# GROWTH AND SURVIVAL OF *PENAEUS MONODON* IN MONOSEX AND MIXED-SEX CULTURE UNDER LABORATORY CONDITIONS

By

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B.F.Sc

THESIS

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Dedicated
To
My family
&
Dr.C.M.Nair

## DECLARATION

I hereby declare that this thesis entitled **"GROWTH AND SURVIVAL OF *PENAEUS MONODON* IN MONOSEX AND MIXED-SEX CULTURE UNDER LABORATORY CONDITIONS"** is an authentic record of the work done by me and that no part there of has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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## CERTIFICATE

Certified that this thesis entitled "**GROWTH AND SURVIVAL OF *PENAEUS MONODON* IN MONOSEX AND MIXED-SEX CULTURE UNDER LABORATORY CONDITIONS**" is a record of research work done independently by Mr. Bajaniya Viralkumar Chhaganlal under my guidance and supervision and that it has not previously formed the basis for award of any degree, fellowship or associateship to him.

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**BAJANIYA VIRALKUMAR CHHAGANLAL**

# CONTENTS

# LIST OF TABLES

## LIST OF FIGURES



## PLATE

# INTRODUCTION

# 1. INTRODUCTION

Aquaculture has immense potential to augment production to partially meet the growing demand for animal protein. It has emerged as one of the fastest growing food production sectors. Aquaculture obviously has the potential to grow rapidly and to make a substantial additional contribution to employment and food security particularly in the rural area.

Marine shrimp farming is an age-old practice in many Asian countries. Dr. Motosaku Fujinaga, the pioneer of shrimp culture, successfully spawned and partially reared the larvae of *Marsupenaeus japonicus* in Japan for the first time in 1934. This technology led to the development in the shrimp farming.

Modern shrimp farming, the production of marine shrimp in impoundments, ponds, raceways and tanks started in early 1970s, and today, in over fifty countries commercial shrimp farming is in practice. In the Eastern Hemisphere, Thailand, Vietnam, Indonesia, India and China are the leaders and Malaysia, Taiwan, Bangladesh, Sri Lanka, the Philippines, Australia and Myanmar have large industries. In the Western Hemisphere, Mexico, Belize, Ecuador and Brazil are the leading producers, and there are shrimp farms in Honduras, Panama, Colombia, Guatemala, and Venezuela. In the Middle East, Saudi Arabia and Iran produce the most farmed shrimp.

The shrimps cultured in Asian countries belong to the family Penaeidae: genera *Penaeus*, *Marsupenaeus*, *Fenneropenaeus* and *Metapenaeus*. Among the dozen species of these genera *Penaeus monodon*, *Marsupenaeus japonicus*, *Penaeus merguensis*, *Fenneropenaeus indicus*, *Penaeus orientalis* and *Metapenaeus ensis* are the important ones.

Penaeid shrimp culture has emerged as a highly profitable investment alternative. *P.monodon* is one of the most commercially important shrimp distributed throughout the Indo-Pacific region. This

species has got tremendous importance in aquaculture because of its high survival, growth and market demand. It is the fastest growing of all shrimps tested for culture. It is euryhaline and grows well in salinity range of 15-30 ppt. Its commercial scale culture is in practice in countries such as Japan, Taiwan, Indonesia, Thailand, Malaysia, the Philippines, China, India, Bangladesh, Sri Lanka and many countries in the Indo-Pacific region.

Although considerable information has been gathered in the past few decades on biology, production and culture of many commercially important shrimps, very little attention has been paid to monosex culture.

Monosex culture of commercially important species has taken advantage of sex-related differences to overcome the adverse effects associated with mixed-sex culture.

Differential growth between the sexes has been reported in many cultured species of fish and crustaceans. In mixed-sex culture, *Macrobrachium rosenbergii* males grow significantly larger than females (Smith *et al.*, 1978; Cohen *et al.*, 1981; Sagi *et al.*, 1986) while in *P.monodon* females generally achieve a larger size than males (Motoh, 1981; Garcia, 1985; Somers *et al.*, 1987). Growth superiority of female shrimp thus provides an incentive to investigate the potential for producing and culturing all female population. So as to enable decreasing the growout period and/or increasing pond yield. There has been very few studies have focused on the monosex culture of penaeid shrimps unlike in the case of *M. rosenbergii.*

The present study envisages a comparative assessment of the growth performance of the giant tiger shrimp, *P. monodon* under three stocking strategies *viz.,* all male, all female and mixed-sex. The study also involved elucidation of the minimum size of sex differentiation as well as the sex linked growth profile.

# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

Shrimp is by far the most important commodity by value in the international fish trade. Shrimp ranks high in consumers favorite seafood and is consumed in all parts of the world. The output from shrimp aquaculture has more than doubled over the last few decades and a growing share of internationally traded shrimp comes from aquaculture.

Dr. Motosaku Fujinaga made some of the most important contributions to the development of shrimp culture when he first accomplished captive spawning of mature *M. japonicus* females and reared the resulting larvae to sub adults (Fujiinaga, 1969).

## 2.1. SEX DETERMINATION

The presence of different male and female body forms is referred to as sexual dimorphism. Sexual dimorphism in a cultured species can make one sex more commercially valuable than the other. In general, it is seen that one of the sexes grows faster than the other, in most species of fish (Hansford, 1991).

Research on sex determination and adoption of female monosex culture in future shrimp aquaculture will require determining the gender at early juvenile stages. In shrimp, it is not known whether sex differentiation is size or age dependent. The easiest way to sex shrimp is by just observing the presence or absence of the endopodite modification, the male petasma in the first pair of pleopods that develops from the early juvenile (postlarval) stage. However, to do this by eye with confidence, shrimp must be cultured for at least four months (Perez-Rostro and Ibarra, 2003a). Although it is known that sex in gonochoric crustacean is primarily genetic (Charniaux-Cotton, 1960), little is known about sex determination of penaeid shrimp species (Benzie, 1998).

According to Yin *et al.* (1986) and Li and Xiang (2002) on *F. chinensis* and Charniaux-Cotton and Payen (1985) and Nakamura *et al.*

(1992) on *M. japonicus*, external differentiation between female and male takes place during the second month after post larval transformation.

Campos-Ramos *et al.* (2006) studied the environmental sex determination in *Litopenaeus vannamei.* From day 50, the development of endopodite of the first pair of pleopods revealed the first external differentiation, showing a triangular structure with three setae in females, while a tubular structure remained in males. Juvenile shrimp sex differentiation takes place from days 50-90, independent of size, only if postlarvae reached a development threshold of 150 mg of body weight and 20 mm of body length.

There is no information on when (size or age), or how to sex young postlarvae of *P. monodon.*

## 2.2. ALL MALE CULTURE

Growth in culture systems may be affected by a wide variety of factors, including gender, sexual maturity and age (Hartnoll, 1982; Botsford, 1985; Aiken and Waddy, 1992). By the late 1970s, monosex culture strategy had become a common practice in fish-based aquaculture (Mires, 1977; Tayman and Shelton, 1978), and more recently attempts have been made to apply this technology to crustacean culture (Curtis and Jones, 1995; Sagi *et al.,* 1997). It was soon realized that differences between male and female, in terms of growth rate, alimentary needs and behavioral patterns dictated the need to establish management systems to control one sex or the other. One of the inherent advantages of non-breeding monosex culture populations is that energy is diverted from reproduction to growth.

### 2.2.1. Giant freshwater prawn

The giant freshwater prawn, *M. rosenbergii* widely cultured in many tropical and sub-tropical countries is sexually dimorphic and shows a highly variable growth pattern between the sexes (Sandifer and Smith, 1985). Growth of females slows once they become sexually mature at about 4-5

months of life, as energy is diverted for reproduction, while males continue to grow at a faster rate. Thus, the yield from an all male population should be higher than that from a mixed-sex population and would be economically advantageous.

A number of crustacean species exhibit bimodal growth patterns in which males exhibit superior growth to females or vice versa (Hartnoll, 1982). The first attempt for monosex culture of *M. rosenbergii* was carried out in a small scale, intensive, cage culture system (Sagi *et al.,* 1986). An all male population yielded 473 g/m$^2$ within 150 days, whereas all female and mixed populations produced 248 and 260 g/m$^2$ respectively, in the same growth period. In addition to giving higher yields, the prawns of the all male population reached market size at a faster rate, a factor that prolonged the fresh product marketing period and enabled the now vacant pond to be used for further production (Sagi *et al.,* 1986). Similar results were obtained when monosex culture was carried out under intensive monoculture condition in earthen ponds (Cohen *et al.,* 1988). The all male stocking gave higher marketable yields, increased average weights, higher calculated income per unit area and shorter rearing periods. Hulata *et al.* (1988) reported an increase of 18% in net income with all male culture of *M. rosenbergii* .

Siddiqui *et al.* (1997) evaluated the effect of stocking density and monosex culture on growth, survival, yield and feed conversion ratio (FCR) of freshwater prawn reared in concrete tanks. The all male showed better growth than the all female and mixed-sex populations and the FCR of all female populations was poor than the all male and mixed-sex populations.

In Bangladesh, Mazid *et al.* (2004) compared the growth of male and female *M. rosenbergii*. The study lasted for 6 months. The average final body weight of male was 80.8 g while it was 51.9 g in the female. The net live weight gain and specific growth rate of males were also significantly higher than that of the females. The growth rate of the males was 55% higher than in the females.

Pillai *et al.* (2006) reared advanced juveniles (10±2 g) of *M. rosenbergii* as monosex populations at two stocking densities (1 and 2 no./$m^2$) in outdoor cement cisterns for two month. The final mean weight of the all male and all female populations at 1/$m^2$ stocking density was 41.27 g and 35.42 g respectively. On the contrary, the stocking density of 2/$m^2$ led to inferior growth with both populations recording only 36.96 g (all male) and 27.50 g (all female) respectively. However, the all female population showed a higher survival rate than the all male population.

Nair *et al.* (2006) compared the economics of all male, mixed-sex and all female culture and showed that the average weight, productivity and specific growth rate were superior in all male culture. The economic analysis revealed that all male monosex culture of *M.rosenbergii* was 63.12% and 60.20% more profitable than mixed and all female culture respectively.

These studies clearly indicate advantages associated with the all male culture of *M.rosenbergii*.

**2.2.2. Tilapia**

Sexual dimorphism in growth has been reported for several fishes of commercial interest (Hunter and Donaldson, 1983; Malison *et al.*, 1988).

Poor performance of mixed-sex Nile tilapia (*Oreochromis niloticus*) in semi- intensive systems has been a major constraint in the commercial development of this species (Okorie, 1975; Pillay, 1979; Hepher and Pruginin, 1982; Hulata *et al.*, 1983; Teichert-Coddington *et al.*, 1997). Use of monosex male juveniles has been identified as the answer to the problem and has been widely promoted and adopted (Green *et al.*, 1997).

Monosex production strategy is successfully applied to aquaculture as well, in the intensive production of *Tilapia* spp. (Mires, 1977; Taymen and Shelton, 1978; Beardmore *et al.*, 2001). Since the monosex culture is

non-breeding, energy is diverted to growth. Males are used for monosex culture because male tilapia grows faster than females.

Ita and Ekeoyo (1988) evaluated the growth performance of all male, all female and mixed populations of *O. niloticus* cultured in hapas installed in fertilized concrete ponds. The best growth was obtained with all male fed on compounded diet followed by all male fed on palm kernel cake and rice bran respectively.

Little and Edwards (2004) studied the impact of nutrition and season on pond culture and performance of monosex and mixed-sex Nile tilapia (*O. niloticus*). In results, the fish growth and net yield were significantly affected by nutrient level, but not by sex.

Dan and Little (2000) compared the culture performance of monosex and mixed-sex, new season and over-wintered fry in three strains of Nile tilapia (*O. niloticus*). The monosex fish of the three strains grew significantly faster than the mixed-sex fish.

Tuan *et al.* (1998) evaluated the growth performance of sex reversed male tilapia (SRT) versus genetically modified tilapia (GMT) and noted that SRT grew significantly faster than GMT.

### 2.2.3. Mud crab

High mortality due to cannibalism is a common problem in mud crab culture. It may occur due to overcrowding (Baliao *et al.*, 1981) and mixed-sex culture (Cholik and Hanafi, 1992). Poovachiranon (1992) and Jayamane (1992) reported that male mud crabs gain more weight than females.

Studies on the effect of three levels of stocking (0.5, 1.5 or 3.0$m^{-2}$) in the monosex pond culture of the mud crab *Scylla* spp. in the Philippines showed that, the growth rates in the different stocking densities were not significantly different even though the males attained significantly higher weight and specific growth rate than females (Trino *et al.*, 1999). Trino and

Rodriguez (2001) also reported that in mud crab fattening, monosex male crabs grew significantly larger than females.

### 2.2.4. Crayfish

The crayfish (*Cherax albidus*), indigenous to central and eastern Australia which has received considerable aquaculture interest (Smallridge, 1990; Medly *et al.,* 1994; Rouse, 1995; Brown *et al.,* 1997; Lawrence, 1998) also show sexual dimorphism. The male is larger than the female with prominent claws with a distinctive red patch (Curtis and Jones, 1995; Karplus *et al.,* 1995 and Jones and Ruscoe, 2001).

Trials involving monosex culture of redclaw crayfish (*Cherax quadricarinatus*) evidenced improved growth rates in male only populations (1.12 g/ week) over mixed-sex populations (0.52 g/ week) and female only populations (0.27 g / week) (Curtis and Jones, 1995).

Sagi *et al.* (1997) also reported improved growth rates for all male populations (2.93 g/week) over mixed-sex populations (1.31 g/ week) and all female populations (1.06 g / week) in redclaw crayfish.

Lawrence *et al.* (2000) studied the growth rates of crayfish (*C.albidus*) in monosex and mixed-sex populations using three feeding regimes to assess whether preventing reproduction in ponds and improving feed quantity or quality could reduce stunting. Male monosex populations grew on an average 68% faster than female monosex populations and 53% faster than mixed-sex populations. Female yabbies in monosex culture grew 9% slower than mixed-sex (50:50) populations. Both males (17%) and females (31%) in monosex culture grew faster than males and females in mixed-sex culture.

Cortes-Jacinto *et al.* (2004) conducted a study to evaluate the effect of different protein levels on growth, FCR, survival and biomass of redclaw crayfish monosex culture and reported that there was no significant difference between treatments.

Rodgers *et al.* (2006) carried out an experiment to determine if there were advantages in rearing male and female redclaw crayfish at two densities in monosex cultures as opposed to the traditional stocking of mixed-sex populations. At harvest male cray fish were significantly larger than the females.

### 2.2.5. Channel catfish

Males channel catfish (*Ictalurus punctatus*) grow faster than females (Beaver *et al.*, 1966; Brooks *et al.*, 1982; Bondari, 1985) when stocked together in ponds. Sex related growth differences increased with increase in size, with marketable sizes of 450-700 g, males being 16-37% heavier and 5-10% longer than females (Simco *et al.*, 1989). Some catfishes shown differences in sex-related growth at sizes as large as 500 g (Simco *et al.*, 1989), while in some others differential growth may be expressed in fish as small as 3 g (Goudie *et al.*, 1993).

Goudie *et al.* (1994) studied the growth of channel catfish in mixed-sex and monosex culture. In the results, the average weight of channel catfish harvested from monosex male ponds was about 8.5% higher than fish in mixed-sex ponds and 15% higher than fish in monosex female ponds.

Monosex culture could also increase processing efficiency by decreasing the size variation among cultured fish. Exploitation of sex-related growth differences could have a significant impact on economics of catfish culture.

## 2.3. ALL FEMALE CULTURE

Penaeid shrimp species exhibit sexual dimorphism, and as observed in wild populations, females are larger than males. Studies on shrimp culture emphasize the possible advantages of the sexual size dimorphism in improving production. In Pacific white shrimp *L. vannamei* (Boone), sexual size dimorphism begins at about 10 g (Chow and Sandifer, 1991) and becomes a significant around 17 g (Perez-Rostro *et al.*, 1999; Perez-Rostro

and Ibarra, 2003a), which is the usual size at harvest in culture systems. In a study on the brown shrimp *Farfentipenaeus californiensis* (Holmes), Campos-Ramos (1997) showed that after eight month of culture period females attained 18 g versus 14 g recorded by the males. Possibly the larger size of females is influenced by faster female growth during the winter season (16-20 $^{0}$C). Investigations on sexual dimorphism as a possible advantage in shrimp farming include Asian species such as *M. japonicus* (Nakamura *et al.*, 1992; Anon. 1999; Li *et al.*, 2003), *P. monodon* Fabricius (Hansford and Hewitt, 1994; Lumare *et al.*, 1998), the Indian species *F. indicus* (Mohan and Siddek, 1995) and *F. chinensis* (Yin *et al.*, 1986; Li and Xiang, 1997; Li *et al.*, 2003).

Moss *et al.* (2002) also reported potential for all female culture by observing sexual dimorphism in penaeid shrimps.

### 2.3.1. Pacific white shrimp

The Pacific white shrimp, *L.vannamei* is sexually dimorphic for growth, with sub-adult and adult females being typically larger than males of the same age.

Though heritability estimates for growth or size traits have been previously obtained for shrimp species (Carr *et al.*, 1997; Hetzel *et al.*, 2000), no study had addressed the effects of sexual dimorphism on heritability estimates. In *L.vannamei*, the differences between sexes in growth occur at or close to harvest size, or at a total weight of over 10 g (Chow and Sandifer, 1991; Perez-Rostro *et al.*, 1999) and it has been also reported to occur for other shrimp species (Diaz *et al.*, 2001).

A captive population of Pacific white shrimp replicated in different environments evaluated for genetic variability and co-variability of size traits, showed that the females were significantly larger than the males for all traits, except for abdominal (tail), length and weight (Perez-Rostro and Ibarra, 2003b).

Moss[1] and Moss (2006) studied the effects of gender and size on feed acquisition in the Pacific white shrimp, *L. vannamei*. The study was meant to ascertain whether growth differences were a result of more aggressive feeding behavior by females. But the results showed that gender is more important than size as males out-competed females for feed even when they were smaller than the competing females. This suggests that sexual growth dimorphism is not the result of more aggressive feeding by females, probably males have a competitive advantage over females in acquiring feed.

### 2.3.2. Pacific oyster

The manipulation of sex ratio may prove to be a management tool to increase bivalve growth and condition. Baghurst and Mitchell (2002) studied the possibility of gaining commercial benefit from culturing one sex of the Pacific oyster (*Crossostrea gigas*). Comparative data on the growth rate and condition of male and female oyster showed that mean shell growth of female oyster was significantly faster than that of the male. This suggests the potential commercial benefits from increasing the proportion of female oysters in culture.

### 2.3.3. Yellow perch

Malison *et al.* (1988) found that female yellow perch (*Perca flavescens*) grew larger than males where sexes were maintained together or separately, and intraspecific competition for food between the sexes was not a factor contributing to sexually dimorphic growth.

Juell and Lekang (2001) studied the effect of feed supply rate on growth of juvenile perch (*Perca fluviatilis*). The results showed that females grew about 20% faster than males, with final mean weights of 87g and 58 g respectively.

Schmitz (2002) observed the comparative growth of all female versus mixed-sex yellow perch (*P. flavescens*) in recirculated aquaculture

systems. Nine production-scale recirculating aquaculture systems were utilized to compare the growth parameters between all female and mixed-sex yellow perch stocks. All female stocks grew more uniformly than the mixed-sex yellow perch stock.

### 2.3.4. Cat fish

Babas (1999) reported that in *Heterobranchus longifilis*, the females show faster growth than the males. All female diploid and triploid eastern cat fish, *Silurus asotus* (Linnaeus) were produced by crossing phenotypic gynogenetic males with normal females, and the survival and growth performance were examined. Both all female diploid (89-91%) and triploid (91-93%) groups survived significantly better than normal diploid group of mixed-sex (73-76%) ($P<0.001$). Mean body weight of all female was heavier than those of all female diploids and mixed-sex diploids (Nam *et al.*, 2001).

### 2.3.5. Salmon

In salmonids, the ability to produce all female stocks presents a tremendous economic and marketing advantage. Female chinook salmon mature one year later than male. Production of all female or all female triploid salmonids circumvents precocious sexual maturation of males and their associated decreased marketability, and thus benefits from the late sexual maturation and larger size of females (Hunter and Donaldson, 1983; Donaldson, 1986; Donaldson and Benfey, 1987). Devlin (1993) used oestrogen treatment to produce all female populations of chinook salmon. A comparison on the economic aspects of monosex chinook salmon production versus mixed-sex stocks in culture was carried out by Solar and Donaldson (1991) and on Pacific salmon by Seeb (1987).

### 2.3.6. Giant tiger shrimp

The giant tiger shrimp (*P. monodon*), the dominant penaeid in shrimp culture, has the fastest growth rate among all penaeids in aquaculture (Cheng and Chen, 1990).

Researchers at Bribie Island examined the potential for manipulating the sex ratio of shrimps in culture systems. They observed that producing more of faster growing females could substantially increase shrimp farm productivity. Besides, higher proportion of more females in shrimp ponds could scale down the production costs (Hansford, 1991).

Hansford and Hewitt (1994) studied the growth and nutrient digestibility by male and female *P.monodon.* Quadruplicate groups of 10 *P. monodon* were grown in tanks in monosex and mixed-sex populations from 8.6 to 17.0 g (mean wet weight) over a period of 69 days. Measurements of feed intake and the apparent digestibility of dietary protein and energy were made. Monosex female shrimps grew significantly faster than monosex males and male shrimp in mixed-sex tanks. Feed intake of monosex females tended to be greater than that of monosex males but not significantly so. No significant difference between the sexes was detected for either apparent digestibilities of dietary protein and energy or feed conversion.

In the coastal waters of Bagamoyo in Tanzania which constitute an important penaeid shrimp trawling ground, the average size at first maturity was different within sexes in *P.monodon* it was 3.58 cm and 4.3 cm for males and females respectively (Telkwa and Mgaya, 2003).

Otoshi *et al.* (2003) compared the growth and reproductive performance of brood stock shrimp reared in a biosecure recirculated aquaculture system versus a flow-through pond and it was seen that, the females grew larger than the males.

Kenway *et al.* (2006) studied the heritability and genetic correlations of growth and survival in black tiger shrimp, *P. monodon*. The shrimps were

reared in captivity in tanks over three generations with full pedigree information. Weights of animals were recorded at six ages between 7 and 54 weeks along with the corresponding survival in each period. Females were more variable in weight than males after 16 weeks, and variances between each sex were standardized prior to estimation of heritability and genetic correlations

Hansford (1991) suggested that the research on sexual dimorphism in *P.monodon* could be organized in two main directions:

1. Investigate the mechanisms allowing female shrimps to grow faster than males. The basic control of differing growth rates is genetic, but the way this influences the animal (behavioral and/or physiological differences) is unknown. Do females eat more? Do they use food more efficiently?
2. Elucidate the mechanisms of controls of sex determination and sexual differentiation.

## 2.4. PRODUCTION TECHNIQUES OF MONOSEX FISH POPULATION

Females of many fishes used or proposed for aquaculture have the potential to be more valuable than males because, depending upon the species in question, they have faster growth rates as juveniles, are older at sexual maturity, reach a larger ultimate size, and/or are a source of roe or caviar (Benfey *et al.*, 2000). A number of techniques are employed to produce monosex fish populations. These techniques include manually sorting juveniles and removing unwanted sex, manipulating the embryonic chromosome sets (gynogenesis and polyploidy), sex reversal of juveniles with appropriate hormones and using sex reversed animals as brood stock (Hansford, 1991).

Hormonal induction of sex reversal may serve as a valuable tool to understand the process of sex determination and to produce monosex populations for the aquaculture industry (Pandian and Sheela, 1995). Oral

administration of androgens to sexually undifferentiated fry has become the standard commercially adopted technique to produce monosex tilapias (Popma and Lovshin, 1996). Phenotypical feminization is induced successfully by using estradiol-17 β or estrone diethylstilbesterol or ethylestradiols. The treatment with steroids are usually given along with feed. In salmon, where male sex differentiation is initiated before feeding commences, other procedure such as immersion of alevins in treatment solution is employed. Chromosome manipulation also has great possibilities for sex control. Artificial gynogenesis in species having female homogamety produced all female fry. Triploid induction produces sterile fish in rainbow trout and this could be achieved easily by applying hydrostatic pressure or temperature shock. Yamazaki (1983) suggested that fruitful techniques for control of sex in aquaculture will involve the combination of steroid treatment with chromosome manipulation

Recent advances in molecular biology, such as microsatellite markers and sex-specific genetic markers, have facilitated application of these technologies to the aquaculture for the production of monosex fish populations (Benfey *et al.*, 2000).

It is thus became obvious that an efficient biotechnology for producing monosex shrimp or prawn populations is required if the monosex culture strategy is to be economically viable (Sagi and Affalo, 2005).

# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

The collection and rearing of postlarvae of *Penaeus monodon*, their feeding regimes and other experimental details followed for determining the gain in weight and survival are presented sequentially. The experiment was conducted at the College of Fisheries, Panangad, Cochin for a period of 100 days.

## 3.1. Experimental animals

The postlarvae of *P. monodon* were procured from the government shrimp hatchery (ADAK), Varkala and transported to the College in oxygen filled polythene bags. Two thousand postlarvae were introduced into an oval, flat bottom fiber glass tank of 3 ton capacity half filled with filtered sea water of salinity 20 ppt and provided with gentle aeration. The postlarvae were fed *ad libitum* with pelleted commercial feed. Fifty percent of the water in the tank was renewed daily. After 7 days the postlarvae were transferred to an earthen pond.

## 3.2. Experimental rearing facilities

The experiment was conducted under two culture conditions as follows:

### 3.2.1. Earthen pond

A brackish water pond (Plate-1) in the instructional farm of the College was used for rearing the postlarvae for first 50 days to obtain sex differentiated male and female shrimps for the growth experiment.

Pond specifications:

Area - 80 $m^2$

Water depth - 3 ft

The pond was filled with the tide fed water of salinity ranging from 18 to 20 ppt.

Plate 1. Earthen pond

### 3.2.3. Circular cement tanks

Circular, flat bottom cement tanks (Plate 2) with the following specifications were used for the experiments to rear the animals for a further period of 50 days.

Capacity of the tank -250 litres
Diameter – 91 cm
Height – 60 cm
Thickness of wall – 7cm
Colour - Slate black

Clear seawater of 20 ppt filtered through a close meshed nylon bolting silk was used for filling the tanks up to a height of 30 cm. Mild uniform aeration was provided in the tanks with air diffusion stones and control valves. PVC pipes were kept in the tanks for providing shelter to the animals. The tanks were kept outside and covered with net to protect against insect and dust and prevent escaping of shrimps by jumping.

## 3.3. Experimental diet

Starter 2 and Starter 3 of super tiger premium shrimp feed (Godrej Gold Coin Feed) were used in the experiment (Plate 3).

The feed ingredients and proximate composition of feed used as declared by the manufacturer on the packaging cover are as follows:

**Feed ingredients:**

Fish meal, shrimp meal, soya flour, squid liver powder, wheat flour, minerals and vitamins.

Plate 2. Circular cement tank with animals and experimental set up

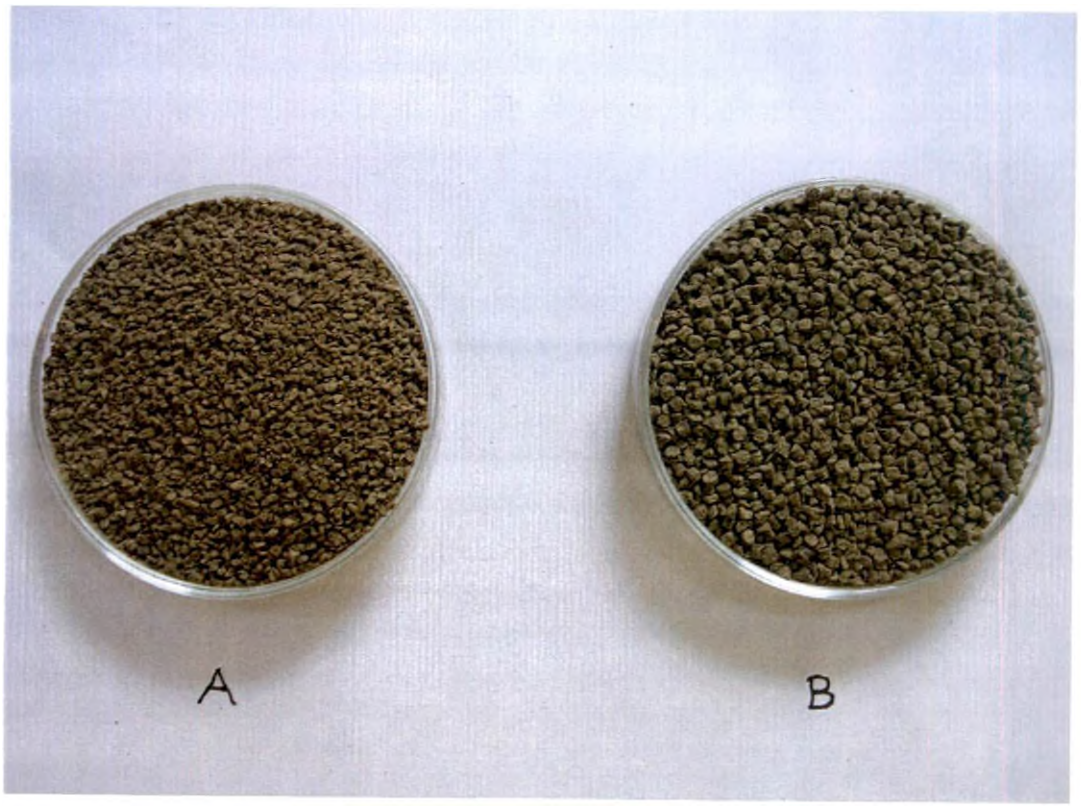



Plate 3. Experimental feed (A: Starter 2 shrimp feed and B: Starter 3 shrimp feed)

**Table 1. Biochemical composition of experimental diets used in the experiment**

| Product | Crude protein (%) | Fat (%) | Crude fibre (%) | Ash (%) | Moisture (%) | Days of use |
|---|---|---|---|---|---|---|
| Starter 2 | 40 | 6 | 3 | 12 | 11 | First 25 days |
| Starter 3 | 40 | 6 | 3 | 12 | 11 | Second 25 days |

## 3.4. Experimental design and procedure

The acclimatized PL of *P. monodon* was transferred to earthen pond. The pond was stocked with 2000 PL (Average weight 0.04 g and average length 1.8 cm). The PL was fed with artificial pelleted feed at the rate of 10% of biomass for 50 days. Optimum water quality parameters were maintained. Sampling was done at weekly intervals to check the minimum size of sex differentiation.

After 50 days of culture in the earthen pond, the shrimps were collected with the help of cast net. Then males and females were separated by manual sorting and 60 males and 60 females of healthy, more or less uniform size were selected. Shrimps having an average weight of 4.46±0.54 g were selected. The selected shrimps were randomly distributed @ 8 numbers/ tank in 15 experimental tanks. Flat bottom circular, 250-L cement tanks were used for rearing the shrimp for a further period of 50 days. The experiment was conducted in a Completely Randomized Design with 3 treatments and 5 replications; the three treatments being all male, all female and mixed-sex (1:1). The 1$^{st}$ treatment was stocked with 8 males in each tank, 2$^{nd}$ treatment with 8 females in each tank and 3$^{rd}$ treatment with 4 males and 4 females.

Before starting of the experiment the shrimps were conditioned to the confined conditions for 2 days. Then eight shrimps from each tank were collected and length and weight was measured. The initial average length and weight of male, female and mixed-sex (1:1 ratio) were 4.774 g, 4.642 g and 3.87g and 4.06 g respectively.

Each treatment group of animals was fed with Starter 2 shrimp feed at the rate of 5% biomass for the first 25 days, twice a day. The leftover feed and faecal matter was collected daily before providing the next day's feeding for 10 days for estimation of FCR. Water quality was maintained by exchanging 50% of the water once in two days.

After 25 days, the sampling was done. In sampling, all the shrimps stocked were taken and length and weight measured.

The quantity of feed was adjusted based on the increased weight at the rate of 4% of biomass. The shrimp was fed with Starter 3 shrimp feed for the next 25 days twice in a day. Water quality was maintained by exchanging the 50% of the water once in two days.

After the second phase of 25 days all the shrimps stocked were taken and the final length and weight measured and the survival ascertained.

## 3.5. Water quality parameters

During the experiment, the water quality parameters such as temperature, pH, Dissolved oxygen, salinity, ammonia and alkalinity were measured at weekly interval by using the following methods.

Temperature: By using mercury thermometer of 0.1°C

pH: By using universal indicator solution

Dissolved oxygen: By using DO meter

Ammonia: By using Ammonia testing kit (BIOSOL)

Alkalinity: By using Alkalinity testing kit (BIOSOL)

## 3.6. Evaluation criteria

The parameters used for evaluation were growth (Average gain in weight), average gain in length, average percentage weight gain, Specific growth rate (SGR), Percentage survival, Food Conversion Ratio (FCR) and Protein efficiency ratio (PER).

### 3.6.1. Average gain in weight

It gives the increase in weight of the animals during the experimental period. It was calculated using the formula.

Average gain in wt. (g) = Average Final wt. (g) – Average Initial wt. (g)

### 3.6.2. Average gain in length

This gives the increase in standard length during the experiment period. It was calculated applying the formula.

Average gain in length (cm) =Average Final length (cm) – Average Initial length (cm)

### 3.6.3. Average percentage weight gain.

Percentage gain in weight of the animal was calculated using following formula.

$$\text{Weight gain (\%)} = \frac{\text{Final Wt. (g)} - \text{Initial Wt. (g)}}{\text{Initial Wt. (g)}} \times 100$$

### 3.6.4. Specific growth rate

In the present study, growth performance was also measured in terms of specific growth rate (SGR) since it is more effective growth index than

absolute weight gain or percentage growth rate (Hepher, 1988). In the present study, SGR was calculated using the following formula.

$$SGR\ (\%) = \frac{\ln (W2) - \ln (W1)}{\text{Time interval in days}} \times 100$$

Where,

W1= Initial weight of animal (g)

W2= Final weight of animal (g)

The calculated value gives the average percentage increase in the body weight per day over a period of 50 days.

### 3.6.5. Survival rate

The survival rate of shrimps is expressed in terms of percentage. This was calculated as follows:

$$\text{Survival}\ (\%) = \frac{\text{Final Number}}{\text{Initial Number}} \times 100$$

### 3.6.6. Food conversion ratio

Food conversion ratio (FCR) is the ability with which an animal can convert the feed consumed into edible and other products (Devendra, 1989). FCR gives an idea about the amount of feed required to produce unit increase in weight of animals. It is the commonly used index to measure the efficiency of a diet used in the experiment. FCR of the experimental diet was calculated using following formula.

$$FCR = \frac{\text{Average weight of food consumed in dry weight}}{\text{Average live weight gain}}$$

### 3.6.7. Protein efficiency ratio

Protein efficiency ratio is defined as the weight gain per unit intake of protein (Paulraj, 1982). It was calculated by employing the formula of Hepher (1988).

$$PER = \frac{\text{Wet weight gain of shrimp (g)}}{\text{Crude protein consumed (g)}}$$

## 3.7. Statistical analysis

The experiment was carried out by using the Completely Randomized Design (CRD). The data pertaining to biological parameters were statistically analyzed. The means of the three treatments T1 (all male), T2 (all female) and T3 (mixed-sex) were compared using One-Way ANOVA. Multiple comparisons of mean values were carried out using Tukey's HSD test. The means of T1 (monosex male) and T3(M) (mixed-sex male) and T2 (monosex female) and T3(F) (mixed-sex female) were compared using two sample Student's t-test (Rangaswami, 2002).

# RESULTS

# 4. RESULTS

The growth and survival of *P. monodon* in three different treatments were evaluated. The details of the observations made during the study period are presented below. The all male, all female, mixed-sex, mixed-sex male and mixed-sex female denoted as T1, T2, T3, T3(M) and T3(F) respectively.

## 4.1. Size at sex determination

The sex differentiation was carried out by observing the presence or absence of the thelycum.

From the day 35, the development of the endopodite on the first pair of pleopods revealed the first external differentiation, showing the presence of a triangular structure in females and a tubular structure in males.

In juvenile shrimp, sex differentiation started after a period of 35-40 days when postlarvae reached 2.5 g of body weight and 7.0 cm of body length.

## 4.2. Biological parameters

### 4.2.1. Gain in weight

The average live weight gain and percentage weight gain of shrimps in different treatments presented in Table 2.

The average live weight gain of *P. monodon* juveniles in the three treatments T1, T2 and T3 was found to be 5.906 g, 8.244 g and 5.780 g respectively. The highest average live weight gain was obtained in treatment T2 i.e., all female and lowest observed in treatment T3 i.e., mixed-sex.

Analysis of variance of the data (Table 3a) showed gain in weight significantly ($P<0.01$) different among the three treatments. Multiple

comparisons as per Tukey's HSD test (Table 3b) revealed that weight gain was highest for T2 (all female) followed by T1 (all male) and T3 (mixed-sex). T2 showed significantly higher weight gain than T1 and T3.There was no significant difference between T1 and T3. Comparison of average gain in weight for T1 (all male) and T3(M) (mixed-sex male) using Student's t-test (Table 3c) revealed no significant difference. But the average gain in weight for T2 (all female) and T3(F) (mixed-sex female) using Student's t-test (Table 3d) revealed that the average gain in weight for T2 (all female) is significantly ($P< 0.01$) higher than T3(F) (mixed-sex female).

The growth observed in different treatments graphically presented in Fig 1a and Fig 1b.

The average percentage weight gain of juveniles from their initial size in treatments T1, T2 and T3 was 125.12%, 178.42% and 146.29% respectively. The highest percentage gain was obtained in treatment T2 with 178.42% and lowest in treatment T1 with 125.12%.

**4.2.2. Gain in length**

The average gain in standard length of shrimps in different treatments presented in Table 4.

Initial and final length of shrimps in the three treatments is shown in plate 4 and 5 respectively. The average gain in length of *P. monodon* juveniles in the three treatments T1, T2 and T3 was found to be 2.896 cm, 3.636 cm and 2.446 cm respectively. The highest average gain in length was obtained in treatment T2 i.e., all female and lowest in treatment T3 i.e., mixed-sex.

Analysis of variance of the data (Table 5a) showed highly significant ($P<0.01$) difference in the average gain in standard length among the three treatments. Multiple comparisons as per Tukey's HSD test (Table 5b) placed

## Table 2. Gain in weight of *Penaeus monodon* in different treatments

| Treatment | Replication | Average initial weight (g) | Average final weight (g) | Gain in weight (g) | Average live weight gain (g) (Mean±SD) | Percentage weight gain | Average percentage weight gain (Mean±SD) |
|---|---|---|---|---|---|---|---|
| T1 | 1 | 4.49 | 10.78 | 6.29 | 5.906 ± 0.567 | 140.09 | 125.12 ± 16.660 |
| | 2 | 5.76 | 11.33 | 5.57 | | 96.70 | |
| | 3 | 3.99 | 9.07 | 5.08 | | 127.32 | |
| | 4 | 4.84 | 11.28 | 6.44 | | 133.06 | |
| | 5 | 4.79 | 10.94 | 6.15 | | 128.39 | |
| T2 | 1 | 4.28 | 12.58 | 8.30 | 8.244 ± 0.566 | 193.93 | 178.42 ± 19.009 |
| | 2 | 4.94 | 13.50 | 8.56 | | 173.28 | |
| | 3 | 4.46 | 12.42 | 7.96 | | 178.48 | |
| | 4 | 4.54 | 13.48 | 8.94 | | 196.92 | |
| | 5 | 4.99 | 12.45 | 7.46 | | 149.50 | |
| T3 | 1 | 3.95 | 9.83 | 5.88 | 5.780 ± 0.479 | 148.86 | 146.29 ± 16.061 |
| | 2 | 4.19 | 9.24 | 5.05 | | 120.53 | |
| | 3 | 3.94 | 9.88 | 5.94 | | 150.76 | |
| | 4 | 3.86 | 10.22 | 6.36 | | 164.77 | |
| | 5 | 3.87 | 9.54 | 5.67 | | 146.51 | |
| T3(M) | 1 | 3.89 | 9.30 | 5.41 | 5.736 ± 0.597 | 139.07 | 148.97 ± 21.027 |
| | 2 | 4.13 | 9.07 | 4.94 | | 119.61 | |
| | 3 | 3.84 | 9.53 | 5.69 | | 148.18 | |
| | 4 | 3.74 | 10.13 | 6.39 | | 170.86 | |
| | 5 | 3.74 | 9.99 | 6.25 | | 167.11 | |
| T3(F) | 1 | 4.01 | 9.84 | 5.83 | 5.800 ± 0.626 | 145.39 | 143.11 ± 17.640 |
| | 2 | 4.25 | 9.41 | 5.16 | | 121.41 | |
| | 3 | 4.05 | 10.24 | 6.19 | | 152.84 | |
| | 4 | 3.99 | 10.60 | 6.61 | | 165.66 | |
| | 5 | 4.00 | 9.21 | 5.21 | | 130.25 | |

## Table 3. Analysis of gain in weight of *Penaeus monodon* in different treatments

**Table 3a. ANOVA Table**

| Source of variation | Sum of squares | Degrees of freedom | Mean sum of squares | F Value | P value |
|---|---|---|---|---|---|
| Treatment | 19.256 | 2 | 9.628 | 33.132** | 0.000 |
| Error | 3.487 | 12 | 0.291 | | |
| Total | 22.743 | 14 | | | |

**Significant at 1 % level

**Table 3b. Tukey's HSD test**

| Treatment | Average gain in weight(g) |
|---|---|
| T1 | $5.906^{a}$ |
| T2 | $8.244^{b}$ |
| T3 | $5.780^{a}$ |

Values with different superscripts are significantly different ($P< 0.05$).

**Table 3c. Student's t-test for comparing growth in weight for T1 and T3(M)**

| | T1 | T3M |
|---|---|---|
| Mean | 5.906 | 5.736 |
| Variance | 0.322 | 0.358 |
| Replication | 5 | 5 |
| df | 8 | |
| t Stat | 0.491 | |
| P(T<=t) two-tail | 0.657 | |

**Table 3d. Student's t-test for comparing growth in weight for T2 and T3(F)**

| | T2 | T3F |
|---|---|---|
| Mean | 8.244 | 5.800 |
| Variance | 0.321 | 0.392 |
| Replication | 5 | 5 |
| df | 8 | |
| t Stat | 6.475 | |
| P(T<=t) two-tail | 0.0002 | |

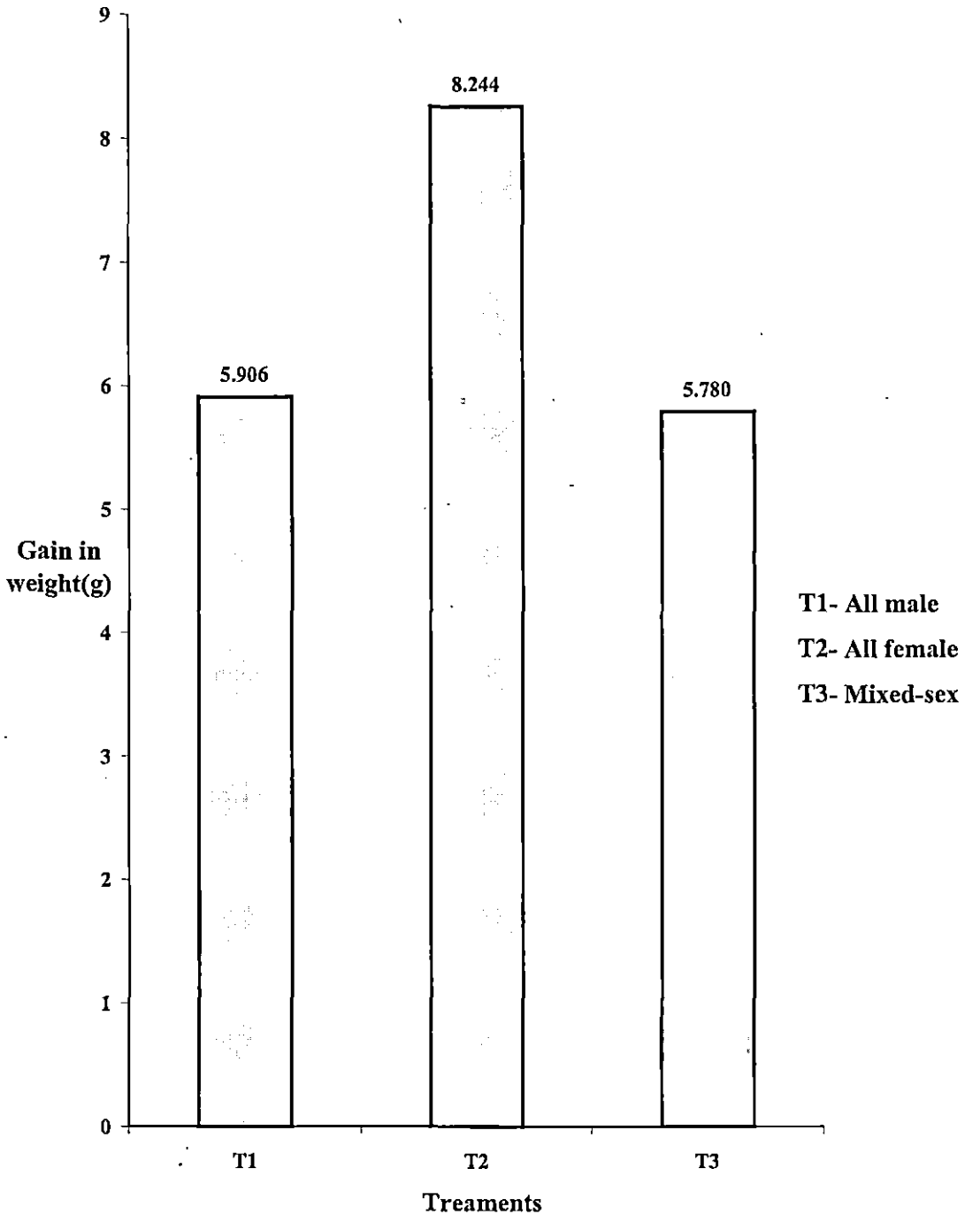



**Fig 1a. Gain in weight of *Penaeus monodon* in three treatments**

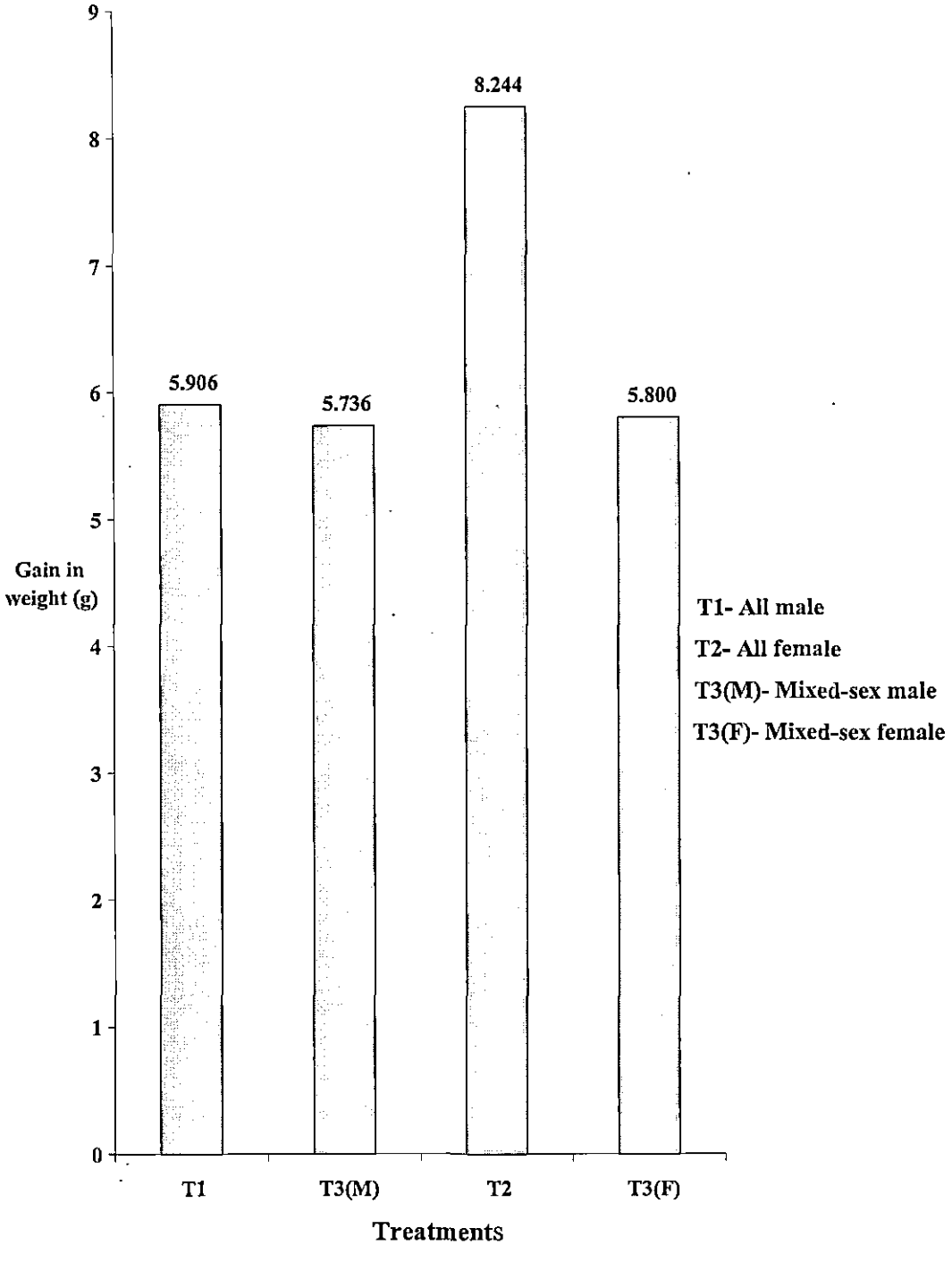



**Fig 1b. Gain in weight of *Penaeus monodon* in different treatments**

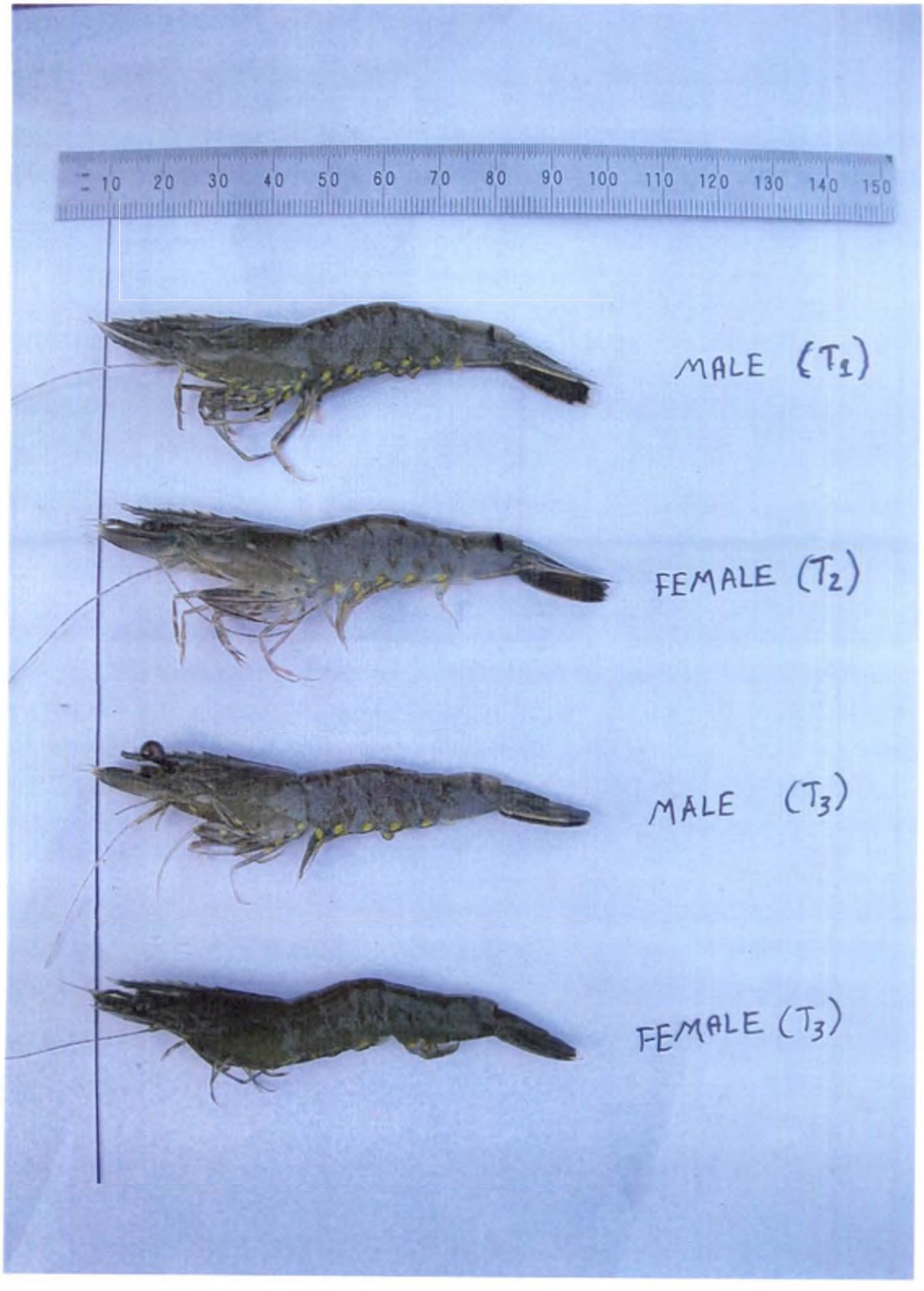



Plate 4. Initial size of the animal at stocking


Plate 5. Final size of the animal at harvest

T1 and T3 in one homogenous group and T3 in the other group. Highest growth in length was observed in T2 (all female).

The growth in standard length observed in different treatments graphically presented in Fig 2.

### 4.2.3. Specific growth rate (SGR)

Specific growth rate of shrimps under various treatments is given in Table 6.

The maximum SGR was recorded in T2 i.e., all female and minimum in T1 i.e., all male. The average SGR values in the three treatments T1, T2 and T3 was found to be 1.618%, 2.044% and 1.799% respectively.

Analysis of the data on specific growth rate using ANOVA (Table 7a) revealed that three treatments are significantly ($P < 0.01$) different. Multiple comparisons as per Tukey's HSD test (Table 7b) showed that the mean SGR of treatment T2 is significantly higher than T1 and T3. There is no significant difference between T1 and T3.

The SGR of the *P.monodon* in different treatments graphically presented in Fig 3.

### 4.2.4. Percentage survival

The percentage survival of *P.monodon* in three different treatments is given in Table 8. Highest average percentage survival of 97.5% was obtained in treatment T2, followed by 95% for both T1 and T3.

The analysis of variance (Table 9a) of the data on percentage survival showed no significant difference among the three treatments.

Graphical presentation of percentage survival rates for different treatments is shown in Fig -4.

## Table 4. Gain in standard length of *Penaeus monodon* in different treatments

| Treatment | Replication | Average initial length (cm) | Average final length (cm) | Gain in length (cm) | Average gain in length (cm) (Mean±SD) | Percentage length gain | Average percentage length gain (Mean±SD) |
|---|---|---|---|---|---|---|---|
| T1 | 1 | 8.73 | 11.51 | 2.78 | 2.896 ± 0.361 | 31.844 | 33.030 ± 4.061 |
| | 2 | 9.26 | 12.17 | 2.91 | | 31.425 | |
| | 3 | 8.49 | 10.84 | 2.35 | | 27.680 | |
| | 4 | 8.76 | 11.98 | 3.22 | | 36.758 | |
| | 5 | 8.60 | 11.82 | 3.22 | | 37.442 | |
| T2 | 1 | 8.59 | 12.14 | 3.55 | 3.636 ± 0.367 | 41.327 | 41.841 ± 4.250 |
| | 2 | 8.86 | 12.91 | 4.05 | | 45.711 | |
| | 3 | 8.61 | 12.19 | 3.58 | | 41.580 | |
| | 4 | 8.59 | 12.49 | 3.90 | | 45.402 | |
| | 5 | 8.81 | 11.91 | 3.10 | | 35.187 | |
| T3 | 1 | 8.39 | 10.74 | 2.35 | 2.446 ± 0.307 | 28.010 | 29.260 ± 3.905 |
| | 2 | 8.44 | 10.44 | 2.00 | | 23.697 | |
| | 3 | 8.38 | 10.94 | 2.56 | | 30.549 | |
| | 4 | 8.25 | 11.09 | 2.84 | | 34.424 | |
| | 5 | 8.36 | 10.84 | 2.48 | | 29.665 | |
| T3(M) | 1 | 8.28 | 10.60 | 2.32 | 2.446 ± 0.335 | 28.019 | 29.412 ± 4.182 |
| | 2 | 8.45 | 10.38 | 1.93 | | 22.840 | |
| | 3 | 8.35 | 10.90 | 2.55 | | 30.539 | |
| | 4 | 8.30 | 11.05 | 2.75 | | 33.133 | |
| | 5 | 8.30 | 11.00 | 2.70 | | 32.530 | |
| T3(F) | 1 | 8.50 | 10.93 | 2.43 | 2.456 ± 0.311 | 28.588 | 29.301 ± 4.058 |
| | 2 | 8.43 | 10.50 | 2.07 | | 24.555 | |
| | 3 | 8.40 | 10.98 | 2.58 | | 30.714 | |
| | 4 | 8.20 | 11.10 | 2.90 | | 35.366 | |
| | 5 | 8.43 | 10.73 | 2.30 | | 27.284 | |

## Table 5. Analysis of gain in standard length of *Penaeus monodon* in different treatments

**Table 5a. ANOVA Table**

| Source of variation | Sum of squares | Degrees of freedom | Mean sum of squares | F Value | P value |
|---|---|---|---|---|---|
| Treatment | 3.610 | 2 | 1.805 | 15.062** | 0.001 |
| Error | 1.438 | 12 | 0.120 | | |
| Total | 5.048 | 14 | | | |

**Significant at 1 %level

**Table 5b. Tukey's HSD test**

| Treatment | Average gain in standard length(cm) |
|---|---|
| T1 | $2.896^{a}$ |
| T2 | $3.636^{b}$ |
| T3 | $2.446^{a}$ |

Values with different superscripts are significantly different ($P< 0.05$).

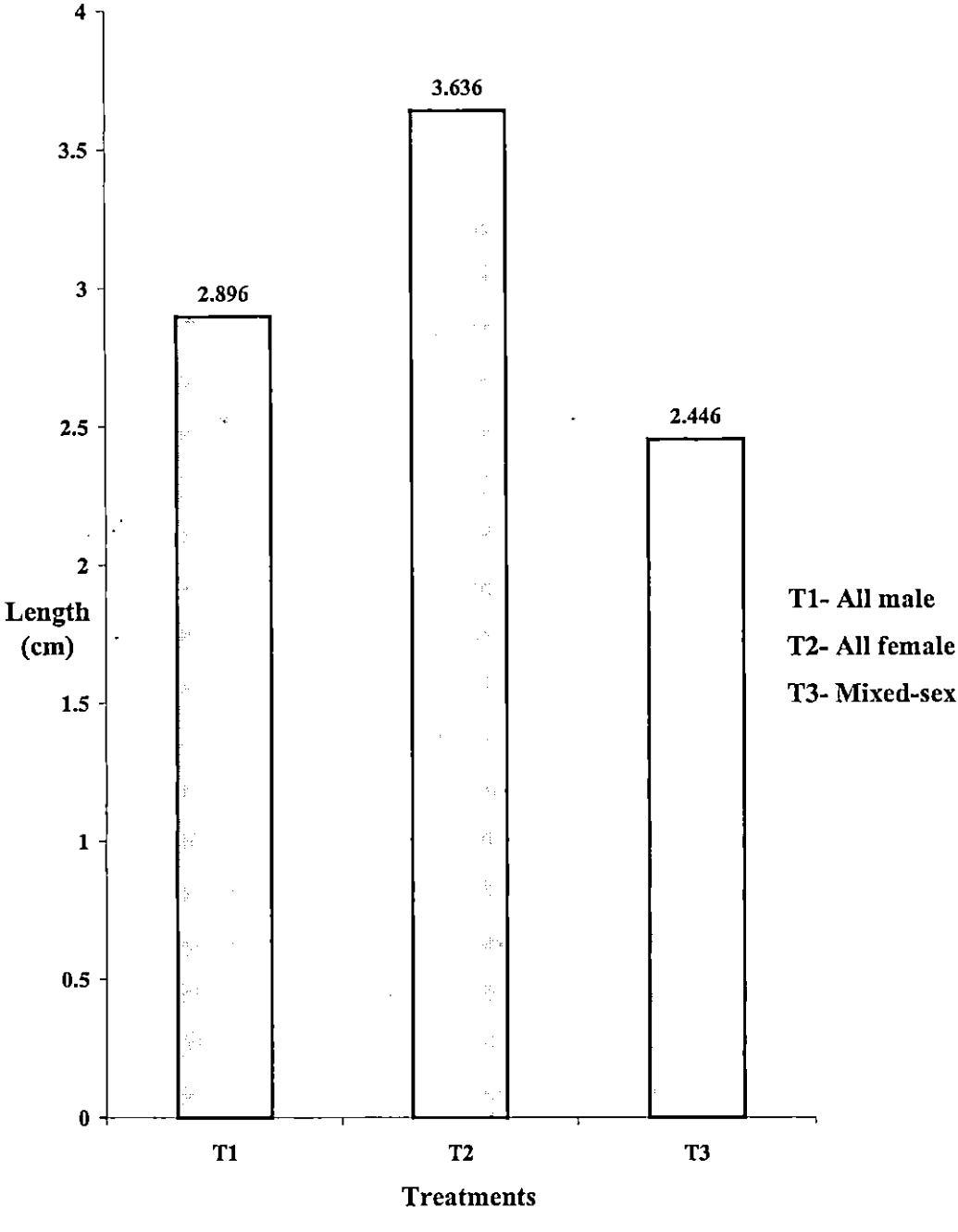



**Fig 2. Gain in standard length of *Penaeus monodon* in different treatments**

**Table 6. Specific growth rate of *Penaeus monodon* in different treatments**

| Treatment | Replication | Average initial weight (g) | Average final weight (g) | Specific growth rate (%) | Average specific growth rate(%) (Mean±SD) |
|---|---|---|---|---|---|
| T1 | 1 | 4.49 | 10.78 | 1.752 | 1.618 ± 0.154 |
| | 2 | 5.76 | 11.33 | 1.353 | |
| | 3 | 3.99 | 9.07 | 1.642 | |
| | 4 | 4.84 | 11.28 | 1.692 | |
| | 5 | 4.79 | 10.94 | 1.652 | |
| T2 | 1 | 4.28 | 12.58 | 2.156 | 2.044 ± 0.139 |
| | 2 | 4.94 | 13.50 | 2.011 | |
| | 3 | 4.46 | 12.42 | 2.048 | |
| | 4 | 4.54 | 13.47 | 2.175 | |
| | 5 | 4.99 | 12.45 | 1.829 | |
| T3 | 1 | 3.95 | 9.83 | 1.823 | 1.799 ± 0.134 |
| | 2 | 4.19 | 9.24 | 1.582 | |
| | 3 | 3.94 | 9.88 | 1.839 | |
| | 4 | 3.86 | 10.22 | 1.947 | |
| | 5 | 3.87 | 9.54 | 1.804 | |
| T3(M) | 1 | 3.89 | 9.30 | 1.743 | 1.818 ± 0.171 |
| | 2 | 4.13 | 9.07 | 1.573 | |
| | 3 | 3.84 | 9.53 | 1.818 | |
| | 4 | 3.74 | 10.13 | 1.993 | |
| | 5 | 3.74 | 9.99 | 1.965 | |
| T3(F) | 1 | 4.01 | 9.84 | 1.795 | 1.772 ± 0.145 |
| | 2 | 4.25 | 9.41 | 1.590 | |
| | 3 | 4.05 | 10.24 | 1.855 | |
| | 4 | 3.99 | 10.60 | 1.954 | |
| | 5 | 4.00 | 9.21 | 1.668 | |

## Table 7. Analysis of SGR of *Penaeus monodon* in different treatments

**Table 7a. ANOVA Table**

| Source of variation | Sum of squares | Degrees of freedom | Mean sum of squares | F value | P value |
|---|---|---|---|---|---|
| Treatment | 0.456 | 2 | 0.228 | 11.237** | 0.002 |
| Error | 0.244 | 12 | 0.020 | | |
| Total | 0.700 | 14 | | | |

** Significant at 1% level

**Table 7b. Tukey's HSD test**

| Treatment | Average specific growth rate (%) |
|---|---|
| T1 | $1.618^{a}$ |
| T2 | $2.044^{b}$ |
| T3 | $1.799^{a}$ |

Values with different superscripts are significantly different ($P< 0.05$).

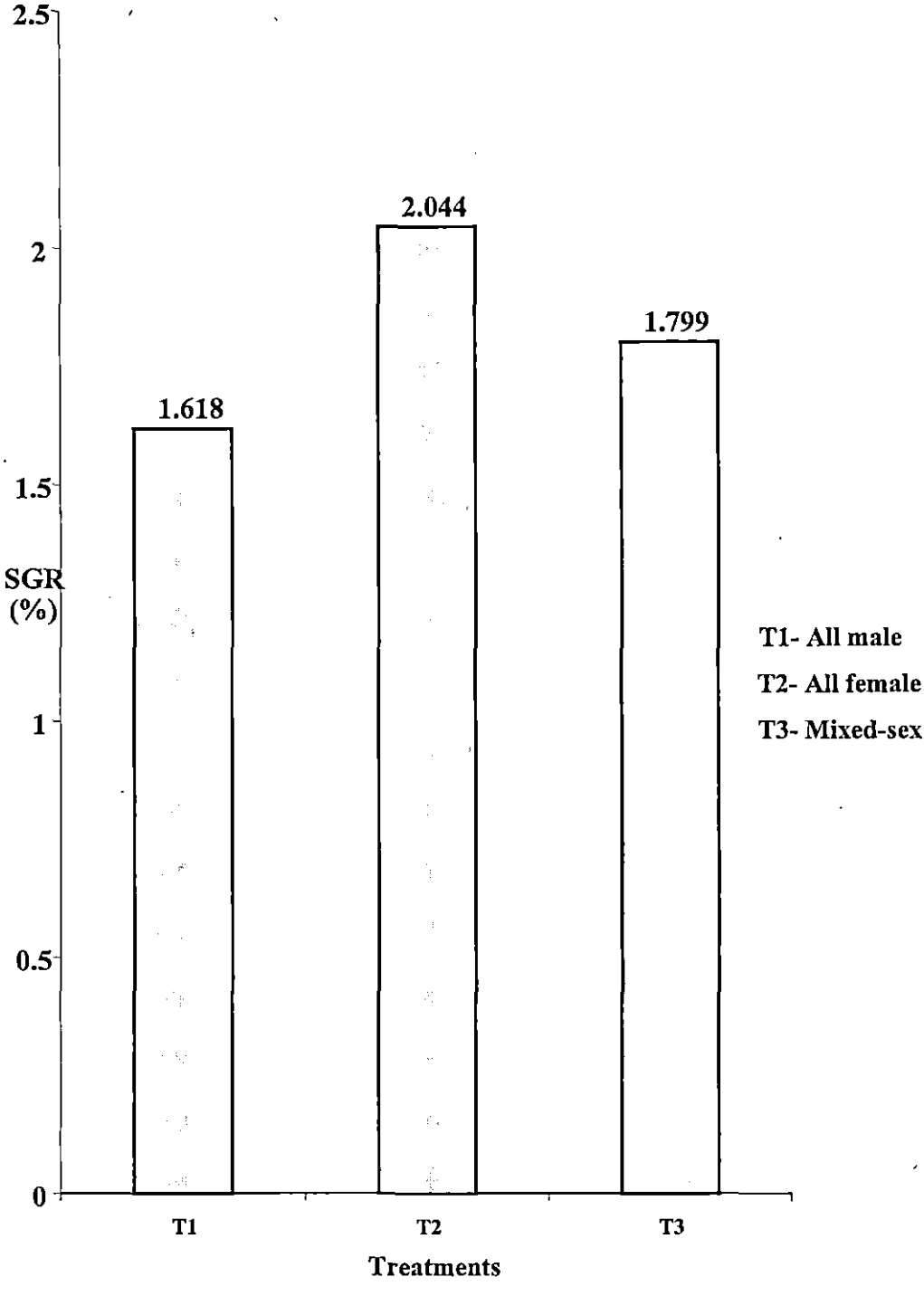



**Fig 3. Specific growth rate (SGR) of *Penaeus monodon* in different treatments**

## Table 8. Percentage survival of *Penaeus monodon* in different treatments

| Treatment | Replication | Initial stocking no. | Final survival | Survival (%) | Average percentage survival (Mean±SD) |
|---|---|---|---|---|---|
| T1 | 1 | 8 | 8 | 100.0 | 95.00 ± 6.85 |
| | 2 | 8 | 8 | 100.0 | |
| | 3 | 8 | 8 | 100.0 | |
| | 4 | 8 | 7 | 87.5 | |
| | 5 | 8 | 7 | 87.5 | |
| T2 | 1 | 8 | 8 | 100.0 | 97.50 ± 5.59 |
| | 2 | 8 | 8 | 100.0 | |
| | 3 | 8 | 8 | 100.0 | |
| | 4 | 8 | 8 | 100.0 | |
| | 5 | 8 | 7 | 87.5 | |
| T3 | 1 | 8 | 7 | 87.5 | 95.00 ± 6.85 |
| | 2 | 8 | 8 | 100.0 | |
| | 3 | 8 | 8 | 100.0 | |
| | 4 | 8 | 8 | 100.0 | |
| | 5 | 8 | 7 | 87.5 | |

## Table 9. Analysis of Percentage survival* of *Penaeus monodon* in different treatments

**Table 9a. ANOVA Table**

| Source of variation | Sum of squares | Degrees of freedom | Mean sum of squares | F Value | P value |
|---|---|---|---|---|---|
| Treatment | 57.132 | 2 | 28.566 | $0.250^{(NS)}$ | 0.783 |
| Error | 1371.168 | 12 | 114.264 | | |
| Total | 1428.300 | 14 | | | |

NS: Statistically not significant ($P > 0.05$)
*Data subjected to Angular transformation.

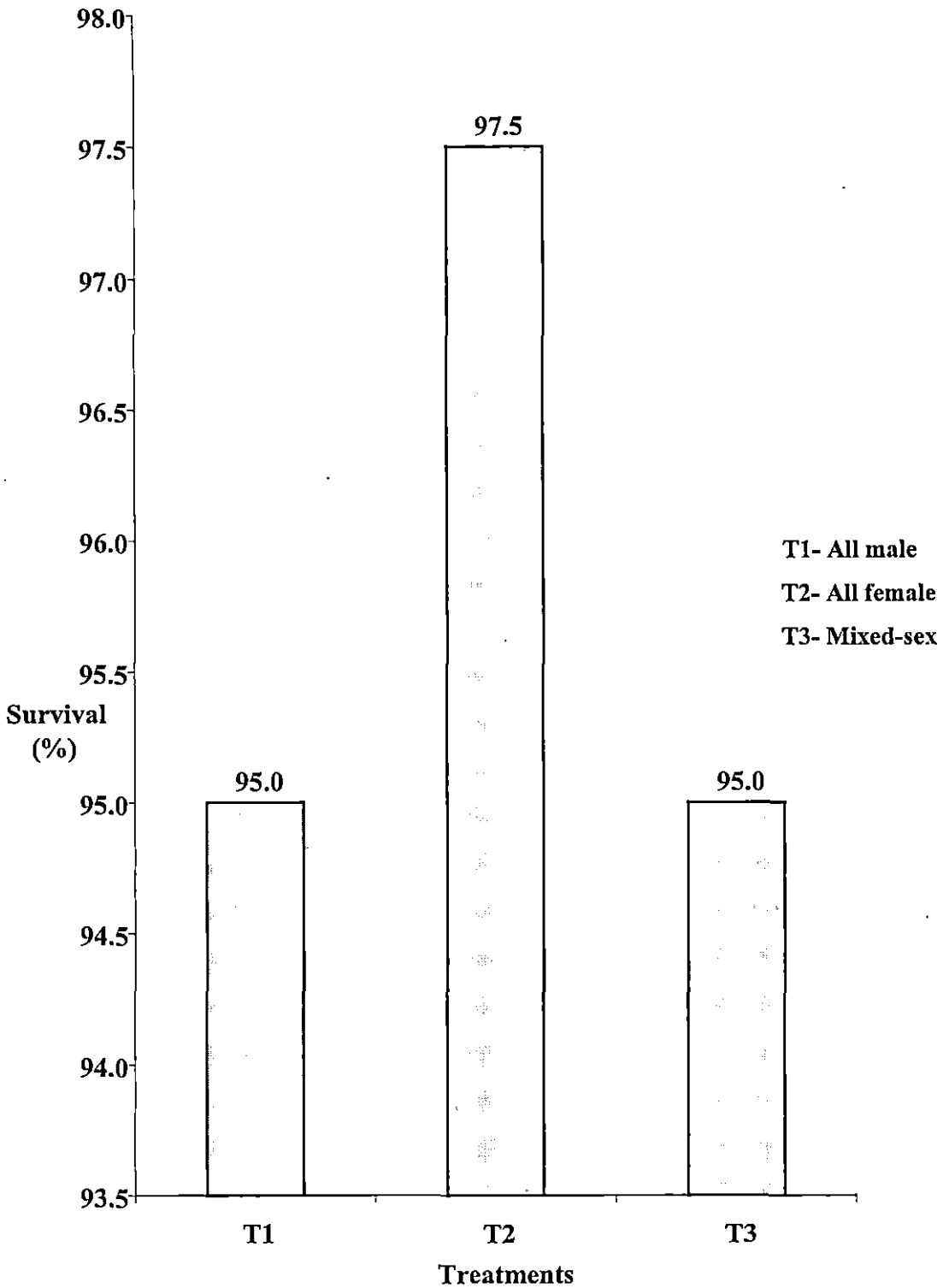



**Fig 4. Percentage survival of *Penaeus monodon* in different treatments**

### 4.2.5. Food conversion ratio

The food conversion ratios (FCR) of *P.monodon* in various treatments given in Table 10.

The mean FCR values in the three treatments T1, T2 and T3 were 2.01, 1.43 and 1.79 respectively. The best FCR was obtained in treatment T2 (1.44) i.e., all female and poorest in T1 (2.01) i.e., all male.

Analysis of variance of FCR values showed highly significant difference ($P<0.01$) among the three treatments (Table 11a). Multiple comparisons using Tukey's HSD test (Table 11b) gave two homogenous groups *viz.*, (T1, T3) and (T2, T3).

Graphical presentation of FCR values in various treatments is given in Fig 5.

### 4.2.6. Protein efficiency ratio

The protein efficiency ratio of the different treatments is given in Table 12.

The average PER values obtained in treatments T1, T2 and T3 were 1.27, 1.75 and 1.41 respectively. The highest PER value was recorded in T2 (1.75) and the lowest in T1 (1.27).

Analysis of variance (Table 13a) of the data on PER values showed highly significant ($P<0.01$) difference among the three treatments. Multiple comparisons as per Tukey's HSD test (Table 13b) placed T1 and T3 in one group and T2 in other group.

The PER values of the different treatments graphically presented in the Fig 6.

## Table 10. Food conversion ratio of *Penaeus monodon* in different treatments

| Treatment | Replication | Average initial weight (g) | Average final weight (g) | Average live weight gain(g) | Average of feed consumed (g) | FCR | Average FCR Mean±SD |
|---|---|---|---|---|---|---|---|
| T1 | 1 | 4.49 | 10.78 | 6.29 | 11.843 | 1.88 | 2.01 ± 0.33 |
| | 2 | 5.76 | 11.33 | 5.57 | 14.399 | 2.59 | |
| | 3 | 3.99 | 9.07 | 5.08 | 10.020 | 1.97 | |
| | 4 | 4.84 | 11.28 | 6.44 | 11.401 | 1.77 | |
| | 5 | 4.79 | 10.94 | 6.15 | 11.359 | 1.85 | |
| T2 | 1 | 4.28 | 12.58 | 8.30 | 11.471 | 1.38 | 1.43 ± 0.11 |
| | 2 | 4.94 | 13.50 | 8.56 | 12.425 | 1.45 | |
| | 3 | 4.46 | 12.42 | 7.96 | 11.899 | 1.49 | |
| | 4 | 4.54 | 13.47 | 8.94 | 11.463 | 1.28 | |
| | 5 | 4.99 | 12.45 | 7.46 | 11.734 | 1.57 | |
| T3 | 1 | 3.95 | 9.83 | 5.88 | 10.540 | 1.79 | 1.79 ± 0.23 |
| | 2 | 4.19 | 9.24 | 5.05 | 10.861 | 2.15 | |
| | 3 | 3.94 | 9.88 | 5.94 | 9.909 | 1.67 | |
| | 4 | 3.86 | 10.22 | 6.36 | 9.673 | 1.52 | |
| | 5 | 3.87 | 9.54 | 5.67 | 10.318 | 1.82 | |

## Table 11. Analysis of FCR of *Penaeus monodon* in different treatments

**Table 11a. ANOVA Table**

| Source of variation | Sum of squares | Degrees of freedom | Mean sum of squares | F Value | P value |
|---|---|---|---|---|---|
| Treatment | 0.850 | 2 | 0.425 | 7.242** | 0.009 |
| Error | 0.704 | 12 | 0.059 | | |
| Total | 1.555 | 14 | | | |

**Significant at 1% level

**Table 11b. Tukey's HSD test**

| Treatment | Average FCR |
|---|---|
| T1 | $2.01^{a}$ |
| T2 | $1.43^{b}$ |
| T3 | $1.79^{a,b}$ |

Values with different superscripts are significantly different ($P< 0.05$).

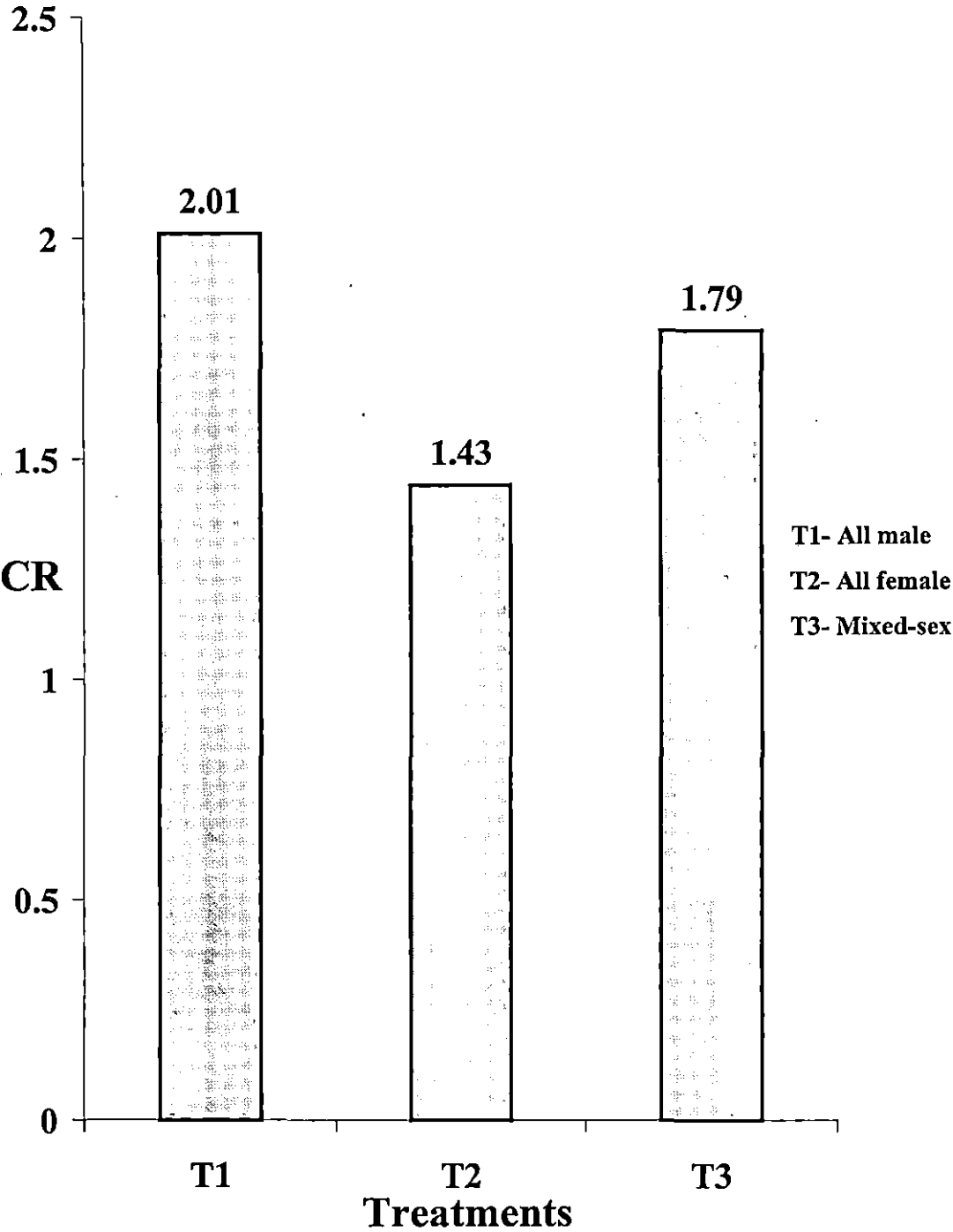



**Fig 5. FCR of *Penaeus monodon* in different treatments**

## Table 12. Protein Efficiency Ratio (PER) of *Penaeus monodon* in different treatments

| Treatment | Replication | Average initial weight (g) | Average final weight (g) | Average live weight gain (g) | Average of protein consumed (g) | PER | Average PER (Mean±SD) |
|---|---|---|---|---|---|---|---|
| T1 | 1 | 4.49 | 10.78 | 6.29 | 4.74 | 1.33 | 1.27 ± 0.17 |
| | 2 | 5.76 | 11.33 | 5.57 | 5.76 | 0.97 | |
| | 3 | 3.99 | 9.07 | 5.08 | 4.01 | 1.27 | |
| | 4 | 4.84 | 11.28 | 6.44 | 4.56 | 1.41 | |
| | 5 | 4.79 | 10.94 | 6.15 | 4.54 | 1.35 | |
| T2 | 1 | 4.28 | 12.58 | 8.30 | 4.59 | 1.81 | 1.75 ± 1.14 |
| | 2 | 4.94 | 13.50 | 8.56 | 4.97 | 1.72 | |
| | 3 | 4.46 | 12.42 | 7.96 | 4.76 | 1.67 | |
| | 4 | 4.54 | 13.47 | 8.93 | 4.59 | 1.95 | |
| | 5 | 4.99 | 12.45 | 7.46 | 4.69 | 1.59 | |
| T3 | 1 | 3.95 | 9.83 | 5.88 | 4.22 | 1.39 | 1.41 ± 0.18 |
| | 2 | 4.19 | 9.24 | 5.05 | 4.34 | 1.16 | |
| | 3 | 3.94 | 9.88 | 5.94 | 3.96 | 1.50 | |
| | 4 | 3.86 | 10.22 | 6.36 | 3.87 | 1.64 | |
| | 5 | 3.87 | 9.54 | 5.67 | 4.13 | 1.37 | |

## Table 13. Analysis of PER of *Penaeus monodon* in different treatments

**Table13a. ANOVA Table**

| Source of variation | Sum of squares | Degrees of freedom | Mean sum of squares | F value | P value |
|---|---|---|---|---|---|
| Treatment | 0.609 | 2 | 0.305 | 11.323** | 0.002 |
| Error | 0.323 | 12 | 0.027 | | |
| Total | 0.932 | 14 | | | |

**Significant at 1% level

**Table 13b.Tukey's HSD test**

| Treatment | Average PER |
|---|---|
| T1 | $1.27^{a}$ |
| T2 | $1.75^{b}$ |
| T3 | $1.41^{a}$ |

Values with different superscripts are significantly different ($P< 0.05$).

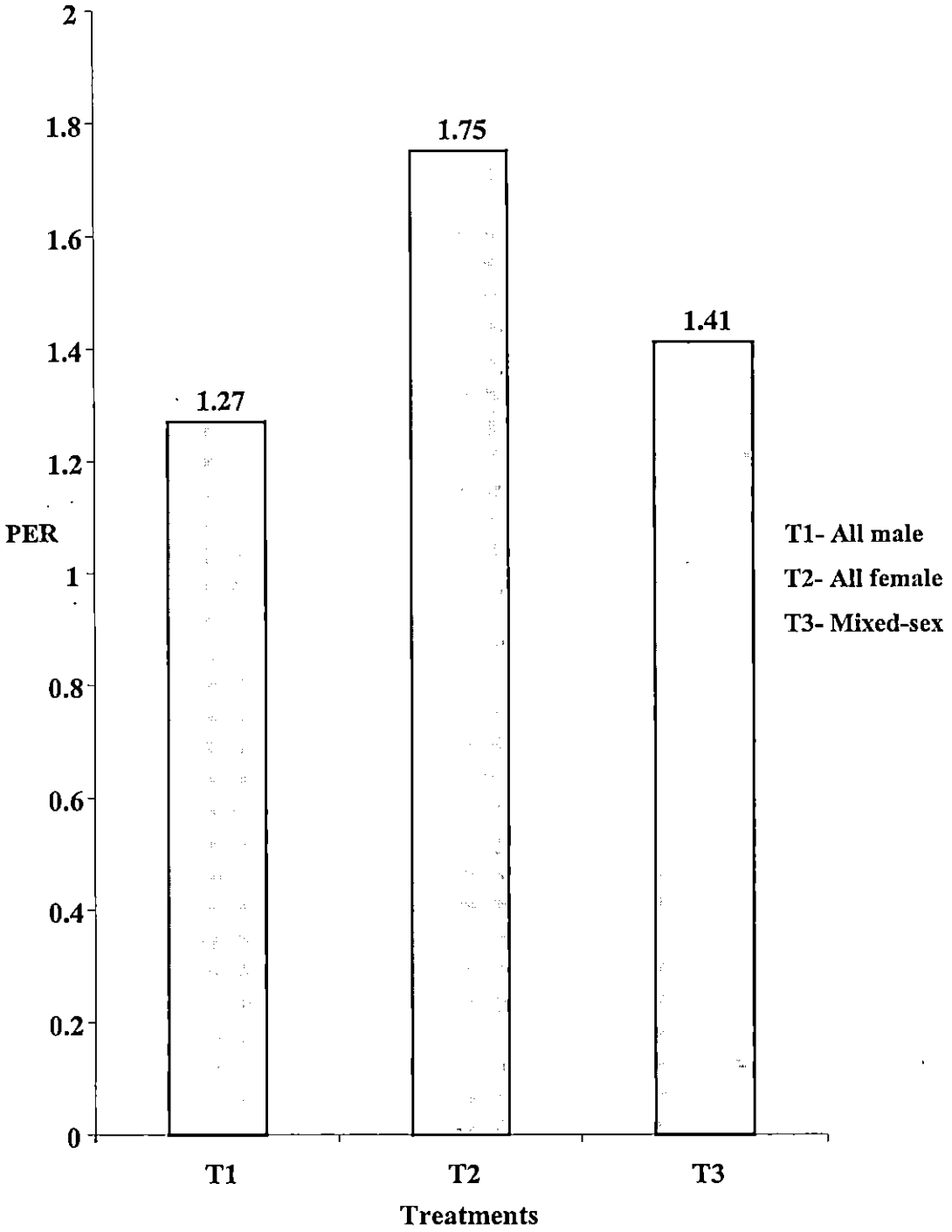



**Fig 6. Protein efficiency ratio of *Penaeus monodon* in different treatments**

## 4.3. Water quality parameters

### 4.3.1. Water temperature

The summary statistics of water temperature in the experimental tanks during the study period is given in Table 14. Minimum temperature recorded was 27.8°C and maximum temperature was 32.1°C. Weekly mean temperature values ranged from 28.4 to 30.6°C.

### 4.3.2. pH

The summary statistics of pH in the experimental tanks is given in the Table 15. Minimum and maximum pH values observed during the study period were 7.0 and 8.0 respectively. Weekly mean pH values ranged from 7.6 to 7.9.

### 4.3.3. Dissolved oxygen

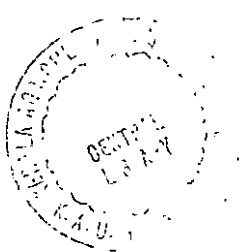
The summary statistics of dissolved oxygen (D.O) in the experimental tanks is given in Table 16. A minimum of 5.7 ppm and a maximum DO content of 7.8 ppm were obtained during the study period. Weekly mean values ranged from 6.8 to 7.4 ppm.

### 4.3.4. Salinity

The summary statistics of salinity in the experimental tanks is given in Table 17.A minimum of 16.0 ppt and a maximum of 20.0 ppt were obtained during the study period. Weekly mean values ranged from 16.0 to 20.0 ppt

### 4.3.5. Total alkalinity

The summary statistics of total alkalinity in the experimental tanks is given in Table 18. A minimum of 80 ppm and a maximum of 110 ppm were obtained during the study period. Weekly mean values ranged from 82 to 104 ppm.



### 4.3.6. Ammonia

The summary statistics of ammonia in the experimental tanks is given in Table 19. A minimum of 0.00 ppm and a maximum of 0.01 ppm were obtained during the study period. Weekly mean values ranged from 0.00 to 0.07 ppm.

**Table 14. Water temperature (C°) in the experimental tanks during the study period**

| Temp. | Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Mean ± SD | 28.6 ± 0.00 | 30.3 ± 0.15 | 30.4 ± 0.17 | 30.6 ± 0.48 | 28.4 ± 0.42 | 28.4 ± 0.41 | 28.4 ± 0.53 |
| Range | 28.6-28.6 | 30.1-30.6 | 30.1-30.6 | 30.1-32.1 | 27.8-28.9 | 27.8-29.1 | 27.8-29.7 |

**Table 15. Water pH in the experimental tanks during the study period**

| pH | Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Mean ± SD | 7.7 ± 0.31 | 7.7 ± 0.32 | 7.8 ± 0.32 | 7.8 ± 0.26 | 7.8 ± 0.32 | 7.6 ± 0.30 | 7.9 ± 0.23 |
| Range | 7.0-8.0 | 7.0-8.0 | 7.0-8.0 | 7.5-8.0 | 7.0-8.0 | 7.0-8.0 | 7.5-8.0 |

**Table 16. Dissolved oxygen (ppm) in the experimental tanks during the study period**

| Dissolved oxygen | Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Mean ± SD | 7.3 ± 0.30 | 7.2 ± 0.30 | 7.4 ± 0.31 | 6.8 ± 0.70 | 7.3 ± 0.33 | 7.0 ± 0.70 | 7.0 ± 0.70 |
| Range | 6.8-7.7 | 6.7-7.7 | 6.7-7.8 | 5.7-7.8 | 6.7-7.8 | 5.9-7.8 | 5.7-7.8 |

**Table 17. Salinity (ppt) in the experimental tanks during the study period**

| Salinity | Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Mean ± SD | 20.0 ± 0.00 | 18.0 ± 0.00 | 18.0 ± 0.00 | 18.0 ± 0.00 | 16.0 ± 0.00 | 16.0 ± 0.00 | 18.0 ± 0.00 |
| Range | 20.0-20.0 | 18.0-18.0 | 18.0-18.0 | 18.0-18.0 | 16.0-16.0 | 16.0-16.0 | 18.0-18.0 |

**Table 18. Alkalinity (ppm) in the experimental tanks during the study period**

| Alkalinity | Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Mean ± SD | 92.67 ± 11.00 | 98.00 ± 8.62 | 102.67 ± 7.04 | 82.00 ± 3.69 | 104.0 ± 7.37 | 88.67 ± 2.29 | 92.00 ± 4.14 |
| Range | 80.0-100.0 | 90.0-110.0 | 90.0-110.0 | 80.0-90.0 | 90.0-110.0 | 85.0-90.0 | 90.0-100.0 |

**Table 19. Ammonia (ppm) in the experimental tanks during the study period**

| Ammonia | Weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Mean ± SD | 0.00 ± 0.00 | 0.05 ± 0.06 | 0.00 ± 0.00 | 0.06 ± 0.06 | 0.07 ± 0.05 | 0.06 ± 0.17 | 0.05 ± 0.06 |
| Range | 0.0-0.0 | 0.0-0.1 | 0.0-0.0 | 0.0-0.1 | 0.0-0.1 | 0.0-0.1 | 0.0-0.1 |

# DISCUSSION

# 5. DISCUSSION

Monosex culture, which puts to advantage the size difference in sexual dimorphism, is a regular practice in commercial aquaculture (Mires, 1977; Tayman and Shelton, 1978; Curtis and Jones, 1995; Sagi *et al.*, 1997).

In *M. rosenbergii*, the males grows faster than the females (Sandifer and Smith, 1985; Sagi *et al.*, 1997; Pillai *et al.*, 2006) and its all male culture is found to be 63.12% and 60.20% more profitable than mixed and all female culture respectively (Nair *et al.*, 2006). In mud crab too Cholik and Hanafi (1992) and Trino *et al.* (1999) reported that culturing male mud crabs is more economical than mixed-sex culture or monosex culture of females. In cray fish (*C. albidus*), all male populations grows 68% and 53% faster than all female and mixed-sex populations respectively (Curtis and Jones, 1995; Lawrence *et al.*, 2000; Cortes-Jacinto *et al.*, 2004; Rodgers *et al.*, 2006).

In channel catfish, the males grow faster than females and this growth differences in two sexes is usually taken advantage in commercial farming of channel catfish (Simco *et al.*, 1989; Goudie *et al.*, 1993).

Contrary to the faster growth of males in case of *M .rosenbergii*, mud crab, cray fish and channel catfish, in penaeids, it is the females which grows faster than the males.

In tilapia, monosex culture is routinely adopted to prevent the breeding and divert energy for growth (Mires, 1977; Taymen and Shelton, 1978; Little and Edward, 2004). In mixed-sex culture, males *O. niloticus* typically grows faster than females (Dixon, 1994; Tave, 1995). However, Schreiber *et al.* (1998) found that under individual rearing conditions, females *O. niloticus* grew faster than males.

In a growth study on *Litopanaeus vannamei* conducted by Moss[1] and Moss (2006), it was seen that the females grows faster than the males. Campos-Ramos *et al.* (2006) reported that the sex differentiation in *L. vannamei* starts at size above 10g.

In the present study, the females grew faster than the males both in length and weight and the sex differentiation started at 2.5 g size i.e., a size much less than the one reported by Campos-Ramos *et al.* (2006) for *L. vannamei.* In *L. vannamei,* sex differentiation is evident only at 50-90 day of rearing, whereas in the present study on *P. monodon* the sex differentiation took place much earlier after 35 days of rearing.

In *M. rosenbergii,* the sexual differentiation is done looking into the gape between the walking legs of the juvenile at an early stage even much before the sexual appendages become functional. In *P. monodon,* it may be possible to identify the sexes at early stage much less than the 2.5 g obtained in the present study by looking the gape between the walking legs at the region where the thelycum develops later.

Workers in Taiwan have reported no evidence of sexually dimorphic growth in pond grown *P.monodon* below a mean size of 28 g wet weight (Liao, 1977; Cheng and Chen, 1990) while, for an Australian shrimp farm, Hansford (1991) demonstrated a significant difference in the size of male and female shrimps at a mean size around 13 g wet weight. Hansford and Hewitt (1994) reported that for *P.monodon*, where females grew 16% faster than those in mixed-sex culture females.

In the present study, 2.3% difference in growth is seen between the all male culture and mixed-sex culture and all female culture 43% faster than the mixed-sex culture over a period of 100 days. It is also seen that the growth of the females in the monosex culture was significantly higher than the females grown along with the males in mixed-sex culture (42%).

It is interesting to see that given the same conditions when female and male were grown together in mixed-sex culture the females grows 1.1% faster than the males. But when the females grown separately in monosex culture it showed 40% faster growth than the males monosex culture.

The faster growth of females in monosex culture may be because of the more efficient utilization of feed by the females. Similar observation have also been reported for the other crustaceans that are sexually dimorphic in growth, including the *Idota balthica* (Strong and Daborn, 1979) and the fairy shrimp, *Branchinecta gigas* (Daborn, 1973). The more efficient use of food by female shrimps would also make monosex culture highly attractive, as feed costs account for upto 40% of the overall production costs (Hardman *et al.*, 1991). The length increment was highest in the all female populations and lowest in the mixed-sex culture.

The specific growth rate (SGR) of shrimps was significantly different between various groups *viz.*, all male, all female and mixed-sex populations. The maximum SGR was recorded for all female populations followed by all male and mixed-sex populations.

Survival rate in the present work ranged 95% to 97.5%. There were no significant differences in survival among treatments, allowing yields to be affected more by animal size than number.

Further more, FCR was lower for females than for males, mainly because females grew faster. Feed conversions in the present study were lower than those reported by Hansford and Hewitt (1994) where FCR of males tended to be lower than for females. PER values in different groups was significantly different. The highest PER value was recorded for all female populations followed by mixed-sex and all male populations.

Shrimps used in this study were (4.46±0.54 g) which is smaller than the size of shrimps taken by the Hansford and Hewitt (1994).

The temperature range observed during the present study was 27.8°C to 32.1°C which was within the optimum range (24-35°C) suggested by Chakraborti *et al.* (1986) for the optimum growth of *P.monodon.*

The pH range observed during the present study was 7 to 8 which was within the optimum range (7.3-8.8) reported to be suitable for the *P.monodon* culture (Parado-Estepa *et al.*, 1990).

In the present study, Dissolved oxygen (DO) was ranged between 5.70 to 7.80 ppm which was within the limit reported by Chakraborti *et al.* (1986) for *P.monodon* culture though they can tolerate DO as low as 4.8 ppm.

The salinity in the present study was ranged between 16 to 20 ppt which was within the optimum range (12-24 ppt) suggested by Motoh (1981) for optimum growth for *P.monodon.*

In the present study, total alkalinity recorded ranged from 80 to 110 ppm which was within the range (40-150 ppm) suggested by Chakraborti *et al.* (1986) for *P.monodon* culture.

In the present study, the ammonia ranged between 0.0 to 0.1 ppm. The growth of *P.monodon* was found impaired at 0.01 to 0.5 ppm (Smith *et al.*, 2002). The values of ammonia in present study were within the limits.

Results of this study provide further support for all female culture of *P.monodon.* In addition, results indicate that females may have physiological advantage with respect to growth (e.g., higher SGR and PER and lower FCR), which could also confer positive benefits during growout. Thus, the present study gives clear indication that farming of monosex females could be much more economical than the present practice of mixed-sex culture. However, further studies are warranted before all female culture can be adopted as a routine practice in commercial shrimp farming.

# SUMMARY

# 6. SUMMARY

1. The black tiger shrimp (*P. monodon*), a dominant penaeid in shrimp culture, has the fastest growth rate among all penaeids in aquaculture

2. The presence of different male and female body forms is referred as sexual dimorphism. Penaeid shrimp species exhibit sexual dimorphism as observed in wild populations. In *P. monodon* the females achieve larger size than the males. This study aimed to investigate the potential of *P.monodon* monosex culture for increasing the yield and/ or decreasing the growout period.

3. The present research was performed at the College of Fisheries, Cochin, Kerala. The postlarvae used in the present study were procured from government shrimp hatchery, ADAK, Varkala, Kerala. Two thousand postlarvae was stocked in the earthern pond (80 $m^2$) and reared for 50 days before initiation of the experiment. The PL was fed with Starter 2 shrimp feed twice daily at the rate of 10% of biomass.

4. In juvenile shrimp sex differentiation took place from 35 days when PL reached 2.50 g in body weight and 7.00 cm in body length respectively.

5. An experiment was carried out in outdoor circular cement tanks at the College of Fisheries, Cochin and maintained for 50 days. The shrimps (4.46±0.54 g) were hand sexed to segregate in to male and female.

6. Animals were stocked in the three treatments: all male, all female and mixed-sex (1:1 ratio). The shrimps were introduced into the 250-L circular cement tanks @ 8 nos./ tank. The experiment was conducted using Completely Randomized Design with 5 replicates

for each treatment. Starter 2 shrimp feed was used as diet and fed twice a day at the rate of 5% of biomass. After 25 days of rearing, the shrimps were sampled to monitor their growth.

7. The shrimps was fed using Starter 3 shrimp feed and fed twice daily at the rate of 4% of biomass. The creek water of salinity 18±2 ppt was used for experiment. Continuous aeration was provided to the tanks. Fifty percent of water was exchanged once in 2 days throughout the experiment. PVC pipes were provided in each tank as shelter for shrimps.

8. Physico-chemical parameters in the tanks were maintained throughout the experiment. Feed remanants and excreta were removed daily before the next feeding.

9. Upon completion of the experiment, performance under each treatment was analyzed with regards to average gain in weight, average gain in standard length, specific growth rate (SGR), percentage survival, FCR and PER.

10. Final mean gain in weight and length, SGR, FCR and PER was significantly different between the treatments. The gain in weight and length, SGR and PER of all female treatment was significantly higher than the all male and mixed-sex treatment. There was no significant difference in the survival among treatments. The FCR in all female treatment was significantly lower than FCR in all male and mixed-sex treatments.

11. Results of the present study demonstrate a significant advantage in all female culture of *P.monodon* over the both all male as well as mixed-sex culture.

12. Advantages of all female culture of *P.monodon* are many. Apart from rapid growth, higher yield and better FCR observed in the present study, a larger proportion of harvested animals are of bigger size and therefore of higher value in female only populations as compared to male only or mixed-sex populations. Thus culture of all female may be commercially more viable and attractive to entrepreneurs.

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# GROWTH AND SURVIVAL OF *PENAEUS MONODON* IN MONOSEX AND MIXED-SEX CULTURE UNDER LABORATORY CONDITIONS

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## ABSTRACT OF THESIS

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# ABSTRACT

Sexual dimorphism is apparent in the black tiger shrimp, *Penaeus monodon* with females achieve larger size than the males. This character may be attributed to behavioral and/or physiological differences between the sexes. An experiment was developed to determine if there were advantages in rearing all female or all male *P. monodon* as opposed to mixed-sex populations. Juvenile shrimps (4.46±0.54 g) were collected from earthen pond and individually hand sexed and stocked in the circular cement tanks. Treatments all male, all female and mixed-sex were stocked @ 8 nos./tank. Each treatment had five replicates. The shrimps were offered commercial shrimp feed. The experiment was conducted for a period of 50 days. At harvest, all female shrimps had shown significantly higher growth than all male and mixed-sex treatment. Survival was not significantly different among treatments. FCR of all female was significantly lower than the all male and mixed-sex treatment. Result of the present study demonstrates a benefit to all female culture of *P.monodon* against the all male or mixed-sex culture. Thus culture of all female may be commercially more attractive to entrepreneurs. Although additional research is required to find a reliable and quick procedure for separation of the sexes or techniques for the production of all female populations.