.8 Total Bacteria:Water

Typical water samples stained with acridine orange and viewed with epifluorescence microscopy are illustrated in Plate 6.2. The bacterial count data are presented in Table 6.9 and Figure 6.4. At the commencement of the trial bacterial counts were very similar in all treatments. Over the experimental period to sampling day 58 inclusive, each treatment exhibited a general increase in the number of bacteria per ml. This upward trend was interrupted temporarily in FO, with a slight decrease on day 29, but recovery by day 44.

On day 72 the general increasing trend continued in FO, DFIM and MO, but a decrease was evident in FPM. At the next sampling on day 86, a decrease was also seen in DFIM and MO, whilst FO continued an upward trend. Throughout the experimental period, bacterial density was consistently highest in DFIM. Up to and including day 58, bacterial density was on average second highest in FPM, followed by MO, and lastly FO. Beyond day 58 FO increased rather rapidly so that it had more bacteria than either FPM or MO. Analyses of variance at each sampling time did not reveal any significant differences between treatments.

Plate 6.3 illustrates the stages in the image processing of photographic negatives of water samples. Table 6.10 and Figure 6.5 present the bacterial cell volume data. Volumes were similar between treatments at the beginning of the trial. Unlike the constant upward trend to day 58 evident in the count data, the cell volumes were inconsistent in each

Table 6.7. Pearson correlation coefficients between the water parameters total phosphorus (TP), chlorophyll (CHL), and chemical oxygen demand (COD).

Parameter	FPM		FO		DFIM		MO	
	TP	CHL	TP	CHL	TP	CHL	TP	CHL
CHL	0.836*[1]	-	0.848*	-	0.917*	-	0.800*	-
COD	0.924*	0.875*	0.846*	0.813*	0.904*	0.921*	0.821*	0.847*

[1] = F level of probability of 0.01

Table 6.8. Regression equations between the log of the water parameters total phosphorus (TP) and chlorophyll (CHL).

Treatment	Regression equation	R^2	F
FPM	CHL = -1.42 + 1.50 TP	0.67	0.000
FO	CHL = -2.09 + 1.81 TP	0.70	0.000
DFIM	CHL = -2.01 + 1.81 TP	0.83	0.000
MO	CHL = -3.79 + 2.83 TP	0.61	0.001

R^2 = R^2 adjusted
F = F statistic of probability

Plate 6.2.

Samples stained with acridine orange and viewed with epifluorescence microscopy;

a) water (magnification = 2025x; bar represents 5 µm), and

b) sediment (magnification = 1800x; bar represents 5 µm).

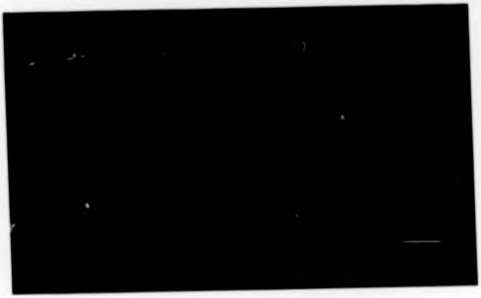


Table 6.9. Total bacterial count ($x10^6$ ml^{-1}) of water; arithmetic mean and standard error.

Sampling day	FPM	FO	DFIM	MO
3	4.72 (0.13)	5.04 (0.09)	4.97 (0.33)	4.40 (0.14)
16	6.36 (0.71)	5.45 (0.19)	8.15 (0.62)	7.05 (0.66)
29	9.63 (4.44)	5.21 (0.46)	12.32 (0.29)	8.00 (0.56)
44	7.70 (0.42)	7.80 (2.44)	12.51 (1.34)	7.26 (0.49)
58	12.49 (3.61)	9.03 (2.14)	17.44 (1.99)	10.25 (0.19)
72	10.86 (3.65)	13.98 (2.16)	19.05 (1.74)	13.13 (3.82)
86	10.87 (3.67)	14.17 (3.61)	16.20 (0.73)	11.98 (2.80)

Figure 6.4.
Total bacterial count ($x10^6$ ml^{-1}) of water.

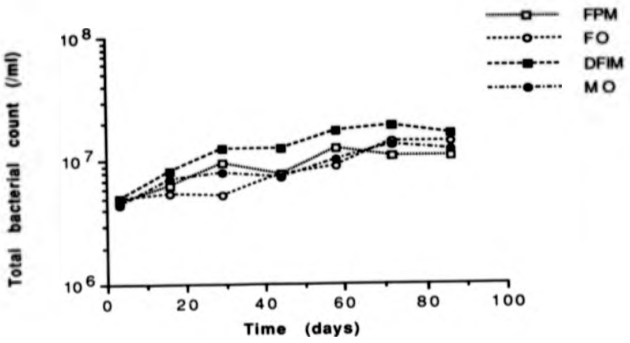

FPM
FO
DFIM
MO
Total bacterial count (/ml)
10^8
10^7
10^6
0
20
40
60
80
100
Time (days)

Plate 6.3.

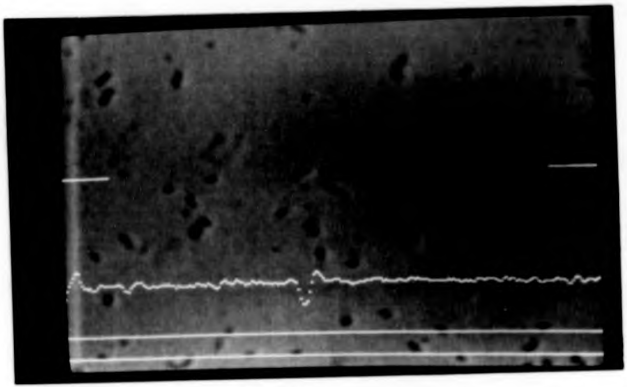
Image analysis processing of photographic negatives of water bacteria;

a) photographic negative,

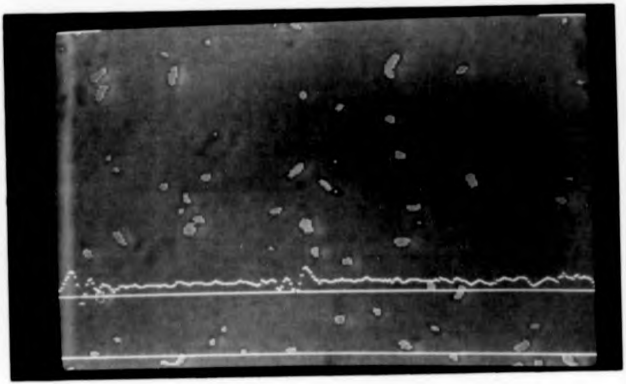
b) grey shade image enhanced and thresholded, showing oscillograph cursor position, oscillograph plot of grey levels, and upper and lower threshold cursor bars, and

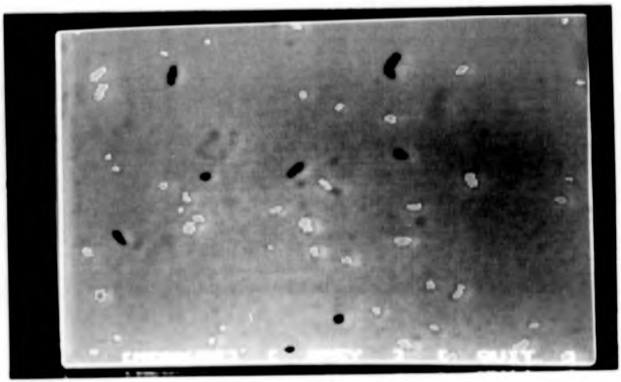
c) binary image with interactive thresholding

d) edited binary image indicating selected and measured bacteria in dark blue, and showing action options.



196 A



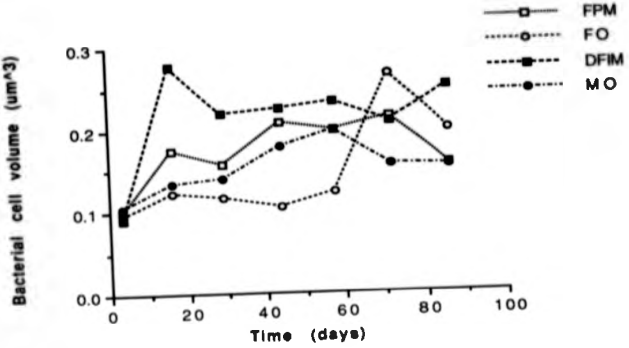


196 B

Table 6.10. Bacterial mean cell volume (μm^3) of water; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
3	0.098^a (0.004)	0.095^a (0.004)	0.092^a (0.004)	0.105^a (0.005)
16	0.175^a (0.015)	0.122^a (0.010)	0.277^b (0.025)	0.133^a (0.011)
29	0.157^b (0.012)	0.116^a (0.013)	0.219^c (0.017)	0.139^b (0.008)
44	0.207^b (0.019)	0.105^a (0.005)	0.225^b (0.015)	0.179^b (0.010)
58	0.198^b (0.019)	0.122^a (0.007)	0.233^c (0.009)	0.198^b (0.008)
72	0.212^b (0.016)	0.265^c (0.011)	0.207^b (0.009)	0.154^a (0.009)
86	0.155^a (0.010)	0.198^b (0.010)	0.249^c (0.013)	0.153^a (0.008)

Figure 6.5.
Bacterial mean cell volume (μm^3) of water.


FPM
FO
DFIM
MO
Bacterial cell volume (um^3)
0.3
0.2
0.1
0.0
0
20
40
60
80
100
Time (days)

treatment except MO where indeed they increased with time. Beyond day 58, rather extreme results were evident in FO, and to a lesser extent FPM, as can best be seen in Figure 6.5.

Analyses of variance indicated that up to and including day 58, FO bacteria were smallest in volume, significantly and consistently so from day 16 as compared to DFIM and from day 29 compared to both MO and FPM. Beyond day 58, FO bacteria had volumes significantly larger than MO and FPM, and on day 72 they were even significantly larger than DFIM bacteria. The latter had the largest volumes with the exception noted above. DFIM volumes were significantly larger than all others on days 16, 29, 58, and 86. The very rapid increase in DFIM bacterial volumes at the start of the trial is notable. FPM bacteria were almost always larger than MO bacteria, but only significantly so on day 72.

The product of bacterial count and cell volume are presented as biovolume in Table 6.11 and Figure 6.6. Initial biovolume values were similar in all treatments. As best seen in the figure, biovolume clarified and indicated more consistent and distinct trends between treatments than did either count or volume. All treatments behaved in parallel with a generally positive slope up to day 58 inclusive, beyond which FO was markedly dissimilar. The constant trends continued until the final sampling on day 86 between DFIM and MO, and with FPM until day 72. In general, DFIM biovolume was largest, followed by FPM, then MO, and lastly until day 72, FO.

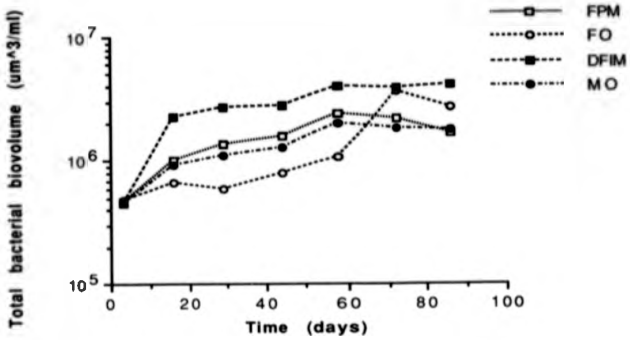
ANOVAs revealed that DFIM biovolume was significantly greatest throughout the experiment. FO biovolume was significantly smallest up to and including day 58, after which it became significantly larger than both MO and FPM. The biovolume of FPM, although generally larger than that of MO, was rarely significantly so.

Table 6.11. Total bacterial biovolume ($x10^6 \mu m^3\ ml^{-1}$) of water; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference ($P<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
3	0.46^{a} (0.02)	0.48^{a} (0.02)	0.46^{a} (0.02)	0.46^{a} (0.02)
16	1.10^{b} (0.10)	0.67^{a} (0.05)	2.26^{c} (0.20)	0.94^{b} (0.08)
29	1.35^{b} (0.08)	0.60^{a} (0.07)	2.69^{c} (0.21)	1.11^{b} (0.06)
44	1.59^{c} (0.14)	0.79^{a} (0.04)	2.79^{d} (0.18)	1.29^{b} (0.08)
58	2.40^{b} (0.18)	1.09^{a} (0.06)	4.00^{c} (0.17)	2.03^{b} (0.08)
72	2.21^{a} (0.15)	3.61^{b} (0.16)	3.91^{c} (0.16)	1.86^{a} (0.09)
86	1.68^{a} (0.09)	2.69^{c} (0.14)	4.04^{d} (0.21)	1.79^{b} (0.09)

Figure 6.6.

Total bacterial biovolume ($x10^6 \mu m^3\ ml^{-1}$) of water.


Total bacterial biovolume (um^3/ml)
10^7
10^6
10^5
0
20
40
60
80
100
Time (days)
FPM
FO
DFIM
MO

As a function of biovolume and a constant, biomass exhibited the same trends and significant differences as biovolume. Values for biomass are presented in Table 6.12.

Table 6.13 presents maximum and minimum values of cell and population sizes for each treatment over the entire experimental period. Minimum values between treatments are quite alike, tending to represent the similar initial characteristics. Maximum values for width also remained quite constant between treatments suggesting little capacity for growth in that dimension. Of particular note are the much smaller maximum values in the FO treatment for the parameters of area, perimeter and length. FPM maxima for volume and biovolume are especially large, somewhat unexpectedly given the much less remarkable mean values noted in the previous tables and figures. Maximum values for biovolume and biomass are intermediate in DFIM, and equally small in FO and MO. For both biovolume and biomass maxima, there exists approximately a 100% increase from either MO or FO to DFIM, and a further 50% increase to FPM.

Due to the exceptional values for FO on days 72 and 86, resulting from both size and count data, new range values were calculated up to and including day 58 only. The maximum values of FO alone were affected, but all treatments are presented in Table 6.14. FO values for area, perimeter, length, biovolume and biomass decreased. This re-evaluation resulted in maximum population indices 400% greater in FPM as opposed to FO.

6.3.9 Total Bacteria:Sediment

Typical sediment samples stained with acridine orange and viewed with epifluorescence microscopy are illustrated in Plate 6.2. Table 6.15 and Figure 6.7 present the sediment bacterial counts. Initial counts were quite similar between treatments. In MO and DFIM regimes, a trend of increasing density with time was evident. FO and FPM counts fluctuated, and a very high count was recorded in FO on day 30. On days 59 and 87,

Table 6.12. Total bacterial biomass (µgC l^{-1}) of water; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
3	143.41^{a} (5.64)	148.65^{a} (5.93)	141.59^{a} (6.36)	142.37^{a} (6.36)
16	341.59^{b} (30.44)	206.74^{a} (16.34)	699.94^{c} (62.88)	289.70^{b} (23.19)
29	419.65^{b} (24.11)	185.32^{a} (20.98)	832.47^{c} (63.87)	344.06^{b} (19.10)
44	491.40^{c} (43.07)	245.83^{a} (11.39)	866.23^{d} (56.64)	400.17^{b} (23.12)
58	743.90^{b} (56.63)	338.28^{a} (18.59)	1240.17^{c} (52.56)	629.43^{b} (23.71)
72	686.00^{a} (47.52)	1120.23^{b} (48.15)	1212.84^{c} (49.86)	574.90^{a} (28.47)
86	521.29^{a} (28.83)	834.82^{c} (42.09)	1253.68^{d} (65.29)	553.39^{b} (27.43)

Table 6.13. Range of bacterial cell size and population size in water; minimum and maximum.

Dimension	FPM	FO	DFIM	MO
Area (μm^2)	0.040 - 7.166	0.031 - 4.811	0.054 - 9.133	0.041 - 8.149
Perimeter (μm)	0.687 - 40.580	0.638 - 28.620	0.807 - 41.820	0.738 - 37.460
Width (μm)	0.173 - 1.661	0.161 - 1.527	0.170 - 1.422	0.123 - 1.308
Length (μm)	0.219 - 20.080	0.219 - 14.110	0.257 - 20.650	0.235 - 18.510
Volume (μm^3)	0.005 - 7.303	0.004 - 3.243	0.007 - 4.399	0.004 - 3.277
Biovolume ($x10^4 \mu m^3 ml^{-1}$)	0.03 - 64.85	0.03 - 22.98	0.04 - 46.91	0.02 - 20.94
Biomass ($\mu gC\ l^{-1}$)	8.76 - 20103.70	9.37 - 7122.75	11.13 - 14541.85	7.45 - 6491.41

Table 6.14. Range of bacterial cell size and population size up to and including day 58 in water; minimum and maximum.

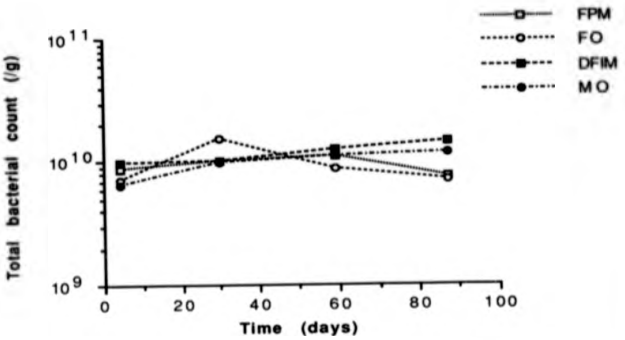
Dimension	FPM	FO	DFIM	MO
Area (μm^2)	0.040 - 7.166	0.031 - 2.981	0.054 - 9.133	0.041 - 8.149
Perimeter (μm)	0.687 - 40.580	0.638 - 8.577	0.807 - 41.820	0.738 - 32.530
Width (μm)	0.173 - 1.661	0.161 - 1.527	0.170 - 1.422	0.123 - 1.308
Length (μm)	0.219 - 20.080	0.219 - 4.091	0.257 - 20.650	0.235 - 15.970
Volume (μm^3)	0.005 - 7.303	0.004 - 3.243	0.007 - 4.399	0.004 - 3.277
Biovolume ($x10^6 \mu m^3\ ml^{-1}$)	0.03 - 64.85	0.03 - 16.00	0.04 - 46.91	0.02 - 20.94
Biomass ($\mu gC\ ml^{-1}$)	8.76 - 20103.70	9.37 - 4958.47	11.13 - 14541.85	7.45 - 6491.41

Table 6.15. Total bacterial count ($x10^9$ g^{-1}) of sediment; arithmetic mean and standard error.

Sampling day	FPM	FO	DFIM	MO
4	8.70 (3.07)	7.14 (1.40)	9.84 (4.06)	6.55 (0.12)
30	10.01 (2.93)	15.36 (4.46)	10.04 (4.14)	9.91 (0.25)
59	10.94 (2.68)	8.71 (0.10)	12.42 (6.30)	11.15 (1.35)
87	7.54 (0.15)	7.20 (1.98)	14.58 (2.23)	11.75 (1.48)

Figure 6.7.

Total bacterial count ($x10^9$ g^{-1}) of sediment.


FPM
FO
DFIM
MO
Total bacterial count (/g)
10^{11}
10^{10}
10^{9}
0
20
40
60
80
100
Time (days)

counts were greatest in DFIM, followed by MO, FPM and finally FO. Analyses of variance did not indicate any significant differences between treatments.

Figure 6.8 illustrates a typical set of projected sediment bacterial outlines for image analysis. Bacterial cell volumes are presented in Table 6.16 and Figure 6.9. Volumes were significantly dissimilar between treatments at the start of the experimental period. During the first 30 days, volumes were seen to both increase and decrease in different treatments. Values for day 30 were more similar to one another than they had been at day 4. After day 30 there was a general decrease in volume in every treatment. A constant decreasing trend throughout was evident in MO. DFIM and FPM had significantly larger cell volumes over MO and FO on days 59 and 87.

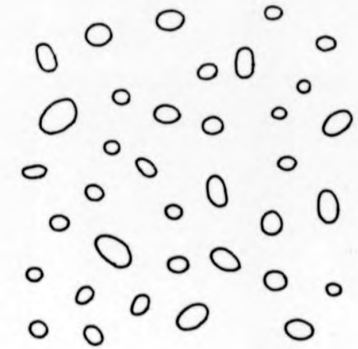
Table 6.17 and Figure 6.10 present the bacterial biovolume data. Initial values were similar between treatments. Fluctuations over time were evident in FPM and FO, the latter having a large increase on day 30 followed by a large decrease on day 59. MO values were quite constant, whilst those of DFIM increased over time. With the exception of day 30, FO data was significantly smallest. On days 59 and 87, DFIM was significantly largest. FPM and MO held intermediary positions, with MO generally lower than FPM.

Biomass exhibited the same trends and significant differences as biovolume. Values for biomass are presented in Table 6.18.

Table 6.19 presents maximum and minimum values of cell and population sizes for each treatment over the entire experimental period. Minimum values between treatments are quite alike, with the exception of biovolume and biomass which are extraordinarily low in FPM. Maximum values for width remained quite constant between treatments. Maximum values for area,

Figure 6.8.

Typical outlines of projected bacteria used for image analysis of sediment samples (magnification = 20,000x).



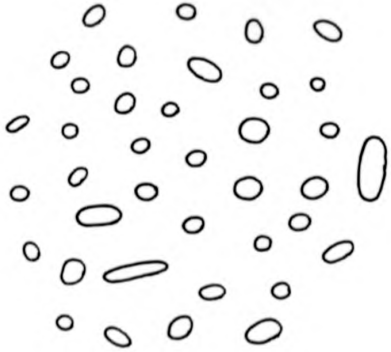


Table 6.16. Bacterial mean cell volume (μm^3) of sediment; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
4	0.090^{b} (0.004)	0.099^{bc} (0.005)	0.071^{a} (0.003)	0.107^{c} (0.005)
30	0.115^{b} (0.006)	0.102^{ab} (0.005)	0.116^{ab} (0.006)	0.097^{a} (0.004)
59	0.105^{c} (0.006)	0.057^{a} (0.003)	0.113^{c} (0.005)	0.081^{b} (0.004)
87	0.091^{b} (0.004)	0.067^{a} (0.003)	0.094^{b} (0.004)	0.065^{a} (0.002)

Figure 6.9.
Bacterial mean cell volume (μm^3) of sediment.

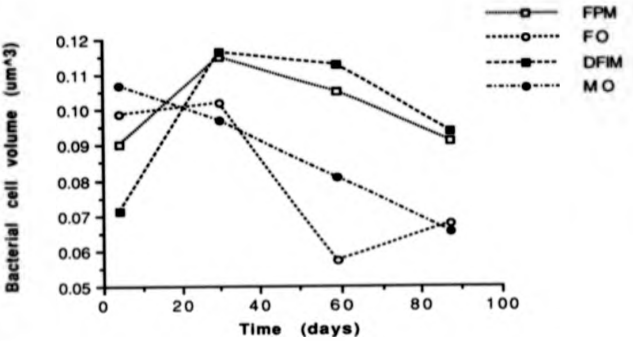

Bacterial cell volume (um^3)
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0
20
40
60
80
100
Time (days)
FPM
FO
DFIM
MO

Table 6.17. Total bacterial biovolume ($x10^9 \mu m^3\ g^{-1}$) of sediment; arithmetic mean and standard error. (Different superscripts in common rows denote statistical significance ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
4	0.77^a (0.04)	0.70^a (0.03)	0.68^a (0.03)	0.70^a (0.04)
30	1.19^a (0.07)	1.50^b (0.07)	1.00^a (0.04)	0.96^a (0.04)
59	1.17^c (0.08)	0.50^a (0.02)	1.30^c (0.06)	0.89^b (0.05)
87	0.69^b (0.03)	0.47^a (0.02)	1.37^d (0.05)	0.75^c (0.02)

Figure 6.10.
Total bacterial biovolume ($x10^9 \mu m^3\ g^{-1}$) of sediment.

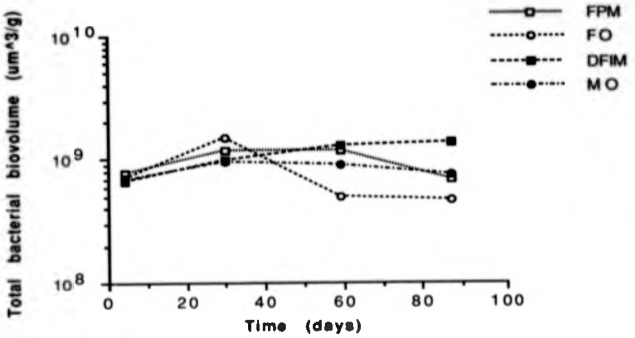

Total bacterial biovolume (um^3/g)
10^{10}
10^{9}
10^{8}
0
20
40
60
80
100
Time (days)
FPM
FO
DFIM
MO

Table 6.18. Total bacterial biomass ($\mu gC\ g^{-1}$) of sediment; arithmetic mean and standard error. (Different superscripts in common rows denote statistical difference ($F<0.05$) and superscripts in uncommon rows are unrelated).

Sampling day	FPM	FO	DFIM	MO
4	238.02^{a} (11.11)	217.30^{a} (10.18)	209.21^{a} (8.86)	216.95^{a} (10.97)
30	368.11^{a} (22.26)	463.28^{b} (20.58)	309.96^{a} (12.37)	297.46^{a} (12.33)
59	363.74^{c} (25.27)	154.71^{a} (7.34)	402.47^{c} (19.33)	276.24^{b} (14.68)
87	213.09^{b} (9.53)	145.98^{a} (6.68)	425.17^{d} (16.81)	233.52^{c} (7.22)

Table 6.19. Range of bacterial cell size and population size in sediment; minimum and maximum.

Dimension	FPM	FO	DFIM	MO
Area (μm^2)	0.057 - 1.524	0.062 - 0.979	0.061 - 1.609	0.064 - 1.153
Perimeter (μm)	0.876 - 6.839	0.894 - 4.727	0.878 - 8.208	0.912 - 6.564
Width (μm)	0.210 - 0.889	0.234 - 0.804	0.199 - 0.794	0.234 - 0.809
Length (μm)	0.318 - 3.145	0.313 - 2.218	0.292 - 3.860	0.321 - 3.107
Volume (μm^3)	0.009 - 0.673	0.010 - 0.526	0.011 - 0.688	0.011 - 0.683
Biovolume ($x10^9 \mu m^3\ g^{-1}$)	0.05 - 9.16	0.09 - 4.72	0.12 - 5.92	0.12 - 4.89
Biomass ($\mu gC\ g^{-1}$)	14.98 - 2840.72	27.67 - 1462.93	35.75 - 1835.16	36.00 - 1517.06

perimeter, length and volume were lowest in FO and highest in DFIM. There was almost a 100% increase in the maxima of each of biovolume and biomass from FO to FPM. MO biovolume and biomass maxima were also low.

6.3.10 Viable Bacteria:Water

Viable count data are presented in Table 6.20 and Figure 6.11. Initial values at the commencement of the trial were very similar between treatments. DFIM and MO each exhibited a constant decreasing trend over time. FPM count increased slightly at the second sampling before decreasing at the third sampling. A dramatic increase in FO count was evident at the second sampling, followed by an almost equally dramatic decrease. Values for FO counts were roughly 400% and 100% greater on days 44 and 86 respectively than those in any other treatments on the same days.

Analyses of variance revealed no statistical difference at either sampling day 3 or 86. However a significant difference existed on day 44 due to the high count value for the FO treatment.

6.3.11 Nutritional Data

The proximate analysis of the prawn diet and the chicken manure are presented in Table 6.21, and that for the fish and shrimp meals in Appendix 6.9.

6.4 DISCUSSION

Prawn weights at sampling and at harvest, and prawn growth rate, were overall quite low. The present growth rates of 0.03 - 0.10 g day^{-1} were much smaller than 0.11 - 0.23 g day^{-1} for Experiment 1, which however was stocked with younger prawns, and also smaller than 0.15 - 0.17 g day^{-1} for Experiment 2, which had similar initial stock size as the present experiment. Hence it would seem possible that an enclosure induced space

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Table 6.20. Viable bacterial count ($x10^3$ ml^{-1}) in water; arithmetic mean and standard error.

Sampling day	FPM	FO	DFIM	MO
3	9.45^a (3.65)	10.00^a (4.70)	10.65^a (0.50)	11.90^a (3.22)
44	10.80^a (0.10)	51.50^b (9.20)	8.52^a (0.35)	7.90^a (0.33)
86	5.15^a (0.12)	13.75^a (3.25)	7.39^a (3.72)	4.40^a (1.87)

Figure 6.11.
Viable bacterial count ($x10^3$ ml^{-1}) of water.

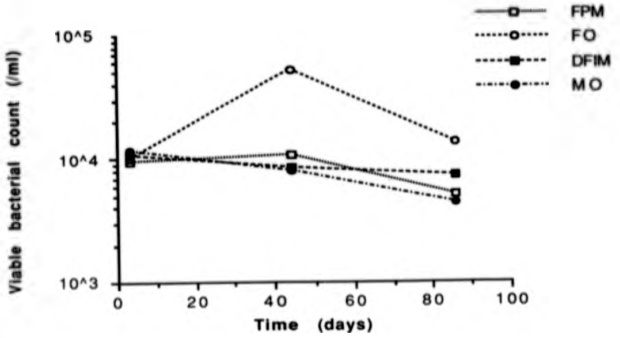
FPM
FO
DFIM
MO
10^5
10^4
10^3
Viable bacterial count (/ml)
0
20
40
60
80
100
Time (days)

Table 6.21. Proximate analysis of the prawn diet and the chicken manure; all data expressed on a percent dry matter basis except percent moisture.

Component	Prawn diet	Chicken manure
Percent moisture (%)	8.78	7.67
Percent crude protein (%)	31.89	18.83
Percent crude lipid (%)	5.57	0.81
Percent ash (%)	15.88	39.66
Percent crude fibre (%)	3.62	12.62
Percent crude carbohydrate (%)	46.66	40.70
Gross energy (kcal g^{-1})	416.27	276.25

effect on prawn growth was in operation in the present trial. This finding is consistent with that for DFIM prawns in Experiment 3, but given that FO prawns in that experiment actually grew better in the enclosures as opposed to in a comparable pond trial (Experiment 1) no consistent effect could be concluded.

The initial stock in Experiment 4 of 0.67 g (3.52 cm) per 0.1 m^2 did not seem to be affected by the enclosures nor did growth up to Day 21. DFIM prawns in Experiment 4 had a mean weight of 2.32 g on Day 21 as opposed to 2.15 g for pond reared prawns sampled on Day 21 from Experiment 2 treatment M14. However by Day 35 the enclosure prawns were 3.79 g as compared to 5.75 g for the pond reared prawns suggesting that the possible space effect occurred soon after Day 21. Translating Experiment 4 DFIM results to standing biomass on the assumption that at both Days 21 and 35 there was 85.5% survival, suggests that at 18.6 g m^{-2} (Day 21) there was no space effect as opposed to 32.4 g m^{-2} (Day 35) at which point there may have been such an effect. The data of Experiment 3 did not indicate consistency concerning the question of a space effect when biomass density was 11 - 14 g m^{-2}, however, Juarez *et al.* (1987) indicated the critical point in their studies with *M. rosenbergii* may have been between 2 - 21 g m^{-2}.

Alternative to the possibility of a space effect, the mesocosms may have influenced prawn growth by affecting the frequency with which prawns encountered feed. However, the frequency of feed encounter probably would have increased in the restricted area and hence promoted prawn growth.

Another possibility is that the enclosures affected prawn behaviour, particularly as aggregation or dispersion. According to Peebles (1979), some individual *M. rosenbergii* demonstrate wide nighttime ranging activity. Male prawns roamed over a significantly smaller area than did females, mean values from sonic tagging experiments were 1.55 m^2 and 29.55

m^2 respectively per eight days. Similarly the mean cumulative distance traversed over a 2-night period was significantly different between the sexes, 6.72 m for males and 92.81 m for females. Peebles (1979) attributed this difference to courtship and reproductive behaviour. Females tend to search for mates in order to gain protection during moulting, and in natural riverine habitats to migrate after successful mating. Indeed female nocturnal movement increased as the time to ecdysis approached. Males tend to roam less, and make depressions in the sediment in which they and their females gain some protection. Hence males established a stationary home range and base to which they returned whereas females were less settled and had a shifting home range.

Furthermore, Peebles (1979) described the typical nocturnal pattern of travel in both sexes as toward the closest pond bank and then parallel to it for 0 - 5 m. Moult related behaviour was also identified by the aggregation of animals near ecdysis in areas of deep, soft mud. Intermoult animals were infrequently found in this microhabitat and the preference by the premoult animals was probably protective. Such a shift in microhabitat preference has been noted for only a few other crustaceans (Hazlett *et al.* in Peebles, 1979; Jachowski in Peebles, 1979).

The present enclosures prevented both long distance unidirectional movement typical of migration and any travel toward the pond banks. However, with an internal area of 10 m^2, multidirectional travel probably was not hampered as even the most adventurous female in Peebles (1979) experiment travelled a mean daily distance of 7.84 m^2. Furthermore, as the habit of extensive roaming is a female phenomenon (Peebles, 1979), an inability of movement should be most apparent in the female population whereas in the present experiment it was the male population which was less similar to pond raised males. An absolute requirement to approach the pond bank, although possible, would seem unlikely as it would impose a falsely high density increasing the possibility of aggression, injury, and

mortality. Furthermore, at least for males cultured in large ponds, it would seem that roaming tendencies would not greatly advance them in the direction of the bank relative to the size of the pond. Finally, although the benthos of the present experiments were not analysed for physical particle size, they were typical of the other ponds on site so that there would have been certain areas which provided some soft sediment as preferred by moulting individuals. Hence, there is little support for the theory of a specific aggregation/dispersion effect on the prawn population caused by the mesocosms.

The argument in favour of the space effect is supported by documented evidence of the phenomenon in finfish. Several fish species including *C. idella*, *Tilapia mossambica* and *Puntius gonionotus*, have each illustrated reduced mean growth rates, weights at harvest, and yield, when stocked at similar densities in small as opposed to large ponds (TFCRI, 1960, 1963, 1965; Chen and Prowse, 1968). Chen and Prowse (1968) reported increasing yields of *T. mossambica* of 242, 460, and 803 kg ha^{-1} $six\ months^{-1}$ with increasing pond sizes of 40.5 m^2, 405 m^2, and 4050 m^2. The differences in yield were attributed to increasing mean weight rather than survival. Similarly, Zhang *et al.* (1987) concluded that ponds stocked with *C. idella*, *H. molitrix*, *Aristichthys nobilis* and *Cyprinus carpio* had a critical area of 1334 m^2 (adapted from 2 mu), below which fish yield was much reduced. Although large ponds appear favourable for both fish and prawn growth, the efficiency of harvesting a very large area is lower (Lee and Wickins, 1992), so that optimum pond size must be determined as a balance of several factors.

Prawn survival in Experiment 4 was high in every treatment suggesting that the enclosures may have afforded some protection from predators. However given that the highest survival occurred concomitantly with the smallest prawns (MO) is evidence for the previously noted phenomenon of an inverse relation between survival and size, which thus implies that part of the

good survival in this experiment may have been due to the small mean prawn size. Biomass and yield of Experiment 4 were, with the exception of MO, moderate and approximately 65% of that achieved in Experiment 2 under similar stocking conditions. As discussed above, this may have been due to a space effect.

Variation between the treatments which received feed may have been masked by this same effect. However the extremely poor prawn data from the MO regime indicated the value of the formula pelleted feed in prawn growth and yield. No prawns in the MO treatment developed either into adult males or into berried females. Unusually berried females outweighed adult males in FPM and FO, probably due more to the small mean size of males compared with a large mean size of females when comparison is made with harvest weights in the previous experiments. This would suggest that a space effect may influence males more given that female growth slows once sexual maturity is achieved (Cohen *et al.*, 1981). Ra'anan and Cohen (1984) described increased population size asymmetry with decreased competition (as stock density) between individuals, a trend which was opposite of that described by Wohlfarth (1977) for various finfishes under restricting growth conditions. However as before, adult males were responsible for a high percent of harvest number and yield.

A comparison of dawn DO concentrations suggested a stronger relation to prawn biomass than to algal biomass. The similar DO levels in FPM, FO, and DFIM may have reflected similar prawn growth rather than pond productivity, especially given the higher DO in the MO treatment which had much higher chlorophyll concentrations and thus overnight algal respiration, but very much smaller prawn production. High TN and quite high TP concentrations suggested great capacity to support phytoplankton. The low values for all the inorganic nitrogen compounds indicated rapid

utilisation of the available nutrients, and certainly chlorophyll concentrations were moderate to high. Unfortunately the lack of nitrogen data prior to the final two sampling days prevents determination of whether or not chlorophyll was more closely tied to phosphorus or nitrogen. The correlations between CHL and TP were high but as in Experiments 1 and 2 may have been due to an intercorrelation between TP and TN. Similarly the regression equations for CHL, although very strong with the single predictor of TP may have been improved upon with the inclusion of TN. The high ratio between CHL:TP in MO indicated that this treatment was the most efficient at algal production per unit of phosphorus. The ratios of TN:TP as can be very roughly derived from the mean values of Table 6.6, are consistently > 15:1 thereby suggesting possible phosphorus limitation (see Chapter 3). Nonetheless, available nitrogen compounds in DFIM were sufficiently low to suggest nitrogen limitation (see Chapter 4), even at the end of the fertilisation trial when their analyses were possible. Temporal shifts in limiting environmental factors were likely (see Chapter 4).

Water bacteria volume, particularly in FO, were noted as unusual after the sampling on day 58. The nature of this effect will be discussed below. However comparative discussion will continue with consideration for the data prior to sampling on day 72. Water bacterial counts were within previously reported ranges and may be illustrative of general trophic state as Pedrós-Alió and Guerrero (1991) suggested densities of 10^6 cells ml^{-1} and 10^7 cells ml^{-1}, reflected very oligotrophic and eutrophic systems respectively. Mean cell volumes, also similar to other literature reports (Fry, 1988), were minimal in FO enclosures and maximal in DFIM to day 58. Volume may be interpreted as generally indicative of bacterial activity in that Krambeck and Krambeck (1984) reported that larger cells are more actively growing and dividing. In all treatments bacterial biomass grew very slowly given the potential for increasing several times within a few days (Güde, 1989). The slow doubling rate could be interpreted as poor

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population growth due to insufficient carbon or oxygen, but given the organic inputs and recorded levels of dissolved oxygen this would seem most unlikely. Grazing, as a major bacterial loss factor (Güde, 1989), probably balanced growth and prevented a rapid increment in count and biomass. A comparison between treatments on day 58 indicated DFIM biomass as 3.7 times that of FO, 2.0 times that of MO, and 1.7 times that of FPM. Given the superiority of DFIM as based on bacterial cell size as well as count, extending the theory of Krambeck and Krambeck (1984) would suggest that the DFIM biomass was not only largest but also most active.

Sediment bacterial counts in the DFIM and MO treatments illustrated a general increase with time, but more erratic values were noted for the feed dominated trials, especially FO. The dramatic rise and subsequent fall in FO bacterial count may have resulted from overfeeding during the first month of the experiment in that pellet accumulating on the pond benthos may have served as substrate for bacterial association. Sediment cell volumes, as in pond water, were generally greatest in the DFIM treatment. Also sediment biomass of DFIM was greatest on later sampling days, but the dramatic early counts of FO initially raised the FO biomass above DFIM.

Viable counts of water samples by the culture technique employed were approximately 10^3 times lower than water total counts, a result typically reported when data using culture and direct counting methods are reported simultaneously (Jones, 1977; van Es and Meyer-Reil, 1983; see Atlas and Bartha, 1987). In no treatment were similar trends between viable counts and total counts evident supporting the view that viable techniques are very limiting, particularly so in systems which may be detrital driven. This limitation originates from the culture conditions of the viable method which possess, to varying degrees of specificity, factors which may effect survival and limit growth on the basis of aerobism, medium composition and substrate concentration, temperature preparation

procedures, incubation conditions and the period of incubation. Furthermore, dilution associated with viable count procedures may result in a loss of cells from the sample should there be any adherence to the pipettes or tubes, and bacterial clumping on the plate may cause undercounting.

The extreme water bacterial cell volume in FO after day 58, was most likely to have been primarily a function of treatment. Given the reported effect of manure in stimulating microbial productivity (Moriarty, 1986; Schroeder, 1978), and the selective grazing by protozoa, irrespective of predator size over a wide range of both flagellates and ciliates, on larger bacterioplankton cells (Andersson *et al.*, 1986; Gonzalez *et al.*, 1990), it follows that the FO treatment should have generally fewer protozoa, hence less cropping of larger bacteria, and thus an increased planktonic bacterial mean size. For the first two months of the experiment the protozoal population may have been maintained in the FO treatment by pond preparation procedures and the proposed initial overfeeding which would have provided organic matter to support balanced food web dynamics. Later as these food resources diminished, the protozoal population may have decreased, causing an increase in the bacterial mean cell size.

A possible additional or alternative explanation for an increase in bacterial volume may be associated with an increase in storage compounds accompanying a decrease in nutrient resources. Pedrós-Alió *et al.* (1985) reported an increase in cell volume, influenced by the accumulation of the storage compound poly-B-hydroxybutyrate (PHB), during nitrogen limitation in cell culture of *Alcaligenes eutrophus*. Similarly, Nickels *et al.* (1979) reported an increase in PHB estuarine detritus exposed to unbalanced growth conditions, but not, an accompanying increase in biomass. The content of certain important storage polymers has been proven to vary not only on a seasonal basis (Pedrós-Alió *et al.*, 1990), but also during the diel cycle (Van Gemerden *et al.*, 1985), and their use as indicators of

bacterial physiological status would require detailed calibration (Pedrós-Alió *et al*, 1990). Given the likely presence, in the prawn enclosures, of many varied bacterial populations each with different growth requirements, it would seem unlikely that there was an accumulation of storage compounds sufficient to affect a dramatic increase in population mean cell volume of a single treatment (FO), particularly at a time when the TP and CHL levels were not especially outstanding relative to a least one other treatment (FPM; Figure 6.3).

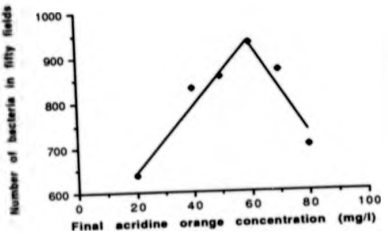
In summary, the significantly lower growth and production of MO prawns provided evidence for the benefit of feed application. However, possible overfeeding in FO during the early stages of the experiment may have caused an initial dramatic increase in sediment bacterial counts. In order to clarify the role of feed, simultaneous measurements of both microbial population growth and loss, particularly as grazing, are required. Moriarty (1987) recommended the determination of bacterial productivity and indicated that only recently have techniques developed to allow satisfactory measurement of heterotrophic bacterial growth rates. Furthermore, the recent identification of size selective grazing on bacterial populations in the natural environment (Andersson *et al.*, 1986; Gonzalez *et al.*, 1990), and of size related activity (Krambeck and Krambeck, 1984), underlines the necessity to quantify cropping in studies on the role of organic matter and food web dynamics.

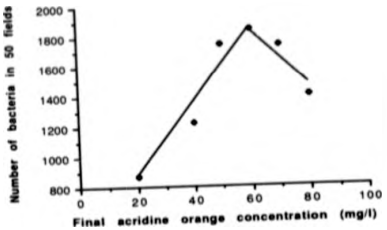
Appendix 6.1.

Preliminary test to determine the optimum final acridine orange concentration for;

a) water, and

b) sediment.


Number of bacteria in fifty fields
1000
900
800
700
600
0
20
40
60
80
100
Final acridine orange concentration (mg/l)


Number of bacteria in 50 fields
2000
1800
1600
1400
1200
1000
800
0
20
40
60
80
100
Final acridine orange concentration (mg/l)

Appendix 6.2. Image analysis command procedure for water samples using Task Programming Language on the Solitaire Plus.

```
Live                          (captures image from negative)
High Pass                     (gives a sharper variation
                               in grey shades)
Number 8                      (defines a number for the
                               following enhancement)
  Smear_Num                   (creates a second image by
                               averaging neighbouring pixels)
  Subtract With Offset        (subtracts second image, which
                               is a background, from first)
Interactive Threshold         (allows the user to alter the
                               thresholding levels)
Edit Threshold                (entry to editing menu)
  Reduce Binary Noise         (entry to reducing binary noise
                               menu)
    Kill Small                (removes tiny specks of thresholded
                               red in blue areas: <2 minimum)
  Draw in Red                 (allows the user to draw in red
                               thereby thresholding manually)
  Draw in Blue                (allows the user to draw in blue
                               thereby unthresholding manually)
Measure Objects               (measures objects at the users
                               selection)
Results                       (entry to results menu)
  Save Results                (saves results onto floppy disk)
Confirm Clear                 (clears the temporary memory in
                               the processor to allow for the
                               next live image)
```

Appendix 6.3. Image analysis command procedure for sediment samples using Task Programming Language on the Solitaire Plus.

```
Live                          (captures image from drawing)

Define Background             (defines a white page background;
                               only required to reenter daily)
Shade Correct                 (adjusts the image by removing
                               the stored background)
Interactive Threshold         (allows the user to alter
                               thresholding levels;rarely used)
Edit Threshold                   (entry to editing menu)

  Draw in Red                 (allows the user to draw in red
                               thereby thresholding manually)
  Draw in Blue                (allows the user to draw in blue
                               thereby unthresholding manually)
  Erode Without Splitting     (effectively narrows the red
                                  thresholded line of pixels)
  Fill Holes                  (thresholds all pixels enclosed
                               within a thresholded ring)
Measure All and Show          (measures all thresholded objects
                               indicating such one by one)
Results                       (entry to results menu)

  Save Results                (saves results onto floppy disk)

Confirm Clear                 (clears the temporary memory in
                               the processor to allow for the
                               next live image)
```

Appendix 6.4. Climatic conditions during the experimental period day 1 - day 100 (27th July 1990 - 3rd November 1990).

Parameter	Mean and standard error	Minimum	Maximum
Relative humidity (%)	95 (1)	87	100
Maximum air temperature (°C)	32.0 (0.1)	28.8	35.1
Minimum air temperature (°C)	22.7 (0.1)	20.6	24.0
Rainfall (mm day^{-1})	6.02 (1.39)	0.00	88.40
Rainfall duration (hr day^{-1})	0.73 (0.12)	0.00	5.10
Sunshine duration (hr day^{-1})	5.48 (0.27)	0.00	10.15
Wind speed (m s^{-1})	0.88 (0.02)	0.40	1.51
Evaporation (mm day^{-1})	4.27 (0.15)	1.37	8.23
Water balance[1] (mm day^{-1})	+1.68 (1.37)	-7.22	+84.4

[1] water balance (mm day^{-1}) = rainfall (mm day^{-1}) - evaporation (mm day^{-1})

Appendix 6.5. Selected climatic conditions during each period between water sampling days; arithmetic mean and standard error.

Time period	Sunshine duration (hr day^{-1})	Water balance[1] (mm day^{-1})
Day 3 - 15	5.48 (0.70)	+1.35 (3.75)
Day 16 - 27	6.00 (0.70)	-3.29 (0.95)
Day 28 - 43	6.16 (0.69)	-2.33 (1.13)
Day 44 - 57	5.90 (0.72)	-0.95 (2.25)
Day 58 - 71	4.31 (0.85)	+6.25 (5.41)
Day 72 - 85	6.94 (0.48)	-0.18 (1.67)

[1] water balance (mm day^{-1}) = rainfall (mm day^{-1}) - evaporation (mm day^{-1})

Appendix 6.6. Cumulative TN[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FPM	FO	DFIM	MO
Day 1 - 11	0.51	0.51	0.34	-
Day 1 - 23	1.63	1.28	1.28	0.35
Day 1 - 37	3.32	2.61	2.68	0.70
Day 1 - 51	4.51	3.45	3.57	1.05
Day 1 - 65	6.07	4.61	4.77	1.40
Day 1 - 79	8.00	6.10	6.07	1.75
Day 1 - 99	9.94	7.55	7.33	2.11

[1] based on percent TN values of 5.04 and 3.05 for feed and manure respectively.

Appendix 6.7. Cumulative TP[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FPM	FO	DFIM	MO
Day 1 - 11	0.055	0.055	0.037	-
Day 1 - 23	0.34	0.14	0.34	0.21
Day 1 - 37	0.70	0.29	0.70	0.41
Day 1 - 51	0.99	0.38	0.99	0.61
Day 1 - 65	1.33	0.50	1.33	0.82
Day 1 - 79	1.71	0.67	1.71	1.02
Day 1 - 99	2.09	0.82	2.09	1.23

[1] based on percent TP values of 0.55 and 1.78 for feed and manure respectively.

Appendix 6.8. Cumulative OC[1] load from pond inputs of prawn feed and chicken manure (dry g m^{-2} unit $time^{-1}$).

Unit time	FPM	FO	DFIM	MO
Day 1 - 11	3.29	3.29	2.19	-
Day 1 - 23	11.21	8.30	9.11	2.91
Day 1 - 37	22.85	16.96	18.96	5.81
Day 1 - 51	31.19	22.38	25.48	8.73
Day 1 - 65	41.99	29.98	34.02	11.64
Day 1 - 79	55.15	39.65	43.37	14.55
Day 1 - 99	68.39	49.07	52.48	17.46

[1] based on percent OC values of 32.75 and 25.30 for feed and manure respectively.

Appendix 6.9. Proximate analysis of the fish and shrimp meals; all data expressed on a percent dry matter basis except percent moisture.

Component	Fish meal	Shrimp meal
Percent moisture (%)	5.70	18.95
Percent crude protein (%)	56.39	51.68
Percent crude lipid (%)	9.84	1.62
Percent ash (%)	31.20	36.72
Percent crude fibre (%)	1.03	5.11
Percent crude carbohydrate (%)	2.57	9.98
Gross energy (kcal g^{-1})	408.52	335.38

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSIONS

7.1. General Discussion

Pelleted formula feed provided an important source of nutrients for *M. rosenbergii* in the present set of experiments. The proximate analysis (Table 6.21) of the pelleted feed indicated an ideal protein content for the prawn based on the established 30% requirement (Chapter 1). However, pellet lipid content was slightly below the suggested 7.5-10.0%. The high pellet carbohydrate may not be inappropriate for the prawn, as previously tested levels up to 40% were suitable. However, fibre content could have been higher, up to 20%, to stimulate growth as previously reported. The lack of evidence for the dietary ash requirement in the diet of the prawn prevents comment on the suitability of the pellet ash, or that of any other food item.

Exclusion of feed resulted in significantly reduced prawn yields in both Experiments 1 and 4. Correlation analyses conducted on the combined data of Experiments 1, 2 and 4 between the loading variables and the prawn production parameters at harvest (Table 7.1), indicated that feed was positively correlated with mean weight ($F<0.01$), growth rate ($F<0.05$) and yield ($F<0.10$). However, the coefficients were strengthened when input (feed and manure) was correlated to mean weight ($F<0.01$), growth rate ($F<0.01$) and yield ($F<0.10$). The strong negative correlation between feed and percent survival ($F<0.05$) may be interpreted as the effect of feed on encouraging growth and weight increment, which, due to the bimodal growth of male *M. rosenbergii*, actually magnified the natural hierarchy, aggression and cannibalism of the species. By extending this hypothesis, it might be expected that the marketable percent be positively correlated with feed. However, although a valid economic indicator of the value of the harvest, the marketable criteria of 20.0 g is artificial to the prawn population structure.

The role of the pelleted formula feed may be considered as both a source of nutrition through direct consumption by the prawn, and also a substrate

Table 7.1. Pearson correlation coefficients between the loading variables of feed, manure and input (feed and manure), and the prawn production parameters for Experiments 1, 2 and 4 combined.

	Feed	Manure	Input
Growth rate	+0.469[b][1]	+0.413[b]	+0.634[a]
Weight	+0.534[a]	+0.419[b]	+0.697[a]
Yield	+0.305[c]	+0.104	+0.332[c]
Percent survival	-0.370[b]	-0.307[c]	-0.492[b]
Percent marketable	-0.093	-0.013	-0.092
Marketable yield	+0.264	+0.483[b]	+0.479[b]

[1] = a, b and c refer to F levels of probability of 0.01, 0.05 and 0.10 respectively

for contribution to the pool of decaying organic matter in the pond. The characteristic external manipulation and mastication of food particles inevitably results in the loss of some small particles or fines to the water and sediment, either dropping directly below the feeding animal or being carried away by currents induced by exhalant gill activity, prawn movement, or purely physical factors such as wind. A further case for the variable role of feed may be expected based on moult induced distribution and behaviour. As animals approaching ecdysis aggregate in soft muddy patches and postmoult individuals feed at least in part on their shed exuviae (Nelson *et al.*, 1977c; Peebles, 1979; Sandifer and Smith, 1985), pelleted feeds applied to such microenvironments may be expected to contribute more as a microbial substrate than those applied simultaneously to microenvironments associated with intermoult animals. The balance between the two roles of pelleted feed may vary within a single pond both in time and in space due to such factors as prawn standing stock density, hierarchy, and nutritional and physiological condition, and environmental conditions including the availability of natural food and water and sediment physico-chemistry. The very dynamic nature of the environment and the rates of grazing and productivity will influence natural feed availability over a short time period and thus affect the role of pelleted feed.

Further evidence for the direct consumption of pelleted formula feed was suggested by the relatively constant density of the sediment macroinvertebrate population in FO of Experiment 1. The application of pellets may have spared prawn grazing on macrobenthos. However, pellet decomposition and hence contribution to the whole pond environment may be inferred from the eutrophic water chemistry values in the FO treatment which were not significantly different from any other treatment in Experiments 1, 2 and 4. Furthermore, there was no evidence (Experiments 1 and 2) to suggest that the FO water trophic condition was maintained from sedimental nutrient sources. High sediment bacterial biomass in the

feed dominated treatments, at the early stage of Experiment 4, also suggested that all feed was not directly consumed. This may be interpreted as overfeeding in the feed dominated regimes in the first month of the experiment.

Fair and Fortner (1981) concluded that formula feed was directly consumed by *M. rosenbergii*. However, several factors of methodology and results encourage caution regarding their conclusion. The authors applied feed, either pelleted or pulverised, at only 3% prawn biomass per day to prawns weighing 0.15 g at stocking. Although the low feeding rate may be justified by the apparent early overfeeding of the present trial, it is not reflective of commercial conditions and prevents the extrapolation of their conclusion to existing farm practices. Nonetheless, within their trial, a final harvest weight of 4.8 g for prawns receiving pelleted feed and 2.6 g for prawns receiving the same but pulverised feed, suggested that more of the pelleted feed was directly consumed. However, Fujimura and Okamoto (1970) suggested that the prawn is able to locate and use small feed particles, and Stanley and Moore (1983) reported no difference in growth between prawns fed bound or unbound diets. Furthermore, the water chemistry data reported by Fair and Fortner (1981), did not indicate any differences between treatments, suggesting a fertilisation effect by the pelleted feed as in the present experiments. Unfortunately, the initial sediment phosphorus levels of the Fair and Fortner (1981) experiment were significantly greater in the ponds receiving pelleted rather than pulverised feed, and may have contributed to the improved prawn growth in the former treatment.

Stahl (1979) reported that pelleted feed significantly improved prawn growth in plastic pools supplied with natural foods, namely phytoplankton and a prepared earthen substrate containing 12% cattle manure. However, natural foods alone significantly encouraged prawn growth over formulated feed alone. The beneficial growth from the natural feeds was attributed

to the sediment, and particularly its manure component. Sediment chemistry data indicated a substantial increment in both N and C in experiments to which feed was applied, indicating some nutrients from the feed were entering the sediment. Unfortunately, the water quality data were not sufficiently consistent over time or within treatments to further elucidate the role of fertilisation. Stahl (1979) considered the possibility that feed played a role in fertilising the detrital pathway, and, New (1980) and New and Singholka (1982) suggested that supplemental feeds, including compounded prawn diets, acted mostly as pond fertilisers.

The role of applied manure has been attributed almost entirely to fertilisation in finfish culture. Fish directly consumed only a small amount of animal manure (Schroeder, 1977), intestinal analysis revealed manure only rarely (Spataru 1976, 1977), and fish fed directly on manure had poor growth (Lu and Kevern, 1975; Kerns and Roelofs, 1977; Schroeder, 1978). Although such specific studies in *M. rosenbergii* are lacking, data for other benthic omnivores, including crustacea, would suggest that the species is probably coprophagic (Chapter 1). However, the applied chicken manure would have provided insufficient protein, if consumed alone, to satisfy the defined requirement of the prawn. The protein content of the chicken manure was slightly lower than some previously reported data (Table 1.3), but nonetheless both present and tabular values were below 30%. Based on the preceding notes concerning suitable nutrient content, the carbohydrate and fibre content of the manure would seem most appropriate although the lipid content was far below requirement.

In Experiment 1, the DFIM treatment produced substantial benefits in terms of prawn mean growth rate, weight at harvest, yield, marketable percent and marketable yield. Furthermore, significantly greater mean weight at harvest, and non-significantly higher biomass and survival, were evident when manure was applied and feed input reduced in Experiment 3 after only 25 days of exposure to manure. This improvement may have been associated

with either the reduction in pelleted feed or the increment in manure. Although the digestion and assimilation of a high protein animal diet is more expensive than that of a mixed diet (Nelson *et al.*, 1977c), the UPM pellet diet contained both animal and plant material and had a moderate 32% protein content. Hence, the improvement in production parameters must have been associated with the increased manure load, due to both direct consumption as discussed above and pond fertilisation. The role of manure as a stimulant of natural productivity was supported by both the water quality data of Experiment 1, which revealed that DFIM had the highest concentrations of total nutrients and chlorophyll, and the water and sediment bacterial biomass data of Experiment 4, which were generally largest with greatest manure input.

The improvement of the correlation discussed above by combining the feed and manure load, rather than considering feed alone, illustrated the importance of manure inclusion in prawn culture management. Furthermore, manure input was positively correlated with marketable yield ($F<0.05$), the critical economic parameter. Feed was not correlated with marketable yield, and the combined input actually reduced the strong correlation of manure alone. The manure to survival correlation was weaker ($F<0.10$) than that between feed and survival reflecting the stronger influence of feed on prawn growth and weight increment. Nonetheless, the combined input had the strongest negative correlation with percent survival ($F<0.05$).

Malecha *et al.* (1981) considered that prawn growth was stimulated both by increased production of natural foods, notably benthic microbes and meiofauna, through the application of swine manure to polyculture ponds including *M. rosenbergii*, and by phytoplankton-rich faeces of silver carp. The authors concluded that detrital driven productivity contributed significantly to fulfilling the nutritional requirements of the prawn.

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However, Fair and Fortner (1981) achieved better prawn growth when ponds were treated with pulverised formulated feed as opposed to composted cow manure, when both were applied at 3% biomass per day. As there were no differences in water or sediment chemistry data, it would appear that the prawns directly consumed some of the pulverised diet, although they may also have directly consumed the manure but benefited less. The methodology provided unequal additions of nutrients, specifically N and P, and was inappropriate for comparing the effectiveness of the inputs as fertilisers.

Results of the isophosphorus experimental design of the FPM and DFIM treatments of Experiments 1 and 4, indicated that despite the often quoted limitation by phosphorus to freshwater phytoplankton production, prawn production and both water and sediment quality and bacterial biomass data were influenced by more than just the absolute total phosphorus load.

A limiting nutrient to the production of organisms at the centre of the aquatic foodweb, namely bacteria and algae, is a temporary condition in a dynamic eutrophic environment because new allochthonous detritus is constantly available and species dominance can shift to suit conditions. As has been discussed, algae in freshwaters tend to be classified as phosphorus or nitrogen limited. However, in highly manured ponds, algae may become light limited such that an excess of water nutrients do not promote further algal production (Schroeder, 1977). Despite the eutrophic nature and high chlorophyll concentrations of the water of the present experiments, calculations indicated that light limitation was unlikely (Chapter 4). According to Colman and Edwards (1989), oxygen and organic substrate limit heterotrophic productivity, although both Boyd (1979) and Anderson (1987) reported that nitrogen is in practical terms the key element determining decomposition and heterotroph nutrition. Nonetheless, Gude (1989) considered that bacterial production is limited by carbon, and Schroeder (1977) reported the levels of both nutrients and carbon

substrates were critical. Zooplankton are not solely dependent upon phytoplankton, but can grow well in algal free aerated medium supplied with animal manure (Schroeder, 1977). Bacteria serve as a facultative food for crustacean zooplankton, although the extent of exploitation varies amoung species (Pedrós-Alió and Brock, 1983; Güde, 1989). Amino acids appear to limit detritivore growth (Bowen, 1987), and the benthic detritivore *M. rosenbergii* may fulfil some of its requirement for amino acids from bacteria (Stahl and Ahearn, 1978).

The suitability of bacteria as a nutrient source for the freshwater prawn is dependent upon chemical composition as well as availability. However, bacterial chemical composition is highly variable, being dependant as in algae upon such factors as the species, the strain, the phase of growth and the environmental conditions. Neidhardt (1987) determined the content of *Escherichia coli* (B/r cells cultured at 37°C under aerobic conditions in glucose minimal medium and when the average cell was 44% through it's growth cycle) as 55% protein and 9.1% lipid, and Simon (1973) recorded total protein for *Anabaena cylindrica* as 46% and 66% in the exponential and stationary growth phases respectively. Generally, bacteria are able to synthesize most of the protein amino acids (Lehninger, 1979), and have a high affinity for amino acids (Sondergaard, 1988), but may be deficient in the sulphur amino acids (Schroeder, 1978). The cell walls are rich in the compound peptidoglycan which is based on a repeating disaccharide unit, and gram negative bacteria have an outer layer of complex lipopolysaccharide. Bacteria contain relatively few and simple fatty acids, as C_{12}-C_{18} saturated acids and C_{16} and C_{18} monounsaturated acids but are lacking in long chain polyunsaturated fatty acids and sterols (Lehninger, 1979). Microorganisms are very rich sources of vitamins, much more so than more complex plants and animals (Bowen, 1987). Hence, assuming good assimilation, high bacterial protein levels would provide protein excess to requirements for the freshwater prawn. The lack of the essential amino acid methionine indicates that an alternative dietary

protein source containing sulphur amino acids would be required. Based on the prawn's dietary requirement for lipid and their seeming lack of a requirement for sterol (Chapter 1), bacterial lipid content would appear suitable. However, a deficiency of dietary polyunsaturated fatty acids would be deleterious to the prawn, and although a large supply of vitamins may be beneficial to *M. rosenbergii*, hypervitaminosis of riboflavin has been reported (Chapter 1).

The suitability of other naturally occurring pond biota may also be considered on the basis of their chemical composition. Hepher (1988) collated the work of various authors and reported proximate analysis of organisms which serve as food for pond fishes (Table 7.2). Although Schroeder (1977) observed that phytoplankton from manured fish ponds consistently contained 45% protein, the broader ranging Table 7.2 indicates that both protein and total lipid content varied greatly between different groups of phytoplankton. However, plants can synthesize all of the protein amino acids (Lehninger, 1979), and the fatty acid 18:2n-6 is often most abundant in freshwater phytoplankton, and 18:3n-3 is frequent but longer chain lengths of both series are less evident (Sargent *et al.*, 1989). Hence, the suitability of phytoplankton as a complete diet for *M. rosenbergii* is species determined, but some species actually may be damaging. Certain blue-green algae which produce toxins (Moore, 1977) may affect growth in some crustacean species (Lightner *et al.*, 1978).

According to Schroeder (1977), zooplankton from manured fish ponds consistently contain 55% protein, a figure which agrees well with the collated data in Table 7.2. The high content of both protein and lipid suggest that zooplankton would not be a suitable single source of nutrient, due to the cost of digestion and assimilation. Similarly, both crustacean and insect protein and lipid content are well above the requirement for the prawn. However, freshwater insect consumption would be advantageous due to their being rich sources of 18:3n-3, 20:5n-3,

Table 7.2. Proximate analysis of pond organisms (% dry matter); percent values are the mean of several figures (n) collected from different sources and hence the proximate components do not sum to 100% (Hepher, 1988).

Organism group	Dry matter (n)	%	Protein (n)	%	Carbohydrate (n)	%	Lipid (n)	%	Ash (n)	%	Energy (n)	kcal kg^{-1}
Bacteria									(2)	5.4	(2)	4710
Algae												
Cyanophyta			(1)	31.3					(5)	46.7	(8)	2213
Chlorophyta	(13)	16.8	(8)	17.6			(7)	3.7	(21)	26.9	(29)	3773
Bacillariophyceae			(4)	30.7			(3)	9.9	(5)	38.3	(4)	3654
Macrophytes	(51)	15.8	(64)	14.6			(29)	4.5	(62)	13.9	(107)	3906
Protozoa											(1)	5938
Rotifers	(15)	11.2	(11)	64.3			(13)	20.3	(5)	6.2	(4)	4866
Oligochaetes	(4)	7.3	(2)	49.3			(1)	19.0	(1)	5.8	(9)	5569
Crustacea												
Anostraca	(6)	11.0	(6)	61.6			(6)	19.5	(6)	10.1	(2)	5835
Cladocera	(18)	9.8	(30)	56.5	(20)	28.2	(28)	19.3	(57)	7.7	(75)	4800
Copepoda	(10)	10.3	(35)	52.3	(36)	9.2	(29)	26.4	(29)	7.1	(39)	5445
Ostracoda	(2)	35.0	(1)	41.5							(2)	5683
Malacostraca	(32)	24.6	(16)	49.9	(7)	18.4	(10)	20.3	(50)	19.6	(112)	5537
Insects	(1)	23.2	(1)	55.9	(1)	20.1	(1)	18.6	(6)	4.9	(6)	5075
Odonata	(6)	21.1	(4)	51.9					(12)	5.8	(14)	4985
Trichoptera	(2)	14.8	(3)	34.7					(6)	11.8	(11)	5019
Diptera	(1)	16.0	(1)	55.3					(1)	6.9	(3)	5177
Chironomids	(5)	19.1	(4)	59.0	(1)	22.5	(2)	4.9	(17)	5.8	(31)	5034
Molluscs	(15)	32.2	(19)	39.5	(15)	7.5	(15)	7.8	(26)	32.9	(84)	3889
Aquatic detritus	(59)	91.5							(68)	12.4	(67)	4701

18:2n-6,and 20:4n-6 (Hanson *et al.*, 1985). The carbohydrate content of crustacea, insects and molluscs, would not be detrimental to the prawn, although higher values up to 40% are possible. Molluscan protein and lipid levels are more appropriate for the prawn, and molluscs would supply calcium and magnesium for the carapace.

Nelson *et al.* (1977c) constructed partial dietary energetic budgets for juvenile freshwater prawns by calculating the rate of caloric assimilation as the caloric sum of the rates of growth, respiration, and excretion, and the increase in respiration by the specific dynamic effect following feed ingestion. Three different diets representative of a plant diet, an animal diet and a mixed diet were calculated to determine growth efficiency values (growth rate/assimilation rate) of -3.32%, +1.75%, and +3.50% respectively for a prawn of 0.1 g dry weight. The variable effects of the diets was associated with the specific dynamic effect (13.74, 68.67 and 34.34 cal g^{-1} day^{-1} for the plant, animal and mixed diets respectively) and the growth rate (-6.10, +4.57 and +7.62 cal g^{-1} day^{-1} for the plant, animal and mixed diets respectively). The expense of digesting and assimilating the animal diet and the negative growth rate of prawns consuming the plant diet, identified the mixed diet as the most efficient for growth and supported the omnivorous feeding habit of the prawn. Unfortunately, as the energy compartments for the rates of respiration, growth, and excretion are influenced by such factors as environmental conditions including temperature and salinity, prawn body weight, and probably the character and density of all interacting biota (Nelson *et al.*, 1977a, 1977b, 1977c), the application of energy budgeting to the present grow-out pond study is unsuitable based on the available literature. Indeed, Nelson *et al.* (1977c) conceded that the energetic expense of moulting was not considered, and that respiration data obtained from individually maintained animals would not be representative of the natural or pond environment where intra- and inter- specific interactions would probably increase metabolism.

An assessment of the functional efficiency of the whole pond system on the basis of aquaculture production may be determined by employing mass balance equations. The amount of nitrogen, phosphorus and carbon applied to each treatment in the form of feed and chicken manure may be compared to the content of those elements in the cultured prawn biomass in order to illustrate the relation between elemental input and output and to determine the elemental load to the system. Mass balance equations have been previously calculated for intensive finfish culture (Beveridge, 1984; Gowen and Bradbury, 1987). In the present semi-intensive systems stocked with a bottom feeding omnivore, feed and manure not directly consumed may temporarily enter the environmental pool without in effect being "lost".

Mass balance determinations were conducted on the Experiment 1 data and are presented in Table 7.3. Clearly the greatest environmentally directed loads of nitrogen and carbon were in FPM, and of phosphorus in MO. The lowest values of nitrogen and carbon in MO, and of phosphorus in FO, indicated those management techniques which minimised the environmental load of each element. Indeed, the FO treatment contributed least combined nitrogen and phosphorus to the environment (63 kg), followed by MO (85 kg), DFIM (105 kg) and FPM (155 kg) per tonne of prawns. Beveridge summarised the work of Pencsak *et al.* (in Beveridge, 1984) and reported nitrogen, phosphorus and carbon nutrient losses to the environment respectively as 100, 23 and 750 kg per tonne of trout cultured in intensive cage farms. Calculations by Beveridge *et al.* (reported in Beveridge, 1984) determined phosphorus environmental losses per tonne of fish as 17-25 kg from intensive trout cages, 23-29 kg from intensive tilapia cages, and 9-113 kg from intensive land-based salmonid culture operations. Hence the mass balance results of the FPM and DFIM treatments of the present work, were not dissimilar from those in intensive cage culture of the carnivorous trout. However, prawn culture under the FO treatment caused a marked reduction in environmental loading compared with the Pencsak study which also applied feed pellets only, and thus suggested

Table 7.3. Estimates of mass balance, defined as the difference between the elemental load contributed by both pelleted feed and chicken manure[1], and the calculated elemental sink into *M. rosenbergii* [2]; the weight (kg) of nitrogen, phosphorus and carbon directed into the environment per tonne of prawns produced in Experiment 1.

Element	FPM	FO	DFIM	MO
Nitrogen	124	56	78	37
Phosphorus	31	7	27	48
Carbon	907	436	610	40

1 based on the TN, TP and OC content of feed and manure presented in Table 3.3, and on the conversion ratios of each of feed and manure to prawns in Table 3.17.
2 based on *M. rosenbergii* moisture content of 74.13% (MacLean, unpublished data), TN and TC content of 10.40% and 39.43% dry matter whole carcass *M. rosenbergii* (Nelson *et al.*, 1977), and TP content of 1.06% dry matter whole carcass *M. rosenbergii* (MacLean, unpublished data).

that there was less feed wastage in prawn culture under the conditions of the FO regime.

The prawn mass balance determinations could be more detailed if unavailable data, such as for feed consumption and faecal production and mineral content, were estimated. However, processes of dynamic prawn behaviour such as regurgitation, cannibalism, and exuviae consumption introduce factors which are not satisfactorily quantified at present.

However, the data in Table 7.3 may be further examined as environmental C:N ratios. Calculations reveal environmental load ratios of 7.3:1, 7.8:1, 7.8:1 and 10.8:1 for the treatments FPM, FO, DFIM and MO respectively. Combined feed and manure calculated inputs (per tonne of prawns) were in ratios of 6.7:1, 6.5:1, 6.8:1 and 7.8:1 in the same treatments respectively. Hence, in all treatments the carbon:nitrogen ratios of combined inputs increased by 9-38%, which is not unexpected given the low carbon:nitrogen ratio of 3.8:1 for whole *M. rosenbergii* (based on Nelson *et al.*, 1977c) and the high protein carcass content (Chapter 1). The low feed and manure carbon:nitrogen ratios, respectively 6.5:1 and 7.8:1, will have encouraged rapid breakdown as organic matter with a low C:N ratio decomposes more quickly (Boyd, 1979).

Decomposition of dead organic matter sustains heterotrophic production in the aqueous environment and the "close functional integration" of the decomposer and herbivore systems effectively transfers microbial production to other trophic levels (Anderson, 1987). The conversion efficiency of detrital organic matter into bacteria, or the growth efficiency of bacteria, varies with temperature, pH and nutrient consumption (Payne and Wiebe, 1978). Payne (1970) reported an average value of 60%, and Moriarty (1987) quoted values of 50-60% (for the data of Koop *et al.*) and 35% (for the data of Bauerfiend) given an improved bacterial biovolume-biomass conversion factor (Bratbak and Dundas, 1984).

Based on his own data Moriarty (1987) determined that the bacterial growth efficiency in aquaculture ponds may have been 40-50%. According to Doetsch and Cook (in Schroeder, 1978), 20-50% of detrital organic matter is fixed by bacteria under aerobic conditions and considerably less under conditions of anaerobic digestion.

However, bacterial biomass and production may be affected by the top-down process of predation (Andersson *et al.*, 1986; Riemann and Sondergaard, 1986; Geertz-Hansen *et al.*, 1987; Gonzalez *et al.*, 1990), as well as the bottom-up process of resource supply (Güde, 1989; Pace and Funke, 1991). Dispute over whether the heterotrophic or autotrophic system preceeds overlooks the rate at which all processes occur and their interdependence (Edwards and Colman, 1987), which is both complimentary and competitive (Rheinheimer, 1985; Güde, 1989). Complex aquatic food webs determine substrate supply and predation pressure at every level. Fish, zooplankton, phytoplankton and bacteria influence the character of the plankton community (Carmichael and Gorham, 1977; Newhook and Briand, 1987; Carpenter and Kitchell, 1988; Mazumder *et al.*, 1989) and water quality parameters (Wright and Shapiro, 1984; Newhook and Briand, 1987; Mazumder *et al.*, 1989). For example, a shift from small to large herbivorous plankton can cause a reduction in epilimnetic total phosphorus (Wright and Shapiro, 1984; Mazumder *et al.*, 1989), although, the introduction of planktivorous fish can increase the epilimnetic TP by favouring smaller plankton which contribute more to total biomass and less to sedimentation (Mazumder *et al.*, 1989).

Food webs can not only affect the trophic status of the planktonic environment, but also then the efficacy of fertilisation procedures (Mazumder *et al.*, 1989), as may be adopted in aquacultural management. The introduction into the community of a different species (or at least if previously present than at greater density), as is the nature of semi-intensive aquaculture, will affect the entire biotic and abiotic community

and necessitates a broad approach to determining the factors affecting the marketable production of the stocked organisms. Food availability is a critical component affecting aquaculture yields, and the effects of the applied inputs of feed and fertiliser should ideally be considered on both biomass and production throughout the food web.

Biomass data of a species occurring within the environment is a valid indicator of the prevalence of that species at any point in time but does not necessarily reflect the amount of available food for a predator. Biomass is the result of the rate of production and the rate of mortality, both natural and predated. Hence, productivity is the most appropriate criterion to identify feed availability, and strong correlations between fish yield and gross primary productivity have been reported (Chapter 1). In Experiment 2, mean values of silver carp yield increased with increasing gross primary productivity. However, high variation within treatments prevented correlation based on the data from all ponds individually. The prawn yield of Experiment 2 was also unrelated to primary productivity, which is not unexpected given the feeding habit of the species.

Accurate determinations of secondary productivity require estimation of the rates of reproduction and mortality, and knowledge of the population structure in terms of age and size, if the approach of population dynamics is adopted. Alternately, if the physiological approach is followed, data is required on the rates of feeding, assimilation, respiration, excretion and other metabolic processes (Edmondson, 1971). Estimates of secondary production can be made from biomass only in cases without continuous reproduction, or in cases with continuous reproduction only if the population mortality is similar for all age classes or development stages (Hepher, 1988). The assumption of non-selective predation is not possible to accept in aquacultural conditions, and the estimation of secondary production on the basis of biomass measurements is unreliable (Hepher,

1988). Assessment of the availability of feed alternately may be determined by measuring or estimating feed intake. The direct estimation of food consumption, requiring both accurate gut analysis and gut turnover rates, is variously affected in fish by feeding rhythms, physiological state, environmental conditions and diet composition (Hepher, 1988) and further complicated in the freshwater prawn due to both physical and behavioral characteristics (Chapter 1). The indirect estimation of food consumption, based on the requirements for energy as determined individually in the laboratory, may be extended to natural populations if information is available on population size, individual growth rate, reproductive losses, metabolic losses, and the relative fraction of feed intake which is assimilated (see Hepher, 1988). At the present time, application of this approach to *M. rosenbergii* is fraught with potential inaccuracies related to the assumption that individual energetics in a confining tank represent population energetics.

Hence, both nutrients and predators may be important regulators in the natural aquatic environment and detailed simultaneous analyses of the whole community and the individual components are required to best understand the dominant processes in a single system. Under the husbandry conditions of semi-intensive aquaculture, the introduction of a target animal must be viewed as a new predator (active or passive), which is in part dependent for its nutrition upon the natural biota. The introduction of a predator extends the complexity of the direct and indirect interactions between species, and the biogeochemical processes of the environment. In the case of the introduction of a benthic omnivore and detritivore, water and sediment chemical analyses must be coupled with determinations of both biomass and production of, and carbon flow between, bacteria, protozoa, phytoplankton, zooplankton, benthic meiofauna and macroinvertebrates, and the target animal. Thereafter, manipulation of the environment through appropriate management techniques can direct, by the shortest route, the carbon and nutrients toward the species of

aquacultural interest. Only by such an exhaustive approach can the nutritional benefit of the environmental biota be maximally matched to the dietary requirements of the target animal, and the necessity for formulated feed minimised. The practical reality of such an approach would require multiple, frequent and detailed sampling and analyses, an enormous number of man-hours, and preferably the involvement of several researchers with complimentary expertise.

7.2. Conclusions

Pelleted formula feeds provided a suitable and important source of nutrition for the cultured prawns, and the exclusion of feed significantly reduced prawn yield (Experiments 1 and 4). The mechanism by which feed affected the prawns likely varied both temporarily and spatially. Some of the applied pellet was directly consumed as evidenced by the visible presence of pellets in the oesophagi (Experiment 2), and implied by both the constant macrobenthic density in the feed only treatment (Experiment 2), and the strong correlations (this chapter) between feed input and each of prawn mean growth rate, weight at harvest and yield (Experiments 1, 2 and 4). However, some pellet was not directly consumed, and probably contributed in the feed only treatment to the non-significant difference of the water nutrient and chlorophyll concentrations (Experiments 1, 2 and 4), and the high sediment bacterial biomass in the early stages of grow-out (Experiment 4).

Chicken manure contained insufficient levels of both protein and lipid to constitute the sole nutrient source for the prawn. However, given the omnivorous behaviour of the species and the reported coprophagy of other crustaceans, manure was probably directly consumed. Enrichment of the manure by bacteria during microbial decomposition would have increased the nutrient value of the manure. Direct consumption was suggested by the non-significant difference between manure only and feed only treatments in terms of prawn mean growth rate, weight at harvest, marketable percent and

marketable yield (Experiment 1). Manure contributions to the pond system were evident in the treatment with increased manure load (DFIM) from the high mean concentrations of water nutrients and chlorophyll (Experiments 1 and 4), and the high mean water and sediment bacterial biomass (Experiment 4).

The addition of both pelleted feed and chicken manure improved prawn marketable yield (Experiment 1). Correlation coefficients (this chapter) were strengthened when both feed and manure were compared to prawn growth rate, mean weight at harvest and yield. Decreased feed and increased manure applications resulted in improvements in all prawn production parameters (Experiment 1), and the benefit evident may be attributed principally to the manure (this Chapter). The manure advantage was evident even during brief exposure, and indeed caused a significant increase in mean prawn weight (Experiment 3). The addition of both inputs (FPM) did not result in either prawn or environmental data reflecting simple additive effects of the single input treatments (FO and MO) (Experiments 1 and 4) due to the ecological complexity of the systems and the variable mechanisms of feed and manure utilisation.

Enclosures inflicted a space effect on prawn growth. The critical point may have been between 11 - 14 g m^{-1} (Experiment 3), although consideration of the effect over a longer period of exposure suggested a higher critical point between 19 - 32 g m^{-1} (Experiment 4).

In pond trials (Experiments 1 and 2), both feed conversion ratios and the ratios describing financial input to marketable prawn output favoured the treatment wherein feed was partially replaced by manure, particularly when the manure was applied every 14 days.

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