STUDIES ON GROWTH AND SURVIVAL IN NURSERY REARING PHASE OF GREY MULLET (*Mugil cephalus*) FOR MARICULTURE”

BY

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON GROWTH AND SURVIVAL IN NURSERY REARING PHASE OF GREY MULLET (*Mugil cephalus*) FOR MARICULTURE"** Submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF FISHERY SCIENCE** in **AQUACULTURE** in the **Faculty of Fishery Science** of Sri Venkateswara Veterinary University, Tirupati is a record of the bona fide research work carried out by **Mr. SANTOSH KUMAR TADANGI,** ID. No. MFM/2014-007 under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for the award of any other degree or diploma. All assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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DECLARATION

I **Mr. SANTOSH KUMAR TADANGI,** hereby declare that the thesis entitled **"STUDIES ON GROWTH AND SURVIVAL IN NURSERY REARING PHASE OF GREY MULLET (*Mugil cephalus*) FOR MARICULTURE"** submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of **MASTER OF FISHERY SCIENCE** majoring in **AQUACULTURE** is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.

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CONTENTS

Chapter NO.	*TITLE*	*Page No.*
1	*INTRODUCTION*	*1-13*
2	*REVIEW OF LITERATURE*	*14-27*
3	*MATERIALS AND METHODS*	*28-41*
4	*RESULTS*	*42-74*
5	*DISCUSSION*	*75-92*
6	*SUMMARY*	*93-95*
7	*REFERENCES*	*96-140*

LIST OF TABLES

S.No.	*TITLE*	*Page No.*
1	*Proximate composition of the feed ingredients (g/100g dry diet)*	*32*
2	*Feed formulation of the diets for studies on optimum CP% for grey mullet fry: (Ingredients g/100g):*	*33*
3	*Proximate composition of experimental diets (g/100g dry diet) for studies on optimum CP% for grey mullet fry:*	*34*
4	*Water quality parameters in floating net cages with grey mullet (*Mugil cephalus*)) at different stocking densities:*	*51*
5	*Water quality parameters in floating net cages with grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels.*	*51*
6	*Growth performance (g) of grey mullet (*Mugil cephalus*) at different stocking densities.*	*52*
7	*Statistical analysis for Growth performance of grey mullet (*Mugil cephalus*) at different stocking densities.*	*52-53*
8	*Survival rate of grey mullet (*Mugil cephalus*) at different stocking densities.*	*53*
11	*Statistical analysis for survival rate of grey mullet (*Mugil cephalus*) at different stocking densities.*	*54*
12	*Feed Conversion Ratio in grey mullet (*Mugil cephalus*) fed on experimental diet at different stocking densities.*	*55*
13	*Statistical analysis for Feed Conversion Ratio in grey mullet (*Mugil cephalus*) fed on experimental diet at different stocking densities.*	*55*
14	*Specific Growth Rate of grey mullet (*Mugil cephalus*) at different stocking densities.*	*56*
15	*Average Daily Weight Gain of grey mullet (*Mugil cephalus*) at different stocking densities*	*57*
16	*Biomass (g) of grey mullet (*Mugil cephalus*) at different stocking densities.*	*57*
17	*Growth performance (g) of grey mullet (*Mugil cephalus*) fed with different % of crude protein (CP) experimental diets.*	*57*
18	*Statistical analysis for growth performance (g) of grey mullet (*Mugil cephalus*) fed with different % of crude protein (CP) experimental diets.*	*58*
19	*Survival rate of grey mullet (*Mugil cephalus*) fed with different % of crude protein (CP) experimental diets.*	*59*
20	*Statistical analysis for survival rate of (*Mugil cephalus*) fed with different % of crude protein (CP) experimental diets.*	*59*
21	*Feed Conversion Ratio of grey mullet (*Mugil cephals*) fed with different % of crude protein (CP) experimental*	*60*

	diets.	
22	*Statistical analysis for feed conversion ratio of grey mullet (*Mugil cephalus*) fed with different % of crude protein (CP) experimental diets.*	*60*
25	*Specific Growth Rate of milkfish (Chanos chanos) at fed with different % of crude protein (CP) experimental diets.*	*61*
26	*Average Daily Weight Gain of grey mullet* (Mugil cephalus*) fed with different % of crude protein (CP) experimental diets.*	*62*
27	*Biomass (g) of grey mullet (*Mullet cephalus*) fed with different % of crude protein (CP) experimental diets.*	*62*

LIST OF ILLUSTRATIONS

Fig. No.	***TITLE***	*Page No.*
1	*Weekly variation of Temperature (^{0}C) in floating net cages with grey mullet at different stocking densities.*	*63*
2	*Weekly variation of Salinity (ppt) in floating net cages with grey mullet at different stocking densities.*	*63*
3	*Weekly variation of pH in floating net cages with grey mullet at different stocking densities.*	*64*
4	*Weekly variation of Dissolved Oxygen (D.O) in floating net cages with grey mullet at different stocking densities.*	*64*
5	*Weekly variation of Total Alkalnity (mg/l) in floating net cages with grey mullet at different stocking densities.*	*65*
6	*Weekly variation of Ammonia (mg/l) in floating net cages with grey mullet at different stocking densities.*	*65*
7	*Weekly variation of Nitrite (mg/l) in floating net cages with grey mullet at different stocking densities.*	*66*
8	*Weekly variation of Nitrate (mg/l) in floating net cages with grey mullet at different stocking densities.*	*66*
9	*Weekly variation of Temperature (^{0}C) in floating net cages with grey mullet fed with different % of dietary protein levels.*	*67*
10	*Weekly variation of Salinity (ppt) in floating net cages with grey mullet fed with different % of dietary protein levels*	*67*
11	*Weekly variation of pH in floating net cages with grey mullet fed with different % of dietary protein levels*	*68*
12	*Weekly variation of Dissolved Oxygen (DO) in floating net cages with grey mullet fed with different % of dietary protein levels.*	*68*
13	*Weekly variation of Total Alkalnity (mg/l) in floating net cages with grey mullet fed with different % of dietary protein levels.*	*69*
14	*Weekly variation of Ammonia (mg/l) in floating net cages with grey mullet fed with different % of dietary protein levels.*	*69*
15	*Weekly variation of Nitrite (mg/l) in floating net cages with grey mullet fed with different % of dietary protein levels.*	*70*
16	*Weekly variation of Nitrate (mg/l) in floating net cages with grey mullet fed with different % of dietary protein levels.*	*70*
17	*Growth performance (g) of grey mullet at different stocking densities.*	*71*
18	*Survival rate of grey mullet at different stocking densities.*	*71*
20	*Feed Conversion Ratio in grey mullet fed on experimental diets at different stocking densities.*	*72*
21	*Specific Growth Rate of grey mullet at different stocking densities.*	*72*
22	*Growth performance (g) of grey mullet fed with different % of crude protein (CP) experimental diets.*	*73*

23	*Survival rate of grey mullet fed with different % of crude protein (CP) experimental diets.*	*73*
24	*Feed Conversion Ratio of grey mullet fed with different % of crude protein (CP) experimental diets.*	*74*
25	*Specific Growth Rate of grey mullet at fed with different % of crude protein (CP) experimental diets.*	*74*

LIST OF PLATES

Plate No.	
1	*Grey mullet (*Mugil cephalus*)*
2	*Experimental site at suryalanka in Guntur district.*
3	*Experimental setup at site.*
4	*Wild seed grey mullet (*Mugil cephalus*) collection*
5	*Seed acclimatization*
6	*Experimental set up*
7	*Grey mullet (Mugil cephals) length measurement*
8	*Cages nets checking and cleaning.*

ABBREVIATIONS

%	*Percentage*
/	*Division Sign*
<	*Less than*
>	*Greater than*
°C	*Degree Centigrade*
C	*Control*
ANOVA	*Analysis of Variance*
AOAC	*Association of official analytical chemists*
APHA	*American Public Health Association*
M	*Meter*
Mm	*Millimeter*
Cm	*Centimeter*
CP	*Crude Protein*
Ppt	*Parts per thousand*
Ppm	*Parts per million*
h	*Hour*
DO	*Dissolved Oxygen*
K	*Condition factor*
FAO	*Food and Agriculture Organization*
FCR	*Food Conversion Ratio*
PER	*Protein Efficiency Ratio*
IPF	*Inland fisheries project*
HAMO	*Hours after initial mouth opening*
NFE	*Nitrogen free Extract*
FM	*Fish meal*
DOB	*De oiled rice bran*
G	*gram*
g%	*Gram per 100 grams*
HDFE	*High density poly ethelene*
GNC	*Ground nut cake*
Kg	*Kilogram*
L	*Litre*
Ln	*Natural Logarithm*
DDGS	*Distillers Dried Graining with soluble*
IRASs	*Integrated Recirculating Aquaculture Systems*
RAS	*Recirculating Aquaculture System*
CG	*Compensatory Growth*
KJ	*Kilo Joule*
K Cal	*Kilo calorie*
ha	*hector*
Mg	*Milligram*
mg/l	*Milligram per Litre*
P	*Phosphorus*
Ppm	*parts per million*
SBM	*Soybean Meal*
^{0}E	*Degree East*

^{o}W	*Degree west*
^{o}N	*Degree North*
^{o}S	*Degree South*
SGR	*Specific Growth Rate*
spp.	*Species*
WG	*Weight Gain*
CIBA	*Central Institute of Brakishwater Aquaculture*
CMFRI	*Central Marine Fisheries Research Institute*
RGCA	*Rajeev Gandhi Centre for Aquaculture*
MPEDA	*Marine Products Exports Development Authority*
GIFT	*Genetically Improved Farmed Tilapia*
BAC	*Brakishwater Aquaculture Centre*
LC	*Lethal Concentration*
mtHb	*Methemoglobin*
NRC	*National Research Council*
FRS	*Fisheries Research Station*

ABSTRACT

Author : *SANTOSH KUMAR TADANGI*

Title of the thesis : ***STUDIES ON GROWTH AND SURVIVAL IN NURSERY REARING PHASE OF GREY MULLET (*Mugil cephalus*) FOR MARICULTURE.***

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*The present studies were conducted at the closed bay near Suryalanka, Bapatla, Guntur district on "Studies on growth and survival in nursery rearing phase of Grey mullet (*Mugil cephalus*) for mariculture".*

Total two (2) studies were conducted. The first study was on the effect of stocking density on growth and survival of grey mullet, (*Mugil cephalus)* in floating net cages in closed bay, Bapatla. Triplicate groups of mullets with an average initial weight of 0.95 g were randomly stocked in floating net cages (1m×1m×2m) at 20, 40, 60 and 80 fish/m^3 designed as D20, D40, D60 and D80 respectively. Mullets in the cages were fed with 25% CP formulated diet at 10% of body weight twice daily. Sampling was done weekly. At the end of the experiment, growth in terms of body weight of fish was high in D20 than those in D40, D60 or D80, and in terms of weight gain, and specific growth rate of fish was high in D40 than those in D20, D60 and D80. There was no significant difference in the survival rates of mullets at different stocking densities. At the end of the experiment survival rates was almost similar in D20, than those in D40, D60 and D80.

Total Feed Conversion Ratio was lower in D20 and D40, followed by D60 and D80. The cages stocked with 20 fish/m^3 have highest growth performance and 80 fish/m^3 showed highest biomass performance. Water quality parameters were at optimum level during study period. Results suggest that 20 fish/m^3 could be recommended for producing better quality mullet fingerlings and 80 fish/m^3 for highest number of seed production.

Another experiment was conducted to study the optimum crude protein for grey mullet, *Mugil cephalus* seed in floating net cages. Mullet fry were obtained through natural seed collection. Rectangular floating net cages made of bamboo poles were provided inside with 1×1×2m hapas. Predatory net and bird net were provided. PVC drums filled with air were fixed to the net cage for floating. Mullets with mean body weight of 0.95 g were stocked at the rate of 40 fish/m^3 in each hapa in the rectangular cages. Triplicates were maintained for each treatment. Fish fry were fed with 5 artificial dry diets containing 12.5% (Control – C (T1)), 15% (T2), 25% (T3), 35% (T4) and 45% (T5) crude protein. The diets were fed at the rate of 10% of the biomass for a period of 63 days (9 weeks). Water quality parameters like D.O, pH, salinity, NH_3, NO^{-2}, NO^{-3} and temperature were estimated at weekly intervals and were within the optimum range for culture of mullet. At the end of 63 days growth experiment, fishes fed on 25% (T3) crude protein feed exhibited highest growth and survival performance at 1% and 5% level of significance. The present study showed that Mullets seed reared in floating net cages in the closed bay performed best at 25% crude protein.

1. Introduction

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. World per capita fish supply reached a new record high of 20 kg in 2014, thanks to vigorous growth in aquaculture, which now provides half of all fish for human consumption, and to a slight improvement in the state of certain fish stocks due to improved fisheries management. Growth in the global supply of fish for human consumption has outpaced population growth in the past five decades, increasing at an average annual rate of 3.2 percent in the period 1961–2013, double that of population growth, resulting in increasing average per capita availability. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 14.4 kg in the 1990s and19.7 kg in 2013, with preliminary estimates for 2014 and 2015 pointing towards further growth beyond 20 kg (FAO., 2016).

Trends in Aquaculture

Aquaculture is defined as the farming of aquatic organisms, including fish, mollusks, crustaceans, and aquatic plants. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. (FAO., 2008).

The historic origin of aquaculture dated back to the eastern civilization before 4,000 years. The Bible refers to fish pond and sluices (Isaiah 19:10), and ornamental fish ponds appear in paintings from ancient Egypt. European aquaculture began sometimes in the middle age and transformed the ‘art’ of Asian aquaculture into a science that studied spawning, pathology, and food web (Swann, 1992; Asfaw, 2011). De Silva and Davy (2007) reported aquaculture, though considered to have over a

2,500 years history, was mostly practiced as an art. It began to be transformed into a modern science in the second half of the 20th century *(*Asfaw, 2011).

Fishing and Aquaculture in India has a long history. Kautilyas Arthashastra (321-300B.C) and king Someswara's manasottara (1127 A.D.) each refers to fish culture. Fish has certain Mythological importance in India. The very first of ten incarnations of God called "MATHSYAVATHARA" is believed to have taken the form of fish. For centuries, India has had a traditional practice of fish culture in small ponds in eastern India. Fish culture received notable attention in Tamil Nadu (formerly the state of Madras) as early as 1911, subsequently, states such as West Bengal, Punjab, Uttar Pradesh, Gujarat, Karnataka and Andhra Pradesh initiated fish culture through the establishment of Fisheries Departments. Fishing was practice even in pre-historic Karnataka, as can be seen from a copper fish hook at, the Brahmagiri, excavation. A vachanakara, a contemporary of Basaveswara called Galada Kannappa is known, gala being hook (Mulla and Shiralashetti, 2015).

Recently, aquaculture is developing, expanding and intensifying in almost all regions of the world. It is the fastest growing sector of the world food economy increasing by more than 10% per year and currently accounts for more than 30% of all fish consumed. This may be due to increased global population demand for aquatic food products, the production from capture fisheries has leveled off, and most of the main fishing areas have reached their maximum potential. Sustaining fish supplies from capture fisheries, therefore, will not be able to meet the growing global demand for aquatic food. Aquaculture appears to have the potential to make a significant contribution to this increasing demand for aquatic food in most regions of the world (FAO, 2008).

CULTURE IN CAGES

A number of different types of aquaculture practices exist; that means, its practices are diverse, ranging from single pond subsistence farming to highly intensive pump-ashore abalone farms. Among these practices, the simplest is the releasing of young organisms into the wild to increase the natural population size. The next level of aquaculture contains the organisms in a pen or cage open to water and nutrients, however, that restrains them from leaving the site. The most complex aquaculture type is a closed system. In this situation, there is no exchange between the aquaculture facility and the environment.

The on-growing and production of farmed aquatic organisms in caged enclosures has been a relatively recent aquaculture innovation. Although the origins of the use of cages for holding and transporting fish for short periods can be traced back almost two centuries ago to the Asian region (Pillay and Kutty, 2005) (Tacon, A.G.J. and Halwart, M., 2007).

Cage culture is commonly used in water bodies that cannot be drained, such as lakes, estuaries, reservoirs or coastal marsh areas. Cage culture practices have numerous advantages over other culture systems. By integrating the cage culture system into the aquatic ecosystem the carrying capacity per unit area is optimized because the free flow of current brings in water and removes metabolic wastes, excess feed and faecal matter (Beveridge, 1984; Asfaw, 2011).

Over the last 20 years, marine finfish culture, predominantly cage farming, has spread throughout Asia. In recent years, finfish mariculture has been growing rapidly on a global basis especially with the development and expansion of sea cage farming. One of the major reasons for the growth of sea cage farming is the availability of

breeding techniques that can produce sufficient quantity of seeds of different high value marine finfish.

Culture of marine finfishes such as mullets, milkfish and seabass has been practised in India for a very long time. However, most of the culture is done in the traditional pattern along the coastline in the brackish water systems or on an experimental level to evaluate the feasibility of the culture system.

Advantages and disadvantages of cage culture

Cage culture includes low investment costs, easy management, easy and low cost of harvesting, opportunities for close observation of feeding and health. Application of cage culture practice has also some disadvantage including vulnerability to poachers and structural damages, less tolerance of fish to poor water quality, more dependence on commercial diets and the increased risk of disease out breaks (Mc Ginty and Rakocy, 2005). Furthermore, the risk of escaping from cages has potential impacts on wild fish population, including potential gene pool and social impacts (Ferguson et al., 2007). Other risks might be mentioned as cage culture solely depends on the natural catch of the fingerlings where hatchery development is new or production is not currently sufficient to meet the demand (Ottolenghi et al., 2004).

MULLETS

The popular name ‘mullets’ generally refers to the species of the family Mugilidae, the name grey mullet is used to distinguish them from the red mullets of the family Mullidae. The main species of aquacultural interest are euryhaline and can be reared both in brackish- and salt-water environments. They can easily be acclimatized for culture in fresh water (Pillay and Kutty, 2005).

Among mullets, the striped mullet (Mugil cephalus) is a fast growing species and is commonly available on the east and west coasts of India (Lazarus et. al., 1991). M. cephalus (Linnaeus, 1758) inhabits estuarine, freshwater, coastal and marine water

bodies (Rivas, 1980). It occurs in lagoon, with juvenile fishes most common in impounded areas, around mangroves, in seagrass beds, and offshore (Burgess, 1980).

The flathead grey mullet is catadromous, frequently found coastally in estuaries and freshwater environments. Adult mullet have been found in waters ranging from zero salinity to 75‰, while juveniles can only tolerate such wide salinity ranges after they reach lengths of 4–7 cm. Adults form huge schools near the surface over sandy or muddy bottoms and dense vegetation and migrate offshore to spawn in large aggregations. The larvae move inshore to extremely shallow water, which provides cover from predators as well as a rich feeding ground. After reaching 5 cm in length, the young mullets move into slightly deeper water (De Silva, 1980).

Flat head grey mullet (M. cephalus) can withstand salinity from 0 to 35 ppt and grows to 500gm size in about six months under pond conditions. Those are diurnal feeder, consuming mainly zooplankton, dead plant matter, and detritus. Adults migrate to the sea to spawn and the larvae migrate to inshore waters and estuaries to grow. Among the mullets, M. cephalus grows faster than other species. It can be mono cultured or poly cultured successfully with many other species both in brackish water and freshwater condition. Flathead grey mullet has a good market in many Asian countries including India and in the southern and eastern Mediterranean region. It can withstand salinity from 0 to 35 ppt (Nordlie et al., 1982). The fish can grow upto500gm size in about six months period under pond conditions with low cost feed such as rice bran and ground nut oil cake (Oren, 1981). Among the mullets, M. cephalus grows faster than other species (De Silva, 1980). In India mullet is being cultured by traditional method. Presently semi-intensive farming is growing with rapid pace (Munilkumar et al., 2013).

In recent years, research on the grey mullet has increased because of its potential of being cultured in a number of tropical countries. The advantage of culturing mullet have been well documented and most of the studies on mullet culture/growth in India, and elsewhere, have been undertaken in coastal areas using seawater (Walsh et al., 1994; Anon 1996; Barman et al., 2005).

Mullet have been recognized as very desirable species for pond culture in China, Egypt, Hawai, Italy, Japan, Philippine, Taiwan and other parts of the world, where they are mainly grown in polyculture with carps, tilapia, and milkfish (Perlmulter et al., 1957; Blanco and Acosta, 1958; Yashov, 1966; Lin, 1968 and Bardach et al., 1972). Mullets (Mugil cephalus and Liza ramada, Family Mugilidae) are considered highly esteemed in Egypt. They are also the most important marine fish, used for culture either in brackish of fresh water (Eissawy et al., 1974 and Sarig, 1981). Because of their great productivity for aquaculture, there is a worldwide interest in mullet culture (El-Dahhar et al., 2013). They are recognized as highly wanted fish for pond culture in Italy, Japan, Philippines and other parts of the world (Yashouv, 1966; Yashouv and Ben-Shachar 1968, Bardach et al., 1972, Benetti and Fugundo Netto, 1991a,b and El-Dahhar, 2000a,b; El-Dahhar et al., 2014).

Despite the economic importance of mullet as a major farmed fish species, no special feeds are commercially available for them in many countries. Apart from dependence on fry availability, further development of mullet farming depends on the development reliable mass propagation techniques and on the availability of reliable low cost and efficient feeds that could enhance production. However, our knowledge of their nutritional requirements is limited (Karapanagiotidis et al., 2014).

Euryhaline fish can live in a wide range of environmental salinities due to their ability to synthesize new salt-transporting proteins as they move from salt to

fresh water and vice versa (Kidder et al., 2006). Therefore, water from different sources can be used to cultivate euryaline fish (Lisboa et al., 2015).

The rearing of grey mullets in combination with milkfish, Indian major carps and shrimps in the coastal tanks of West Bengal has been described by Pakrasi et al., (1975). Mullet is a filter feeder, which usually swim near the top of the water as school and swallows the scum of the water surface (Anil et al., 2010). Also, Anil et al. (2010) investigated the economic feasibility of polyculture of the giant tiger shrimp with mullet. All these reports clearly indicate that shrimp performance is enhanced by the presence of a secondary species as mullet (Hosseini et al., 2017).

Brackishwater aquaculture sector in India which is shrimp dependent has witnessed set backs of late due to disease outbreaks and environmental problems and therefore the need of the hour is to adopt eco-friendly farming with emphasis on species diversification. The striped mullet, M. cephalus is one of the most popular and fast growing brackish water finfish being cultured in tropical and subtropical regions (Thompson, 1963). The advantages of this species for aquaculture have been well documented (Odum, 1970; Pillai et al., 1984 and Rajyalakshmi and Chandra, 1987). In India farming of this species is being done mainly by polyculture method using seed collected from the wild and is restricted to traditional system (Abraham et al., 2000).

Biology of Mugil cephalus

Grey mullets are mainly catadromous fish family, excluding a few member species. They live in schools on the coasts, lagoons and rivers of tropical and subtropical waters (Mc Dowal, 1988). They are important food fishes. The euryhalinity, eurythermality and their simpler diet, as well as the rapid growth of some species, have made them the object of aquaculture in many parts of the world (Ali, 2010).

Feeding Habit:

Flathead grey mullet is a diurnal feeder, consuming mainly zooplankton, dead plant matter, and detritus. Mullet have thick-walled gizzard-like segments in their stomach along with a long gastrointestinal tract that enables them to feed on detritus. They are an ecologically important link in the energy flow within estuarine communities. Feeding by sucking up the top layer of sediments, flathead grey mullet remove detritus and microalgae (Goldstein and Simon, 1999). They also pick up some sediment which functions to grind food in the gizzard-like portion of the stomach. Mullet also graze on epiphytes and epifauna from seagrasses as well as ingest surface scum containing microalgae at the air-water interface (Etnier and Starnes, 1993). Larval flathead grey mullet feed primarily on micro crustaceans. Copepods, mosquito larvae, and plant debris have been found in the stomach contents of larvae under 35 mm in length. The amount of sand and detritus in the stomach contents increases with length, indicating that more food is ingested from the bottom substrate as the fish matures (De Silva, 1980; Munilkumar et al., 2013).

Flathead grey mullet (M. cephalus) is an economically important euryhaline and eurythermal marine teleost contributing to sizable fisheries of estuarine and coastal regions in many countries and has a cosmopolitan distribution between latitude 40˚N and 40˚S covering all the oceans. The overall share of Aquaculture was 2.6% in the total production of marine fishes and it was contributed substantially by flathead grey mullet (*M. cephalus*) as one of the species. It is an excellent candidate species for both mono and polyculture as it feeds at the lower trophic level grazing on plant detritus and microflora. This species is widely cultured in both brackish and freshwater semi-intensive fish ponds and can utilize both supplemental feed and /or natural food. In India, M. cephalus occurs in the shallow costal waters, lagoons, the

coastal lakes and estuaries and is mainly reared by polyculture method using seed collected from the wild and is restricted to traditional system in extensive poly-farming impoundments (Mondal et al., 2015).

Significance of Finfish Nursery Phase

Closed life-cycle aquaculture involves a thorough understanding of the behaviour, habitat and environmental requirements, reproductive biology, nutritional requirements, and larval and juvenile physiology of each species, as well as its susceptibility to disease under culture conditions. Moreover, it involves the development of all aspects of fish husbandry, such as the facilities required for the various life-cycles stages (brood stock holding tanks/sea cages, nursery tanks/cages, grow-out facilities), feed development, fish handling systems, and disease control. Such procedures and techniques have been developed for several diadromous and marine fishspecies – notably salmonids, seabass, seabream, and more recently for cod. the development of seed production in hatcheries on an economically viable commercial scale, and the refinement of grow-out technology to ensure that the fattening phase is environmentally acceptable are the critical issues for the future. Failure to address these matters successfully would have severe consequences for both aquaculture and capture fisheries (Ottolenghi et al., 2004).

Since nursery rearing is the most critical phase in seabass culture, Philipose et al., (2010) showed that Asian seabass juveniles can be reared in indoor cement tanks fed on artificial fish feed.

Effect of Stocking Density On Growth And Survival

Tidwell and Webster (1992) and Tidwell et al., (1994) found a consistent decrease in growth with increasing stocking density, indicating the possibility of competitively reduced feed utilization (Anderson et al., 2002). In cage culture finding

out the optimal stocking rate becomes an important part because stocking density directly influences the growth rate of the cultured species (Kilambi et al., 1977).

Stocking density is one of the most important variables in aquaculture because it directly influences survival, growth, behavior, health, water quality, feeding and production. Increasing stocking density results in stress (Leatherland and Cho, 1985) which leads to enhanced energy requirements causing reduced growth and food utilization (Hengsawat et al., 1997). Optimum stocking densities need to be determined for each species and production phase to enable efficient management and to maximize production and profitability (Rowland et al., 2006; de Oliveira et al., 2012).

Importance of Dietary Protein:

Feed is widely regarded as becoming a major constraint to the growth of aquaculture production in many developing countries.

Protein is the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. Protein is the most expensive macronutrient in fish diet (Pillay,1990).

The amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets to be unnecessarily expensive (Ahmad, 2000). Reducing feeding costs could be a key factor for successful development of aquaculture. Protein requirements for optimum growth of the fish seem to be affected by numerous factors such as temperature, salinity, fish age and size, etc. (Cowey, 1976; Ahmad *et al.*, 2004).

Protein is the major macronutrient in fish which provides essential and non-essential amino acids for protein synthesis and energy for maintenance and growth (Kim et al., 2002). However, protein is the most expensive component in fish feed

(NRC, 1993; Mohseni et al., 2013). For successful aquaculture practices, it is needed to determine the minimum level of protein at which fish can attain maximum growth as well as the operational costs can be saved (NRC, 2011). It is well documented that dietary protein requirements for most of the fish species are found to be between 30 and 55 % of the diet; however, it depends on the fish species, fish size, dietary protein sources and environmental conditions (Hepher, 1988; NRC, 1993) (Kim et al., 2016).

It is well known that formulation of balanced diets and their adequate feeding are important for successful aquaculture. Protein is one of the major dietary nutrients effecting growth performance of fish (Lovell, 1989). Dietary protein provides essential and non-essential amino acids to synthesize body protein and energy for maintenance. According to fish species, fish size, dietary protein sources and environmental conditions protein requirements ranges from 300 to 550 g kg^{-1} (NRC, 1993) (Schulz et al., 2007).

The intensification of fish culture has led to dependence on artificial feeds. Protein is the most expensive component in fish feeds and also the most important factor affecting growth performance of fish and feed cost (Lovell, 1989) (Luo et al., 2004; Siddiqui et al., 2009).The artificial feeding is considered supplementary feeding to help the natural growth of fish quickly during a culture period which varies depending on the type of fish farmed. Macfadyen et al. (2011) reports that there are 25 fish feed mills in Egypt, producing more than 3,00,000 Mt of fish feed each year. Increased world market prices of raw materials resulted in an increase of feed prices with 200-250% over the last 6-7 years (Macfedyen et al., 2012; Abou- Zied, 2015).

Farming of omnivorous fish, such as mullet, in brackish water under semi-intensive conditions, should allow under-utilized trophic resources to be fully exploited for animal production (Ravagnan, 1990; 1992). Practical diets used in Italy still rely on a

significant inclusion of expensive and limited marine raw materials such as fishmeal. In Italian semi-intensive farming systems, mullet are intensively reared during the first year and later released into open ponds (Ravagnan, 1990; 1992; Luzzana et al., 2005).

Studies are confined fry and young grey mullets (M. cephalus) understanding the protein requirement during Nursey phase is important thing in fish culture. The objective of this study was to assess optimal stocking density and the optimum protein level leading to optimum growth and feed utilization of grey mullet in nursey phase.

The objectives of present study are:

1. To study the growth rate in nursery phase.
2. To study the effect of water quality on growth of species.
3. To record the survival at nursery phase.

*Plate : 1 Grey mullet, (*Mugil cephalus*)*

2. Review of literature

Faced with one of the world's greatest challenges – how to feed more than 9 billion people by 2050 in a context of climate change, economic and financial uncertainty, and growing competition for natural resources – the international community made unprecedented commitments in September 2015 when UN Member States adopted the 2030 Agenda for Sustainable Development. The 2030 Agenda also sets aims for the contribution and conduct of fisheries and aquaculture towards food security and nutrition in the use of natural resources so as to ensure sustainable development in economic, social and environmental terms.

Many millennia after terrestrial food production shifted from hunter-gatherer activities to agriculture, aquatic food production has transitioned from being primarily based on capture of wild fish to culture of increasing numbers of farmed species. A milestone was reached in 2014 when the aquaculture sector's contribution to the supply of fish for human consumption overtook that of wild-caught fish for the first time. Meeting the ever-growing demand for fish as food in conformity with the 2030 Agenda will be imperative, and also immensely challenging (FAO, 2016).

Fisheries in India a very important economic activity and a flourishing sector with varied resources and potentials. Mariculture in India, although limited to the farming of mussels and edible oysters undertaken in some coastal region of Kerala over the years, has successfully produced sea cage farming in recent years, initially with seabass and most recently cobia, which has shown the prospects of commercial mariculture in the country (FAO, 2005).

Inland fishery is a time old tradition of most early civilizations. However, in recent times, inland waters have been increasingly impounded for hydropower generation,

irrigation, and other purposes (Mc Cully, 1995 ; De Silva, 1998), with devastating impacts on nearby agrarian and other communities (IEA, 2000 ; IRN, 2003) (Gurung et al., 2010) (De silva and Davy, 2010).Cage fish culture is considered to be an old tradition that has developed into a major sector in aquaculture only in the recent past (De Silva and Phillips 2007 ; Tacon and Halwart, 2007).

2.1 Finfish culture practices in cages:

Cage culture systems employed by farmers are currently as diverse as the number of species currently being raised, varying from traditional family-owned and operated cage farming operations (typical of most Asian countries) to modern commercial large-scale salmon and trout cage farming operations in northern Europe and the Americas (Tacon and Halwart, 2007).

Cage aquaculture is an important technology to increase fish production. A widespread and profitable culture of fish and prawns in cages has already been developed successfully in Asia, Europe and America. This technique in South-East Asia first started from late 1800s, since then, many countries in this area were practicing cage culture in freshwater and marine environments, including open sea, estuaries, lakes, reservoirs, ponds and river. However, in Bangladesh, aquaculture activities are still concentrated largely in pond culture system. Cage aquaculture in open water bodies could provide an opportunity for mitigating protein demand in the nation (Moniruzzaman et al., 2015).

Marine cage aquaculture is getting popular in Taiwan where land and freshwater resources are limited (Su and Liao, 2001). Among many species of cultured marine fish, cobia is a prominently popular species for cage aquaculture because of its fast growth rate and comparatively low production cost. Survival rate at all stocking densities tested was above 95%. Results show that optimum stocking density using

this system is 370/m^3 based on survival, specific growth, and food conversion rates (Liao et al., 2004).

In cage aquaculture technical simplicity with which farms can be established or expanded, lower capital cost, feeding, growth and health of stocked fishes can be monitored on a daily basis without much disturbance, cultured species are well and harvesting of cultured species would be easier and cost effective. As caged fish are generally fed with high protein diets, wastes derived from feed are either directly or indirectly released to the surrounding environment, causing accelerated eutrophication in the receiving waters (Beveridge, 1984; Ackefors, 1986; Lin, 1990; Mondal et al., 2010).

Cage culture techniques are well-established for many fish species and cages are becoming a significant contributor to production in some countries (Beveridge, 1996; Wu et al., 2000; Marte et al., 2000; Takashima and Arimoto, 2000; Chua and Tech, 2002; Watanabe et al., 2002; Liao et al., 2004: Mariojouls et al., 2004; Wray, 2004). Cage culture would reduce or eliminate losses from bird predation, as well as facilitate husbandry and harvesting (Beveridge, 1996; Chua and Tech, 2002). Cages enable the use of existing water bodies (Beveridge, 1996; Rowland et al., 2006).

Weight gain and survival rate are the most important issues for a successful cage aquaculture because they determine the production performance and profit of the system. Yi and Lin (2001) reported that higher biomass of caged tilapia had a significant negative effect on final body weight. Feeds and feeding are the major costs of production in cage culture (Moniruzzaman *et al.*, 2015). Cages are easy to manage, produce fish of high quality, and utilize existing water bodies (Beveridge, 1996). Cage fish culture as a family activity has been largely applied in Asia, particularly in the northeastern region of Thailand where there are many reservoirs suitable for

culturing fish in cages (Huchette and Beveridge, 2003; Liao et al., 2004: Jiwyam, 2011).

Fermin et al. (1996) showed that nursery rearing of sea bass fry in illuminated floating net cages installed in coastal areas is practicable and can be an alternative to the traditional method of pond nursery. Anil et al. (2010) indicated that cage culture of seabass, L. calcarifer, in the sea can provide significant advantages in terms of faster growth and effective utilisation of water volume as compared to pond systems thus making it an alternative culture approach to enhance production.

However, optimum feeding frequency and rate vary depending on the fish species, size and rearing system (Cho et al., 2003). Although several studies at their early life stages (Chiu et al., 1987; Abud, 1990; Tsevis et al., 1992; Folkvord and Ottera, 1993: Wang et al., 1998, 2009; Lee et al., 2000; Hossain et al., 2001; Dada et al., 2002; Dwyer et al., 2002; Cho et al., 2003; Schnaittacher et al., 2005; Tucker et al., 2006; Biswas et al., 2006 a,b; Silva et al., 2007; Booth et al., 2008), lack of information exists in this regard for Asian seabass fry reared in net cages (Biswas et al., 2010).

Oliveira et al. (2012) explained cage culture is one of the most productive aquaculture systems widely used for intensive fish culture (Chua and Teng, 1980; Guo and Li, 2003). Cage culture techniques are well-established for many fish species and cages are becoming a significant contributor to production in some countries (Beveridge, 1996; Marte et al., 2000; Chua and Tech, 2002; Liao et al., 2004; Mariojouls et al., 2004). There were several factors affecting economic return of the system such as yield, sales price, fed cost, fingerlings or juveniles cost, system investment and operating cost (Muangkeow et al., 2007). Karapanagiotidis et al. (2014) given that mullets such as M. cephalus are not only cultured as food fish but also for their roe, highly prized as a nutritious delicacy and beneficial to human health

(Kalogeropoulous et al., 2008). Liza aurata in particular is processed into a highly prized smoked product, locally called “lykourinos”. Most importantly mullets feed low in the food chain, either as direct primary consumers and detrivores, or at a secondary level feeding on small macrofauna. Their nutritional requirements therefore could be met by natural sources and low cost supplementary feeds. Several studies in feeding and nutrition indicate that fish regulate their feed intake with respect to the energy content of the diet (Lee and Putnam, 1973; Kaushik and Médale, 1994; Kentouri et al., 1995; Paspatis and Boujard, 1996; De la Higuera, 2001; Hernández et al., 2001; Boujard et al., 2002; Lee and Kim, 2005; Kousoulaki et al., 2007).

Water quality refers to all the physical, chemical and biological factors that influence its use (FAO, 1994; Amoah, 2011). Water quality management remains one of the most important operations in the successful culture of any fish species and most periods of poor growth, disease and parasite outbreaks or fish kills are often associated with poor water quality (Boyd, 1990; Mwachiro et al., 2012).

Water quality plays a significant role in the biology and physiology of fish and may impact on the health and productivity of the culture system (Boyd, 1997; Landau, 1992; M. M’balaka et al., 2012). Kheriji, et al. (2003) reported that both high temperature and low salinity are stressful to mullet. Ali, (2010) indicated that golden grey mullet fry (Mugil aurata) show best performance when reared at a salinity 18%. Kalidas et al. (2012) indicated that the culture of juvenile pompano might be feasible in salinities up to 15 ppt in tropical conditions. Resley et al. (2006) found that juvenile cobia, at an initial size of 6-7 g can be reared successfully at salinities ranging from 5 to 30 ppt.

2.2 Effect of stocking density on growth and survival of fish:

In intensively cultured fish species, there are demands for more efficient early stages production due to low survival rates (Baras, 1999; Baskerville-Bridges and Kling, 2000). To meet current needs, farmers resort to increasing stocking density. Density is one of the most deterministic factors in larviculture, affecting social interactions such as aggressiveness (Kaiser et al., 1995; Sakakura and Tsukamoto, 1999), hierarchical phenomena (Schreck, 1981) and cannibalism (Katavic et al., 1989; Moore and Prange,1994), resulting in variations in size, survival and growth performance in fish populations (Sheikh-Eldin et al., 1997; Hatziathanasiou et al., 2002). It is an undisputed fact that larval rearing remains the most critical and crucial phase in brackish water fish seed production. Development of suitable protocols for the mass rearing of larval fish represents one of the last barriers for the successful propagation of a variety of marine species (Tamaru et al., 1993 and Abraham et al., 1999).

Growth of common sole Solea solea is negatively correlated to density, which affects productivity in culture and hence commercial success (Lund et al., 2013).

These herbivorous cum-omnivorous fish have a good aquaculture potential because they are exceptionally hardy and prolific, easy to farm and thus ideal for both small farmers and industrial sized aquaculture (Chirwa, 2009; Maluwa and Gjerde, 2006). Fish stocking density is one of the most important parameters affecting fish growth and health in a number of ways (Garr et al., 2011; Mazlum, 2007; Zhu et al., 2011). Growth performance and survival rate are adversely affected by high stocking densities (Sorphea et al., 2010; Pouey et al., 2011) but in some cases this effect is either temporary (Garr et al., 2011) or absent (Gokcek and Akyurt, 2007; Southworth et al., 2009). Some species can tolerate extreme crowding although competition for

food will then limit their growth and lead to poor weight gain (Stickney, 1994; M'balaka et al., 2012). Consequently, optimum stocking densities need to be determined for each species and production phase to enable efficient management and to maximize production and profitability (Rowland et al., 2006).

Stocking density is one of the factors that could potentially affect survival and production performance of aquatic organisms. Thus, the use of an appropriate density can increase the profitability of farming systems, by maximising the utilisation of water and the other resources in the rearing system (Fairchild and Howell, 2001; Ardiansyah et al., 2016).

Stocking density is one of the most important variables in aquaculture because it directly influences survival, growth, behaviour, health, water quality, feeding and production. In cage culture, optimum stocking densities and carrying capacities vary with species, size of fish, size of cages, rate of water exchange, size of ponds and length of growing season (Kilambi et al., 1977; Chua and Teng, 1979; Coche, 1982; McGinty, 1991; Duarte et al., 1994; Beveridge, 2002; Chua and Tech, 2002; Masser, 2004; Rowland et al., 2006). Quinitio and Juario, (1980) showed in floating cages, juvenile milkfish grow faster in 5-m diameter cages than in 3-m diameter cages at stocking densities of 1, 2, and 4 kg/m^3; likewise faster at 4 kg/m^3 than at 1-2 kg/m^3 in both cage sizes. Hwang et al. (2014) studied on the effects of stocking density on Korean rockfish (Sebastes schlegeli) culture and reported that rockfish reared in low density were growing faster than those in higher density and output per unit area of high density culture was higher than those of low and medium density cultures. The ability to tolerate a wide range of salinities is present in M. cephalus from the juvenile stage (Young et al., 1997; Kheriji et al., 2003). Mc Ginty and Rakocy, (1989) suggested that stocking rate of tilapia fingerlings depends on cage volume, desired

harvest size and production level, and the length of the culture period for cage culture. Survival rates were not affected by stocking density which is consistent with Daungsawasdi et al. (1986) who reported that mortality in Nile tilapia raised in cages was not dependent on stocking density. Stocking density had negative effects on both growth and feed utilization efficiency, but positively sustains fish production. High densities improve yield, production and profitability (Abou et al., 2007). Bakeer, (2006) given that in monoculture system of grey mullet stocking rate 2 fish/m^3 is required for better yield.

Ahmad et al. (1999) examined that the stocking densities used in the nursing and early yearling's experiments did not have significant effects on growth or survival up to 400 g in body weight. However, higher stocking densities in the 400-800 g size range resulted in significant reduction of growth in cages. (Abdel-hakim et al., 2006) recommended a feeding level of 2.5% for tilapia, mullet and carp cultured together at higher rate in earthen ponds without supplemental aeration.

Watanabe, et al. (1990) shown that in Florida red tilapia fingerlings efficiency of production is increased and size variation minimized by stocking at higher densities up to 300/m^3. Benetti et al. (2010) indicated that grow out of cobia is technically feasible in submerged open cages and suggest that growth rates are negatively influenced by decreasing temperatures and/or increasing stocking density. The rearing of fish under cages has several advantages among them increased or improved stocking density, easiness in harvesting as well as assessment of fish growth, higher maturity rates, there is free contact with the normal environment among others (Mwachiro et al., 2012).

2.3 Optimum protein requirement:

Protein is the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. Protein is the most expensive macronutrient in fish diet (Pillay, 1990). So, the amount of protein in the diet should be just enough for fish growth where the excess protein in fish diets may be wasteful and cause diets to be unnecessarily expensive (Ahmad, 2000). Protein is the main determinant of fish growth and is also the most expensive nutrient in fish diet. When low amounts of energy are included in the diets fish may be forced to use protein as energy source causing a reduction in protein synthesis and growth (Cho, 1990; Kousoulaki et al., 2007).

(Ojaveer, 1996) given elevating the protein content of a diet to its optimum level generally results in a higher growth rate. Increasing the dietary energy or protein content beyond this threshold will not further improve the performance of fish (Garling and Wilson 1976). Protein is a major component in fish feeds. Increasing protein level in feeds can lead to improved fish production, but excessive dietary protein level is not economical for fish culture. Many studies have been carried out to determine protein requirements for fish, with estimated protein requirements ranging from 30% to 55% according to fish species, fish size, dietary protein sources and environmental conditions (NRC, 1993;. Yang et al., 2003). To be economically feasible, this later option would require the correct combination of dietary protein and energy source as well as good primary sources of the dietary protein itself (Santiago et al.,1983; Seneriches and Chiu, 1988; Sumagaysay et al., 1991). Therefore, the growing interest in milkfish farming in brackish water ponds requires an insight into the nutritional requirements of the milkfish and into various aspects of its physiology during culture operations The current lack of sufficient information on these aspects

of milkfish aquaculture is a major technical constraint in developing an aquaculture system in which the production diet(s) provides adequate nutrition at the lowest cost (Benitez, 1984).Dietary protein levels and ration size are two major factors influencing fish growth, feed efficiency and also water quality (Sumagaysay and Borlongan, 1995; Sumagaysay1999). The experiments of Sumagaysay et al., (1991) and Sumagaysay and Borlongan, (1995) demonstrated that milkfish production increases with increasing levels of dietary protein. The amount of ammonia excreted and orthophosphate produced by the fed fish appears to be directly related to the source and levels of protein intake (Kaushik and Cowey, 1991; Kalla et al., 2003a; Jana et al., 2006). Unfortunately, there is lack of information on nutrient requirements of mullet, and different species (M. cephalus, Chelon labrosus, Liza aurata, Liza ramada, Liza saliens) could have different nutritional requirements according to their feeding habits (Yashouv and Ben-Sachar, 1967; Albertini-Berhaut and Vallet 1971; Papaparaskeva, 1986; Benetti and Fagundes Netto, 1991; Argyropoulou et al., 1992; Ojaveer et al., 1996; Luzzana et al., 2005).

El – dahhar et al. (2014) stated the poor survival rates of the wild mullet larvae is a limiting factor in mullet production and many investigations were made to determine environmental and nutrition requirements (Brusle, 1981; Alexis and Papaparaskeva and Alexis, 1986; Benetti and Fagundes Netto, 1991a,b and El-Dahhar, 1999).

Fish feeding is one of the most important factors in commercial fish farming because feeding regime may have consequences on both growth efficiency and feed wastage (Tsevis et al., 1992 and Azzaydi et al., 2000). Moreover, knowledge of the optimum feeding rate is important not only for promoting best growth and feed efficiency, but also for preventing water quality deterioration as a result of excess

feeding (Ng et al., 2000; Mihelakakis et al., 2002 and Webster et al., 2002; El-Dahhar et al., 2013). The cost of the fish production depends mainly on the cost of feed which constitute most of the economical expenses, and dietary protein is the most important factor affecting growth performance of fish and feed cost (Lovell, 1989). Reducing feeding costs could be a key factor for successful development of aquaculture. .Papaparaskeva and Alexis (1986) found that 24% dietary crude protein level was required for maximum growth for young grey mullet (M. capito) of 2.5 g initial body weight. It was also found that the dietary protein content of 15% resulted in sufficient growth equal to 26, 37 and 50% dietary crude protein levels for grey mullet (M. capito) of 12.5 initial BW (Papaparaskeva and Alexis, 1986; El-Dahhar et al., 2011).

Protein requirements of fish vary with species, size or age, protein quality, dietary level of energy, water quality, presence of natural food and feeding and culture management (NRC, 1993). Protein requirements for optimum growth of the fish seem to be affected by numerous factors such as temperature, salinity, fish age and size, etc. (Cowey, 1976; Ahmad et al., 2004). El-Dahhar, 2000 (a, b) are found for striped mullet (M cephalus) larvae 0.2g, maintained at 26% dietary crude protein and 245 kcal ME / 100g in diet possessed superior growth characteristics, feed efficiency and survival rate. Elnady et al. (2014) indicated that the best growth and feed performance during secondary rearing were obtained when feeding mullet fry with 25% - crude protein diet while the best economic efficiency was obtained when feeding grey mullet fry with ground corn (6.58 L.E/kg fingerlings), followed by the use of 25%-crude protein diet (10.16 L.E / kg fingerlings). Karapanagiotidis et al. (2014) examined that L. aurata reared on diets of varying protein levels exhibited relatively low growth parameters and poor feed utilization consistent with the slow growth rate of the species. The species is known to grow slower than other mullets,

such as M. cephalus and C. labrosus (Gautier and Hussenot, 2005). The growth performance and feed utilization of L. aurata was not significantly affected by dietary protein level. These results are applicable to lagoon cage culture of golden grey mullet and thus may not be valid for other environments.

The dietary protein levels influences the growth, conversion efficiency, and body composition of the fish, and that the inclusion of dietary protein in the range of 40–43% of the diet is optimum for the growth and efficient feed utilization of protein for growth of young H. fossilis (Siddiqui and Khan, 2009). Level of dietary protein is of fundamental importance, because it is significantly influences growth, survival, and yield of fish as well as economics of a farming industry by determining the feed cost which is typically the largest operational cost. Increase in dietary protein has often been associated with higher growth rate in many species However, there is a certain level beyond which further growth is not supported, and may even decrease (Mohanty and Samantary, 1996; Shiau and Lan, 1996; McGoogan and Gatlin, 1999; Gunasekera et al., 2000; Kim and Lall 2001; Yang et al., 2002; Abbas et al., 2005; Debnath et al., 2007; Kvale et al., 2007; Siddiqui et al., 2009).

Biswas et al. (2010) suggested that in brackish water net cage rearing, Asian sea bass fry can achieve maximum growth, survival and better feed conversion when they are fed a commercial diet containing approximately 55% crude protein, at a given ration with three times feeding dialy.

Otchoumou et al. (2011) showed that it is possible to incorporate local carbohydrate sources as corn, millet, and sorghum in diets for African catfish, reducing the cost of feed production. Carbohydrate sources are of interest in aquaculture, particularly in developing countries. Lipids, proteins, and carbohydrates are energy sources for animals, however, protein is the single most expensive

ingredient in fish diets. Further, high levels of dietary protein may lead to consumption of protein for energy purposes, thus, non-protein energy sources are being sought for fish diets (De Silva et al., 1991; Erfanullah and Jafri, 1995). Gonzalez-Rodrıguez et al. (2014) summarized that juvenile T. tinca require 52 % dietary protein for optimum growth and feed utilization.

Karapanagiotidis et al. (2014) concluded that L. aurata reared on diets of varying protein levels exhibited relatively low growth parameters and poor feed utilization consistent with the slow growth rate of the species. The species is known to grow slower than other mullets, such as M. cephalus and C. labrosus (Gautier and Hussenot, 2005). The growth performance and feed utilization of L. aurata was not significantly affected by dietary protein level. These results are applicable to lagoon cage culture of golden grey mullet and thus may not be valid for other environments. Lisboa et al. (2015) reported that energy demand for osmoregulation in juvenile M. liza can be minimized in water salinity close to the isosmotic condition. However, the amount of energy spared under this condition is not enough to improve growth in juvenile mullets.

Najafi et al. (2016) indicated that feeding times did not influence the growth performance, blood parameters, muscle proximate composition, and diet digestibility.

El-Dahhar *et al*. (2013) recommended that sea bass larvae (0.08g initial BW) require 3.75mg protein / g BW and 28.14 cal gross energy /g BW daily as maintenance requirements. Vergara, *et al,* (1996) indicated that highest specific growth rates were obtained in fish fed 55% protein at a fixed dietary feeding rate of 6% body weight per day. The minimum dietary protein level producing maximum growth of S. *aurata* fry was found to be 55%. Elangovan *et al.* (1997) showed that the weight gain, specific growth rate and feed efficiency indicated that 41.7% dietary protein was the optimum

requirement for maximum growth of *B. ultus* at a feeding rate of 5% body weight per day. Hebb *et al.* (2003) revealed that a crude protein level of 50% with a DP/DE ratio of 26 mg Kj^{-1} is optimal for growth of juvenile flounder (0.9–5 g). Jana *et al.* (2006) documented that milkfish should be fed diet containing about 40% protein, preferably of plant origin. Jover *et al.* (1999) given that Mediterranean yellowtail have good growth when fed dry extruded diets containing 50% protein. Yang, *et al.* (2003) concluded that percentage weight gain of fish increased with increasing dietary protein level in juvenile *Spinibarbus hollandi* up to a point, and decreased slightly thereafter with further increases in dietary protein level.

3. Materials and Methods

3.1. Experimental site:

The present study conducted in the closed bay near Suryalanka, Bapatla, Guntur district. It lies between latitude- 15◦51' 04.54''N and longitude 80◦31'58.87''E. Plate no:2.

3.2. Experimental animals and their acclimatization:

Grey mullet (Mugil cephalus) seed were collected from wild sources in Ramapuram village 20km away from Suryalanka village, Bapatla mandal Guntur district. Fish seed were brought in aerated, closed bags and released into hapas (2m×2m×1.5m) in brakish water fish pond at suryalanka and acclimatized for one hour. Before transfer into hapas the seed was slowly acclimatized to water of experimental site.

3.3. Experimental Design:

Experiments:

Two studies were conducted:

1. To study the effect of stocking density on growth and survival of Grey mullet, Mugil cephalus.

2. To study the effect of different protein levels on growth and survival of Grey mullet, Mugil cephalus

3.3.1. Experimental cages for studies (1 to 2):

The floating net cages used for experiments were of $20^1 \times 10^1$. (Hapas of 1m × 1m × 2m size, fine-meshed polyethylene (PE) net cages (1.25 mm) were fixed in the cages). Outer cage made up of high density polyethylene (HDPE) was used as protection from predators (Predatory net). The net cages were fixed to a bamboo raft. The bamboo raft was used for easy movement, feeding and sampling of the experimental fishes on the cage structure. Sealed and air filled plastic drums of 200 litre size were used as cage float for floating of cage structure. Each cage was covered at the top with a piece of small mesh

size (2.5 cm) net to prevent escape of fish by jumping and predation of birds. The top of portion of each experimental hapa was covered with a fine mesh net up to 20 cm depth to prevent floating feeds from escaping the hapa and the whole structure was tied with anchors at each corner by nylon rope to make easy movement of floating cages depending on water level and flow. The cages were positioned in closed bay 500 m. The submerged volume of the cages was invariably 1 m^3.

3.3.2. Studies on optimum stocking density for Grey mullet seed in floating cages:

Prior to start of the experiment, the transported fish were acclimated with the sea environment by rearing them in hapa net for one week. Fish with an average initial weight of 0.95 ± 0.005 g were randomly stocked in the net cages at 20 fish/ m^3, 40 fish /m^3, 60 fish/m^3, 80 fish/m^3 as D20, D40, D60 and D80, respectively, in triplicates.

3.3.2.1. Feed:

Formulated floating feed with the 25% crude protein was used for feeding. The feed was prepared with groundnut cake (GNC) at 18.82%, fish meal (FM) at 18.82%, de oiled rice bran (DOB) at 30.18%, wheat flour at 30.18% and 2% of vitamin and mineral mixture were added to the diets (Table 2). The diet was estimated for proximate composition (AOAC, 1995).

3.3.2.2. Feeding:

Feed was applied at the rate of 10% of body weight. Fish were fed twice a day at 8:00 hr. and at 16:00 hr. with daily ration divided into two equal halves. Feeding was done manually to ensure ingestion of feed completely by the fish. Fish in each treatment was sampled weekly to obtain weight of fish and the feed amount was adjusted accordingly.

3.4. Studies on optimum % (CP) crude protein for Grey mullet seed in floating cages:

Fish with an average initial weight of 0.95±0.010 g were randomly stocked in the net cages at 40 fish/cage and de-oiled rice bran is used as control feed (C) and four formulated feeds with different protein levels as CP 15% (T1), CP 25% (T2), CP 35% (T3) and CP 45% (T4), respectively were prepared. For each treatment triplicates was maintained.

3.4.1. Feed preparation:

Formulated feed with various crude protein levels were used. Feed formulation was done on the basis of Pearson's square method. Experimental diets were prepared with Ground Nut Cake (GNC), Fish Meal (FM), De Oiled rice Bran (DOB) and wheat flour. 2% of vitamin and mineral mixture were added to experimental diets. De-oiled rice bran was used as control diet (C). Four practical diets were formulated to contain protein levels of 15, 25, 35, and 45% named T1, T2, T3, and T4 respectively (Table 2). All the ingredients like fish meal, groundnut cake, de-oiled rice bran and wheat flour, used in feeds were obtained from local market. Ingredients used in the feed and all the experimental diets were estimated for proximate composition (AOAC, 1995).

3.4.2. Feeding:

A feed ring (0.5 × 0.5 m) was fixed at middle of each cage. Feed rings are enclosures that float at the water surface. They hold floating feeds and prevent the escape of feed out of cage and thus reduce wastage of feed. Feed was applied at the rate of 10% of body weight. Fish were fed twice a day at 8:00 hr. and at 16:00 hr. with each daily ration divided into two equal halves. Feeding was done manually to ensure ingestion of feed completely by the fish.

3.5. Management of cages:

The cages were removed from water at every 15 days interval to check the net and cleaning purpose. Cages were cleaned regularly to remove algae, polychaetes and other organisms. Dead fish were removed from cages immediately and disposed off in a pit. Ancillary works like mending of torn nets and realignment/readjustment of sinkers and anchors were also performed for proper management of cages (Plate. No 8).

3.6. Statistical Analysis:

The data obtained on Growth, Weight Gain, Survival, Feed Conversion Ratio and Protein Efficiency Ratio was treated statistically by applying analysis of variance ANOVA.

3.7. Proximate composition:

Proximate analysis of the feed was estimated by the method of AOAC, 1995.

Table no.1. Proximate composition of the feed ingredients (% on dry matter basis):

Ingredients / *Composition*	Fish meal	Groundnut cake (GNC)	De-oiled rice bran	Wheat flour
Moisture	**7.04**	**8.80**	**8.20**	**5.72**
Crude Protein	**55**	**38.40**	**12.50**	**11.30**
Crude fibre	**3.70**	**7.30**	**22.40**	**00.60**
Ether extract	**4.03**	**7.20**	**3.90**	**4.02**
Total ash	**3.46**	**5.60**	**15.80**	**01.55**
Acid insoluble ash	**3.59**	**5.39**	**5.78**	**3.27**
NFE	**2.01**	**2.21**	**2.42**	**1.23**

Table.2: Feed formulation of the diets for studies on optimum CP% for Grey mullet fry: (Ingredients g/100g):

Dite / ***Composition***	**De-oiled Rice bran CP (12.5)** ***T1 (control)***	**CP (15%)** ***T2***	**CP (25%) T3**	**CP (35%)** ***T4***	**CP (45%)** ***T5***
Fish meal	**_**	**4.0**	**18.82**	**33.19**	**47.0**
GNC	**_**	**4.0**	**18.82**	**33.19**	**47.0**
De oiled rice bran	**98**	**45.0**	**30.18**	**15.81**	**2.0**
Wheat flour	**_**	**45.0**	**30.18**	**15.81**	**2.0**
Vitamin and mineral mix	***2.0***	**2.0**	**2.0**	**2.0**	**2.0**
Total	**100**	**100**	**100**	**100**	**100**

Control T1 = Diet with 12.5% crude protein

T2 = Diet with 15% crude protein

T3 = Diet with 25% crude protein

T4 = Diet with 35% crude protein

T5 = Diet with 45% crude protein

Table.3: Proximate composition of the diets (g/100g dry diet) for studies on optimum CP% for Grey mullet fry:

Feeds / *Composition*	*Control (T1)*	T2 (15%)	T3 (25%)	T4 (35%)	T5 (45%)
Dry matter	**89.90**	**92.81**	**91.91**	**93.11**	**91.81**
Moisture	**8.20**	**07.19**	**08.09**	**06.89**	**08.19**
Crude protein	**12.50**	**14.94**	**24.99**	**35.00**	**44.89**
Crude Fat	**8.11**	**06.14**	**06.37**	**06.32**	**06.13**
Crude fibre	**22.40**	**05.43**	**13.59**	**11.00**	**09.00**
Ash	**15.80**	**02.54**	**02.57**	**02.57**	**02.47**
NFE	**32.99**	**63.76**	**44.39**	**38.22**	***70.78***

3.7.1. Moisture:

A known weight of feed sample was taken and dried in the hot air oven at 105˚C to constant weight and moisture content was calculated by using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Wet weight of the sample} - \text{Dried weight of sample}}{\text{Wet weight of sample}} \times 100$$

3.7.2. Crude Protein:

Nitrogen content of the sample was estimated by Micro kjeldahl method using titration of the means for determining nitrogen percent and then crude protein was estimated by multiplying nitrogen percent by a constant factor 6.25.

$$\text{Crude Protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

3.7.3. Ether extract:

Ether extract was estimated by soxhlet apparatus using petroleum ether as a solvent.

$$\text{Ether extract (\%)} = \frac{\text{Weight of initial sample – weight after extraction}}{\text{Weight of initial sample}} \times 100$$

3.7.4. Crude fibre:

Crude fibre estimation was based on treating the moisture and fat free sample successively with dilute acid (1.25%) and alkali.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of crude fibre}}{\text{Original weight of sample}} \times 100$$

3.8. Assessment of water quality parameters:

Water quality parameters like temperature (Celsius glass thermometer), salinity (Hand held Refractometer), dissolved oxygen (Titrimetric, Winkler's method APHA, 1995) and pH (Digital pH meter) were measured at 8.00 hrs. on weekly basis. Water samples were transported to the laboratory after collection and analysed.

3.8.1. Physico-Chemical parameters of water:

The water quality parameters were observed weekly during the experimental period. Parameters studied during the period are:

SI.NO.	***Parameter***	***Method***
1.	pH	Digital pH meter (model-HI 98107 pHep® HANNA Instruments, Carrollton, TX, USA).
2.	Temperature	Celsius glass thermometer.
3.	Salinity	Hand held Refractometer (Erma, Tokyo).
4.	Dissolved oxygen	Titrimetric Winklers method (APHA, 1995).
5.	Total alkalinity	Titrimetric Winklers method (APHA, 1995).
6.	Ammonia	(APHA, 1995).
7.	Nitrite	(APHA, 1995).
8.	Nitrate	(APHA, 1995).

3.9. Growth performance:

The growth performance of all the fishes of each floating cages was individually estimated by taking their total body length and weight at 7 days interval.

3.10. Weight increment:

Weight increment was obtained by subtracting initial body weight from the final body weight.

Weight increment (gm) = final body weight (gm) – initial body weight (gm).

3.11. Specific growth rate:

Specific growth rate was calculated by the formula

$[(L_n\,FBW - L_n\,IBW) / day]$ **x** 100

FBW -- Final body weight

IBW -- Initial body weight

Ln -- Logarithm

Day -- duration of experiment (63days)

3.12. Survival rate:

Survival of the fishes at each fortnight was noted down and survival rates are calculated as below

$$\text{Survival (\%)} = \frac{\text{Total number of fish survived}}{\text{Total number of fish stocked}} \times 100$$

3.13. Food conversion ratio (FCR):

Food Conversion Ratio was calculated by dividing feed given (dry weight) by body weight gain (wet weight)

$$\text{Food Conversion Ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight (wet weight)}}$$

3.14. Average Daily Weight Gain (ADWG):

$$\text{Average Daily Weight Gain (ADWG)} = \frac{\text{Final fish weight (g)} - \text{Initial fish weight (g)}}{\text{Number of days}}$$

3.15. Biomass:

Biomass = No. of fish × average body weight (g).

Plate no : 2. Experimental site at suryalanka in Guntur district.

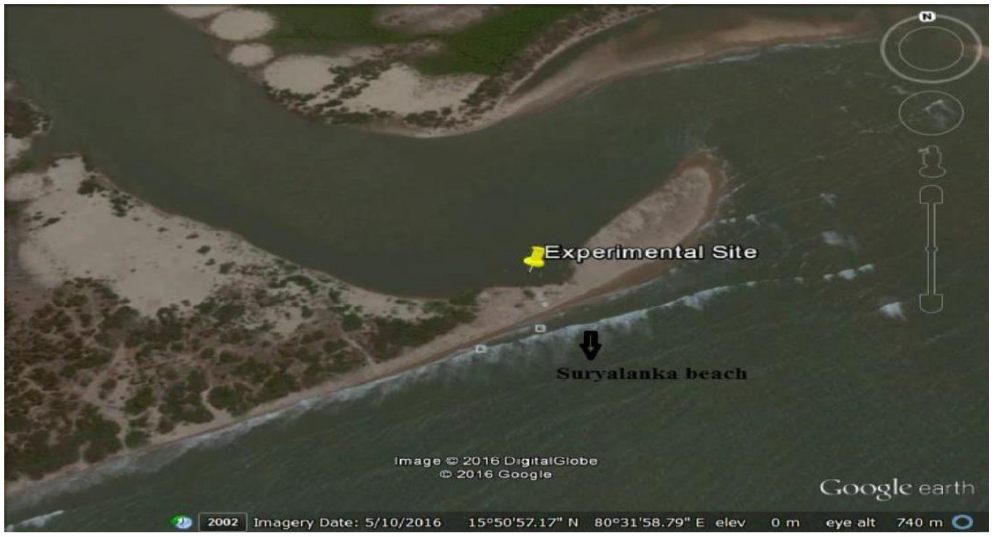


Plate no : 3. Experimental setup at site.

Plate no : 4. Wild seed Grey mullet (*Mugil cephalus*) collection.

Plate no : 5. Seed acclimatization.

Plate no: 6. Experimental Setup

Plate no. 7. Grey Mullet (*Mugil cephalus*) length measurement

Plate no.8. Cage nets checking and cleaning

4. RESULTS

4.1. Water quality parameters:

4.1.1. Grey mullet (Mugil cephalus) with different stocking density, for all floating net cages:

4.1.1.1 Temperature (°C):

The initial day temperature was recorded as 28.5°C. The highest and lowest values of temperature recorded were 32.3°C and 27.5°C respectively. On the 14th day sampling the temperature recorded was 32.3°C which was highest and 56th day sampling the temperature was 27.5°C which was lowest. At the end of the experiment the temperature recorded was 28.9°C. (Table 4)

4.1.1.2 Salinity:

The initial day salinity was recorded as 24 ppt and the highest and lowest values of salinity recorded were 30 ppt for 35th, 49th day and 21 ppt for 14th day. On the initial and 21th day sampling the salinity recorded was same as 24 ppt. At the end of the experiment the salinity was recorded as 28 ppt. (Table 4).

4.1.1.3 p^H :

The Initial day the pH value were recorded as 8.2. The highest and lowest values of pH, recorded during the entire period of experiment were 8.4 (on 28th day) and 7.6 (on 42nd day). On the initial day sampling the pH was recorded (8.2) same as on 56th day sampling. Likewise on the 35th day sampling the pH was recorded (8.0) same as on 63th day sampling. At the end of the experiment the pH was recorded as 8.2. (Table 4).

4.1.1.4 Dissolved Oxygen (mg/l):

The dissolved oxygen was calculated as 6.4 mg/l initially after the fishes were introduced. The highest and lowest values of dissolved oxygen, recorded during the entire period of experiment were 6.5 mg/l (on 1st day) and 4.1 mg/l (on 56th day). At the end of the experiment the dissolved oxygen value was recorded as 4.4 mg/l. (Table 4)

4.1.1.5 Total Alkalinity (mg/l):

The Initial day the alkalinity value was recorded as 137 mg/l. The highest and lowest values of total alkalinity, recorded during the entire period of experiment were 168 mg/l (on 49th day) and 135 mg/l (on 35th day). At the end of the experiment total alkalinity value was recorded as 154 mg/l. (Table 4).

4.1.1.6 Ammonia (mg/l):

The initial day ammonia value recorded was 0.02 mg/l. The highest and lowest values of ammonia, recorded during the entire period of experiment were 0.35 mg/l (on 63rd day) and 0.01 mg/l (on 14th, 21st, and 35th day). At the end of the experiment ammonia value was recorded as 0.35 mg/l. (Table 4).

4.1.1.7 Nitrite (mg/l):

Initially the nitrite value was recorded as 0.02 mg/l and the highest value of nitrite, recorded during the entire period of experiment were 0.03 mg/l (on 21st, 63rd day) and the lowest value 0.01 mg/l (on 7th, 35th day) and 0.02 mg/l (on 1st, 14th, 28th, 42nd , 49th, 56th). (Table 4).

4.1.1.8 Nitrate (mg/l):

Initially the nitrate value was recorded as 2.25 mg/l. The highest and lowest values of nitrate, recorded during the entire period of experiment were 3.85 mg/l (on 21st day) and 1.95 mg/l (on 14th, 49th day). On 35th day and 56th day of sampling the nitrate values recorded were same (3.50 mg/l). (Table 4).

4.1.2 Grey mullet (Mugil cephalus) fed with different % of dietary protein levels, for all floating net cages:

4.1.2.1 Temperature (°C):

The initial day temperature was recorded as 27.9°C. The highest value of temperature recorded was 30.9 °C on 42nd day and lowest was 27.2°C on 35th and 56th day respectively. At the end of the experiment the temperature recorded was 29.5°C. (Table 5)

4.1.2.2 Salinity :

The initial day salinity was recorded as 27 ppt and the highest and lowest values of salinity recorded were 32 ppt on 21st day and 26 ppt on 42nd day. On the initial day sampling the salinity 27 ppt recorded was same as on 14th, 35th and 49th day sampling, likewise on 7th day and 63rd day salinity recorded was same (29 ppt). At the end of experiment salinity recorded was 29 ppt. (Table 5)

4.1.2.3 p^H :

The Initial day the pH value was recorded as 7.8. The highest and lowest values of pH, recorded during the entire period of experiment were 8.3 (on 49th day) and 7.8 (on 1st day). On the 14th day sampling the pH was recorded (7.9) same as on 35th day and likewise on the 21st day sampling the pH was recorded (8.0) same as on 56th day sampling, likewise 42nd day and the last day (63rd day) of the experiment the pH was recorded (8.1) were same. (Table 5).

4.1.2.4 Dissolved Oxygen (mg/l):

The dissolved oxygen was calculated as 6.2 mg/l initially after the fishes were introduced. The highest and lowest values of dissolved oxygen, recorded during the entire period of experiment were 6.2 mg/l (on 1st day) and 4.6 mg/l (on 63rd day). During 7th day the dissolved oxygen recorded (5.2) was same as 49th day sampling. (Table 5).

4.1.2.5 Total Alkalinity (mg/l):

Initially the total alkalinity value recorded was 145 mg/l. The highest and lowest values of total alkalinity, recorded during the entire period of experiment were 163 mg/l (on 63rd day) and 139 mg/l (on 2nd day). At the end of the experiment total alkalinity value recorded was 163 mg/l. (Table 5)

4.1.2.6 Ammonia (mg/l):

The ammonia value initially recorded was 0.01 mg/l. The highest and lowest values of ammonia, recorded during the entire period of experiment were 0.25 mg/l (on 21st and 56th day) and 0.01 mg/l (on 1st , 42nd and 49th day). At the end of the experiment ammonia value recorded was 0.02 mg/l. (Table 5).

4.1.2.7 Nitrite (mg/l):

Initially the nitrite value was recorded as 0.02 mg/l and the highest and lowest values of nitrite, recorded during the entire period of experiment were 0.03 (on 7th , 28th , and 42nd day) and 0.01 mg/l (on 14th , 35th , and 49th day). (Table 5).

4.1.2.8 Nitrate (mg/l):

Initially the nitrate value was recorded as 2.85 mg/l. The highest and lowest values of nitrate, recorded during the entire period of experiment were 3.26 mg/l (on 21th day) and 2.15 mg/l (on 14th day). At the end of the experiment nitrate value recorded was 3.20 mg/l. (Table 5).

4.2. Growth parameters:

4.2.1. Grey mullet (Mugil cephalus) with different stocking density in floating net cages:

4.2.1.1. Growth performance:

Over all weight gain of the fish in grams were observed 63 experimental days for different treatments and they were presented in the Tables 6, 7 and figure 17.The Initial weight of the fish in different treatments were recorded between 0.94g and 0.96g. Observations on the growth of Mugil cephalus during the last experimental day (63rd day) revealed that oveall average weight gain varied between 11.19g and 9.07g for the D20 and D80 respectively. On the final day of the experiment average highest weight increment was observed in the D20 (11.19g), followed by D40 (11.08g), D60 (10g) and the lowest was observed in the D80 (9.07g), they stood in first, second, third, and fourth positions respectively.

The growth data was subjected to Analysis of Variance (ANOVA) at 1% and 5% level of significance and the observations are given in table 7. The statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair wise comparison of any one Treatment could be done by computing CRD one way classification. The Treatment D20 ($20/m^3$) is found to be significantly superior when compare to other Treatments. Treatment D20 has shown significantly different from all other treatments. The second, third and fourth positions occupied by D40, D60, and D80 respectively. There was a significant difference between the culture periods also.

4.2.1.2. Survival:

Survival percentages of Grey mullet (Mugil cephalus) in various experimental treatments are presented in the Table 8 and figure 18. Overall survival observed for the 63 experimental days. At the end of the experiment the highest average survival was observed in D20 (76.66%) and lowest survival was recorded in D20 (70%). The treatments D60 and D40 stood second and third respectively.

The survival data was subjected to Analysis of Variance (ANOVA) and presented in the Table 9. Statistical analysis has shown that F- value is found to be non-significant among treatments.

4.2.1.3. Feed Conversion Ratio (FCR):

Feed Conversion Ratios in different treatments of Grey mullet (Mugil cephalus) with different stocking densities were calculated and are presented in the Table 10 and in Figure 19. The treatments D20, D40 were recorded average FCR was 2.31, D60 recorded 2.50 and D80 recorded 2.55.

The Feed Conversion Ratio was subjected to Analysis of Variance (ANOVA) and presented in the Table 11. Statistical analysis has shown that F- value is found to be non-

significant among treatments. In the present study there is no significant difference between FCR and stocking density in grey mullet.

4.2.1.4. Specific Growth Rate (SGR):

Specific growth rate for Grey mullet (Mugil cephalus) with different stocking densities was calculated and presented in the Table 12 and Figure 20. The specific growth rate was calculated for the total experimental period (i.e. 63 days). The maximum average SGR was recorded in the D40 (1.75) and the minimum average SGR was observed in the D80 (1.63). The other treatments D20 and D60 recorded SGR of 1.74 and 1.67.

4.2.1.5. Average Daily Weight Gain (ADGR):

The maximum ADGR was recorded in D20 (0.177) and the minimum ADGR was observed in D80 (0.143). The other treatments D40 and D60 recorded ADGR of 0.175 and 0.158. Observations are given in table 14.

4.2.1.6. Biomass:

The maximum biomass was recorded in D80 (560.5) and the minimum biomass was observed in D20 (194.4). The other treatments D40 and D60 recorded biomass of 360.9 and 460.3. Observations are given in table 15.

4.2.2. Grey mullet fed with different % of crude protein (CP) level diets:

4.2.3.1. Growth performance:

Over all Weight gain of Mugil cephalus in grams for different treatments at different protein percentages were presented in table 16 and figure 21.

The highest average weight gain values (12.69g) and lowest average weight gain values (6.73g) were recorded for both treatments T3 (25% CP) and T1(Control) respectively at the end of the 63 experimental days.

The treatments T4 (11.08g); T5 (8.90g) and T2 (7.91g), they stood second, third and fourth positions respectively. At the end of the experiment the highest weight values

was observed in T3 (13.64g) and lowest value was recorded in T1 (Control) (7.68g). The treatments T4 (12.02g); T5 (9.86g) and T2 (7.68g), they stood second, third and fourth respectively.

The growth data was subjected to analysis of variance (ANOVA) at 1% and 5% level of significance and the observations are given in the tables 18, 20. The statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair wise comparison of any one treatment could be done by computing CRD one way classification. The Treatment Crude Protein 25% (T3) is found to be significantly superior when compare to other treatments. The second, third, fourth, and fifth positions were occupied by Crude Protein 35% (T4), Crude Protein 45% (T5) and Crude protein 15% (T2) and Crude protein 12.5% (T1) respectively. There was a significant difference between the culture periods also.

4.2.3.2. Survival:

Overall Survival of Grey mullet in various experimental treatments are presented in the Table 18, and in Figure 22. Observations on 63 experimental days at the end of the experiment the highest average survival was observed in T3 (83.33%) and lowest survival was recorded in remain all treatments T1, T2, (70.83%).

The survival data was subjected to Analysis of Variance (ANOVA) and presented in the Table 19. Statistical analysis has shown that F- value is found to be significant among treatments at 1% and 5% level of significance. Since F- value is found to be significant, the pair wise comparison of any one treatment could be done by computing CRD one way classification. The treatment T3 (25%CP) is found to be significantly superior when compare to other Treatments.

4.2.3.3. Feed Conversion Ratio (FCR):

Feed Conversion Ratios in different treatments of Grey mullet fed with different experimental diets were calculated and are presented in the Table 20 and in Figure 23.Over all at the end of the 63 experimental days the least average FCR was recorded for T3 (2.14) and the highest FCR for T1(3.06).

The Feed Conversion Ratio was subjected to Analysis of Variance (ANOVA) and presented in the Table 21. Statistical analysis has shown that F- value is found to be significant among treatments at 1% and 5% level of significance. Since F- value is found to be significant, the pair- wise comparison of any one treatment could be done by computing CRD one way classification. Treatment T3 was found to be significantly lower FCR when compared to the other Treatments. Treatment T1(control), T2, T5, T4 and T3- occupied first, second, third, fourth and fifth positions.

4.2.3.5. Specific Growth Rate (SGR):

Specific growth rate for Grey mullet (Mugil cephalus) fed with different experimental diets were calculated and presented in the Table 22 and Figure 24. The specific growth rate was calculated for the total experimental period (i.e. 63 days). The maximum SGR was recorded in the T3 (1.83) and the minimum SGR was observed in the T1 (Control) (1.43). The other treatments SGR is T4 (1.75), T5 (1.60) and T5 (1.54).

4.2.4.6. Average Daily Weight Gain (ADGR):

The maximum ADGR was recorded in T3 (0.225) and the minimum ADGR was observed in T1 (control) (0.156). The other treatments T2, T4and T5 recorded ADGR of 0.189, 0.204 and 0.168. Observations are given in table 22.

4.2.5.7. Biomass:

The maximum biomass was recorded in T3 (606.8) and the minimum biomass was observed inT1 (control) (432.8). The other treatments T2, T4 and T5 recorded biomass of 514.4, 552 and 462.4. Observations are given in table 23.

Table .4 Water quality parameters in floating net cages with Grey mullet for different stocking densities (D20 to D80):

Parameters / days	Temperature (°C)	Salinity (ppt)	pH	DO (mg/l)	Total alkalinity (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)
initial	**28.5**	**24**	**8.2**	**6.5**	**137**	**0.02**	**0.02**	**2.25**
7	**27.9**	**22**	**8.1**	**5.1**	**140**	**0.25**	**0.01**	**2.15**
14	**32.3**	**21**	**7.9**	**4.9**	**145**	**0.01**	**0.02**	**1.95**
21	**29.4**	**24**	**7.8**	**4.5**	**154**	**0.01**	**0.03**	**3.85**
28	**27.8**	**26**	**8.4**	**5.6**	**138**	**0.25**	**0.02**	**3.65**
35	**30.2**	**30**	**8.0**	**5.8**	**135**	**0.01**	**0.01**	**3.50**
42	**27.6**	**29**	**7.6**	**4.9**	**150**	**0.25**	**0.02**	**2.85**
49	**29.5**	**30**	**8.3**	**5.2**	**168**	**0.24**	**0.02**	**1.95**
56	**27.5**	**27**	**8.2**	**4.1**	**160**	**0.02**	**0.02**	**3.50**
63	**28.9**	**28**	**8.0**	**4.4**	**154**	**0.35**	**0.03**	**3.65**

Experiment no-2

Table .5 Water quality parameters in all floating net cages with Grey mullet fed with different % of dietary protein levels:

Parameters / days	Temperature (°C)	Salinity (ppt)	pH	DO (mg/l)	Total alkalinity (mg/l)	Ammonia (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)
initial	**27.9**	**27**	**7.8**	**6.2**	**145**	**0.01**	**0.02**	**2.85**
7	**28.7**	**29**	**8.2**	**5.2**	**139**	**0.03**	**0.03**	**3.00**
14	**29.3**	**27**	**7.9**	**5.8**	**148**	**0.02**	**0.01**	**2.15**
21	**29.5**	**32**	**8.0**	**6.1**	**156**	**0.25**	**0.02**	**3.26**
28	**30.1**	**28**	**8.1**	**4.8**	**160**	**0.05**	**0.03**	**3.20**
35	**27.2**	**27**	**7.9**	**5.1**	**158**	**0.02**	**0.01**	**2.28**
42	**30.9**	**26**	**8.1**	**4.7**	**145**	**0.01**	**0.03**	**2.45**
49	**30.1**	**27**	**8.3**	**5.2**	**160**	**0.01**	**0.01**	**3.10**
56	**27.2**	**30**	**8.0**	**5.5**	**161**	**0.25**	**0.02**	**2.28**
63	**29.5**	**29**	**8.1**	**4.6**	**163**	**0.02**	**0.02**	**3.20**

	D20			D40			D60			D80		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Initial weight	0.96	0.97	0.96	0.95	0.96	0.95	0.96	0.97	0.96	0.94	0.95	0.94
Final weight	12.13	12.16	12.18	12.01	12.04	12.05	10.94	10.96	10.99	10.05	10.02	9.98
Growth	11.17	11.19	11.22	11.06	11.08	11.10	9.98	9.99	10.03	9.11	9.07	9.04

Table .06: Growth performance (g) Grey mullet (Mugil chephalus) at differentstocking densities:

Table: 07 Statistical analysis for growth performance (g) of Grey mullet (Mugil chephalus) at different stocking densities:

Treatment means	
S.No	Average
Treatment 1	11.193
Treatment 2	11.080
Treatment 3	10.000
Treatment 4	9.073

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	8.983	2.997	4039.259	4.663
Error	8	0.009	0.007	-	-
Total	11	-	-	-	-

Coefficient of Variation = 0.264

Treatments found Significant at 1% and 5% level of significance
CD(0.01) = 0.076 CD(0.05) = 0.052

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3	T 4
Treatment Average	11.193	11.080	10.000	9.073
Critical Difference (CD) Compared	a	b	c	d

Table .08: Survival rate of Grey mullet (*Mugil chephalus*) at different stocking densities:

	D20			***D40***			***D60***			***D80***		
	R1	***R2***	***R3***	***R1***	***R2***	***R3***	***R1***	***R2***	***R3***	***R1***	***R2***	***R3***
Initial	***20***	***20***	***20***	***40***	***40***	***40***	***60***	***60***	***60***	***80***	***80***	***80***
Final	***15***	***16***	***15***	***32***	***30***	***28***	***40***	***44***	***43***	***53***	***58***	***57***
Survival rate(%)	***75***	***80***	***75***	***80***	***75***	***70***	***66.66***	***73.33***	***71.66***	***66.25***	***72.50***	***71.25***

Table: 09 Statistical analysis for survival of Grey mullet (Mugil chephalus) at different stocking densities:

Treatment means	
S.No	**Average**
Treatment 1	76.666
Treatment 2	75.000
Treatment 3	70.550
Treatment 4	70.000

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	3	97.306	32.432	2.307	0.156
Error	8	112.632	14.072	-	-
Total	11	-	-	-	-

Coefficient of Variation = 5.132

Treatments found to be Non Significant

Table .10: Feed Conversion Ratio of Grey mullet (Mugil chephalus) at different stocking densities:

	D20			D40			D60			D80		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
FCR	2.23	2.42	2.28	2.26	2.41	2.26	2.30	2.40	2.80	2.30	2.55	2.80

Table: 11 Statistical analysis for Feed Conversion Ratio in Grey mullet (*Mugil chephalus*) at different stocking densities:

Treatment means	
S.No	Average
Treatment 1	2.310
Treatment 2	2.310
Treatment 3	2.500
Treatment 4	2.550

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	3	0.144	0.044	1.265	0.347
Error	8	0.294	0.034	-	-
Total	11	-	-	-	-

Coefficient of Variation = 8.002

Treatments found to be Non Significant

Table .12: Specific Growth Rate of Grey mullet (*Mugil chephalus*) at different stocking densities:

	D20			D40			D60			D80		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
SGR	1.75	1.75	1.73	1.76	1.74	1.75	1.65	1.67	1.68	1.62	1.63	1.65

Table: 13 Statistical analysis for Specific Growth Rate of Grey mullet (Mugil chephalus) at different stocking densities:

Treatment means	
S.No	Average
Treatment 1	1.743
Treatment 2	1.750
Treatment 3	1.666
Treatment 4	1.633

Anova Table					
Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F cal	F prob
Treatments	3	0.027	0.009	56.694	9.838
Error	8	0.003	0.001	-	-
Total	11	-	-	-	-

Coefficient of Variation = 0.779

Treatments found Significant at 1% and 5% level of significance
CD(0.01) = 0.032 CD(0.05) = 0.029

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3	T 4
Treatment Average	1.743	1.750	1.666	1.633
Critical Difference (CD) Compared	a	a	b	c

Table:14: Average Daily Weight Gain of Grey mullet (*Mugil cephalus*) at different stocking densities:

TREATMENTS	D20	D40	D60	D80
Initial average body weight (g)	0.96	0.95	0.96	0.94
Final average body weight (g)	12.15	12.03	10.96	10.01
ADGR	0.177	0.175	0.158	0.143

Table:15: Biomass of Grey mullet (*Mugil cephalus*) at different stocking densities fed with 25% crude protein:

TREATMENTS	D20	D40	D60	D80
Initial biomass(g)	19.2	38	57.6	75.2
Final biomass(g)	194.4	360.9	460.3	560.5

Experiment No: 2

Table .16: Growth performance (g) Grey mullet (Mugil chephalus) fed with different % of crude protein (CP) experimental diets:

	T1			T2			T3			T4			T5		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Initial wt	0.96	0.95	0.96	0.95	0.94	0.95	0.96	0.95	0.95	0.94	0.94	0.95	0.96	0.95	0.96
Final wt	7.69	7.66	7.71	8.85	8.83	8.89	13.61	13.69	13.63	12.03	12.01	12.04	9.76	10.06	9.76
Total weight gain	6.73	6.71	6.75	7.90	7.89	7.94	12.65	12.74	12.68	11.09	11.07	11.09	8.80	9.11	8.80

Table. 17:Statistical analysis for Growth performance (g) of Grey mullet (*Mugil chephalus*) at different % of crude protein (CP) experimental dites:

Treatment means	
S.No	**Average**
Treatment 1	6.730
Treatment 2	7.910
Treatment 3	12.690
Treatment 4	11.083
Treatment 5	8.903

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	4	69.700	17.425	2463.478	6.420
Error	10	0.077	0.000	-	-
Total	14	-	-	-	-

Coefficient of Variation = 0.887

Treatments found Significant at 1% and 5% level of significance

CD(0.01) = 0.216 CD(0.05) = 0.159

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3	T 4	T 5
Treatment Average	6.730	7.910	12.690	11.083	8.903
Critical Difference (CD) Compared	a	b	c	d	e

Table .18: Survival rate of Grey mullet (*Mugil chephalus*) fed with different % of crude protein:

	T1			T2			T3			T4			T5		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
Initial	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
survilval	28	29	28	28	27	30	33	34	33	30	29	27	29	29	28
Survival Rate (%)	70	72.5	70	70	67.5	75	82.5	85	82.5	75	72.5	67.5	72.5	72.5	70

Table. 19: Statistical analysis for survival rate of Grey mullet (*Mugil chephalus*) fed with different % of crude protein:

Treatment means	
S.No	**Average**
Treatment 1	70.833
Treatment 2	70.833
Treatment 3	83.333
Treatment 4	71.666
Treatment 5	71.666

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	4	352.500	88.125	12.441	0.006
Error	10	70.833	7.083	-	-
Total	14	-	-	-	-

Coefficient of Variation = 3.618

Treatments found Significant at 1% and 5% level of significance
CD(0.01) = 6.884 CD(0.05) = 4.845

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3	T 4	T 5
Treatment Average	70.833	70.833	83.333	71.666	71.666
Critical Difference (CD) Compared	a	b	b	b	b

Table .20: Feed Conversion Ratio of Grey mullet (Mugil chephalus) fed with different % of crude protein (CP) experimental dites:

	T1			T2			T3			T4			T5		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
	3.06	3.07	3.06	2.56	2.68	2.74	2.12	2.18	2.13	2.28	2.37	2.40	2.99	2.49	2.99

Table. 21: Statistical analysis for Feed Conversion Ratio of Grey mullet (Mugil chephalus) fed with different % of crude protein (CP) experimental dites:

Treatment means	
S.No	**Average**
Treatment 1	3.063
Treatment 2	2.660
Treatment 3	2.143
Treatment 4	2.350
Treatment 5	2.823

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	4	1.616	0.401	20.896	7.618
Error	10	0.194	0.013	-	-
Total	14	-	-	-	-

Coefficient of Variation = 5.333

Treatments found Significant at 1% and 5% level of significance
CD(0.01) = 0.358 CD(0.05) = 0.259

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3	T 4	T 5
Treatment Average	3.063	2.660	2.143	2.350	2.823
Critical Difference (CD) Compared	a	ab	b	c	c

Table .22: Specific Growth Rate of Grey mullet (Mugil chephalus) fed with different % of crude protein (CP) experimental diets:

	T1			T2			T3			T4			T5		
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
SGR	1.41	1.47	1.42	1.55	1.52	1.55	1.80	1.84	1.85	1.71	1.73	1.81	1.40	1.60	1.80

Table: 21: Statistical analysis for Specific Growth Rate of Grey mullet (Mugil chephalus) fed with different % of crude protein (CP) experimental diets:

Treatment means	
S.No	**Average**
Treatment 1	1.433
Treatment 2	1.540
Treatment 3	1.830
Treatment 4	1.750
Treatment 5	1.600

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Treatments	4	0.302	0.075	8.539	0.009
Error	10	0.086	0.009	-	-
Total	14	-	-	-	-

Coefficient of Variation = 5.809

Treatments found Significant at 1% and 5% level of significance
CD(0.01) = 0.240 CD(0.05) = 0.172

Comparison of Treatment Means with Critical Difference (0.05)

Treatment No.	T 1	T 2	T 3	T 4	T 5
Treatment Average	1.433	1.540	1.830	1.750	1.600
Critical Difference (CD) Compared	a	ab	bc	c	c

Table:22: Average Daily Weight Gain of Grey mullet (Mugil cephalus) at fed with different % of crude protein (CP) experimental diets:

TREATMENTS	T1 (Control)	T2 (CP-15%)	T3 (CP-25%)	T4 (CP-35%)	T5 (CP-45%)
Initial average body weight (g)	**0.96**	**0.94**	**0.95**	**0.94**	**0.96**
Final average body weight (g)	**10.82**	**12.86**	**15.17**	**13.80**	**11.56**
ADGR	**0.156**	**0.189**	**0.225**	**0.204**	**0.168**

Table: 23: Biomass of Grey mullet (Mugil cephalus) at fed with different % of crude protein (CP) experimental diets:

TREATMENTS	T1 (Control)	T2 (CP-15%)	T3 (CP-25%)	T4 (CP-35%)	T5 (CP-45%)
Initial biomass(g)	**38.4**	**37.6**	**38**	**37.6**	**38.4**
Final average body weight (g)	**432.8**	**514.4**	**606.8**	**552**	**462.4**

Water quality parameters in floating net cages with Grey mullet for different stocking densities (D20 to D80):

Fig. 1 Weekely variation of temperature °C in floating net cages with grey mullet (*Mugil cephalus*) for all stocking densities

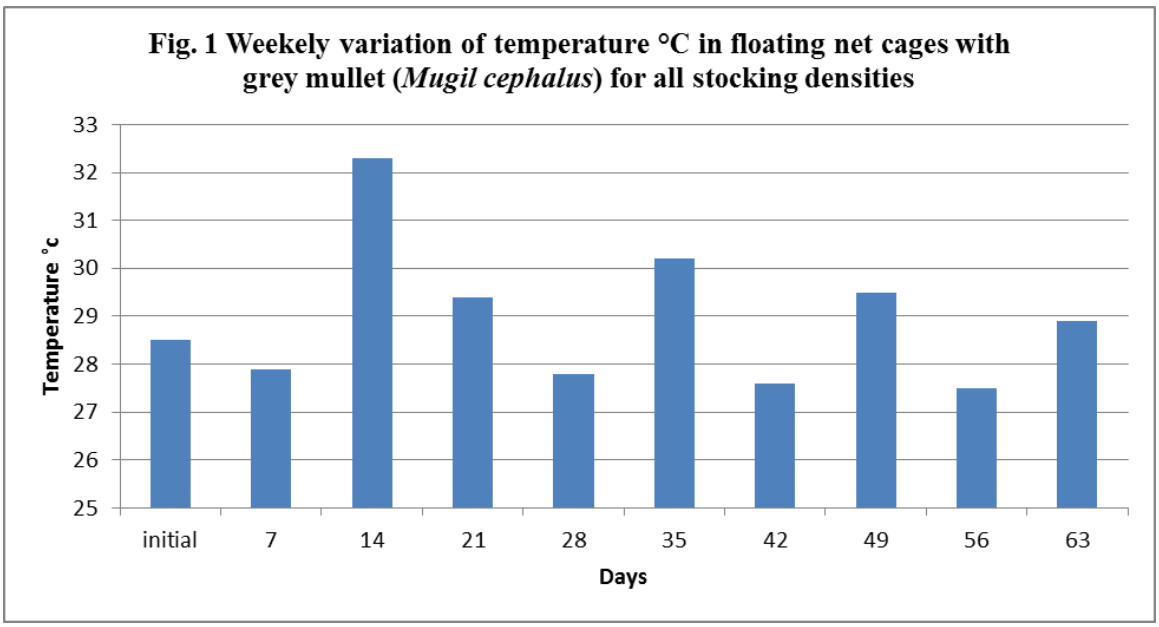


Fig.2 Weekely variation of salinity (ppt) in floating net cages with Grey mullet (*Mugil cephalus*) for all stocking densities

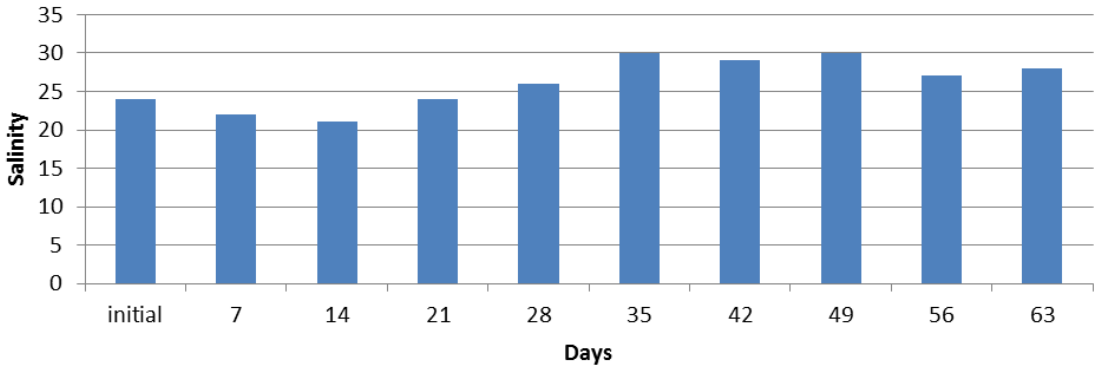


Fig.3 weekly variation of pH in floating net cages with grey mullet (*Mugil cephalus*) for all stocking densities

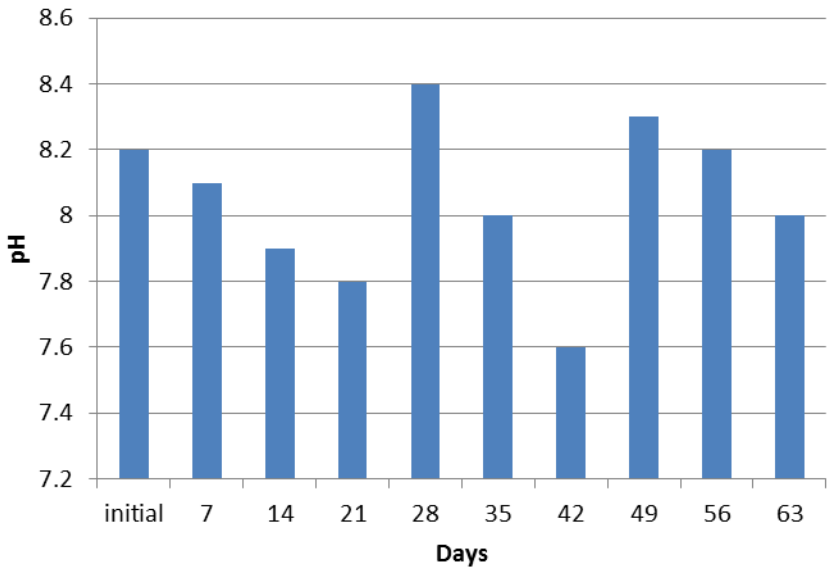


Fig.4 Weekely variation of DO (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) for all stocking densities

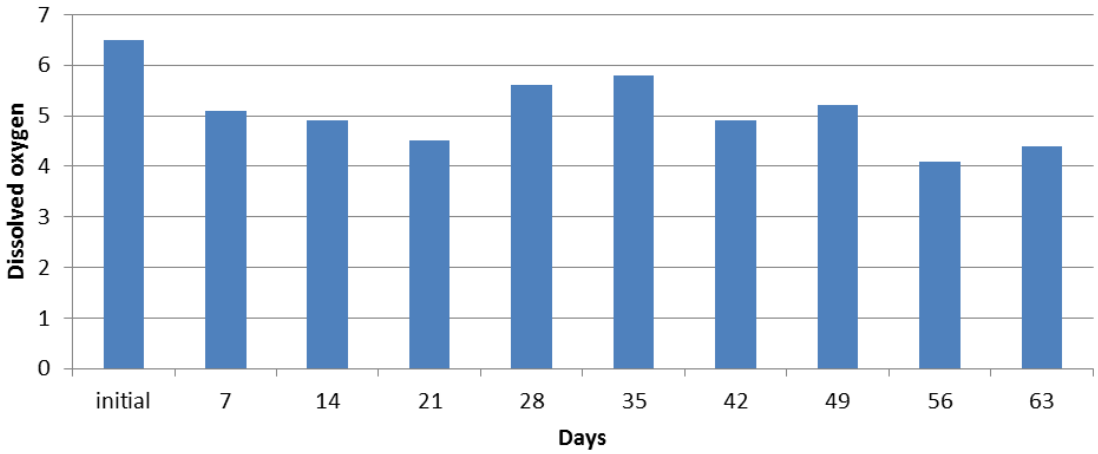


Fig.5 Weekely variation of Total alkalinity (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) for all stocking densities

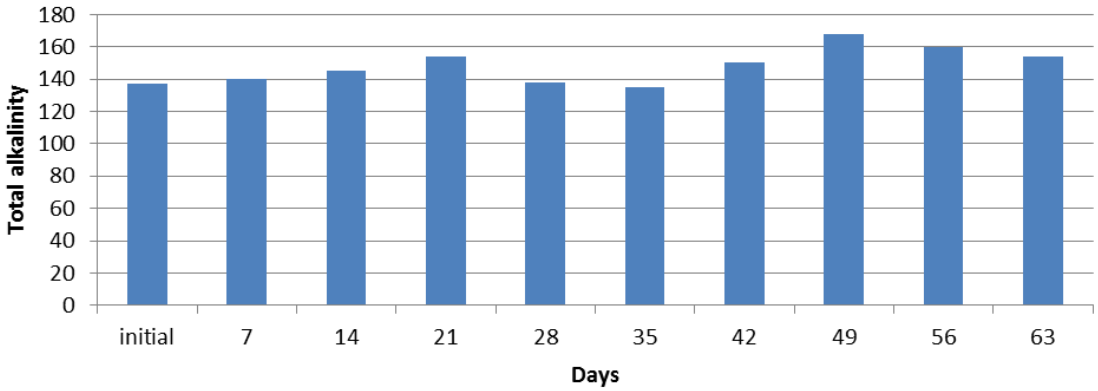


Fig.6 Weekely variation of Ammonia (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) for all stocking densities

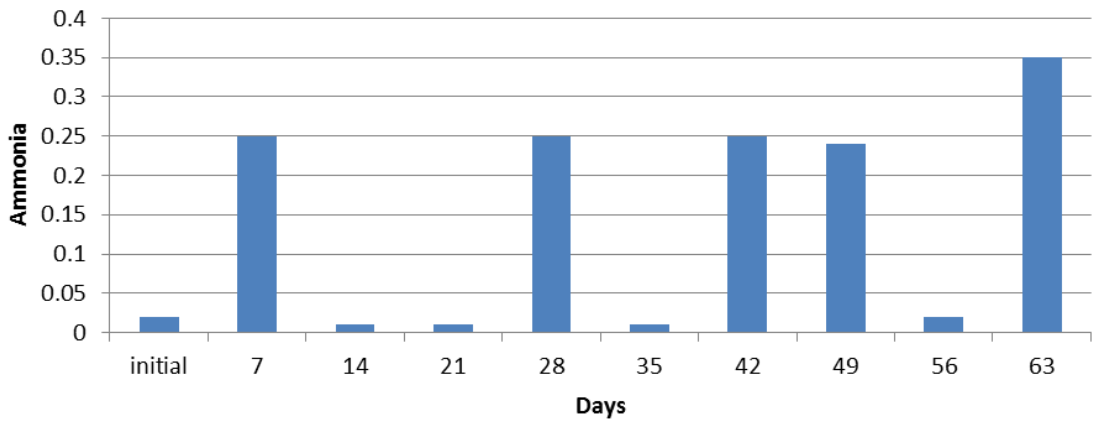


Fig.7 Weekely variation of Nitrite (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) for all stocking densities

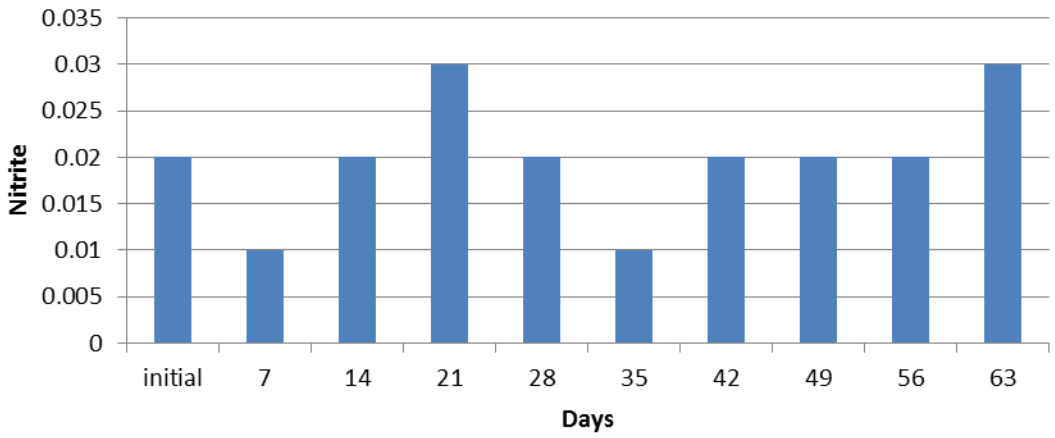
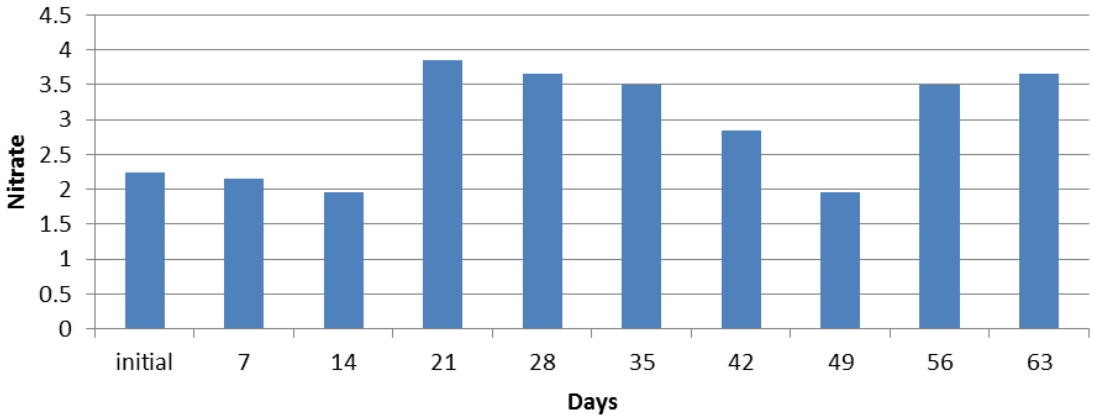


Fig.8 Weekely variation of Nitrate (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) for all stocking densities



Experiment no-2

Water quality parameters in all floating net cages with Grey mullet fed with different % of dietary protein levels:

Fig. 9 Weekely variation of temperature (°C) in floating net cages with Grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels:

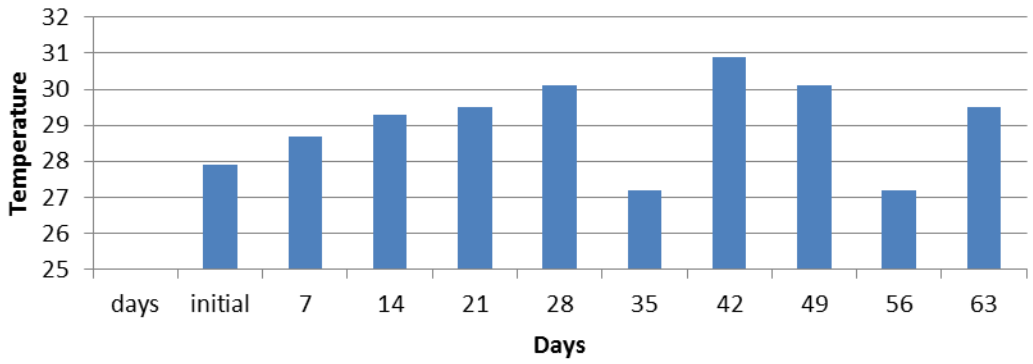


Fig. 10 Weekely variation of Salinity (ppt) in floating net cages with Grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels:

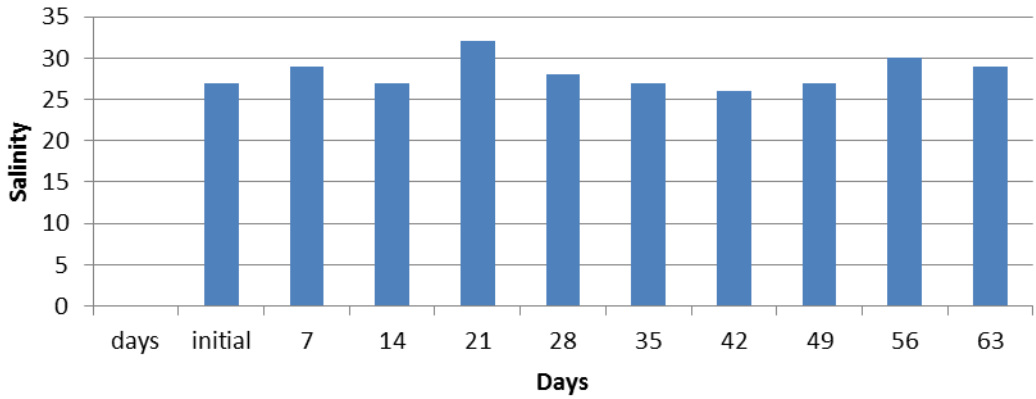


Fig. 11 Weekely variation of pH in floating net cages with Grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels:

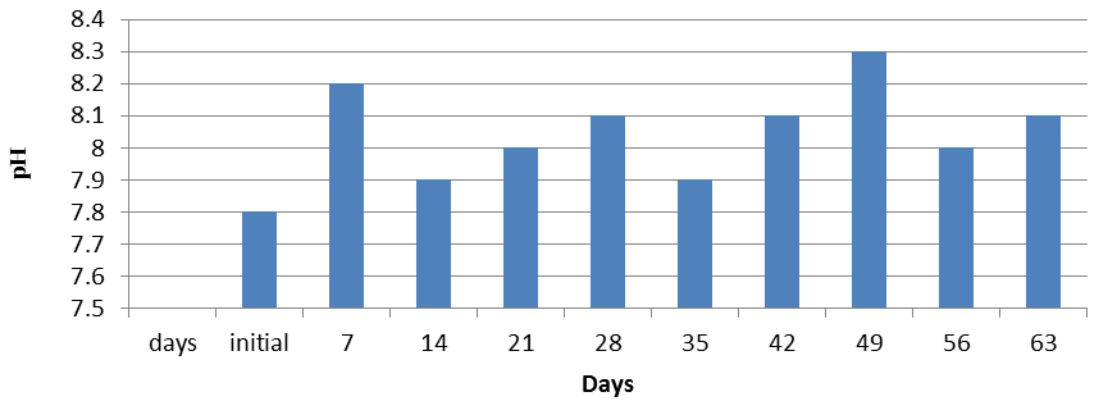


Fig. 12 Weekely variation of DO (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels:

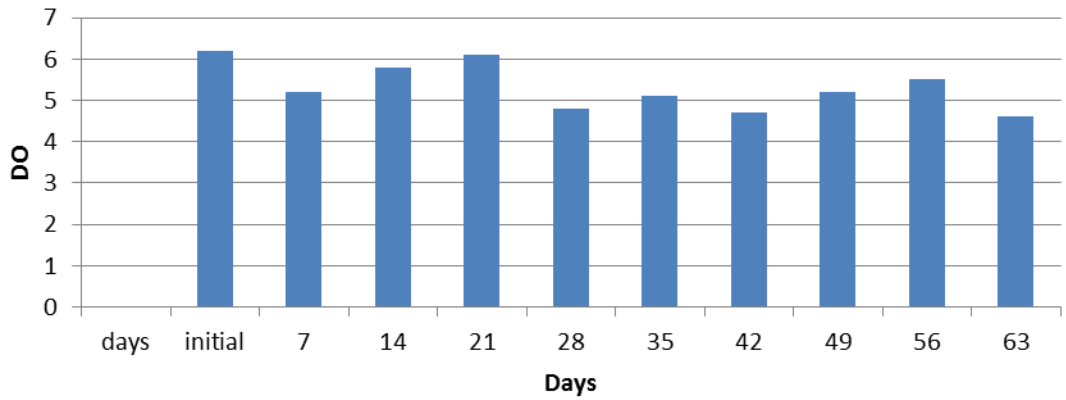


Fig. 13 Weekely variation of Total alkalinity (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels:

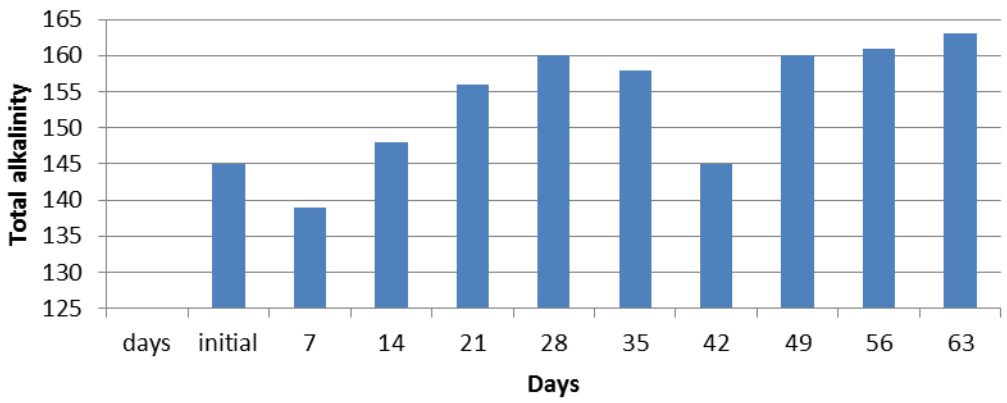


Fig. 14 Weekely variation of Ammonia (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels:

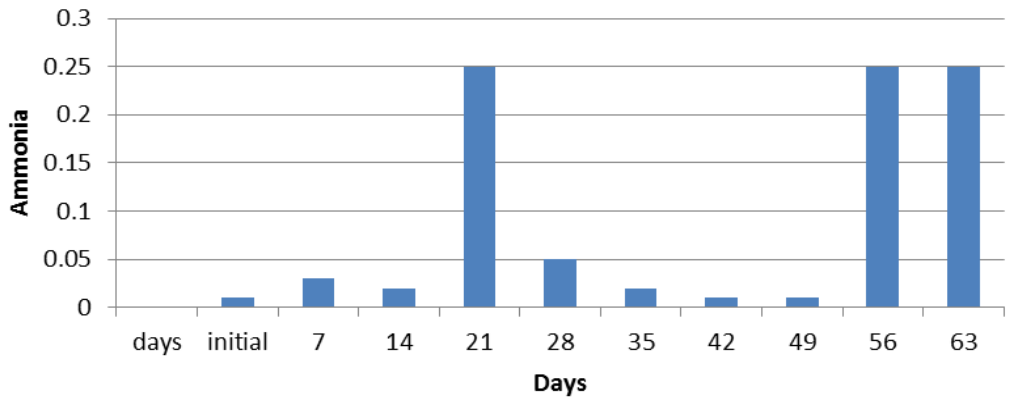


Fig. 15 Weekely variation of Nitrite (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) fedwith different % of dietary protein levels:

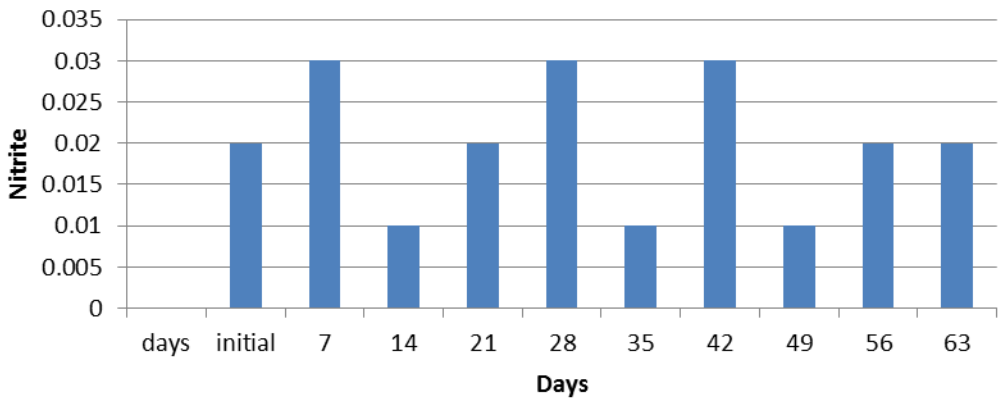
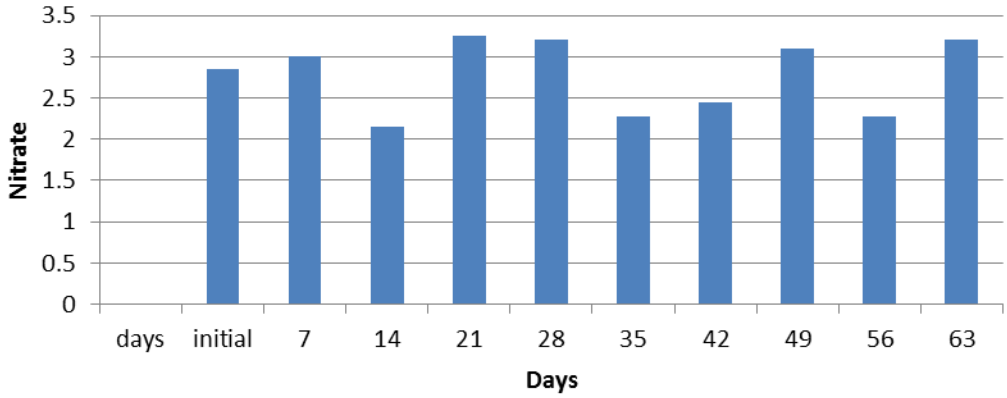


Fig. 16 Weekely variation of Nitrate (mg/l) in floating net cages with Grey mullet (*Mugil cephalus*) fed with different % of dietary protein levels:



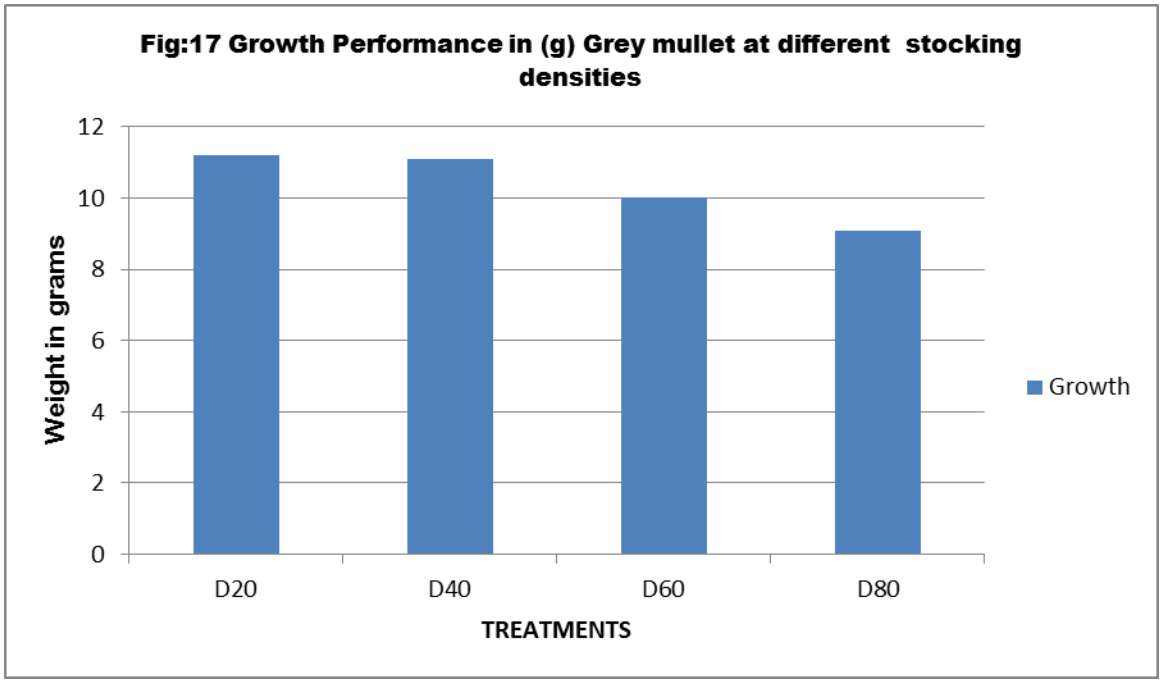


Fig:17 Growth Performance in (g) Grey mullet at different stocking densities
12
10
8
6
4
2
0
Weight in grams
D20
D40
D60
D80
TREATMENTS
Growth

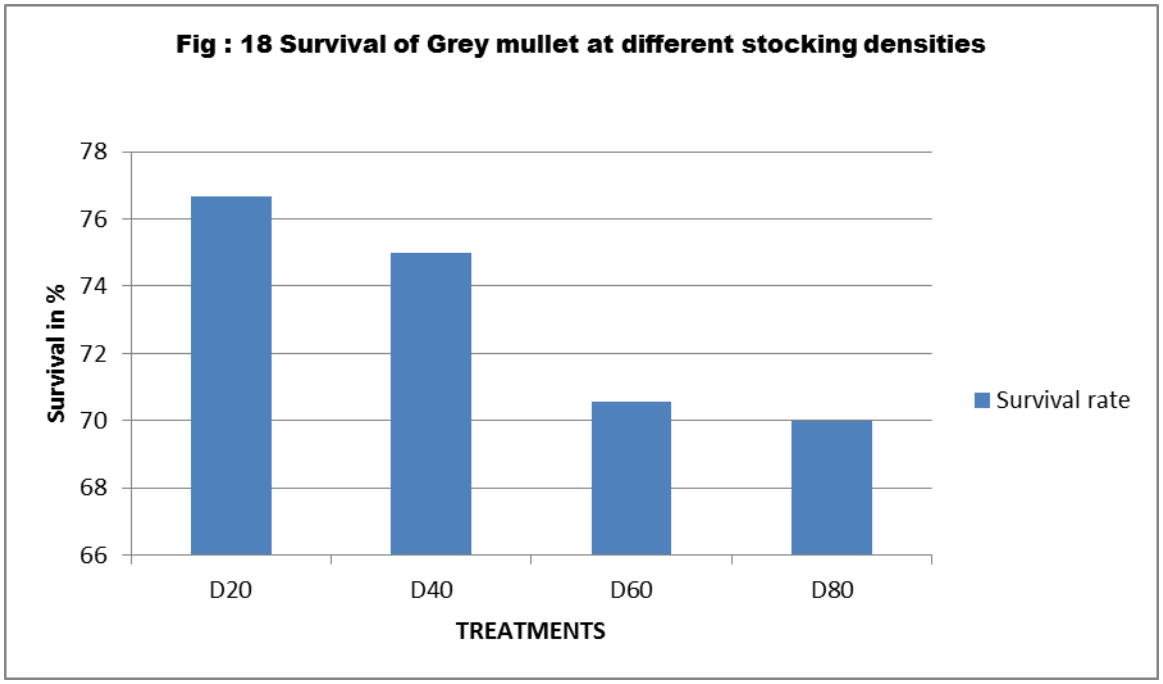


Fig : 18 Survival of Grey mullet at different stocking densities
78
76
74
72
70
68
66
Survival in %
D20
D40
D60
D80
TREATMENTS
Survival rate

Fig :19 Feed conversion ratio in Grey Mullet at Different Stocking Densities

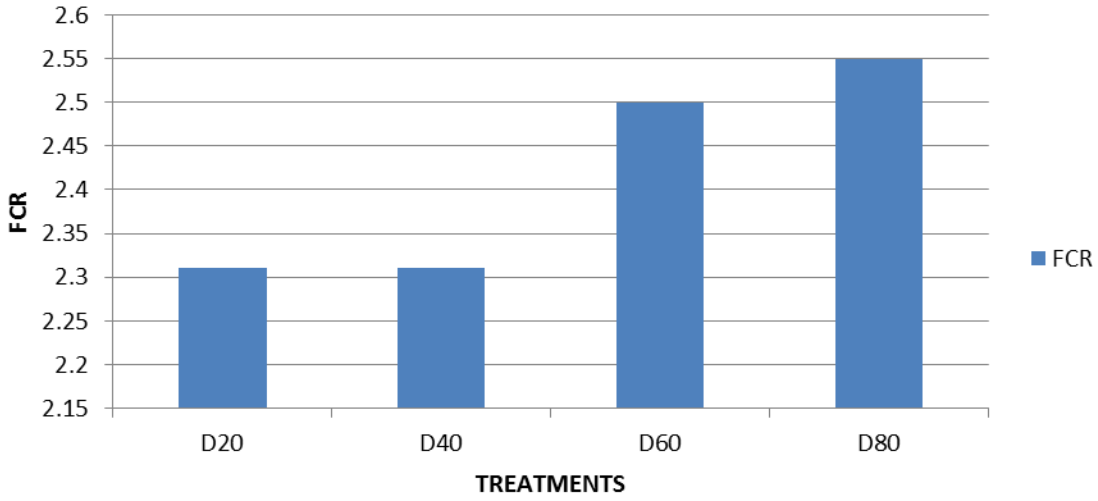


Fig :20 Specific growth rate of grey mullet at different stocking densities

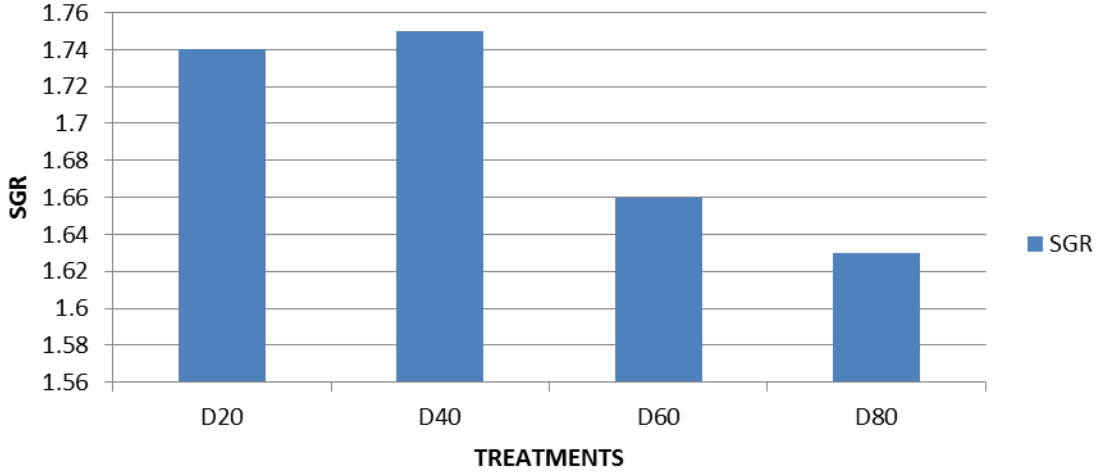


Fig: 21 Growth performance of (g) Grey mullet fed with different % of crude protein (CP) experimental dites

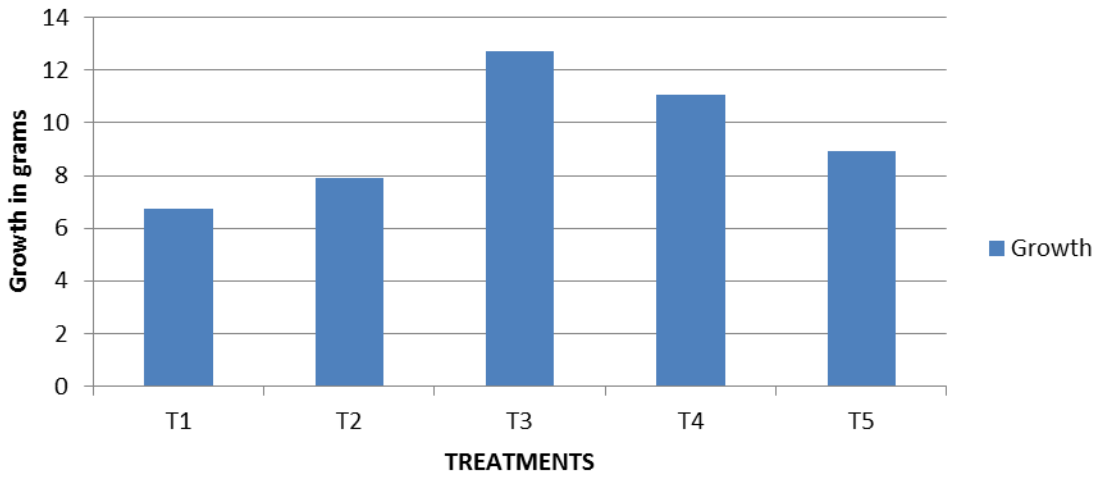


Fig: 22 Survival of Grey mullet fed with different % of crude protein (CP) experimental dites

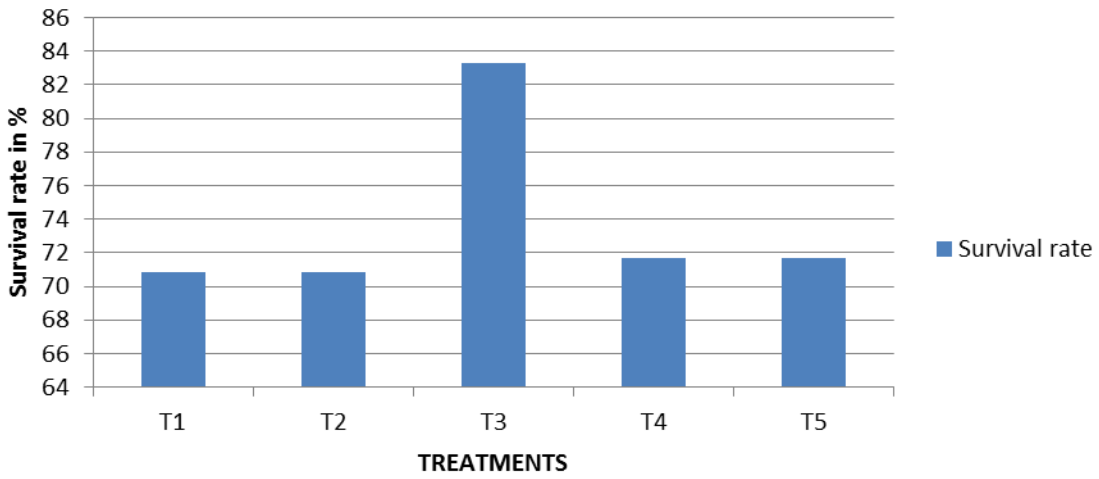
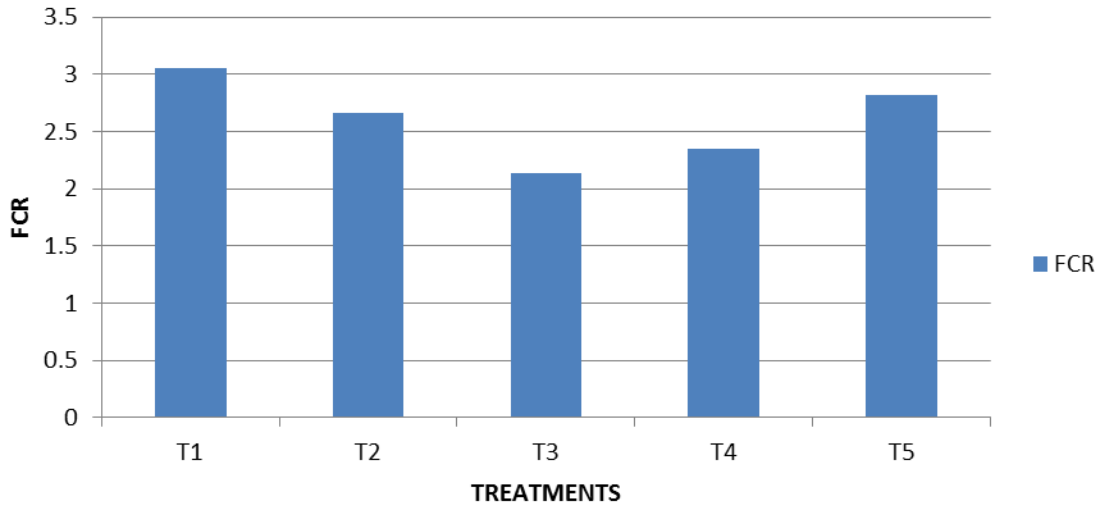
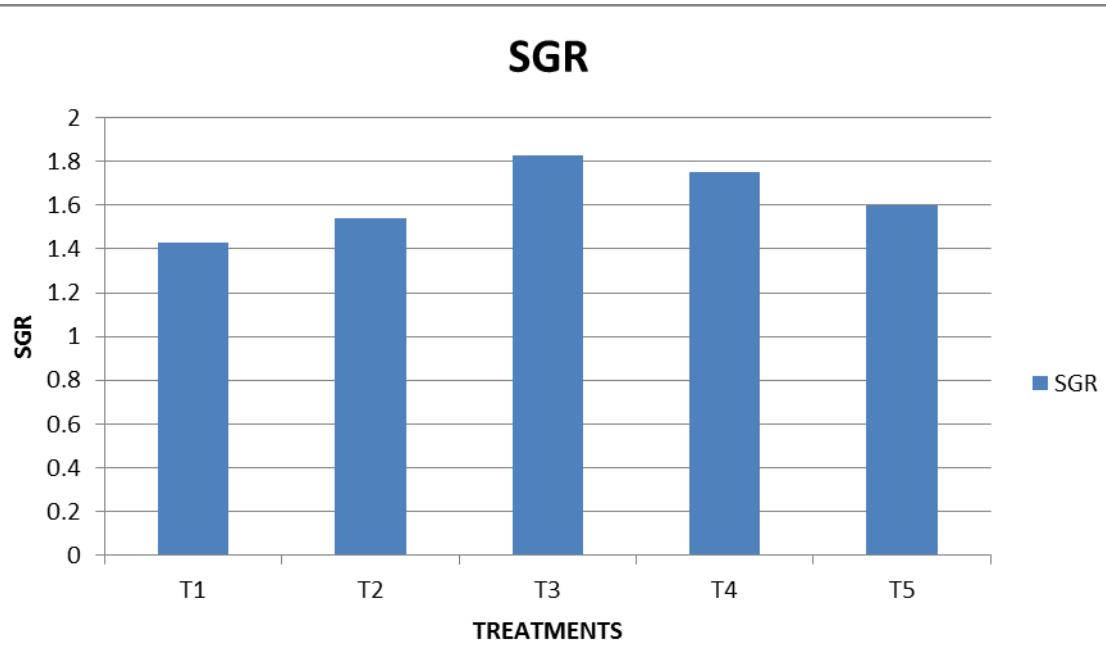


Fig: 23 FCR of Grey mullet fed with different % of crude protein (CP) experimental dites





5. Discussion

In cage aquaculture, fish stocking density has great impact on growth, survival, health, water quality and production. Consequently, optimum stocking densities need to be determined for each species and production phase to enable efficient management and to maximise production and profitability (Costa *et al.*, 2013).

Stocking density and growth rates are often reported to be related, however, the relationships between both parameters may not be uniformly, positively or negatively, linear for a given species.

5.1. Water quality

According to Lin (1985) temperature fluctuations can be a significant factor in the growth of milkfish in nursery and culture ponds that are small or shallow. In the present study temperature is slightly fluctuated with a difference of 1.0 to 2.0 °C and no adverse effect on growth and survival.

In the present study recorded temperature values (27.2^0C to 32.3^0C) are at acceptable level. Low temperatures (<23°C) decrease activity, responsiveness, food intake, growth, and development of milkfish fry and juveniles; high temperatures have the opposite effect (Villaluz and Unggui, 1983). In the present study temperature is recorded more than the above value (>27.6°C).

Grey mullet are able to tolerate hypersaline conditions. Their tolerance limits are at salinities ranging from 0 to 158 ppt (Crear, 1980). The salinity readings in the present study obtained in the net cages were within the tolerance limits of the fish. Growth and FCR of grey mullet fry was significantly affected by salinity. According to Alava (1998) highest growth was observed in milkfish

reared in freshwater and as salinity was increased, growth decreased. Results of present study is in agreement with Holliday (1969). He reported that survival and growth of larvae could be improved by rearing at lowered salinities because low levels were iso-osmotic with body fluids, reducing the energy costs of osmoregulation.

In the present study (Study 1) water temperature was recorded between 27.5 °C to 32.3 °C, pH 7.6 to 8.4, dissolved oxygen 4.1 to 6.5 mg/l, and salinity 21 to 30 ppt. In (Study 2) water temperature was recorded between 27.2 °C to 30.1°C, pH 7.8 to 8.3, dissolved oxygen 4.6 to 6.2 mg/l, and salinity 27 to 32 ppt. The water quality parameters were within the limits cited by Rimmer and Russel (1998) for the rearing of Asian seabass nursery and grow-out. Bardach et al. (1972) found water temperature 24–38°C, pH 7.1–7.9 and salinity 10–35 ppt with good growth of sea bass. In this study, water quality parameters are in the suitable range for grey mullet. In the view of aquaculture, grey mullet culture is feasible in inland saline ground waters and the best results are obtained at a salinity of 10 ppt, as higher salinity levels appear to be detrimental to growth and metabolism of the fish (Barman *et al.*, 2005).

In this study, salinity and temperature evidently influenced fish growth, since dissolved oxygen and other water quality parameters, NH_3, NO_2, pH, alkalinity were within tolerable ranges. In the present study salinity of water is slowly decreased. In initial days, this may be due to rains in that particular period and riverine inflow into the bay.

Ammonia toxicity increases with the increase in pH and temperature. In the present study on 28^{th}, 42^{nd} and 63^{rd} day ammonia is recorded as 0.25 and 0.35 mg/l respectively on these days some mortality of grey mullet was observed

except 63rd day. Increased ammonia level may be the reason for the mortality. In the present study the nitrite and nitrate values recorded were within the suitable and safe range for grey mullet.

5.2. Growth:

Size and water temperature may also influence the SGR. In the present study, SGR of Grey mullet ranged between 1.63 (SD 80) to 1.75 (SD 40). SGR decreased at the highest stocking density of 80 fish/m^3.

In the present study average daily growth rate between 0.177 to 0.143 is recorded in grey mullet. ADGR in the present study was found to be high in low stocking density (SD20) throughout the study period. Earlier studies have also reported decreased ADGR with increasing stocking densities in largemouth bass, *Micropterus salmoides* (Petit *et al*., 2001) in seabass (Philipose *et al.*, 2010) and in silver perch, *Bidyanus bidyanus* (Rowland *et al*., 2004).

Moniruzzaman *et al.* (2015b) reported that in Thai silver barb, *Barbonymus gonionotus* higher weight gain was recorded at a stocking density of 30 fish/m^3 compared to fish stocked at 50, 70, or 90/m^3. Likewise in red sea bream (*Pagrus major*), lower stocking densities resulted in superior weight gain over higher densities (Biswas *et al*., 2007). Hwang *et al*., (2014) also observed slow growth in Korean rockfish (*Sebastes schlegeli*) reared in low density than those in higher density. Higher stocking densities have been reported to inhibit the growth of European seabass (*Dicentrarchus labrax*) cultured in RASs (Sammouth *et al*., 2009) and other fish species cultured in various production systems (Montero *et al*., 1999; McKenzie *et al*., 2012).

Decreasing growth and SGR with increasing stocking density of largemouth bass, Micropterus salmoides, stocked at 5, 10 or 15 fish per aquaria was reported by Petit *et al.* (2001). Developing offshore cage farming is a new way providing employment for fishermen transferring from fish capture to aquaculture. From 2003 to 2010, there are 300 000 fishermen leaving their fishing vessel and looking for other jobs. Thereby, developing offshore cage farming creates the chance for them. Developing offshore cage farming is not only a matter of pushing mariculture or fishery industry, but it will create significant social-economic influences in future (Chen *et al.,* 2006).

Growth in terms of final weight, and SGR of grey mullet was higher in SD 20 compare to fish in higher stocking densities.

5.3. Feed:

Fishes at stocking density of 20/m^3 and utilized the feed very efficiently among all stocking densities. Highest FCR was recorded at highest stocking density of 80/m^3. It was also observed that at stocking density of 20/m^3 feed conversion efficiency was not the best. At very low stocking density, some of the feed might have been wasted and not utilized full.

Feed conversion ratio (FCR) was significantly affected by fish stocking densities in this study where the FCR SD 20 and SD 40 are similar. This finding was similar with that obtained by Moniruzzaman *et al.* (2015b) in caged Thai silver barb, *Barbonymus gonionotus* and Mondal *et al.* (2010) in caged Thai climbing perch, *Anabas testudineus* and Gibtan *et al.* (2008) in caged Nile tilapia, *Oreochromis niloticus* at 50 and 100 fish/m3. However, Mondal *et al.* (2010) observed lower FCR of 0.79 in combined cage culture of Thai climbing perch and tilapia after 120 days culture. Liti *et al.* (2005) opined

that high density stocked fishes might have lower ability to convert given feed to flesh than fish stocked with low density in terms of growth.

The feed was well accepted by grey mullet from 1st day itself. Fishes were approaching the feed within 5 min. Fish fed the diets containing CP 25%,35%, 45%(T3,T4,T5) exhibited a schooling behavior during feeding. No such behavior was observed in fish given diets containing 12.5% (D.O.B), 15% CP (Control (T1) and T2). The feeds given in all treatments were consumed within 20 min after feeding but fish fed the diets containing 25 and 35% protein (T3 and T4) consumed the feed within a shorter period of time.

5.4. Survival rate:

In the present study highest survival was recorded in lowest stocking density (SD 20) in grey mullet Survival rate decreased with increasing stocking density. These results are similar with the findings of Sayeed *et al*. (2008) in Thai pangus and Cremer *et al*. (2002) in pangasius catfish. Lower density gave larger size and higher survival rate in *Clarias macrocephalus* as reported by Mollah (1985) and Ita *et al.* (1989). Barua (1990) stated that Survival rates were higher in the larvae of *C. batrachus* raised at the stocking densities of 2, 4 and 8 fish per liter as compared to those obtained 16 fish/litre.

In the present study survival rate of grey mullet ranged between 80% and 70% with SD 20 having highest survival rate (76.66%) and SD 80 was lowest (70%). These results are in agreement with observations of Moniruzzaman *et al.,* (2015b) in Thai silver barb and Liti *et al.* (2005) and Abou *et al.* (2007) where they found 70 and 75% survival rate in tilapia, respectively. In contrast, some researchers found high survival rate at high stocking densitiy of caged tilapia (Osofero *et al.,* 2009; Mondal *et al.,* 2010).

In the present study survival rate of grey mullet was significantly higher in SD 20. Similar observations was made in mahseer (Rahman *et al*., 2005); climbing perch (Kohinoor *et al*., 2009). The highest weight gain and survival rate of *Heteropneustes fossilis* in lower stocking density was reported by Narejo *et al*. (2005 and 2010). In *Trachinotus blotchi* also lower stocking densities resulted in higher growth and survival (Hanibal *et al*., 2011).

In the present study grey mullet at lowest stocking density (SD 20) utilised the feed most efficiently and showed better FCR than the other groups. Philipose *et al.* (2010) reported FCR of 1.15 in 45 day nursery rearing of Asian seabass in indoor cement tanks. As the present studies were conducted in closed bay and there may be some feed wastage in the open system, higher FCR of 2.55 was recorded. Similar results were reported by Siddiqui *et al.* (1997) in hybrid tilapia reared in concrete tanks with 50 fish/m^2 where the FCR was 2.59.

It is well documented that high stocking density could reduce fish growth and increase mortality (Liti *et al.*, 2005; Abou *et al.*, 2007). Moniruzzaman *et al.* (2015a) also found similar results in monosex tilapia with an average weight of 15.20 g. The highest final weight was observed in 50 fish/m^3 followed by 75, 100 and 125 fish/m^3. In the present study grey mullet at 20 fish m^3 exhibited better growth followed by 40, 60 and 80 fish/m^3. Daudpota *et al.* (2014b) also reports highest growth and survival of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) at low stocking densities of 200/m^2 grown in rectangular concrete pond.

Moniruzzaman *et al.* (2015a) reported lower survival of monosex tilapia at 125 fish/m3 than lower stocking densities of 50, 75 or 100 fish/m3. In the

present study also lower survival of grey mullet was found at stocking density of 80 fish/m^3.

Hannibal *et al*. (2011) reported that survival of silver pompano in floating cages was significantly different among different stocking densities. Highest survival was obtained in 25 pcs/ m3 followed by 30 pcs/ m^3 and 35 pcs/m^3.

On-field trials on grey mullet seed rearing in different stocking densities under feeding, fertilization and combined fertilization–feeding systems revealed better performances in the latter. This study infers that combined fertilization–feeding system with a stocking density of 15,000 fry ha^{-1} would be appropriate for production of striped grey mullet fingerlings in brackish water ponds. The findings of the current study have practical significance as a major step for establishing M. *cephalus* seed rearing practice using a cost-effective and resource efficient approach. There is a scope for refinement of this practice by evaluating further higher density rearing and working out the economic return. An understanding of the proportionate contributions of feed and various natural foods to the growth of grey mullet would help in further refining such systems (Biswas *et al.*, 2012).

Open and closed intensive culture systems offer the opportunity to accelerate the growth of mullet fry under optimal conditions in order to provide fish of appropriate size and number for pond stocking purposes. The high stocking densities and the aim for the complete control of the fish producing make artificial feeding necessary in both systems. (Houner et al., 1989).

According to Fairchild and Howell (2001) Stocking density is one of the factors that could potentially affect survival and production performance of

aquatic organisms. Thus, the use of an appropriate density can increase the profitability of farming systems, by maximizing the utilization of water and the other resources in the rearing system.

Fish stocking density is one of the most important parameters affecting fish growth and health in a number of ways (Garr et al., 2011; Mazlum, 2007 and Zhu et al.,2011). Growth performance and survival rate are adversely affected by high stocking densities (Pouey et al., 2011; Sorphea et al., 2010) but in some cases this effect is either temporary (Garr et al., 2011) or absent (Gokcek and Akyurt, 2007; Southworth et al., 2009). Some species can tolerate extreme crowding although competition for food will then limit their growth and lead to poor weight gain (Stickney, 1994). Such may have been the case in this study where fish stocked at higher stocking densities had poor growth. (M'balaka et al., 2012).

Based on growth and survival of sea bass fry grown in illuminated cages, a stocking density of 600 m^{-2} seemed to be optimal when fish were reared on wild zooplankton alone. However, increasing the stocking density to 1200 m^{-2} would indicate the need for supplemental feeding in the form of minced fish flesh (Fermin *et al.*, 1996**)**. In the intensive rearing of the European sea bass, Dicentrarchus labrax, cannibalism induced mortality occurs due to high stocking density resulting in adequate of insufficient food supply. (Katavic *et al.*, 1989; Fermin *et al.*, 1996).

Villegas and Bombeo (1981) recommended a stocking density of 25-50 fry/m^2 for nursery rearing of milkfish. Soomro *et al*. (2015) reported that milkfish were stocked in a tank at a stocking density of 20 pcs/m^2 and reared for three months.

Increasing stocking density results in stress (Leatherland and Cho, 1985) which leads to enhanced energy requirements causing reduced growth and food utilization (Hengsawat *et al*., 1997). They reported that increasing the stocking density from 2000 to 4000/ha reduced the weight gain of juvenile milkfish to about 50%. Haque *et al.* (1984) and Kawamoto *et al.* (1957) also reported best growth at lower stocking densities in java tilapia and carp. In the present study, growth reduced with increasing stocking density beyond 20/m^3. For the studies on optimum stocking density in nursery rearing of grey mullet in floating net cages, formulated feed with 25% CP was used. Grey mullet stocked at 20 fish/m^3 showed better growth performance than fish stocked at 40, 60 or 80 fish/m^3.

There was significant difference between stocking density and survival rate for all treatments 20 to 80 fish/m^3.

The lower growth performance of grey mullet at higher stocking density could have been caused by voluntary appetite dominance, more expenditure of energy because of intense aggressive developmental contact, competition for food and living space and increased stress. Survival rate of grey mullet depends on feeding. In their study survival rate of milkfish fed rice bran was more though the stocking density was high compared to unfed fishes at lower stocking densities, Villegas and Bombeo, (1981).

In the present study results was similar to that observations of Sadhu *et al.* (2015). They reported that growth, survival, average daily weight gain and SGR were higher in fish held in low stocking density (14/m^3) when compared to high stocking density (35/m^3). Seabass larvae reared in 5 Litre capacity

cylindrical plastic containers and maximum growth was observed in intermediate stocking density 8 no.l^{-1}(Sukumaran *et al*., 2011).

There are reports of growth reduction in fish with increasing stocking density. This may be due to crowding. Under crowded conditions at higher stocking densities, fish get stress as a result of aggressive feeding interaction and eat less, resulting in growth retardation (Sinha and Ramachandran, 1985; Bjoernsson, 1994). In the present study also fish growth was decreased with increasing stocking densities.

Cheong, (1989) opined that as the fish grows thinning is practiced in some cases. In Thailand, stocking density followed in cages is between 40-50 m^{-3} initially, later thinning to 10-20 m^{-3} (Kungvankij *et al*., 1984). In Singapore, similar stocking protocol is followed: initially 40-50 m^{-3} for 20-100 g size, reducing to 33 m^{-3} at 100-700 g and some farmers practice a second thinning to 27 m^{-3} at 300 g.

The causes might be competition for food and habitat at higher density. Similar observations was made in mahseer (Rahman et al., 2005); Labeo rohita, Cirrhinus mrigal and Cyprinus carpio (Haque et al., 1991, 1993 and 1994); punti (Kohinoor et al., 1994); mahseer (Islam, 2002); Labeo calbasu (Rahman and Rahman, 2003). Growth of jundia in cubic-shaped cages is density dependent. In terms of weight parameters, the most effective stocking density of jundia´ fry is 100/m^3. However, should the desirable weight be from 30 to 40g, the density of 300/m^3 is most effective in that the fingerling production increases three times (Barcellos *et al.,* 2004). Rowland *et al.* (2006) showed that silver perch can be efficiently grown to market-size in cages and its performance is similar to tilapia and channel catfish, species that

have the advantage of decades of research and genetic improvement. Cage culture is a viable alternative to the use of ponds for the production of silver perch, will reduce or eliminate losses due to bird predation, and facilitate feeding, grading and harvesting. Survival, size variation, food conversion and production rate are density-dependent in silver perch. Sadhu et al. (2015) concluded that significantly better final biomass was obtained by culturing Asian seabass at high density (35 fish/m^3) in open sea cage farming whilst higher values of SGR, ADGR, SR and FCR were recorded in low density (14 fish/m^3). Further studies are required to refine cage farming at this high density by taking into consideration the impact of chronic stress due to high stocking density on the physiological well being and occurrence of diseases in culture organisms

Stocking density may not have much influence on growth in some fish. Merola and Souza (1988a, b) found an absence of density effect on growth of Brazilian native species pacu (Piaractus mesopotamicus), using densities of 50, 100 and 150/m^3, respectively. Biswas et al. (2012) also reported that growth parameters and survival were not affected by stocking density and feeding or pond fertilization in grey mullet fingerlings. This is in contrary with present findings. Hannibal et al. (2011) reported that the growth of silver pompano at three different stocking densities was not significantly different among treatments. The best growth was attained for silver pompano with 25 pcs/m^3. Studies on higher stocking densities over 15 kg/m^3 were conducted in cage culture of seabass (Kissil et al., 2000), salmonids (Roberts and Hardy, 2000), Japanese flounder (Min, 1995) and red sea bream (Ikenoue and Kafuku, 1992). However, studies on growth utilizing lower stocking densities of 5 and

25 fingerlings/m3 have been used for snappers (Benetti *et al.* 2002) and 1.26 to 3.24 fingerlings/m^3 (Castillo et al., 2007). It is reported that juvenile turbot (Irwin et al., 1999) exhibit higher growth rates at relatively low densities (0.7–1.1 kg/m^3). In the present study survival of grey mullet was significantly affected by stocking density. Baliao et al. (1987) reported that highest survival of 86.9% was attained at stocking density of 20 fingerlings/m^2.

In the present study highest survival was recorded in T3 (83.33%) and lowest in control (T1), T2 (70.83%). Similar results was recorded by Soomro et al. (2015). They reported that survival rate in fingerlings of milkfish in different treatments are ranging between 79.5 to 80.5%.

In the present study, there was an increasing trend of biomass with increasing stocking density. In the present study production is more in 80 fish m^3 in terms of biomass. This study shows a strong trend that final production increased with increasing stocking density. These findings are in agreement with those reported by Cruz and Ridha (1989) and Watanabe *et al*. (1990) for tilapia and Suresh (2017) for milkfish and seabass.

Results of study on effect of stocking density on growth and survival of grey mullet revealed that stocking density had significant impact on growth and final mean weight. Survival rate, protein efficiency ratio, average daily growth rate and specific growth rate were higher in fish held at low stocking density (SD20) when compared to high stocking density. Feed conversion ratio was lower in low density group (SD20). However, final production in terms of total biomass was higher in high density group (SD80).

There appear to be no size depensation within populations at different densities. At increased densities growth is little depressed primarily due to a

lower feed intake of all individuals, but also as indicated due to a less efficient utilization of the feed, which is speculated to be a result of increased activity due to social interactions and feed browsing and possible chronic stress.

The results of the present study indicate that cage culture of grey mullet (M. *cephalus*) in the sea can provide significant advantages in terms of faster growth and effective utilisation of water volume as compared to pond systems thus making it an alternative culture approach to enhance production.

5.5. Crude protein:

The level of protein in fish feed is of fundamental importance because it influences growth. Inadequate protein level in fish feed results in a reduction or cessation of growth due to withdrawal of protein from less vital tissues for body maintenance. Increase in dietary protein levels is often associated with higher growth rate in many species until it reaches the level beyond which further growth is not supported and may even decrease.

Protein, the most expensive component in fish feeds, is required by fish for maintenance and growth, and the protein level needed for these functions varies with species and the culture environment (DeLong *et al.,* 1958). Growth can be adversely affected by both low and high protein diets. Excessive protein content is wasteful and causes the diets to be unnecessarily expensive. Hence, knowledge of the optimum dietary protein requirement is essential for the formulation of well-balanced and low-cost artificial diets (Mazid *et al.,* 1979; Elangovan and Shim, 1997).

Dietary protein is always considered to be of primary importance in fish feeding (Jauncey and Ross, 1982), thus sufficient supply of dietary protein is needed for rapid growth (Lovell, 1989).The optimum dietary protein level is

45% for Nile tilapia fry (0.5 g/fish), 35% for fingerlings (20 g/fish) and adult (40 g/fish).The high protein level (45% protein) did not significantly enhance the fish growth at fingerlings and adults.

Furthermore, Wilson (1989), Pillay (1990) and El-Sayed and Teshima (1991) found that dietary protein requirements decreased with increasing fish size and age. Based on various studies, Balarin and Halfer (1982) made a general conclusion that fry of tilapia <1 g requires diet with 35-50% protein, 1-5 g fish requires diet with 30-40%protein and 5-25 g fish requires diet with 25-35% protein. A diet containing 45% protein appears to be economical and suitable for fry of Nile tilapia (0.5 g), and diets containing 35% protein is recommended for fingerlings and adult (grow out) fish (20-40 g), these results may be due to the fact that each fish size has a certain protein limit after which excess protein level could not be utilized efficiently (Ahmad et al., 2004).

The minimum or the maximum amount of protein needed per animal per day is the protein requirement (Guillaume, 1997). Protein requirements changes with biotic, abiotic factors and dietary characteristics. The protein requirement of a given species is often based on the growth, feed efficiency, and protein conversion ratio of the animal to varying levels of dietary protein under a given set of circumstances. Hence, the requirement is generally described as the optimal protein content of the diet. The protein requirement of any organism need, not only to satisfy the organism's need for substrates, for the maintenance and growth, but also, to activate the various processes associated with the growth (Millward, 1989). Protein requirements of fish and shellfish are high, both as a source of amino acids for protein synthesis and also for gluconeogenesis (Azad, 1996). The protein requirement has been

defined more adequately as the amount of protein required per animal biomass per day (Kureshy and Davis, 2002), and it is often based on the response i.e. weight gain, feed efficiency or protein conversion efficiency.

It has also been reported that some freshwater fish species were exclusively reared on artificial diets from the beginning of exogenous feeding (Appelbaum and Damme, 1988; Charlon *et al.,* 1986; Legendre *et al.,* 1995). However, inclusion of excess protein in the diet is undesirable because some of it could be catabolized for energy production (Watanabe, *et al.,* 1987; Ruohonen, *et al.,* 1999; Jahan, *et al.,* 2002). Therefore, the amount of protein included in the diet of fish is a vital consideration, to promote feed efficiency and growth performance. Utilization of commercial pellet feed is now popular, especially for carnivorous fish, in order to reduce the dependence on trash fish, feeding cost and environmental impact. Studies on replacement of trash fish by formulated feed in several species demonstrated better growth rates and more profit. Dietary protein level had a profound effect on fish growth. The optimum dietary protein levels reduce the tendency of cannibalism. This situation did not arise in the present study due to short-term experiment; however, there were no greater differences in minimum and maximum size.

Normally fish size/weight increases with the increase in the protein percentage to a maximum and then starts decreasing in growth even though the protein percentage further increased. In other words we cannot increase the fish growth after certain percentage of protein and there is always an optimum protein percentage beyond which there will decrease or in other words weight decrease of fish. Cremer *et al.,* (2001a) replaced trash fish with formulated diets in cage culture of red drum (Sciaenops ocellata) and Japanese seabass

(Lateolabrax japonicus) and concluded that fish consuming formulated diet (43% crude protein) with 35% soybean meal showed better growth and less feeding cost than fish fed trash fish. In the present study the optimum protein percentage is around 25%.

Acording to Willson (1994), the importance of estimating not just the protein requirement of cultured species but also changes in protein utilization in response to the amount and type of ingested energy. Knowledge of the optimum protein : energy ratio in diets for Chelon labrosus is a prerequisite for the development of balanced aquaculture feeds. Finally, the physiological basis for energy partitioning, and the availability of protein and energy from differing raw materials, must also be investigated (Ojaveer *et al.*, 1996). In fine, based on the broken-line analysis of weight gain, it can be corroborated that the optimum dietary protein level for juvenile parrot fish could be 48.5 % for its maximum growth at the gross energy level of 16.7 kJ/g diet (Kim *et al.*, 2016).

Mohanty and Samantray (1996) observed depressive growth above protein percentage of 55% at 8-5% BW. But in the present study, better growth and survival was observed for crude protein 25% rather than crude protein 50% at 5% BW. Feeding the fish more than the optimum waste food, increase FCR, and deteriorate water quality (Marian *et al.*, 1982).

The growth rate of fish is high at the juvenile stage and decreases with increased body size. There was growth in juvenile Arctic char at all dietary protein levels but highest growth rate was observed in fish fed high dietary protein level (39.89% CP). Similar trends have been observed in similar

studies with increased dietary protein levels (Jindal *et. al.,* 2010, Siddiqui and Khan, 2009, De Borba *et al.,* 2003, Tabacheck, 1986; Amoah, 2011).

However, it is important to remember that determination of the optimum dietary protein level is also affected by the level of other components in feed (Andrews *et al.,* 1972) quality of the protein (Smith *et al.,* 1985), water quality (Shiau *et al.,* 1991; Robertson et al., 1993; Wyban *et al.,* 1995), and experimental factors such as stocking density, daily ration size, and feeding frequency (Robertson *et al.,* 1993), which makes comparison of results difficult (Velasco *et al.,* 2001).

Feed contributes 60 to 75% of the total cost of production in intensive culture. In the present study fishes fed on 25% CP (T3) resulted in high final body weight, weight gain and SGR. The present study is an agreement with the studies of Mwangamilo and Jiddawi (2003).

In the present study fishes fed on supplemental feed with 25% CP (T3) exhibited significantly higher growth than the fishes fed on DOB (12.5% CP-Control, T1) or 15(T2), 35(T4) and 45% (T5) CP. However, there was no significant in growth of fish fed on diet with 45% CP compared to fish fed on with 25% CP. Fish fed diet beyond 25% Crude protein could not produce additional growth. There is some evidence that at very high feeding levels, protein deposition tends to level off (Bureau, 2002). Magondu et al. (2016) reported that feeding the milkfish on formulated diets resulted in significantly higher mean fish weight gain than that of fish fed on non formulated diets like coconut cake and maize bran. In the present study also non formulated feed (DOB – Control (T1)) showed lower growth than the formulated diets (T2, T3, T4 or T5). Marquez, (1987) conducted an experiment on the effects of

supplementary feeding on milkfish growth, production, survival, water quality, and economic benefits in brackish water ponds evaluated by comparing the use of rice bran containing 11.3% protein and two pelleted diets containing 22.0% and 27.4% protein to an unfed control and he reported that the diets containing 22.0% and 27.4% protein had net profits. Papoutsoglou and Alexis (1986) reported that young grey mullet, M. capito require about 24% of protein for maximum growth. Siddiqui et al. (1988) reported that Nile tilapia, Oreochromis niloticus showed maximum growth diet containing 30% protein. From the biological, environmental and economic sustainability points of view, it is now clear that it is more advantageous to farm omnivorous (mullet) rather than carnivorous fish because of lower amount of fishmeal required as well as conversion efficiency (25%) CP is sufficient. It may be concluded that for grey mullet (M. *cephalus*) juveniles a dietary protein level of 25% CP is the optimum for maximum growth and good feed conversion ratio in cages grown in closed bay. In addition, the natural food contribution under cage culture conditions should also be considered. Further, the study provide important evidence that grey mullet fed with 25% CP performed well in net cages at that particular environment. Grey mullet showed better final biomass at high density (80 fish/m^3) in closed bay whilst higher values of growth in terms of final mean body weight, survival rate, ADWG and SGR were recorded in low density (20 fish/m^3).With the recent developments in both nursery and grow-out culture of grey mullet, improvement in many aspects of production is still needed. Automation in terms of feeding, grading, thinning, harvesting, and net washing are still needed to be developed as these activities are highly labor intensive especially in offshore grow-out cages. Globally, the

system of marine cage farming has had an important role in meeting the global demand for fish products and India is endowed with vast fisheries resources in terms of a coast line of 8118 km and 2.02 million km2 of exclusive economic zone, including 0.53 million km2 of continental shelf. A great potential is existing in India for mariculture activities in future and cage farming is the best option for it (Imelda-Joseph *et al.,* 2010). Further studies are required to refine cage farming at this high density by taking into consideration the impact of chronic stress due to high stocking density on the physiological well being and occurrence of diseases in culture organisms (Sadhu *et al.,* 2015).

6. Summary

The present studies were conducted in the closed bay near Suryalanka, Bapatla, Guntur district for "Studies on growth and survival in nursery rearing phase of grey mullet (*Mugil cephalus*) for mariculture". Seed of mullet was collected from wild source in suryalanka village, Bapatla. Two (2) experiments were conducted. 1. To study the effect of stocking density on growth and survival of grey mullet (*M.cephalus*) 2**.** To study the effect of different protein levels on growth and survival of grey mullet (*M.cephalus*).

Grey mullet used for stocking density studies were fed with 25% CP formulated feed. Grey mullet used for optimum crude protein level studies were fed with different % of CP levels, de-oiled rice bran (12.5% CP) is used as control (T1) feed and four formulated feeds with different protein levels as CP 15% (T2), CP 25% (T3), CP 35% (T4) and CP 45% (T5), respectively. For each treatment triplicates were maintained.

The objective of the present studies is to evaluate the impact of stocking density and also growth, survival and FCR of grey mullet at different % of crude protein levels. In addition, specific growth rate, average daily weight gain, and biomass were estimated.

The results obtained from the present studies are summarized:

Study-1: To study the effect of stocking density on growth and survival of grey mullet (*M.cephalus*):

- Water quality parameters were at optimum level during the study period. The parameters were in the following range, Temparature: 27.5 to 32.3^{0}C, Salinity: 21 to 30 ppt, pH: 7.6 to 8.4, DO: 4.1 to 6.5 mg/l, Total alkalinity: 135 to 168

mg/l, Ammonia: 0.01 to 0.35 mg/l, Nitrite: 0.01 to 0.03 mg/l, Nitrate: 1.95 to 3.85 mg/l.

- The highest growth performance was recorded at D20 (12.15g) and lowest was observed in D80 (10.08g) in gery mullet.
- The analysis of variance for growth performance showed significant difference among the treatments at 1% and 5% level of significance and better performance was observed at D20.
- There was no significant difference in the survival rates of gery mullet at different stocking densities. However, highest survival was recorded at D20 (76.66%) and lowest at D60 and D80 (70%).
- D20 and D40 (2.31) has resulted in lowest FCR while highest was observed in D80 (2.55).
- D40 has resulted highest SGR (1.75) and D20 showed ADWG (0.177 g) while lowest SGR (1.63) and ADWG (0.143 g) recorded in D80 respectively.
- D80 showed highest biomass (560.5 g) and lowest biomass (194.4 g) was recorded in D20.

Study-2 To study the effect of different protein levels on growth and survival of gery mullet (*M. cephalus*).

- Water quality parameters were at optimum level during the study period. The parameters were in the following range, Temparature: 27.2 to 30.9^{0}C, Salinity: 26 to 32 ppt, pH: 7.8 to 8.3, DO: 4.6 to 6.2 mg/l, Total alkalinity: 139 to 163 mg/l, Ammonia: 0.01 to 0.25 mg/l, Nitrite: 0.01 to 0.03 mg/l, Nitrate: 2.15 to 3.26 mg/l.

- The highest growth performance of grey mullet was recorded at T3 (13.64 g) and lowest was observed in Control (7.69 g). However, highest weight gain and lowest weight gain was observed at T3 (2.86 g) and Control (1.24 g) respectively.
- The analysis of variance for growth performance showed significant difference among the treatments. Better performance was observed at T3 (35% CP).
- The highest survival of 83.33% (T3) and lowest survival of 70.83% (Control (T1)), T2, were recorded.
- Analysis of variance for survival revealed significant differences (at 1% and 5% level) among the treatments for survival.
- Fish fed on C (12.5% CP, DOB) had the highest Feed Conversion Ratio of 3.06 and the lowest 2.14 recorded in T3 (25% CP). All the treatments have shown better feed utilization than Control.
- The Analysis of variance has shown significant difference for Feed Conversion Ratio at 1% and 5% level of significance.
- T3 (25% CP) has resulted highest SGR (1.83) and ADWG (0.225 g) while lowest SGR (1.43) and ADWG (0.156 g) recorded in (C) control respectively.
- T3 showed highest biomass (606.8 g) and lowest biomass (432.8 g) was recorded in control (T1).
- It can be concluded that 25% CP level is optimum for grey mullet in nursery rearing in floating net cages.

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