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Penaeus indicus

CULTURE CONDITIONS FOR

STUDIES TO OPTIMISE THE

FROM THE SAUDI ARABIAN

COAST OF THE RED SEA

A THESIS SUBMITTED TO THE UNIVERSITY OF WALES

BY

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UNITED KINGDOM

وما يستوى البحران هذا عذب فرات سائغ شرابه وهذا ملح

بسم الله الرحمن الرحيم

IN THE NAME OF ALLAH, MOST GRACIOUS, MOST MERCIFUL

(اسورة فاطر ,آيه ١٢)

تر δ الفلك فيه مواخر لتبتغوا من فضله ولعلكم تشكرون.

اجاج ومن كل تاكلون لحما طريا وتستخرجون حليه تلبسونها و

Nor are the two bodies of flowing water alike, the one palatable, ,
,
, sweet, and pleasant to drink, and the other, salt and bitter. Yet from each (kind of water) do ye eat flesh fresh and tender, and ye extract ornaments to wear; and thou seest the ships therein that plough the waves, that ye seek (thus) of the bounty of Allah that ye may be grateful.

(Glorious Qur'an, sura Fatir, verse 12, translated by Abdullah Y. All, published by Nadim & Co. London. 1983)

To my Mother, Father, Wife, Children; (Farris, Zyad, Khulude) and to all of those who encouraged me and prayed for me.

SUMMARY

On the Red Sea coast of Saudi Arabia ponds utilising seawater drawn from wells have an average salinity of 43% and temperatures ranging from $23-33\degree C$. The present study has demonstrated that yields of up to 4.3 tonnes ha"' of the Indian white shrimp Penaeus indicus at 20 g size may be obtained in these ponds. P. indicus was isolated from local stocks (Gizan) and has now been cultured through several generations at the Fish Farming Centre. Present work has determined that the optimal salinity for larval culture is 30% and for nursery culture 25-30%. The best stage for transfer from nursery to growout pond (43%) is about PL25. Comparison with biological data for P. indicus cultured elsewhere indicates that Red Sea populations may be preadapted to tolerate high salinities. The potential for artificial feeds to replace live feeds in P. indicus larval culture from Z1 to PL1 and PL5 using microencapsulated feeds, Nippai and Frippak has been investigated. Results reveal that 50% replacement with Nippai and Frippak is possible giving comparable growth to the control, but poorer survival. Also comparative growth trials were conducted with post larval P. indicus (PL5-PL30) spawned from Red Sea stock and cultured through larval stages on five feeds (Chaetoceros, Tetraselmis and Artemia). Post larvae were reared on commercial feeds: Taiwanese, Nippai, Frippak and 4 formulated feeds based on locally available ingredients at 28-30°C and at 30% and 42% salinities. Feeding trials at 42% produced poor survival, but at 30% all treatments gave over 50% survival to PL25, at which stage shrimp are ready for stocking in growout ponds. Although the Taiwanese feed produced the overall fastest growth, it was not significantly better (P>0.05) than two locally formulated diets (FFC1,4) or Frippak. Survival rates of over 60% where achieved on all diets with the exception of Nippai and Taiwanese feeds. Yields were significantly higher (P<0.05) on one of the locally prepared diets (FFC1) than Taiwanese and Nippai, and Taiwanese than Nippai. All diets yielded significantly more shrimp biomass at 30% than 42% (P>0.05). The relative costs of imported and locally produced diets are discussed and it is concluded that it is possible

to produce cost-effective nursery diets in Saudi Arabia.

A growout feeding study for P. indicus juvenile utilising 4 locally formulated diets compared with a Taiwanese diet revealed no significant difference (P. >0.05) in survival nor in growth or yield amongst all diets. However growth was slow due to low pH and high ammonia levels.

Finally shrimp density production trials at 20, 40,60 and 80m"2 during winter and summer were conducted in cages placed in a rubber lined pond. For all densities; yields were significantly higher in summer than winter with an overall average of 3.41±1.5 tonnes and 4.04 ± 2.36 tonnes 180 days⁻¹ for winter and summer respectively. Yield at 80m⁻² was highest but was not different from yield at 60m'2. Based on average harvested size and market price the density of $60\pi^2$ was regarded suitable for growout culture.

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

The world's total production of aquatic species increased nearly 100% between 1975 and 1986, from 6.1 million metric tonnes (MT) to 12.1 million MT and is still increasing (Anonymous, 1990). Crustacean

increase during this period was from 16 to 399 thousand MT. The world's total production of crustacean from culture and capture increased from 2.4 to 3.2 million MT between 1975 and 1985, or 34% (Casvas, 1988). Cultured crustacean production increased from 1.2% of the total crustacean production in 1975 to 8.2% in 1985 and in 1988 accounted for 15% or more of the total (Casavas, 1988).

Farmed shrimp has reached more than 0.7 million

tonnes in 1992, up from about 0.1 million tonnes in 1982 (Rosenberry, 1990; Casvas, 1993). These increases are driven by market demands from an increasing world population and decreasing catches. Several earlier technological breakthroughs supported this success, primarily in the seed and feed supply (Hudinaga, 1935, 1942; Jones et al., 1979b). Until the last two decades, all shrimp supplied to the world markets was harvested from wild stock in

bays and open seas. Shrimp fisheries production has

not increased but actually declined in the Gulf of

Mexico, increasing the cost of the wild shrimp fisheries (Tettey and Griffin, 1984). Due to improved techniques for cultured production, economics consideration is increasingly favouring this form of production, making shrimp farming a major source of shrimp within the last decade. In addition,

improvements in processing and marketing have favoured farmed shrimp production giving advantages for cultured over captured shrimp. In terms of production area in 1982, Ecuador was the leading country in commercial shrimp farming, with approximately 12,000 ha, and Taiwan with 3,200 ha. By 1989, the center of shrimp farming in the world had shifted to the Orient. Presently, China leads the world in cultured shrimp production with

145000 ha of production, and 29% of total world supply of cultured shrimp. Indonesia and Thailand each have 16% of total world culture production. Other countries included are the Philippines, Ecuador, Vietnam, India and Taiwan. P. chinensis is cultured primarily in China. P. monodon is the popular species cultured in most of the other Asian countries, while P. vannamei is the principal species grown in Latin America. Shrimp farming can be divided into three

categories which are based on management intensity:

extensive, semi-intensive and intensive. The main

characteristics of the extensive method of shrimp culture is that production depends entirely on natural conditions. The semi-intensive farming method utilizes a nursery phase, carefully laid out ponds, feeding and water pumping. The intensive culture system introduces smaller ponds, high stocking densities and continuous

management of feeding, waste removal and aeration (Rosenberry, 1989)(Table 1). Table 1 Shows the major categories of shrimp farming based on intensity.

In Saudi Arabia yearly fish and shrimp production from the Red Sea and the Gulf reached 45.5 thousand tonnes in 1985, but then declined to a level of around 41 thousand tonnes per year (Fishery statistics bulletin for Saudi Arabia 1986 and personal communication with the Fisheries authority). At the

same time the imports exceeded 59 thousand tonnes for

the same year (FAO, 1987) (Table2). Table 2 Shows the imports of fish products to Saudi Arabia in tonnes and values in U\$ 1000.00 from 1986-1990.

*Q = Quantities in tonnes and $V =$ Values in U\$ 1000.00

Table 3 shows the imports of crustacea in Kg, and values in Saudi Riyals during the period from 1981-91

(Foreign trade statistics of Saudi Arabia 1992).

Recently aquaculture has been considered in an

attempt to close the gap between production and importation (Table 4). Table 4 shows Aquaculture Fish Production in Saudi Arabia in tonnes from 1986 - 1990

(Progress Report of Fish Farming Centre 1991).

Saudi Arabia has endeavoured to increase the

utilization of available natural resources in order to

be self sufficient in food production through

development in agriculture and recently aquaculture. In 1982, the Fish Farming Centre was established as a unilateral trust fund project between the Saudi Arabian Ministry of Agriculture and Water and the Food and Agriculture Organization of the United Nations to direct and support the development of aquaculture in

the Kingdom. The aim was to apply and adapt the existing worldwide technologies and production systems suitable to local. environmental conditions. The Centre has established successful commercial culture for Tilapia Oreochromis spilurus in seawater in cages, pens and tanks. The Centre has also successfully demonstrated the feasibility of culturing other brackish and marine fish such as the Rabbit fish Siganus rivulatus. The tilapia Oreochromis aurea, O.

nilotica, O. spilurus, the Chinese carp Cyprinus carpio and the freshwater prawn Macrobrachium rosenbergii are now successfully cultured in Saudi Arabia. The tiger prawn Penaeus monodon was imported to

start penaeid culture in the Kingdom. However P.

monodon presents some technical problems for commercial culture (Bukhari et al., 1989α). Poor adaptation to local high salinity conditions was

demonstrated by low survival and slow growth which made the culture period too long and yield too low. In

addition seed supply was limited due to poor maturation of cultured specimens and unavailability of local wild seed. Penaeid shrimp have been known to inhabit the Red Sea since the last century (de Man, 1882). Penaeus indicus has been found among other penaeid shrimp in

the Gizan area (Red Sea coast southwest of Saudi Arabia) by Ghamrawi (1978) when a survey took place to study the shrimp fishery and spawning grounds in the Red Sea. Badawi and Cas (1989) collected P. indicus juveniles which are found abundantly on the southwestern coast of the Gizan area. These were brought to the Fish Farming Centre to start preliminary studies of this species. These studies have demonstrated that this species could grow up to

20g in captivity when a quality shrimp feed is provided (Bukhari et al. \mathbf{r} 1994). Survival rates of 76% and growth of up to 16g have been achieved recently after 180 days of pond culture at a density of 30m'2. Postlarvae were produced from the Centre's hatchery from ablated and unablated (Primavera et al., 1982) females harvested from rubber lined earthen ponds with seawater of 43% (Bukhari et al., 1991band 1992). Table 5 shows a comparison between production in

ponds and tank trials for local P. indicus and

imported P. monodon from at the Fish Farming Center

from 1989-1991 fed on imported standard shrimp feed

(Bukhari et al., 1989 k c1990 and 1991a).

The life cycle of Penaeus indicus has now been completed in captivity at the Fish Farming Centre without the need to collect new broodstock from the sea (Bukhari et al., 1991 b). Penaeus indicus is one of the species of the genus Penaeus Fabr. It is characterized by an elevated rostral crest of 7-9 dorsal teeth and 4-5 ventral teeth. The adrostral groove is distinct, but close to

the median groove and reaches almost to the middle of the carapace. The gastro-orbital ridge is well defined

and hepatic ridge is absent. The telson is broadly triangular and has a median longitudinal groove which lacks spines (Cheung 1960; Racek and Dall, 1965). The Indian white shrimp P. indicus is distributed mainly in the Indian ocean, but also has been reported in Australia, Indonesia, Philippines, India, Pakistan,

East coast of Africa (including Madagascar), Gulf of Aden and the Red Sea (Holthuis and Rosa, 1965), and has rare occurrence in the Arabian Gulf and the Gulf of Oman (FAO, 1987; Tseng, 1988). The species has been recorded from as deep as 43m to as shallow as 3m, but it is generally common in water of less than 30m deep. It occurs on both sandy and muddy bottoms, with slight preference to sand (Barnard, 1950). Panikkar and Menon (1956) stated that the species preferred deeper waters for spawning. Eggs of P. indicus have been reported to occur in large numbers in subsurface plankton in Madras, India waters and were obtained from 3m below surface (Subrahmanyam, 1965). Larval stages (protozoea and mysis) and postlarval stages (from 8 to 14mm in total length) have been recorded in areas near Cochin on the southwest coast of India. They are found in nearshore subsurface waters and in the estuaries during all

months except June to September (Menon, 1937; Crosnier, 1965; George, 1962) where they feed and

grow. Juvenile P. indicus (from 30 to 120mm in total length) spend their life mostly in the estuaries and backwaters, on the south-west coast of India. These juveniles support a good commercial fishery in the backwaters and paddy fields where they live until they attain a length of 100 to 120mm after which they go

back into the deep sea (Menon, 1955; Menon and Raman, 1961). Crosnier (1965) captured juveniles and adults, ranging in length from 30 to 200mm, in the intertidal zone of bays in Madagascar in water of 5-10m depth. Sexually mature adults occur in the sea down to 45m deep, but are also found on sandy bottoms in shallow waters of the sea from 3 to llm where favourable physical conditions and nutrients occur from the land and rivers (De Bruin, 1965). The occurrence of this

species in the fishery is subject to seasonal fluctuations (George and Mohamed, 1966) (Table 6).

Table 6 summarizes the distribution of P. indicus stages, sizes (total length in mm) and time of occurrence on the coast of India (from George and Mohamed, 1966).

Similarly $P.$ indicus postlarvae in the Red Sea have been observed to be abundant throughout the year in sandy-muddy shallow lagoons of around 1-2m deep, in the Gizan area (Badawi and Cas, 1989). Penaeus indicus appears to reach an age of more than 1 year old in Singapore prawn ponds, Madagascar intertidal traps and lined ponds at the Fish Farming Center (Hall, 1962; Crosnier, 1965; Bukhari et al.,

199ta) .

The species is heterosexual with morphologically

differentiated male and female sex organs. While the male sex organ, the petasma, is abdominal in position, on the endopodite of the first pleopod, the female sex organ, the thelycum, is a modification of the last thoracic sternite. The presence of an appendix masculina on the endopod of the second pair of

pleopods is another male character. The genital openings of the male are situated on the coxa of the fifth pair of pereiopods and those of the female are on the coxa of the third pair of pereiopods. Females attain relatively larger sizes than males. The sexes of this species in marine and backwater environments of Indian coastal waters are more or less equally distributed (Menon, 1957). A lower ratio of males was reported by Crosnier (1965) which ranged from 35-55% with an average of 45% obtained from samples in Madagascar. This sex ratio is similar to that observed in the culture ponds at the Fish Farming Center (Bukhari et al., 1990). Mature individuals normally measure 150mm or more in total length (Menon, 1957). By examining the nature of the petasmal endopodites, Hall (1962) was able to find mature females at 134mm total length. However, Aquacop (1983) report that P . indicus may reproduce

in captivity at a weight of 6-8g. The age of the species at first maturity has not been precisely

estimated.

During mating, the sperm packs known as spermatheca are deposited by the male in the external genitalia of the female. The female carries the spermatheca in the thelycum and the sperm are dispensed at the time of spawning. Fertilization is

external for as the eggs are extruded from the genital openings of the female the sperm is dispensed from the spermatheca. Fecundity has been estimated as 68,000 in a female of 140mm total length (Rao, 1968). Penaeus indicus may spawn five times during a normal life time with an interval of two months between successive spawnings (Rao, 1968). During the prolonged spawning period breeding extended from October to April in Cochin waters of India (Roa,

1982; Bukhari et al. , 1991() Larvae for culture may be

1968). This species also has an extended spawning period in Madagascar waters of East Africa (Crosnier, 1965). It reproduces throughout the year in the Red Sea with a peak from April to November (Badawi and Cas, 1989). Like other penaeids, P. indicus eggs hatch into a nauplius, with 6 developmental stages, then metamorphose into 3 zoea, 13 mysis stages and finally the postlarva (Subrahmanyam, 1965; Primavera et al.,

wild caught from the sea or artificially propagated in

a hatchery. Wild larvae are caught by various methods such as beach seine net, scissor net, scoop net.. etc. and then transported in plastic bags containing oxygenated water (Badawi and Cas, 1989). Hatchery reared larvae are preferred over wild larvae due to the greater numbers produced at one time, more uniform

size and minimal handling (Apud et al., 1983). Hatchery sourced larvae of P. indicus are easily obtained from ablated and unablated females (Primavera et al., 1982; Bukhari et al ., 1991b). Larval reari systems differ in design and size, but are usually provided with filtered water and, light and temperature control devices (Hudinaga and Kittaka, 1987; Shigueno, 1975; Mock and Neal, 1974; Aquacop, 1975; Planton, 1987 and Mock et al., 1980).

During the nauplius stages N1-N6, larvae depend on their yolk reserves for feeding. Zoeal stages Z1-Z3 prefer phytoplankton, but start to consume zooplankton at the last substage. Larvae at zoeal stages are often weak and mortairey may occur. Mysis stages M1-3 and postlarvae PL1-PL5 prefer zooplankton (Liao, 1984). The extensive use of live foods such as microalgae and zooplankton in shrimp larval rearing, requires appropriate facilities, high maintenance and trained

intensive labour (Kanazawa et al. 1982). Recent work

has demonstrated the feasibility of the replacement of

live foods with microparticulate and microencapsulated diets for crustaceans (Teshima et al., 1984; Amjad and Jones, 1989; Jones et al., 1989,1991,1993). However, little information is available on live foods replacement with microencapsulated diets for P. indicus (Galgani and Aquacop, 1988).

Gopalakrishnan (1952) observed that P. indicus

does not show any significant difference in food habits at different times of the year. Analysis of the food of different sizes also showed no variation. Vegetable matter and crustaceans formed the bulk of the food consumed indicating an omnivorous habit (Gopalakrishnan, 1952). The vegetable matter included diatoms like Coscinodiscus, Pleurosigma, Rhizosolenia planktonic alga Trichodesmium and pieces of sea weeds.

The crustaceans included copepods, ostracods, amphipods and tiny decapods and their larval stages. In addition molluscs, polychaetes and both animal and plant detritus that accumulate on the benthos are eaten (Hall, 1962). Production of cultured prawn larvae in an intensified culture system is dependent upon nutritionally effective and acceptable diets for each of the different developmental stages (New, 1976). The

past decade has witnessed a rapid increase in the

utilization of artificial feeds to replace live feeds

in penaeid culture, and a wide range of microparticulate and encapsulated feeds are now marketed for routine use by hatcheries (Jones et al., 1993). These encapsulated feeds have potential for P. indicus larval culture.

For nursery and growout phases of culture, feed

is one of the major inputs in the semi-intensive and intensive production of prawn (Pascual, 1989,1990). If this is to be economical it should rely on the use of local ingredients such as squid, fish meal and meat and bone meal, as well as cheap by-products such as wheat bran, sesame bran and shrimp head which are now available in Saudi Arabia as a result of the recent development in agriculture and animal production plants.

Prior to any applied study on fish or shrimp culture, the study of culture conditions must be taken into consideration for the concerned species. These studies should optimize physical conditions e.g. temperature, salinity and pH and determine effects of metabolites such as hydrogen sulphide, ammonia and nitrite which may develop in cultured water (Cole and Boyd, 1986).

Table 7, presents the physical data for seawater

from wells as well as from the Red Sea at the Fish

Farming Centre during the period (1990-1992).

Table 7. Water quality from 1990-1992. sourced from Red Sea and wells at the Fish Farming Centre (Progress Reports of the Fish Farming Centre 1990,1991 and 1992).

The primary objective of the present study is to investigate the optimal culture conditions for P. indicus from the Red Sea as all previous studies are from regions where salinities are considerably lower. Chapter 2 investigates the effects of physical conditions on P. indicus larval and postlarval stages, by examining their culture at different salinities. It

includes an osmolality experiment on pond cultured juveniles to determine optimal salinity and salinity

tolerance for the Red Sea P. indicus. Results are compared to those for the Indian strain of P. indicus. In Chapter 3, the use of live and artificial feeds for larval culture of P. indicus from the Red Sea is investigated. The best available algal replacements using microparticulate and

microcapsulated diets for larval rearing to PL1 and PL5 are considered. This chapter also includes a comparative nursery phase study at salinities of 30% and 4396 for P. indicus postlarvae from PL5-PL30 fed on different commercial and locally formulated feeds using ingredients available in the Saudi market. Four feeds formulated from different sources of protein are tested for juvenile growout and compared with an imported Taiwanese diet in chapter 4. This is to determine the possibility of culturing this species to marketable size on a low cost feed. Chapter 5, examines the growth and survival of P. indicus at different densities in rubber lined earthen ponds during winter and summer. This is to determine the optimal culture density and production per unit area on a standard feed. Chapter 6 contains general discussion of the results obtained from this study and focuses on possible future research areas for P.

indicus as well as other locally available penaeid

species.

CHAPTER 2

The determination of some of the optimal physical conditions for the culture of Penaeus indicus from the Red 0ea

Results of this research were presented at the

Aquaculture technology and investment opportunities

symposium. April 11-14,1993q(Abstract p. 37). Riyadh,

Saudi Arabia.

Title: Optimal salinities for culture of P. indicus

from the Red Sea.

Authors: Feisal A. Bukhari, D.A. Jones and A.J. Salama

 \bullet

Introduction

One of the most important criteria for successful aquaculture is the ability to sustain regular production of the species. This requires control over broodstock to ensure regular seed production, well

tried and successful larval and postlarval culture methods, and finally, economic growout under locally prevailing environmental conditions. The desirable range and levels of water quality factors for penaeid species are shown in Table 1 (Van Olst et al., 1980; Wickins, 1981,1982, and Kuo, 1988).

Table 1: Ideal physical conditions for culture of penaeid species.

Sea temperatures on the Red Sea coast of Saudi Arabia range from 20-32°C, which are favourable for penaeid shrimp production, and 12-35°C on the Gulf coast, which are below optimum, especially for a short

period during the winter.

Dissolved oxygen concentrations range from 4-4.5

mg l^{-1} (Robinson, et al., 1979) and are with in the optimal levels desired by penaeid species. Other water quality factors recorded by the Fish Farming Centre are also usually within the acceptable range shown in Table 1 above with pH and ammonia levels from 7.6-8.2 and $0.03-0.09$ mg 1^{-1} respectively.

Higher salinities up to 6016 have been measured th inshore waters of the Red Sea during summer when currents and tidal influences are insignificant (Robinson et al., 1979). In some coastal lagoons of the Red Sea, salinity reaches 78% (Leger, 1983). However even coastal open water salinities at 4316 for the Red Sea and 42-55% for the Gulf coast present problems, as most shrimp have a culture optima of 3516 or below.

With the above criteria in mind, it is possible to review the culture potential for locally available penaeid species. These include Penaeus semisulcatus, which dominates catches on both coasts; P.monodon, only found in low numbers (< 1%) in the south of the Red Sea; P. japonicus, again in low numbers on both coasts; P. indicus in significant numbers in the south (Gizan) of the Red Sea and Metapenaeus species on both coasts. Other species and genera are too infrequent to

be considered as possible sources of broodstock

(Sanders and Kedidi, 1981; Shakraporti et al., 1985;

Badawi and Cas, 1989).

Although P. semisulcatus is an obvious candidate, all culture trials in Kuwait, Bahrain and Saudi Arabia have demonstrated poor survival and growth under culture conditions prevailing in the region (Farmer, 1979). P. japonicus has performed equally badly (Fish

Farming Centre Progress Report, 1990), and the Metapenaeus species have a smaller size and hence lower market price.

P. monodon has perhaps the greatest potential, particularly if the Red Sea race were to be adapted to high salinities. However, this species is difficult to mature and spawn, survival in ponds is not good, and broodstock is difficult to obtain on a regular basis. Although there is a commercial farm operating in

Saudi Arabia based on this species, there is as yet no information as to the success of this operation. In contrast to the above, P. indicus from the Red Sea appears to show distinct biological advantages. The most important of these is the ability to close the life cycle. At the Fish Farming Center in Jeddah this species has been reared through several seasons from broodstock selected from culture ponds (Bukhari et al., 1991). This confirms experience from elsewhere

(Kumlu & Jones, 1993), where this species has been successfully reared and spawned through several

generations in recirculation systems in Europe. Once the life cycle is closed, it becomes possible to obtain broodstock on demand without reliance upon unpredictable wild stocks. The potential also exists to exclude disease brought in with wild stock. Manipulation of maturation is possible to

produce seed throughout the growing season, and hence to ensure harvest coincides with maximum market demand.

Finally, and most importantly, domesticated pond broodstock allows genetic selection for the first time. Hence it should be possible to select for traits such as fast growth and tolerance to high salinity. Higher average size, growth rates and production were obtained when utilizing saline ground water of 28.5-

3016 for prawn and fish farming when compared with previous studies of Hora and Pillay (1962), Subrahmanyam (1973), Ranoemihardjo et al., (1975), Dwivedi and Reddi (1976) and Rao and Raghavalu (1982) utilizing brackish water with fluctuating salinities. Whilst the potential for P.indicus is exciting, it should not be forgotten that another important criterion is "market viability". Although P. indicus has repeatedly demonstrated potential yields

of 4-5 tonnes per ha over a 180-day growout period (equivalent to 9-10 tonnes ha-1 year-' with 2 crops) in

lined ponds with 20% daily water change, final average size at 15-20g individual⁻¹ is small (Bukhari et al., 1993). Hence investors are strongly urged to investigate the market potential for this species, both within the Kingdom and Europe before proceeding beyond pilot scale culture.

These were conducted at the Fish Farming Center using 2 L round-bottom flasks with 100 Nauplius

Present work investigates the ideal salinities for larval and nursery culture and the best time for transfer to growout ponds for Red Sea P. indicus. Results are compared with similar data for P. indicus originating from elsewhere.

Materials and methods

All larvae used in experiments were obtained

from pond cultured broodstock held at the Fish Farming Center, Jeddah. These broodstock were bred originally from wild P. indicus obtained from the Red Sea in the Gizan region.

Larval Salinity Experiments

 \bullet

6 (N6) L'1 in U/V-irradiated seawater diluted where

necessary with chlorine free fresh water to 10,15,25,

Penaeus indicus larval culture in 2 litre round-bottom flasks, fed on algae and Artomia from Z1-PL5 at different salinities, at Fish Farming

Centr , Jeddah, Saudi Arabi

Penaeus indicus nursery culture at different salinities in 10 litre basins, at Fish Farming Centre, Jeddah, Saudi Arabia.

 \bullet

30,35,43,509% salinity. All trials were at 28*C and triplicated, and all flasks were supplied with 50 cells µl"' of mixed Tetraselmis chuii and Chaetoceros calcitrans until mysis (Ml) stage was reached, when larvae were fed Artemia (Artemia 90 brand) at 20 ml"' until postlarva (PL) 5 stage. All flasks were gently

aerated and culture medium changed every second day, when length measurements (total length in mm, tip rostrum to end telson), staging and counts of larvae were made. Larvae placed in 10 and 50% sea water died within 24h.

larval stages at 30% salinity were initially placed at a density of 50 in 10-litre plastic basins at 10,20, 25,30,35 and 4316 salinities with gentle aeration and fed on a standard Taiwanese PL diet (President Enterprises Corp.). All trials were at 28'C and water was exchanged completely when sampling for measurement every two days. Postlarvae at PL1, PL5, PL10 and PL15 were tested for survival rate by transfer from 30% then for survival and growth at 10,20,35 and 43%..

Postlarval Salinity Experiments

Postlarvae previously cultured through the early

In a separate trial the LD50 (time for 50% mortality) was tested for PL4,9,14 and 19 by

transferring postlarvae into 0 and 55%. from culture salinities of 10,20,35 and 43%. Finally, postlarvae cultured at 30%. until PL15 were transferred into basins with 5,10,15,25,30, 35, 43 and 50%. Physico-chemic factors (Salinity). Temperature'C, Dissolved oxygen mgl⁻¹, pH and NH₄-N)

Å.

were measured (Spotte, 1970) as follows: Salinity was measured to prepare new culture water before every sampling by using an Automatic Temperature Compensated Hand Refractometer (ARGENT Laboratories). Salinity was read from a drop or two of sample, the instrument is self-compensating for temperatures 60-100 'F (15.6-37.8'C) by means of a hollow glass prism filled with a stable liquid. This prism is hermetically sealed so that it may completely

immersed in liquids or cleaned without danger of leaking or damage to the instrument under prescribed operating conditions.

Temperature was measured daily using a water thermometer.

Dissolved oxygen was measured three times each day using an oxygen meter. To measure dissolved oxygen the probe was placed in the sample and which was stirred and the salinity knob was adjusted to the

salinity of the sample. Sufficient time for probe to

stabilize to sample temperature and dissolved oxygen

was allowed and dissolved oxygen read in mgl⁻¹. PH was measured on a scale from 0-14 with 7 as the neutral point, water with pH less than 7 at 25'C is acidic and higher than 7 is basic. pH was measured using an electrode (pH meter). The electrode responds to hydrogen ions present by developing an electrical

potential at the glass/liquid interface. At constant temperature, this potential varies linearly with pH of solution being measured. The potential is then amplified, translated and displayed as pH reading. Ammonia in mgl⁻¹ is a very important factor for measurement because it is the most toxic form of inorganic nitrogen produced in culture water. Ammonia in culture water originates from mineralization of organic substances by heterotrophic bacteria and also by excretion from the animals. Ammonia is the main form of nitrogen excreted by most aquatic organisms. The degree of toxicity of ammonia varies according to its chemical state, unionized ammonia (NH3) is significantly more toxic than ionized ammonia (NH_4+) . The toxicity of ammonia is influenced by the pH of the water which controls the hydrolysis of NH4+. The percentage of free ammonia increases in proportion to ammonium ions with increasing pH. Both NH_3 and (NH_4+)

can cross tissue barriers but NH₃ is considered more toxic at higher values. Ammonia toxicity is

exacerbated by a low dissolved oxygen level, but the mechanism is obscure. Elevated levels of ammonia in the environment interfere with the ability of haemoemocy winto retain oxygen. The effects of sublethal levels of ammonia to aquatic animals are: a. to increases the susceptibility of the animals

to other unfavourable conditions (fluctuating temperature, lack of oxygen, etc.). b. to inhibit normal growth. c. to decrease fecundity. d. to decrease resistance to diseases. Ammonia-nitrogen can be determined spectrophotometrically by the indophenol method. Kits (e. g. Hack, Lamotte) are available at the Fish Farming Centre. Ammonia reacts with phenol and hypochlorite

2. Sodium nitroprusside or nitroferricyanide solution. Dissolve 1 g. Na₂Fe (CN) NO.2H₂O in 200 ml

under alkaline conditions to form indophenol blue, the colour intensity being proportional to ammonia concentration. Sodium nitroprusside functions as a catalyst to facilitate colour development at room temperature. The blue indophenol formed is then measured at 640 nm. The ammonia reagents are as following :

1. Phenol solution. Dissolve 20 g. phenol crystals in 200 ml 95% v v'1 ethanol.

 $NH₃$ - free water.

3. Alkaline reagent. Dissolve 100 g. sodium citrate and 5g. NaOH in 500 ml NH3 - free water. 4. Sodium hypochlorite solution commercial, 1.5 N Chlorox or Purex.

5. Oxidizing solution. 4:1 mixture of alkaline

reagent and sodium hypochlorite solution (to be prepared as needed). 6. Standard ammonia solutions. Dissolve 0.472 g. of (NH₄) 2SO₄ 1⁻¹ in deionised water to give a 100 mg NH. -N solution. To 990 ml of deionised water add 10 ml of the 100 mg solution to give a 1.0 mg NH₄-N 1⁻¹ solution. Use a 1.0 1 volumetric flask. To 45 ml of deionised water, add 5.0 ml of the 1.0 mg solution to give a 0.1 mg NH. -N l-' solution.

7. NH, - free water. Remove ammonia from distilled water by passing it through a small column (e.g. 30 cm long by 1-2 cm internal diameter) of cation exchange resin just before use and store the water in a tightly stoppered glass flask. A demineralizer bottle can also be used (such as the Hach). The procedure for ammonia analysis is as follows: 1. Add 40 ml of sample to a 50 ml volumetric

flask.

2. Add 2 ml of phenol-alcohol solution from a

pipette.

3. Swirl the flask and add in order, 2 ml of sodium nitroferricyanide solution and 5.0 ml of oxidizing solution. Swirl the flask after each addition. Fill to mark with deionised water and mix well.

4. Prepare a blank with 40 ml of ammonia free or

deionised water and do again steps 2 and 3. 5. Pipette in 40 ml of standard containing 0.1 mg NH4-N 1-1. Do again steps 2 and 3. 6. Cover the flask to prevent contamination by atmospheric ammonia and let stand for 1 hour. 7. Fill a sample cell of thickness greater than 1.0 cm with blank and set the absorbance at 0.00 for 640 mm. The turbidity blank contains 40 ml of sample diluted to 50 ml with deionized water. Use this blank

if necessary.

8. Read the absorbances of the standard, unknown and turbidity blank.

9. Calculate the concentration of total NH. -N as

follows;

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Cu =
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Where; Cu = concentration of the unknown in mg

 $NH_{4}-N$ 1⁻¹.

 $NH_{4}-N$ 1^{-1} .

 $Vu = volume of unknown pipetted in ml.$

 $Au = absorbance$ of unknown.

 $As = absence of the standard.$

At $=$ absorbance of the turbidity blank.

10. If the absorbance of the unknown is too high,

a new sample solution and turbidity blank can be prepared using a small volume.

Survival and length of shrimp were also measured

over 60 days at PL20, PL25, PL30, PL40, PL50 and PL60.

Yields were calculated by multiplying mean survival by

length in each treatment.

All results were analyzed for significance using

ANOVA and the appropriate statistical tests (Fisher

PLSD, Scheffe F-test with MacIntosh statistical

package, Statview 512 TM).

Adult Osmolality Experiments

P. indicus of a mean length of 11.65cm and 10.50g weight were taken from culture ponds (salinity 43% at the Fish Farming Center, Jeddah and 5 each placed in 0, 10, 20, 35, 43, 55, 60 and 65% salinity seawater for 24h at 28*C. Total mortality occurred after 315 min in 0Y6 and 465 min in 6516. After 24h haemolymph

samples were taken from all other animals and osmolality (milliosmoles) measured using an advanced

digimatic osmometer model 3D11 at the Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

Results

Larval Salinity Tolerances

Tables 1 a, b and Figures la, b and 2a, b show results of P. indicus larvae cultured from PZ1 to PL5 at different salinities. From both experiments best survival rates ranged from (25.7%±7.42 to 41.05%±23) and (26.2%±8.81 to 39.0% ±11.17) at PL1 obtained from 25% and 30% respectively, followed by (6.3%±1.76 to 38.3%±5.92) cultured at 3516, (1.80%±0.56 to 36.5%±40)

and the lowest survival rate averaged (9.3%±3.40) cultured at 1516.

Similarly from both experiments at the PL5 stage best survival rates ranged from (11.3%±5.06 to 24.20% ±11.93) and (8.8%±3.25 to 21.0%±17.09) cultured 3016 and 25% respectively, followed by (3.0%±2.29 to 8.8%± 9.78) cultured at 35%, (0.6%±0.12 to 19.5%±1.80) and the lowest survival rate averaged (2.30%± 2.40) for

15%.

From both experiments best length in mm ranged from (5.23±0.34 to 6.30±1.76) and (5.17±0.26 to

5.60±0.28) at PL1 obtained from 35% and 30% respectively, followed by (4.77±0.38 to 5.60±0.02) for 25% , $(4.40\pm0.31$ to $4.85\pm0.42)$ and length in mm averaged (4.90 ± 0.19) at PL1 for culture at 15%. Similarly from both experiments at the PL5 stage best length in mm ranged from (7.20±0.61 to 7.21±0.38)

and $(6.26\pm0.20 \text{ to } 6.56\pm0.16)$ at PL5 obtained from 30% and $25%$ respectively, followed by $(6.56±0.38$ to 6.96 ± 0.28) for 25% , (5.57 $\pm 0.0.47$ to 7.1 ± 0.25) and length in mm averaged (6.50 ± 0.29) for PL \cdot cultured at 15%. Statistical analysis shows that; at PL1 and PL5 stages survival rate and length were significantly better at 25 and 30% than at 15, 35 and 43% ($p<0.05$) (Tables 1 a, b and Figures la, b.) (Appendix 2a). Size at PL1 (total length 5.5 mm) at the

salinities of 25 and 30% is similar to growth achieved elsewhere with other Indian Ocean strains of P. indicus. Survival was significantly better at 25% than 15, 35 and 43% but not 35% and no significant difference occurred between 25,30% or 30,35% nor between 35 and 43% (p>0.05) (Kumlu et al., 1993) (Appendix 2a). Poor overall survival was due to over handling

when sampling took place. Sampling mortality was

estimated to be 25% higher than mortality obtained during mass culture in large tanks at the hatchery of

the Fish Farming Center. From the above results salinities of 25 and 30% appear to be best for P . indicus larval rearing from N6-PL5.

Postlarvae previously cultured through the early larval stages at 30% salinity were transferred to salinities of 10%-42% in a series of nursery trials (Tables 2,3, and Figures 3a, b, 4a, b, 5a, b, c). Survival rates at PL10 for P. indicus postlarvae reared from PL2-PL10 averaged 33.3%±3.1,24.0%±7.2 and 18.0±8.7% for 20,35 and 42% salinities respectively (Table 2 and Figure 3a). No survival was obtained from salinity of 10%, at this postlarval stage. Likewise

Postlarval salinity tolerances

survival at PL 20 averaged 28.7%±21.6,13.3%±7.57, 12.0% \pm 17 and 5.3% \pm 2.31 for 25, 30, 35 and 42 μ

for other postlarvae that were obtained at stage 10 sourced from same culture and reared upto PL20 at the same density of 50 postlarvae basin ⁻¹ and in the same 10 litre plastic basins, survival rates at PL20 averaged 52.0%±11.1,26.7%±18.15 and 23.3%±9.24 for 20%, 35% and 4216 salinities respectively (Table 2 and Figure 4a). Survival rate at 1016 was as low as 0.04%±0.8. In the other postlarval salinity trial, P. indicus postlarvae reared from PL2 through to PL20,

salinities respectively (Table 3 and Figure 5a). From the first trial survival rates at PL10 for all higher salinities of 20, 35 and 42% were significantly better than in 10% salinity (p<0.05), and no significant difference in survival at PL10 among the salinities of 2016 and 3516,35% and 42% was found, but survival rate

in 20% salinity was significantly higher than in 42% salinity (p<0.05) (Appendix 2b). Likewise from the trial of PL10-20 survival rate at PL20 was significantly higher in 20% than in salinities of 10%, $35%$ and $42%$ ($p<0.05$) (Appendix 2b). From the second trial, P. indicus survival rates at PL17 when reared form PL5 through to PL17 were significantly higher in salinity of 25% than survival in 4216 (p<0.05) (Appendix 2c), At PL20 no significant differences in survival were seen when animals were in salinities of 43% in comparison with the lower salinities of 35%, 30% and 2596 (Figure 5a) (p<0.05) (Appendix 2c). Length in mm at PL10 of P. indicus from the first trial averaged 6.47±0.25,6.33±0.12 and 6.26±0.13 for the salinities 20%, 35% and 43% respectively (Table 2 and Figure 3b). No length was recorded at PL10 in 10% salinity because complete postlarval mortality

occurred in this salinity at PL4. Average final length

in mm for postlarvae reared from PL10-PL20 at PL20

were 9.50±0.00,10.34±1.00,8.84±0.32 and 8.82±0.27 for the culture salinities 10% , 20% , 35% and 43% (Table 2 and Figure 4b). In the second postlarval salinity trial where P. indicus postlarvae were reared from PL2 through to PL20, lengths in mm at PL 20 were 9.86±0.91,8.46±0.18,9.95±1.12 and 10.44±0.33 for the

culture salinities 25%, 30%, 3516 and 43*. (Table 3 and Figure 5b).

Length measurement results showed no significant difference at PL5 nor at PL7, but length of PL10 was significantly better at the higher salinity of 43% in comparison with the lower salinities of 35,30 and 25%. Similar results were achieved at PL14 (p<0.05) (Appendix 2c).

Length for PL17 was significantly better at 43%

salinity in comparison to the postlarval length for PL17 at 30% salinity, there were no significant differences in lengths among other salinities at this stage. For PL20 lengths were significantly better in salinities of 43% and 25% as compared to 30% salinities (p<0.05) (Appendix 2c). In separate experiments postlarvae at PL4,9,14 and 19 were transferred from the above culture salinities of 10,20,35 and 43%, where they had been

kept for 4 days in 10-litre plastic basins, into 0 and

55% (Table 4 and Figure 6).

Time taken for 50% mortality to occur was monitored (LD50). At 0% salinity, when postlarvae were transferred from salinities of 43, 35, 20 and 10%, LD50s for PL4s were 28,16,9 and 26 min. respectively. For PL9 they were 14,19,81 and 85 min. respectively. For PL14 they were 15,17,135 min. and

13 hrs. and 49 min. respectively and finally for PL19 they were 14,44,174 min. and 22 hrs. and 25 min. respectively (Table 4 and Figure 6). At 55.0% salinity, when postlarvae were transferred from salinities of 43, 35, 20 and 10%, the LD50s for PL4s were 72, 35, 25 and 35 min. respectively. For PL9 they were 61,33 and 24 min. from salinities of 35,20 and 10% respectively. Postlarvae were surviving from 43% salinity at 55% for more than 2 days when experiment

obtained from the hatchery at PL5 stage (by which time they had been transferred from 30 to 43% salinity),

was terminated. For PL14 survivals were 58,31 and 16 min. from salinities of 35,20 and 1016 respectively, but postlarvae from 43% survived at 55% for more than 2 days until the experiment was terminated. Finally for PL19 survivals were 168, 51 and 31 min. from salinities of 35, 20 and 10% respectively, but postlarvae from 4316 survived for more than 2 days when the experiment was terminated (Table 4 and Figure 6). In a further set of experiments postlarvae

were further acclimated over a 10 day period to salinities of 50,43,35,30,25,15,10 and 5%. These animals at PL15 were then grown over a 45 day period at each of these salinities. Physical data including temperature, dissolved oxygen, pH and ammonia are recorded at each of the above salinities (Table 5).

For all salinities, temperature, dissolved oxygen, pH and NH_{4} -H ranged from 26-28 \degree C. , 3.5-6.6 mgl 7, 7. and 0.10-0.80 mgl⁻¹ respectively. Sampling incorporated records of survival rates (%), length measurements in (mm) and yields in grammes per 1000 postlarvae for stages PL20, PL25, PL30, PL40, PL50 and PL60 (Table 6 and Figure 7a, b, c). At PL60, survival rate was highest in 50% (83.4%±15.3) followed by 50.2%±10.31,47.9%±8.62,

45.9%±10.04,30.1%±4.35,11.0%±10.99 and 9.6%±9.41 for salinities of 43%, 35%, 30%, 25%, 15% and 10% respectively (Table 6 and Figures 7a). No survival occurred for this postlarval stage in 5%. Survival rate in 50% was significantly higher than in all other lower salinities of 43%, 35%, 30%, 25%, 15%, 10% and 5% (p. <0.05) (Appendix 2d). At PL60, length in mm for all salinities ranged from 28.5±0.58 - 41.0±24.88 (Figure 7b), with no

significant difference among all the salinities

mentioned above.

25,30,35,43 and 5016 contrast to results for PL2-20. Highest yields were obtained at the higher salinities of 35-50% (Figure 7c), although there is no significant difference between yields in 43 and 50% (p. <0.05) (Figure 7c) (Appendix 2d) in these ongrow experiments. Statistical analysis revealed

significantly better yields at 50fa than 35%, but not significantly different from yields at 4316 (p<0.05) (Figure 7c) (Appendix 2d). Table 7 and Figure 8 show the results of haemolymph osmolality in milliosmoles for adult Red Sea P. indicus kept in the following salinities for 24 h: 0,5.0,10.0,20.0,35,43,55,60 and 6516. Complete mortality occurred only at 0% after 315 mins. and 65% at 465 mins. No mortality was observed at

culture temperature range is 26-30°C (Kuo) Pas; Van Olst et al., 1980; Wickins, 1981,1982) and it is likely

salinities of (10-55%).

Discussion

From present work it appears that optimum salinity for larval culture of P. indicus from the Red Sea is 25-30%, as any deviation from this salinity range results in reduced growth and survival. Previous studies have shown that optimum larval

that any deviation from this temperature range will result in an even narrower salinity tolerance range (Mantel and Farmer, 1983).

From the experiments with postlarvae it is clear that during early PL stages (2-20) there is a preference for lower salinities with survival rapidly

decreasing to below 20% at ambient Red Sea salinities of 42% (Figure 5a,b). Although growth appears to be best at this salinity, this is due to the faster growth achieved by the few survivors at this salinity. Hence salinities of 25-3096 should be maintained at least until PL20. However from Figure 6 it is clear that some acclimation to higher salinities is achieved before acclimation to lower salinities. This resistance is

seen as early as PL9 where postlarvae from 43% acclimate to 55%, but postlarvae from 10% do not survive in 016. By PL14 stage acclimation to both high and low salinities is seen (Figure 6). This adaptation to a wide range of salinities, even for a short period, would be very important in the coastal waters of the Red Sea where lagoon and pool salinities may reach 78fa. \blacksquare

As expected, later stage postlarvae showed better

resistance to salinity stress with acclimation first occurring at PL9. These trials also demonstrate that

acclimation to higher salinity (PL9) occurs before acclimation to lower salinity (PL14). Physical data from the longer term salinity trial show that similar levels of ammonia, oxygen and pH were found in all triplicated trials, hence salinity was the sole factor influencing survival and growth

Therefore survival and growth results in which postlarvae were cultured in a range of salinities for 45 days (Figure 7a, b) confirm the euryhaline ability of P. indicus, but in contrast to early PL stages (PL2-20) demonstrate increasing tolerance of salinities in the higher range. By PL40 stage survival at the highest salinity of 5016 was better than at all other salinities and final

35%, but not significantly different from yields at 4316 (p<0.05)(Figure 7)(Appendix 2d), demonstrating

survival at PL60 was significantly better at this salinity (p<0.05)(Appendix 2d). There was no difference in survival at 30%, 35% and 43% salinities, but salinities of 5-25% produced the lowest survival rates (Figure 7a). Length was apparently best at the lower salinities (Figure 7b), but this is possibly due to lower stocking densities resulting from higher mortality.

Yields were significantly better at 50% than at

that the Red Sea P. indicus prefer full strength or even higher salinity water once juvenile stages are reached. From Figure $7c$ it can be seen that this preference is first exhibited at the PL30 -40 stage. Overall yields decreased with decrease in salinities, due to poorer survival at lower salinities.

From a physiological standpoint it is difficult to explain why cultured postlarvae at 50% (Figure 7c). gave greater yields than at 35%.. The Red Sea P. indicus show osmoconformity at 30%. (Figure 8) and hence in hyperosmotic external media may be expected to expend energy to maintain a hypoosmotic haemolymph, this expended energy should ultimately be reflected in a reduction in length.

When present length and survival rates for

postlarvae are compared to those obtained by Raj and Raj (1982) for Indian Ocean P. indicus (Table 8), it is clear that the Red Sea P. indicus show physiological adaptation to higher salinity. Survival rates of over 90% were obtained during 60 days of nursery culture in India for salinities of 5-30% and survival only dropped at higher salinities of 35-43%. Nair and Krishnankutty (1975) reported that growth rate of P. indicus was significantly higher at

a salinity of 10%. for postlarval stages and the optimal salinity for juvenile prawn was 36%.. In

contrast, Red Sea P. indicus showed high mortalities at lower salinities and best survivals at salinities in excess of 35%.

Larval culture results up to PL1 for P. indicus from India reveal that the growth, best survival rate of 90% and final length of 6.0mm were obtained at 25%

et al. (1993) in their trial for P. indicus from India was 0.43 mm at 20%, which was almost in the same range

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(Kumlu et al. 1993). Similar results were achieved from the present study on Red Sea P. indicus with 41.0% survival rate and 5.6 mm growth, therefore P. indicus larvae from Red Sea appear not to adapt for Red sea high salinity conditions and still show a preference to lower salinities. Results of postlarval culture trials for P. indicus from India by Kumlu at al. (1993) agree with Raj & Raj (1982), but contradict results of the present study. The highest survival rate of 70% at PL60 was obtained at 2016, which was significantly better than the 20% survival rate obtained at the same salinity and stage for P. indicus from the Red Sea. Also at 5096 the PL60 survival rate of 50% was significantly lower for P. indicus from India when compared to survival rate of 83.4% for P. indicus the Red Sea.

The highest daily growth rate obtained by Kumlu

of P. indicus from Red Sea daily growth of 0.65 mm. However both rates of growth were less than 0.93 mm daily growth rate of Raj & Raj (1982) (different larval source) at the same salinity. The lowest daily growth rate of 0.15 mm was

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obtained by Kumlu at 50% and lowest daily growth rate obtained by Raj & Raj was 0.47 mm at 4516. The daily growth rate of 0.50 mm does not change much with different salinities in the present study of P. indicus from the Red Sea.

Daily rate of growth ranged from 0.45-0.65 mm day-

and was within the same range for all salinities experimented on with the Red Sea P. indicus.

When Grajcer and Neal (1972) obtained a better

growth rate for P. aztecus at 5016 he attributed this to the poor consumption of food at this salinity. Although data on food intake was not recorded, a similar trend of poor consumption of food at 45% was observed during experiments by Raj (1982). Although the food consumption was not recorded in the present study; it can be seen from the above results (Figure 7b) that no change in daily rate of growth occurred at salinities exceeding 3596 for P.

indicus from the Red Sea.

This confirms that these prawns are extremely good hyperregulators in dilute seawater and

hyporegulators in high salinity media. Although 100% mortality was observed in 0% after 315 min and in 6516 after 465 min, at all other salinities there was no mortality over the 24h period of immersion.

The results of this study demonstrate the

adaptation of P. indicus PL30 and older postlarvae to the high saline environment of Red Sea and it is recommended that future investigations concentrate upon the culture potential of Red Sea P. indicus, rather than considering importations. Similar research is required on local races of P. monodon, providing sufficient broodstock can be located.

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Table 2: P. indicus average postlarval length in (mm) and survival rates from
(PL2-10) and from (PL10 30) at different calinities. Initial survival (PL2-10) and from (PL10-20) at different salinities. Initial survi rate were 100% and length were 5.65±0.25 and 7.01±0.36mm at PL2 and
PL10 respectively in and dividend and the final product to respect include PL10 respectively. (Experiment 1)

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Table: 5 Physical data analysis of P. indicus Postlarval culture (PL15-60) at different
Postlarval culture (PL15-60) at differ salinities.

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Table 6: P. indicus average postlarval survival rate (ö), length in (mm) and yields (PL15-60) at different salinities. (Experiment 3)

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Table 7:

Haemolymph osmolality (milliosmoles) of Red Sea P. indicus* obtained from five individuals at each salinity after 24 h in a range of 10-55%.

*The shrimp samples were supplied by the Fish Farming Center, averaged 11.95cm and 10.50g. Complete mortality occurred only at 0% after 315 mins and at 65% after 465 mins. No mortality was observed at salinities of $(10\text{m} - 55\text{m})$.

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Figure 1 a: *P. indicus* average larval survival rate (*)
from 71-PLS at different calinities (Experi) from Z1-PL5 at different salinities (Experiment 1)

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Figure 1 b: P. indicus average larval and length in mm from Z1-PL5 at different salinities (Experiment 1).

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\star \star 15 0/00 0 0 0 25 0/00 Å 4 30 0/00 **E** 4 30 0/00 E 35 0/00 Å Figure 2 a: P. indicus average larval survival rate (%)
from Z1-PL5 at different salinities (Experiment 2).

Stages

Figure 2 b: *P. indicus* average larval length in mm
Example 21-PL5 at different calinities (EX from Z1-PL5 at different salinities (Experiment 2).

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Figure 4 a&b: P. indicus average postlarval survival rate (3) and length in (mm) from PL10-20 at different salinities $(Experiment 1).$

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CHAPTER 3

Development of artificial feeds for larval and nursery culture of Penaeus I ndi cus from the Red Sea

Part of these research (postlarval) results were

presented at the Aquaculture technology and investment opportunities symposium. April 11-14,19936(Abstract p. 38). Riyadh, Saudi Arabia. Title: Development of nursery feeds for Penaeus indicus cultured in Saudi Arabia. Authors: Feisal A. Bukhari, D. A. Jones and A. J. Salama

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Introduction

In 1934, Dr. Fujinaga, the world's acknowledged father of shrimp culture, successfully spawned and partially reared larvae of Penaeus japonicus in Japan (Hudinaga, 1942). In 1963, Mr. Harry Cook of the Galveston Laboratory in Texas, U. S. A. in collaboration

with Dr. Fujinaga, successfully spawned and reared the larvae of two American species, P. setiferus and P. aztecus (Cook and Murphy, 1966). Since this time reproduction and culture has been widely practised, especially for the penaeids: P. semisulcatus (AQUACOP, 1975), P . vannamel (Chamberline C_{\bigcup} , 1981) and P. indicus (Muthu and Laxminarayana, 1977; Alikunhi and Hameed Ali, 1978; Emmerson, 1980). Larval production techniques have been adopted in Taiwan, Philippines, Thailand and Malaysia for Asian species such as P. monodon, P. merguiensis, P. indicus and P. orientalis (Kungvankij, 1984) and in the Americas for P. vannamei and P. stylirostris (Fast & Lester 1992). P. indicus from the Red Sea was spawned and larvae cultured upto PL30 successfully for the first time at the Fish Farming Centre, Jeddah (Bukhari et al., 1991).

Live foods such as microalgae and zooplankton

have been extensively used in shrimp larval rearing,

but these practices require large facilities, high maintenance expenses and trained intensive labour to produce the live food organisms (Kanazawa et al., 1982). Further more, the quantity and quality of live food produced may vary greatly and is often unreliable (Teshima et al. 1982; Langdon et al., 1985; Kanazawa, et al., 1990; Fegan, 1992).

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The development of artificial larval feeds, including microparticulate and microencapsulated diets for crustaceans and other suspension feeders is a significant advancement for aquaculture during the last decade (Jones et al., 1974; Latscha, 1990; Lee and Wickins, 1992).

Recent work has demonstrated the replacement of live foods with microparticulate diets in laboratory scale penaeid larval rearing (Teshima et al., 1982; Kurmaly et al. 1989b), and also at the hatchery level (Jones et al., 1987; Kanazawa, 1990). Microcapsules are readily accepted by penaeid larvae (Kurmaly et al., 1989b), highly stable (Amjad and Jones, 1989) and nutritionally adequate (Jones et al., 1991). However for all penaeid larvae tested including P. monodon, the total replacement of live food by microencapsulated diets has always produced slower

growth than controls fed on microalgae and Artemia nauplii (Amjad et al., 1989; Jones et al., 1989,1991).

However little information is available on survival and growth of P. indicus when live foods are replaced with microencapsulated diets (Galgani and AQUACOP, 1988). The first part of this chapter aims to investigate the possibility of algal replacement by artificial microencapsulated diets fed to Red Sea P.

indicus larvae up to PL5.

The second part of the chapter attempts to replace commercial nursery feed with locally produced feeds. Nutritional studies on shrimp were initiated in the early 1970's as feed must be nutritionally adequate and economical for successful culture (Fast and Lester 1992). The most researched area in shrimp nutrition has been proteins, and recommended protein levels vary from 30-57% depending on species and size of marine shrimp. Shrimp will utilize not only protein but lipids and carbohydrates as energy sources, because the use of protein for energy is not economically efficient. Therefore, adequate nonprotein energy sources must be maintained in shrimp feeds. Dietary and tissue lipids are important in the nutrition of shrimp, as they form a concentrated and highly digestible source of energy, and supply

essential fatty acids necessary for normal growth and survival of all animals. Dietary lipid serves also to

act as an attractant and can affect the texture of the feed. The following table shows the recommended levels of protein, lipid and cholesterol in commercial shrimp feeds (Fast and Lester, 1992).

Four fatty acids have been demonstrated to be essential for shrimp (Kanazawa et al., 1979; Jones et al., 1979), these are given in the following table (Tacin, 1987).

Cholesterol is also considered to be an

essential nutrient which must be provided in the diet,

because of it's role in absorbing and transporting of

fatty acids (Teshima and Kanazawa, 1971).

Carbohydrates are not a dietary essential for

shrimp feeds, because of their limited utilization and

metabolism by shrimp (Fast and Lester 1992), but are

usually included to spare protein.

Vitamins must be supplied in the diets to achieve

normal growth, metabolism and reproduction, and

minerals have different roles such as to balance

osmotic pressure, form essential components of enzymes, vitamins and pigments (New, 1987). The best natural ingredient is squid meal as it

contains an unknown growth factor which increases the digestive efficiency of shrimp but, also enhances growth rates as it supplies the highest concentration of cholesterol phospholipids and fatty acids, 20:5n3 and 22: 6n3 found in any natural source. In addition it acts as an excellent attractant (Cuzon pers. comm.). Shrimp head meal is the ground dried waste from shrimp and includes the head and exoskeleton, it is usually steamed or sun-dried and serves as a good source of minerals, chitin, cholesterol, phospholipids and the fatty acids (20: 5n3 and 22: 6n3), beside being an excellent attractant. Binders are a necessary feed additive to increase water stability of feeds, and wheat gluten is a commonly used binder for shrimp feeds because of its cost effectiveness.

In the second part of this chapter a

comparative study is designed in which P. indicus postlarvae are fed on commercial and locally

formulated nursery diets. Due to the high local price of squid meal, feeds were formulated with different levels of cheap shrimp head meal as a substitute for squid (Table 2). The aim was to reduce the cost of rearing postlarvae through the nursery stage until they are ready for ongrow in ponds.

Materials and Methods

All larvae used in experiments were obtained from pond cultured broodstock held at the Fish Farming Centre, Jeddah. These broodstock were bred originally from wild P. indicus obtained from the Red Sea in the Gizan region.

These three experiments were conducted at the Fish Farming Centre using 21, 2 litre round-bottom flasks for the first experiment and 9 flasks for each of the other two experiments. Each flask contained 200 P. indicus larvae stocked at nauplius 6-zoea 1 in 2000 ml of UV-irradiated seawater $(S=30\), T=28^{\circ}C)$. As a control in the first experiment the first treatment

incorporated 3 flasks where the larvae were fed at a l
* $\mathop{\mathtt{concation}}$ or 50 cells μ $\!$ mixed Algae Tetrasel

Larval feeding Experiments

chuii and Chaetoceros for 4-6 days. From Mysis stage onwards the larvae were fed with Artemia sp. at a density of 20 individuals ml⁻¹ in all treatments. In an other three flasks 50% of the algae was replaced with Nippai artificial plankton (B. P.) at a rate of 8.0 and 16.0 mg l⁻¹ for zoea and mysis stages respectively, and artificial plankton (A. S.) for postlarval stages from

1-5 at a rate of 30 mg l^{-1} .

Similarly in an other treatment of 3 flasks; 50%

of algae was replaced with Frippak microcapsulated ا۔
ع diets (CAR 1) of 10-30 pifor zoea stages 1-3, (2CD) of $\frac{1}{30-90}$ $\mu\bar{l}$ for mysis stages and (3CD) of 80-150 $\mu\bar{l}$ for postlarval stages from 1-5 at the same density as the Nippai (Jones et al., 1987; Kurmaly et al., 1988). In another 6 flasks 80% of the algae was replaced by Nippai and Frippak for each 3 flasks respectively receiving the same feeding rates of the above. Complete algal replacement with Nippai and Frippak was attempted in the last 6 flasks, with no algae provided in these treatments. Artemia were added at same rate as in the control. Algae and artificial diets were given to the larvae in 4 feeds per day i. e. 0700,1400,1800 and 2200 h.

Gentle aeration using countable bubbles was

provided to each flask for the 12-14 days duration of

the experiments and every second day the culture medium was changed when sampling took place for measurement of survival, staging and growth. Due to poor results treatments of complete algal replacement by the artificial feeds, were not repeated in the further two larval experiments conducted later.

Postlarval feeding Experiments

A comparative study was conducted at the Fish Farming Centre in 21,10 litre basins of Sea water (4216) with a high rate of exchange of 500 ml min. -' and repeated in still water at (3016) with a 50% water exchange day'.

Optimal salinities for postlarval culture of P. indicus from Red Sea was determined to be from 25 to 30% salinities (Chapter 2, Bukhari et al. 1993). A satisfactory performance (survival rate and growth) was observed at salinity 42%, which happened to be the salinity of the sea water at the Fish Farming Centre (Chapter 2, Bukhari et al., 1993). Therefore the above salinities were selected for this study for testing postlarvae.

The postlarvae of P. indicus at PL5 were obtained

from the Centre hatchery (Bukhari et al., 1990) and

were reared at a rate of 50 animals basin' fed on the

following commercial feeds: Taiwanese, Nippai, Frippak, and 4 formulated feeds based on locally available ingredients (FFC1, FFC2, FFC3 and FFC4) (Table 4a) at 28-32'C. FFC stands for Fish Farming Centre, and the animal protein source for the first locally formulated diet FFC1 was squid meal, the other local diets FFC2, FFC3 and FFC4 contained partial or complete substitution by cheaper alternatives for this ingredient. All diets commercial and local were tested at both 4216 and 30%, salinities. Diet preparation involved selecting and purchasing ingredients from Grain Silos and Flour Mills Organisation (G. S. F. M. O.), and Jeddah Central Fish Market (Tables 2, 4a and b). The ingredients

obtained from the last source (squid and shrimp heads)

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were sun dried for 2 days, cooked and completely dried in an oven for 24-36 at a low temperature of 40'C. Grinding was by small laboratory grinder (Laboratory mill 3303) and the product was sieved through a 100 micron siever (Ro-Tap testing sieve shaker, model b). Each ingredient was weighed and dry mixed manually before adding vitamins and minerals dissolved in tap water. Pellets were made using a pelletizer of 3mm diameter and these were steamed using a domestic

steamer for 5 min. This cooked the binder and gave the pellets a firm structure, these were in an oven at

40-C. All of the equipment was available at The Fish Farming Centre (New, 1987; Pascual, 1983). Protein was analyzed at the Grain and Silo organization (using Kjeldal method (1963) by multiplying the concentration of detected nitrogen in the samples by a factor of 6.25).

isolating total lipid from the samples with a mixture of chloroform methanol which acts as an antioxidant (the double bonds in fatty acids are easily oxidised). About 10% of the dry weight was then lipid and for the analysis of fatty acids 0.02 volume of 0.017% aqueous magnesium chloride was added to the measuring cylinder containing the extract. It was then sealed with a glass stopper. The cylinder was shaken briefly to mix the reagents and the stopper eased slightly to release any generated pressure. The emulsion was decanted into a centrifuge tube and covered with aluminium foil. Centrifuge was spun at 2000 rpm for 5 min. The emulsion separated into two phases. The upper phase was removed by aspiration using a pasteur pipette connected to a vacuum pump. The lower phase was then washed with Folch upper phase (chloroform : methanol : water, 3: 48: 47 $v/v/v$ and gently swirled. The

Lipid extraction and fatty acids were analyzed by

upper layer was removed again and the washing step was

repeated. The extract was filtered through filter

paper treated by anhydrous sodium sulphate to remove any traces of upper phase and to dehydrate the extraction. The remaining solvent was removed by evaporation at 30°C using a Rotary Evaporator. The sample was poured through a funnel into a special thick walled pear-shaped flask (to avoid the neck of the flask) and fixed onto the evaporator. The dried extraction (lipid) was then dissolved in a little chloroform and transferred using a clean pasteur pipette to a reactivial to which was added 2ml of the methylating reagent (14% boron triflouride in methanol). The reactivial was flushed with oxygen-free nitrogen (OFN), sealed then placed in a heating block at 100°C for 1h. The mixture was then allowed to cool at room temperature and washed into a 14ml glass vial using 3m1 of water and 6m1 of pentane. The vial was sealed, shaken and allowed to settle. The extraction was repeated and the pentane evaporated under (OFN) and the fatty acid methyl esters (FAME) dissolved in hexane. The samples were analyzed using Gas Chromatography (Folch et al., 1957). Ash and carbohydrate analysis took place when samples of different diets were dried in an oven at 80*C to constant weight. Approximately 50 - 200mg

duplicate samples of the test diets were ignited using

a furnace at 550*C for 5h. The resultant was assumed

to represent the ash-free dry weight. The percentage of carbohydrate contents determined by substraction from the dry weight. Water stability analyses were done at Fish Farming Centre by placing diet samples in the postlarval culture facilities basins in triplication for 1,3,7 and 14 hours. Gentle aeration was provided

to each basin and the residue of the diets were weighed and compared with initial weight as follows

Initial weight - Final weight

Water stability = ----------------------------- X 100

Initial weight

Results were statistically analysed by (ANOVA)

and are shown in Table 5. Pellets were ground (Using

a small laboratory grinder) and graded (using an automatic siever (available at Grain, Sailo and Flour Mills Organization, Jeddah) to match the required food particle sizes (New, 1987; Pascual, 1983), see the

following table;

Daily feeding rate was 50-100% of total shrimp

biomass to ensure feeding to satiation, and was

equivalent 50mg day-' divided in 3 portions given at 7.00,15.00 and 20.00pm. Gentle aeration was provided to each basin for the 26 days duration of the experiment. Sampling took place at PL5, PL10, PL15, PL20, PL25 and PL30 for measurement of growth and survival, physical data; (Temperature, DO, pH, NH4 H and turbidity) were also

measured, using standard methods for examination of

water and wastewater (APHA, 1980).

calculations gave the formula; $(Y = 9.2605 - 2.6389X)$ + 0.23308 x2) for post larval P. indicus PL1-40 with a strong correlation between length and weight of $R=$ 0.996 (X = length, Y = weight). The following table shows the P. indicus obtained from the hatchery of Fish Farming Centre - length (mm), wet and dry weight (g) at different postlarval stages.

Survival percentage and growth (length) were converted to yield for each diet (weight of 1000 postlarvae in grams). Weight was the calculation of wet and dry weight regressed against length for samples taken at PL1, PL5, PL10, PL15, PL20, PL25, PL30 and PL40 (Table 7 and Figure 5a, b). These

Yields of P. indicus in this study were calculated at

PL25 and presented per 1000 postlarvae.

Results

Larval feeding Experiments

(Tables la, D, C and Figures la...) . Similarly from all

For all experiments the best survival rates at PL1 from live feed treatments (control) were 59.7%±10.77,39.0%±11.17 and 26.2%±8.81 with an overall average of 41.6 GHz .9. These were followed by the treatment with 50% algae replacement with Nippai 55.5%±12.13,34.0%±8.89 and 13.3%±6.11 giving an over all average of 34.3%±21.1. Survival rate for Frippak replacement of 50% algae were 8.0st6.61 , 31.7st7.18 and 29.7%±9.39 with an over all average of 23.1%±13.14

experiments the best survival rates at PL5 were from the live feed treatment (control) with 18.0%±1.3, 24.2%± 11.93 and 11.3%±5.06 giving an overall average of 17.8%±6.46. This was followed by the 50% algae replacement with Nippai treatment (20.0%±2.56, 21.8%±3.69 and 8.3%±5.48) giving an overall average of 16.7±7.33. The survival rates for Frippak 50%

replacement algae were 1.5%±2.18, 8.3%±3.69 and 11.2±9.81 with an over all survival of 7.0±4.98. Statistical analysis shows that for the first experiment survival rate at both PL1 and 5 stages were significantly better on algae and Nippai than on Frippak (p<0.05) (Appendix 3a). There was no significant difference between algae and Nippai (p>0.05) (Appendix 3a). In the second experiment the survival rate at PL1 was not significantly different between all the treatments (p>0.05) (Appendix 3a), but the control treatment survival rate was significantly better than Frippak at PL5 (p<0.05) (Appendix 3a). No significant difference between algae and Nippai nor between Nippai and Frippak replacement treatments (p>0.05) was found (Appendix 3a). No significant difference in survival rate was found among all treatments at PL1 and 5 (p>0.05) in the last

experiment (Appendix 3a).

For all experiments the best length at PL1 was

from the live food treatment (control) with 4.58±0.09 mm, 5.2±0.26 mm and 5.6±0.26 mm giving an overall average of 5.13±0.51 mm, followed by the 50% Nippai replacement treatment giving 3.81±0.17 mm, 5.20±0.52 mm and 5.20±0.20 mm with an overall average of 4.74±0.80 mm. Length for Frippak 50% replacing was 2.87±0.2 mm , 4.95±0.35 mm and 5.0±0.13 mm with an overall average of 4.23±1.27 mm. In the first experiment it was noticed that P. indicus larvae reached the M3 stage when sampling took place and did not reach PL1 on the 50% Nippai and 50% Frippak replacements at the time when the control treatment had metamorphosed to PL1. Similarly best length at PL5 was from the live food treatment (control) with 5.64±0.9 mm, 7.21±0.38

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mm and 7.2±0.61 mm and an overall average of 6.68±0.90 mm. This was followed by the 50% Nippai algae replacement (5.35±0.42 mm, 6.53±0.86 mm and 6.40±0.41 mm with an over all average of 6.1±0.65 mm.). Length on Frippak was 5.64±1.24 mm, 5.96±0.30 mm and 5.60±0.62 mm with an overall length of 5.73±0.20 mm. Statistical analysis of the data from the first experiment shows that at PL1 length on the live feed treatment was significantly better than length results

of all of the other treatments (p<0.05) (Appendix 3a). Length on 50% Nippai replacement was also

significantly better than Frippak (p<0.05) (Appendix 3a). At PL5 there was no significant difference in length between the live feed (control) treatment and all of the other algae replacement treatments (p>0.05) (Appendix 3a). For the second experiment there was no significant difference at PL1 between all the treatments in length (p>0.05) (Appendix 3a). However the control treatment length was significantly better than Frippak at PL5 (p>0.05) (Appendix 3a), but there was no significant difference between live feed and Nippai, nor between Nippai and Frippak 50% algae replacement treatments (p<0.05) (Appendix 3a). In the last experiment length in the live feed control treatment was significantly better at PL1 than for Nippai and Frippak. At PL5 live feed was significantly

Water temperature and other physical data; Dissolved oxygen and pH levels during culture period from PL5-30 at both salinities 42% and 30% were within

better than for Frippak (p<0.05) (Appendix 3a), but no

difference in length between Nippai and Frippak

treatments (p>0.05) (Appendix 3a).

Postlarval feeding Experiments

the satisfactory limits (Table 3a, b). Ammonia levels

were higher during the 30% study than the 42% study

due to less water exchange. But generally there appears to be little difference between local diets and commercial diet in the effect on water quality (Tables 3a & b).

proximate analyses (Table 4a-d) and ingredients of local diets demonstrate that these are within the same range as seen in commercial diets. The local diet

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 $\frac{1}{2}$.

FFC1 had the highest production price because of the inclusion of a high percent (40%) of the expensive squid meal. The price of the commercial diets Frippak and Nippai were similar to FFC1, and the cheapest diets were the Taiwanese and FFC4. Local diets were also as stable, if not more so, than commercial diets when placed in water for 1 and 3 hours (Table 5).

The survival rates in a salinity of 42% at PL30 for P. indicus fed on local diets FFCl, FFC2 and FFC4 were not significantly different (p<0.05) (Appendix 3b) from those on the commercial diets (Taiwanese, Frippak and Nippai). However although the local Diet FFC3 was not significantly different from the Taiwanese (p>O. 05) (Appendix 3b), it was significantly better than Frippak and Nippai (p<0.05) (Appendix 3b) (Table 6a and Figure 3a, b). For survival on the

local diets ; FFC1 was not significantly different

from FFC1, FFC3 or FFC4 (p>0.05) (Appendix 3b). FFC3 was significantly better than FFC1 (p<0.05) (Appendix 3b), but was not significantly different from FFC2 and FFC4 (p>0.05) (Appendix 3b), and the last local diet FFC4 was not significantly different from FFC1, FFC2 and FFC4 ($p>0.05$) (Appendix 3b).

Survival rate in a salinity of 30% at the same stage PL30 was significantly better for local diet FFC1 than Taiwanese and Nippai, but not from Frippak (p<0.05) (Appendix 3b). FFC2 was significantly better than Frippak (p<0.05) (Appendix 3b), but not better than Taiwanese nor Nippai (p>0.05) (Appendix 3b). FFC3 did not produce significantly better survival than any of the commercial diets (Taiwanese, Nippai and Frippak). FFC4 was not significantly better than Nippai (p>0.05) (Appendix 3b), but other commercial diets (Taiwanese and Frippak) were significantly worse than FFC4 (p>0.05) (Appendix 3b). Survival rate on FFC1 was significantly better than FFC2, FFC3 and FFC4 (p<0.05) (Appendix 3b). Survival on FFC2 was not significantly different from that on FFC3 and FFC4 (p>0.05) (Appendix 3b). FFC3 was not significantly different from FFC2 and FFC4 (p>0.05) (Appendix 3b).

Length in a salinity of 4296 at PL30 for P.

indicus fed on the local diet FFC1 was not

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significantly different from Nippai, but other commercial diets (Taiwanese and Frippak) gave significantly better length than FFC1 (p>0.05) (Appendix 3b). FFC2 and FFC3 were not significantly different from commercial diets (Taiwanese, Nippai and Frippak) (p>0.05) (Appendix 3b). FFC4 gave significantly better length than Nippai and was not significantly different from other commercial diets (Taiwanese and Frippak) (p>0.05) (Appendix 3b) (Table 6a, b and Figure 3a, b).

Length on FFC1 was significantly different from that on FFC2 and FFC4 (p<0.05) (Appendix 3b), but was not significantly different from that on FFC3 (p>O. 05) (Appendix 3b). FFC2 was not significantly different from FFC3 and FFC4 (p<0.05) (Appendix 3b). FFC3 was not significantly different from FFC1 and FFC2 (p>0.05) (Appendix 3b). FFC4 was significantly better than FFC3 (p<0.05) (Appendix 3b) and was not significantly different from FFC2 (p>0.05) (Appendix 3b) (Table 6a and Figure 4a). Length in a salinity of 30% at the same stage PL30 for shrimp fed on local diets FFC1, FFC2, FFC3 and FFC4 was not significantly different from that achieved on commercial diets; Taiwanese, Frippak and

Nippai (p>0.05) (Appendix 3b). Length on local diets

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FFC1, FFC2, FFC3 and FFC4 was not significantly

different between diets (p>0.05) (Appendix 3b) (Table 6b and Figure 4b).

Yields in salinities of 42% and 30% at PL25 for P. indicus fed on the local diet FFC1 were significantly better than for Nippai only at the salinity of 30% (p<0.05) (Appendix 3c) (Table 7 Figure 5. a, b). Yields from all other local diets did not differ significantly from those commercial diets (p>0.05). Yields on local diets did not significantly differ from each other at either salinities (p>0.05) (Appendix 3c) (Table 7 and Figure 5).

Discussion

It was not feasible in this study to consider

testing algal replacement diets up to the PL5 stage due to an overall low average survival rate in all treatments which ranged from 7.0-17.8%. At PL1 reasonable survival rates were achieved ranging from 13.33-55.5% with 50% algal replacement by Nippai which is comparable with the control range of 26.2-59.7%. The overall survival rate of Frippak 50% algal replacement ranged from 8.0-31.7%. This compares poorly with P. indicus in Tahiti where PL1 survival

rate ranged from 60-66% when fed on live food, and 37-

40% on an artificial diet given at a rate of 1250 g l⁻

15 times day"' and 5% when fed on the same diet at the same density but two times day⁻¹ (Galgani and AQUACOP. 1988). Jones et al. (1987) have achieved a 9-47% survival rate to PL7 when P. monodon was fed with microcapsules, algae and no Artemia, but a lower survival rate for the same species was obtained (3- 26%) when fed only on microcapsules (Jones et al., 1987). Kurmaly et al. (1989) achieved a 51-64% survival rate to PL1 with P. monodon when fed on microcapsules only, but a higher survival rate of 76% was obtained when 10 cells μ 1⁻¹ algae were added (Jones et al., 1989). P. vannamei produced a higher survival rate of 90% when Artemia was added and 80% when fed microcapsules and algae without Artemia (Jones et al., 1987). In order to ensure good larval survival it is recommended that Artemia be added prior to M3 to avoid cannibalism during the M3-PL1 stages (Emmerson, 1984). The poor survival rate in the present study was due to handling when sampling took place. Estimated survival rate in mass culture for P. indicus at PL5 in large 10m' tanks the Fish Farming Centre, Jeddah is 45%±15. In addition Kumlu (pers. comm. 1993) reports considerable success with artificial diets in the larval culture of P. indicus.

The daily overall mean growth rate between M3 and

PL1 was 0.51mm for control, 0.47mm for 50% algal
due to less water exchange. But generally there appears to be little difference between local diets and commercial diet in the effect on water quality (Tables 3a & b).

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highest production price because of the inclusion of a high percent (40%) of the expensive squid meal. The price of the commercial diets Frippak and Nippai were similar to FFC1, and the cheapest diets were the Taiwanese and FFC4. Local diets were also as stable, if not more so, than commercial diets when placed in water for 1 and 3 hours (Table 5). The survival rates in a salinity of 42% at PL30

Approximate analyses (Table 4c) of local diets demonstrate that these are within the same range as seen in commercial diets. The local diet FFC1 had the

for P. indicus fed on local diets FFC1, FFC2 and FFC4 were not significantly different (p<0.05) (Appendix 3b) from those on the commercial diets (Taiwanese, Frippak and Nippai). However although the local Diet FFC3 was not significantly different from the Taiwanese (p>O. 05) (Appendix 3b), it was significantly better than Frippak and Nippai (p<0.05) (Appendix 3b) (Table 6a and Figure 3a, b).

For survival on the local diets; FFC1 was not

significantly different from FFC2 and FFC4 (p>0.05)

(Appendix 3b). FFC2 was not significantly different

replacement with Nippai and 0.42mm for 50% algal replacement with Frippak. This compares well with P. monodon which had a daily growth at PL1 of 0.38mm (Kurmaly et al., 1988), 0.45mm (Motoh and Buri, 1979) and 0.35mm (Silas et al., 1978). There is a wide range of microparticulate, and encapsulated feeds now marketed for routine use by hatcheries. In most cases, these feeds are still fed only as partial replacements (50-70%) for live feeds at the hatchery level, although total replacement using microencapsulated feeds has been demonstrated both at the laboratory (Jones et al. , 1989) and hatchery level (Jones et al. \cdot 1987). Recently Ottogalli (1991) reported the total replacement of cultured algae with microcapsules for the culture of several penaeid species in hatcheries

in New Caledonia, with similar growth and survival to controls on live feeds. While total replacement of algae is also possible with microparticulate feeds (Kanazawa, 1990), growth may be significantly lower than live feed controls (Galgani and AQUACOP, 1988). Liao et al. (1988) and Jones et al. (1989) have demonstrated success with a wide range of different compounded diets.

The lack of water stability in most

microparticulate diets leads to rapid leaching, bacterial buildup and water pollution. Under 70

laboratory conditions the culture of P. monodon larvae solely on microncapsulated diets has been possible for several years, but size at metamorphosis is usually less than that achieved on live diets (Jones et al., 1989). Similarly in the present study when bacterial buildup and water pollution occurred high mortality results with microparticulates.

The postlarval experiments demonstrate that shrimp head meal is a good substitute for squid meal. The local diet FFC3 supported the best survival rate of 35.33% ± 14.19 and FFC1 produced the best growth of 16.06mm ± 4.12 at PL25 in 4216 salinity. Also this diet at the same postlarval stage in salinity of 30%, gave the best survival and growth of 72.67% ± 12.06 and 14.07mm ± 1.82, (the latter is close to that achieved by the Taiwanese feed (14.38mm ± 1.17)). Yields of PL25 grown in salinities of 42fa were best when fed with local diets FFC2 (7.49g ± 3.50) and FFC1 (6.39g \pm 6.72). These were followed by the other

Based on results of this study at the present time it is not advisable yet to depend on microcapsules solely for larval rearing at the Fish Farming Centre, and further research is required in this area.

local diets FFC3 (5.79g ± 0.83) and FFC4 (5.05g ±

0.67). Yields of the commercial feeds (Taiwanese 4.94g

± 1.51, Nippai 4.32g ± 0.75 and Frippak 3.54g ± 0.82) were not significantly better from each other (p>0.05) (Appendix 2c) (Table 7). Although the best yield of 12.34g ± 4.35 at PL25 in 30% was obtained on the expensive local diet FFC1 (no squid replacement by shrimp head meal), the local diet FFC4 containing complete squid replacement by shrimp head yielded 10.75g ± 1.96, which was not significantly different from the Taiwanese feed (11.02g ± 3.46). This compares well with other studies; Ali et al. (1982), Sandifer and Joseph (1976), Venkataramaiah et al. (1978) who report that use of waste shrimp head meal diets for P. indicus, P. aztecus and P. setiferus because they are a good source of fatty acids.

However, there are no nutritional limits for the

use of other good quality ingredients (Table 2). Fish meal is palatable to shrimp, serves as a desirable attractant and should contain a minimum of 60% protein (Table 2). Fish meal levels in commercial feeds usually range from 10% to 40%, and the only limitation is digestibility and price (Fast & Lester, 1992). Squid meal is an excellent ingredient due to the quantity of lipids and because it has the highest

concentration of cholesterol, phospholipids and the

essential fatty acids, 20: 5n3 and 22: 6n3 of any

natural source (Table 4d). Squid meal also normally contains a minimum of 40% protein and 5% lipid (Table 2). Although it is not nutritionally limiting, its use is restricted by price and availability (Table 2). Shrimp meal is the ground dried waste of shrimp including the head, exoskeleton. It is usually steamed or sun-dried, and it is an excellent source of minerals, chitin, cholesterol, phospholipids and the fatty acids, 20: 5n3 and 22: 6n3. Shrimp meal should contain a minimum of 32% protein and 4% lipid (Table 2) and a maximum of 14% fibre. Shrimp meal levels in commercial feeds usually range 5% to 15%. Soyabean meal has the best protein profile of all plant sources (Table 2). Soyabean meal is usually a cost-effective source of protein (Table 2) and should contain a minimum of 44% protein. Soyabean meal levels in commercial feeds usually range from 10% to 25%. The maximum level of soyabean meal in feeds should not exceed 40% (Akiyama, 1988). Soyabean meal is usually limited by processing constraints to produce water stable feeds. Wheat products are usually given as supplement or

as a binder and filler of shrimp feeds. Wheat gluten

is an excellent binder and a good source of protein,

containing a minimum of 60% protein. However wheat flour may contain a minimum of 12% protein. Other

wheat products such as wheat bran, wheat pollards and wheat middlings are not commonly used because of their high fibre contents and low protein values (Table 2). Inadequate protein in a diet results in a reduction or cessation of growth, on the other hand, if too much protein is supplied in the diet, only part of it will be used to make new protein and the remainder will be converted to energy or excreted. Protein levels recommended for commercial feeds are listed in the introduction of this chapter. Amino acids considered as dietary essentials for shrimp are methionine, arginine, threonine, tryptophan, histidine, isoleucine, leucine, lysine, valine and phenylalanine (Cowey and Forster, 1971; Shewbart et al., 1972; Kanazawa and Teshima, 1981).

The nutritional value of synthetic amino acids has been demonstrated with fish and terrestrial animal feeds, but not yet for shrimp feeds. Due to different rates of absorption of synthetic amino acids, and because shrimp are slow feeders the synthetic amino acids quickly leach out of feed, and hence these amino acids may no longer be present when feed is consumed. Excessive leaching of amino acid may also present a secondary problem of eutrophication in the culture

environment.

Dietary and tissue lipids are important in the

nutrition of shrimp. Dietary lipids serve as a carrier of fat soluble vitamins and provide other compounds, such as sterols and phospholipids, which are essential for the normal metabolic function of shrimp. Recommended lipid levels for commercial, feeds range from 6% to 7.5% and should not exceed 10%.

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The four essential fatty acids mentioned in the introduction of this chapter are important because of their roles as components of phospholipids and as precursors of prostaglandins. Essential fatty acids are found in the highest concentration in phospholipids and as such are important in maintaining the flexibility and permeability of biological membranes, in lipid transport, and in activation of certain enzymes (Teshima and Kanazawa, 1980 a and b). As precursors of prostaglandins they are probably involved in many diverse physiological and metabolic functions (Lehhinger, 1984; Teshima and Kanazawa, 1980 a). Chen (1990) achieved a survival rate of 36.67% for P. indicus when he fed a diet containing a lipid level of 10%. Highest survivals in present work were on diets of 6-8% lipid. Similar studies with P. japonicus have revealed the essentiality of lipid for

proper survival and growth (Kanazawa et al., 1970;

Kanazawa, 1985). Villegas and Kanazawa (1980) reported

good survival of larval P. japonicus on a diet containing 8% lipid. Mohamed et al., (1983) report very good survival of larval P. indicus when fed a compounded diet containing 10.1% lipid. Higher survival rates were obtained in the present study for local diets ranging from 59.33±13.61% to 77.33±6.43%

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at PL25 (Table 6b).

The experimental diets used in this study appear to have adequate protein and lipid levels (Table 4c). This table shows that despite the high protein percentage of 47.9% in diet FFC1, growth did not differ significantly from that achieved on the lowest protein level 40.2% in the diet FFC4. Also the highest lipid level of (8.9%) in diet FFC3 did not give significantly better results when compared with FFC1

containing (6.7%) lipid. Other analyses of local diets were also with in the range seen in commercial diets (Table 4c).

Despite low HUFA levels found in the local diets FFC1-4 than in commercial diets (Table 4a) little difference in survival and growth was seen suggesting that lower levels rather than higher levels are acceptable.

It may be concluded that a formulation combining

that of local diets FFC3 and 4 may be recommended for

use at the present time at the Fish Farming Centre,

whilst continuing with further research to improve these diets. Generally local diets formulated with local ingredients have demonstrated that it is possible to obtain similar growth and yields for P. indicus postlarvae to those achieved on imported commercial diets. It has been possible to replace expensive local protein sources with cheaper local protein, and to produce diets for approximately the same price as the cheapest imported feed. In addition postlarval results obtained in these experiments agree with results in the previous chapter confirming the salinity preference of P. indicus from Red Sea. This strain at early post larval stages still prefers a salinity of 30% and is not physiologically capable of tolerating higher salinities. The following

chapter will discuss the use of local ingredients for production of ongrow feeds fed to P. indicus juveniles.

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Table: lc P. indicus larval survival rate (%) and length (mm) fed on algae, Artemia and microen capsulated diets (Zl-PL5). Int: survival rate (%) and length were 100% and 0.98t0.08mm. (Experiment 3)

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Table 3(a): Water temperature for P. indicus nursery feeding experiments at 42% , and 30% , from PL5-30.

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Table 4a: Ingredients of locally formulated diets fed to P. indicus larvae from (PL5-30) cultured in salinity of 42b, quantity of ingredients as percentages.

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Table 4b: Vitamin and mineral content used in the locally formulated diets above.

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Table 6 (a): P. indicus postlarval survival rate (%) and length in (mm) from PL 5-30 on different commercial and locally formulated diets at a salinity of 42%. Initial survival rate and average length at PL 5 were 100% and (6.05 mm)
respectively (mean±0.38).

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Table 6 (b): P. indicus Postlarval survival rate (\$) and length in (mm) from PL 5-30 on different commercial and locally formulated diets at a salinity of 30%. Initial survival rate and average
length at PL 5 were 100% and (6.02 mm)
respectively (meant0.30) respectively (mean±0.30).

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Table 7: Yields of P. indicus in (g)/100 & at PL25 fed on commercial and
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Figure 5a & b:P. indicus yield data in (g) 1000 postlarvae⁻¹ at PL 25 from feeding experiments, fed on different commercial and locally formulat diets at 42% and 30% .

CHAPTER 4

Development of artificial feeds for culture of juvenile Penaeus indicus

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Introduction

Stomach analysis has shown that Penaeus indicus predates on vegetable matter, molluscs, crustaceans and detritus (Gopalakrishnan, 1952; Rao, 1971). Therefore this species is an omnivore with a wide range of

adaptability in feeding habits, which makes it a

desirable culture species.

Nutritional studies of shrimp were initiated in the

early 1970's, but due to differences in research methodologies and lack of standard diet, comparisons between early and recent studies are difficult. In any case, variables such as species, size, source, physiological state of the shrimp, environmental conditions, experimental design, facilities and diet

form, composition and processing often make comparisons

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invalid (Akiyama, 1988).

Fresh diets of animal source as well as compounded diets are used. Frog flesh waste has been used as a main ingredient in a diet for Penaeus monodon (Ali et al., 1982), whereas Kanazawa et al. (1970) reported that the fresh diet of short-necked clam (Tapes philippinarum) gave superior growth in P. japonicus compared to the compounded diets in P. monodon. Mohammed Sultan, (1982)

had used frog flesh waste as a main ingredient in diets. Similar results were obtained by Forster and Beard (1973) for the common prawn Palaemon serratus. Although fresh or live food such as Artemia nauplii, shrimp meat and fresh clam gave superior growth in brown shrimp P. aztecus and P. indicus, a high rate of chitinoclastic

bacterial infection, leading to heavy mortality occurred

(Venkataramaiah et al., 1975; Ali et al., 1982).

Economic use of feeds in aquaculture can increase both production and profits considerably. To achieve these objectives, feed must be nutritionally adequate and developed from low cost ingredients. The difficulty in procuring and storing live and fresh food has led to the development of artificial diets. In addition fresh food easily deteriorates

resulting in the reduction of its nutritive value (Pascual, 1989). Replacement of live foods by artificial diets is the major task in developing successful aquaculture (Jones et al., 1993).

Nutritionally adequate feed must contain appropriate

levels of protein, fats, carbohydrates as well as major

and minor (trace) minerals and vitamins.

Not only the protein level in the diet but also the

protein source has its effects on diet quality.

Optimum protein level in diets compatible with maximum growth for penaeid prawns has been determined to lie between 35-40% (Forster and Beard, 1973; Skick et al., 1973; Venkataramaiah et al., 1975) and in some studies it was determined to be 43% (Colvin, 1976; Ali et al., 1982). Studies on P. indicus protein requirements have also varied considerably. A protein level as high as 60% has been reported to yield higher conversion efficiency and higher growth but not the highest survival (Sambasivam et al., 1982). While Ali (1982) reported a progressive increase in the weight gain of P. indicus with the increase in protein level up to 43%, it declined thereafter and the protein efficiency ratio was highest at 20.5% crude protein level. In general, for shrimp sizes from first feed to 40g and above the percent of

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recommended protein level in the diet varies between 36

and 45% (Akiyama et al., 1988).

Diets containing the same protein content and energy

level may yield different growth under the same experimental conditions. The protein source is therefore an important factor. Ali (1982) fed Penaeus indicus four diets containing the same protein level, but from different sources namely; prawn waste meal, mantis shrimp protein,

clam meat powder and fish meal. Feeds with mantis shrimp protein and clam meat powder gave the highest increase in weight gain and good food conversion values. Also, Raman et al. (1982) fed P. indicus diets containing different protein levels and sources. They found that a diet containing 29.4% protein from fish meal resulted in better growth than a diet containing 40% protein from a prawn factory waste. Clearly then, the protein amino acid content, digestibility or palatability may have played a role in these studies. A diet containing an appropriate level of protein is not necessarily efficient unless it contains the right ammount and proportions of essential amino

acids. The amino acids considered dietary essential for shrimp are similar to those reported for other aquatic

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animals (Cowey and Foster, 1971; Shewbant et al., 1972; Kanazawa and Teshima 1981). For example, poor growth obtained on diets containing fish meal protein was thought to be due to relative deficiency of the amino acids, typosine and phenylalanine in fish meal (Colvin, 1976).

An essential component of shrimp meal is fat or lipids. Dietary lipids are a concentrated and highly digestible source of energy. Lipids also include sterols

and phospholipids which are essential for normal metabolic function. Also dietary lipids serve as carriers of fat soluble vitamins. The recommended level of lipid in shrimp feed is from 5-10% (Pascual, 1989), but should not exceed 10% (Akiyma et al., 1988). As mentioned for protein, not only the lipid level

in the diet but the fatty acid content is important. Four unsaturated fatty acids are considered essential for shrimp, linoleic (18: 2n6), linolenic (18: 3n3), eicosapentaenoic (20: 5n3) and decosahexaenoic (22: 2n6) (Kanazawa et al., 1979; Jones et al., 1979). In general, plant oils are high in 18: 2n6 and 18: 3n3, while the marine animals oils are high in 20: 5n3 and 22: 6n3 (Akiyama et al., 1988). Dietary lipids therefore should incorporate both plant and marine animals oil.

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The other major component of shrimp feed is carbohydrates, which is a major source of energy, but its metabolism by shrimp is not adequately studied. Carbohydrates are also utilized in feeds as fillers in form of fiber. The content of carbohydrate in the diet affects their digestibility. The apparent dry matter digestibility of soya bean meal is lower than squid meal and fish meal probably due to lower protein and higher carbohydrate content of soyabean meal.

Minor components of shrimp feeds are vitamins and minerals. Vitamins are organic catalysts needed in minute amounts. Because very little is known about vitamins in feed nutrition, their vitamin content varies a great deal. Recommended levels of vitamins in shrimp feeds are given by Akiyama et al. (1988). Minerals are essential

components of enzymes, hormones and vitamins are

metabolic co-factors, catalysts and enzyme activators.

Since shrimps can absorb minerals from their environment,

their dietary requirement depends on their availability

in the culture facility. Recommended level of minerals in

commercial shrimp feeds are also given by Akiyama (1988).

Local ingredients are always recommended to use for

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diet formulation because they are cheaper than imported

ones, readily available and suitable to local conditions

(New, 1987; Pascual, 1989).

This study was designed to investigate the feasibility of using local or cheap ingredients in formulating diets for growout of Penaeus indicus juvenile and to test these diets under local culture conditions .

Materials and Methods

Feeding experiments were carried out to test the efficacy

of four local, formulated shrimp diets on growth of juvenile Penaeus indicus. A commercial Taiwanese pelleted type shrimp diet (President Enterprises Corp.) was used concurrently as control. Juvenile shrimp for these experiments were obtained

from a stock maintained by the Fish Farming Centre. Diets

were formulated so that their final protein content was approximately 44% and final lipid content was 5-10%. The selection of protein and fat levels is within fairly arbitrary limits the levels were selected because they were perferred for P. indicus by some authors (Colvin, 1976; Pascual, 1989). Soya bean, fish meal, squid meal and shrimp head meal were used as protein source, while fish oil was used as the main source of lipid (Chapter 3, Table 2). The

diets were named according to their protein source namely: Soya bean meal, fish meal, squid meal and shrimp head meal. As in chapter 3, the preparation of diets involved drying of meals in an oven at 40°C to allow easy grinding. The meals were then ground and seived through 40µm mesh (Santiago, 1987). Other ingredients were then added and the diet was mixed and pelletized. Pelletized diets were dried in an oven at 40'C to attain a moisture

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Penaeus indicus culture, fed on five different diets for two 12 weeks growout periods in fiberglas tanks in the Red Sea water, Jeddah, Saudi Arabia.

level of approximately 14%. Pellet size was 3x5 mm, this was within the pellet size recommended for juvenile penaeids (Chen and William, 1988). All diets were stored in tight plastic bags in a freezer at 20°C until used. To verify the level of ingredients in final products, proximate nutritional analysis of test, as well as of the control diets, was carried out. Proteins were determined using the K j eldahl method, fat by the petroleum ether extact method, fatty acids by the Gas Chromatography method of Folch et al. (1957) and Morrison and Smith (1964) (Methods were described in chapter 3). Moisture was determined by drying pelleted feeds samples at 100'C for 5 h. and ash was determined by ashing samples in a furnace at 550*C after treatment of samples with nitric acid. The resultant was assumed to represent the ash-free dry weight. Water stability of pellets was determined after floating dried pellet.. samples in water for 1,3,7 and 14 h. using wire baskets, and then drying and reweighing the samples. Feeding was carried out in fiberglass tanks of 10001 size. For each feed, 10 P. indicus juveniles were stocked in each of 3 tanks. Each tank was provided with continuously flowing and gently aerated seawater with a flow rate of 201 min-' and a salinity of 43%. The seawater used was sourced via a well

drilled into the ground some 30m from the sea. Tanks were covered with plywood to keep temperature variations minimal. Water physical data (temperature, salinity, DO, pH and NH. -N) were measured as described in chapter 2. Shrimp were fed at 3-4% of total biomass and to ensure feeding satiation and efficiency the daily ration was given in 2 portions, the first fed at 15: 00 h. and the second at 20: 00 h. (Chen and Willams, 1988). The starting shrimp size for all feeds ranged between 5.9- 6. Og. Sampling for survival and growth measurements were carried out weekly and during sampling, tanks were cleaned.

Physical factors were measured monthly. The experiment was terminated after a growing period of 12 weeks.

The above described experiment was later repeated

for a similar culture period to verify results. In the

later experiment, average shrimp size used was 6.0g,

sampling and physical data measurements were carried out

every other week.

Results

Diets formulations and analysis

Percent ingredients of the commercial Taiwanese feed which was used as control and the four locally prepared diets is given in Table (la). The vitamins and minerals mix added to local diets is presented in Table (lb). Proximate nutritional analysis of all diets is given in Table (2). It could be seen that protein levels were close to the 44% level which was thought during local diets formulation. The largest variation in protein level revealed by analysis was about 2% between the soya bean meal diet (46.4%) and the shrimp head meal (42.2%). The lipid content of diets varied between 7.0 and 10.0% which was within the limits of 5-10% recommended for shrimp feeds. Carbohydrate level ranged from 27-35% for the five diets and ash content was highest for shrimp head meal at 16.24% and lowest for the soya bean diet at 7.38%. The price of locally prepared diets is given in Table 2. The fatty acid composition for each diet are presented in Table 3. Since there are four fatty acids considered to essential for shrimp diets, their level in the diets were calculated. These fatty acids and their recommended levels in shrimp feed are; linoleic (18: 2n6) 0.4%, linolenic (18: 3n3) 0.3%, eicosapentaenoic (20: 5n3) 0.4% and decosahexaenoic (22: 6n3) 0.4% (Akiyama, 1992). The level of these acids were calculated from levels in

the lipid portion of the diet and the percent of lipid in each diet (Table 3a), and the values obtained for each of these essential fatty acids are given in Table 3b. It can be seen from this table that all diets were deficient in the 18: 3n3 as values were less than the recommended levels of 0.3%. The diets contained sufficient amounts of

The water stability test revealed that there were significant differences amongst the diets, (A) was significantly higher than all other diets (B, C, D and E), over the tested times of $1, 3, 7$ and 14 h. (p, <0.05)

the other acids except diet E (Shrimp head meal) which

contained less than the recommended levels of 20: 5n3 and

22: 6n3.

Water stability test

(Table 4 and Appendix 4a). For the other diets, all were relatively stable over 1,3,7 and 14 h. respectively (Table 4 and Appendix 4a). Generally all diets were very stable and after 14 hours of immersion into water none of the diets had lost more than 18% of its weight. Loss of weight after the first hour in water was only 6% or less for all diets (Table 4 and Appendix 4a).

Physical data

The water quality parameters of the sea water source which is a well were as follows; DO 4.96±0.58 mg l⁻¹, NH₄H 0.07±0.014 mg 1'1, pH 7.29±0.12 as an average of several determinations. Temperature was 28-30°C and salinity was 43%.

Water quality parameters for the first 12 week growout period are given in Table 5. Dissolved oxygen was consistently higher than 3.9 mg 1-1 and ranged between 3.9-5.3 mg 1⁻¹. NH₄N increased progressively from 0.06 mg 1^{-1} at the start of experiment to 0.90 mg 1^{-1} at the end, but this increase was not correlated to shrimp growth. The pH also decreased with time and was the lowest at 7.15 in the last growing month. Temperature was constant at $28-30$ °C and salinity was $42-44%$.

Water quality parameters for the second growing period are presented in Table 6. The D.O. values were always greater than 3.9 mg 1^{-1} . Lowest NH₄N value was 0.09 and highest value was 0.5 mg 1^{-1} . The pH values were never greater than 7.4 or lower than 7.2. Temperature and salinity were constant in the range 27-31°C and 41-43% respectively. The relations between pH, ammonia NH.N and P. indicus biomass are shown in Figures la&b and 2a&b for the first and second 12 week culture periods

respectively.

Survival and growth for the first growing period are given in Table 7 and Figures 3a and 4a. Survival rates were highest for the commercial Taiwanese diet, which was used as a control and designated (Diet A) at 70.0±10.0% followed by the soya bean diet designated (Diet B) at

63.3±5.77%, squid meal (diet D) and Fish meal (diet C)

at 53.3±28.87% and 53.3±30.55% respectively. The lowest

was for shrimp head meal (diet E) at 50.0±10%. However

these differences were not significant (P<O. 05) (Appendix 4b).

Survivals for the second growing period were 86.7± 5.77%, 66.7±20.82%, 63.3±5.77%, 56.7±5.77% and 63.3±25.17% for the diets A, B, C, D and E (as named above) respectively (Table 8, Figures 3b and 4b).

Differences between survivals were not significant (p<0.05) (Appendix 4b). The final average shrimp weights for the five diets A, B, C, D and E in the first growing period were 7.57±0.63 g, 7.20±0.7 g, 7.81±1.02 g, 5.61±1.93 g and 7.48±2.55 g respectively (Table 7 and Figure 4a). Differences were not significant (P<0.05). Final biomass from the five diets in this growing periods (Table 7 and Figure 5a) were 53.0± 3.61 g, 45.6± 6.57 g, 41.6±27.62 g,

29.9±11.45 g and 37.4± 19.76 g respectively. Differences in biomass were not significant (p<0.05) (Appendix 4b). The final average shrimp weights in the second growing period for diets A, B, C, D and E were 11.07±2.20 g, 9.44±2.70 g, 9.77±2.64 g, 11.47±1.33 g and 9.20±1.06 g respectively (Table 8 and Figure 4b). Again differences were not significant. Final biomass in this growing period were 96.5±23.88 g, 63.10±24.13 g, 62.60±20.25 g, 65.20±11.92 g and 60.0±30.66 g for diets A, B, C, D and E respectively (Table 8 and Figure 5b). No significant difference was seen between these values (p<0.05) (Appendix 4b). The relations between weight, biomass and survival rates throughout the two 12 culture weeks are plotted in Figures 6a&b and 7a&b respectively.

For all diets, protein, lipid and carbohydrate levels ranged from 42-47%, 7-10% and 27-35% respectively (Table 2) were within the recommended levels by other workers (Colvin, 1975; Ali et al., 1982; Pascual, 1989). The recommended vitamin and mineral mixes (Table 1b)

Discussion

Diets formulations and analysis

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(New, 1987) were added precisely to diets B, C, D and E,

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when diet preparation took place except for the vitamin (Inositol) which was unavailable. This may have a role in the slow growth of P. indicus in these trials, because the absence of Inositol can cause slow growth and anorexia as this vitamin is a component of the insitol phosphoglycerides and insitol phospholipids that are

found in the tissues.

The fatty acid composition for each diet is presented in Table 3a, and compares well with the recommended levels of Akiyama (1988). The level of these acids was calculated from their level in the lipid portion of the diet and the percent of lipid in this diet (Table 3a). The values obtained for each of these essential fatty acids are given in Table 3b. It can be seen from this table that all diets were deficient in 18: 3n3 since all values were less than the recommended level of 0.3%. The diets contained sufficient amounts of the other acids except diet E (Shrimp head meal) which contained less than the recommended levels of 20: 5n3 and 22: 6n3 (Table 3b). This diet gave the poorest results among all other diets in the two 12 weeks culture periods, but was not significantly different from other diets (Appendix 4b).

Water stability test

Despite the significant differences among the tested diets, all diets were generally very stable (Table 4), after 14 hours of immersion in water none of the diets lost more than 18% of its weight. Loss of weight after the first hour of the water stability test was only 6% or

Although temperature and salinity measured throughout the two 12 week culture periods, ranging from 27-31 'C and 41-44 % respectively (Tables 5 and 6) and were within the satisfactory limits for penaeid species (Van olst et al. 1980; Wickins, 1981,1982; Kuo, 1988) (Table 1, Chapter 2). However pH levels below 7.6 and ammonia concentrations as high as 0.9 and 0.4 mg 1^{-1}

less in all diets (Table 4 and Appendix 4a). However, pellets of the shrimp head meal diet (E) floated on the water surface as they contained higher undigestable fibre (A) levels of 16±2.1% compared to the other sinking pelleted diets of 11.2±0.1, 7.4±0.0, 9.1±0.0 and 10.4±0.0% fibre (Ash) for the diets A, B, C, and D respectively. Total fibre levels in commercial feeds are recommended not to exceed 4% (Akiyama, 1988). The shrimp head meal diet (E) gave the poorest results.

seen in both of the two 12 weeks culture periods exceed the recommended levels for penaeid shrimp $(> 0.1$ mq $1^{-1})$ (Van Olst et al., 1980). The cause of the low pH may be attributed to the oxidation of ammonia to nitrite and nitrate by nitrifying bacteria growing within culture tanks (Wickins, 1976). Oxidation of ammonia increases the hydrogen-ion concentration and lowers the pH of the medium (Muthu, 1982; Wickins 1976). The autotrophic micro-organisms cause ammonia oxidation, and the oxidation of dissolved and finely suspended organic materials is by populations of heterotrophic microorganisms. P. indicus as a crustacean is a heterotroph and like the microbes, also produces ammonia and carbon dioxide wastes. The autotrophs, on the other hand, feed on the ammonia and produce hydrogen ions and nitrate as waste products (Wickins, 1984). The hydrogen ions (acid) produced by -Nitrosomonas are normally neutralized or buffered by the alkaline reserve of sea water. The loss of bicarbonate and associated decline in pH is likely to prevent proper mineralization of the exoskelton (Wickins, 1984). In addition nitrification may lead to a loss of inorganic carbon from the medium which could also affect the moulting process in prawns (Wickins, 1984). In the present studies Figures la&b and 2 a&b reveal

no correlations between pH nor ammonia (NH4N) and the biomass of P. indicus among the different five diets A, B, C, D and E for either the first or the second 12 week culture periods.

At the time when the present study was undertaken it

was expected that the high through flow of sea water

would compensate for the above effects. Hence the use of a rate of 20 1 min⁻¹ which is similar to systems used by Aquacop (1975) and Primavera et al. (1982). In both culture periods survival rates averaged 50% or higher at the end of the two 12 culture weeks of each experiment which compares well with the work by $\texttt{All} \rightarrow \mathbb{R}$ (1982), who achieved 70-100% survival when experimenting for 30 days with several feeds with different protein levels (Table 9).

Growth rates ranged from 0-29.2 mg day-' and from 37.9-62.0 mg day-' for first and second 12 week culture periods respectively which compares with 21.3 and 10.8 mg day"' achieved by Raman et al. (1982) from a two month P. indicus feeding experiment. Also 84-107 mg day⁻¹ was achieved by Colvin (1976) from a 3-5 week feeding experiment for P. indicus fed on different protein levels. All et al. (1982) reported a lower daily growth of 5-13.8 mg than found in the present study also using

4 different protein sources.

It is not feasible to discuss feed conversion ratio in the present studies because of poor total weight gained (biomass) over the duration of the 12 week culture periods due to water conditions mentioned above.

The locally formulated feeds in these studies need

to be retested when water quality is improved; especially

as results of previous work contradict each other.

Deshimaru and Shigeno (1972) and Colvin (1976)

report that fish meal gives comparatively poor results

because its amino acid composition is not similar to that

of P. japonicus. However Raman et al. (1982) and Robinette and Dearing (1978) found fish meal gave a

better performance producing 35.5 mg day-1 in comparison

to prawn factory wastes which gave a growth of 21.3 mg

day-'. This is probably because the latter ingredient is not as digestible or as palatable as fish meal. The fish meal diet (C) in the present study produced growth of 29.2 and 41.8 mg day⁻¹ respectively (averages from both the first and second 12 weeks trials). Ali et al. (1982) reported 9.3 mg day⁻¹ in comparison to the shrimp head meal diet in present studies of 26.3 mg day-'. Venkataramaiah et al. (1978) and Sandifer and Joseph (1976) found that shrimp heads waste of P. indicus, P.

aztecus and P. setiferus were a good source of fatty acids and pigments for diets and gave good results. Therefore aside from the water quality problems the diet quality requires futher evaluation.

Table la: Percent ingredients of a commercial Taiwanese and four locally prepared diets fed to Penaeus indicus in growout fiberglass tanks in Red Sea water, Jeddah, Saudi Arabia at 43% salinity.

A= commercial Taiwanese diet, B= Soya bean, C= Fish meal, $D =$ Squid meal. $E =$ Shrimp head meal.

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* DIETS:

Table 1b: Vitamin and mineral mix used in the local formulated diet

* Insitol not added, because of not being available.

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Table 2: Proximate nutritional analysis and cost of a commercial Taiwanese and four locally formulated diets feed to Penaeus indicus in growout fiber glass tanks in the Red Sea water, Jeddah, Saudi Arabia at 43% salinity.

* DIETS: A= commercial Taiwanese diet, B= Soya bean, C= Fish meal,

D= Squid meal, E= Shrimp head meal.

** Values are average of 2 determinations.

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* DIETS

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A= commercial Taiwanese diet, B= Soya bean, C= Fish meal, D= Squid meal, E= Shrimp head meal.

* * Values have been adjusted to be equivelant to 100%

Table 3b: Percent of essentional fatty acids of a commercial Taiwanese diet and four locally prepared diets fed to *Penaeus indicus* in growout fiberglass tanks in Red Sea wate;
Isaach, Coudi Americ of 10* selinitus Jeddah, Saudi Arabia at 43<mark>%</mark> salin

* Akiyama 1988

** DIETS: A= commercial Taiwanese diet, B= Soya bean, C= Fish meal, D= Squid meal, E= Shrimp head meal.

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Table 4: Water stability % in different times of a commercial Taiwanies and four local prepared diets fed to Penaeus indicus in grwout fiberglass tanks in Red Sea wate
Isdaab Seredi washin 1964 Jeddah, Saudi arabia at 43% salin

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Table 5: Water quality analysis during the first 12 week growout period of Penaeus indicus fed on five different diets in fiberglas tanks in the Red Sae water, Jeddah Saudi Arabia.

* DIETS:

A= commercial Taiwanese diet, B= Soya bean, C= Fish meal,
D= Squid meal, E= Shrimp head meal.

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Table 6: Water quality analysis during the second 12 week growout period of Penaeus indicus fed on five different diets in fiberglas tanks in the Red Sae water, Jeddah Saudi Arabia.

* DIETS:

A= commercial Taiwanese diet, B= Soya bean, C= Fish meal,
D= Squid meal, E= Shrimp head meal.

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Table 7: Survival rate (%), average and total weight of Penaues indicus fed on five different diets during the first 12 week growout period in fiberglass tanks in Red Sea water, Jeddah, Saudi Arabia at 43% salinity (Initial survival rate and wieght were 100% and 6±0.1 respectively).

=10.0 ±0.63 -3.01 -5.77 -30.55 **1.02** 127.62 -60.87 -10.0

Su = Survival % $Aw = Average weight$ Tw . Total weight

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* DIETS:

A= commercial Taiwanese diet, B= Soya bean, C= Fish meal, D= Squid meal, E= Shrimp head meal.

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Of Saudi sate
Vely) diets \mathbf{u} weight $\ddot{}$ survival
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7.94 8.29 \bullet 10.9 æ $\frac{3}{5}$ verage Survival Total ▾ ಜ್ಗ $\mathbf{\mathbf{\#}}$ 8 n R $\ddot{\ast}$ \mathcal{L} \triangleleft $\frac{3}{100}$ 70.0021 100 800 $\tilde{\mathbf{z}}$ $H = H$ 100 $\overline{\mathbf{3}}$ H $\ddot{\bullet}$ $\ddot{\bullet}$ $\frac{3}{4}$ \mathbf{S} œ m $.88$ Й m \sim 98.376 19.8 89.6 87.3
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Table 9: Penaeus indicus and other Penaeid and Metapenaeid spieces fed on different diets.

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Figure la&b: PH and Ammonia (NH'H) levels in relation to biomass (g) of Penaeus indicus fed on five different diets during the first 12 week growout period in fiberglas tanks in the Red Sea water, Jeddah Saudi Arabia.

Taiwanese Soya Fish NH4-N
Control A pean pean meal a meal AD A meal A E

Figure 2a&b: PH and Ammonia (NH'H) levels in relation to biomass(g) of Penaeus indicus fed on five different diets during the second 12 week growout period in fiberglas tanks in the Red Sea water, Jeddah Saudi Arabia.

Culture weeks

Soya C \mathbf{D} \mathbf{E} \mathbf{B} **A** Taiwanese **Fish** Shrimp head **Squid** $A \longrightarrow A$ meal Dentrol D-Chean B----E meal A----A meal

Figure 3a&b: Penaeus indicus survival rate (%) fed on five different diets during the first and second 12 week growout periods (a) and (b) respectively, in fiberglas tanks in the Red Sea water, Jeddah, Saudi Arabia.

Culture weeks

Shrimp head

> Figure 4a&b: Penaeus indicus average weight(g), fed
on five different diets during the first and second 12 week growout periods (a) and (b) respectively, in fiberglas tanks in the Red Sea water, Jeddah, Saudi Arabia.

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Culture weeks

Taiwanese Soya Fish Squid Shrim

o o o (control) \Box o (control) \Box o o \Box o Squid Shrimp head

Figure 5a&b: Penaeus indicus biomass(g), fed on five
different diets during the first and second 12 week growout periods (a) and (b)
respectively, in fiberglas tanks in the Red Sea water, Jeddah, Saudi Arabia.

Figure 6a&b: Survival rate (%) of Penaeus indicus in relation to average weight and biomass in (g), fed on five different diets during the first 12 week growout period in fiberglas tanks in the Red Sea water, Jeddah, Saudi Arabia.

CHAPTER 5

Growth and survival of Penaeus indicus under different densities in rubber lined earthen ponds during Winter and Summer

Results of this research were presented at The World Aquaculture Symposium . May 26-28, 1993C, Torremolinos,

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Spain (Abstract p. 117).

Title: The potential for the culture of white shrimp Penaeus indicus in high saline ponds on the Saudi Arabian coast of the Red Sea.

Authors: Feisal A. Bukhari, D.A. Jones and A.J. Salama

Introduction

Large potential profits for shrimp culture have led

for many centuries; the Romans practised brackish water pond culture (Brown, 1983), and they learned the techniques from earlier civilizations. Egyptian artwork also depicts pond culture, but Asia has the oldest history of pond culture (Ling, 1977; Atkinson, 1983). Modern shrimp culture incorporates intensive as well as older extensive pond systems (Apud et al., 1983; Nailon, 1985). In Ecuador, large irregular shaped extensive ponds of more than 5 ha⁻¹ in size, with shallow

to both private and government interest in investment in shrimp farming.

Marine shrimp have been cultured in earthen ponds

depths (30-40 cm) and low stocking densities of 3000-8000 ha⁻¹ (Apud et al., 1983; Hirasawa, 1985), will not be able to meet the future market needs for marine shrimp. Yields from this system are low even if sufficient land is devoted for this practice (Hirasawa, 1985). In the Philippines, rectangular or square ponds ranging in size from 1-20 ha" of 80-100 cm depth and with stocking densities of $10-15x10^{-3}$ ha⁻¹ are used for semi-intensive production (Apud et al., 1983; Fast and

Lester, 1992). In this system, production can be doubled when compared with extensive ponds (Kungvankij et al., 1986). Intensive Taiwanese shrimp culture systems use ponds with a size ranging from $0.25-2$ ha^{\cdot}, a depth of 1.5-2 m and a density of $20-40$ (X10³) ha⁻ (Apud et al., 1983; Fast and Lester, 1992.). This intensive shrimp

culture is only practised where seed to stock the ponds are available. Seed are cultured in nursery ponds for a growth period of 20-40 days, when fry is available from an adequate hatchery (Liao and Chao, 1983; Liao, 1984). But due to pond over stocking. shrimp farmers suffered great setbacks when in 1988, environmental degradation brought about by their own activities resulted. in severe disease outbreaks and the near collapse of the industry in Taiwan (Lin, 1989).

In Japan, the round pond ultra-intensive culture of Shogun for P. japonicus and other concrete aqua cells in Hawaii by Marine Culture Enterprises (MCE) (Mahler et al., 1974), have evolved a very high water exchange of 300-400% day". This enables culture of high densities of shrimp yielding 150 tonnes ha⁻¹ γ ⁻¹ using only very small areas of land with tanks or raceways of less than 0.25 ha . However, these ultra-intensive culture systems, whilst technically successful, are not economically

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profitable (Salser et al., 1978; Colvin, 1985 ; Liao, 
1986; Wyban et al., 1988).
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Two other major producing nations Ecuador (Guayas estuary) and Thailand (Bight of Bangkok) also seem likely to experience significant pollution problems in the near future, which will affect productivity (Aiken, 1990).

In Jeddah, Saudi Arabia, located along the Red Sea eastern coast; rainfall averages only 50 mm a^{-1} , temperature and salinity range from 20-32°C and from 38- 41% respectively (Robinson et al., 1979). Higher salinities up to 60% have been measured in inshore waters during summer when currents and tidal influences are insignificant (Robinson et al., 1979). In some coastal lagoons of the Red Sea, salinity may reach 78% (Leger, 1983). Other physical data reveals low levels of

dissolved oxygen concentration from 4.0-4.5 mg 1-1 because of the high temperature.

Penaeid shrimps are found in the limited shallow

coastal waters of the Red Sea with an estimated annual

catch from 800-1000 tonnes declining to 400 tonnes

(Sanders and Kedidi, 1981; Shakraporti et al., 1985). Al

Kholy and El Hawary (1970) provided a review of Red Sea penaeids.

Tiger Prawn, Penaeus monodon culture is popular

world wide because of the rapid growth of this species in pond environments, attaining 35 g after 120 culture days with a survival rate of 85% (Aquacop, 1983; Chen and William, 1988). However, P. monodon postlarvae are rare and not found easily along the Red Sea coast (Badawi and Cas, 1989). Initially postlarvae of P. monodon were

imported from Malaysia to carry out growth, survival and production studies in the high saline waters of the Red Sea in different culture facilities at the Fish Farming Centre of Jeddah (Bukhari et al., 1989). Results showed poor growth averaging from 10.8-23.1 g after 120 culture days and survival from 42-49%, except for a survival in rubber-lined ponds of 70%. This was thought to be because of the very different environmental conditions operating locally, as compared to those where the experimental

The desirable temperature and salinity range and levels of water quality factors for penaeid species are given in Table 1, chapter 2. Penaeus indicus juveniles which are found abundantly on the southern coast of the Red Sea were collected and transported alive (Badawi and Cas, 1989). P. indicus is an Indian white prawn originating from the Indian ocean and south east Asia, and has been cultured in water

animals originated (Bukhari et al., 1989).

temperatures from 22-33°C and salinity from 15-25% (Chen and William, 1988; Lee and Wickins, 1992). This red tailed white shrimp is one of the penaeid species which has a strong potential for aquaculture (Aquacop, 1985). Preliminary studies conducted at the Fish Farming Centre, demonstrated the ability of this species to

survive and grow in captivity providing an adequate diet is supplied (Bukhari et al., 1989). As the supply of post larvae from the wild can be unpredictable due to its dependance upon environmental factors (Jones, 1988), a hatchery at the Fish Farming Centre was set up to spawn and culture P.indicus larvae. It is now possible to produce seed on demand for research or production in lined pond culture (Bukhari et al., 1990). Previous preliminary ongrow experiments gave

promising results with shrimp reaching 15 to 23.5 g in lined ponds after 6 months culture period (Bukhari et al., 1990,1991).

In the present study stocking densities were selected to be within the range of other semi-intensive and intensive systems, since the high densities used in ultra-intensive systems are likely to lead to serious stress problems (Lin, 1989). In addition stocking at more than 100m'2 has been reported to be a waste of shrimp fry

(Kurata and Shogun, 1979).

The experimental production trial was designed to investigate the growth and survival of P.indicus juveniles at densities of 20m'2 , 40m'', 60m-2 and 80m'', and was conducted for 180 culture days, which included winter and summer at the Fish Farming Centre, Jeddah.

Materials and Methods

The study was conducted at the Fish Farming Centre (Jeddah, Saudi Arabia) in an experimental lined earthen pond; (sized 12m x 12m x 1.75m).

Twelve net cages of 5mm mesh size in diameter; were

placed on the pond bottom, each was tied from corners to a wooden frame with plastic barrels to help suspension.

Each cage was 2m x 2m x 1.75m, and small sinkers of average 500g each were used to keep the nets spread and protected from wind drift. The nets and the wooden frame were fixed by ropes to tent pegs at the pond edges. Cages were checked and repaired biweekly and cleaned, dried and repaired between the two separate experiments in winter and summer. Initially the pond was dried and lime was added at

a rate of 0.8 tonnes ha⁻¹. This disinfected the pond and

Penaeus indicus pond culture at different densities for two 180 days during winter and summer in the Red Sea water, Jeddah, Saudi Arabia.

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helped to enhance natural productivity in the pond water, which in addition to supplying some nutrition, provided necessary shading needed by the cultured animals. Sea water Of 4196 was added to the pond from a coastal well at the site to give 1.5m depth. Air was also injected into the pond water in order to keep

dissolved oxygen rates within the satisfactory levels for

Penaeid sp. (above 3.0 mg/1) (Seidman and Lawrence, 1985),

water exchange was 20% day ⁻¹.

The juveniles were stocked randomly in the net cages at densities of $20m^{-2}$, $40m^{-2}$, $60m^{-2}$ and $80m^{-2}$. Each density was replicated thrice for winter and summer experiments.

Experimental animals

Penaeus indicus juveniles originating from the southern coast of Red Sea were obtained from the hatchery station of the Fish Farming Centre. Juveniles were 82 and 66 days old averaging 5.6cm, 1.16g and 5.56cm, 0.94g, in

length and weight for winter and summer experiments respectively.

Food and feeding

A standard Taiwanese pelleted diet sized 2 mm of 40%

protein was used to feed the shrimp. Feeding rates were

based on shrimp biomass as following: Daily amount of feed $=$ Ave. Wt. X Total No. X Estimated Sur. % X Feeding % $Wt. = Average weight, No. = Number and Sur. = Survival$. This amount was fed daily until the next sampling after 30 culture days where it was changed for each cage

(Chen and William, 1988; Pascual, 1989; Chen, 1990). Feed was added to the cages twice a day; one third at 15.00 h and two thirds at 20.00. The initial feeding rate was 7% of the total biomass at each shrimp density. After a growing period of 30 culture days, feeding was reduced to 5% of total biomass until the last two months when feeding was reduced to 4%.

Water quality

Temperature and dissolved oxygen in the pond were monitored randomly in cages three times each week with a Yellow Spring Polarographic Dissolved Oxygen meter. Salinity, turbidity, pH , $NH_{-4}N$ and $NO_{-2}-N$ were measured biweekly using APHA Standard Methods for the Examination of Wastewater (1980).

Sampling and harvesting

Penaeus indicus were sampled for weight and length

gains (length measured from the beginning of the rostrum to the end of the tail) at monthly intervals. A sample of at least 10% of shrimps in each cage was collected at in each sampling data using a scoop net and returned to the corresponding cage following measurements. After 180 days all shrimp were harvested from each

cage and weights, length, survival rates, yields and feed

conversion ratio were calculated for each treatment. Data

were analyzed using ANOVA.

Results

Physical factor data during the two six months culture periods of winter and summer (Table 1 and Figure 1), reveal correlation between temperature and some other

factors; lowest temperature correlates positively with highest O₂, but highest temperature does not with lowest $0₂$, because $0₂$ drops in winter and summer at the end of growth period due to the increase in biomass which correlates positively with O₂ demand. For winter period, the temperature ranged between a low of 19.0°C and a high of 27.0°C. For the summer period, the lowest temperature recorded was 23.0°C while the highest was 32.0°C and the avereage was 28.0°C-

29.6°C. The D. O. lowest and highest values were 3.4 and 5.3 mg 1^{-1} for winter 2.9 and 4.7 mg 1^{-1} for summer. The average D.O. values were 4.8 and 4.0 for winter and summer respectively. The salinity did not fluctuate by more than 1.0% throughout the growing periods except for one measurement during the winter period where the

difference between period measurements was 2.0%. The salinity ranges were 42-43% and 43-45% for winter and summer respectively. Highest pH values were obtained during winter when the pH range was 7.2-8.1 with an average of 7.7. For the summer period, the pH range was 7.2-7.8 with an average of 7.5. The ammonia values were 0.02-0.04 mg 1^{-1} (NH, K) for winter period and 0.01-0.23 (NH, N) for summer period. Nitrite (NO₂) was recorded during only 2 months of growth in the winter and 3 months

of growth during summer. Higher values and greater variation in turbidity were obtained in the summer period (Table 1 and Figure 1). It appears that temperature does not correlate with pH neither with ammonia nor with nitrite (Figures 2a, b and c), also the relation between these physical factor data and average biomass per m⁻² for all densities is shown in Figures 3a, b, c and d. Results of survival, length and weight gains, yields and food conversion ratio are given in Table 2. Survival

at each density was compared to its conterpart in the other period and to other density in the two periods. Hence the only significant difference (P<0.05) was found between summer density 20m⁻² and winter density 60m⁻² (Appendix 5a). When the sum of survival at each density during the two growing periods were compared to other

densities i.e. regardless of winter or summer, there was no difference in survival between densities (P<0.05) (Appendix 5b). Growth in length and weight gains are shown in Table

of means were compared at which 24 were significantly different and 4 were not (P<0.05) (Appendix 5a). The means which were not significantly different are winter 20m⁻² vs summer 80m⁻², winter 40m⁻² vs winter 60⁻ $2.$ winter $40m^{-2}$ vs summer $80m^{-2}$ and winter $60m^{-2}$ vs summer 80m⁻². However, when the six replicates for each density in the two growing periods were compared to replicates of three densities (Appendix 5b) a significant difference was found between the densities of $20m^{2}vs$ 60m⁻², 20m⁻² vs

3 and 4 respectively, and length only in Figures 4a, b,

c, d and 5, a, b, c, while length and weight relationship

is shown in Figure 6. The mean gain in length for each

density was compared to all other densities in each

growing period (Appendix 5a). Thus, a total of 28 pairs

80m⁻² and 40m⁻² vs 80m⁻², while differences between 20m⁻² vs $40m^{-2}$. $40m^{-2}$ vs $60m^{-2}$ and $60m^{-2}$ vs $80m^{-2}$ were not significant $(P>0.05)$. Highest average gain in length was 8.4±0.1 cm and was recorded in the density of 20m⁻² in the summer (Table 2 and Figures 4,5). This was significantly higher than

all other densities, including the same density in the winter (P<0.05). The same is true for lowest average length gain which was 6.60 cm and was recorded in the density of 80m⁻² during the winter (Table 2 and Figures 4,5).

When pairs of densities were compared in terms of weight gain as was done for length gain, 22 pairs had different weight increments of which 6 were not significantly different (P<O. 05). The 6 pairs which were

not different including the 4 pairs which were also not different for length gain plus two other pairs which were winter 40m'2 vs winter 80m'2 and summer 60m'2 vs summer 80m-2.

When densities were compared irrespective of winter and summer (Appendix 5b), the only significant differences in weight gain were the densities of 20m⁻² vs $60m^{-2}$ and $20m^{-2}$ vs $80m^{-2}$ (P<0.05). These two same densities were different in length gain.

As for length gain, the highest average gain in weight was 16.5±0.45 g and was recorded in summer 20m⁻² and the lowest gain was 10.9±0.66 g in the winter 80m-2 (Table 2). Growth in terms of length and weight corresponded well with each other (Figure 6), while the relations between survival rates and length and weight at

densities 20m⁻², 40m⁻², 60m⁻² and 80m⁻² in winter and summer are presented in Figures 7a and b. The yield in terms of tonnes ha⁻¹ is presented in Table 2 and Figures 8a, b, c). The yield was highest (6.59 \pm 2.63 tonnes ha⁻¹) in the summer density of 80m⁻² and was lowest $(1.34\pm0.73$ tonnes ha⁻¹) in the summer density of $20m⁻²$ (Table 2 and Figures 8a, b, c). Yield was not different between similar densities whether in summer or winter (P<0.05) (Appendix 5a). Where yields at

the four densities were compared, the following yields were different: 20m⁻² vs 60m⁻², 20m⁻² vs 80m⁻², 40m⁻² vs 60m⁻ ² and 40m-2 vs 80m⁻² while the following yields were not different: $20m^{-2}$ vs $40m^{-2}$ and $60m^{-2}$ vs $80m^{-2}$ (P<0.05) (Appendix 5b). The food conversion ratio (F . C.R.) (Table 2) was not different between different densities when means were compared in any manner (Appendix 5a and 5b).

Discussion

Difference in survival between all treatments was

not significant except between summer 20m"' and winter

latter. However in general, winter survival is higher than summer survival, even though the summer temperature average of 28.0-29.6°C was within the temperature range of 26-30*C which is favourable for this species (Van Olst et al., 1980; Wickins 1981,1982; Kuo, 1988). Some other physical factors appear to have favoured better winter survival. The ammonia levels in the last 2 months of summer period were 0.17 and 0.23 mg 1⁻¹ which were well above the desired level of $0.09-0.11$ mg 1^{-1} stated by Van

60m2. This may be attributed to exceptional low

survival in the former density and high survival in the

Olst et al. (1980), Wickins (1981,1982) and Kuo (1988). Also during these 2 months turbidity was exceptionally high and may reflect water column disturbance and sediment turnover, which might have brought some other unmeasured toxic metabolites in to the cages e.g. H_2S . Also in the last summer months the dissolved oxygen was recorded at lowest values of 2.9 mg 1⁻¹. The desired dissolved oxygen value for penaeid shrimps is about 5.0 mg 1-1 (Van Olst et al., 1980; Wickins, 1981,1982; Kuo,

1988) and 3.0-3.5 mg 1^{-1} or above (Tsang, 1988). The NO₋₂-N values were recorded for the last 2 months of the winter period and the last 3 months of the summer period. However, measurements in winter were higher and therefore may not have contributed to difference in survival, although they might have influenced growth in general.

The pH values for two periods overlapped with each other and the average for winter of 7.7 was close to that of summer average of 7.5. This difference may not be enough to have an influence on survival, but its possible impact on growth will be mentioned later. Normally the pH for shrimp culture should be around 8 to 9 and lower pH can stress the shrimp and cause soft shell and low survival (Tseng, 1988). The favourable pH range for shrimps is 7.8-8.3 (Van Olst et al., 1980; Wickins, 1981,1982; Kuo,

1988). In the last summer month the pH was at its lowest (7.2) and coupled with high ammonia, low oxygen and high turbidity may have caused survival to be lower than winter. The lowest survival rate was recorded in the summer density of 20m⁻². This inverse correlation between density and survival is in part due to an exceptional high mortality (87.7%) in one of the treatment replicates. This particular replicate is located in the pond corner where water circulation was probably low

creating a dead spot. Therefore some detrimental water qualities like higher ammonia, higher nitrite, lower D. O., plus some other unmeasured toxic factors probably built up in this remote corner and caused this mortality. It was noticed that this mortality was caused when water in the pond was exchanged. The pond in which the

experiment was carried out was a square pond and it has been noticed that rectangular and square ponds are inferior to circular ponds in uniformity of water flow which causes stagnant areas in rectangular pond collecting excrets, and other wastes.

Overall survival rate was within the range or mostly

superior to that reported by other workers (Table 5).

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It has been argued above that difference in

survival, though mostly non significant between densities, could be attributed to physical factors. When growth is considered physical factors apart from temperature, do not appear have an influence judging by the observed differences in growth. This is particularly evident as growth in the summer was always better than in winter (Table 2), despite high levels of ammonia, high turbidity and lower pH values during the summer growing period. A general trend is apparent when comparing

treatment pairs, that differences in growth were either, temperature dependent, density dependent or both temperature and density dependent. Difference between the same density for winter and summer are attributable to differences in temperature. The desirable temperature range for penaeids is 26-30°C (Van Olst et al., 1980;

Wickins, 1981, 1982; Kuo, 1988), which covers the summer range of 28.0-29.6°C recorded in this study. The range for the winter period in this study was 22.7-25.2°C which is below this desirable range. Differences between densities during the same growing period may be attributed to differences in stocking density. The length gain decreased as density increased which is to be expected. Differences between different densities in winter and summer may be due to differences in stocking

density and temperature i.e. density and temperature dependent.

It is difficult to compare the results achieved from the present study with previous P. indicus production grow-out studies due to differences in culture environments used. These include lower salinities of 4-189x, and seed source from the wild, the level of management of extensive pond culture, low inputs and quality of feed, rate of water exchange resulting in poor

survival and low production. Whilst the literature on various aspects of the biology and fisheries of the common penaeid prawns of India is abundant, information on growth is much more restricted and based mostly on length frequency studies.

However, in a short-term mixed culture experiment

for 75 days, P. indicus were reared extensively with other prawns in brackish water ponds in different densities of 0.5-10m⁻² (Das and Chakrabarti, 1979). In these experiments, growth rates ranged from 0.8-1.27mm day-'. and survival rates were 10-40%, which are typical results of extensive systems (Fast and Lester, 1992). In the present study, overall average growth rates were 0.37-0.47mm day⁻¹ and survival rates were 40.3-70.1%. These growth rates are below these from the extensive

system as survival is so low in the extensive system thus accounting for a stocking density far below that stated at the start of their experiments. A growth rate of 1.06 mm day-' from a mixed culture experiment was reported by Habib-Ul-Hassan (1988). This again may be attributed to the low starting stocking density of 2m'2. The actual stocking density at the end of this experiment was obviously below this since survival which was not mentioned and cannot have been 100%. Therefore growth and

survival from present studies cannot be regarded as inferior to the above mentioned studies and compares well with results from semi-intensive and intensive systems (Fast and Lester, 1992). In 450m² polythene film lined ponds, P. indicus

produced daily growth rates of 0.78mm and 0.64mm at a

density of 5m⁻² during 158 and 272 days with survival rates of 44.0 and 37.2% respectively (Nandakumaran, 1982). Arvindakshan et al. (1982), reported that for 6 month culture in cages of 50 x 50 x 30 cm at different densities of 5m⁻², 10m⁻², 20m⁻² and 40m⁻² daily growths were 0.33mm, 0.25mm, 0.21mm and 0.1mm respectively. Higher daily growth rates were obtained in the present study (0.45mm, 0.42mm, 0.41mm and 0.39mm for densities of 20m-², 40m⁻², 60m⁻² and 80m⁻² respectively). In another density

experiment P. indicus were stocked in ponds at 5m⁻², 10m⁻ 2,25M-2 and 50m'2 for 110 days and attained an average daily growth of 1mm, 0.82mm, 0.64mm and 0.42mm respectively, but no survival data were recorded (Muthu et al ., 1982). When daily growth rates from present study were calculated for the same culture period they were 0.57mm, 0.53mm, 0.51mm and 0.47mm for densities of 20m⁻², 40m⁻², 60m⁻² and 80m⁻² respectively. it is evident that growth from present study

compares well with other studies and that it is unlikely that water quality generally affected the overall growth. The yield in the present study ranged from 1.34±0.73-6.59±2.63tonnes ha-', but it should be noted that the lowest yield was obtained from a summer density of 20m⁻² which produced the highest growth but lowest

survival. Because the low survival at this density was attributed to physical factors, improvement in these physical factors may have resulted in better yields. The survival at this density was 40.3%±21.8 which was average of survival in 3 replicate cages of 58.8,45.9 and 16.3%. If one ignores the survival of 16.3% in this cage, then the average of the 2 other cages (58.8% and 45.9%), would have been 52.3% which would have resulted in a yield of 1.74 tonnes ha-'. In typical Penaeus indicus culture in

Taiwan, stocking densities of 20-30m⁻² are used and annual harvest is 2-10 tonnes ha $^{-1}$ from 1.5-2 crops a $^{-1}$ (Liao and Chao, 1983; Apud et al., 1983). In the present study, the yield of 1.34±0.75-6.59±2.63 tonnes stated above is for only 180 days of growth. The production for one year i. e. the combined growing periods of the present study gives 3.21-11.07 tonnes ha⁻¹ (the average yield of the lowest and highest densities in the two growing periods). This corresponds well or even exceeds that of

P. monodon in Taiwan. Penaeus indicus yields from salt pan areas of 42-46%, when prawns were stocked in densities of $121,410$ and $149,578$ ha⁻¹ (equivalent to 12 and 15m-2), show daily rates of growth after 198 days of 0.45-0.5mm and 0.05-0.06g month-1 with a survival rate of 73.2%-95.4%, a harvest size of 106-123mm, at 8-13.2g and

production ranging from 881-1604kg ha⁻¹crop⁻¹(Marichamy and Matha, 1982). Production elsewhere is generally poor (Nandakumar., 1982) only achieving 231.5Kg ha⁻¹ 5 month $\bar{ }$ 1, whilst present study production achieved an overall average of 3.76 tonnes 6 month⁻¹. However level of feed input and F.C.R. are unknown in these low density extensive cultures. Production levels for P. indicus have fluctuated

during the 1950's through 1960's and 1970's from 300-1131

k ha⁻¹ yr⁻¹(Menon, 1954; Gopinath, 1956; Gopalan et al., 1980; Mamman, 1978). Das and Chakrabarti (1979) reported 666-850kg ha-1 270 days-' from a mixed prawn farming in West Bengal which was the highest at that time from that region. It has been mentioned that temperature has influenced growth at the same density between the two

growing periods because summer growth was usually higher.

It appears that this effect is not great enough to
influence the overall yield, because yield at the same density was not different in winter and summer (Appendix 5a). Nor did other water quality appear to affect yield, but it is probably important and improvement in certain water quality factors, especially pH would have increased the yield. Higher pH could be obtained by addition of

calcium carbonate (Muthu et al., 1977). Cost of shrimp production for P.indicus on the Red Sea coast of Saudi Arabia is presented in Table 7. Based on above production figures a profit of 18% of operational cost is obtained. It can be seen from table 7 that feed constitutes 75% of the total operational cost. Generally in intensive systems 40-60% of operational cost is attributed to feed cost (Pascual et al., 1990).

The following table shows the average sizes and

their corresponding prices per kg in Saudi Arabia (Bukhari, 1990). Table 6 shows shrimp prices per kg in Saudi Arabia in relation to weight per individual shrimp. Table 6. Market prices for shrimp taken from the

Saudi market.

Table 7 Shows an operational cost budget for P. indicus in ponds on the Red Sea coast of Saudi Arabia.

Categories Cost Cost % Manpower

A) Pond techinican \bullet 1 Manx1500 SR Month-'x12 Months 18,000 SR

B) Pond helper

Feed cost

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Amount of required feed is 100 tonnes year<sup>-1</sup>
(feed conversion ratio is from 3.5-6.5: 1 and
 production is 5 tonnes ha<sup>-1</sup> X 5 ha = 25 tonnes)
(4,000 SR tonne<sup>-1</sup>)<br>Total feed cost
                                       400,000 SR 75.0 %
Medicine, Chemicals 
for water quality and Others. 4,000 SR 0.75 %<br>Total Operational cost 532,400 SR 100 %
Total Operational cost<br>
Total Selling price<br>
625,000 SR
Total Selling price
(25 tonnes X 25,000 SR) 
Total Gross Profit 92,600 SR
```
Profit \approx 18 %

In the present intensive study, the cost of feed is

too expensive. This is indicative of low food conversion ratio. The food conversion ratio for P. monodon in Taiwanese semi-intensive systems was 1.8-3.3 and for intensive culture of P. japonicus in Japan was 2.5 (liao and Chao, 1983). For prawns and shrimps in general an F. C. R. of 1.5-2.5 is acceptable for an artificial diet

(Apud et al., 1983). For P. indicus F.C.R. obtained by Ali (1982) ranged from 1.5-4.2 in a feeding experiments using different protein sources of 30 culture days, also Colvin (1976) reported a range of F. C. R. from 2.4-5.3 in other feeding experiments testing different protein levels over 3-5weeks, Raman et al. (1982) have achieved F. C. R. ranging from 1.7-3.2 after 2 months in a feeding study using different combinations of a number of ingredients. F.C.R. in the present study ranged from

3.8±2.31-8.0±0.90 which compares well with some previous

studies. Hence feeding experiments have been carried out

to investigate this problem (Chapter 4)

Since difference in yields between the same density

in summer and winter were not significant (P>0.05)

(Appendix 5a), densities were compared irrespective of

summer and winter. This comparison revealed no difference

between 20m'2 and 40m72 and between 60m'2 and 80m'2. Hence,

which density is to be recommended depends on certain

variables. In very rare occasions has a density of more than 40m'2 been used in semi-intensive ponds (Muthu et al., 1982). Therefore since there was no difference in yields between the densities of 60m⁻⁴ and 80m⁻⁴ , and since there is more biomass at the density of 80m⁻² making it more susceptible to water quality deterioration, a

density of $60m^{-2}$ would be favoured over the $80m^{-2}$. In Taiwan when tiger prawn (P. monodon) farmers increased their stocking density in semi-intensive pond culture to 100m2, they experienced mass mortality which was attributed to water quality deterioration caused by man which could have been averted (Lin, 1988). Also in the present study the 60m⁻² density resulted in a final shrimp size of 15+g (Table 4), which commands a better market value than the size obtained at the 80m⁻² density.

The price for size $10-14$ g is SR. 20.00 kg⁻¹ and for the $size$ 15-19 g is SR. 30.00 kg^{-1} (Table 6). Both the densities of 20m⁻² and 40m⁻² resulted in shrimp sizes of 17.5±0.5 g and 16.5±0.06 g (Table 4). So they command the same price of SR. 30.00 kg⁻¹. Since production is significantly higher in the 60m⁻² density than both 20m⁻² and 40m⁻², it is recommended that a density of 60m⁻² P. indicus is advisable under local culture conditions in Saudi Arabia. In addition, when

statistical analyses were carried out for weight gain vs culture periods between five and six months at the densities 20m⁻, 40 , 60nr-2 and 80m 2, there was a significantly higher weight gain at six months vs five months only at the 60m⁻² density (Appendix 5c). Therefore it is recommended that the culture of P.

indicus in ponds be extended to six months in order to achieve the desirable marketable size of 14g+ to obtain the price mentioned above (Tables 4 and 6). Also it is essential that water quality be improved, possibly by increasing water exchange rate per day, as well as aeration.

	$\mathbf{\hat{z}}$	26.0	2.9	45	7.22		38	0023
eriod Jan.93) Summer per	\overline{S}	28.0 26.0 4.0		$\frac{1}{4}$		$7.3 - 17$	OZ	D.02
		0.002	4.2		7.3			\bullet
	3	32.0 31.0	0, 4	4 ₃	7.8	0.02		
	2	32.0	4.1	4 ₃		0.02		
		30.0 29.0	4.7	43	N	\bullet	∞	
	$\overline{6}$	27.0	3.4	43	7.2	\ddot{O}	\sim	
2) $\boldsymbol{\sigma}$ Winter period (25/Nov./91-23Ma) Winter		25.5 24.5	4.8	43	さ・	0.03		
	さ	22.5 20.5	5.3	4	$\bar{\mathcal{L}}$	0.02		
	3	19.0 24.0	O S	$\bar{\mathbf{t}}$	∞ 7	0.02		
	2	25.0 22.0	\mathbf{v}	42	ထံ	0.02		
		27.0	5.1	42	$\overline{8.1}$	0.02	L	\vec{c}

 $3f$

180 days of Penaeus indici in the Red Sea water, Jeddal data during two periods on winter and summer 1: Pond physical factor Saudi Arabia. culture Table

Salinity (ppt.) Temp. Max
(C) Min.
D.O.(mg/l) Turbidity ZS(1)
ZS(1)
ZE NO₂ $\frac{1}{\mathbf{p}}$

Months

Table 2: Penaeus indicus length gain, weight gain, survival rate, yield, feed consumption and feed convertion ratio after two 180 days of cage/pond culture in the Red Sea of Saudi Arabia at different stocking densities during winter and summer (inital winter length and weight; 5.60 ± 0.16 cm., 1.16g. and for summer 5.56 ± 0.11 cm., 0.94g respectivly).

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1982

 $\frac{1}{2}$

Nandakuma -ran
1982 Aravindaksh 1982 $\overline{\mathbf{a}}$ ne- \mathbf{c}

Muthu et al Marichamyr
and J. Maleha Gopalan, et al

Charkrabarty
and Das Hassan 1988 Habib-ut 1988

Bukhari, $\frac{1}{2}$

Extensive
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ponds of 60m⁻² though -semi-intensive
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In 0.3-1.0 Extensive
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paddy-cum shri
culture fields 2 Semi-intensive

Extensive
in 50mx50mx2. Extensive ponds ponds

Semi-intensive

Penaeus indicus water, Jeddah,

Sea σ the Red two 180 days \mathbf{m} summer data during and winter factor 5^o

physical periods rabia Pond culture Saudi \vdots Figure

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$\frac{10}{10}$ $\frac{1}{4}$ $\frac{10}{10}$ $\frac{11}{10}$ $\frac{11}{10}$ $\frac{11}{10}$

$\mathbf{2}$ $3⁷$ 5 6 $\mathbf{1}$ $\overline{\mathbf{4}}$ $\boldsymbol{0}$

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Summer culture months

10 5 6 Summer culture months

 ~ 0.1

I days of Penaeus
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$\frac{0}{2}$ $\frac{5}{10}$ $\frac{5}{2}$ $\frac{0}{3}$ \mathbf{O}

Ln weight (g)

Figure 7a and b: Penaeus indicus survival rates in relation to length in cm and weight in grams during two 180 days of culture periods at different densities during winter and summer in the Red Sea water, Jeddah, Saudi Arabia.

Contract Contract $\langle \bullet \rangle$.

General discussion

General discussion

culturing this species, the present investigations will open the door for the study of other local races of penaeid species such as P. monodon. Experiments were carried out over the complete life cycle of this species in captivity and to optimize culture conditions for each developmental stage. It was found that optimal salinity for larval culture upto PL5 is 30%, while for nursery culture (PL5

and above) the optimal salinity is $25-30%$.

The growing interest in shrimp culture for investment in Saudi Arabia, together with recent spawning and larval productions success for the local shrimp species, Penaeus indicus from the Red Sea, necessitated rapid investigations of optimal culture conditions for this species. In addition to establishing guidelines for

The earliest nursery stage for transfer from nursery to growout pond at 43% is about PL25. However, it has been observed that a preference for higher salinities of 35-5046 occurs at the PL35-50 stage. Results from hemolymph osmolality experiments with adult Red Sea P. indicus confirm that these prawns are extremely good hyperregulators in dilute seawater and hyporegulators in high salinity media. Therefore, it appears that the Red

Sea strain of P. indicus is preadapted to tolerate high

salinities when compared to the Indian Ocean P. indicus

(Raj and Raj, 1982). Future research into ongrow diets should examine optimal levels of minerals required to minimize osmotic stress in pond culture. Artificial feeds to replace live feeds in P. indicus larval culture from Z1 to PL5, using microencapsulated feeds Nippai and Frippak have been investigated. Results reveal that 50% replacement with Nippai and Frippak is

possible giving comparable growth to controls which are fed live feeds, but poorer survival. However, research on live feed replacement should not be discouraged by this low survival because total replacement of live feeds using microencapsulated has been demonstrated at laboratory and hatchery level elsewhere (Jones et al., 1987). In addition Kumlu (pers. comm., 1993) reports considerable success with artificial diets in the larval culture of P. indicus from Red Sea at the School of Ocean

Sciences (Menai Bridge).

However, based on results of this study at the present time it is not advisable yet to depend on microcapsules alone for larval rearing at the Fish Farming Centre, and further research is required in this area as expertise is gained in handling larvae and operating artificial diet systems. The postlarval comparative growth trials from PL5-PL30 reared on commercial feeds: Taiwanese, Nippai,

Frippak and 4 locally formulated feeds at $28-30$ °C at 30%

and 42% demonstrate that it is possible to get similar growth and yields from P. indicus postlarvae fed local diets. Comparable yields were achieved to those on imported commercial diets. It has been possible to replace expensive local protein sources with cheaper local protein and to produce diets for approximately the same price as the cheapest imported feed. In addition

postlarval results obtained in these experiments agree with the results of the previous salinity studies confirming the salinity preference of P. indicus from the Red Sea. This strain during early postlarval stages still prefers a salinity of 30 ppt. and is not physiologically capable of tolerating higher salinilies. Local ingredients were also used to prepare growout feeds for P. indicus juveniles utilizing 4 locally formulated diets and compared with a Taiwanese diet. Unfortunately water quality factors including, low pH and high ammonia resulted in poor growth and in consequence poor food conversion ratios. These circumstances make comparison of these feeds unrealistic. Hence further comparisons remain essential for P. indicus growout. These trials have highlighted serious water quality problems with the present system of wells used to obtain sea water for the Fish Farming Centre. In particular attention should be given to the periodic low pH of water

drawn from this system.

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Finally shrimp density production trials at 20,40, 60 and 80m'2 during winter and summer were conducted in cages placed in a rubber lined earthen pond. For all densities yields were significantly higher (p<0.05) in summer than winter, which is attributed to favourable temperatures resulting in faster growth during summer. But survival in the summer is generally lower than

winter. This again may be attributed to poor water quality especially low pH, high ammonia and water column turnover during parts of the summer growing period. Results achieved from this study showed that the best density was at a density of 60m'2 due to highest recorded survival rate of 62.5, 70.1 % and high yields equivalent to 4.73, 5.31 tonnes for winter and summer respectively with shrimp reaching the biggest sizes commanding the highest total revenue.

Feed conversion ratios are poor ranging from 4.3-5.7 during winter and summer trials due to low harvested size in winter and low survival in summer. Again the negative influence of low pH on growth is stressed. More research is required to improve Food conversion ratio verses weight gain and to optimise pond water quality.

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APPENDICES

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APPENDIX 2

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Appendix 2a

One Factor ANOVA X1: Salinities Y2: Survival PL1

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(Experiment 1)

Analysis of Variance Table

Model II estimate of between component variance = 19.56

One Factor ANOVA X1: Salinities Y2: Survival PL1

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One Factor ANOVA X1: Salinities Y4: Survival PLS (Experiment 1)

Analysis of Variance Table

Model II estimate of between component variance $= 20.65$

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One Factor ANOVA X1: Salinities Y4: Survival PLS

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One Factor ANOVA X1: Salinities Y1 Length mm PL1 (Experiment 1)

Analysis of Variance Table

Model II estimate of between component variance $= -009$

One Factor ANOVA X1: Salinities Y1: Growth PL1

One Factor ANOVA X1: Salinities Y3 Length mm PLS (Experiment 1) Analysis of Variance Table

Model II estimate of between component variance = .421

One Factor ANOVA X1: Salinities Y3: Growth PL5

One Factor ANOVA X1: Salinities Y3: Growth PL5

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One Factor ANOVA X1: Salinities Y4: Survival % PL1 (Experiment 2) Analysis of Variance Table

Model II estimate of between component variance = 168.119

One Factor ANOVA X1: Salinities Y4: Survival % PL1B

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* Significant at 95%

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One Factor ANOVA X1: Salinities Y6: Survival % PLS

(Experiment 2)

Analysis of Variance Table

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Model II estimate of between component variance = 38.412

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One Factor ANOVA X1: Salinities Y6: Survival % PL5B

· Significant at 95%

One Factor ANOVA X1: Salinities Y3 Length mm PL1

(Experiment 2)

Analysis of Variance Table

Model II estimate of between component variance $= .197$

One Factor ANOVA X1: Salinities Y3: Grwoth PL1B

One Factor ANOVA X1: Salinities Y₃: Grwoth PL1B

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. Significant at 95%

One Factor ANOVA X1: Salinities Y5 Length mm PL5

(Experiment 2)

Analysis of Variance Table

Model II estimate of between component variance $= .04$

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Appendix 2b

		One Factor ANOVA X 1: Salinity Y 1: Survival PL 2 Analysis of Variance Table	$survival$ (Experiment 1)	
Source:	DF:	<u>Sum Squares: Mean Square:</u>		F -test:
Between groups 3				
Within groups	18			$D = 0$
Total				

Model II estimate of between component variance = 0

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Model A estimate of between component variance = 1543.778

One Factor ANOVA X 1 : Salinity Y 2: PL 4

Contract Contract

Model II estimate of between component variance = 906.185

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One Factor ANOVA X1: Salinity Y1: Survival PL 10

Analysis of Variance Table

Model II estimate of between component variance = 186

* Significant at 95%

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One Factor ANOVA X 1 : Salinity Y 3 : PL 16

One Factor ANOVA Y + Callotty V 4 + DI 10

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Model II estimate of between correctional variance = .418

Appendix 2c
SURVIVAL (*)
One Factor ANOVA X1: Salinities Y1: *PL5 (Experiment 2)

Analysis of Variance Table

Model II estimate of between component variance = 334.407

One Factor ANOVA X1: Salinities Y1: SAPL5

SURVIVAL

One Factor ANOVA X1: Salinities Y1: WPL7

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Analysis of Variance Table

Model II estimate of between component variance = 551

One Factor ANOVA X1: Salinities Y1: %PL7

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SURVIVAL %

One Factor ANOVA X1: Salinities Y_1 : SPL10

Analysis of Variance Table

Model II estimate of between component variance = 278.519

One Factor ANOVA X1: Salinities Y1: %PL10

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SURVIVAL (%)

One Factor ANOVA X1: Salinities Y_1 : XPL14

Analysis of Variance Table

Model II estimate of between component variance = 133.333

One Factor ANOVA X1: Salinities Y1: XPL14

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SURVIVAL (%)

One Factor ANOVA X1: Salinities Y1: %PL17

Analysis of Variance Table

Model II estimate of between component variance = 65.556

One Factor ANOVA X1: Salinities Y1: WPL17

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SURVIVAL (*)

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One Factor ANOVA X1: Salinities Y1: WPL20

Analysis of Variance Table

Model II estimate of between component variance = 40.963

One Factor ANOVA X1: Salinities Y1: %PL20

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One Factor ANOVA X1: Salinities Y1: PLS Length mm

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(Experiment 2)

Analysis of Variance Table

Model II estimate of between component variance $= -019$

One Factor ANOVA X1: Salinities Y1: PLS

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One Factor ANOVA X1: Salinities Y1: PL7 Length mm

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Analysis of Variance Table

Model II estimate of between component variance $= .003$

One Factor ANOVA X1: Salinities Y1: PL7

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One Factor ANOVA X1: Salinities Y1: PL10 Length mm

Analysis of Variance Table

Model II estimate of between component variance = .162

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One Factor ANOVA X1: Salinities Y1: PL10

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One Factor ANOVA X1: Salinities Y1: PL14 Length mm

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Analysis of Variance Table

Model II estimate of between component variance = .477

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One Factor ANOVA X1: Salinities Y1: PL14

. Significant at 95%

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One Factor ANOVA X1: Salinities Y1: PL17 Length mm

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Analysis of Variance Table

Model II estimate of between component variance $= .346$

One Factor ANOVA X1: Salinities Y1: PL17

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One Factor ANOVA X1: Salinities Y1: PL20 Length mm

Analysis of Variance Table

Model I estimate of between component variance = .754

One Factor ANOVA X1: Salinities $Y_1:$ PL20

· Significant at 95%

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One Factor ANOVA X1: Salinities Y1: Su. PL40 Appendix 2d

Analysis of Variance Table

Model II estimate of between component variance = 190.476

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One Factor ANOVA X1: Salinities Y3: Su. PL 50

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Analysis of Variance Table

Model II estimate of between component variance = 373.784

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* Significant at 95%

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups 7		13501.336	1928.762	24.66
Within groups		1251.413	78.213	$ p=.0001$
Total	23	14752.75		

Model II estimate of between component variance $=$ 264.364

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and the state of the state of the

Significant at 95%

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One Factor ANOVA X1: Salinities Y2: Yields PL 40

Analysis of Variance Table

Model II estimate of between component variance = 65.37

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Analysis of Variance Table

Model II estimate of between component variance = 160.636

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One Factor ANOVA X1: Salinities Y₂: Yield PL 60

Analysis of Variance Table

Model II estimate of between component variance = 998.421

+ Significant at 95%

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APPENDIX 3

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Appendix 3a One Factor ANOVA X1: diets Y1: Survival % PL 1 (Experiment 1) Analysis of Variance Table

Model II estimate of between component variance = 633.056

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One Factor ANOVA X1: diets Y1: Survival % PL 1

* Significant at 95%

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One Factor ANOVA X1: diets Y1: Survival % PL 5 (Experiment 1)

Analysis of Variance Table

Model II estimate of between component variance $= 74.025$

One Factor ANOVA X1: diets Y1: Survival % PL 5

One Factor ANOVA X1: diets Y1: Survival % PL 5

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* Significant at 95%
One Factor ANOVA X1: diets Y1: LENGTH (mm) PL 1 (Experiment 1)

Analysis of Variance Table

Model II estimate of between component variance = .649

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One Factor ANOVA X1: diets Y1: Growth (mm) PL 1

. Significant at 95%

One Factor ANOVA X1: diets Y1: LENGTH (mm) PL 5

(Experiment 1)

Analysis of Variance Table

Model II estimate of between component variance = 1.675

One Factor ANOVA X1: diets Y1: Growth (mm) PL 5

· Significant at 95%

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One Factor ANOVA X1: Feeds Y4: PL1 SURVIVAL %

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(Experiment 2'

Analysis of Vanance Table

Model II estimate of between component variance $= -21.5$

One Factor ANOVA X1: Feeds Y4: PL1 Sur 2

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One Factor ANOVA X1: Feeds Y6: PLS SURVIVAL %

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(Experiment 2)

Analysis of Variance Table

Model II estimate of between component vanance = $81,347$

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One Factor ANOVA X1: Feeds Y6: PL5 Sur 2

. Significant at 95%

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One Factor ANOVA X1: Feeds Y3: PL1 LENGTH mm

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(Experiment 2)

Analysis of Variance Table

Model II estimate of between component variance $= -047$

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One Factor ANOVA X1: Feeds Y3: PL1 Gr 2

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One Factor ANOVA X1: Feeds YS: PLS LENGTH mm (Experiment 2)

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Analysis of Variance Table

Model II estimate of between component variance = .423

One Factor ANOVA X1: Feeds Y5: PL5 Gr 2

. Significant at 95%

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One Factor ANOVA X1: Feeds Y4: PL 1 SURVIVAL %

(Experiment 3)

Analysis of Variance Table

Model II estimate of between component variance = 77.097

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One Factor ANOVA X1: Feeds Y4: PL 1 Su.

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One Factor ANOVA X1: Feeds Y6: PL 5 SURVIVAL % (Experiment 3)

Analysis of Variance Table

Model II estimate of between component variance $= -21.069$

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One Factor ANOVA X1: Feeds Y6: PL 5 Su.

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One Factor ANOVA X1: Feeds Y3: PL 1 LENGTH mm (Experiment 3)

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Analysis of Variance Table

Model II estimate of between component vanance = .156

One Factor ANOVA X1: Feeds Y3: PL 1 Gr.

One Factor ANOVA X1: Feeds Y3: PL 1 Gr.

* Significant at 95%

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One Factor ANOVA X1: Feeds Y5: PL 5 LENGTH mm

(Experiment 3)

Analysis of Variance Table

Model II estimate of between component variance = .712

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One Factor ANOVA X1: Feeds Y5: PL 5 Gr.

· Significant at 95%

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Appendix 3b One Factor ANOVA X1: DIETS Y1: SURVIVAL % PL30

Analysis of Variance Table

Model II estimate of between component variance = 86.342

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+ Significant at 95%

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* Significant at 95%

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One Factor ANOVA X1: DIETS Y1: LENGTH GAIN PL30

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Source:	DF:	Sum Squares:	Mean Square:	F-test:
I Between groups 13		171.891	13.222	2.71
<i><u>Within groups</u></i>		136.632	4.88	$p = .0132$
Total		1308.524		

Analysis of Variance Table

Model II estimate of between component variance = .642

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" Significant at 95%

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Appendix 3c One Factor ANOVA X1: Feeds Y1: Yields in grammes 1000 PL ⁻¹ at PL25

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Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups 13		300.593	23.123	2.519
Within groups	28	1257.043	9.18	$ p = .0198$
Total	41	557.636		

Model II estimate of between component variance = 1.072

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+ Significant at 95%

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. Significant at 95%

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APPENDIX 4

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Appendix 4a One Factor ANOVA X1: Feeds Y1: % leaching 1 hr

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Analysis of Variance Table

* Significant at 95%

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Analysis of Variance Table

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· Significant at 95%

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Analysis of Variance Table
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* Significant at 95%

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Analysis of Variance Table

One Factor ANOVA X1: Feeds Y1: %leaching 14 hr

" Significant at 95%

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Appendix 4b One Factor ANOVA X1: DIETS Y1: SURVIVAL (%)

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups] 4		840	210	.525
Within groups	10	4000	400	1057. – q
Total	14	4840		

Model II estimate of between component variance $= -47.5$

One Factor ANOVA X1: DIETS Y1: Weight gain (g)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
i Between groups 14		4.184	1.046	.515
Nithin groups		20.296	2.03	1p = .7265
Total		24.48		

Analysis of Variance Table

Model II estimate of between component variance $= -246$

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APPENDIX 5

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Appendix 5a

One Factor ANOVA X1: Densities Y3: Survival%

Analysis of Variance Table

Model II estimate of between component variance = 8.484

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One Factor ANOVA X1: Densities Y₂: L. gain mm

Source:	DF:	Sum Squares:	Mean Square:	F-test:
/ Between groups 7		7.592	1.085	142.036
Within groups	16	.413	.026	$p = .0001$
Total	23	8.005		

Analysis of Variance Table

Model II estimate of between component variance = .151

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* Significant at 95%

Significant at 95%

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One Factor ANOVA X1: Densities Y1: W. gain (g)

Source:	DF:	Sum Squares:	Mean Square:	F-test:
, Between groups 7		85.864	12.266	12.524
I Within groups		15.671	.979	$p = .0001$
Total	23	101.535		

Analysis of Variance Table

Model II estimate of between component variance = 1.612

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* Significant at 95%

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* Significant at 95%

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One Factor ANOVA X1: Densities Y4:Yields/tons/ha

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Source:	DF:	Sum Squares:	Mean Square:	F-test:
Between groups 7		70.529	10.076	5.908
Within groups		27.284	1.705	$ p=.0016$
Total	23	97.814		

Analysis of Variance Table

Model II estimate of between component variance = 1.196

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* Significant at 95%

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the contract of the

One Factor ANOVA X1: Densities $Y₅: FCR$

Source:	DF:	Sum Squares:	Mean Square:	F-test:	
Between groups J		129.688	18.527	.713	
Within groups		1415.915	25.995	$p = .6626$	
Total	23	545.603			

Analysis of Variance Table

Model II estimate of between component variance = -1.067

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Y3: Sur. % One Factor ANOVA X1: Density

Appendix 5b

Analysis of Variance Table

Model II estimate of between component variance = -12.048

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One Factor ANOVA X1: Density Y3: Sur. %

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One Factor ANOVA X1: Density Y1: L. Gain

Analysis of Variance Table

Model II estimate of between component variance = .296

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One Factor ANOVA X1: Density Y1: L. Gain

Contract Contract

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One Factor ANOVA X1: Density Y2: W. gain

Analysis of Variance Table

Model II estimate of between component variance = 1.939

والمتحدث المالي الأربيبين

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One Factor ANOVA X1: Density Y2: W. gain

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One Factor ANOVA X1: Density Y4: Yield

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Analysis of Variance Table

Model II estimate of between component variance = 5.965

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One Factor ANOVA X1: Density Y4: Yield

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One Factor ANOVA X1: Density Y4: Yield

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One Factor ANOVA X1: Density Y5: FCR

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Analysis of Variance Table

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Model II estimate of between component variance = -2.277

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One Factor ANOVA X1: Density Y5: FCR

One Factor ANOVA X1: Density Y5: FCR

Appendix 5C

One Factor ANOVA X1: Cultre month Y1: Wi.g.20/m2

Analysis of Variance Table

Model II estimate of between component variance $= -0.667$

One Factor ANOVA X1: Cultre month Y2: Wi.g40/m2

Analysis of Variance Table

Model II estimate of between component variance = -1.885

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One Factor ANOVA X1: Cultre month Y3: Wi.g.60/m2

Analysis of Variance Table

Source:	DF:	Sum Squares:	Mean Square:	F-test:	
, Between groups J		1.815	1.815	'24.75	
Within groups		.293	.073	$p = .0076$	
Total		2.108			

Model II estimate of between component variance = 1.742

Group:	Count:		Mean:		Std. Dev.:	Std. Error:
Five month			11.233	.252		.145
/Six month			12.333	.289		.167
Comparison:		Mean Diff.:	Fisher PLSD:		Scheffe F-test:	Dunnett t:
Five month vs. Six month		\sim 1.1	$.614*$		$24.75*$	4.975

* Significant at 95%

One Factor ANOVA X1: Cultre month Y4: Wi.g.80/m2

Analysis of Variance Table

Model II estimate of between component variance = . 928

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