Effects of monospecific and mixed algal diets on survival, development and biochemical composition of *Penaeus monodon* larvae

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By

Cilla Abraham

Department of Marine Biology, Microbiology and Biochemistry

Cochin University of Science and Technology

Cochin - 682 016, Kerala, India.

November 2012

Dedicated To Abisha

DECLARATION

I hereby do declare that the thesis entitled ***"Effects of monospecific and mixed algal diets on survival, development and biochemical composition of Penaeus monodon larvae"*** *is an authentic record of research work done by me under the guidance of* ***Dr. C. K. Radhakrishnan****,* Professor Emeritus, *Dept. of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology for the doctorate degree in marine biology and that no part thereof has been presented for the award of any other degree in any University.*

CILLA ABRAHAM

November, 2012

CERTIFICATE

This is to certify that the thesis entitled ***"Effects of monospecific and mixed algal diets on survival, development and biochemical composition of Penaeus monodon larvae"*** *is an authentic record of research work carried out by* ***Mrs. Cilla Abraham*** *under my supervision and guidance in the School of Marine Sciences, Cochin University of Science and Technology in partial fulfillment of the requirements for the degree of* ***Doctor of Philosophy*** *and no part there of has been presented before for the award of any other degree, diploma or associateship in any University.*

Dr. C. K. RADHAKRISHNAN
Professor Emeritus
Dept. of Marine Biology,
Microbiology and Biochemistry.
Cochin University of
Science and Technology,
Kochi- 682016.

November, 2012.

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ABSTRACT

The brackishwater aquaculture sector is supported by shrimp production mainly the giant tiger shrimp *Penaeus monodon*. *P. monodon* is a candidate species for aquaculture in India and the demand is ever-growing in both local and international markets. One of the major bottlenecks in aquaculture is the rearing of fish and crustacean larvae. Larvae of the most fish and crustaceans encounter problems to accept inert/dry diets. Hence, live food (phyto and zooplankton) remains an important food source for the start feeding of early larval stages. This live food should be easily available, reproducible and economical. Problems occurring in supply with this live food may prevent successful larval rearing, which limits the whole production system. The choice of algal strains for aquaculture use is constrained by a number of factors such as toxicity of the alga, nutritional profile, size and palatability and finally the selected algae should be relatively robust and easy to culture.

Quality prawn seed is a critical input required for successful shrimp aquaculture. In order to ensure availability of quality shrimp seeds commercial shrimp hatcheries are established in the public as well as private sector. The success of shrimp culture depends largely on the availability of adequate quantity of the seeds of desired species at the appropriate time.

The main objective of the present work is to acquire information regarding the growth responses of *P. monodon* larvae (from PZ1 upto PL1) to various mono specific and mixed diets. Evaluate the nutritional quality of selected species of micro algae viz. *Chaetoceros calcitrans, Dunaliella salina, Isochrysis galbana* and *Nannochloropsis salina,* to *P. monodon*

larvae at three cell concentrations $10x10^4$ cells/ml, $25x10^4$ cells/ml and $50x10^4$ cells/ml.

The *P. monodon* larvae were transported, at the Nauplius stage, to the laboratory. The larvae were stocked at density of 150 larvae per litre in 5 litre FRP tanks with 3 litres of sea water. The algal cell density given to the larvae varied. The larval stages were fed with increasing densities of algae to evaluate the relationship between the food densities, ingestion rates, development and growth of the larvae. The water quality parameters, the percentage of survival rate, the growth estimation and the algal cell count were done. Each experiment was carried out in triplicate with a control group of larvae fed with *Chaetoceros calcitrans*. For the estimation standard procedures were used.

Effect of mono algal diet on growth responses of *P. monodon* larvae were carried out. The length and percentage survival of all the three cell concentrations were evaluated, whereas for cell concentration of $50x10^4$ cells/ml, along with length and percentage survival the ingestion rate, developmental index, growth rate and feed efficiency were also calculated. The results of the experiments conducted were explained. The relative ingestion rate increases through each substage, reaching its maximum during larval development in PZ III. The results from these studies show that the practice of feeding the protozoeal and mysis substages of penaeid shrimps with expensive *Artemia* nauplii is not necessary when the early larval stages are still filter feeders and can still benefit on a cheaper but nutritionally sufficient natural diet of phytoplankton. *D. salina* was found to be an efficient feed among the selected algal species.

Effect of mixed algal diet on growth responses of *P. monodon* larvae were carried out. The length and percentage survival of all the three cell concentrations were evaluated, whereas for cell concentration of $50x10^4$ cells/ml, along with length and percentage survival the ingestion rate, developmental index, growth rate and feed efficiency were also calculated. The results of the experiments conducted were explained. The biochemical composition of each microalgae species varies, hence the use of monoalgal diets could produce a shortage of essential nutrients needed for the adequate development of penaeid shrimp. The mixed algae fed *P. monodon* had better developmental rate with *P. monodon* larvae fed with *D. salina* which was found to be very efficient in combination with *N. salina* as well as that of *C. calcitrans*. Higher survival rate of 71.3% was obtained with the combination of *N. salina* and *D. salina*.

The protein, carbohydrate, lipids from the larval samples were determined. The gross lipid and carbohydrate compositions of the algal diets could explain the observed differences in growth and development of the larvae corresponding to the gross composition of the larvae. For algae the protein, carbohydrate, lipid, pigments, amino acid quantification and fatty acid quantification were carried out. The differences in the composition of fatty acids in the algal diets seemed to be the factor most likely to explain the differences in larval survival and development.

From the larval rearing experiment, the *Dunaliella salina* was found to be a suitable food organism for the black tiger shrimp, *Penaeus monodon.* The survival rate and growth performance was found to be high in those larvae which fed on *Dunaliella salina* and the next was *Chaetoceros calcitrans. Nannochloropsis salina* was found to be a suitable food during the

protozoeal stages but not during the late mysis stages. It might be due to the small size of the algae which may not be preferable for the mysis which has wider mouth gape than that of protozoea. In this experiment the growth and survival rate of the prawn larvae fed on mixed algae (*Dunaliella salina* + *Nannocholopsis salina*) was high compared with that of monospecific algal trials. It was found that the relative ingestion rate increases through each substage, reaching its maximum during larval development in PZ III. To avoid food waste, under feeding, and water fouling, optimum feeding response to a particular food during the particular larval stage must be known. The application of food levels based on the ingestion rates for each larval stage is an effective strategy if larval growth, development and survival are consequently maximized.

CONTENTS

Chapter 1 : Introduction.............................. 1-5

Objectives of the Present Study 6

Chapter 2 : Literature Review................ 7-40

2.1 Historical Perspective 7
2.2 Necessity of Live Feed 8
2.3 Micro algae for aquaculture 9
2.4 Mono Algal Diets for Shrimp 13
2.5 Mixed Algal Diets for Shrimp 22
2.6 Micro algae as food for Other Organisms 24
2.7 Biochemical Composition of Algae 29
2.8 Biochemical Composition of Larvae 35
2.9 Energy 37

Chapter 3 : Materials and Methods...... 41-55

3.1 Maintenance of *P. monodon* larvae in the laboratory 42
3.2 Live Feed 43
3.3 Composition and preparation of Walne medium 45
3.4 Replacement Amount of Pure Culture 47
3.5 Water quality monitoring 47
3.6 Siphoning of wastes 48
3.7 Aeration 48
3.8 Larvae swimming activity 48
3.9 Faecal string 48

3.10 Length 49
3.11 Percentage Survival 50
3.12 Weight 50
3.13 Specific Growth Rate 50
3.14 Ingestion Rate 50
3.15 Developmental Index 51
3.16 Dry weight of algae 51
3.17 Ash 51
3.18 Biochemical Composition Larvae 52
3.19 Biochemical Composition Algae 53
3.20 Pigments TLC Analysis 54
3.21 Amino acid quantifcation by HPLC 54
3.22 Composition of fatty acids by GC 55

Chapter 4 : Effect of mono algal diet on growth responses of P. monodon larvae…… 57-109

Introduction
Results
4.1. Length of *P. monodon* larvae 57
4.2. Survival rate of *P. monodon* larvae 66
4.3. Developmental index of *P. monodon* larvae 74
4.4. Ingestion rate of *P. monodon* larvae 77
4.5. Weight gain of *P. monodon* larvae 80
4.6. Percentage increment on length of *P. monodon* larvae 81
4.7. Growth rate (length) of *P. monodon* larvae 84
4.8. Percentage increment on weight of *P. monodon* larvae 87

4.9. Growth rate (weight) of *P. monodon* larvae 88
4.10. Water quality parameters 90
Discussion 97-109

Chapter 5 : Effect of mixed algal diet on growth responses of P. monodon larvae 111-186

Introduction
Results
5.1. Length of *P. monodon* larvae 112
5.2. Survival rate of *P. monodon* larvae 128
5.3. Developmental index of *P. monodon* larvae 143
5.4. Ingestion rate of *P. monodon* larvae 149
5.5. Weight gain of *P. monodon* larvae 154
5.6. Percentage increment on length of *P. monodon* larvae 156
5.7. Growth rate (length) of *P. monodon* larvae 161
5.8. Percentage increment weight of *P. monodon* larvae 166
5.9. Growth rate (weight) of *P. monodon* larvae 168
5.10. Water quality parameters 170
Discussion 181-186

Chapter 6 : Biochemical composition of P. monodon larvae …………..187-205

Introduction
Results
6.1. Biochemical composition of mono-algae fed *P. monodon* larvae at Z3 stage 188

6.2. Biochemical composition of mono algae fed
P. monodon larvae at M3 stage 190
6.3. Biochemical composition of mixed algae fed
P. monodon larvae at Z3 stage 192
6.4. Biochemical composition of mixed algae fed
P. monodon larvae at M3 stage 195
Discussion 199-205

CHAPTER : 7 Biochemical composition of micro algae…………………..207-227

Introduction
Results
7.1. Biochemical composition of algae 208
7.2. Amino acid composition of algae 209
7.3. Fatty acid composition of algae 212
7.4. Correlations Analysis - Protein 214
7.5. Correlations Analysis - Carbohydrate 215
7.6. Correlations Analysis - Lipid 215
7.7. Correlations Analysis - Ash 216
Discussion 217-227

Chapter 8 Summary and Conclusion………. ..229-231

Chapter 9 References.....………..………233-271

ABRREVIATIONS

%	Percentage
AFW	Ash free dry weight
AW	Dry weight
C	*Chaetoceros calcitrans*
C+D	*Chaetoceros calcitrans* + *Dunaliella salina*
C+N	*Chaetoceros calcitrans* + *Nannochloropsis salina*
D	*Dunaliella salina*
D+N	*Dunaliella salina* + *Nannochloropsis salina*
DI	Developmental Index
DNA	De oxy ribonucleic acid
FCE	Feed Conversion Efficiency
FCI	Feed Conversion Index
Fi	Feed ingested
FRP	Fibre reinforced tanks
Fs	Feed supplied
GC	Gas chromatography
GR	Growth rate
Ha	Hactre
HPLC	High power liquid chromatography
I	*Isochrysis galbana*
I+C	*Isochrysis galbana* +*Chaetoceros calcitrans*
I+D	*Isochrysis galbana* + *Dunaliella salina*
I+N	*Isochrysis galbana* + *Nannochloropsis salina*
IR	Ingestion rate
M 1	Mysis 1
M 2	Mysis 2
M 3	Mysis 3
MBD	Micro Bound Diet
MED	Micro encapsulated diet
mg	Milligrams

ml	Millilitre
MT	Metric Tonnes
MT/ha/Yr	Metric tons per hactre per year
N	*Nannochloropsis salina*
^oC	degree celcius
P. m	*Penaeus monodon*
Pl 1	Post larvae 1
ppm	Parts per million
Pz 1	Protozoea 1
Pz 2	Protozoea 2
Pz 3	Protozoea 3
RNA	Ribonucleic acid
SD	Standard deviation
SDLA	Single Dose of Live Algae
SGR	Specific Growth Rate
SPM	*Spirullina platensis* meal
TL	Total length
TLC	Thin layer chromatography
TW	Total weight
Wf	Final Weight
Wi	Initial Weight
µg/L	micrograms per litre
NO_2-N	nitrite nitrogen
NO_3-N	Nitrate nitrogen
NH_3-N	ammonia nitrogen
PO_4	Phosphate

List of Tables

Table No	Title	Page No.
4.1.	Length of P. m larvae at cell conc. 10 x 10^4 cells/ ml	58
4.2.	Length of P. m larvae at cell conc. 25 x 10^4 cells/ ml	61
4.3.	Length of P. m larvae at cell conc. 50 x 10^4 cells/ ml	64
4.4.	Survival rate of P. m larvae at cell conc. 10 x 10^4 cells/ ml	67
4.5.	Survival rate of P. m larvae at cell conc. 25 x 10^4 cells/ ml	69
4.6.	Survival rate of P. m larvae at cell conc. 50 x 10^4 cells/ ml	72
4.7.	Developmental index of P. m larvae at cell conc. 50 x 10^4 cells/ ml	75
4.8.	Ingestion rate of P. m larvae at cell conc. 10 x 10^4 cells/ ml	78
4.9.	Weight of P. m larvae at cell conc. 10 x 10^4 cells/ ml	80
4.10.	Percentage increment on growth (length) of P. m larvae at cell conc. 10 x 10^4 cells/ ml	82
4.11.	Growth rate (length) of P. m larva at cell conc. 10 x 10^4 cells/ ml	85
4.12.	Percentage increment (weight) of P. m larva at cell conc. 10 x 10^4 cells/ ml	87
4.13.	Growth rate (weight) of P. m larvae at cell conc. 10 x 10^4 cells/ ml	89
4.14.	Water quality parameters of P. m larvae fed C at cell concentration (50 x 10^4 cells/ ml)	91
4.15.	Water quality parameters of P. m larvae fed I at cell concentration (50 x 10^4 cells/ ml)	92
4.16.	Water quality parameters of P. m larvae fed D at cell concentration (50 x 10^4 cells/ ml)	94
4.17.	Water quality parameters of P. m larvae fed N at cell concentration (50 x 10^4 cells/ ml)	95
5.1.	Length of P. m larvae at cell conc. (10x10^4 cells/ ml)	113

5.2.	Length of P. m larvae at cell conc. ($25x10^4$ cells/ ml)	117
5.3.	Length of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	124
5.4.	Survival rate of P. m larvae a at cell conc. ($10x10^4$ cells/ ml)	129
5.5.	Survival rate of P. m larvae at cell conc. ($25x10^4$ cells/ ml)	134
5.6.	Survival rate of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	139
5.7.	Developmental index of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	145
5.8.	Ingestion rate of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	150
5.9.	Weight of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	154
5.10.	Percentage increment on growth (Length) of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	157
5.11.	Growth rate (Length) of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	162
5.12.	Percentage increment on growth (weight) of P. m larvae at cell conc. ($50x10^4$ cells/ ml)	166
5.13.	Growth rate (weight) of P. m larvae at cell conc. ($50x\ 10^4$ cells/ ml)	168
5.14.	Water quality parameters of P. m larvae fed with C	170
5.15.	Water quality parameters of P. m larvae fed with I+C	172
5.16.	Water quality parameters of P. m larvae fed with I+N	173
5.17.	Water quality parameters of P. m larvae fed with I+D	175
5.18.	Water quality parameters of P. m larvae fed with C+N	176
5.19.	Water quality parameters of P. m larvae fed with C+D	178
5.20.	Water quality parameters of P. m larvae fed with D+N	179
6.1.	Biochemical composition of mono-algae fed P. m larvae at Z3 stage	189
6.2.	Biochemical composition of mono algae fed P. m larvae at M3 stage	191
6.3.	Biochemical composition of mixed algae fed P. m larvae at Z3 stage	194
6.4.	Biochemical composition of mixed algae fed P. m larvae at M3 stage	198
7.1.	Proximate composition of algae	208
7.2.	Amino acid composition of algae	210
7.3.	Fatty acid composition of algae	213

7.4.	Correlations Analysis – Protein	214
7.5.	Correlations Analysis – Carbohydrate	215
7.6.	Correlations Analysis – Lipid	215
7.7.	Correlations Analysis - Ash	216
7.8	Pigments of the Micro Algae	216

List of Figures

Figure No.	Title	Page No.
4.1	Length of P.m larvae fed I at 10x10^4 cells/ml	59
4.2	Length of P.m larvae fed D at 10x10^4cells/ml	59
4.3	Length of P.m larvae fed N at 10x10^4 cells/ml	60
4.4	Length of P.m larvae fed I at 25x10^4cells/ml	62
4.5	Length of P.m larvae fed D at 25x10^4 cells/ml	62
4.6	Length of P.m larvae fed N at 25x10^4cells/ml	63
4.7	Length of P.m larvae fed I at 50x10^4cells/ml	65
4.8	Length of P.m larvae fed D at 50x10^4 cells/ml	65
4.9	Length of P.m larvae fed N at 50x10^4cells/ml	65
4.10	Survival rate of P. m larvae fed I at 10x10^4cells/ml	67
4.11	Survival rate of P. m larvae fed D at 10x10^4cells/ml	68
4.12	Survival rate of P. m larvae fed N at 10 x10^4cells/ml	68
4.13	Survival rate of P. m larvae fed I at 25 x10^4cells/ml	70
4.14	Survival rate of P. m larvae fed D at 25x10^4cells/ml	70
4.15	Survival rate of P. m larvae fed N at 25x10^4cells/ml	71
4.16	Survival rate of P. m larvae fed I at 50x10^4cells/ml	73
4.17	Survival rate of P. m larvae fed D at 50x10^4 cells/ml	73
4.18	Survival rate of P. m larvae fed N at 50x10^4cells/ml	73
4.19	Developmental index of P. m larvae fed I at 50x10^4cells/ml	76
4.20	Developmental index of P. m larvae fed D at 50x10^4cells/ml	76
4.21	Developmental index of P. m larvae fed N at 50x10^4cells/ml	76
4.22	Ingestion rate of P. m larvae fed I at 50x10^4cells/ml	79
4.23	Ingestion rate of P. m larvae fed D at 50x10^4cells/ml	79
4.24	Ingestion rate of P. m larvae fed N at 50x10^4cells/ml	79

4.25	Weight of P. m larvae fed at $50x10^4$ cells/ml	81
4.26	Percentage increment on growth (length) of P. m fed I	83
4.27	Percentage increment on growth (length) of P. m fed D	83
4.28	Percentage increment on growth (length) of P. m fed N	83
4.29	Growth rate of P. m larvae fed I	86
4.30	Growth rate of P. m fed D	86
4.31	Growth rate of P. m fed N	86
4.32	Percentage increment in weight of P. m larvae at 50x 104 cells/ml	88
4.33	Growth rate (weight)of P. m at 50x 104 cells/ml	90
4.34.a	Water quality parameters of P.m fed on C	91
4.34.b	Water quality parameters of P. m fed on C	92
4.35.a	Water quality parameters of P. m fed on I	93
4.35 b	Water quality parameters of P. m fed on I	93
4.36 a	Water quality parameters of P. m fed on D	94
4.36 b	Water quality parameters of P. m fed on D	95
4.37.a	Water quality parameters of P. m fed on N	96
4.37.b	Water quality parameters of P. m fed on N	96
5.1.	Length of P.m larva fed I + C at $10x10^4$ cells/ml	114
5.2.	Length of P.m larva fed I+N at $10x10^4$ cells/ml	114
5.3.	Length of P.m larva fed I + D at $10x10^4$ cells/ml	118
5.4.	Length of P.m larva fed C + N at $10x10^4$ cells/ml	118
5.5.	Length of P.m larva fed C+ D at $10x10^4$ cells/ml	119
5.6.	Length of P.m larva fed D+ N at $10x10^4$ cells/ml	119
5.7.	Length of P.m larva fed I + C at 25 $x10^4$ cells/ml	120
5.8.	Length of P.m larva fed I + N at 25 $x10^4$ cells/ml	120
5.9.	Length of P.m larva fed I + D at 25 $x10^4$ cells/ml	121
5.10.	Length of P.m larva fed C + N at $25x10^4$ cells/ml	121

5.11.	Length of P.m larva fed C + D at 25 x10^4 cells/ml	122
5.12.	Length of P.m larva fed D + N at 25x10^4 cells/ml	122
5.13.	Length of P.m larva fed I + C at 50x10^4 cells/ml	125
5.14.	Length of P.m larva fed I + N at 50x10^4 cells/ml	125
5.15.	Length of P.m larva fed I + D at 50x10^4 cells/ml	126
5.16.	Length of P.m larva fed C + N at 50x10^4 cells/ml	126
5.17.	Length of P.m larva fed C + D at 50x10^4 cells/ml	127
5.18.	Length of P.m larva fed D + N at 50x10^4 cells/ml	127
5.19.	Survival rate of P. m larva fed I + C at 10x10^4 cells/ml	130
5.20.	Survival rate of P. m larva fed I + N at 10x10^4 cells/ml	130
5.21.	Survival rate of P. m larva fed I + D at 10x10^4 cells/ml	131
5.22.	Survival rate of P. m larva fed C + N at 10x10^4 cells/ml	131
5.23.	Survival rate of P. m larva fed C + D at 10x10^4 cells/ml	132
5.24.	Survival rate of P. m larva fed D + N at 10x10^4 cells/ml	132
5.25.	Survival rate of P. m larva fed I + C at 25x10^4 cells/ml	135
5.26.	Survival rate of P. m larva fed I + N at 25x10^4 cells/ml	135
5.27.	Survival rate of P. m larva fed I + D at 25x10^4 cells/ml	136
5.28.	Survival rate of P. m larva fed C + N at 25x10^4 cells/ml	136
5.29.	Survival rate of P. m larva fed C + D at 25x10^4 cells/ml	137
5.30.	Survival rate of P. m larva fed D + N at 25x10^4 cells/ml	137
5.31.	Survival rate of P. m larva fed I + C at 50x10^4 cells/ml	140
5.32.	Survival rate of P. m larva fed I + C at 50x10^4 fed I + N at 50x10^4 cells/ml	140
5.33.	Survival rate of P. m larva fed I + D at 50x10^4 cells/ml	141
5.34.	Survival rate of P. m larva fed C + N at 50x10^4 cells/ml	141
5.35.	Survival rate of P. m larva fed C + D at 50x10^4 cells/ml	142

5.36.	Survival rate of P. m larva fed D + N at $50x10^4$ cells/ml	142
5.37.	Developmental index of P. m larva fed I + C at $50x10^4$ cells/ml	146
5.38.	Developmental index of P. m larva fed I + N at $50x10^4$ cells/ml	146
5.39.	Developmental index of P. m larva fed I + D at $50x10^4$ cells/ml	147
5.40.	Developmental index of P. m larva fed C + N at $50x10^4$ cells/ml	147
5.41.	Developmental index of P. m larva fed C + D at $50x10^4$ cells/ml	148
5.42.	Developmental index of P. m larva fed D + N at $50x10^4$ cells/ml	148
5.43.	Ingestion rate of P. m larva fed I + C at $50x10^4$ cells/ml	151
5.44.	Ingestion rate of P. m larva fed I + N at $50x^4$cells/ml	151
5.45.	Ingestion rate of P. m larva fed I + D at $50x10^4$ cells/ml	152
5.46.	Ingestion rate of P. m larva fed C + N at $50x10^4$ cells/ml	152
5.47.	Ingestion rate of P. m larva fed C + D at $50x10^4$ cells/ml	153
5.48.	Ingestion rate of P. m larva fed D + N at $50x10^4$ cells/ml	153
5.49.	Weight of P. m larva (Z3) fed at $50x10^4$ cells/ml	155
5.50.	Weight of P. m larva (M3)fed at $50x10^4$ cells/ml	155
5.51	Percentage increment on growth (length) of P. m larva fed I + C	158
5.52	Percentage increment on growth (length) of P. m larva fed I + N	158
5.53	Percentage increment on growth (length) of P. m larva fed I + D	159
5.54	Percentage increment on growth (length) of P. m larva fed C+ N	159
5.55	Percentage increment on growth (length) of P. m larva fed C + D	160
5.56	Percentage increment on growth (length) of P. m larva fed C + D	160
5.57	Growth rate (length) of P. m fed I +C	163
5.58	Growth rate (length) of P. m fed I + N	163
5.59	Growth rate (length) of P. m fed I + D	164
5.60	Growth rate (length) of P. m fed C +N	164
5.61	Growth rate (length) of P. m fed C + D	165
5.62	Growth rate (length) of P. m fed D + N	165

5.63	Percentage increment (weight) of P. m larva Z3	167
5.64	Percentage increment (weight) of P. m larva M3	167
5.65	Growth rate (weight) of P. m larva at Z3	169
5.66	Growth rate (weight) of P. m larva at M3	169
5.67.a	Water quality parameters of P. m fed on C	171
5.67.b	Water quality parameters of P. m fed on C	171
5.68.a	Water quality parameters of P. m fed on I+C	172
5.68.b	Water quality parameters of P. m fed on I+C	173
5.69.a	Water quality parameters of P. m fed on I+N	174
5.69.b	Water quality parameters of P. m fed on I+N	174
5.70.a	Water quality parameters of P. m fed on I+D	175
5.70.b	Water quality parameters of P. m fed on I+D	176
5.71.a.	Water quality parameters of P. m fed on C+N	177
5.71.b	Water quality parameters of P. m fed on C+N	177
5.72.a	Water quality parameters of P. m fed on C+D	178
5.72.b	Water quality parameters of P. m fed on C+D	179
5.73.a	Water quality parameters of P. m fed on D+N	180
5.73.b	Water quality parameters of P. m fed on D+N	180
6.1	Biochemical composition of P. m larva fed mono-algae at Z3	185
6.2	Biochemical composition of P. m larva fed mono-algae at Z3	191
6.3	Biochemical composition of P. m larva fed mixed- algae at Z3	195
6.4	Biochemical composition of P. m larva fed mixed- algae at Z3	199
7.1	Biochemical composition of algae	209
7.2	Essential Amino acids	211
7.3	Non Essential Amino acids	212
7.4	Fatty acid composition of microalgae	214

List of Plates

Plate No. 1 Experimental Setup

Plate No. 2 Micro Algae Culture

Plate No. 3 Protozoea 1

Plate No. 4 Protozoea 2

Plate No. 5 Protozoea 3

Plate No. 6 Mysis 1

Plate No. 7 Mysis 2

Plate No. 8 Mysis 3

Plate No. 9 Protozoea with Long Faecal String

Plate No. 10 Fully Fed Protozoea

Plate No. 11 Pigment TLC of Micro Algae

Chapter- I

INTRODUCTION

Aquaculture is the fastest growing food producing sector that is developing, expanding and intensifying worldwide to meet the increasing demand of global population. 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 is considered to be an opportunity to bridge the supply and demand gap of aquatic food around the world. The aquaculture sector is expected to contribute more effectively to global food security, nutritional well-being, poverty reduction and economic development by producing, with minimum impact on the environment and maximum benefit to society (Pratoomyot, 2005).

The reported global production of food fish from aquaculture, reached 68.3 million tonnes with a first-sale value of US$106 billion in 2008. In the period 1970–2008, the production of food fish from aquaculture increased at an average annual rate of 8.3 percent, while the world population grew at an average of 1.6 percent per year. The combined result of development in aquaculture worldwide and the expansion in global population is that the average annual per capita supply of food fish from aquaculture for human consumption has increased by ten times. Notwithstanding the slower growth rate, aquaculture still remains one of the fastest growing sectors when compared to other food-producing systems. The share of aquaculture in total fish production expected to grow from an average 38% for 2008-10 to 45% in 2020.

Among the Asian countries, India ranks second in aquaculture and third in capture fisheries production and is one of the leading nations in export of marine products. Aquaculture in India has a long history; there are references to fish culture in Kautilya's Arthashastra (321–300 B.C.) and King Someswara's Manasoltara (1127 A.D.). The traditional practice of fish culture in small ponds in eastern India is known to have existed for hundreds of years ago.

The overall aquaculture production from export oriented aquaculture in India during the year 2009-10 has given a hope that with proper management technique, the production would definitely be a viable venture. The total production from aquaculture is estimated to be 1, 04, 217.81 MT with a total value of Rs.2530.70 crores. The production has shown an increase of 15,414.81 MT in volume and Rs.615.70 crores in value respectively over the previous year's production and value which is 17.36% improvement in volume and 32.15% in value. It is mainly contributed from shrimp production. During the current year of 2009-10, shrimp production was estimated to be around 95,918.89 MT from an area of 1, 02,259.98 ha.

Regarding the state wise shrimp farming Kerala has a production of 1, 581.00 (MT) and Productivity of 1.07(MT/ha/Yr). The value of shrimp produced during the year is estimated as Rs. 2, 398 crores, which has registered an increase of around Rs.688 crores over the previous year. Kerala, has a sprawling brackish water area of nearly 65, 000 ha suitable for shrimp farming. Out of this about 14, 500 ha has been utilised for shrimp aquaculture. Major portion of this area is under the traditional prawn filtration fields, locally known as Chemmeen kettu. This culture

operation is an age-old avocation, wherein shrimp culture and paddy cultivation is practiced in rotation during summer and monsoon seasons respectively.

The brackishwater aquaculture sector is mainly supported by shrimp production mainly the giant tiger shrimp (*Penaeus monodon*), which is internationally known as tiger shrimp, has been and continues to be the leading candidate species. *P. monodon* is also the largest (maximum length 363 millimeters) and fastest growing of the farmed shrimp species. In India, other than *P. monodon*, species such as *Fenneropenaeus indicus* (white shrimp), *P. penicillatus* (like white shrimp), *P. semisulcatus* (green tiger prawn) and *P. merguiensis* (banana shrimp) are also farmed (but the two shrimp species – *P. monodon* and *Fenneropenaeus indicus* form the mainstay of shrimp aquaculture in the country.

One of the major bottlenecks in aquaculture is the rearing of fish and crustacean larvae. Larvae of the most fish and crustaceans encounter problems to accept inert/dry diets. Even if they accept the diets, their low enzyme activity and non functional stomach do not allow them to digest the existing formulated diets. Improving the acceptance of the dry diets remains a central task for aqua culturists. Hence, live food (phyto and zooplankton) remains an important food source for the start feeding of early larval stages. This live food should be easily available, reproducible and economically viable. Problems accounting in supply with this live food may prevent successful larval rearing, which hamper the whole production system. The choice of algal strains for aquaculture use is constrained by a number of factors such as toxicity of the alga, nutritional profile, size and

palatability and finally the selected algae should be relatively robust and easy to culture.

Food and water quality are important parameters affecting the success of aquatic larval culture. The introduction of microalgae in the culture tanks has been reported to improve the water quality and also supply food for many species of cultured larvae. From an economic perspective, the application of algal diet is an important factor in aquaculture. The production of microalgae in culture is commonly considered to be the major constraint on productivity and expansion of a farm. Furthermore, it is the most expensive part of the process, accounting for 30 million dollar of hatchery costs (Borowitzka, 1999).

Quality prawn seed is a critical input required for successful shrimp culture. In order to ensure availability of quality shrimp seeds commercial shrimp hatcheries are established in the public as well as private sector. At present an area of more than 15000 ha is identified as suitable for shrimp culture. At the rate of stocking density of 40,000 seeds per ha as directed by Aquaculture Authority of India, the total seed requirement will be in the tune of 600 million of *Penaeus monodon*. The success of shrimp culture depends largely on the availability of adequate quantity of the seeds of desired species at the appropriate time.

To determine which components provide adequate nutrition to prawn larvae, along with the growth response in various stages for which the biochemical composition of algae and of larvae should be estimated. There is a continuous need to evaluate the effect of algal food on the survival, growth and chemical composition of the prawn in order to

establish good working criteria for the production of *P. monodon* larvae. Hence it is imperative that feeding schemes for artificial rearing, behavioral response of each larval stage toward food etc are to be highlighted. Hence the present study was undertaken to evaluate the growth responses of *Penaeus monodon* larvae to various monospecific and mixed algal diets.

OBJECTIVES OF THE PRESENT STUDY

1. Compare the growth rate of *P. monodon* larvae fed with varying concentration of monospecific algal diets of *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina.*
2. Compare the growth rate of *P. monodon* larvae fed with mixed algal diets ie. Combinations of *Chaetoceros calcitrans* + *Dunaliella salina, Chaetoceros calcitrans* + *Isochrysis galbana*, *Chaetoceros calcitrans* + *Nannochloropsis salina, Dunaliella salina* + *Isochrysis galbana, Isochrysis galbana* + *Nannochloropsis salina, Nannochloropsis salina* + *Dunaliella salina.*
3. Evaluate the Protein, Carbohydrate and Lipids in mono and mixed algae fed *P. monodon* larvae during the PZ3 and M3 stages.
4. Evaluate the Protein, Carbohydrate and Lipids in *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina*.
5. Evaluate the amino acid and fatty acid composition of the micro algae.

Chapter- 2

LITERATURE REVIEW

2.1 Historical Perspective

It is difficult to determine exactly where and when marine aquaculture began. Milkfish culture has been conducted in Asia for centuries, based on the capture of fry from the wild (Liao, 1992), so that modern rearing methods and live feed in the hatchery were not required. Spawning and successful larval culture of mussels was not achieved until the early 1950s. Investigations of algal feeds for the rearing of molluscan larvae took place in the 1930s at both the Conway, Wales, Fisheries Experiment station and the Milford, USA, Bureau of Commercial Fisheries Biological Laboratory. Fertilization of large tanks of filtered seawater to induce mixed phytoplankton blooms as food for molluscan larvae was carried out continuously beginning in 1938. Decades of work at the Conway and Millford laboratories paved the way for hatchery production of mollusks for commercial aquaculture in which natural settling of larvae was either impossible or undesirable.

The culture of algae seems to have its origin in the late 1800^{s}. A significant advance marine algal culture was reported, who tried to culture copepods. Little did he know that they would also be of great importance in aquaculture. Methods for marine algal culture continued to advance during the middle of the twentieth century with the development of artificial media and the devepment of 'F' medium for the enrichment of sea water (Guillard and Ryther 1962). Improved methods for monospecific algal cultures

allowed expansion of hatcheris for molluscan aquaculture and enabled culture of live invertebrates as feed for larval fish and crustaceans.

The last quarter of the twentieth century saw the explosion of the marine aquaculture, both the shrimp and fish. The Japanese work on Kuruma prawn led ultimately to the culture of numerous species around the world. Although post larval shrimp for stocking into grow out ponds were for years collected from the wild, the recent trend has been towards hatchery production, which is heavily dependent on micro algae and *Artemia* nauplii as live feeds (Lavens and sorgeloos, 1996).

2.2 Necessity of Live Feed

Live feeds are able to swim in the water column and are thus constantly available to the larvae. Formulated diets tend to aggregate on the water surface or more commonly, sink quickly to the bottom, and are thus normally less available the the larvae than are the live feeds. In addition the movement of live feed in the water is likely to stmulate larval feeding responses, since they are adapted to attack moving prey in nature. Formulated diets sre capable of moving only in a downward direction, towards the bottom. Finally, live prey, with a thin exoskeleton and high water content, may be more palatable to the larvae, once taken into the mouth, compared to the hard, dry formulated diets. Larvae quickly either accept or reject food on the base of palatability.

Crustacean larvae such as shrimp are qualitatively different from fish larvae. They are filter feeders as early larvae and by the time they can feed on live zooplankton, they possess not only feeding appendages with

which to manipulate the prey organisms captured, but also a gut morphology and physiology with which to digest formulated diets more effectively (Jones *et al* 1997 b)

2.3 Micro algae for aquaculture

Ocean phytoplankton, with a several hundred billion tones of dry weight per, forms the back bone of the aquatic food chain, contributing to the production of some 100 million tones of renewable resources per year from fishing. Hence it is hardly surprising that the micro algae composing phytoplankton play a crucial role in marine animal aquaculture, especially for mollusks, shrimp and fish. Aquaculture hatcheries often need to include a micro algal production system and, in the case of marine fish larvae, a live prey production system.

Micro algae and Cyanobacteria are a major component of the plant kingdom and play a major role in building and maintaining the earth's atmosphere by producing oxygen and consuming carbon dioxide. Micro algae species selected for aquaculture are generally free living. All are pelagic and in the nannoplankton range (2-20µm).

Microalgae are required for larval nutrition of aquatic animals during a brief early period, either for direct consumption (molluscs and penaeid shrimp) or indirectly as food for live prey fed to small marine fish larvae. Even when necessary for a short period only, microalgae are crucial as they determine (to various extents) the supply of juveniles available for production. The main microalga-consuming aquaculture groups include filtering molluscs, penaeid shrimps and small larva fish (Muller-Feuga 2003).

Most marine invertebrates have a distinct larval phase in their early life histories and can be divided into species whose larvae feed in the plankton (planktotrophic) and species whose larvae can develop and metamorphose with out feeding (lecithotrophic) (Thorson, 1950; Strathmann, 1985). A number of studies have used mathematical models to explore the ecological and Evolutionary forces that select for plank totrophy or lecitho-trophy (Vance, 1973; Christiansen and Fenchel, 1979; Caswell, 1981; Pechenik, 1987). Lecithotrophic development is believed to have repeatedly evolved from plank totrophic larval forms in echinoderms and many other phyla (Strathmann, 1985; Emlet, 1990, 1995).

Many changes associated with developmental mode have been reported for eggsize (Emlet *et al* 1987), biochemical composition (Hoegh-Guldberg and Emlet, 1997; Byrne and Cerra, 2000), patterns of embryogenesis (Raff, 1987; Emlet, 1995; Mar-tindale and Henry, 1995) and larval morphology (Olson *et al* 1993; Emlet, 1995). While there are many suites of morphological and eco-logical characters associated with planktotrophy and leci-thotrophy, less is known about the biochemistry and physiology of larval stages. A number of studies have examined the energetics of development of plank totrophic and lecithotrophic species (Crisp *et al* 1985; Gallager *et al* 1986; Dawirs, 1987; Anger *et al* 1989; Nates and Mc-Kenney, 2000; Marsh *et al* 2001) and some have compared the physiological energetics of development of congeneric plank totrophic and lecithotrophic species (e.g., Hoegh-Guldberg and Emlet, 1997; Moreno and Hoegh-Guldberg, 1999) as a means of exploring the evolutionary changes that are associated with the switch from feeding to non feeding development.

Marine larviculture without live feed or crustacean cultures without micro algae are rarities in commercial aquaculture. The development of commercial formulated feeds remains today's upcoming challenge. In the meantime, the industry continues the struggle to produce stable quantities of high quality live feeds. The different species used in marine aquaculture differ in their biology and culture requirements, providing ample challenges for the novice and requiring expertise in a commercial enterprise.

In aquaculture, the production of larvae and juveniles in good nutritional and health conditions is one of the most important factors to assure success. Larvae generally feed from different species of micro algae and the amount and the quality of food are some of the most critical factors affecting larval development. Thus, the biochemical composition of the food used for feeding the larvae provides the basic element for their present and future metabolism, growth and reproduction.

The nutritional value of a micro algal species depends on the main molecular components such as proteins, carbohydrates and lipids (specially fatty acids), qualifying them as good or bad quality (Napolitano *et al* 1990). At the same time the biochemical content of the microalgae depends on several factors, such as the strain, type and amount of nutrients used in the culture medium, temperature and light conditions, stages of the growth curve at the harvest and water quality (Abalde *et al* 1994; Duerr *et al* 1998).

In general the nutritional elements of a microalgale required for high survival of penaeid larvae are protein content higher than 25% of its dry weight, 8-30% carbohydrate content and around 10% lipid content, specially including certain types of lipids such as fatty acids C20: w3 and

C22:6w3 (Tobias Quinito and Villegas, 1982). Several species of Chaetoceros have shown to meet these requirements have shown to meet this requirements and serve as an excellent food for penaeid larvae (Simon 1978; Tobias and Villegas 1982; D' Souza and Loneragan 1999).

Several studies found that the indigenous species of microalgae can perform better, than some of the exotic species commonly used for culture. Sancez (1986) found that the larvae of *Litopenaues vannamei* cultured in Florida and fed with a native microalgae *Isochrysis sp.* exhibited the highest survival from larval stages of protozoea I to postlarvae I, compared to those fed with other non-indigenous species. Renaud *et al* (1994) evaluated the biochemical components, compared to non-indegenous species used for culture.

Martin *et al* (1994) compared the growth rate, dry biomass and bacteriological quality of 18 strains of exotic microalgae and 7 native species. They found that, from the exotic strains, only *Tetraselmis chuii* showed satisfactory results for mass culture under 'tropical' condition. They also found that among the native species, *Tetraselmis sp. G1, Chaetoceros sp. A1* and *Skeletonema sp. Ch1,* showed good growth and high dry biomass. They suggested some native species, could be cultured as food for larvae of marine organism such as fish, molluscs and crustaceans.

Penaeid larvae are generally cultured on live unicellular algae during the protozoeal stages and animal preys are added along with algal feeds during the mysis and early postlarval stages (Hudinaga 1942, Cook and Murphy 1969). It has been reported that several species of algae, e.g. the phytoflagellates *Tetraselmis* (Samocha and Lewinsohn 1977, Kurmaly *et al* 1989) and *Isochrysis* (Aquacop 1983), and the diatoms *Skeletonema*

(Yúfera *et al* 1984, Preston 1985), *Thalasiossira* (Emmerson 1984 Kuban *et al* 1985)and *Chaetoceros* (Aquacop 1983 Kuban *et al* 1985,Tobias Quinitio and Villegas 1982) are adequate food for penaeid larvae.

2.4 Mono Algal Diets for Shrimp

Feed formulation and water quality are important parameters influencing the growth potentiality of shrimps. Experimental shrimps and the post larvae (PL) reared in the nursery ponds were provided with cultured unicellular algae (*Skeletonema and Chaetocerous*) for PL (1-10) and cultured live feed (*Artemia* and *Cyclops*) for PL (10-30). Among the phytoplankton, *Skeletonema* appeared promising and among the other feed *Artemia* promoted substantial growth in the post larvae (Devi 2004). The influence of protein and energy levels on growth rate, survival, pre- and post-prandial oxygen consumption, ammonia excretion, haemolymph glucose (HG), glycogen in digestive gland and osmotic pressure (OP) in white shrimp *L.vannamei* and *L. setiferus* juveniles was studied (Rosas *et al* 2001).

The presence of *Tilapia hornorum* alone was more efficient in controlling the growth of luminous bacteria than the co-existence of tilapia and *Chlorella* sp. Nevertheless, the presumptive Vibrio count was lowest in control tanks that had the highest shrimp survival rate, which was attributed to the presence of other micro-algae such as *Chaetoceros, Thalassiosira, Navicula, Nitszchia, Melosira,* and *Fragilaria* (Tendencia 2005).

The total ash, chlorophyll, phaeopigment, lipid, and fatty acid content of investigated isolate were compared with the diatom *Skeletonema costatum* and the prymnesiophyte *Isochrysis galbana* Parke. Considerable

amounts (2-3% of the total fatty acid) of 20:5w3 eicosapentaenoic acid and very large amounts of 18:3w3 linolenic acids (16-20%) were present. The three algal species contained the w3-polyunsaturated fatty acids necessary for the growth and survival of penaeid prawn larvae grew well under the conditions of temperature and salinity in the hatchery. (Shamsudin 1999)

A study was done by Ronquillo (1997) to determine the different optimum culture conditions for *T. tetrathele*, and to evaluate its application in the hatchery production of different penaeid species. The dietary value of *T. tetrathele* was evaluated by feeding it to different shrimp larvae from protozoea-1 (PZ-1) until postlarva-1 (PL-1). There was no significant difference ($P>0.05$) in the growth of *T. tetrathele* at 25 ^{0} C and 30^0 C in acidic media; but, there were significant differences ($P<0.05$) in the range favoring fast growth at 25 ^{0} C.

Different diet series were used on different stages of *L. vannamei's* larvae and post larvae. The results showed that from zoea to the second stage of mysis, survival rate of those fed with genus *Skeletonema* and artifical compound feed is 18. 5% and 26.7% higher than those fed only with genus *Skeletonema* or artifical compound feed, respectively. There was no distinct difference of survival rate while feeding only with artifical compound feed or artemia nanplii after the second stage of mysis. In the later period of artifical breeding, the survival rate is constant while the artifical diet replaced the Artemia nauplii (Luo *et al* 2004).

Growth rate, soluble-protein content and digestive-enzyme activities were studied in *L. vannamei* (Boone, 1931) early post-larvae under six feeding regimens, which included combinations of freshly hatched *Artemia* nauplii, an artificial diet and algae. No obvious relationship was found

between enzyme activity and growth in any feed combination. Based on growth and soluble-protein content, we determined that partial substitution (50%) of *Artemia* nauplii by artificial diet and the use of algae co-fed beyond the first post-larval stage benefits growth and the nutritional state of *L. vannamei* post-larvae (Brito 2001).

Sangha, (2000) evaluated the effectiveness of partial or total replacement of microalgae with artificial diets administered together with a single dose of live algae (SDLA) for the protozoeal stages of the Pacific white shrimp *L. vannamei.* A significantly better total length was achieved by larvae fed the live microalgae control (2.51mm) or the above artificial diet regime (2.44 mm) than in all other treatments.

Chaetoceros, microparticulated A, microparticulated B and frozen *Spirulina* were fed to the zoea stage of the tiger prawn (*P. monodon* Fabricius). The lowest concentration of ammonia and the highest concentration of nitrite including the highest survival rate of the tiger prawn larvae are observed by feeding on *Chaetoceros*. Development period of the zoea stage to the first stage of mysis on microparticulated A and B application are longer than *Chaetoceros* application. There is no significant of survival rate from mysis stage to the first stage of post larvae found in all kinds of food application but the shortest development period and the active movement of the larvae were found in *Chaetoceros* application (Chote Sahakichrungruang 2000).

Gallardo (2002) focused on an adequate level of replacement of *Artemia* nauplii and microalgae by a microbound diet for rearing *L. setiferus* larvae. In the presence of algae, maximum growth and survival may be obtained in 40-60% (5.5-6.5 mg MBD L super (-1) day super (-1))

of *Artemia* nauplii replacement levels. In the absence of algae, the *Artemia* nauplii replacement resulted in slower development, less salinity resistance, lower growth and lower survival than was obtained in larvae fed with algae.

Moss (1994) studied on the changes in whole body weight, RNA and DNA concentrations, and RNA/DNA ratios of juvenile white shrimp, *Penaeus vannamei* Boone, fed different algal species were compared to assess the nutritional contribution of each species to shrimp growth. Shrimp fed a diatom culture composed primarily of *Chaetoceros sp*. were significantly heavier ($p < 0.05$) than shrimp fed a monoculture of the green alga, *N. oculata*, fronds from the leafy macroalga, *Ulva* sp., or fronds from the filamentous macroalga, *Enteromorpha* sp. after 5 days. Diatoms can contribute substantially to short-term shrimp growth, and are probably important in coastal nursery grounds and aquaculture ponds when other food resources are scarce or to supplement available food.

The food organisms commonly used in the Malaysian mariculture of penaeid shrimp *P. monodon*, consist of algae (diatom *C. calcitrans*; prymnesiophyte *I. galbana*) and zooplankton species (rotifer *Brachionus plicatilis*, local copepod species (mostly *Oithona nana*) and brine shrimp (*Artemia salina*). The algae were fed to the early stages of the penaeid larvae (Z (1)-Z (2)) while the later stages (M (1)-PL (3)) were given rotifer, copepod species and *Artemia salina* (Shamsudin 1993). Improvements in rearing larvae penaeid shrimp by the Galveston Laboratory method are presented. The use of frozen algae and frozen *Artemia* nauplii as food for larval penaeid shrimp in hatchery systems is discussed (Mock, 1980).

Dunaliella, a unicellular green alga, offers enormous potential benefits to the commercial mariculture industry. Tolerant of very wide

ranges of temperature, salinity and pH, this organism accumulates high levels of nutrients such as β-carotenoids, making it a favorable source of live feed. One of the stress-induced responses in *Dunaliella* is the production and accumulation of the carotenoid, β-carotene. *Dunaliella* is one of the richest natural producers of carotenoid, producing up to 15% of its dry weight under suitable conditions. (Ben-Amotz *et al* 1989).The carotenoids are stored as intracellular oil droplets near the outer membrane and it has been suggested that carotenoid and triacylglycerol biosynthesis are interrelated (Rabbani *et al* 1998). The commercial cultivation of *Dunaliella* began in the 1960's once it was realized that their halotolerance allowed for monoculture in large brine ponds.Till today, β-carotene remains the major natural product harvested from *Dunaliella.* Common uses of β-carotene include food coloring, additives to multivitamin preparations, health food products, cosmetics, and animal feed as provitamin A. The ease of maintaining *Dunaliella* in culture its ability to grow in very high salt concentrations, tolerance to high temperature and to extreme pH changes - makes this species a highly desirable target for exploitation as a biological factory for the large-scale production of foreign proteins.

Artemia are non-selective filter feeders and therefore will ingest a wide range of foods. The main criteria for food selection are particle size, digestibility, and nutrient levels (Dobbeleir *et al* 1980). Possibly the best foods for Artemia are live microalgae such as *Nannochloropsis, Tetraselmis, Isochrysis, Dunaliella* and *Pavlova*. Combinations of live phytoplankton fed to *Artemia* cultures have demonstrated superior enrichment characteristics over feeding single phytoplankton species (D'Agostino 1980). However, not all species of unicellular algae are

appropriate for sustaining *Artemia* growth. For example, *Chlorella* and *Stichococcus* have a thick cell wall that cannot be digested by *Artemia.*

Larval cultures of *Fenneropenaeus indicus* have received considerably less attention than other commercially important penaeid species. The larvae of this species have been cultured successfully on various single algal species, such as *Thalassiosira weissflogii* (Emmerson 1980), and on mixed algal diets, such as *Chaetoceros gracilis, Platymonas sp*., and *Isochrysis galbana*, and *Artemia salina* nauplii after the PZ3 stage (Galgani and Aquacop 1988, Aquacop1983) suggests the use of mixed algae, *Isochrysis* and *Chaetoceros*, in rearing *F. indicus* larvae. The optimal cell concentration for the growth and survival of penaeid larvae varies with the larval developmental stages and the cell size of the algal species used. Emmerson 1980 obtained 96% survival at PL1 when he maintained the algal cell density of T. weissflogii at 7 cells mL-1 between the PZ1 and PZ3 stages. Aquacop 1983 recommends 100 cells mL-1 of mixed algae *Chaetoceros* (20%) and *Isochrysis* (80%) between the PZ1 and PZ3 stages. Galgani and Aquacop 1988 report that an algal cell density of 30-40 cells mL^{-1} of *C. gracilis, Platymonas sp.,* and *I. galbana* was sufficient to rear *F. indicus* larvae during the protozeal stages. Emmerson and Andrews 1981 studied the effect of stocking density on the growth, survival and development of *F. indicus* and concluded that levels decrease with increasing larval stocking density.

Feed formulation and water quality are important parameters influencing the growth potentiality of shrimps. Experimental shrimps and the post larvae (PL) reared in the nursery ponds were provided with cultured unicellular algae *(Skeletonema* and *Chaetoceros*) for PL sub(1-10)

and cultured live feed (*Artemia* and *Cyclops*) for PL sub (10-30). Among the phytoplanktons, *Skeletonema* appeared promising and among the other feed *Artemia* promoted substantial growth in the post larvae (Devi 2004).

The two principal methods of culturing penaeid larvae and post larvae are briefly described by (John 2009). In the "indoor" method algae to feed the larval shrimp are cultured separately and added to the rearing tank when needed, while in the "outdoor" method sunlight is utilized to grow phytoplankton as food in the same water with the larvae. Based on growth and soluble-protein content, Brito *et al* 2002 determined that partial substitution (50%) of Artemia nauplii by artificial diet and the use of algae co-fed beyond the first post-larval stage benefits growth and the nutritional state of *L. vannamei* post-larvae.

Chu, 2008 studied on the feasibility of completely replacing live foods with an artificial diet for rearing the larvae of *Metapenaeus ensis* (de Haan) and *P. chinensis* (Osbeck). The highest survival and development rates were always obtained with live foods. Larvae fed with artificial diets had retarded development and their survival to post-larvae was always lower than those fed live foods. Total replacement of live foods with artificial diets resulted in a reduction in the body length of post-larvae I in *P. chinensis*. It was concluded that the two artificial diets are not satisfactory complete substitutes for live foods in raising the two penaeids but can be used as a convenient supplement when algal diet is limited.

The effects of the density and type of food on oxygen consumption and ingestion rate of larvae of the white shrimp *P. setiferus* fed diatoms *C. ceratosporum*, flagellates *T. chuii* and *Artemia franciscana* nauplii were analysed by Rosas *et al* 2006. The oxygen consumption in three

experiments increased with larval stage a maximum ingestion peak in MI was recorded in larvae fed diatoms than in larvae fed flagellates.

In low-quality water after algal blooms, survival of white shrimp (*F. indicus*) larvae was poor when reared in both sterile and 5 mm filtered water. The addition of bacteria filtered from xenic algal cultures together with the algal exudates to such water gave significant improvements in survival to larvae fed MED in 5 mm filtered water but not in autoclaved culture water. Live algal diets promote high larval survival and growth irrespective of the inclusion of bacteria.

Artificial and natural feeds for shrimp larvae cultures (*L. vannamei*) were tested in a series of containers. The control groups was fed natural diets and were cultured in large tanks that are normally used in postlarvae hatchery laboratories Results show differences in ammonia concentration and larval survival between the different diets and also between containers. Survival rate with natural feeds was almost four times higher, than with artificial diets. (Meyer, 2004)

Studies were done on the survival and development rates of larvae fed on *N. oculata* were found obviously inferior to those of larvae fed on other algal species. Most larvae in tank N (-1 to -4) diedat Z1 and Z2 stages, and the few surviving larvae were Z3 and M1 stages at the end of the experiment. It is found that *N. oculata* seemed to be inadequate as a food organism for the shrimp larvae. (Helm and Laing 1987, Okauchi 1990). The algal fatty acids were shown to be essential for *P. japonicus* larvae (Kanazawa *et al* 1978). Therefore, the nutritive values of *T. tetrathele* and *Isochrysis sp.* seem to be inferior to that of *C. gracilis.*

Larvae, fed *Artemia salina* nauplii, were provided with supplements of *Pseudoisochryois paradoxa, D. tertiolecta* or *Phaeodactylum tricornutum* for 32 days. Fatty acid analysis of the algae showed that each species of alga contained unusual fatty acids, not normally present in larvae, which might be expected to appear in the larval lipids if the algae contribute to larval nutrition. Fatty acids of the larvae from the different dietary treatments were remarkably similar and none of the unusual algal fatty acids were observed. (Jeanne 2009).

Sangha *et al* 2000, evaluates the effectiveness of partial or total replacement of microalgae with artificial diets administered together with a single dose of live algae (SDLA) for the protozoeal stages of the Pacific white shrimp *L. vannamei*. A significantly better total length was achieved by larvae fed the live microalgae control or the above artificial diet regime than in all other treatments. Larvae administered this artificial diet treatment were significantly heavier than those fed the algal control or any other treatment. The results indicate that the administration of Artemia enriched with DIS during mysis 1–post-larvae 1 for *L. vannamei* larvae results in a significant increase in survival total length and weight in comparison with other enrichments or non-enrichment.

The penaeid culture water requires a stable bacterial community if total replacement of algae is to be achieved successfully on a routine basis. In the absence of ocean-quality water, the inclusion of a single dose of microalgae (SDLA) (20 cells L−1) fed in conjunction with 5-µm-filtered sea water at the N6-PZ1 stage allows microencapsulated feed to be used without further addition of algae (Jones, *et al* 1997). Sources of DHA occur naturally in both *I. galbana* and *C.gracilis*, and may be as high as 95% and 10% of their n-3 HUFA respectively (Barclay and Zeller 1996).

2.5 Mixed Algal Diets for Shrimp

Phytoplankton are the main food of larval stages of some crustaceans (Preston *et al* 1992), of all the growth stages of bivalves (Frankish *et al* 1991), and of the early growth stages of some fishes (Reitan *et al* 1994). Detailed studies have been carried out on the nutritional value of microalgae to bivalve larvae (Thompson and Harrison 1992). Differences in the composition of protein, lipid, and particular fatty acids in the algal diet were associated with different growth rates and different biochemical compositions of the larvae. However, little work has been done on the nutritional requirements of prawn larvae. There are large differences between the survival and growth of prawn larvae fed different species of algae (Chu and Lui 1990; Naranjo *et al* 1995). For example, both are high when the larvae are fed *C. gracilis* (Simon 1978), but low when they are fed *D. tertiolecta* (Kurmaly *et al* 1989). Some mixed-algae diets have resulted in higher survival and faster development of larvae than the component species alone (Kurmaly *et al* 1989). Assuming that the toxicity, size, shape, and digestibility of the cells are the same or are not a contributing factor, the difference has been attributed to the nutritional compositions of the algae (Webb and Chu 1983). To determine which components in an alga provide nutritional value to prawn larvae, three major parameters should be measured: the simultaneous biochemical compositions of algae and of larvae, and the growth response of the larvae. Several studies have measured one or two of these parameters, but not all three (Tobias and Villegas 1982).

A study was carried out to investigate the effects of various diets: 4 monoalgal diets: *N. oculata* (N), *Isochrysis galbana* (I), *C. calcitrans* (C), and *T. tetrathele* (T); 4 mixed algal diets: N+I+C+T(NICT), N+I+C(NIC), C+T(CT), and I+T(IT); and 2 nonalgal diets: baker's yeast (BY) and prepared shrimp feed (SF) on population growth and density of *Apocyclops dengizicus* (Omidvar *et al* 2009). The type and density of diet had significant effects on the growth and density of *A. dengizicus* ($P < 0.01$). The results of the present study illustrated that *T. tetrathele* was the most suitable food for the culture of *A. dengizicus*.

According to Ali, 1999 a microparticulate diet (passed through 45 micron sieve), consisting of fish (Anchovy), squid, clam, soyaflour, wheat floor, egg (duck) and other additives and prepared by freeze drying was tested alone and in combination with diatoms in 1:1 ratio on protozoea 1 larvae of tiger shrimp *Penaeus monodon* in 500L tanks stocked with 25,000 larvae. A mixed culture of diatoms *Chaetoceros sp.* and *Skeletonema sp.* formed the control. The larvae fed with mixed diet (0.04 mg diet per larvae per day + 20,000 cells of diatoms per ml) and control diet (40,000 cells/ml of diatoms), metamorphosed to post-larvae 1 (PL1) in 8 days, while those fed the microdiet alone reached PL1 in 9 days. The mixed diet resulted in higher survival of 85.7%, compared to that of control diet of 62.5%. The microdiet alone produced a survival of 40 % ().

Larval stages of the Pacific white shrimp, *L. vannamei* were fed standard live diets of mixed microalgae from the first to the third protozoea (PZ1 to PZ3), followed by Artemia nauplii until post-larvae 1 (PL1). *L.vannamei* larvae transferred to a diet of Artemia at the beginning of the second protozoea (PZ2) stage were significantly heavier on reaching the

first mysis stage (M1) than those fed algae, while survival was not significantly different between treatments. At both PZ2 and PZ3 stages, trypsin content in larvae feeding on Artemia was significantly lower than in those feeding on algae. The rapid decline in trypsin content from PZ1 and the flexible enzyme response from PZ2 suggest that *L. vannamei* is physiologically adapted to transfer to a more carnivorous diet during the mid-protozoeal stages (Puello-Cruz 2002)

Four species of microalgae (*C. muelleri, T. suecica, Tahitian Isochrysis sp*. (T-iso) and *D. tertiolecta)* with distinctly different fatty acid profiles were grown in continuous culture and fed to prawn larvae (*Marsupenaeus japonicus, P. semisulcatus and P. monodon*) as monospecific diets. The best two diets (*C. muelleri and T. suecica*) were also fed as a mixed diet (D'souza and Loneragan 1999). Experiments were run until the larvae fed the control diet of *C. muelleri* metamorphosed to Mysis 1.

2.6 Micro algae as food for Other Organisms

The present study investigated the effects of microalgae *Nannochloropsis sp*. addition and concentration on larval survival, development and growth of an Australian strain of *M. rosenbergii* (lineage II). The results have shown that the addition of *Nannochloropsis sp.* at appropriate levels substantially improved performance of larval culture of the Australian strain of *M. rosenbergii*, suggesting that the Australian native strain has a promising potential for aquacultural development (Malwine and Chaoshu 2009).

Gireesh and Gopinathan 2008 studied the effects of food availability on the larval growth and survival of *Paphia malabarica* in two experiments by feeding the larvae with six algal diets. Newly hatched larvae of *P. malabarica* were fed with six different marine microalgae species, singly and in a combination of two species. The best growth was with *I. galbana and N. salina* as a single species of diet. The nutritional value of single-species diets was in the order of *N. salina, I. galbana, Dicrateria inornata, C. calcitrans, Tetraselmis gracilis and D. salina*. Of the mixtures tested, 50% *I. galbana* and 50% *N. salina* supported growth and metamorphosis equivalent to those of the *I. galbana* control.

Six densities of the micro-alga *Scenedesmus acuminatus* fed to the cladoceran, *Moina micrura*, in 40-litre glass aquaria indicates that it is a satisfactory micro-alga food for *M.micrura*. *Moina* population increased with increasing cell densities of *Scenedesmus* only up to treatment 3. *Moin*a population growth was inhibited at higher algal densities. The percentage of egg-bearing females and the number of eggs per egg-bearing females followed a similar pattern.

Studies were undertaken (Nuria and carmen 1998) to test whether freeze-dried microalgae are nutritionally adequate for rearing rotifers as food for gilthead seabream larvae. No significant differences were observed between the biochemical composition of larvae with treatment A (with freeze-dried microalgae) and the composition of larvae in treatment D that were obtained with the acceptable methods for culture systems with live microalgae (Soutoa *et al* 2008).

Mixed microalgal diets provide essential nutrients for animal growth and development and are more nutritionally balanced than single microalgal diets (Brown, Jeffrey & Garland 1989). Moreover, microalgae stimulate the digestive processes in marine fish larvae and contribute to the establishment of the early gut flora (Reitan *et al* 1997). The microalgae also served as food for the rotifer *Brachionus rotundiformis*, which is used to feed the larvae from day 4 to 11 post-hatch at a density of 5 rotifers per ml, and from day 11 to 20 post-hatch at a density of 10 rotifers per ml. The rotifers are grown in a mix of *Isocrhysis sp.* and *N. oculata*.

Southgate *et al* (1998) assessed the nutritional value of three species of tropical microalgae (Tahitian *I. galbana, P. salina* and *C.simplex*) for larvae of *Pinctada margaritifera.* They reported significant differences in the nutritional value of the three species tested. The nutritional value of microalgae to bivalve larvae is influenced by many factors, including size, morphology and chemical composition. Particle capture by bivalve larvae depends on morphological characteristics of the larvae, such as length and velocity of the preoral cilia and the length of the velar edge (Strathmann and Leise 1979; Gallager 1988; Riisgard *et al* 2000).

This study was carried out to investigate the population growth and egg hatching success of *A. sinjiensis* when fed a range of mono-and binary algal diets, including algae in the form of frozen paste. Of the diets tested, the binary algal diets were more successful than monoalgal diets, while the frozen algae had little dietary value (Michael *et al* 2007). Omidvar et *al* 2007; Cruz *et al* 2009 evaluated the nutritional values of *Apocyclops dengizicus* (Copepoda: Cyclopoida) fed Chaetocerous calcitrans and

Tetraselmis tetrathele. Pedro *et a*l 2008; Richard *et al* 2005 on effect of algal species Artemia.

The potential impact of selective grazing by filter-feeding bivalves was studied on the relative composition of both planktonic and benthic algae that are commonly suspended in coastal areas. Different feeding behaviour was observed in the oyster *Crassostrea gigas* and the mussel *Mytilus edulis. C. gigas* preferentially filtered and rejected (as pseudofaeces prior to ingestion) diatom species relative to flagellates. (Bougrier *et al* 1997)

For hatcheries to be able to produce mussel spat all year round, good broodstock conditioning is essential. Although it is possible to use a commercial formulated diet for *Mytilus* spat (Nevejan *et al* 2007), no such diet is available yet for mussel broodstock conditioning or larval rearing. Because of this, hatcheries still greatly depend on the use of micro-algae (Muller 2000; Brown 2002). In a study by Bayne *et al* 1978 raw seawater with added diatoms was used for broodstock conditioning but resulted in a low spawning success. Other studies described the laboratory spawning and larvae rearing of several *Mytilus* sp. (Beaumont *et al* 1993, Matson *et al* 2003). But even though *M. edulis* is one of three commonly cultured mussel species in China (Zhang 1984) no information is available on the conditioning of mussel broodstock under fully controlled conditions.

It has been reported that the haptophyte *I. galbana* .produced higher growth in *Crassostrea gigas* than the yeast-based diet Microfeast® or the dried thraustochytrids diet known commercially as Algamac 2000® (Brown and McCausland 2000). Pearl oyster postlarvae *Pinctada fucata martensii* also exhibited minimal growth when fed microencapsulated cod

liver oil (Numaguchi 2002). More consistent and positive results were reported with dried algae (Knauer and Southgate 1996), gelatin-acacia microcapsules (Numaguchi 2002), algal pastes (Brown and Robert 2002), or wheatgerm flour (Albentosa *et al* 1999, 2002) used as supplements for live microalgae. Mc Causland *et al* (1999) also demonstrated that *C. gigas* postlarvae (500–700 µm) had similar growth rates in a flow-through system supplemented with live microalgae *Shizochrytium costatum* and microalgal pastes of *C. calcitrans.*

More recent studies reported comparable growth of abalone postlarvae when fed a mixture of diatoms or two types of balanced microparticulated feed (Stott *et al* 2002). The authors also reported better growth of postlarval abalone with balanced microdiets, as opposed to diatom films.

The Prymnesiophycea *Pavlova lutheri* was produced using a traditional technique and by an innovative technique (Emanuele *et al* 2003). No differences in terms of gross composition and larval performance were noticed between the fresh algae biomass produced by the two techniques. In contrast, when *C. calcitrans* forma pumilum was used as a mono specific diet, good growth performance (>4.5 µmday−1) and a high survival rate (>86%) were observed in larvae. A substitution of 50% (trial 1) or 80% (trial 2) of fresh *C.calcitrans* forma pumilum with the preserved *P. lutheri* concentrates did not adversely affect growth rate or survival of *C. gigas* larvae.

It is now established that intensive rearing on monoalgal diets does not result in severe deterioration of the nutritional value of the copepods, at

least in terms of their highly unsaturated fatty acid (HUFA) content (Støttrup *et al* 1999). In culture, copepods are usually fed monospecific algal diets to reduce costs, but mixed diets may be more appropriate, in terms of both copepod productivity and effects on their nutritional value for fish larvae. When *A. tonsa* females were solely fed *D. tertiolecta*, they ceased feeding and producing eggs, although the reason was unclear (Støttrup and Norsker 1997).

2.7 Biochemical Composition of Algae

Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications (Pulz and Gross, 2004). They can be used to enhance the nutritional value of food and animal feed, due to their well balanced chemical composition. Moreover, they are cultivated as a source of highly valuable molecules such as polyunsaturated fatty acids, pigments, antioxidants, pharmaceuticals and other biologically active compounds. The application of microalgal biomass and/or metabolites is an interesting and innovative approach for the development of healthier food products. Microalgal biotechnology is similar to conventional agriculture, but has received quite a lot of attention over the last decades, because they can reach substantially higher productivities than traditional crops and can be extended into areas and climates unsuitable for agricultural purposes (e.g. desert and seashore lands).

Microalgae are an indispensable food source for all growth stages of bivalves and for the larvae of some crustacean and fish species in aquaculture. They are also eaten by zooplankton reared as food for the larvae and juveniles of some crustacean and fish species. In these latter

aquaculture food chains, important nutrients from microalgae are transferred to higher trophic levels via the intermediary zooplankton (Watanabe *et al* 1983). The nutritional value of microalgae is influenced by their size, shape, digestibility and biochemical composition (Webb and Chu, 1983; Brown *et al* 1989). Many investigations have examined the gross composition of microalgae, but in only a few instances has it been correlated with nutritional value (Enright *et al* 1986; Utting, 1986; Whyte *et al* 1989).

Microalgae are a major natural source for a vast array of valuable compounds, including a diversity of pigments, for which these photosynthetic microorganisms represent an almost exclusive biological resource. Yellow, orange, and red carotenoids have an industrial use in food products and cosmetics as vitamin supplements and health food products and as feed additives for poultry, livestock, fish, and crustaceans.

In algae and higher plants, carotenoids play multiple and essential roles in photosynthesis. They contribute to light harvesting, maintain structure and function of photosynthetic complexes, quench chlorophyll triplet states, scavenge reactive oxygen species, and dissipate excess energy (Demming and Adams 2002). The demonstrated antioxidant activity of carotenoids is the basis of the protective action of these compounds against oxidative stress in many organisms and situations.

The potential of scientific and technological advances for improvements in yield and reduction in production costs for carotenoids from microalgae is also discussed. Effects of carotenoids on human health are, in general, associated with their antioxidant properties (Guerin *et al* 2003; Higuera-Ciapara *et al* 2006; Hussein *et al* 2006).

The pigmentation properties of carotenoids have granted to some of them extensive application in the food and feed industry (Borowitzka and Borowitzka 1988; Todd and Cysewski 2000). The requirement in aquaculture and animal farming for these compounds also rests on their additional positive effects on adequate growth and reproduction of commercially valuable species.

Microalgae are expensive to produce, although many efforts are under way addressed to achieve cost-efficient modes for mass cultivation of these organisms. Different systems have been designed for the growth and handling of microalgae on a large scale (Borowitzka 1999; Gudin and Chaumont 1980; Molina-Grima *et al* 1999; Pulz 2001; Richmond 2004; Tredici 2004; Weissman *et al* 1988).

The Chlorophyceae is the largest group of green algae, most of the species being unicellular freshwater forms. The best known microalgae like *Chlorella, Chlamydomonas, Dunaliella*, and *Haematococcus* belong to this group (Pulz and Gross 2004). Some chlorophycean microalgae accumulate carotenoids as a part of their biomass and represent interesting biological alternatives to traditional sources for some of these pigments (Del *et al* 2000; Bhosale and Bernstein 2005).

B-carotene is a pigment of increasing demand and a wide variety of market applications: as food coloring agent (the most important outlet); as pro-vitamin A (retinol) in food and animal feed; as an additive to cosmetics and multivitamin preparations; and as a health food product under the antioxidant claim (Edge *et al* 1997; Johnson and Schroeder 1995). The halophilic green biflagellate microalga *Dunaliella salina* has since long

been recognized as an efficient biological source of this carotenoid (Ben-Amotz and Avron 1990). Many epidemiological studies suggest that humans fed on a diet high in β-carotene from *Dunaliella,* which maintains higher than average levels of serum carotenoids, have a lower incidence of several types of cancer and degenerative diseases (Ben-Amotz 1999). Open ponds, with no or scarce process control, represent the conventional method used in commercial production plants for *Dunaliella* (Ben-Amotz 1999, 2004; Borowitzka 1995).

As other bioactive compounds synthesized by microalgae, amino acids composition, especially the free amino acids, varies greatly between species as well as with growth conditions and growth phase (Borowitzka, 1988). Protein or amino acids may therefore be by-products of an algal process for the production of other fine chemicals, or with appropriate genetic enhancement, microalgae could produce desirable amino acids in sufficiently high concentrations (Borowitzka, 1988).

Microalgae contain essential nutrients which determine the quality, survival, growth and resistance to disease of cultured species. These illustrate the importance of the control of microalgal biochemical composition for the success of aquaculture feed chains, opening new perspectives for the study of fish larval nutrition and the development of microalgae-based feeds for aquaculture (Fábregas *et al* 2001). To support a better balanced nutrition for animal growth, it is often advised to use mixed microalgae cultures, in order to have a good protein profile, adequate vitamin content and high polyunsaturated fatty acids, mainly EPA, AA and DHA, recognized as essential for survival and growth during the early stages of life of many marine animals (Volkman *et al* 1989). One of the

beneficial effects attributed to adding algae is an increase in ingestion rates of food by marine fish larvae which enhance growth and survival as well as the quality of the fry (Naas *et al* 1992). In addition, the presence of algae in rearing tanks of European sea bass larvae has been shown to increase digestive enzyme secretion (Cahu and Zambonino 1998).

The vitamin content in four Australian microalgae, a *Nannochloropsis-like sp., Pavlova pinguis, Stichococcus sp.* and *Tetraselmis sp.,* were examined. Comparison of the data with the known nutritional requirements for marine fish species and prawns suggests that the microalgae should provide excess or adequate levels of the vitamins for aquaculture food chains (Brown *et al* 1999).

Larvae of *C. gigantea* were fed a binary diet of *Isochrysis aff. galbana* (T-iso) and *Chaetoceros calcitrans*, and two ternary diets consisting of the binary diet with either *Tetraselmis suecica* or *Thalassiosira pseudonana*. In a second feeding study, larvae were fed three ternary diets consisting of *I. aff. galbana* (T-iso) and *C. calcitrans* with either *T. pseudonana, Chaetoceros gracilis* or *Skeletonema costatum* (Whyte *et al* 1990). It was concluded that nutritional condition of the larvae correlated with the content of dietary carbohydrate rather than dietary lipid or protein. Determination of macronutrients in algal diets, even when the algae were cultured under conditions considered to be standard, was shown to be essential before any estimate of food value. The importance of carbohydrate in providing a balanced diet for effective conversion of dietary macronutrients to tissue and energy reserves has hitherto been overlooked in larval nutrition (Susana *et al* 2007).

Diatoms *Thalassiosira pseudonana* (Hustedt) Hasle and Heimdal, *C. calcitrans* (Paulsen) Takano and *Chaetoceros sp.* (Aquaculture Research Corporation), and phytoflagellates *I. galbana* Parke, *I. galbana* Green (T-Iso) and *Tetraselmis suecica* (Kylin) Butcher were harvested at exponential and stationary phases of growth and biochemical compositions determined by Whyte. 1987. Content of carbohydrates was generally higher in diatoms than phytoflagellates, with glucose the dominant sugar originating from a reserve glucan considered digestible by bivalves. Caloric content of lipid, carbohydrate and protein in phytoplankton allowed for ranking as sources of energy in the following decreasing order: *Isochrysis aff. galbana* (T-Iso), *I. galbana, C. calcitrans, T. suecica, T. pseudonana, and Chaetoceros sp*. with all but *Thalassiosira* exhibiting higher energy levels in the stationary cells.

The riboflavin enrichment of the marine microalga *Tetraselmis suecica* and the transfer of this vitamin to higher trophic levels of the aquatic food chain such as the rotifer *Brachionus plicatilis* and the larvae of two species of sparids: White Sea bream and gilthead sea bream were studied (Soutoa *et al* 2008). In the present study, riboflavin enrichment of microalgal cultures resulted in higher levels of this vitamin in both rotifers and fish larvae.

Twelve algal strains representing the classes Cyanophyceae, Prymnesiophyceae, Bacillariophyceae, Rhodophyceae, Cryptophyceae, Chlorophyceae, Xantophyceae and Eustigmatophyceae were selected mainly from the culture collection of The Norwegian Institute for Water Research (NIVA). The growth responses and fatty acid composition were

analysed, being the first report on the fattyacid profiles of *N. oceanica, Chroococcus* sp.*, Synechococcus* sp.*and Tribonema* sp. (Vishwanath 2007).

Nannochloropsis is a marine microalga currently cultivated in fish hatcheries as feed for rotifers and to create a green-water effect in the larvae tanks (Fulks and Main 1991; Lubzens *et al* 1995). Because of its high content of eicosapentaenoic acid (EPA, C20:5n3), Nannochloropsis has also been proposed as a source of this important polyunsaturated fatty acidfor human consumption (Malwine and Chaoshu 2009).

The marine microalgae *T. suecica, I. galbana, D. tertiolecta* and *Chlorella stigmatophora* are good biological sources of single cell protein (SCP). Marine microalgae can be used as a potential SCP source. To date, biomass production of microalgae has focused largely on a few freshwater species that were believed to be potential dietary supplements (Becker and Venkataraman 1983; Ciferri 1983). Fabregas and Herrero (1985) suggest that marine microalgae can be used as a potential single cell protein (SCP) source.

2.8 Biochemical Composition of Larvae

Rosas *et al* 2002 studied on the effect of dietary protein and energy levels on growth, oxygen consumption, haemolymph and digestive gland carbohydrates, nitrogen excretion and osmotic pressure of *L.vannamei* (Boone) and *L. setiferus* (Linne) juveniles (Crustacea, Decapoda; Penaeidae). *L. vannamei* is a most tolerant shrimp species with a high capacity to use a wide range of dietary P/E ratios for growth, which may be due to its lower energy requirements. *L. setiferus* showed a lower capacity

to accept different P/E ratios but the optimum P/E ratio obtained with this species shows that *L. setiferus* accept diets with a high carbohydrate level as well. These results demonstrate that there are nutritional and physiological differences that explain the differences that have been observed when both species were cultured in commercial ponds.

Although recent reports had proposed that shrimp can grow well when fed diets with 20% protein (Lawrence *et al* 1998), other researchers have shown higher protein requirement of shrimp of 27% and 60%, depending on the species (Allan and Smith 1998; Rosas, Martinez, Gaxiola, Brito, Díaz-Iglesia and Soto 1998). The optimum levels of the other components of the diet (lipids and carbohydrates) and their relation to the protein/energy ratio (P/E ratio) can be useful in reducing the production costs of feed and its ecological impact. Although dietary carbohydrates are the most economical source of energy in feed, there is little information related to the shrimp carbohydrate requirement (Pascual, Coloso & Tamse 1983; Alava & Pascual 1987; Shiau & Peng 1992). Some researchers demonstrated that the type and level of carbohydrates affected growth rate of *Marsopenaeus japonicus* (Bate) (Deshimaru and Yone 1978; Abdel *et al* 1979), *Farfantepenaeus aztecus* (Ives) (Andrews *et al* 1972), *L. vannamei* (Bonne) (Cousin 1995) and *L. stylirostris* (Stimpson). In *Penaeus monodon* (Fabricius) juveniles, survival was affected by carbohydrate levels and trehalose promoted growth rate better than sucrose and glucose (Pascual *et al* 1983; Alava and Pascual 1987). Using different levels of dietary carbohydrates (1%, 10%, 21% and 33%), Rosas *et al* 2000) showed that 33% of dietary carbohydrates added as starch limited the growth rate of L. stylirostris juveniles.

A brief summary of information on recent advances in the nutrition of *Penaeus monodon,* other aspects concerning the importance of digestive enzymes, dietary requirements of protein, carbohydrate, lipid and vitamin, energy and larval feeding for *P. monodon* were reviewed by Houng Chen, 2007.

Larval stages of the Pacific white shrimp, *L. vannamei* (Boone) were fed standard live diets of mixed microalgae from the first to the third protozoea (PZ1 to PZ3), followed by *Artemia* nauplii until post-larvae 1 (PL1). Litopenaeus vannamei larvae transferred to a diet of *Artemia* at the beginning of the second protozoea (PZ2) stage were significantly heavier on reaching the first mysis stage (M1) than those fed algae, while survival was not significantly different between treatments. The rapid decline in trypsin content from PZ1 and the flexible enzyme response from PZ2 suggest that *L. vannamei* is physiologically adapted to transfer to a more carnivorous diet during the mid-protozoeal stages (Puello *et al* 2002 Barbarito *et al* 2006).

2.9 Energy Budget

Accurate energy values of animals are essential for studies of animal energetics. Dry weight or ash-free dry weight is used to estimate biomass. As the energy content of dry or ash-free dry biomass varies considerably from species to species and within one species from season to season (Schinder *et al* 1971; Wissing and Hasler 1971; Snow 1972), the use of general energy equivalents for dry or ash-free dry weight does not yield very accurate values.

The energy expended in mechanical and biochemical processes, expressed as apparent heat increment (AHI; before called specific dynamic action), and post-prandial nitrogen excretion (PPNE) has been related to the growth rate of shrimp. In *L. setiferus* (Linne), we observed in both post-larvae and juveniles that the lowest AHI and PPNE are related to the best growth of shrimp (Rosas *et al* 1996; Taboada *et al* 1998). In *L. vannamei* (Pedrazzoli *et al* 1998), *L. setiferus* (García *et al* 1998; Taboada *et al* 1998) and *P. monodon* (Chen 1998) it has been demonstrated that protein requirements varied with age, according to the physiological changes that occur during their life cycle (Rosas *et al* 1999).

Although the positive effects of HUFA on crustacean larvae are well documented, a comparative analysis of the energy budget between shrimp larvae fed HUFA-enriched diets and those fed non-enriched diets is lacking. Such a comparison will not only provide information on the partitioning of the energy intake into several measurable variables, but will also elucidate the role of HUFA in shrimp performances. Hence, a comparative energy budget analysis will aid in better understanding the physiology of shrimps, such as gastrointestinal function and mechanisms related to food digestibility (Omidvar *et al* 2009).

The energy budget of *M. japonicus* postlarvae (PL) fed highly unsaturated fatty acid-enriched (EA) and non-enriched (NEA) Artemia nauplii was determined by equating energy intake (EI) with the summation of energy channeled to feces (F), metabolism (M), excretion (U), growth (G) and exuvia (Ev). The energy budget of *M. japonicus* PL was presented based on an input–output model equating energy intake with the energy allocated for metabolism, growth, assimilation, excretion and exuviae.

Growth is a parameter of obvious importance in fish and shrimp culture. It has been the topic of numerous studies yet careful examination of the current scientific and technical literature shows that it is still poorly understood by many scientists and aquaculturists. Growth is defined as a change in magnitude. The change can be in size (weight and/or length), in tissue, chemical composition, number, etc. (Franco *et al* 2006)

Supply of sufficient quantity of a balanced diet in the proper form and at proper timing is, essential to realization of growth potential. It is common for nutritionists to believe that diet quality and quantity is the factor driving growth. It may be more appropriate to recognize that animals have a genetically determined target for body size (and, perhaps, composition) and that they are capable of recognizing whether the target is achieved or achievable given current environmental and nutritional circumstances. Animals will seek to eat a sufficient amount of an appropriately balanced diet to allow them to achieve their target or preferred performance unless limited by constraints or overridden by an externally managed intervention.

Investigation on the effects of feeding level on the growth, energy budget and body biochemical composition of Chinese shrimp *F. chinesis* under different feeding levels was undertaken. The relationship among daily growth coefficient (DGC) (in terms of wet weight, dry weight, protein, and energy), initial body weight (IBW) and feeding level (RL) was well described. The relationship between food efficiency (FE) in terms of dry weight, protein, and energy of shrimp with different body weight and feeding level was described.

The culture of *L. vannamei* in low-salinity waters is now popular in many regions of the world; a factorial experiment was conducted to determine the effects of salinities and dietary carbohydrate levels on survival, growth, food consumption, food efficiency, absorption efficiency, and energy budget of juvenile *L. vannamei (*Lilian *et al* 2007). The results showed that no shrimp survived in tap water at the end of the experiment irrespective of dietary carbohydrate (CBH). At each dietary CBH level, the specific growth rate (SGR), food consumption, and food efficiency generally increased with increasing salinity.

During larval development, physiological demands for dietary changes are often associated with morphological transformation of the feeding apparatus. The ability to grow and survive on such different feed implies a high degree of flexibility in the digestive physiology to offset the nutritional requirements. Hence it is imperative that feeding schemes for artificial rearing, behavioral response of each larval stage toward food etc are to be highlighted. Hence the present study was conducted to evaluate the growth responses of *P. monodon* larvae to various monospecific and mixed algal diets.

Chapter- 3

MATERIALS AND METHODS

The primary aim of this study was simply to ascertain the best of the available algal species and cell concentrations together with the optimum environmental conditions promoting maximum larval growth and survival in *P. monodon. P. monodon*, the fastest growing marine shrimp attains a maximum size of about 229.8 mm with a weight of about 95.1gm (MPEDA, 2003, FAO 2007). *P. monodon* is commonly known as Giant tiger prawn.

Systematic Position of *P. monodon*

Phylum	:	Arthropoda
Sub Phylum	:	Crustacea
Class	:	Malacostraca
Sub Class	:	Eumalacostraca
Super Order	:	Eucarida
Order	:	Decapoda
Sub Order	:	Natantia
Family	:	Penaeida
Genus	:	*Penaeus*
Species	:	*monodon*

3.1 Maintenance of *P. monodon* larvae in the laboratory

The *P. monodon* larvae were transported from the hatchery (Kaippamangalam and Kannamaly), at the Nauplius stage, to the laboratory. The transport of larvae at this stage permitted experiments to begin within 24 hours after arrival at the lab, when they reach the protozoea 1 stage. They were carried to the lab in 10 litre plastic bags with $1/3^{rd}$ Vol. filled with water and $2/3^{rd}$ with oxygen, at a temperature of $25\text{-}27^{oc}$ and a concentration of 1000 larvae per litre. The bags were placed inside the 50 litre tanks for acclimation to the experimental laboratory conditions. After two hours the larvae were counted and placed in different tanks with moderate aeration and the experiment was carried out until the protozoea metamorphosed up to the post larva stage. It took 12 days for the development.

The larvae were stocked at density of 150 larvae per litre in 5 litre FRP tanks with 3 litres of sea water. Incoming seawater was filtered and treated prior to use to minimize the chances of viral, bacterial, fungal, protozoal diseases from the source water. For disinfections 0.3 ml of 10% sodium hypochlorite was used. Aeration was turned on for 5-10 minutes until the chlorine is fully mixed, then turned it off and let the tank stand for 12-24 hours. The aeration is turned off to maintain the chlorine concentration in the water for a long time, so it is able to kill any pathogens. After 12-24 hours, aeration system was turned on and chlorine concentration was measured with a swimming pool chlorine test kit then added sodium thiosulphate crystals dissolved first in water at the rate of 1ppm ($1g/m^3$) for every 1ppm of chlorine left in solution until no yellow colour was seen (FAO 2007, Pablo *et al* 2005).

About 30-50% of the water is being drained daily from the 2nd day onwards and fresh filtered sea water is being added. Water was siphoned out from the bottom of the tank, mesh-net strainers was used to prevent the removal of the larvae along with the water and wastes. Each experiment was carried out in triplicate.

3.2 Live Feed

The larvae were fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* starting from first protozoea stage (PZ-1) and lasted to PL1. The algal cell density given to the larvae varied and was increased at different larval stages. The larval stages were fed with increasing densities of algae to evaluate the relationship between the food densities, ingestion rates, development and growth of the larvae.

The various algal culture inoculums were brought from Central Marine Fisheries Research Institute, Regional Centre, Mandapam Camp and were cultured in the marine botany lab. Walne's medium is used as the nutrient medium for the culture of the algae (Walne 1970). The algae were taken as feed during the exponential phase, the cell count was done using the Neubauer Chamber.

Systematic Position of Algae

Chaetoceros calcitrans

Kingdom	Chromista
Phylum	Bacillariophyta
Class	Mediophyceae
Order	Chaetocerotales
Family	Chaetocerotaceae
Genus	*Chaetoceros*
Species	*calcitrans*

Nannochloropsis salina

Phylum	Ochrophyta
Subphylum	Phaeista
Infraphylum	Limnista
Class	Eustigmatophyceae
Order	Eustigmatales
Family	Monodopsidaceae
Genus	*Nannochloropsis*
Species	*salina*

Dunaliella salina

Phylum	Chlorophyta
Subphylum	Tetraphytina
Class	Chlorophyceae
Order	Dunaliellales
Family	Dunaliellaceae
Genus	*Dunaliella*
Species	*salina*

Isochrysis galbana

Phylum	Chlorophyta
Class	Prymnesiophyceae
Order	Isochrysidales
Family	Isochrysidaceae
Genus	*Isochrysis*
Species	*galbana*

3.3 Composition and preparation of Walne medium

Solution A (at 1 ml per litre of culture)

Constituents for Walnes A medium

Sodium nitrate ($NaN0_3$) 100.0 g

Sodium di-hydrogen orthophosphate ($NaH_2P0_4.2H2_0$) 20.0 g

EDTA di-sodium salt 45.0 g

Boric acid (H_3B0_3) 33.6g

Ferric chloride ($FeCl_3.6H_2O$) 1.3g

Manganous chloride ($MnCl_2$. $4H_20$) 0.36g

Distilled water 1 Litre

Heat to dissolve

Solution B (at 0.5 ml per litre of culture)

Constituents for Walnes B medium

Zinc chloride ($ZnCl_2$) 4.2g

Cobaltous chloride ($CoCI_2.6H_20$) 4.0g

Cupric sulphate ($CuS0_4.5H_20$) 4.0g

Ammonium molybdate ($(NH_4)_6Mo70_{24}.4H_20$) 1.8g

Distilled water 1Litre

Heat to dissolve

Solution C (at 0.1 ml per litre of culture)

Constituents for Walnes C medium

Vitamin B_1 (Thiamin) 100mg in 50 ml distilled water

Vitamin B_{12} (Cyanocobalamine) 5 mg in 50 ml distilled water

Biotin 200 micrograms

Solution D

(For culture of diatoms used in addition to solutions A B and C, at 2 ml per litre of culture)

Sodium metasilicate ($Na_2Si0_3.5H_20$) 40.0 g

Make up to 1 litre with distilled water

Shake to dissolve

Walne, 1970.

In the first experiment, *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* were tested individually at three cell concentrations $10x10^4$ cells/ml, to $25x10^4$ cells/ml and $50x10^4$cells/ml, from the PZ1 to the PL1 stage. In the second experiment, *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* were tested in combination at three cell concentrations at $10x10^4$ cells/ml, $25x10^4$ cells/ml and $50x10^4$ cells/ml, from the PZ1 to the PL1 stage.

The algal cell count in the larval culture tank was taken daily prior to exchange of water to know the amount of algae ingested. Twice daily the number of algal cells in each tank was counted (using a Neubauer Chamber and a microscope). Any deficiency in algal cell numbers was made up by adding appropriate volumes of algal cultures for which the cell density has

already been established. All daily rations were supplied in two equal portions. The amount of algae consumed was calculated as the difference between those supplied the previous day and those remaining in each aquarium, evaluated with direct counts with a Neubauer Chamber of three 10 ml samples concentrated to 1 ml to improve the precision of counting (Pinna *et al* 2005).

3.4 Replacement Amount of Pure Culture

The replacement amount of pure culture was calculated using the formula by (Nunez *et al* 2002).

Va= Vt*Da/ Dt

Where, Va= Vol. of pure culture to be added

Vt = Vol. of water in the larval culture

Da = cell conc. needed for the larval culture

Dt = Cell conc. In the pure culture

3.5 Water quality monitoring

The water quality parameters like temperature, PH (optimum 7.8–8.2), salinity, ammonia, nitrite, were estimated. Total ammonia nitrogen (NH_3 optimum <0.1 ppm NH_3), NO_2N, and NO_3N were monitored every two days according to American Public Health Association (APHA; 1998), using a spectrophotometer prior to water exchange. Water temperature was in the range of 25^{oc} -27^{oc} (26 ± 1^{oc}) and salinity (30–32ppt), was measured daily, and dissolved oxygen (DO) concentration (6.30–6.85 mg L−1) was measured alternate days in each tank using winkler method. Tank water pH (7.66–8.5) was measured using a pH meter and salinity was measured using

a temperature-compensated refractometer (Strickland and Parsons, 1960). All surviving shrimp in each tank were collected after the 12 days of experiment. The percentage of survival rate, the growth estimation was done on alternate days in each tank and the algal cell count was done daily.

3.6 Siphoning of wastes

In addition to daily water exchanges, the bottom of the tanks was siphoned from zoea 2-3 throughout larval rearing. Uneaten food and faeces may was siphoned from the bottom of the tanks periodically by turning off the air and allowing the larvae to come to the surface of the tank.

3.7 Aeration

Uniform aeration in all parts of the tanks was provided through use of a perforated air pipe or air diffuser stones situated at the base of the tank to help promote thorough oxygenation and turn-over of the water in the tank and to keep the larvae and the feed uniformly distributed in the tank.

3.8 Larvae swimming activity

The swimming activity and Phototaxis movement of the larvae was observed to see whether the larvae were healthy. Zoeal stages will swim rapidly and consistently forwards, usually in circles, filter feeding on phytoplankton. Mysis, by comparison, swim backwards with intermittent flicks of their tails, maintaining themselves in the water column and feeding visually. PL again starts to swim rapidly and consistently forward, searching for food while being maintained in the water column by strong aeration. Within these distinct modes of swimming, if 70–95 percent of the

larvae are observed to be swimming actively, they seem to be healthy. Zoeal-stage larvae should retain a strong positive phototaxis and move towards light. To test this, a sample of larvae was placed in a translucent container (glass or beaker) next to a light source and the displacement of the animals is observed. The zoea should move strongly towards the light. Mysis and PL do not show such attraction to light. If 95 percent or more strongly towards the light, the larvae are good and healthy.

3.9 Faecal string

During the zoea 1 stages, when the zoea are feeding almost exclusively on algae, long faecal strings can be seen projecting from the anus and loose in the water column. When 90–100 percent of the larvae have these long, continuous strings all along the digestive tube, through their bodies and continuing outside, they are considered well fed. In older larval stages the intestinal contents (gut contents) can be observed. The intestine was visible as a dark line extending posterior from the hepatopancreas in the larva's head region that can easily observed in larvae held in a clear container such as a glass beaker. Most larvae should appear full and dark and if they do not, they are probably being underfed or are diseased which indicates remedial action.

3.10 Length

To determine the body length of the larvae samples of 15–20 larva were obtained and was measured under the dissecting microscope with a calibrated eyepiece on alternate days. Growth was determined by total length measurement of seven larvae from each experimental tank. Measurements of PZ1 were made from the tip of the cephalothorax

to the end of the telson including the spines. PZ2, and PZ3 were measured from the front of the rostrum to the end of the telson excluding the spines, and mysis were measured from the anterior edge of the cephalothorax to the end of the telson.

3.11 Percentage Survival

Survival was calculated as the final number of larvae in proportion to the initial number of larvae. The final number of larvae was obtained by counting all individuals in in aliquot samples in each treatment. Survival was calculated and expressed in percentage based on the final number of larvae in 5 L tanks.

3.12 Weight

The initial and final weight was measured in an electronic balance.

3.13 Specific Growth Rate

This expresses growth per unit time and is expressed as

SGR% = log final wt. (w2) – log initial wt. (w1)/ T2-T1

Where W2 = weight at time T 2

W1 = weight at time T1

t = time in days

3.14 Ingestion Rate

The ingestion rate was measured according to Paffenhofer's (1981) equation. The ingestion rates were calculated per day and not per hour.

IR= (Co-Ct) (V/nt)

Where, Co and Ct=initial and final cell conc.

n= no. of larvae

V= vol. of water in the beaker

t= expt. time

3.15 Developmental Index

The developmental index was calculated by the equation according to Villegas and Kanazawa 1979. The developmental index was used to determine the stage of development.

DI= A/Total No. Of Larvae Staged

Where, A=£ (assigned stage value *no. of larvae at that stage).

assigned stage value of the larvae are: N6 = 0, Pz1=1, Pz2=2, Pz3=3, M1=4, M2=5, M3=6,Pl 1= 7

3.16 Dry weight of algae

This assay was based on a method described according to Standard Methods (APHA, 1998). The dry weight of the microalgae was determined by filtering 100 mL of microalgae onto precombusted glass-fiber filters (Whatman GF/C 47 mm in diameter) and washing with 0.5 M ammonia formate to remove residual salts from the culture. The filters were then dried at 60°C until a constant weight was reached (total dry weight = TW).

3.17 Ash

The methods of the Association of Official Analytical Chemists (AOAC, 1990) were used for determination of ash. Determination of ash content was done by gravimetrically after heating 550°C for about

16hrs to obtain their inorganic content (AW). All determinations were done in duplicates. The proximate values were reported in percentage.

3.18 Biochemical Composition of Larvae

Protein

Protein was measured by the method of Lowry *et al* (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was proportional to the amount of protein which was read in Spectrophotometer at 660nm.

Carbohydrate

The carbohydrate in the sample was extracted and was determined according to Anthrone method (Hedge, 1962). Crude extracts were used for glycogen analysis mixing 0.1 mL of each component with 1 mL anthrone reagent (0.1% dissolved in 76% sulfuric acid). Absorbance was measured at 630 nm against a reagent blank. Carbohydrate was quantified using dextrose solution as the standard.

Lipid

Lipids were extracted from samples, by homogenization in chloroform/ methanol (2:1,v/v), containing 0.01% (w/v) butylated hydroxytoluene (BHT) as an antioxidant, according to Folch et al. (1957) and were determined according to the Bligh and Dyer (1959) method. Absorbance was recorded at 560 nm.

3.19 Biochemical Composition Algae

The microalgal cultures were filtered through whatman GF/C filter papers. The filters were washed with 0.5 M ammonium formate to remove excess salt traces and dried at 100^{oc} for 24 hours to volatilize the ammonium formate. The microalgae were filtered, rinsed with ammonium formate to eliminate salts, weighed, lyophilized, re-hydrated in 3 mL 35% cold saline solution, and homogenized to obtain a crude extract.

Protein

Protein was measured by the method of Lowry *et al* (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was proportional to the amount of protein which was read in Spectrophotometer at 660nm.

Carbohydrate

The carbohydrate in the sample was extracted and was determined according to Anthrone method (Hedge, 1962). Crude extracts were used for glycogen analysis mixing 0.1 mL of each component with 1 mL anthrone reagent (0.1% dissolved in 76% sulfuric acid). Absorbance was measured at 630 nm against a reagent blank. carbohydrate was quantified using dextrose solution as the standard

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(1957) and were determined according to the Bligh and Dyer (1959) method. Absorbance was recorded at 560 nm.

3.20 Pigments TLC Analysis

The identification analysis of pigments was done by Thin Layer Chromatography method. Samples were extracted with 80% acetone and spotted on TLC and eluted in Mobile phase of Petroleum ether (40-600C): Acetone (7:3v/v). Rf values were compared with the standard Rf values (Reiss, 1994). The Rf values are not reproducible but their order of separation is consistent in a particular solvent system. "Rf values will be unique for each solvent. However, the general order of the Rf values should be the same because the more non polar pigments move farther in non polar organic solvents (Hao Quach, 2004).The color of the bands can be a general guide to identify the pigments. Carotene is orange. Chorophylls are green. Chlorophyll a is a blue-green. Chlorophyll b is a yellow-green. Xanthophylls are yellow (Jeffry, 1997).

3.21 AMINO ACID QUANTIFCATION BY HPLC

1 mg/ml solution of mixed amino acids was taken as reference solution. A C18 column of length of 0.10 m and diameter 4.6 mm was used with The mobile phase was 85% of 15.2 g/l of triethylamine R (pH 3) and 15% of 2:3 (v/v) mixture of propanol and acetonitrile. Flow was maintained at 1.0-1.5 ml/min.

Amino acids were detected from their absorbance at 220 nm. (As per British Pharmacopia, 2007)

3.22 COMPOSITION OF FATTY ACIDS BY GC

Test solution Introduce about 0.45 g of the substance to be examined into a 10 ml volumetric flask, dissolve 0.45 g of the algal sample in *hexane* containing 50 mg/L of *butylhydroxytoluene* and dilute to 10.0 ml with the same solvent. 2.0 ml of the solution is transfered into a quartz tube and evaporated with a gentle current of *nitrogen*. 1.5 ml of a 20 g/l solution of *sodium hydroxide* in *methanol* is added, covered with *nitrogen* and capped tightly with a polytetrafluoroethylene lined cap. It was then mixed and heated in a water-bath for 7 minutes. After cooling, 2 ml of boron *trichloride-methanol solution* was added and then covered with *nitrogen*, It was capped tightly, mixed and heated in a water-bath for 30 minutes. It was later cooled to 40-50 °C and 1 ml of *trimethylpentane* was added, capped and vortexed for at least 30 seconds. Immediately 5 ml of *saturated sodium chloride solution* was added covered with *nitrogen*, capped and vortexed for at least 15 seconds. When the upper layer to become clear, it was transferred to a separate tube. The methanol layer was shaken once more with 1 ml of *trimethylpentane* and combined with trimethylpentane extracts. The combined extracts were then washed twice each with 1 ml of *water* and dried over anhydrous *sodium sulphate*. The fused silica column used for the study is of length 30 m and diameter 0.25 mm. The *stationary phase* was *macrogel 20 000* (film thickness 0.25 µm). Carrier gas hydrogen for chromatography or helium for chromatography, where oxygen scrubber was applied. The eluate is split at 1:200 ratio and detected using a flame ionization detector (Renaud *et al* 1994).

Statistics

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme and SPSS.

Chapter- 4

EFFECT OF MONO ALGAL DIET ON GROWTH RESPONSES OF P. MONODON LARVAE

Growth rate of *P. monodon* larvae fed with varying concentration of monospecific algal diets of *Chaetoceros calcitrans, Isochrysis galbana*, *Dunaliella salina,* and *Nannochloropsis salina* were compared. The algae were tested individually at three cell concentrations $10x10^4$ cells/ml, $25x10^4$ cells/ml and $50x10^4$ cells/ml, from the PZ1 to the PL1 stage. The length and percentage survival of P. m larvae fed the algae at all the three cell concentrations were evaluated. The results of the experiments conducted are shown in the following tables and is explained.

RESULTS

4.1. Length of *P. monodon* larvae

4.1. 1. Effect of mono-algal diet (10×10^4 cells/ ml) on length of *P. monodon* larvae

Table 4.1 summarizes about length of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans*, *Dunaliella salina*, and *Nannochloropsis salina* at the cell concentration of 10×10^4 cells/ ml starting from first protozoea stage (PZ-1). When compared to *C* fed *Pm* larvae, a significant increase was observed in the length of *Pm* larvae fed with *D* on 2nd day ($p<0.05$), 10th day ($p<0.05$), 12th day ($p<0.01$) and also on *Pm* larvae fed with *N* on 2nd day ($p<0.01$), 4th day ($p<0.05$) and 8th day ($p<0.001$) through out the 12 days of study.

No significant change ($p>0.05$) was observed in the length of larvae fed with *I* when compared to *C* fed P. m larvae during the 12 days of study except on the 6th day, when the larvae fed with *I* showed a significant decrease ($p<0.01$) in length compared to the *C* fed *Pm* larvae.

When compared to *I* fed P. m larvae a significant increase was observed in the length of P. m larvae fed with *D* on 2nd day ($p<0.001$), 6th day ($p<0.01$) 8th ($p<0.01$), 10th and 12th day ($p<0.001$) and also on *Pm* fed with *N* on 2nd day ($p<0.001$), 4th day ($p<0.01$), 6th day, 8th ($p<0.001$) and 10th day ($p<0.01$) through out the 12 days of study.

There was a significant increase ($p<0.05$) in the body length of P. m larvae fed with *N* compared to P. m larvae fed with *D* on the 8th day of experiment. But a significant decrease ($p<0.05$) in the body length of P. m larvae fed with *N* compared to P. m larvae fed with *D* on the 12th day of experiment. The greatest growth (4.185 mm TL) however, was obtained with the *D* fed larvae.

Exp Day	P. m fed C	P. m fed I	P. m fed D	P. m fed N
2	1.152±0.03	1.110±0.04	1.217±0.06*$^{@@@}$	1.254±0.07**$^{@@@}$
4	1.242±0.06	1.213±0.07	1.303±0.07	1.342±0.1*$^{@@}$
6	1.983±0.09	1.784±0.10**	2.009±0.18$^{@@}$	2.101±0.18$^{@@@}$
8	3.161±0. 17	3.083±0.10	3.298±0.18$^{@@}$	3.468±0.140***$^{@@@\$}$
10	3.575±0.16	3.439±0.16	3.790±0.19*$^{@@@}$	3.699±0.14$^{@@}$
12	4.049±0.10	4.011±0.07	4.185±0.10**$^{@@@}$	4.091±0.06$^{\$}$

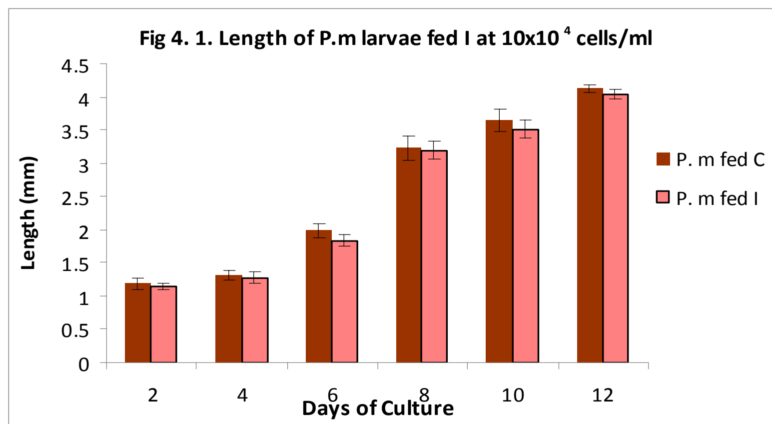
Table 4.1. Length of *P. m* larvae at cell conc. 10 x 10^4 cells/ ml

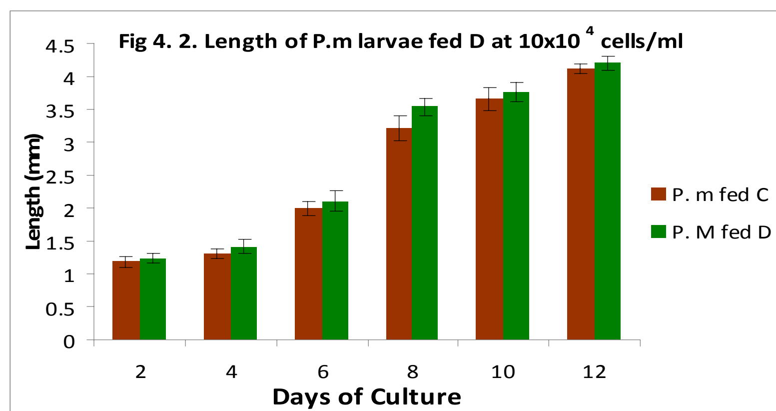
Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

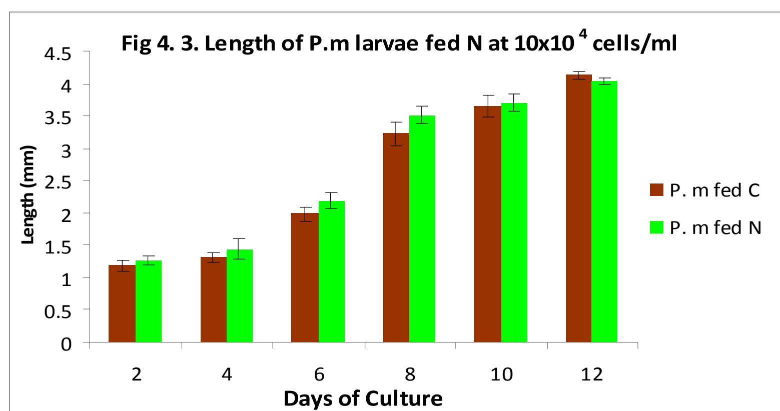
***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to *C. salina* fed *P. monodon*

$^{@@@}p<0.001$, $^{@@}p<0.01$, $^{@}p<0.05$ when compared to *I. galbana* fed *P. monodon*

$^{\$\$\$}p<0.001$, $^{\$\$}p<0.01$, $^{\$}p<0.05$ when compared to *D. salina* fed *P. monodon*







4.1.2. Effect of mono-algal diet (25 x 10^4 cells/ ml) on length of *P. monodon* larvae

Table 4.2 summarizes about length of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 25 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). When compared to *C* fed *Pm* larvae a significant increase was observed in the length of *Pm* larvae fed with *D* on 6th day ($p<0.05$) 8th ($p<0.001$), and 12th day ($p<0.01$) and also with *Pm* larvae fed *N* on 4th day ($p<0.05$), 6th day ($p<0.01$), 8th day, ($p<0.001$) through out the 12 days of study.

When compared to *I* fed *Pm* larvae a significant increase was observed in the length of *Pm* larvae fed with *D* on 2nd day ($p<0.01$), 4th day ($p<0.05$), 6th day ($p<0.001$) 8th day ($p<0.001$) 10th day ($p<0.01$), and 12th day ($p<0.001$) and also larvae fed with *N* on 2nd day ($p<0.01$), 4th day ($p<0.05$) 6th day ($p<0.001$), 8th day ($p<0.001$) and 10th day ($p<0.05$), through out the 12 days of study.

A significant change was observed in the length of larvae fed with *I* compared to *C* fed *Pm* larvae during the 12 days of study on the 6th day (p<0.01) and 10th day (p<0.05) when larvae fed with *I* showed a significant decrease compared to the *C* fed larvae.

There was no significant change (p>0.05) in the body length of *Pm* larvae fed with *N* compared to *Pm* larvae fed with *D* throughout the experiment. But a significant decrease (p<0.05) in the body length of *Pm* larvae fed with *N* compared to *Pm* larvae fed with *D* on the 12th day of experiment was observed. The greatest growth (4.205mm TL) however, was obtained with the *D* fed larvae.

Exp. Day	**P. m fed C**	**P. m fed I**	**P. m fed D**	**P. m fed N**
2	1.186± 0.08	1.134± 0.05	1.238± 0.07$^{@@}$	1.263± 0.07$^{@@}$
4	1.306± 0.07	1.277± 0.08	1.408± 0.11$^{@}$	1.442± 0.16*$^{@}$
6	1.989±0.11	1.834±0.08**	2.103±0.15*$^{@@@}$	2.181±0.12**$^{@@@}$
8	3.224±0.19	3.194±0.13	3.538±0.13***$^{@@@}$	3.508±0.13***$^{@@@}$
10	3.655±0.17	3.511±0.14*	3.770±0.14$^{@@}$	3.707±0.13$^{@}$
12	4.124±0.07	4.033±0.07	4.205±0.11**$^{@@@}$	4.042±0.05$^{\$\$\$}$

Table 4.2. Length of *P. m* larvae at cell conc. 25 x 10^{4} cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to *C. salina* fed *P. monodon*

$^{@@@}p<0.001$, $^{@@}p<0.01$, $^{@}p<0.05$, when compared to *I. galbana* fed *P. monodon*

$^{\$\$\$}p<0.001$, when compared to *D. salina* fed *P. monodon*

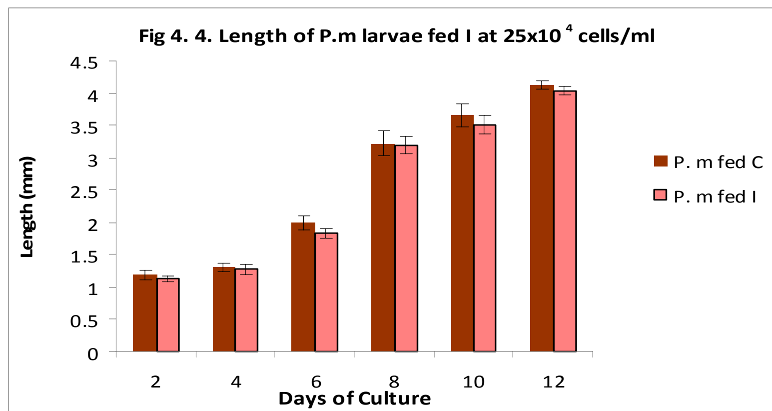


Fig 4. 4. Length of P.m larvae fed I at 25x10^4 cells/ml

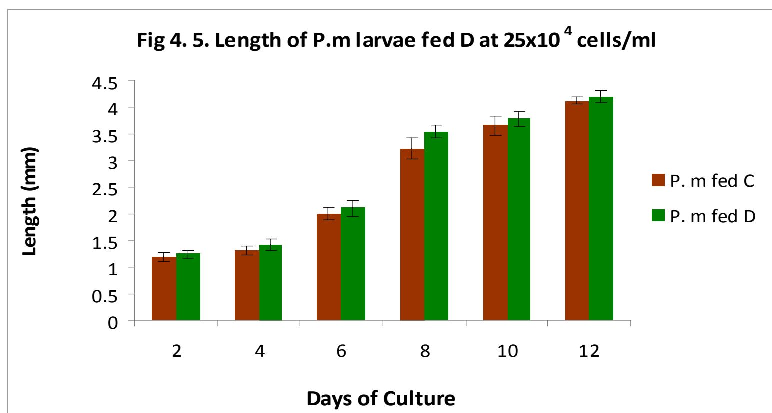
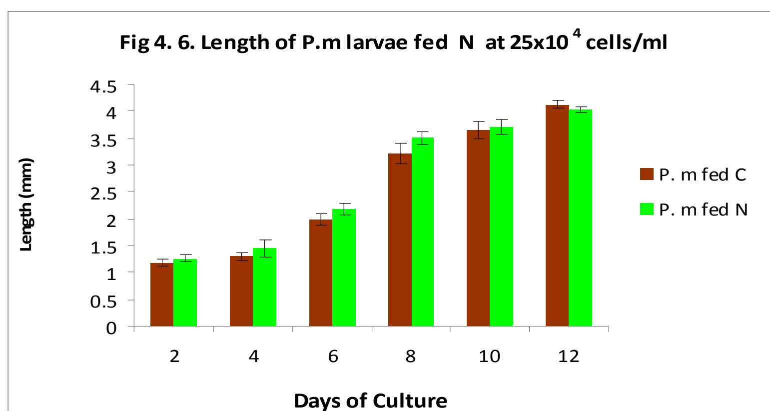


Fig 4. 5. Length of P.m larvae fed D at 25x10^4 cells/ml



4.1.3. Effect of mono-algal diet (50 x 10^4cells/ ml) on length of *P. monodon* larvae

Table 4.3 summarizes about length of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). When compared to *Pm* larvae fed with *C* a significant increase was observed in the length of *Pm* larvae fed with *D* on 4th day ($p<0.05$), 8th day ($p<0.01$) 10th day ($p<0.05$), and 12th day ($p<0.05$) and also with *Pm* larvae fed with *N* on 2nd day ($p<0.01$), 4th day ($p<0.01$) 6th day ($p<0.05$), 8th day ($p<0.001$) through out the 12 days of study.

When compared to *Pm* larvae fed with *I* a significant increase was observed in the length of *Pm* larvae fed with *D* on 2nd day ($p<0.001$), 4th day ($p<0.05$), 6th day ($p<0.001$) 8th day ($p<0.001$) 10th day ($p<0.01$), and 12th day ($p<0.01$) and also larvae fed with *N* on 2nd day ($p<0.001$), 4th day ($p<0.01$) 6th day ($p<0.001$), 8th day ($p<0.001$) and 10th day ($p<0.05$), through out the 12 days of study.

A significant change was observed in the length of larvae fed with *I* compared to P. m larvae fed with *C* during the 12 days of study on the 2nd day and 6th day when larvae fed with *I* showed a significant decrease ($p<0.05$) compared to the *C* fed larvae.

There was no significant change ($p>0.05$) in the body length of P. m larvae fed with *N* compared to P. m larvae fed with *D* throughout the experiment till 12th day. But a significant decrease ($p<0.05$) in the body length of P. m larvae fed with *N* compared to P. m larvae fed with *D* on the 12th day of experiment was observed. The greatest growth (4.245 TL) however, was obtained with the *D* fed larvae.

Exp. Day	**P. m fed C**	**P. m fed I**	**P. m fed D**	**P. m fed N**
2	1.187± 0.05	1.128±0.07*	1.242± .05@@@	1.281±0.06**@@@
4	1.312± 0.07	1.299±0.09	1.435± 0.11*@	1.468± 0.14**@@
6	1.996 ±0.11	1.849±0.07*	2.121±0.14@@@	2.157±0.20*@@@
8	3.284±0.14	3.226±0.12	3.549±0.15**@@@	3.528±0.17***@@@
10	3.664±0.18	3.554±0.17	3.883±0.21*@@	3.806±0.18@
12	4.155±0.09	4.079±0.07	4.245±0.12*@@	4.091±0.06$$

Table 4.3. Length of *P. m* larvae at cell conc. 50 x 10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to *C. salina* fed *P. monodon*

@@@p<0.001, @@p<0.01, @p<0.05, when compared to *I. galbana* fed *P. monodon*

$$p<0.01, $p<0.05 when compared to *D. salina* fed *P. monodon*

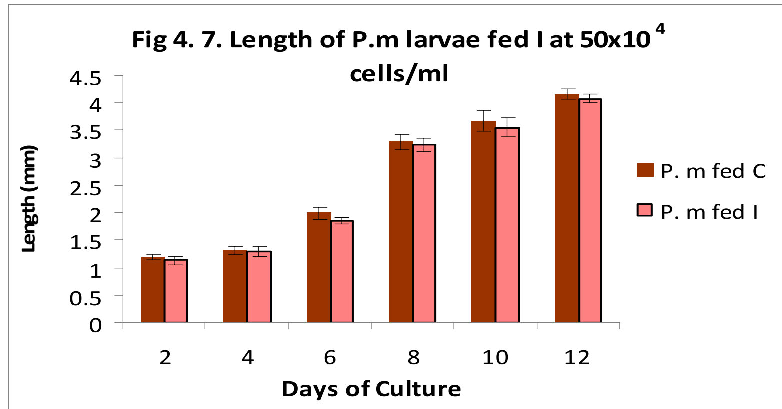


Fig 4. 7. Length of P.m larvae fed I at 50x10 4 cells/ml
Length (mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I

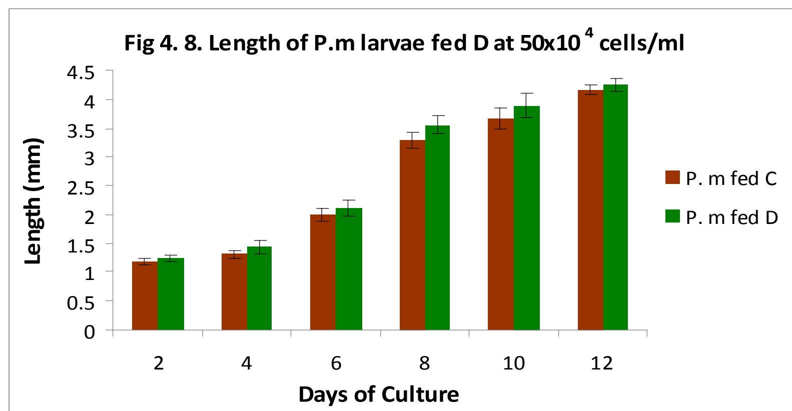


Fig 4. 8. Length of P.m larvae fed D at 50x10 4 cells/ml
Length (mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D

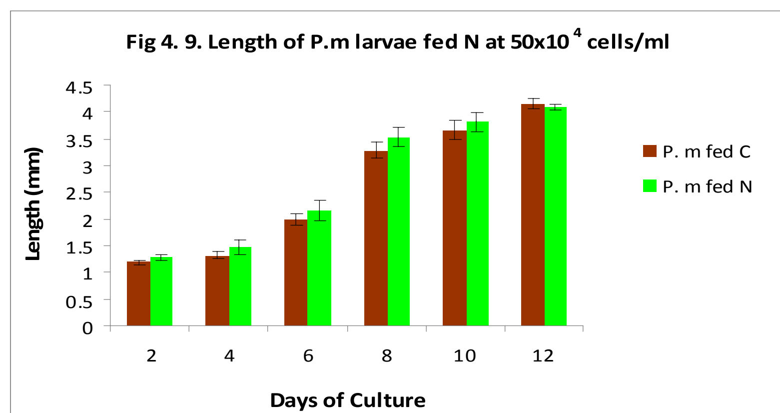


Fig 4. 9. Length of P.m larvae fed N at 50x10 4 cells/ml
Length (mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed N

4.2. Survival rate of *P. monodon* larvae

4.2.1. Effect of mono-algal diet (10 x 10^4 cells/ ml) on survival rate of *P. monodon* larvae

Table 4.4 summarizes about percentage survival of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 10 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). When compared to P. m larvae fed with *C* a significant increase was observed in the survival rate of Pm larvae fed with *D* on 6th day ($p<0.001$), 8th day ($p<0.01$) and 10th day ($p<0.05$) and also on P. m larvae fed with *N* on 2nd day ($p<0.05$), 4th day ($p<0.01$) 6th day ($p<0.001$) 8th day ($p<0.001$) 10th day ($p<0.05$), 12th day ($p<0.01$) through out the 12 days of study.

No significant change ($p>0.05$) was observed in the survival rate of larvae fed with *I* compared to P. m larvae fed with *C* during the 12 days of study except on the 6th day when larvae fed with *I* showed a significant increase ($p<0.01$) compared to the *C* fed larvae. A significant increase was observed in the survival rate of P. m larvae fed with D on day 8th ($p<0.01$), and 10th day ($p<0.01$) and also with N on 4th day ($p<0.01$), 6th day, 8th, ($p<0.01$) and 10th day ($p<0.001$) 12th day ($p<0.01$), when compared to *Ig* fed P. m larvae fed larvae through out the 12 days of study.

The highest survival rate (58.7%), however, was obtained with the N fed larvae. There was a significant increase ($p<0.05$) in the survival rate of P. m larvae fed with *Ns* compared to P. m larvae fed with *D* only on the 2nd day of experiment.

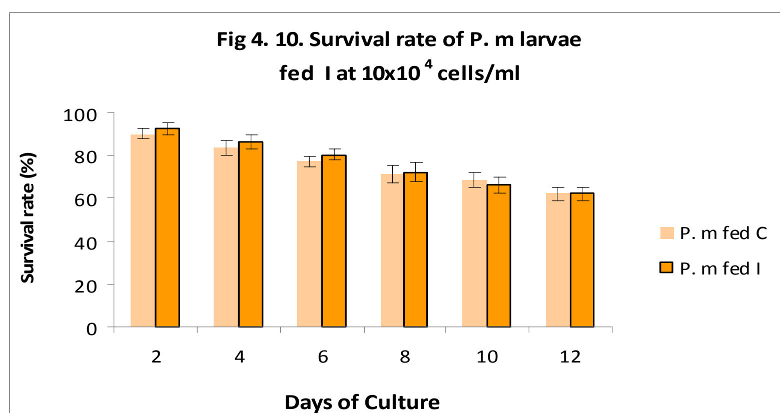
Exp. Day	P. m fed C	P. m fed I	P. m fed D	P. m fed N
2	90.02±3.14	92.45±2.52	91.41±3.28	94.43±3.41*
4	83.63±4.1	86.15±3.58	85.14±4.78	89.950±2.76 **@$
6	77.24±2.63	80.27±2.29**	82.25±1.85***	83.76±2.46***@@
8	71.15±4.4	72.21±4.22	77.49±3.49**@@	78.34±2.96***@@
10	68.47±3.45	66.11±3.457	71.92±3.76*@@	72.75±3.29*@@@
12	62.04±3.52	61.4±3.15	63.31±2.52	66.06±2.77*@@$

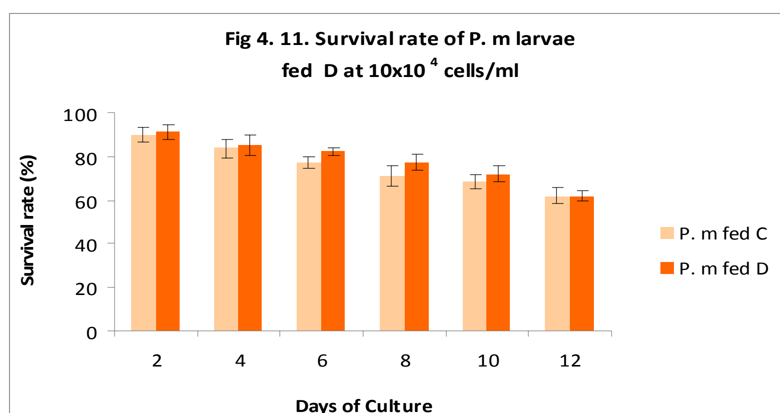
Table. 4.4. Survival rate of P. m larvae at cell conc. 10 x 10^4 cells/ ml

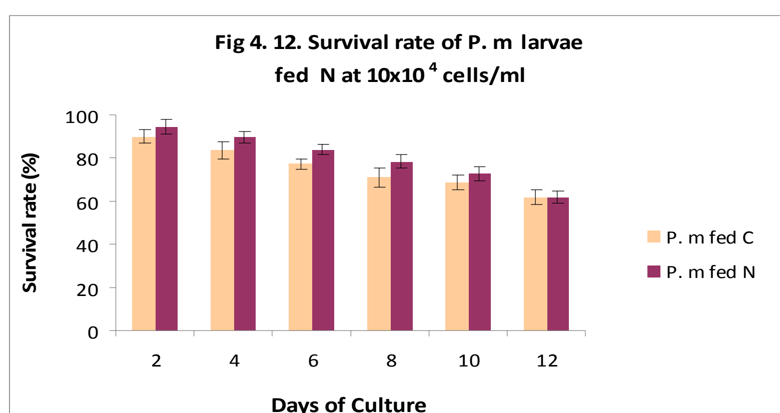
Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to *C. salina* fed *P. monodon*

@@@p<0.001, @@p<0.01, @p<0.05, when compared *I. galbana* fed *P. monodon*







4.2.2. Effect of mono-algal diet (25 x 10^4 cells/ ml) on survival rate of *P. monodon* larvae

Table 4.5 summarizes about percentage survival of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 25 x 10^4 cells/ mL starting from first protozoea stage (PZ-1). When compared to *C* fed *Pm* larvae a significant increase was observed in the survival rate of Pm larvae fed with *D* on 2nd day ($p<0.01$), and 6th day ($p<0.05$) and also on Pm larvae fed with *N* on 2nd day ($p<0.01$), 4th day ($p<0.001$) 6th day ($p<0.01$) 8th day ($p<0.01$) 12th day ($p<0.05$), through out the 12 days of study.

No significant change ($p>0.05$) was observed in the survival rate of larvae fed with *I* compared to *Pm* larvae fed with *C* during the 12 days of study. A significant increase was observed in the survival rate of *Pm* larvae fed with *D* on day 2nd ($p<0.01$), and 6th day ($p<0.05$) and also on *Pm* larvae fed with *N* on 2nd ($p<0.01$), 4th day ($p<0.01$), 8th, ($p<0.001$) and 12th day ($p<0.01$), when compared to *I* fed *Pm* larvae fed larvae through out the 12 days of study.

The highest survival rate (66.067%), however, was obtained with the *N* fed larvae. There was a significant increase ($p<0.05$) in the survival rate of *Pm* larvae fed with *N* compared to *Pm* larvae fed with *D* on the 4th 8th and 12th day of experiment.

Exp. Day	P. m fed C	P. m fed I	P. m fed D	P. m fed N
2	90.02±3.475	88.75±3.615	93.54±3.666*@@	94.85±2.403**@@
4	84.83±2.93	86.15±4.92	87.92±2.81	91.86±3.55***@@$
6	81.17±2.217	83.55±2.751	85.18±3.498*	86.53±3.235**
8	77.04±2.755	75.04±2.416	78.89±2.761@	81.91±4.030**@@@$
10	66.08±3.34	64.44±4.46	68.86±4.56	67.80±3.91
12	61.08±3.5	60.63±4.23	64.62±3.23	66.78±4.03

Table. 4.5. Survival rate of P. m larvae at cell conc. 25 x 10^4 cells/ ml

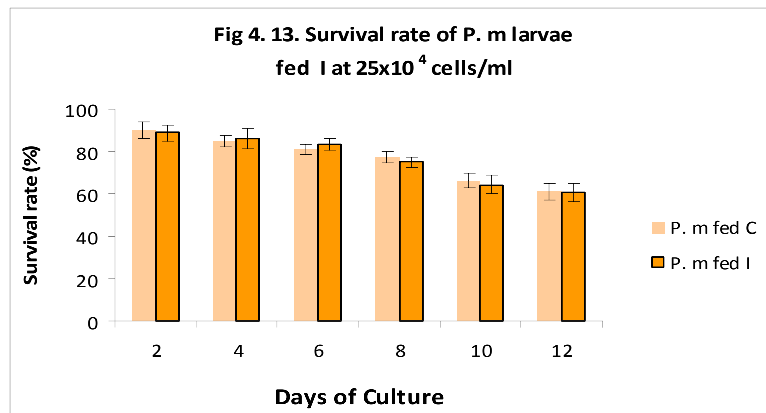
Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

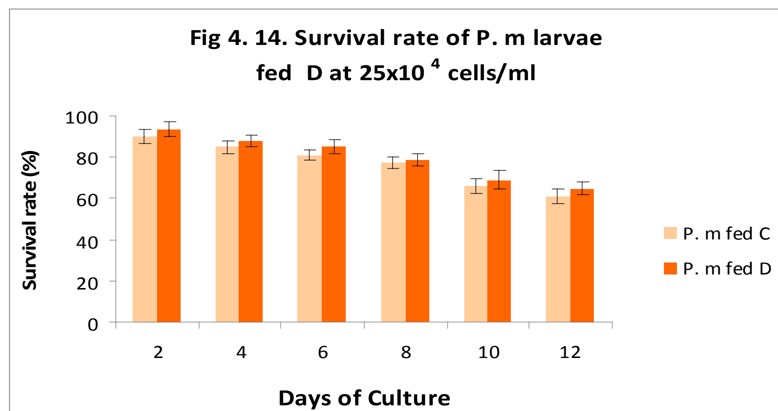
***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to *C. salina* fed *P. monodon*

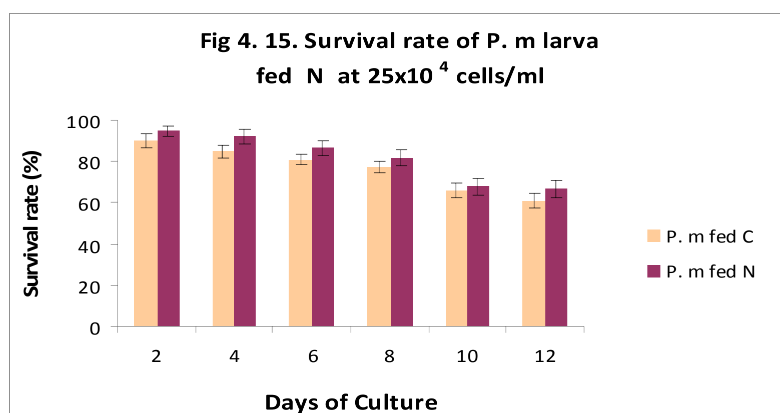
$^{@@@}p<0.001$, $^{@@}p<0.01$, $^{@}p<0.05$, when compared to *I. galbana* fed *P. monodon*

$^{\$\$\$}p<0.001$, $^{\$\$}p<0.01$, $^{\$}p<0.05$ when compared to *D. salina* fed *P. monodon*

$^{\$}p<0.05$ when compared to *D. salina* fed *P. monodon*







4.2.3. Effect of mono-algal diet (50 x 10^4 cells/ ml) on survival rate of *P. monodon* larvae

Table 4.6 summarizes about percentage survival of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). When compared to *C* fed *Pm* larvae a significant increase was observed in the survival rate of *Pm* larvae fed with *D* only on 6th day ($p<0.01$) and also on *Pm* larvae fed with *N* on 2nd day ($p<0.05$), 4th day ($p<0.05$) 6th day ($p<0.001$) 8th day ($p<0.05$), 10th day ($p<0.01$) and on 12th day ($p<0.01$), through out the 12 days of study.

No significant change ($p>0.05$) was observed in the survival rate of larvae fed with *I* compared to *C* fed *Pm* larvae during the 12 days of study. A significant increase was observed in the survival rate of *Pm* larvae fed with *D* on day 6th day ($p<0.05$) and 12th ($p<0.05$), and also on *Pm* larvae fed with *N* on 6th day ($p<0.01$), 8th, ($p<0.01$) and 12th day ($p<0.01$), when compared to *I* fed *Pm* larvae fed larvae through out the 12 days of study.

The highest survival rate (69.47%), however, was obtained with the *N* fed larvae. No significant change (p>0.05) was observed in the survival rate of larvae fed with *N* compared to *Pm* larvae fed with *D* during the 12 days of study.

Exp. Day	P. m fed C	P. m fed I	P. m fed D	P. m fed N
2	93.29±2.02	92.08±2.4	94.42±2.44	95.16±2.44*
4	89.91±2.53	91.32±1.5	91.34±3.89	93.36±1.88*
6	80.32±5.22	82.32±3.8	86.74±2.58**$^{@}$	89.32±5.01***$^{@@}$
8	78.55±4.91	76.85±4.18	79.64±2.89	83.21±3.57*$^{@@}$
10	67.25± 3.07	70.32±4.41	70.55±2.89	72.73±2.97**
12	63.27±4.23	61.58±6.15	67.44±3.72$^{@}$	69.47±4.9*$^{@@}$

Table. 4.6. Survival rate of P. m larvae at cell conc. 50 x 10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to *C. salina* fed *P. monodon*

$^{@@@}$p<0.001, $^{@@}$p<0.01, $^{@}$p<0.05, when compared to *I. galbana* fed *P. monodon*

$^{\$\$\$}$p<0.001, $^{\$\$}$p<0.01, $^{\$}$p<0.05 when compared to *D. salina* fed *P. monodon.*

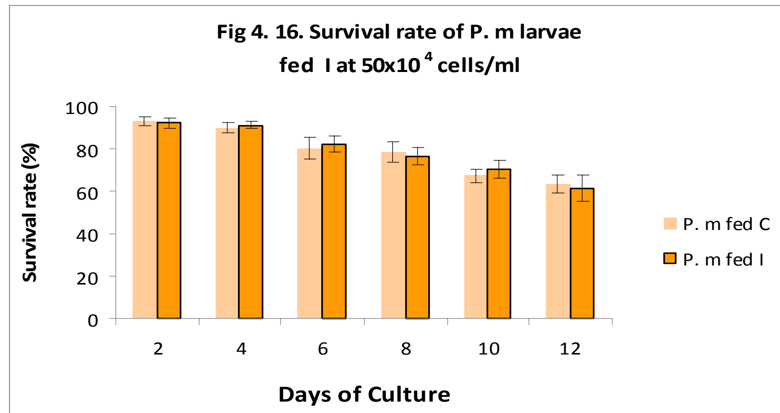


Fig 4. 16. Survival rate of P. m larvae
fed I at 50x10 4 cells/ml
Survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I

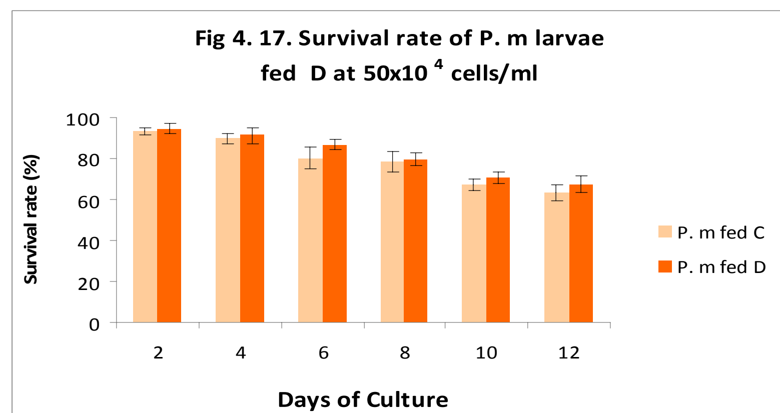


Fig 4. 17. Survival rate of P. m larvae
fed D at 50x10 4 cells/ml
Survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D

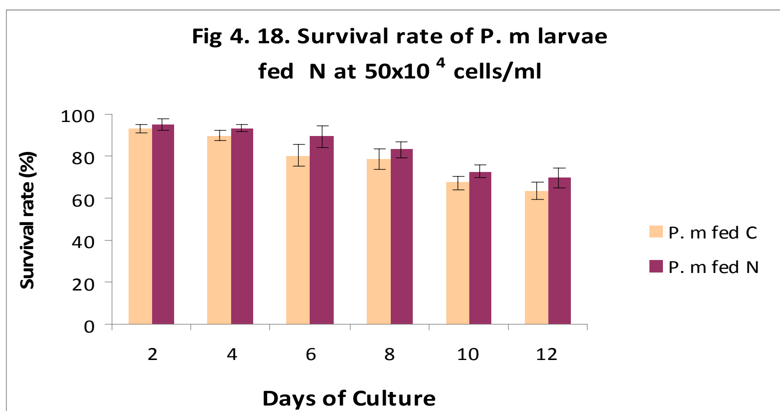


Fig 4. 18. Survival rate of P. m larvae
fed N at 50x10 4 cells/ml
Survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed N

From the above results it could be inferred that the *Pm* larvae fed with the micro algae at cell concentration $50x10^4$ showed better survival and increment in growth. Hence for the algal cell concentration of $50x\ 10^4$ further analysis of developmental index of the larvae indicating the stages of development, ingestion rate of algal cells by the *Pm* larvae, weight of the larvae, percentage increment in length and weight of the larvae specific growth rate and the water quality parameters were discussed.

4.3. Developmental index of *P. monodon* larvae

4.3.1. Effect of mono-algal diet ($50\ x\ 10^4$cells/ ml) on developmental index of *P. monodon* larvae

Table 4.7 summarizes about developmental index of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of $50\ X\ 10^4$ cells/ mL starting from first protozoea stage (PZ-1). When compared to *Pm* larvae fed with *C* a significant increase was observed in the developmental index of *Pm* larvae fed with *D* only on 10th day ($p<0.01$) and also on *Pm* larvae fed with *N* on 4th day ($p<0.05$) 6th day ($p<0.001$), through out the 12 days of study.

No significant change ($p>0.05$) was observed in the developmental index of larvae fed with *I* compared to *Pm* larvae fed with *C* except on 6th day ($p<0.05$) during the 12 days of study.

A significant increase was observed in the developmental index of *Pm* larvae fed with *D* on day 4th day ($p<0.05$), 6th day, and 10th day and 12th day ($p<0.01$), and also on *Pm* larvae fed with *N* on 4th day ($p<0.01$), 6th, ($p<0.01$) and 8th day ($p<0.05$), when compared to *I* fed *Pm* larvae fed larvae through out the 12 days of study.

The highest developmental index (6.34), however, was obtained with the *D* fed larvae. Significant change was observed on the 6th day, 8th day ($p>0.05$), and 10th day and 12th day ($p>0.05$), in the developmental index of larvae fed with *N* compared to *D* fed *Pm* larvae during the 12 days of study.

Exp. Day	P. m fed C	P. m fed I	P. m fed D	P. m fed N
2	1.02±0.08	0.96±0.15	1.06±0.11	1.12±0.21
4	1.92±0.04	1.88±0.08	2.06±0.05$^{@}$	2.20±0.18**$^{@@}$
6	2.84±0.11	2.96±0.05*	3.06±0.05$^{@@}$	3.20±0.07***$^{@@@\$}$
8	4.04±0.08	3.84±0.31	3.94±0.15	4.26±0.05$^{@\$}$
10	5.00±0.15	4.94±0.16	5.36±0.13**$^{@@}$	5.08±0.08$^{\$\$}$
12	6.14±0.13	5.96±0.11	6.34±0.20$^{@@}$	5.98±0.16$^{\$\$}$

Table. 4.7. Developmental index of P. m larvae at cell conc. 50 x 10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 5 in each group. ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to *C. salina* fed *P. monodon*

$^{@@@}p<0.001$, $^{@@}p<0.01$, $^{@}p<0.05$, when compared to *I. galbana* fed *P. monodon*

$^{\$\$\$}p<0.001$, $^{\$\$}p<0.01$, $^{\$}p<0.05$, when compared to *D. salina* fed *P. monodon*

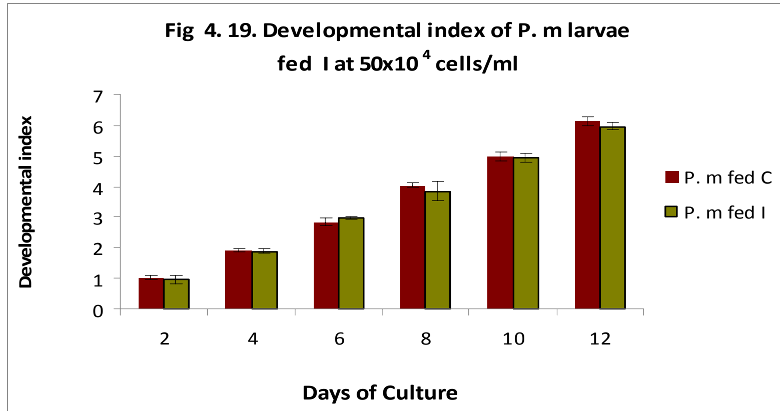


Fig 4. 19. Developmental index of P. m larvae fed I at 50x10 4 cells/ml
Developmental index
7
6
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I

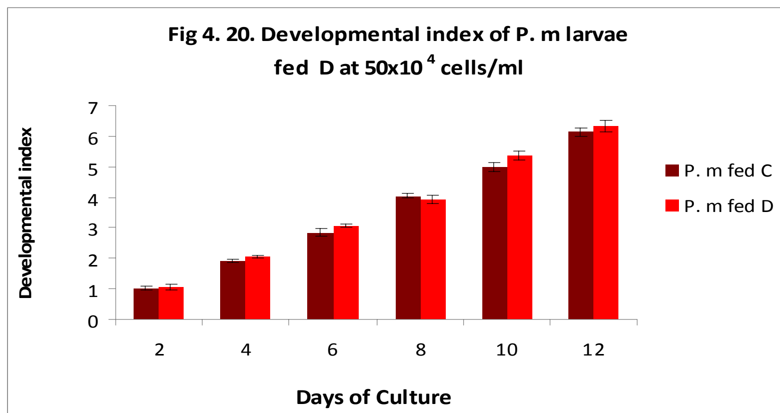


Fig 4. 20. Developmental index of P. m larvae fed D at 50x10 4 cells/ml
Developmental index
7
6
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D

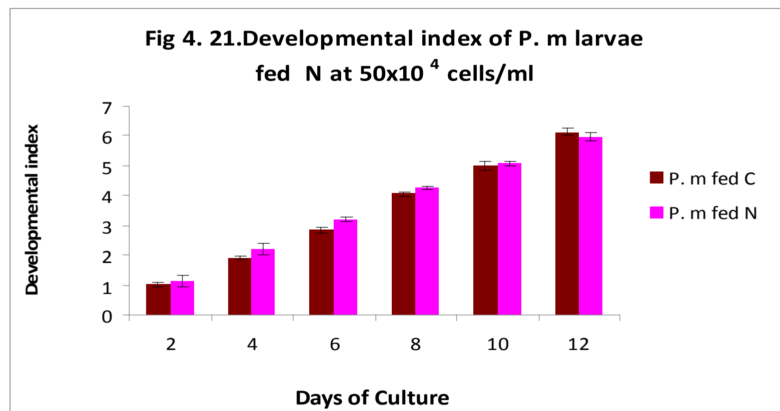


Fig 4. 21.Developmental index of P. m larvae fed N at 50x10 4 cells/ml
Developmental index
7
6
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed N

4.4. Ingestion rate of *P. monodon* larvae

4.4.1. Effect of mono-algal diet (50x 10^4 cells/ ml) on ingestion rate of *P. monodon* larvae

Table 4.8 summarizes about ingestion rate of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). A significant increase was observed in the ingestion rate of *Pm* larvae fed with *D* on 2nd day ($p<0.001$), 6th day ($p<0.01$) and on 10th and 12th day ($p<0.001$), and also on *Pm* larvae fed with *N* on 2nd day ($p<0.001$), 4th day ($p<0.01$) 8th day ($p<0.05$) and 10th day ($p<0.01$) when compared to *C* fed *Pm* larvae through out the 12 days of study.

Significant change ($p>0.05$) was observed in the IR of larvae fed with *I* compared to *Pm* larvae fed with *C* during the 12 days of study on 2nd day ($p<0.01$), 4th day ($p<0.05$) and on 10th day ($p<0.01$).

A significant increase was observed in the IR of *Pm* larvae fed with *D* on day 2nd and 4th day ($p<0.01$), 6th 8th, 10th day ($p<0.001$) and on 12th day ($p<0.05$), and also on *Pm* larvae fed with *N* on 4th day ($p<0.001$), 6th day ($p<0.05$), 8th ($p<0.01$) and 10th day ($p<0.001$), when compared to *I* fed *Pm* larvae fed larvae through out the 12 days of study.

The highest ingestion rate of (32.88 x 10^4cells/ml), however, was obtained in larvae fed with the *D*. A significant increase ($p>0.05$) was observed in the ingestion rate of larvae fed with *N* compared to *Pm* larvae fed with *D* on 2nd and 4th day but a significant decrease was seen ($p>0.05$) 6th, 8th and 10th day ($p>0.05$) during the 12 days of study.

Exp. Day	P. m fed C	P. m fed I	P. m fed D	P. m fed N
2	2.93±1.61	5.08±0.46**	5.8±0.39***$^{@@}$	6.97±0.22***$^{\$}$
4	8.16±1.0	7.21±0.64*	8.83±0.31$^{@@}$	9.7±0.28**$^{@@@\$}$
6	17.19±1.56	15.5±1.92	21.62±2.39**$^{@@@}$	19.05±0.92$^{@\$}$
8	27.89±0.65	26.97±2.41	32.59±0.52***$^{@@@}$	30.2±0.73*$^{@@\$}$
10	28.08±1.36	26.3±0.85**	32.88±0.48***$^{@@@}$	30.33±0.36***$^{@@@\$\$\$}$
12	28.03±0.52	27.36±1.0	29.9±1.62$^{@}$	28.7±1.27

Table. 4.8. Ingestion rate of P. m larvae at cell conc. 10×10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 5 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to *C. salina* fed *P. monodon*

$^{@@@}$p<0.001, $^{@@}$p<0.01, $^{@}$p<0.05, when compared to *I. galbana* fed *P. monodon*

$^{\$\$\$}$p<0.001, $^{\$}$p<0.05, when compared to *D. salina* fed *P. monodon*

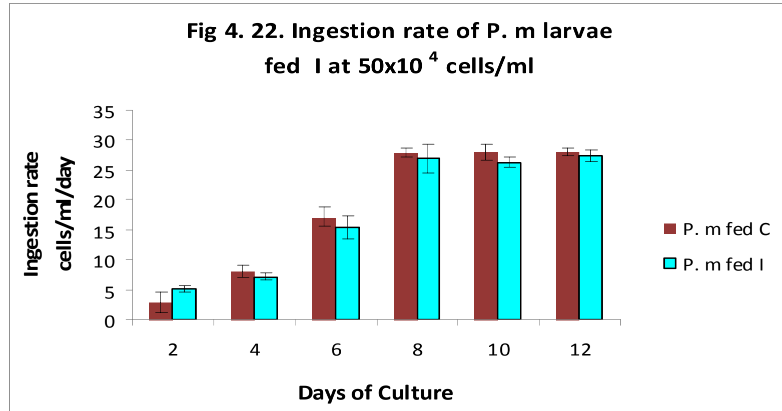


Fig 4. 22. Ingestion rate of P. m larvae
fed I at 50x10 4 cells/ml
Ingestion rate
cells/ml/day
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I

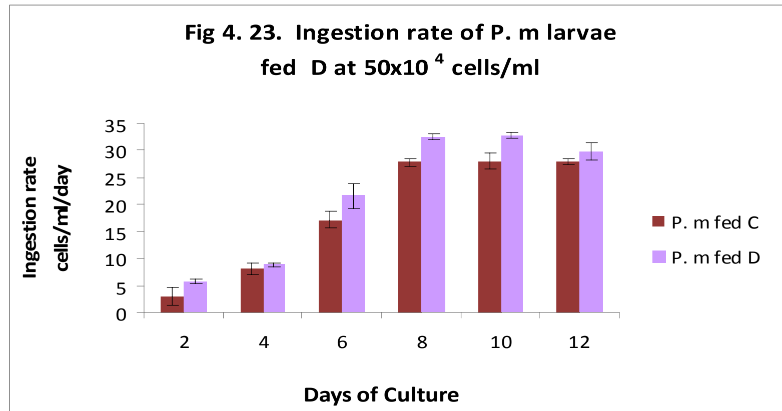


Fig 4. 23. Ingestion rate of P. m larvae
fed D at 50x10 4 cells/ml
Ingestion rate
cells/ml/day
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D

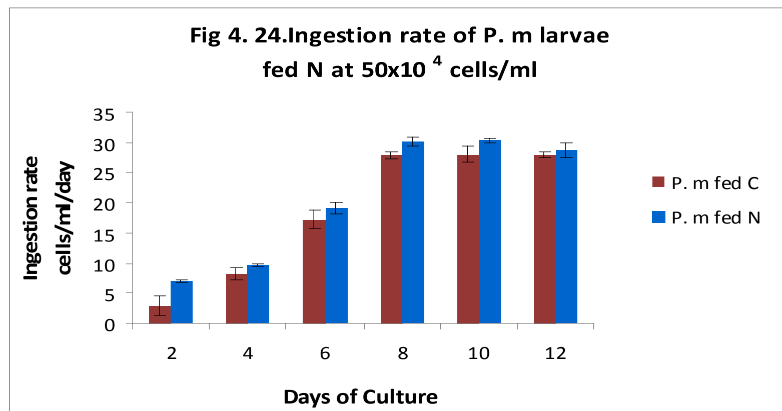


Fig 4. 24.Ingestion rate of P. m larvae
fed N at 50x10 4 cells/ml
Ingestion rate
cells/ml/day
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed N

4.5. Weight gain of *P. monodon* larvae

4.5.1. Effect of mono-algal diet (50 x 10^4 cells/ ml) on weight gain of *P. monodon* larvae

Table 4.9 summarizes about the weight gain of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). No significant changes was observed with the Z3 stages of the larvae whereas a significant increase in the weight gain was observed in the of P. m larvae fed with *N* when compare P. m larvae fed with that of *C* ($p<0.05$), *I* ($p>0.05$), and *D* ($p<0.01$) through out the 12 days of study.

	Larvae Stages	
	Z3(µg)	**M3**(µg)
P. m fed C	21.284±1.055	68.474±0.9703
P. m fed I	21.634±0.5699	68.836±0.6645
P. m fed D	21.984±0.5615	67.948±0.4733
P. m fed N	22.532±0.4515	69.746±0.2682*[@$$]

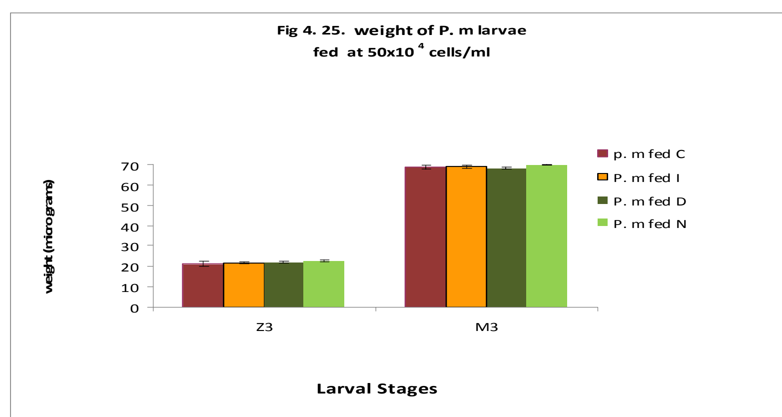
Table. 4.9. weight of P. m larvae at cell conc. 10 x 10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

*$p<0.05$ when compared to *C. salina* fed *P. monodon*

[@]$p<0.05$, when compared to *I. galbana* fed *P. monodon*

[$$]$p<0.01$, when compared to *D. salina* fed *P. monodon*



4.6. Percentage increment on length of *P. monodon* larvae

4.6.1. Effect of mono-algal diet (50×10^4 cells/ ml) on percentage increment on growth (length) of *P. monodon* larvae

Table 4.10 summarizes about percentage increment on growth (length) of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50×10^4 cells/ ml starting from first protozoea stage (PZ1-PL1). A significant increase was observed in the percentage increment on growth of Pm larvae fed with *D* on 4th day ($p<0.05$), 8th day ($p<0.01$) 10th day ($p<0.05$), and 12th day ($p<0.05$) and also with *N* on 2nd day ($p<0.01$), 4th day ($p<0.05$), 8th day ($p<0.001$) when compared to Pm larvae fed with *C* through out the 12 days of study.

A significant increase was observed in the percentage increment on growth of *Pm* larvae fed with *D* on 2nd day ($p<0.001$), 4th day ($p<0.05$), 6th day ($p<0.001$) 8th day ($p<0.001$) 10th day ($p<0.001$), and 12th day ($p<0.01$) and also with *N* on 2nd day ($p<0.001$), 4th day ($p<0.05$) 6th day ($p<0.001$), 8th day ($p<0.001$) and 10th day ($p<0.01$), when compared to *I* fed Pm larvae fed larvae through out the 12 days of study.

A significant change was observed in the percentage increment on growth of larvae fed with *I* compared to *Pm* larvae fed *C* during the 12 days of study on the 2nd day and 6th day, when larvae fed with *I* showed a significant decrease ($p<0.05$) compared to the larvae fed with *C*.

There was no significant change ($p>0.05$) in the percentage increment on growth of P. m larvae fed with *N* compared to P. m larvae fed with *D* throughout the experiment till 12th day. But a significant decrease ($p<0.01$) in the body length of P. m larvae fed with *N* compared to P. m larvae fed with *D* on the 12th day of experiment was observed. The greatest growth (302.65) however, was obtained in larvae fed with the *D*.

Exp. Day	**P. m fed C**	**P. m fed I**	**P. m fed D**	**P. m fed N**
2	12.381±4.8	6.984±5.129 *	17.672±5.494 $*^{@@}$	21.164±6.313 $**^{@@@}$
4	24.021±7.28	22.751±8.825	35.45±11.226 $*^{@}$	37.884±12.597 $*^{@}$
6	91.958±10.2	77.354±6.019 *	104.34±11.982 $^{@@}$	106.03±20.107 $^{@@@}$
8	210.48±12.7	205.4±11.518	235.87±13.586 $**^{@@}$	233.97±16.225 $***^{@@}$
10	244.34±13.9	233.76±11.449	264.44±19.083 $*^{@@}$	257.25±12.755 $^{@@}$
12	294.71±8.70	287.2±6.531	302.65±11.176 $*^{@}$	288.04±4.393 $^{\$\$}$

Table. 4.10. Percentage increment on growth (length) of P. m larva at cell conc. 10 x 10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to *C. salina* fed *P. monodon*

$^{@@@}$p<0.001, $^{@@}$p<0.01, when compared to *I. galbana* fed *P. monodon*

$^{\$\$}$p<0.01, when compared to *D. salina* fed *P. monodon*

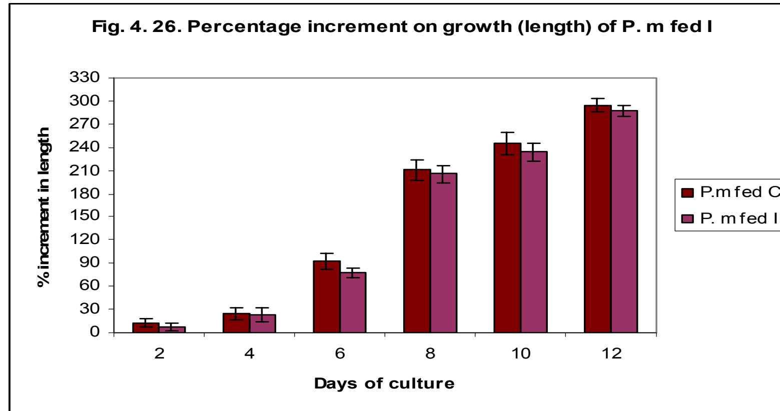


Fig. 4. 26. Percentage increment on growth (length) of P. m fed I

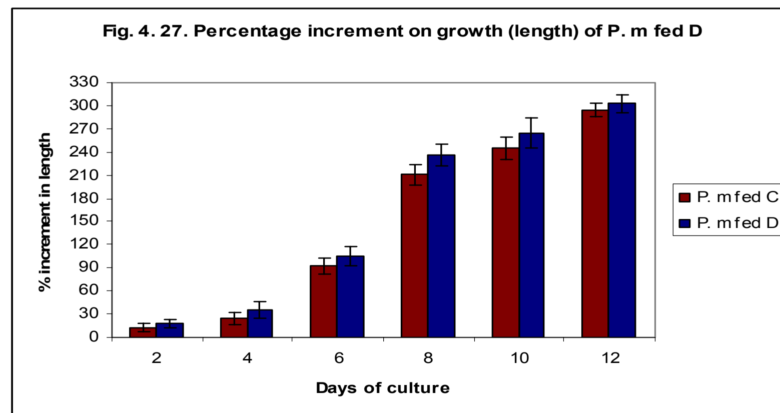


Fig. 4. 27. Percentage increment on growth (length) of P. m fed D

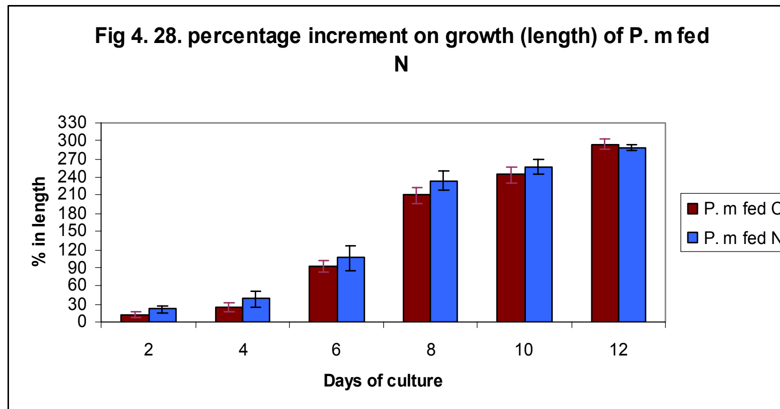


Fig 4. 28. percentage increment on growth (length) of P. m fed N

4.7. Growth rate (length) of *P. monodon* larvae

4.7.1. Effect of mono-algal diet (50 x 10^4 cells/ ml) on growth rate (length) of *P. monodon* larvae

Table 4.11 summarizes about percentage increment on growth (length) of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50 x 10^4 cells/ ml starting from first protozoea stage (PZ1-PL1). A significant increase was observed in the percentage increment on growth of P.m larvae fed with *D* on 4th day ($p<0.05$), 8th day ($p<0.01$) 10th day ($p<0.05$), and 12th day ($p<0.05$) and also on P.m larvae fed with *N* on 2nd day ($p<0.01$), 4th day ($p<0.05$), 8th day ($p<0.001$) when compared to P.m larvae fed with *C* through out the 12 days of study.

A significant increase was observed in the percentage increment on growth of P.m larvae fed with *D* on 2nd day ($p<0.001$), 6th day ($p<0.001$) 8th day ($p<0.001$) 10th day ($p<0.001$), and 12th day ($p<0.01$) and also on larvae fed with *N* on 2nd day ($p<0.001$), 4th day ($p<0.05$) 6th day ($p<0.001$), 8th day ($p<0.001$) and 10th day ($p<0.01$), when compared to P.m larvae fed with *I* through out the 12 days of study.

A significant change was observed in the percentage increment on growth of larvae fed with *I* compared to P.m larvae fed with *C* during the 12 days of study on the 2nd day and 6th day when larvae fed with *I* showed a significant decrease ($p<0.05$) compared to the larvae fed *C*.

There was no significant change ($p>0.05$) in the percentage increment on growth of P.m larvae fed with *N* compared to P.m larvae fed with *D* throughout the experiment till 12th day. But a significant decrease ($p<0.01$) in the body length of P.m larvae fed with *N* compared to P.m larvae fed with *D* on the 12th day of experiment was observed. The greatest growth (11.605) however, was obtained in larvae fed with the *D*.

Exp. Day	P. m fed C	P. m fed I	P. m fed D	P. m fed N
2	0.965±0.36	0.5543±0.39 *	1.348±0.39 $*^{@@@}$	1.589±0.44 $**^{@@@}$
4	1.781±0.49	1.689±0.606	2.501±0.72 $*^{@}$	2.645±0.78 $*^{@}$
6	5.423±0.45	4.771±0.27 *	5.941±0.51$^{@@@}$	5.987±0.83 $^{@@@}$
8	9.435±0.34	9.298±0.31	10.09±0.34 $**^{@@@}$	10.04±0.41 $**^{@@@}$
10	10.298±0.32	10.039±0.28	10.767±0.43 $*^{@@@}$	10.606±0.29 $^{@@}$
12	11.44±0.18	11.28±0.14	11.605±0.23 $*^{@@}$	11.299±0.09$^{\$\$}$

Table. 4.11. Growth rate (length) of P. m larva at cell conc. 10 x 10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

$**p<0.01$, $*p<0.05$ when compared to *C. salina* fed *P. monodon*

$^{@@@}p<0.001$, $^{@@}p<0.01$, $^{@}p<0.05$, when compared to *I. galbana* fed *P. monodon*

$^{\$\$}p<0.01$, when compared to *D. salina* fed *P. monodon*

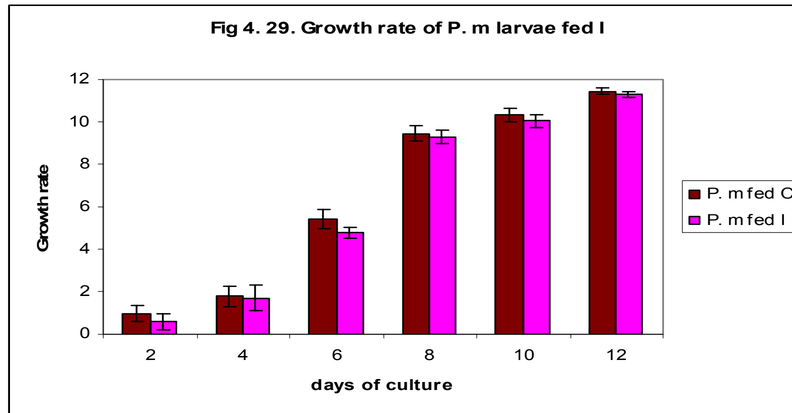


Fig 4. 29. Growth rate of P. m larvae fed I
Growth rate
12
10
8
6
4
2
0
2
4
6
8
10
12
days of culture
P. m fed C
P. m fed I

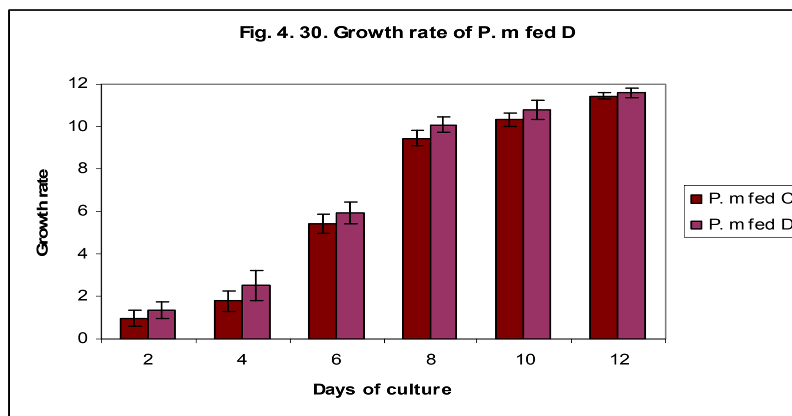


Fig. 4. 30. Growth rate of P. m fed D
Growth rate
12
10
8
6
4
2
0
2
4
6
8
10
12
Days of culture
P. m fed C
P. m fed D

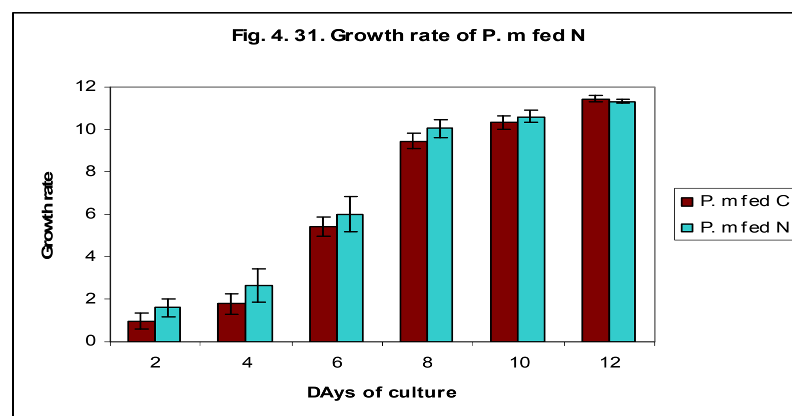


Fig. 4. 31. Growth rate of P. m fed N
Growth rate
12
10
8
6
4
2
0
2
4
6
8
10
12
DAys of culture
P. m fed C
P. m fed N

4.8. Percentage increment on weight of *P. monodon* larvae

4.8.1. Effect of mono-algal diet (50 x 10^4 cells/ ml) on percentage increment (weight) of *P. monodon* larvae

Table 4.12 summarizes about the percentage increment (weight) of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). No significant changes was observed with the Z3 stages of the larvae whereas a significant increase in the weight gain was observed in the *Pm* larvae fed with *I* ($p>0.01$), *D* and N ($p<0.01$) when compared with that of *Pm* larvae fed with C.

A significant increase in the weight gain was observed in P.m larvae fed with *D* and *N* ($p<0.001$) when compared with that of P.m larvae fed with *I.* P.m larvae fed with *N* showed a significant increase in the weight gain ($p<0.001$) when compared with that of larvae fed with *D* through out the 12 days of study.

	Larvae stages	
	Z3	**M3**
P. m fed C	280.07±18.83	976.32±14.76
P. m fed I	286.32±10.17	950.64± 11.86 **
P. m fed D	292.57±10.02	1113.4±8.45 ***@@@
P. m fed N	302.36±8.06	1145.5±4.78 ***@@@$$$

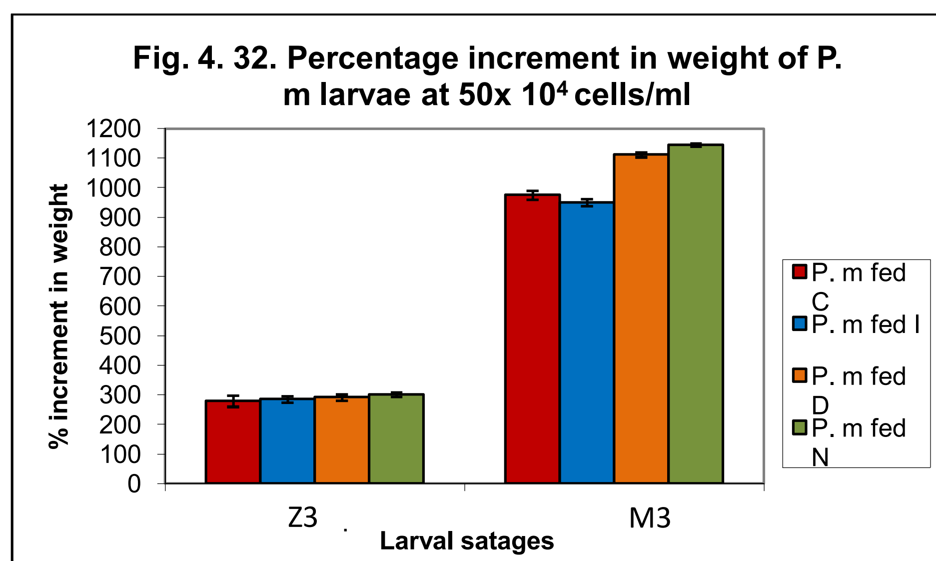
Table. 4.12. Percentage increment (weight) of P. m larva at cell conc. 10 x 10^4 cells/ ml

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01 when compared to *C. salina* fed *P. monodon*

@@@p<0.001, when compared to *I. galbana* fed *P. monodon*

$$$p<0.001, when compared to *D. salina* fed *P. monodon*



4.9 Growth rate (weight) of *P. monodon* larvae

4.9.1 Effect of mono-algal diet (50 x 10^4 cells/ ml) on growth rate (weight) of *P. monodon* larvae

Table 4.13 summarizes about the growth rate (weight) of the rearing experiments of *Penaeus monodon* larvae fed with *Isochrysis galbana*, *Chaetoceros calcitrans, Dunaliella salina,* and *Nannochloropsis salina* at the cell concentration of 50 x 10^4 cells/ ml starting from first protozoea stage (PZ-1). No significant changes was observed with the Z3 stages of the larvae whereas a significant increase in the weight gain was observed in the of P.m larvae fed with *I* (p>0.01), *D* and N (p<0.01) when compared with that of P.m larvae fed with *C*

A significant increase in the weight gain was observed in P.m larvae fed with *D* and N ($p<0.001$) when compared with that of P.m larvae fed with *I.* P.m larvae fed with *N* showed a significant increase in the weight gain ($p<0.001$) when compared with that of larvae fed with *D,* through out the 12 days of study.

	Larvae stages	
	Z3	**M3**
P. m fed C	11.118±0.414	19.8±0.11
P. m fed I	11.26±0.217	19.59±0.093 **
P. m fed D	11.394±0.211	20.8±0.058 $^{***@@@}$
P. m fed N	11.6± 0.167	21.017±0.032 $^{***@@@\$\$\$}$

Table. 4.13. Growth rate (weight) of P. m larva at cell conc. 10 x 10^4 cells/ ml

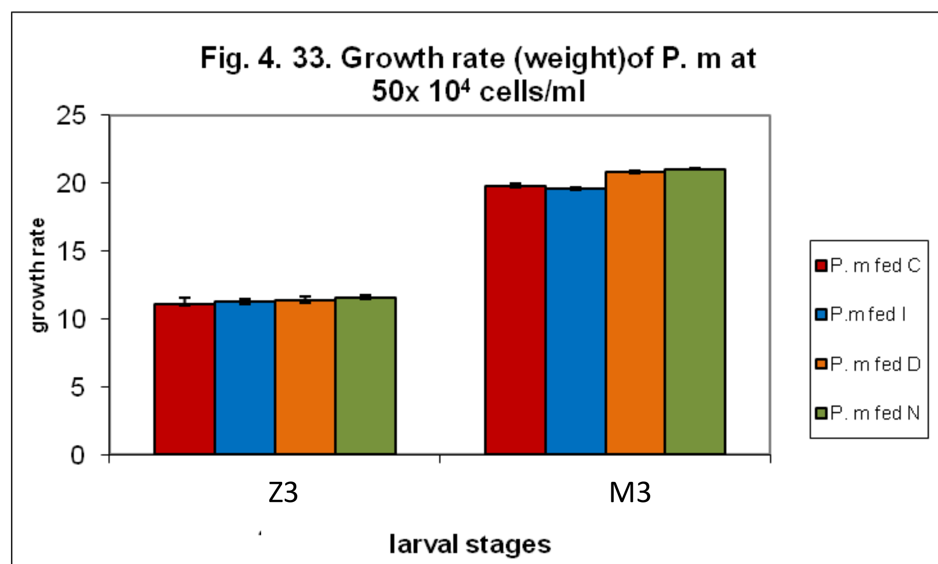
Values are mean ± SD of 4-5 separate experiments; n = 10 in each group.

ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$ when compared to *C. salina* fed *P. monodon*

$^{@@@}p<0.001$, when compared to *I. galbana* fed *P. monodon*

$^{\$\$\$}p<0.001$, when compared to *D. salina* fed *P. monodon*



4.10. Water quality parameters

4.10.1. Water quality parameters of *P. monodon* larvae fed with monoalgal diets at cell concentration (50 x 10^4 cells/ ml)

The water quality parameters like temperature, pH, salinity and dissolved oxygen were maintained throughout the experimental condition. Water temperature was in the range of 25°C -27°C (26±1°C) and salinity (30–32ppt), and dissolved oxygen (DO) concentration (6.30–6.85 mg L−1) and the tank water pH (7.66–8.5). Total ammonia nitrogen (NH_3 optimum <0.1 ppm NH_3), NO_2N, NO_3N and PO_4 were less than 1mg/l.

DOC*	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.9±0.5	31.3±0.6	8.3±0.2	7.1±0.3	2.22±0.08	2.65±0.07	1.12±0.09	3.69±0.06
4	26.8±0.4	31.2±0.4	7.7±0.4	7.1±0.5	3.05±0.03	3.76±0.05	1.14±0.03	2.06±0.07
6	26.7±0.5	31.5±0.5	7.9±0.6	7±0.7	2.49±0.06	3.66±0.08	1.16±0.08	2.68±0.08
8	26.1±0.7	30.8±0.3	7.8±0.4	6.7±0.3	3.11±0.03	4.16±0.04	1.16±0.05	4.16±0.04
10	25.8±0.5	30.8±0.6	8.1±0.7	6.6±0.4	2.90±0.07	2.00±0.07	1.16±0.08	2.34±0.06
12	25.9±0.6	31.8±0.4	7.9±0.5	6.5±0.7	2.82±0.08	2.80±0.09	1.12±0.07	3.06±0.06

Table 4.14. Water quality parameters of *P. monodon* larvae fed C at cell concentration (50 x 10^4 cells/ ml)

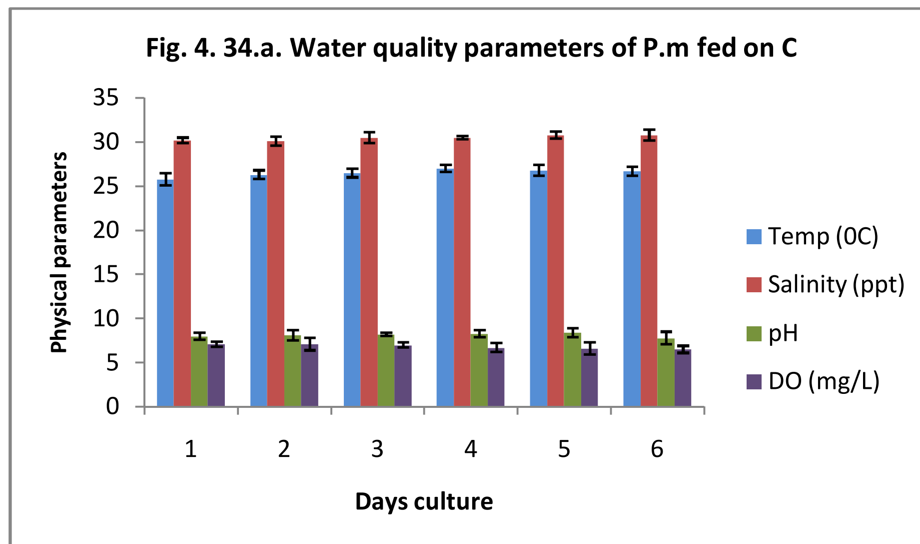
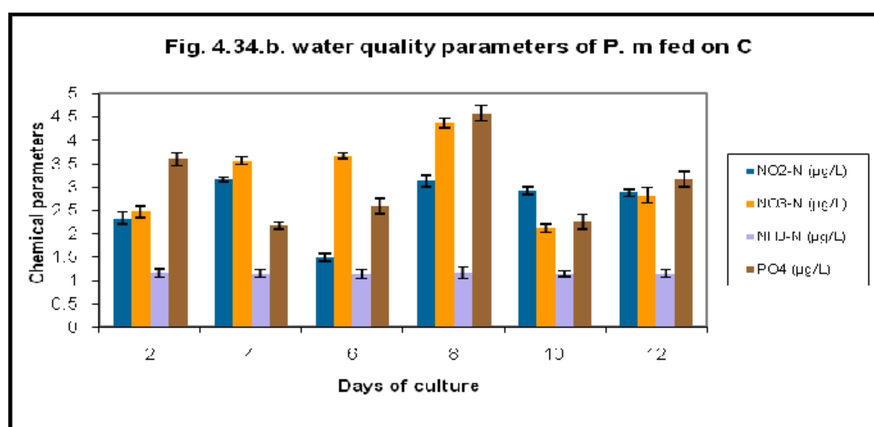


Fig. 4.34.b. water quality parameters of P. m fed on C



DO C*	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.8±0.2	30.2±0.1	7.9±0.4	6.8±0.1	2.65±0.02	3.61±0.06	1.15±0.07	3.16±0.02
4	26.5±0.1	30.7±0.2	8.3±0.3	6.5±0.3	2.01±0.04	3.25±0.05	1.15±0.05	2.58±0.02
6	26.3±0.3	31.5±0.2	8.2±0.2	6.4±0.1	1.82±0.08	4.25±0.06	1.15±0.07	2.30±0.05
8	25.8±0.2	31.5±0.2	8.1±0.3	6.7±0.5	2.09±0.06	2.45±0.07	1.15±0.08	3.40±0.08
10	26.9±0.2	30.9±0.3	8.1±0.1	6.5±0.4	2.61±0.04	3.76±0.07	1.16±0.08	2.78±0.04
12	26.3±0.3	30.8±0.2	7.9±0.4	6.4±0.5	2.76±0.02	4.51±0.08	1.14±0.05	2.92±0.06

Table 4.15. Water quality parameters of *P. monodon* larvae fed I at cell concentration (50 x 10^4 cells/ ml)

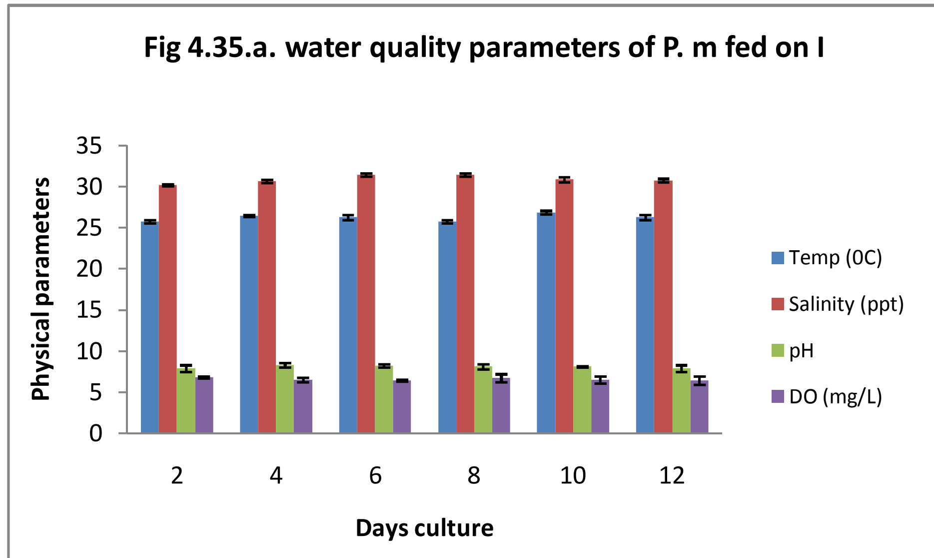


Fig 4.35.a. water quality parameters of P. m fed on I
Physical parameters
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days culture
Temp (0C)
Salinity (ppt)
pH
DO (mg/L)

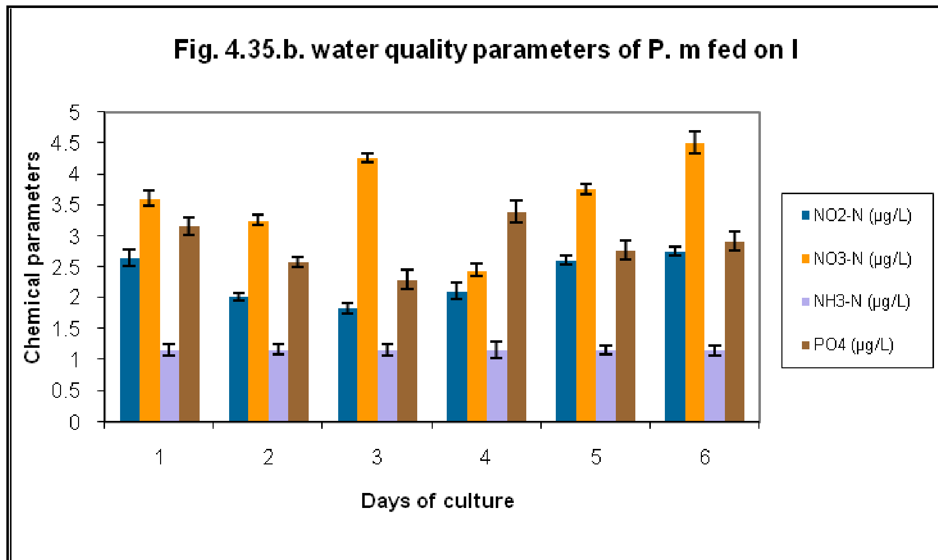
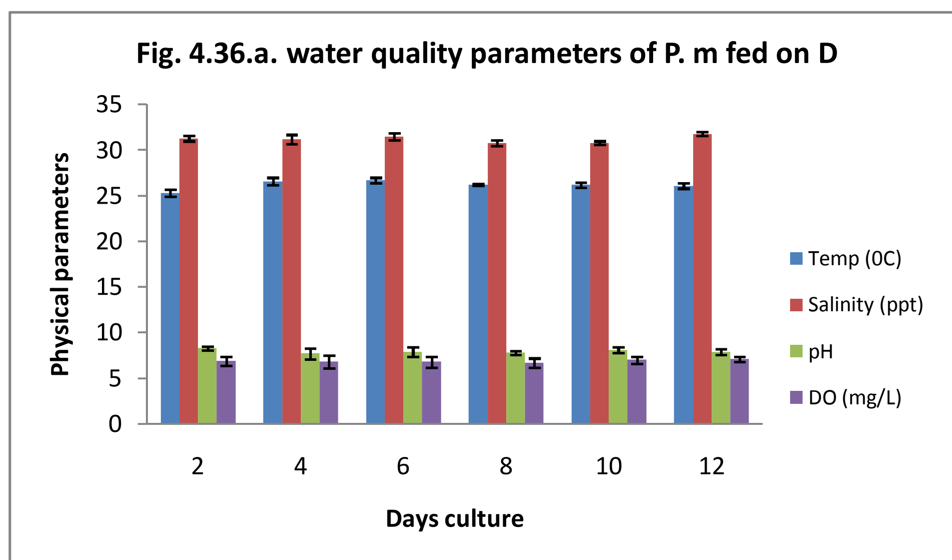
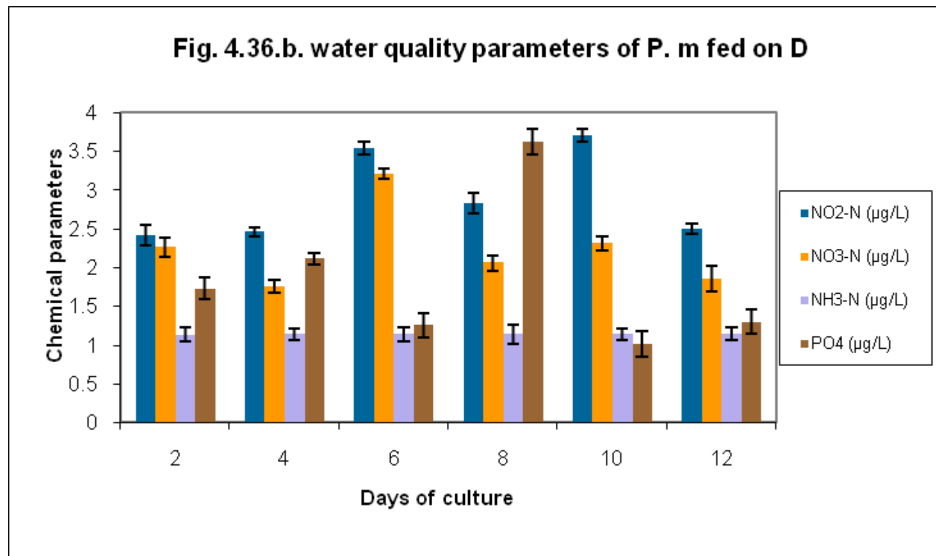


Fig. 4.35.b. water quality parameters of P. m fed on I
Chemical parameters
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
Days of culture
NO2-N (µg/L)
NO3-N (µg/L)
NH3-N (µg/L)
PO4 (µg/L)

DOC*	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.3±0.4	31.3±0.3	8.3±0.2	6.9±0.5	2.41±0.09	2.25±0.07	1.13±0.08	1.72±0.07
4	26.6±0.4	31.2±0.5	7.7±0.6	6.8±0.7	2.45±0.08	1.75±0.04	1.13±0.06	2.11±0.07
6	26.7±0.3	31.5±0.4	7.9±0.5	6.8±0.6	3.53±0.07	3.20±0.08	1.13±0.02	1.24±0.09
8	26.2±0.1	30.8±0.3	7.8±0.2	6.7±0.5	2.82±0.05	2.05±0.06	1.13±0.09	3.62±0.07
10	26.2±0.3	30.8±0.2	8.1±0.3	7±0.4	3.70±0.05	2.30±0.06	1.13±0.07	1.09±0.06
12	26.1±0.3	31.8±0.2	7.9±0.3	7.1±0.3	2.49±0.05	1.85±0.06	1.14±0.07	1.29±0.08

Table 4.16. Water quality parameters of *P. monodon* larvae fed D at cell concentration (50 x 10^4 cells/ ml)





DOC	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.7±0.3	30±0.2	8.2±0.3	6.4±0.5	3.58±0.09	1.90±0.09	1.13±0.08	1.24±0.09
4	26.2±0.4	30.5±0.2	7.9±0.4	6.6±0.5	3.62±0.02	2.09±0.03	1.14±0.04	1.48±0.04
6	26.3±0.5	30.5±0.2	8.2±0.3	6.7±0.4	2.80±0.04	2.50±0.02	1.14±0.03	1.53±0.04
8	26.7±0.3	30.5±0.5	7.6±0.4	6.8±0.2	2.70±0.03	2.75±0.04	1.12±0.05	1.63±0.02
10	25.8±0.3	30.8±0.2	8.3±0.3	6.9±0.2	2.72±0.03	2.25±0.04	1.13±0.05	1.67±0.06
12	25.7±0.2	30.9±0.1	7.8±0.2	6.8±0.3	2.6±0.07	2.30±0.08	1.13±0.03	1.96±0.04

4.17. Water quality parameters of *P. monodon* larvae fed N at cell concentration (50 x 10^4 cells/ ml)

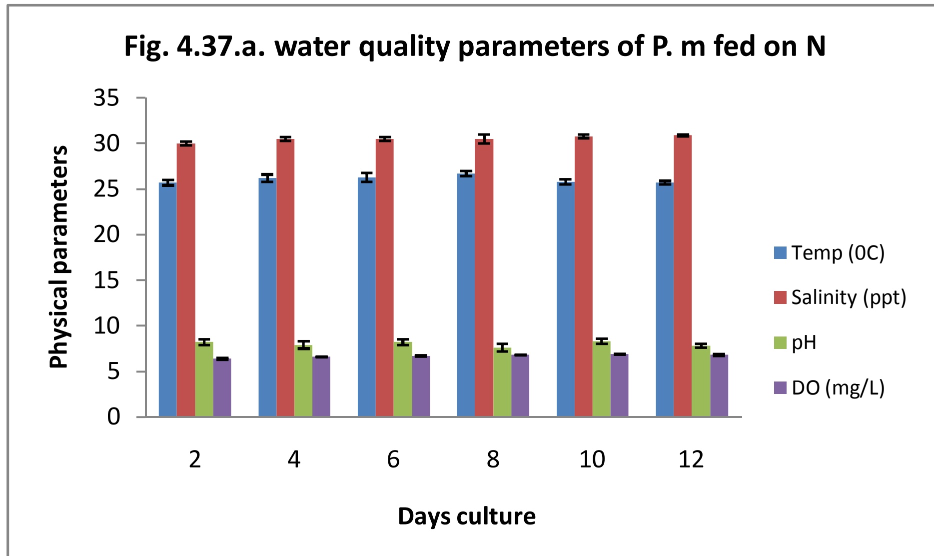


Fig. 4.37.a. water quality parameters of P. m fed on N
Physical parameters
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days culture
Temp (0C)
Salinity (ppt)
pH
DO (mg/L)

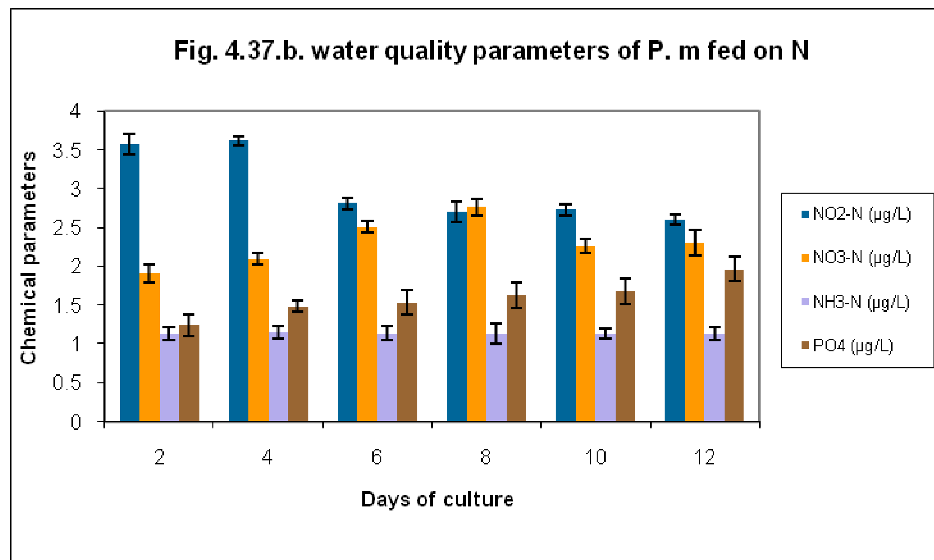


Fig. 4.37.b. water quality parameters of P. m fed on N
Chemical parameters
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of culture
NO2-N (µg/L)
NO3-N (µg/L)
NH3-N (µg/L)
PO4 (µg/L)

DISCUSSION

Scope for growth has been used successfully as an indicator of food suitability in aquaculture, but may not be considered a reliable estimator of body growth, because the percentages of assimilated energy that go into secondary production vary widely according to the species in culture and even for the same species, depending on the experimental conditions.

In protozoea, swimming and feeding are virtually continuous and the relative ingestion rate increases through each substage, reaching its maximum during larval development in PZ III (Emmerson 1980; Gilda 1989). The retention time of food in the gut is lower than in mysis and post larva and feces production is high (Jones *et al* 1993). Such strategies indicate an intense food-energy turnover achieved by higher ingestion and digestive-enzyme activity. Behavioral events also accompany the variety of body forms noted through the ontogenetic development of penaeids, PZ 3 and M 1 are almost morphologically identical (Dall *et al* 1990).

The survival tended to be higher with increasing food concentrations. In this case, the percentages of food ingested were not similar with all rations and the highest gave the better efficiency of food utilization. There was found to a significantly higher final length at higher cell concentrations. Therefore, a ration aiming to improve the efficiency of food utilization should not be considered a viable option, whereas cautious superfluous feeding would seem an appropriate strategy for culture of the PZ larvae of *P. monodon*. But, a negative effect of overfeeding on the larval survival of *Litopenaeus schmitti*, which was tentatively explained as the effect of toxic metabolites of microalgae or of impaired swimming caused by the excessive length of fecal filaments was also reported.

The study by D' Souza (1999) showed that prawn larvae (*Penaeus* spp.) do as well, or better, on a diet of *Chaetoceros muelleri* alone than on a diet of either of the other three algae. However, their survival and development may be better on a mixed diet of *C. muelleri* and *Tetraselmis suecica.*

In the present study it was found that among the four algal species selected the *P. monodon* larvae fed on *N. salina* was found to be having significantly higher length and the development was also faster till the 8th day (table 1). It seems that the *N. salina* is preferred during the protozoeal stages. The survival rate rates were also found to be higher during these stages. But throughout the experiment *D. salina* was found to be the most efficient diet until the mysis 3 stage (table 6).

Preliminary experiments indicated that the daily handling needed for a precise evaluation of survival may increase the mortality by 15% to 20%, but that this effect is independent from the experimental treatment. Several authors pointed out that when there are no differences in mortality, the evaluation of rearing regimes for shrimp larvae should consider growth in body weight rather than rates of development or increases in length (Kuban *et al* 1985; Wilkenfeld *et al* 1984). Although the survival of *Artemia franciscana* was not affected by a particularly poor diet whereas development and total length yielded similar results than weight increase. Similar results were obtained with the species fed two micro algae *D. salina* and *N. salina* that are considered of high food value for filter feeders, but gave significant differences in rates of development (table 7) and different sizes (table 3), as well as different body weights (table 9).

Although the ingestion rate was found to be increasing with increasing larval stages, it was found to be declined after M1 stage i.e day 8 (table 7). The mysid stages exhibited less active swimming behavior, and filter feeding efficiency declines at M II, when a shift to raptorial feeding is typical (Emmerson 1980, 1984). In late larval stages, thoracic appendices are now more specialized, enabling a better manipulation and selection of food particles (Jones *et al* 1992) , leading to the ingestion of more digestible parts of food The retention time of food in the gut is longer and the development of the gastric mill may contribute to the processing of food during early post larval stages (Lovett and Felder 1989). The morphological and behavioral changes in mysis and early postlarvae may compensate for the lower digestive enzyme activity when the transition to the benthic life begins. The reduced metabolic activity, food uptake, and assimilation capacity of early postlarval stages accompany the search for a suitable new habitat after migration into inshore, brackish, nursery grounds.

Guoqiang Huang 2004 was found that the compound relationship among the special growth rate DGC of Chinese shrimp, feeding level (FL) and initial body weight (IBW) were duality linear. It is to say that in every body weight, size, the DGC and FL were significantly linearly correlated. Similar positive correlations between growth and ration size have been found in studies on shrimp and crabs (Venkataramiah *et al* 1975, Caillouet *et al* 1976, Sedgwick 1979, Bartley *et al* 1980, Viayaraghavan *et al* 1982, Maguire and Leedow 1983, Gu *et al* 1996). In the present study though the body weight was high in *P. monodon* fed *N. salina*, the length was high in *P. monodon* fed *D. salina* (table 3, 9).

Some authors have attributed the poor performance of prawn larvae to the large size of the algal cells in the diet (Tobias and Villegas 1982; Sanchez 1986). *Chaetoceros muelleri* and *T-iso* are small and very similar in size (3 to 5 µm in length), while *Tetraselmis sucecia* and *Dunaliella tertiolecta* are larger and similar in size (5 to12 µm in length).

In the current study, the larvae of both the small *N. salina* and the large *D. salina* displayed high survival and development. The species selected for the present study the *C. calcitrans* had a cell size of 4-6 um and *I. galbana* had a cell size of 3-5 um and *D. salina* had a cell size of 4-5 um and the *N. salina* was the smallest had a cell size of only 1-2 um. It is seen that the differences in larval performance between algal diets were due to the differences in size of the algal cells. Increasing the proportion of *D. salina* was found to improve the diet, since *D. salina* alone also performs well.

The biomass consumed by *Litopenaeus vannamei* larvae differed depending on the algae used for feeding, even though all the species tested were ingested by the larvae. Of all the microalgae used in this experiment, the diatoms produced the highest consumable biomass. This demonstrates the advantage of using diatom species as food for larval development of shrimp and other marine organisms (Simon 1978). The variation in the biomass of microalgae consumed by *L. vannamei* could be due to several factors, including the size of the algae.

The adequate cellular diameter for feeding shrimp protozoea ranges from 3 to 30 µm However, detailed studies on the effect of the size of microalgae on feeding (Tobias and Villegas 1982; Okauchi *et al* 1990; Clark *et al* 1987;) have indicated that protozoea prefer diameters smaller

than 10 µm., Nunez *et al* 2002 found that the smallest microalgae (*Chaetoceros gracilis* and *C. calcitrans*) were consumed at a higher rate compared to larger microalgae (*Tetraselmis chuii* and *Tetraselmis sp*. G1). An exception to this was *Skeletonema* sp. Ch1 which had a low consumed biomass in relation to its small size (7–8 µm). These reports agree with our results (table 8) that N. salina showed significantly different changes with *I. galbana* and *C. calcitrans* during PZ stages (till day 8) whereas during mysis stages there was no significant changes with *C. calcitrans.*

It could be related to the small setae size compared to the setae sizes in *Chaetoceros* and *Skeletonema costatum*, making it more difficult for the protozoeae to capture the micro algae. This suggests that cellular morphology as well as volume were factors which affect micro algae consumption. Thus, it is important to take into account the appendages of diatoms when selecting a microalgal diet (Simon 1978; Wyban and Sweeney 1991). Larvae fed with *I. galbana* had a higher survival rate although not significantly different from those fed with *Chaetoceros* sp. (table 4, 5, 6). The 66–76% survival of *L. vannamei* larvae obtained in this study is high, considering the conditions of culture, high density (200 larvae/l) and no water exchange. This may be compared to a survival rate between 70–90% under normal culture conditions of commercial hatchery operations, with densities of 100–150 larvae/l and water exchange (Simon 1978;).

Sanchez (1986) who found that 90% of the *L. vannamei* larvae passed to protozoea II stage when fed with *Isochrysis* sp. which is of similar size to the *Chaetoeros species* used in this study. In our study we found that 80–89% of the larvae reached protozoea III stage in all the four

algae fed p. monodon larvae (table 6) whereas the survival rate was reduced at low cell conc.(table 4). But during mysis stages, after day 6 survival rates declined. Highest survival of 69% obtained with high cell concentration.

Although *C. gracilis* demonstrated the best results as a diet for *L. vannamei* larvae in this study, *Chaetoceros* sp. G1 produced a survival rate which was not statistically different. Previous comparisons between native and exotic microalgae (including *C. gracilis*) have shown that native microalgae produce a higher biomass in a shorter time than the exoticones, when cultured in a large scale and under field conditions (Marín *et al* 1995). This suggests that native micro algae are adequate for large scale culture conditions, saving costs and producing a higher shrimp biomass. Additionally, *Chaetoceros* sp. A1 showed a high division rate, and maintained a high micro algal concentration through out the assay.

Diatoms and prasinophytes are an ideal live diet for penaeid shrimp larvae as they inhibit the growth of virulent bacteria and are rich in nutrients (Parsons *et al* 1961; Watanabe *et al* 1983; Fukusho *et al* 1986; Whyte, 1987; Millamena *et al* 1990), which are limiting in artificial diets and zooplankters (*Artemia* nauplii and *B. plicatilis*).

(D'souza *et al* 2002), found that fresh *C. muelleri* was the best of the four diatoms tested as a diet for *P. monodon* larvae. It also confirmed that the feeding density of fresh *C. muelleri* could be halved to 5×10^4 cells ml^{-1} without affecting the survival and growth of larvae thus potentially reducing algal production costs in Australian prawn hatcheries. The *N. salina* was the best concentrate diet for prawn larvae in the present study. It

produced a high survival rate comparable with the *D. salina* showed a significant change and a faster development rate upto mysis than all of the other diets. When compared to *C. calcitrans* no significant change was seen except in *N. salina* during the PZ stages rather than mysis but when compared to *I. galbana*, *D. salina* had a significant change both in PZ and mysis stages (table 5, 6, 7).

It is not known whether the weight of larvae at the protozoeal/mysis stages influences parameters such as weight, survival or health at later stages of development. Survival alone is not a sensitive measure of the nutritional value of diets for prawn larvae (D'Souza and Loneregan 1999). Larvae fed concentrated *T. pseudonana* or *C. muelleri* had a high survival rate comparable with the best diet of fresh *C. muelleri* but their development rate and dry weight were significantly lower. In the present study, larvae fed the high density of fresh *D. salina and N. salina* had a similar DI (table7), but their average length was vastly different. In a hatchery, the diet producing larger animals would be preferable. Clearly, all three measures of the performance of larvae must be included in an evaluation of diets.

The evaluation of algae as live feeds for penaied larvae is generally based on the selection of species that sustain the maximum growth, survival and development. The present study suggests that, of the three unicellular algal species tested, the diatom *S. costatum* promotes better larval growth, survival and development throughout all larval stages than the *I. gabana* and *C. calcitrans*. Hence the *D. salina* can be used as one of the most suitable live diets for penaied larvae during the mysis stages and therefore

can be commoly used in hatcheries. Whereas during the PZ stages *N. salina* was found to promote better growth survival and development. But a significant change was observed only during the 12th day when compared with that of *D. salina*.

The results from many of these studies shows that the practice of feeding the protozoeal and mysis substages of penaeid shrimps with expensive *Artemia* nauplii is not necessary when the early larval stages are still filter feeders and can still benefit on a cheaper but nutritionally sufficient natural diet of phytoplankton (Ronquillo *et al* 1997). This study confirms and augments the observations reported previously on the larval rearing of penaeid shrimps using algal diets only (Liao *and* Chao 1983).Therefore, this study demonstrates that penaeid larvae show species specific differences between algae. suggest that there may not be any benefit to *P. indicus* larvae in feeding algal diets during the mysis stages as larvae fed only with *Artemia* from the PZ3/M1 to PL stages displayed equal growth and better survival than those fed with algae and *Artemia*. Studies with other penaeid larvae, such as *P. marginatus*, *P. aztecus, P. setiferus* and *P. vannamei* have also shown that the exclusive use of algae throughout all the larval stages results in lower growth and delayed metamorphosis, although comparable survival rates can be achieved. Significantly higher growth and survival obtained when fed *P. japonicus* larvae with alga (*C. gracilis*) throughout the larval stages together with *Artemia* during the mysis stages, as opposed to larvae fed with only the alga. In their study, the survival and growth of larvae receiving either alga or Artemia during the mysis stages did not differ significantly. The study presented here demonstrates that *P. monodon* can be reared successfully from the PZ1 to the PL1 stage within 11-12 days.

The study by Lober and Zeng 2009, clearly demonstrated that the addition of *Nannochloropsis* sp. at appropriate levels led to significantly improved larval survival, development and growth of the Australian strain of *M. rosenbergii*. Larval survival to M3 was significantly higher at the two higher algal levels of 25×10^4 and 50×10^4 cells/ml, suggesting that between the algal levels of 25×10^4 and 50×10^4 cells/ml lays a critical threshold (table 2, 3). At the highest algal concentration of 50×10^4 cells/ml, development to M3 was the shortest and significantly faster than all other treatments except that of the second highest algal density of 25×10^4 cells/ml. This was also reflected in the DI values, with generally higher DI recorded for the higher algal concentrations (table 7). Significant differences in DI were registered as early as on day 4, suggesting that the beneficial effects of algae was seen in PZ2 stages. Furthermore, the improved growth at the two higher microalgae levels was manifested by significantly heavier M3 dry weights. The algal concentrations used for marine shrimp, such as *Penaeus stylirostris* and *P. vannamei*, for which diatom *Chaetoceros gracilis* concentrations were reported to range between 30,000 and 100,000 cells/ml to assure good growth and survival of the zoeal larvae (Simon, 1978).

Moreover adding micro algae to larval culture may help improve water quality via a reduction in ammonia and other nitrogen wastes in the culture medium (New, 1990). However, considering that the water used in the current experiment was totally exchanged daily, a built up of toxic nitrogen compounds seems less likely to be a major limiting factor. However, there is also a possibility that micro algae may secrete unknown bioactive chemicals that inhibit various pathogens or directly benefit the larvae. In the present study though *P. monodon* fed *C. calcitrans* showed

better survival and length when compared with that of *I. galbana* the changes were not significant except with that of day 6 i.e the M1 stage.

Rico-Villa *et al* 2006 found that the nutritional value of microalgae for bivalve larvae depends, above all else, on the cell size and/or the presence of short spines allowing its accessibility and ingestion (Robert and Trintignac,1997) while the nature and thickness of the cell wall will explain its digestibility, some species being ingested but poorly digested. The three microalgae used in the present study are known to have similar sizes and fine cell walls (Chrétiennot-Dinet et al., 1991) but, despite their similarity, a preferential consumption was shown according to the microalgal species and type of diet.

However, it was seen that *Nannochloropsis sp*. is not considered a particularly suitable diet for Artemia nauplii due to toughness and the indigestibility of its cell wall. But in the present study *N. salina* was found to show better survival and length at all cell concentrations during the protozoea stage. The ingestion rate reduced during M2 stage onwards, where the *D. salina* showed a significantly higher ingestion rates (table 8).

The differences in survival and growth rates during the second and third days can probably be attributed to cell size. Villegas and Quinitio 1982 discussed that cells of *C. calcitrans* although bearing setae of considerable length, measure 4-5 µm, while that of *T. chuii* were 12-15 µm. Although measurements of the mouths of early zoeal stage (Z1 and Z2) of *P. monodon* were not made, the mouths were probably not wide enough to take in cells of *T. chuii*. Consequently the larvae could not ingest enough food. It is may be due to this reason that in the present study the *P.*

monodon larvae prefer *N. salina* during the PZ stages rather than mysis stages among all the four algae selected.

This schedule is based on the results obtained in this study, considering growth and DI as the most important indices and survival, QI and performance index as factors to define the optimum concentrations with greater precision. In accordance with Kuban et al. (1985), growth is one of the best ways to evaluate a diet. The physiological state of the organisms can be established through growth. The DI, as a measure of the rate of metamorphosis, has turned out to be an index complementary to growth and gives a good idea of the effects of the food on the larvae that are being cultivated. The growth rate and DI of *P. setiferus* larvae during larval development were affected by the different concentrations of diatoms and flagellates (Tables l-4).

According to the results obtained in relation to the performance index *P. setiferus* larvae can not be fed only with C. *ceratosporum* because survival and quality index were diminished when flagellates were absent. This is in strong contrast to the idea that diatoms constitute the main contribution to the nutrition of the larvae and that flagellates are needed as a complement in the feeding schedule. The fact that flagellates have not produced spectacular results in several species under study might be related to their limited availability during the first phases of development which seems to determine the success of the culture.

Ammonium toxicity is discarded in all trials, since the lethal dosage of this ion for crustaceans is far over 1 mg/L (Chen 1998), being the critical concentration for shrimp larvae between 0.95 and 3.80 mg/L depending on

larvae age. Around 95% of this ammonium ion was excreted by the nauplii through their gills (Regnault, 1981). To keep total ammonium ion under lethal concentration, it was necessary to exchange and replace water in a 50% per day basis.

Nitrite ion was always present in sublethal concentrations at a maximum of 50 µg/l. Wickins 2002 presented lethal concentrations for nitrite between 400 and 2300µg/l depending the range on other substances present in water, as for example ammonium ion. Their presence is due to the addition of nitrate salts in form of f/2 media for algae growth enhancement and nitrite is the by-product of the transformation of nitrate with the help of bacteria. On the other hand the decomposition of the feces of the shrimp larvae, and uneaten diets, also contribute to increase these ions. Their concentration decreased clearly, when partial water exchanges were done daily. These results also coincide with those observed by Wilkenfeld *et al* 1984, Kurmaly *et al* 1989. Costs of larvae fed artificial diets are higher. Survival was another significant parameter that was higher in those experiments with natural feeds, than with the artificial ones.

Thus, even if it is possible to obtain larger animals with a high DI and QI in a particular condition, it does not necessarily mean that there are more postlarvae in the harvest. One often finds a few very resistant and large animals that have survived after being exposed to adverse conditions during the culture stages. In this sense, survival can be an important parameter by which to define the conditions of production with more precision. Survival made it easier to define the optimum conditions of feeding in this study (Table 10).

Although larvae fed 25 X $l0^4$ of diatoms showed a high growth rate (Table 2), survival was low, indicating cell deficiency in that treatment. Biedenbach *et al* 1989 have reported that food in excess can cause stress to the larvae that are being cultured. The excess algae cause formation of long fecal strings, which get caught in the appendages and hinder the free movement of the larvae. As larvae increase the energy used for movement, they get tired and finally die. Only the largest and strongest larvae survive. From the present study the cell concentration beyond $50x10^4$ cells may cause fouling and mortality.

Chapter- 5

EFFECT OF MIXED ALGAL DIET ON GROWTH RESPONSES OF P.MONODON LARVAE

Growth rate of *P. monodon* larvae fed with varying concentration of mixed algal diets of *Chaetoceros calcitrans, Isochrysis galbana*, *Dunaliella salina,* and *Nannochloropsis salina* were compared. Combinations of *Chaetoceos calcitrans* + *Dunaliella salina, Chaetoceros calcitrans* + *Isochrysis galbana*, *Chaetoceros calcitrans* + *Nannochloropsis salina, Dunaliella salina* + *Isochrysis galbana, Isochrysis galbana* + *Nannochloropsis salina, Nannochloropsis salina* + *Dunaliella salina* were taken for the study. The algae were tested individually at three cell concentrations $10x10^4$ cells/ml, $25x10^4$ cells/ml and $50x10^4$ cells/ml, from the PZ1 to the PL1 stage. The length and percentage survival of all the three cell concentrations were evaluated. The results of the experiments conducted are shown in the following tables and is explained.

5.1. Length of *P. monodon* larvae

5. 1.1. Effect of mixed-algal diet ($10x10^4$ cells/ ml) on length of *P. monodon* larvae

Table 5.1 summarizes about length of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $10x10^4$ cells/ ml starting from first protozoea stage (PZ-1). Throughout the 12 days of study when compared to P.m larvae fed with *C* a significant increase was observed in the length of Pm larvae fed with *I+C* on 2nd day (p<0.001), 10th day and 12th day (p<0.01) with *I+N* on 2nd day(p<0.001), 8th day (p<0.01), and 12th day (p<0.01). A significant change was observed in the length of Pm larvae fed with *I+D* on 2nd day (p<0.001), 4th day (p<0.05), 8th day, (p<0.01) 10th day (p<0.001), and 12th day (p<0.001). A significant change was observed in the length of Pm larvae fed with *C+N* on 2nd day (p<0.001), 4th day (p<0.05), 8th day, (p<0.01) 10th day (p<0.001), and 12th day (p<0.001). A significant change was observed in the length of P.m larvae fed with *C+D* on 2nd day (p<0.001), 4th day (p<0.05), 6th day (p<0.05) 8th day, 10th day, and 12th day (p<0.001). A significant change was observed in the length of Pm larvae fed with *D +N* on 2nd day (p<0.001), 4th day (p<0.05), 6th day (p<0.01) 8th day, 10th day, and 12th day (p<0.001)

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the length of P.m larvae fed with *I+N* on 2nd day (p<0.05), and 8th day (p<0.01). A significant change was observed in the length of Pm larvae fed *I+D* on 2nd day (p<0.01), 4th day (p<0.05), 8th day, (p<0.01). A significant change was observed in the length of Pm larvae fed with *C+N* on 2nd day (p<0.001), 4th day (p<0.05), 8th day, (p<0.001) 10th day

($p<0.001$). A significant change was observed in the length of P.m larvae fed with *C+D* on 2nd day ($p<0.001$), 4th day, 6th day ($p<0.05$) 8th day, ($p<0.001$) 10th day ($p<0.001$), and 12th day ($p<0.01$). A significant change was observed in the length of Pm larvae fed with *D +N* on 2nd day ($p<0.001$), 4th day ($p<0.01$), 6th day ($p<0.05$) 8th day, 10th day and 12th day ($p<0.001$)

A significant increase was observed in the length of P.m larvae fed with *C+D* on 6th day and 10th day ($p<0.05$) and also a significant change was observed in the length of P.m larvae fed with *D +N* on 6th day ($p<0.05$) and 10th day ($p<0.001$) when compared with that of *I+D*.

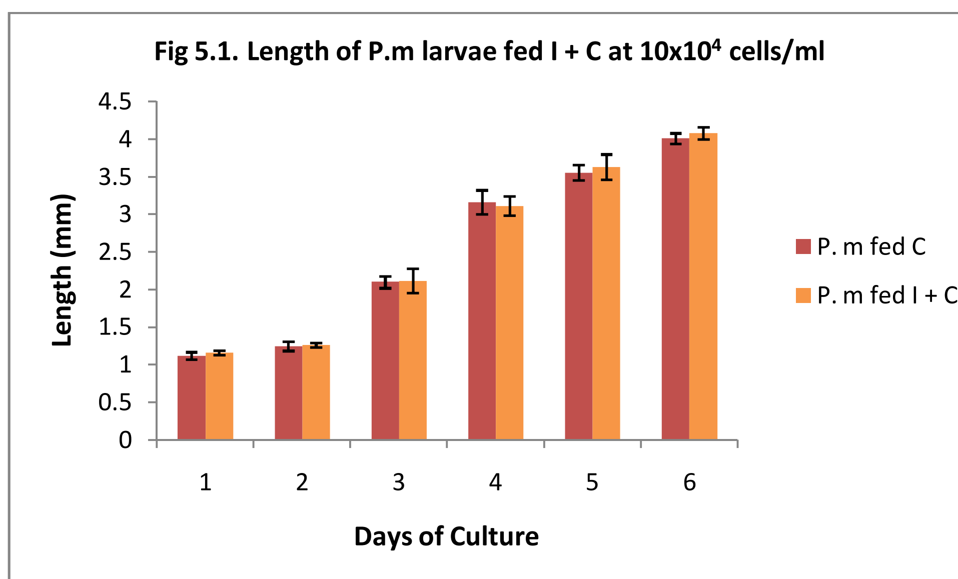
Exp. Day	**2**	**4**	**6**	**8**	**10**	**12**
C	1.118± 0.05	1.246± 0.06	2.101± 0.08	3.163± 0.16	3.558± 0.10	4.011± 0.07
I+C	1.160± 0.03***	1.261± 0.03	2.117± 0.16	3.114± 0.13 @@	3.631± 0.17	4.084± 0.08
I+N	1.217± 0.06 ***@	1.317± 0.07	2.169± 0.09	3.345± 0.16 **@@	3.773± 0.16 **	4.144± 0.05 **
I+D	1.229± 0.04 ***@@	1.337± 0.06*@	2.111± 0.09	3.367± 0.15**@@	3.727± 0.12 *@	4.185± 0.10 ***@
C+N	1.256± 0.03 **@@@	1.352± 0.09*@	2.209± 0.06	3.423± 0.14 ***@@@	3.830± 0.16 ***	4.211± 0.09 ***@
C+D	1.260± 0.06 ***@@@	1.349± 0.03*@	2.249± 0.07 *@£	3.479± 0.11 ***@@@	3.927± 0.13 ***@@@£	4.246± 0.09 ***@@
D+N	1.271± 0.03 ***@@@	1.382± 0.07 ***@@	2.262± 0.06 **@£	3.476± 0.13 ***@@@	3.937± 0.12 ***@@@£	4.255± 0.09 ***@@@

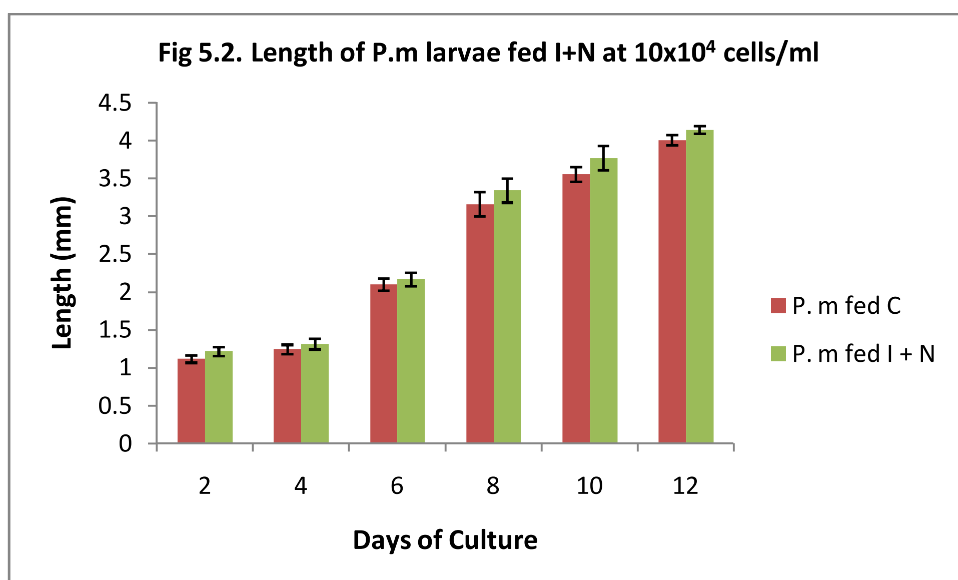
Table 5.1. Length of *P. monodon* larvae at cell conc. ($10x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group.

ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to C
$^{@@@}p<0.001$, $^{@@}p<0.01$, $^{@}p<0.05$, when compared to I+C
£ $p<0.05$ when compared to I+D





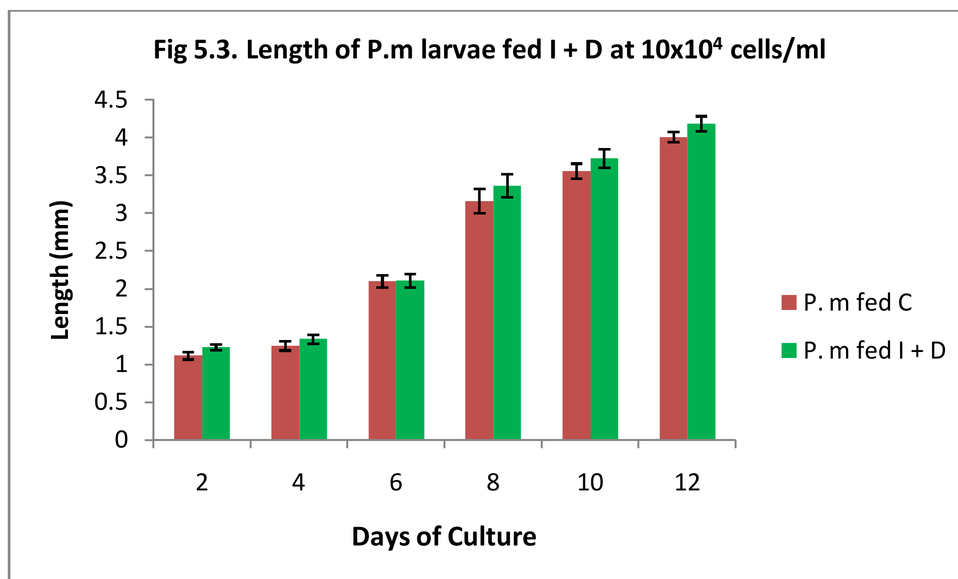


Fig 5.3. Length of P.m larvae fed I + D at 10x10⁴ cells/ml
Length (mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + D

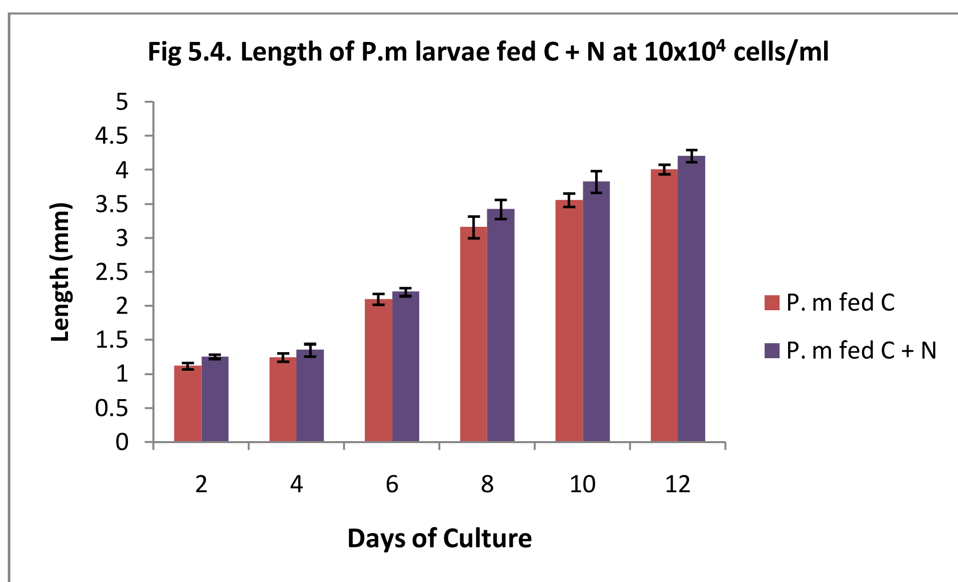


Fig 5.4. Length of P.m larvae fed C + N at 10x10⁴ cells/ml
Length (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + N

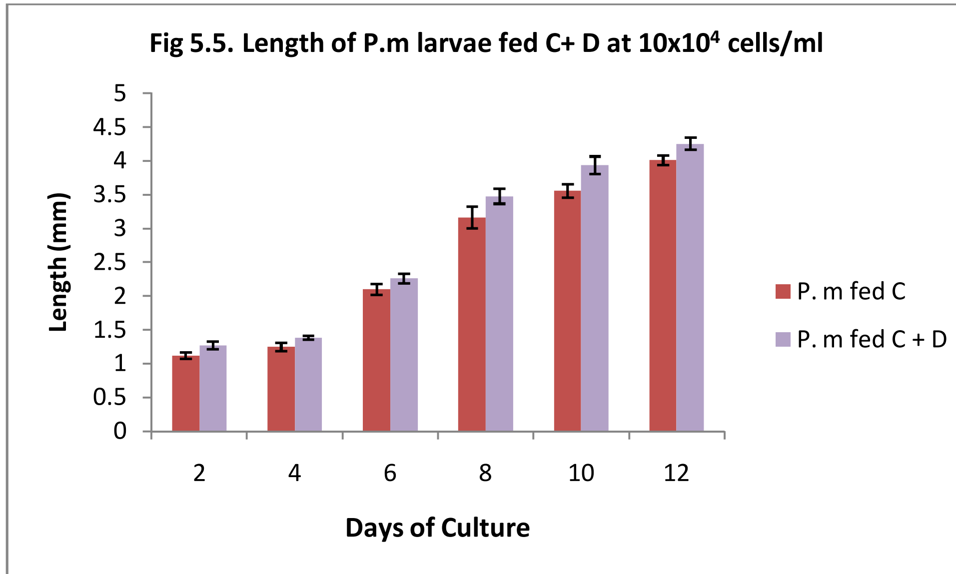


Fig 5.5. Length of P.m larvae fed C+ D at 10x10⁴ cells/ml
Length (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + D

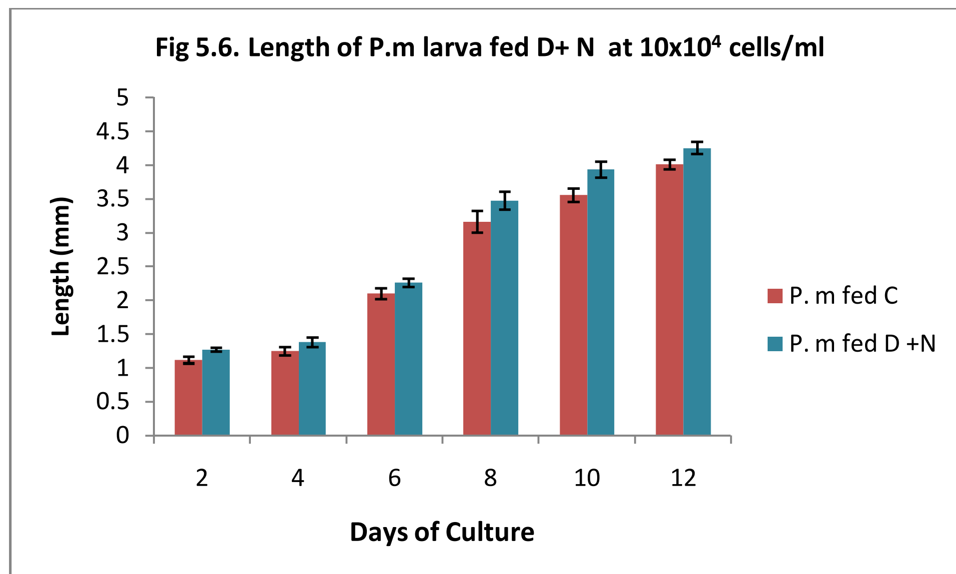


Fig 5.6. Length of P.m larva fed D+ N at 10x10⁴ cells/ml
Length (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.1.2 Effect of mixed-algal diet ($25x10^4$ cells/ ml) on length of *P. monodon* larvae

Table 5.2 summarizes about length of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $25x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to P.m larvae fed with *C* throughout the 12 days of study, a significant increase was observed in the length of P.m larvae fed with *I+C* on 6th day ($p<0.05$). A significant change was observed in the length of P.m larvae fed with *I+N* on 6th day ($p<0.001$), 8th day, 10th day ($p<0.01$) and 12th day ($p<0.01$). A significant change was observed in the length of P.m larvae fed with *I+D* on 4th day ($p<0.05$), 6th day, 8th day, 10th day, and 12th day ($p<0.001$) *C+N* on 2nd day ($p<0.05$), 4th day, 6th day, 8th day, 10th day, and 12th day ($p<0.001$). A significant change was observed in the length of P.m larvae fed with *C+D* and P.m larvae fed with *D +N* on all days ($p<0.001$)

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the length of P.m larvae fed with *I+N* on 4th day ($p<0.05$), 6th day ($p<0.001$), and 12th day ($p<0.01$). A significant change was observed in the length of P.m larvae fed with *I+D* on 4th day ($p<0.05$), 6th day ($p<0.05$), 8th day, and 12th day ($p<0.001$), 10th day ($p<0.01$). A significant change was observed in the length of P.m larvae fed with *C+N* 4th day, 8th day, 10th day, and 12th day ($p<0.001$) but on 6th day ($p<0.05$). A significant change was observed in the length of P.m larvae fed with *C+D* and P.m larvae fed with *D +N* on all days ($p<0.001$)

A significant increase was observed in the length of P.m larvae fed with *C+N* and P.m larvae fed with *C+D*, on 4th day ($p<0.05$) and 12th day ($p<0.001$). A significant change was observed in the length of P.m larvae fed with *D +N* on 4th day ($p<0.01$) and 12th day ($p<0.001$) when compared with that of P.m larvae fed with *I+N*

A significant increase was observed in the length of P.m larvae fed with *C+D* on 8th day and 12th day ($p<0.05$) and also with P.m larvae fed with *D +N* on 8th day ($p<0.05$) and 12th day ($p<0.01$) when compared with that of P.m larvae fed with *I+D*.

A significant increase was observed in the length of P.m larvae fed with *C+D* on 8th day ($p<0.05$) and also with P.m larvae fed with *D +N* on 8th day ($p<0.05$) and 12th day ($p<0.01$) when compared with that of Pm larvae fed with *C+N*.

Exp. Day	2	4	6	8	10	12
C	1.174± 0.03	1.305± 0.06	1.968± 0.07	3.214 ± 0.12	3.549 ± 0.17	4.057 ± 0.08
I+C	1.202± 0.06	1.312± 0.06	2.077± 0.05 *	3.223± 0.09	3.625± 0.10	4.076± 0.06
I+N	1.242± 0.06	1.371± 0.09	2.164± 0.06***@	3.518± 0.10 ***@@@	3.745± 0.15 **	4.188± 0.04*** @@
I+D	1.241± 0.06	1.438±0.05**@@	2.208± 0.10***@	3.443± 0.15 ***@@@	3.829± 0.10 ***@@	4.228± 0.07***@@@
C+N	1.257± 0.04*	1.472± 0.11 ***@@@$	2.216±0.10***@	3.424± 0.17 ***@@@	3.887± 0.12 ***@@@	4.249± 0.08 ***@@@ $$$
C+D	1.299± 0.08 ***@@	1.494± 0.10 ***@@@$	2.246± 0.12 ***@@	3.584± 0.08 ***@@@£Ψ	3.905± 0.14 ***@@@	4.336± 0.05***@@@ $$$£
D+N	1.305± 0.05 ***@@	1.528± 0.10 ***@@@$$	2.273± 0.11 ***@@@	3.604± 0.04 ***@@@£Ψ	4.017± 0.12 ***@@@$$$£	4.372± 0.13***@@@ $$$££ΨΨ

Table 5.2. Length of *P. monodon* larvae at cell conc. ($25x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group.
ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to C
@@@p<0.001, @@p<0.01, @p<0.05, when compared to I+C
$$$p<0.001, $$p<0.01, $p<0.05 when compared to I+N
££p<0.01, £p<0.05 when compared to I+D
ΨΨp<0.01, Ψp<0.05, when compared to C+N

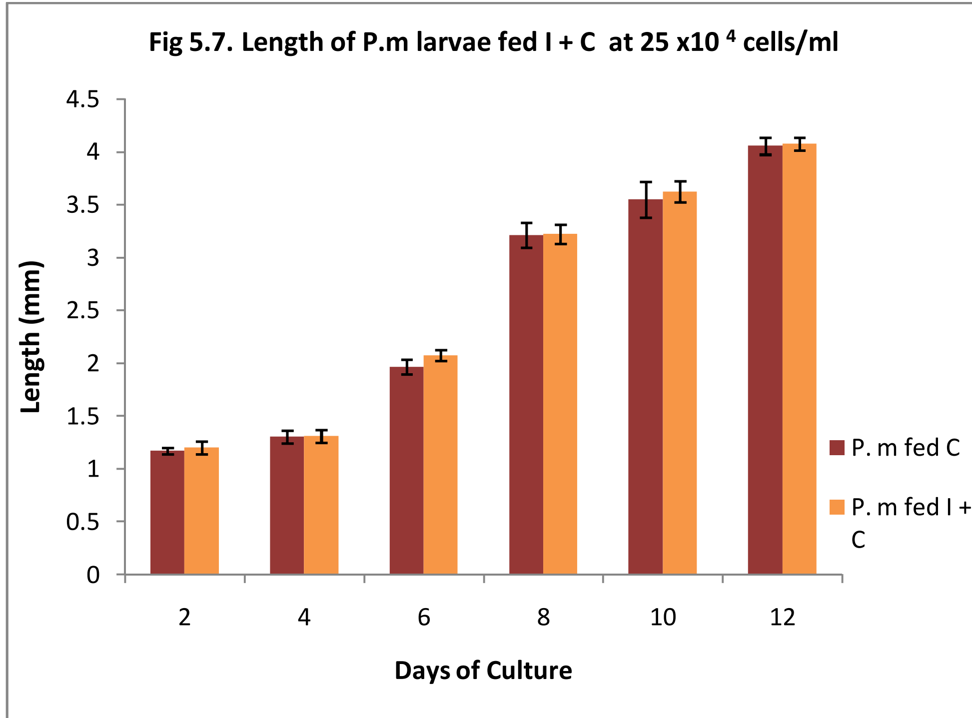


Fig 5.7. Length of P.m larvae fed I + C at 25 x10 4 cells/ml
Length (mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + C

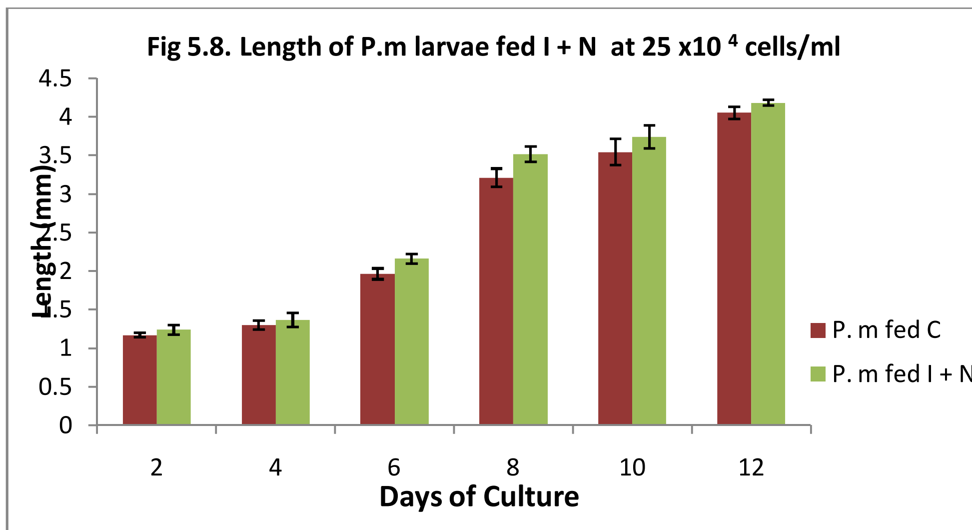


Fig 5.8. Length of P.m larvae fed I + N at 25 x10 4 cells/ml
Length (mm)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + N

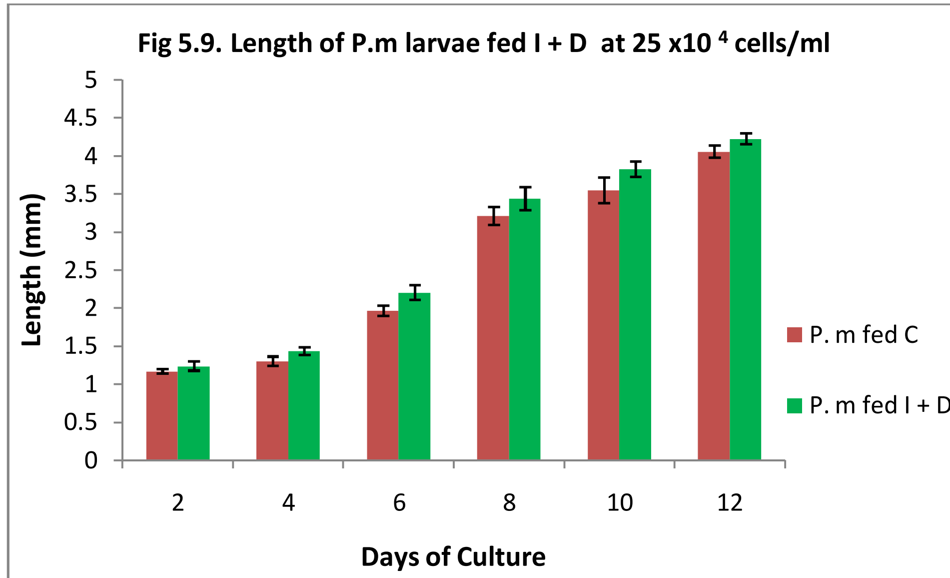


Fig 5.9. Length of P.m larvae fed I + D at 25 x10 ^{4} cells/ml

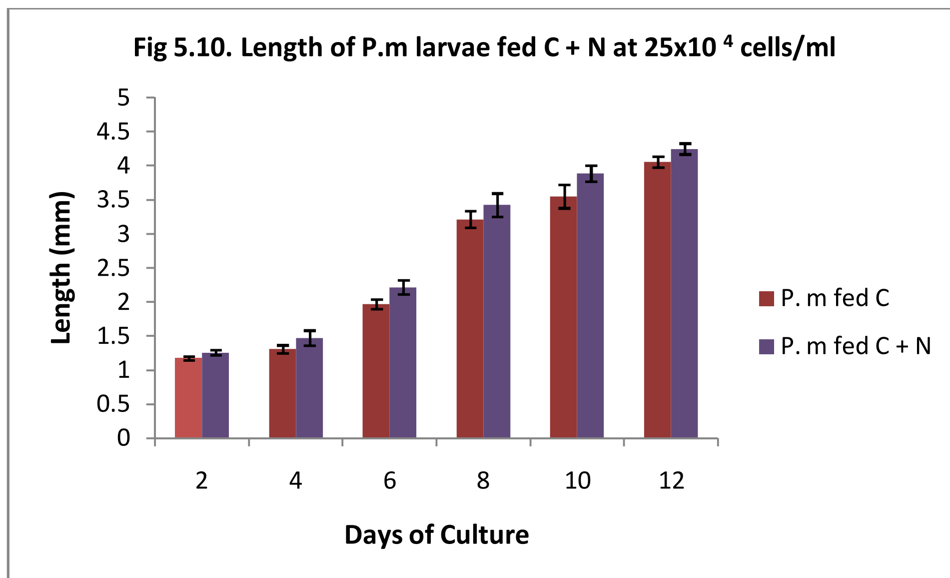


Fig 5.10. Length of P.m larvae fed C + N at 25x10 ^{4} cells/ml

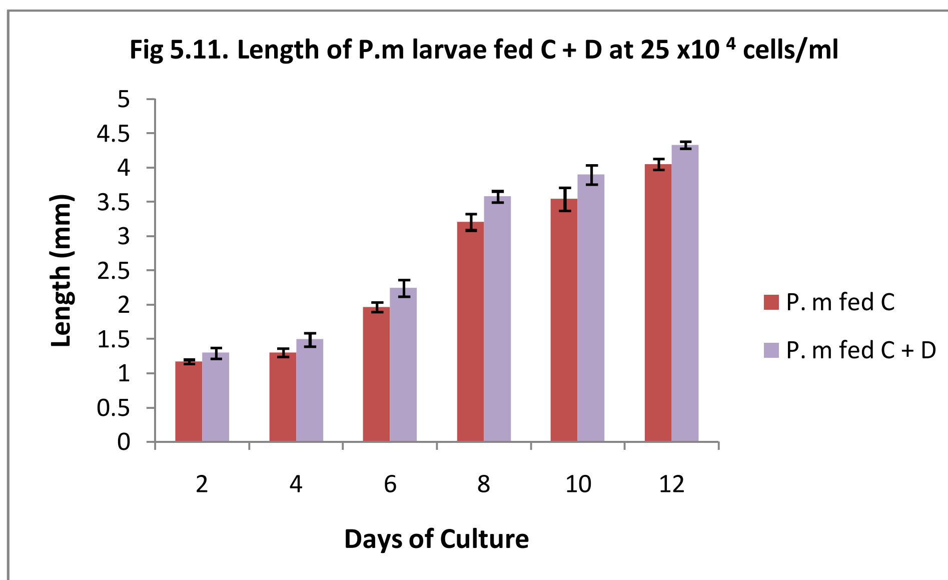


Fig 5.11. Length of P.m larvae fed C + D at 25 x10 4 cells/ml
Length (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + D

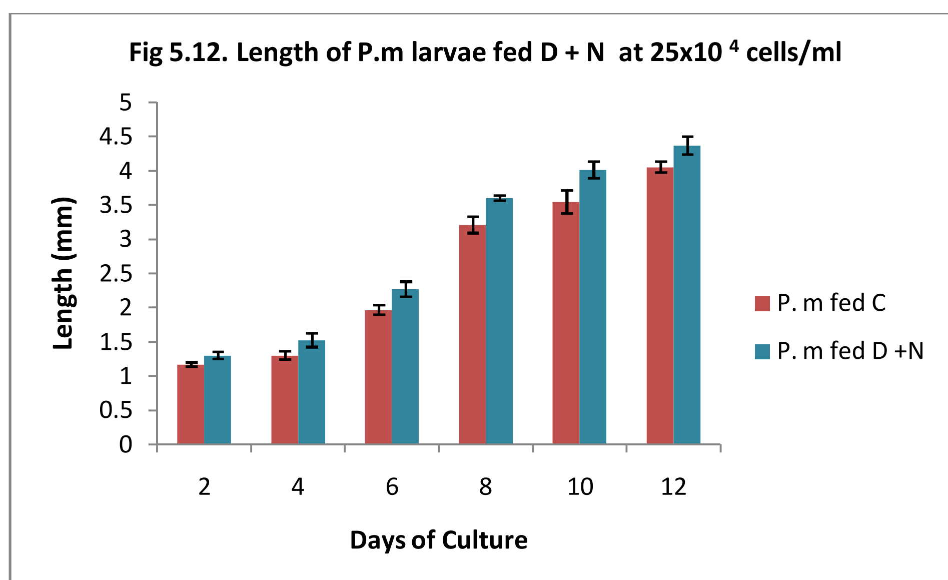


Fig 5.12. Length of P.m larvae fed D + N at 25x10 4 cells/ml
Length (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.1.3. Effect of mixed-algal diet (50x10^4 cells/ ml) on length of *P. monodon* larvae

Table 5.3 summarizes about length of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of 25x10^4 cells/ ml starting from first protozoea stage (PZ-1). When compared to Pm larvae fed with *C* throughout the 12 days of study, a significant increase was observed in the length of Pm larvae fed with *I+C* on 4th day, 6th day ($p<0.05$), 8th day($p<0.001$), 10th day, and 12th day ($p<0.01$). A significant change was observed in the length of Pm larvae fed with *I+N* on2nd day ($p<0.05$), 4th day, 6th day ($p<0.01$), 8th day, 10th day and 12th day ($p<0.01$). A significant change was observed in the length of P.m larvae fed with *I+D, C+N* and *C+D*, on 2nd day($p<0.01$), and all other days ($p<0.001$), and Pm larvae fed with *D +N* on all days ($p<0.001$)

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the length of P.m larvae fed with *I+D* and *C+N* on 2nd day ($p<0.05$). A significant change was observed in the length of Pm larvae fed with *C+D* on 2nd day and 4th day ($p<0.05$) 6th day, 8th day, ($p<0.01$), 10th day and 12th day ($p<0.001$). A significant change was observed in the length of Pm larvae fed with *D +N* on 2nd day 4th day, 8th day, ($p<0.001$) 10th day, and on 12th day ($p<0.001$).

A significant increase was observed in the length of Pm larvae fed with *C+N* and *C+D*, on 4th day ($p<0.05$) and 12th day ($p<0.001$). A significant change was observed in the length of Pm larvae fed with *D +N* on 4th day 6th day, 8th day, ($p<0.05$) and 10th day ($p<0.01$) when compared with that of P.m larvae fed with *I+N*

A significant increase was observed in the length of P.m larvae fed with *C+D* on 10th day (p<0.05) and 12th day (p<0.001) and also on P.m larvae fed with *D +N* on 8th day, 10th day and 12th day (p<0.01) when compared with that of *I+D*.

A significant increase was observed in the length of P.m larvae fed with *C+D* on 12th day (p<0.001). A significant increase was observed in the length of P.m larvae fed with *D +N* on 8th day (p<0.05) 10th day (p<0.05) and 12th day (p<0.001) when compared with that of *C+N*.

Exp. Day	2	4	6	8	10	12
C	1.184±0.04	1.332±0.05	2.044±0.05	3.285±0.07	3.568±0.18	4.091±0.06
I+C	1.207±0.05	1.430±0.05 *	2.147±0.06 *	3.506±0.11 ***	3.765±0.13 **	4.201±0.09 **
I+N	1.254±0.06 *	1.471±0.09 **	2.189±0.09 **	3.535±0.10 ***	3.891±0.17 ***	4.211±0.07 ***
I+D	1.287± 0.07**@	1.519±0.09 ***	2.222±0.11 ***	3.520±0.12 ***	3.853±0.12 ***	4.285±0.08 ***
C+N	1.300± 0.06**@	1.518±0.04 ***	2.227±0.10 ***	3.549±0.15 ***	3.905±0.15 ***	4.353±0.13 ***@@@$$$
C+D	1.281± 0.06**@	1.556±0.10 ***@	2.293±0.09 ***@@	3.640±0.08 ***@@	4.030±0.13 *** @@@£	4.528±0.04 ***@@@$$$£££ ΨΨΨ
D+N	1.314±0.07 ***@@	1.586±0.12 ***@@$	2.322±0.12 ***@@$	3.680±0.04 ***@@$££Ψ	4.138±0.09 ***@@@$$ £££ΨΨ	4.577±0.04 ***@@@$$$£££ ΨΨΨ

Table 5.3. Length of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)
Values are mean ± SD of 4-5 separate experiments; n = 10 in each group.
ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to C
@@@p<0.001, @@p<0.01, @p<0.05, when compared to I+C
$$$p<0.001, $$p<0.01, $p<0.05 when compared to I+N
£££p<0.001, ££p<0.01, £ p<0.05 when compared to I+D
ΨΨΨp<0.001, ΨΨp<0.01, Ψp<0.05, when compared to C+N

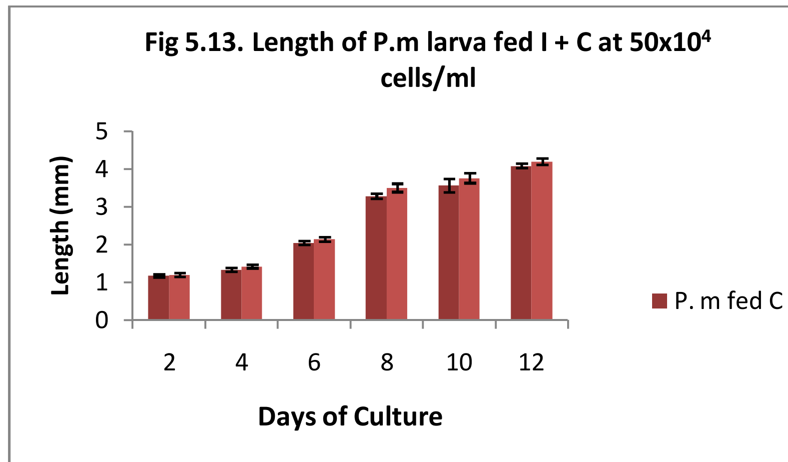


Fig 5.13. Length of P.m larva fed I + C at 50x10^4 cells/ml
Length (mm)
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C

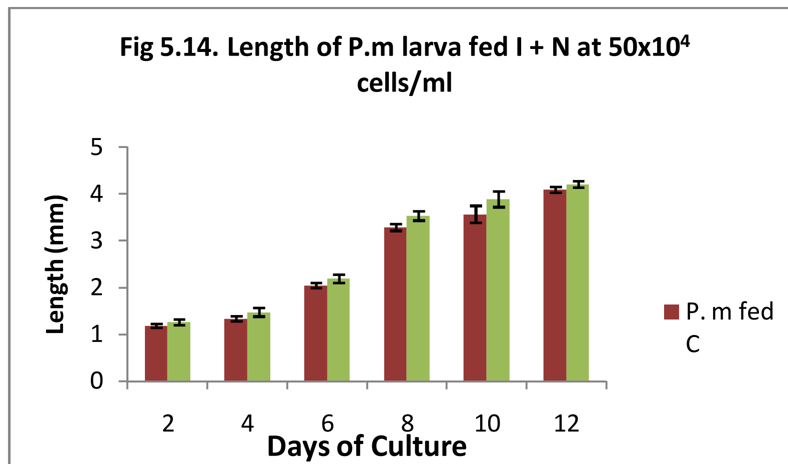


Fig 5.14. Length of P.m larva fed I + N at 50x10^4 cells/ml
Length (mm)
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C

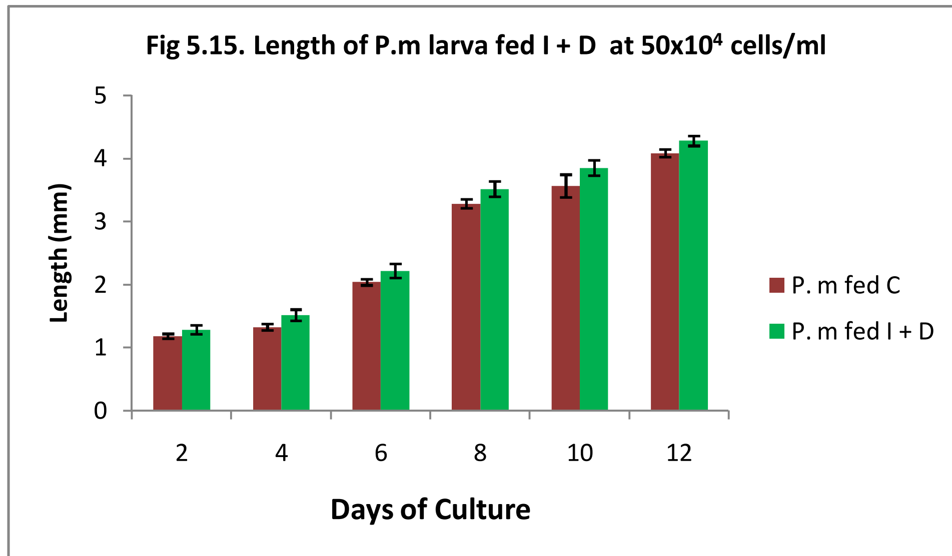


Fig 5.15. Length of P.m larva fed I + D at 50x10^4 cells/ml
Length (mm)
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + D

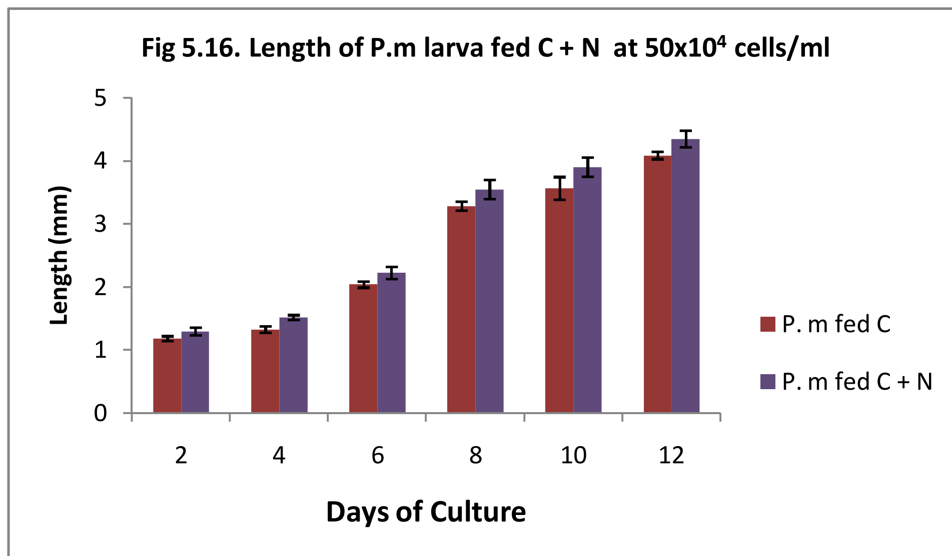


Fig 5.16. Length of P.m larva fed C + N at 50x10^4 cells/ml
Length (mm)
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + N

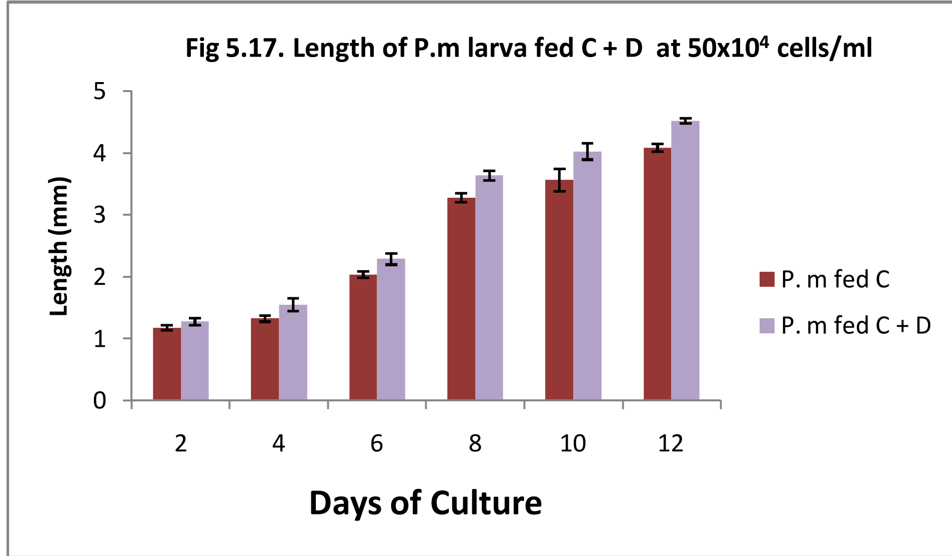


Fig 5.17. Length of P.m larva fed C + D at 50x10⁴ cells/ml
Length (mm)
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + D

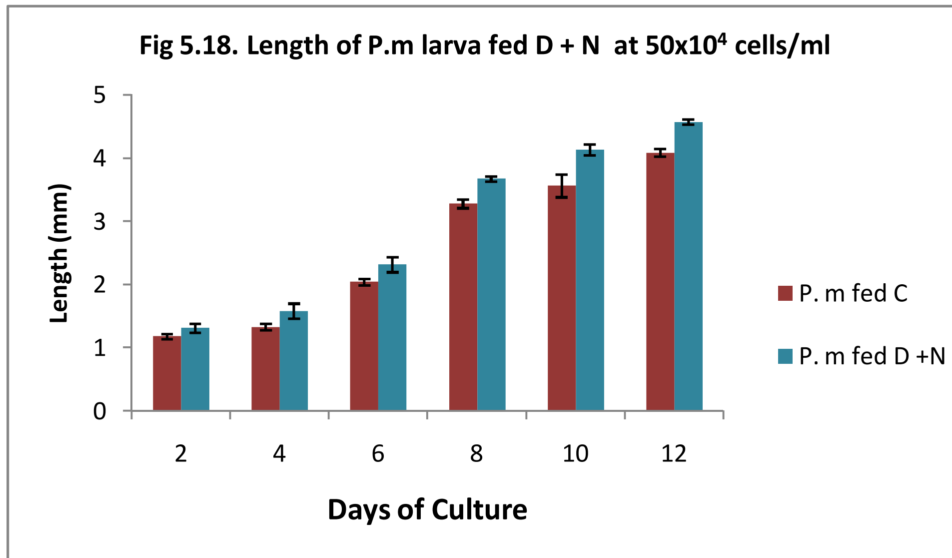


Fig 5.18. Length of P.m larva fed D + N at 50x10⁴ cells/ml
Length (mm)
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.2. Survival rate of *P. monodon* larvae

5.2.1. Effect of mixed-algal diet ($10x10^4$ cells/ ml) on survival rate of *P. monodon* larvae

Table 5.4 summarizes about survival rate of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $10x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to P.m larvae fed with *C* throughout the 12 days of study, a significant increase was observed in the survival rate of P.m larvae fed with *I+C* on 6th day ($p<0.05$) only. A significant change was observed in the survival rate of P.m larvae fed with *I+N* on 6th day ($p<0.001$), 8th day, 10th day and 12th day ($p<0.01$). A significant change was observed in the survival rate of P.m larvae fed with *I+D,* on 6th day, 8th day and 10th day ($p<0.001$). A significant change was observed in the survival rate of P.m larvae fed with *C+N* on 6th day ($p<0.001$), 8th day ($p<0.05$) and 10th day ($p<0.01$). A significant change was observed in the survival rate of P.m larvae fed *C+D*, on 2nd day, ($p<0.01$), 6th day, 8th day($p<0.001$), and 12th day ($p<0.05$), and with *D +N* on all days ($p<0.001$)

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the survival rate of P.m larvae fed with *I+N* on 6th day and 8th day ($p<0.05$) A significant change was observed in the survival rate of P.m larvae fed with *I+D* on 4th day, 6th day and 8th day ($p<0.05$). A significant change was observed in the survival rate of P.m larvae fed with *C+N* on 6th day only, and with *C+D* on 2nd day and 4th day and 6th day, ($p<0.01$) A significant change was observed in the survival rate of P.m larvae fed with *D +N* on 2nd day 4th day ($p<0.05$), 6th day and 8th day, ($p<0.001$).

A significant increase was observed only in the survival rate of P.m larvae fed with *D +N* on 2nd day, 4th day, 6th day and 8th day, ($p<0.05$) when compared with that of Pm larvae fed with *I+N*

A significant increase was observed in the survival rate of P.m larvae fed with *C+D* on 10th day ($p<0.001$) and with *D +N* on 2nd day ($p<0.05$) when compared with that of *I+D*.

A significant increase was observed in the survival rate of P.m larvae fed with *D +N* on 2nd day ($p<0.05$) and 6th day ($p<0.001$) when compared with that of *C+N*.

Exp. Day	2	4	6	8	10	12
C	90.04± 3.2	84.05±3.3	77.42±2.768	71.15±4.40	63.66± 3.41	60.25±3.92
I+C	89.77±3.02	86.15 ±3.58	80.09±2.55 *	73.43±4.07	66.62± 3.12	62.24±4.88
I+N	91.77±3.17	86.37 ±4.16	82.79±2.75 ***@	77.49±3.49 ***	70.56±3.9 **	66.77±3.23 **
I+D	91.71±1.14	90.65±3.3 ** @$	83.80±2.90 ***@	78.34±2.96 ***	72.24±3.89 ***@	65.0±2.81 *
C+N	92.54±2.26	87.92±2.8	83.54±2.86 ***@	75.04±2.41 *	69.93±2.9 **	66.45±3.36 **
C+D	94.41±3.59 **@@£	87.08 ±4.58	85.18±3.49 ***@@	78.89±2.76 ***@@	68.30±4.23 *	64.91±2.05 *£
D+N	95.49± 1.69*** @@@$£Ψ	90.79 ±2.33 **@$	86.92±3.43 ***@@@$	81.91±4.03 ***@@@ $ΨΨΨ	69.47±4.9 **	66.11± 3.95**

Table 5.4. Survival rate of *P. monodon* larvae at cell conc. ($10x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group.
ANOVA followed by Students-Newman-Keuls Test.
***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to C
@@@$p<0.001$, @@$p<0.01$, @$p<0.05$, when compared to I+C
$$p<0.05$ when compared to I+N
£$p<0.05$ when compared to I+D
ΨΨΨ$p<0.001$, Ψ$p<0.05$, when compared to C+N

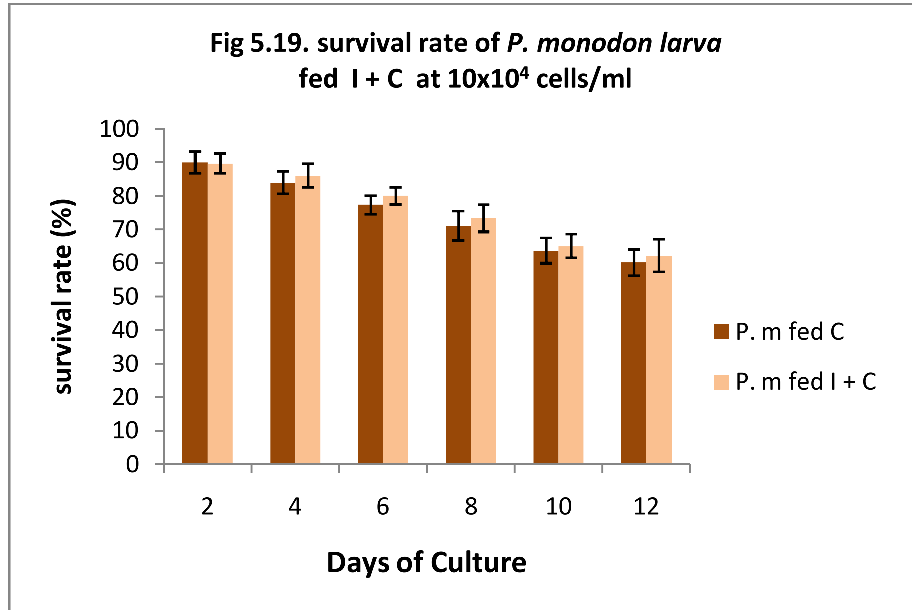


Fig 5.19. survival rate of P. monodon larva
fed I + C at 10x10⁴ cells/ml
survival rate (%)
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + C

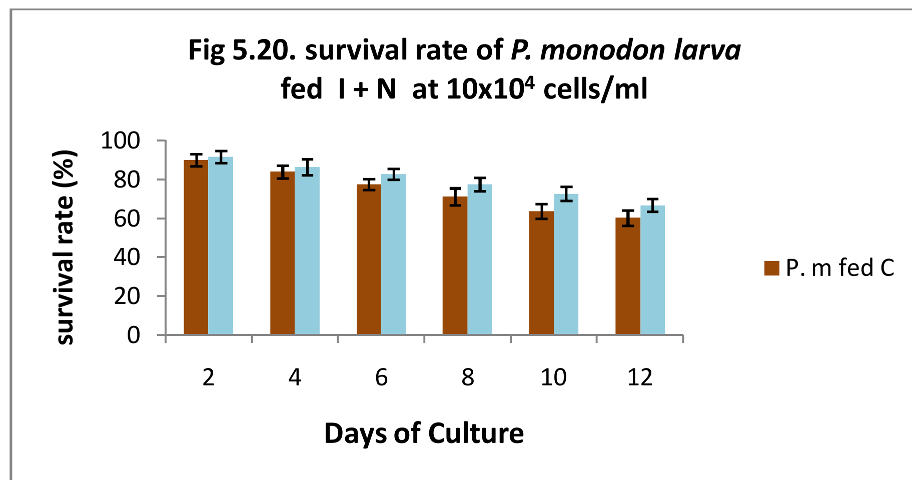


Fig 5.20. survival rate of P. monodon larva
fed I + N at 10x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C

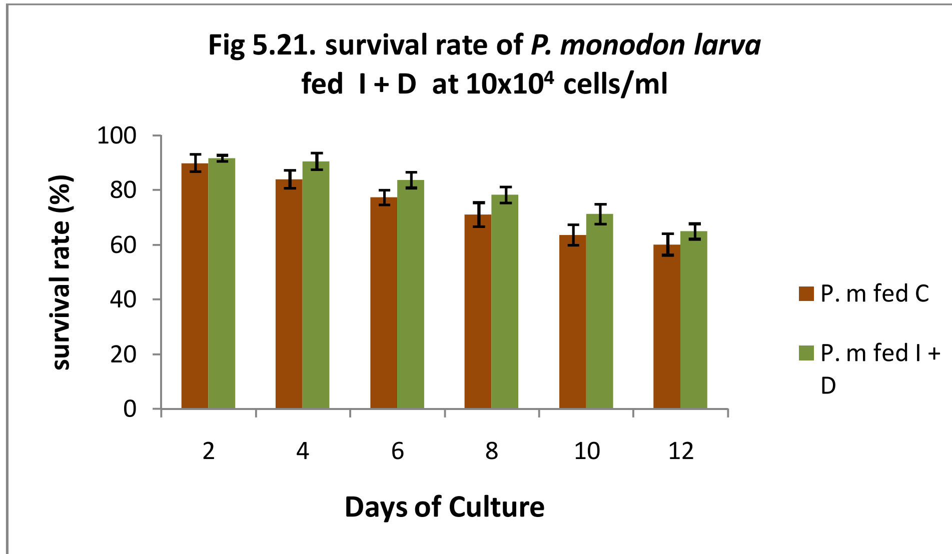


Fig 5.21. survival rate of P. monodon larva
fed I + D at 10x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + D

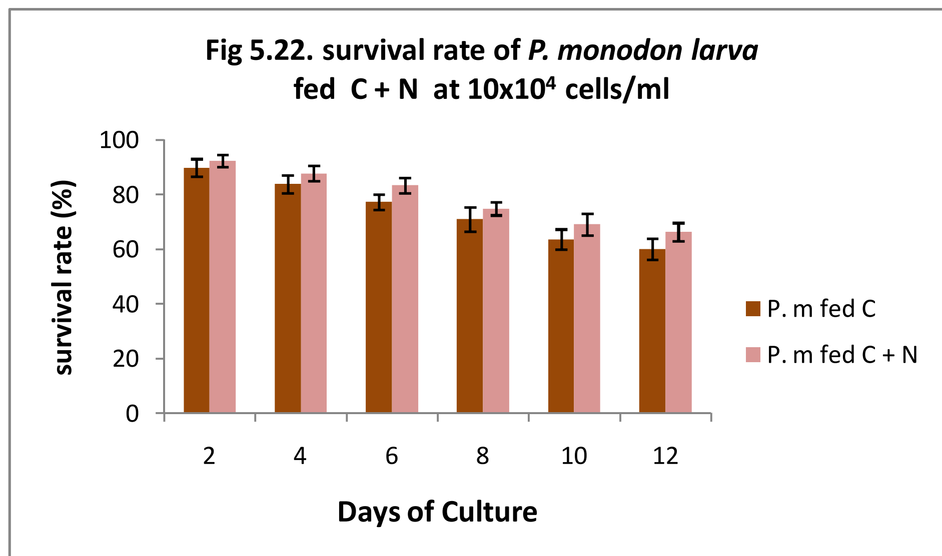


Fig 5.22. survival rate of P. monodon larva
fed C + N at 10x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + N

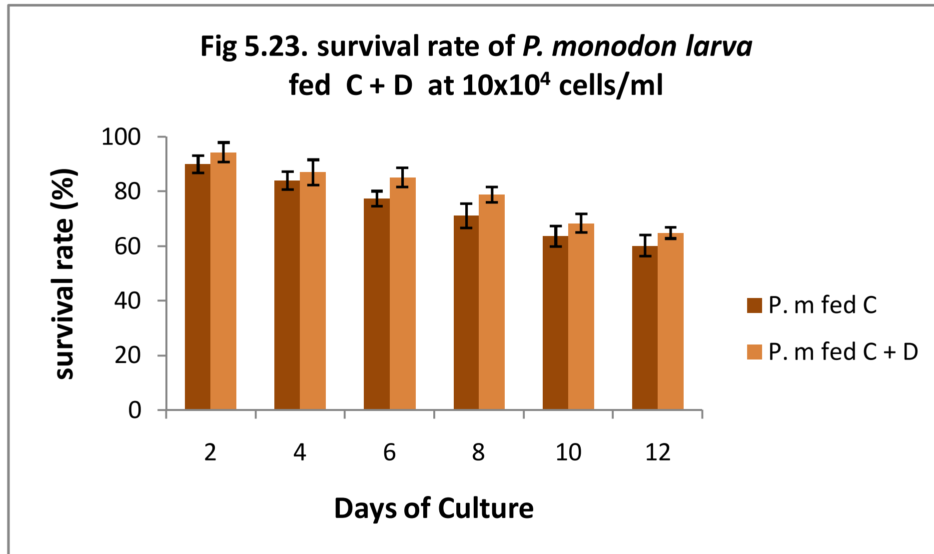


Fig 5.23. survival rate of *P. monodon larva* fed C + D at 10x10^4 cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + D

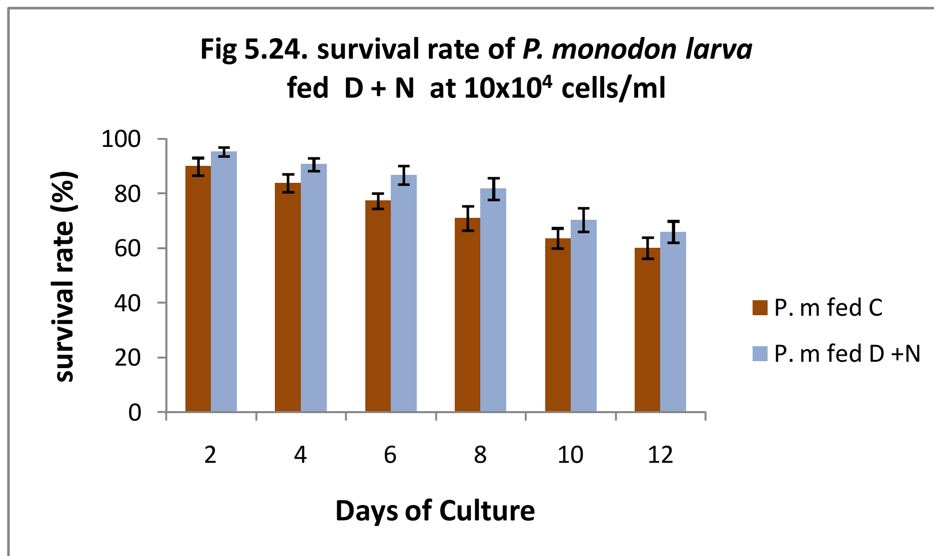


Fig 5.24. survival rate of *P. monodon larva* fed D + N at 10x10^4 cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.2.2. Effect of mixed-algal diet ($25x10^4$ cells/ ml) on survival rate of *P. monodon* larvae

Table 5.5 summarizes about survival rate of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $25x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to P.m larvae fed with *C* throughout the 12 days of study, a significant increase was observed in the survival rate of P.m larvae fed with *I+C* on 8th day, 12th day ($p<0.01$), 10th day ($p<0.001$), and P.m larvae fed with *I+N* on 6th day, 8th day, and 12th day ($p<0.01$). A significant increase was observed in the survival rate of P.m larvae fed with *I+D* on 4th day, 6th day, 8th day, ($p<0.001$), 12th day ($p<0.01$). A significant increase was observed in the survival rate of P.m larvae fed with *C+N* on 6th day, 8th day, 10th day ($p<0.01$), and on 12th day ($p<0.05$). A significant increase was observed in the survival rate of P.m larvae fed with *C+D*, on 2nd day 6th day ($p<0.001$), 8th day, 10th day ($p<0.01$) and on 12th day ($p<0.05$). A significant increase was observed in the survival rate of P.m larvae fed with *D +N* on all days ($p<0.001$)

When compared to P.m larvae fed with *I+C*, a significant increase in survival rate was observed in the survival rate of P.m larvae fed with *I+D* on 4th day and *C+D* on 6th day ($p<0.05$), and P.m larvae fed with *D +N* on 6th day ($p<0.001$), and 10th day($p<0.01$).

A significant increase was observed in the survival rate of P.m larvae fed only with *D +N* on 6th day and 10th day, ($p<0.05$) when compared with that of P.m larvae fed with *I+N*

A significant increase was observed in the survival rate on 4th day of P.m larvae fed with *C+N* ($p<0.01$) and *C+D* ($p<0.001$) when compared to P.m larvae fed with that of *I+D*.

When compared to P.m larvae fed with that of *C+N* a significant increase was observed in the survival rate of P.m larvae fed with *D +N* on 6th day ($p<0.05$). A significant increase was observed in the survival rate of Pm larvae fed with *D +N* on 4th day ($p<0.05$) when compared with that of *C+D*.

Exp. Day	2	4	6	8	10	12
C	90.64±2.61	84.71±2.8	77.84±3.24	77.84±3.24	64.23±4.89	61.530±4.39
I+C	92.95±2.67	87.1±3.84	80.460±2.72	80.460±2.72 **	66.45±3.36	68.210±2.01 **
I+N	92.970±2.59	88.530±3.09	83.19±3.46 **	83.19±3.46 **	67.88±3.79	67.880±3.79 **
I+D	91.310±2.65	91.710±3.69 ***@	84.140±2.71 ***	84.140±2.71 ***	68.860±4.56	65.01±2.81 *
C+N	92.46±3.64	86.32±3.39 ££	83.58±3.47 **	83.58±3.47 **	70.96±3.34 **	66.45±3.36 *
C+D	95.33± 2.48***	85.03±4.62 £££	87.65±3.89 ***@	87.65±3.89 **	70.70± 4.2**	65.96± 4.28 *
D+N	94.06±2.75 *	89.89±2.5 *λ	85.54±3.73 ***@@@Ψ	85.54± 3.73$ **	73.06± 3.23*** @@$	67.88±3.79 **

Table 5.5. Survival rate of *P. monodon* larvae at cell conc. ($25x10^4$ cells/ ml)
Values are mean ± SD of 4-5 separate experiments; n = 10 in each group.
ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to C
@@@$p<0.001$, @@$p<0.01$, @$p<0.05$, when compared to I+C
$$p<0.05 when compared to I+N
£££$p<0.001$, ££$p<0.01$, when compared to I+D
Ψ$p<0.05$, when compared to C+N
λ$p<0.05$ when compared to C+D

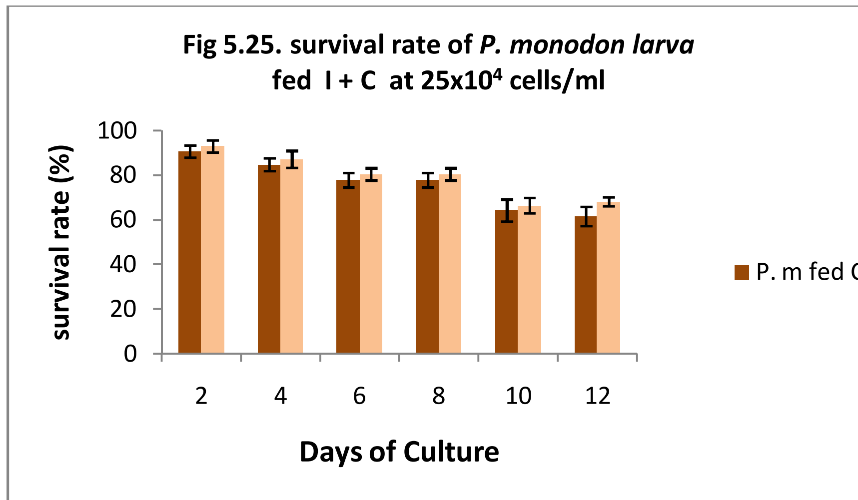


Fig 5.25. survival rate of P. monodon larva
fed I + C at 25x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C

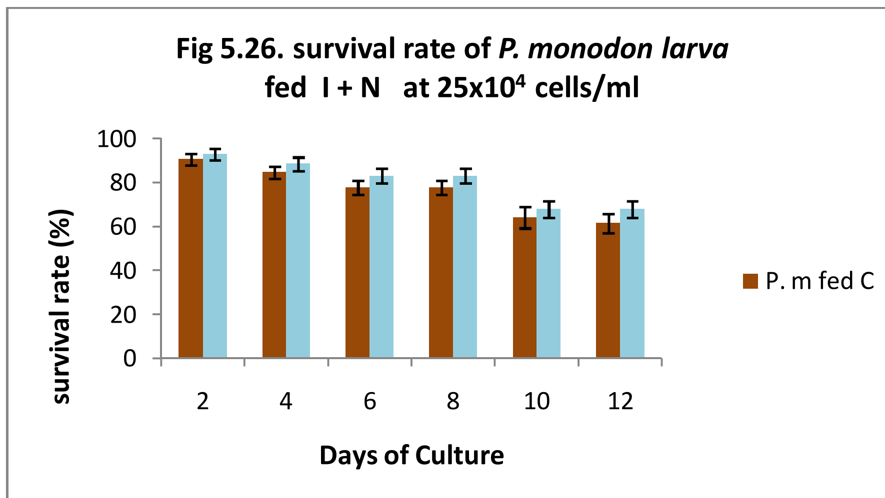


Fig 5.26. survival rate of P. monodon larva
fed I + N at 25x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C

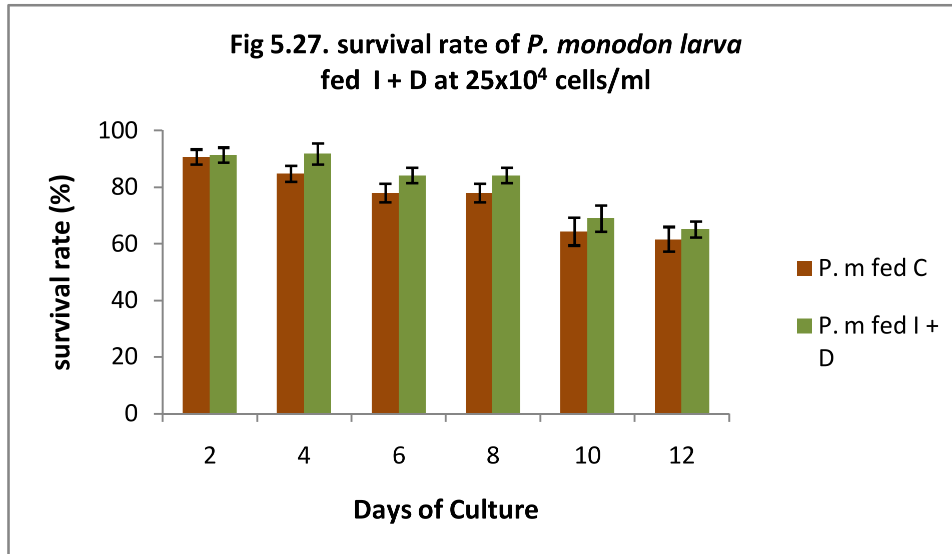


Fig 5.27. survival rate of P. monodon larva fed I + D at 25x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + D

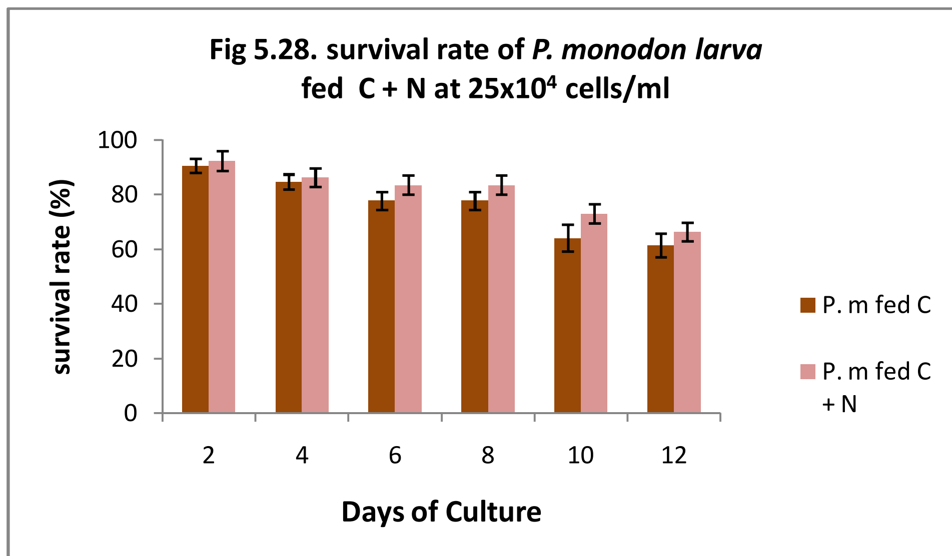


Fig 5.28. survival rate of P. monodon larva fed C + N at 25x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + N

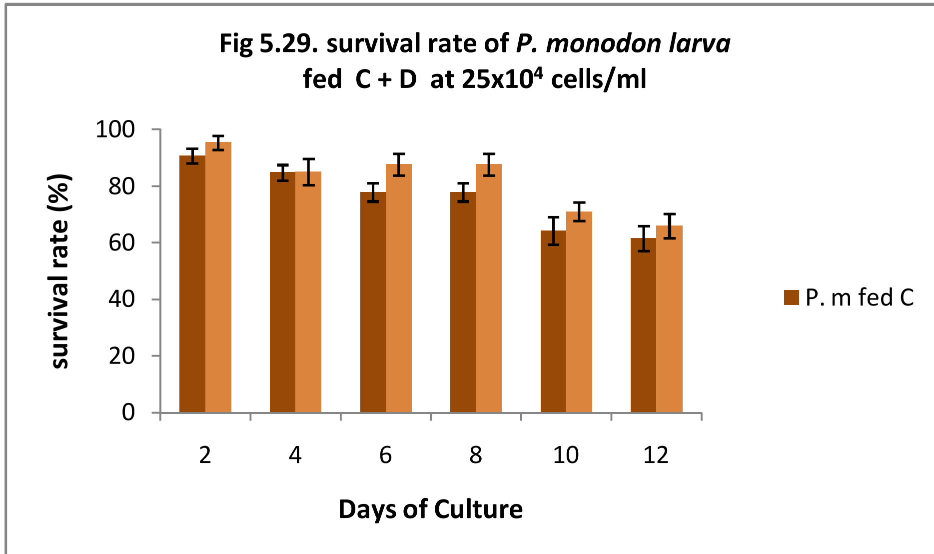


Fig 5.29. survival rate of P. monodon larva
fed C + D at 25x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C

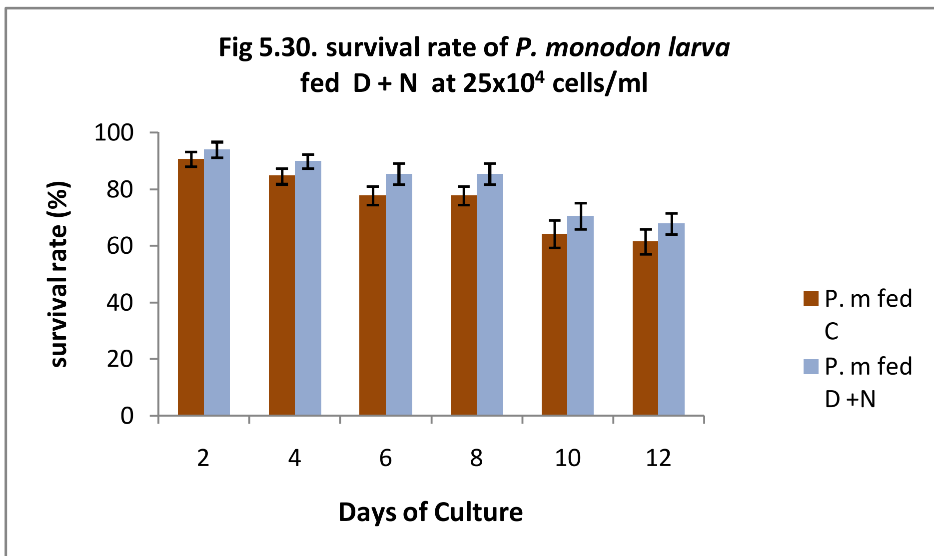


Fig 5.30. survival rate of P. monodon larva
fed D + N at 25x10⁴ cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.2.3. Effect of mixed-algal diet ($50x10^4$ cells/ ml) on survival rate of *P. monodon* larvae

Table 5.6 summarizes about survival rate of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $50x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to P.m larvae fed with *C* throughout the 12 days of study, a significant increase was observed in the survival rate of P.m larvae fed with *I+C* only on 10th day ($p<0.05$). A significant increase was observed in the survival rate of P.m larvae fed with *I+N* on 2nd day 4th day, 6th day ($p<0.05$), and with *I+D* on 2nd day ($p<0.05$), 6th day and 10th day ($p<0.001$), A significant increase was observed in the survival rate of P.m larvae fed with *C+N* on 4th day($p<0.05$), 6th day ($p<0.01$) and with *C+D*, on 2nd day 4th day, 10th day ($p<0.05$), and on 12th day ($p<0.001$) and). A significant increase was observed in the survival rate of P.m larvae fed with *D +N* on all days ($p<0.001$).

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the survival rate of P.m larvae fed with *I+N* and *C+N* on 4th day ($p<0.01$) and 6th day ($p<0.05$). A significant increase was observed in the survival rate of P.m larvae fed with *I+D* on 6th day ($p<0.001$), and with *C+D* on 4th day ($p<0.01$) and 12th day ($p<0.05$). A significant increase was observed in the survival rate of P.m larvae fed with *D +N* on 2nd day ($p<0.05$), 4th day ($p<0.001$), 6th day ($p<0.05$), 8th day ($p<0.01$) and 12th day ($p<0.05$).

A significant increase was observed in the survival rate of P.m larvae fed with *C+N* and *C+D*, on 4th day ($p<0.05$) and 12th day ($p<0.001$). A significant increase was observed in the survival rate of P.m larvae fed

with *D +N* on 4th day 6th day, 8th day, ($p<0.05$) and 10th day ($p<0.01$) when compared with that of P.m larvae fed with *I+N*

A significant increase was observed in the survival rate of P.m larvae fed with *C+D* on 10th day ($p<0.05$) and 12th day ($p<0.001$) and also with *D +N* on 8th day, 10th day and 12th day ($p<0.01$) when compared with that of *I+D.*

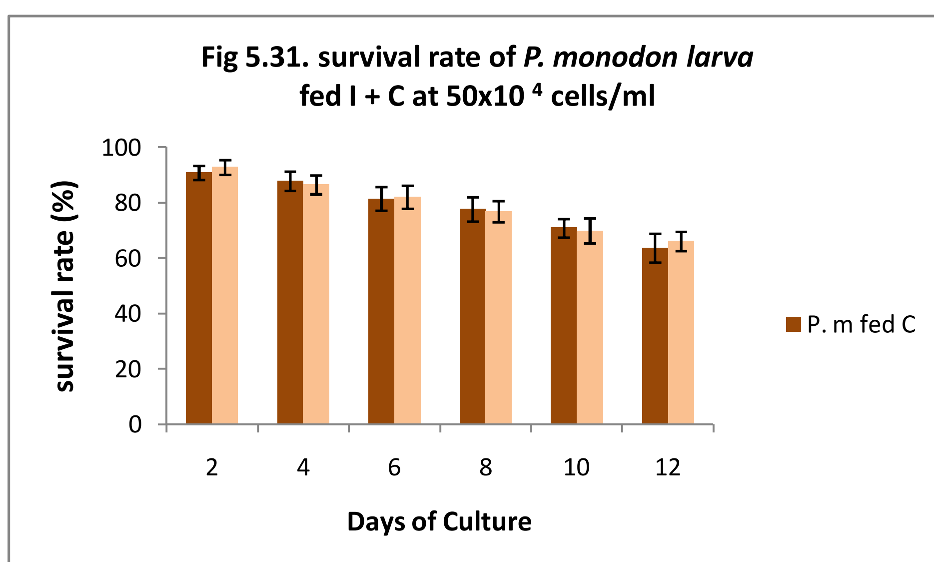
A significant increase was observed in the survival rate of P.m larvae fed with *C+D* on 8th day ($p<0.001$). A significant increase was observed in the survival rate of P.m larvae fed with *D +N* on 8th day ($p<0.05$) 10th day ($p<0.05$) and 12th day ($p<0.001$) when compared to P.m larvae fed with that of *C+N.*

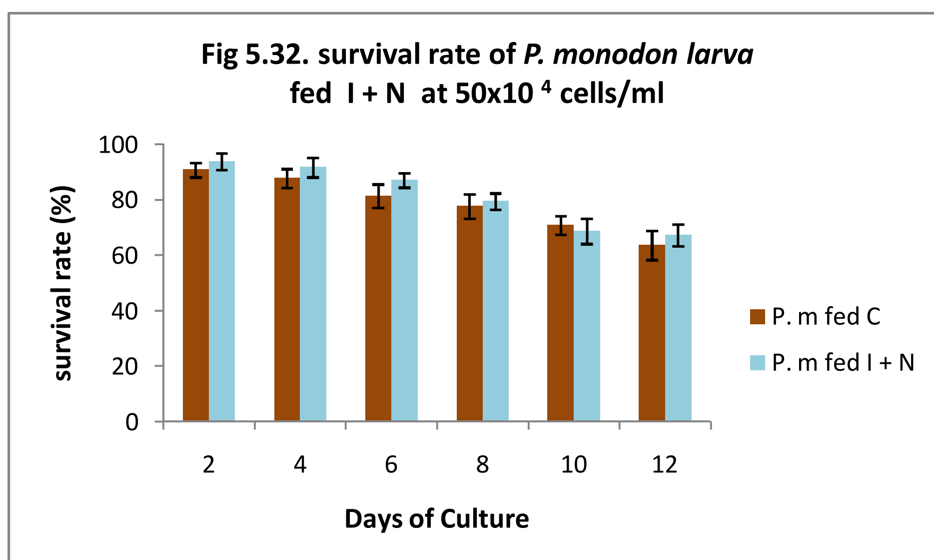
Exp. Day	2	4	6	8	10	12
C	90.94±2.61	87.88± 3.46	81.57±4.23	77.85±4.39	66.18±2.56	63.75±5.21
I+C	92.95±2.67	86.62± 3.44	82.12±4.1	76.9±3.78	68.61±2.76	66.12±3.46
I+N	93.92±2.98 *	91.86± 3.55 *@@	87.23±2.59 *@	79.64± 2.89	70.95±3.35 *	67.44±3.99
I+D	93.23±2.55 *	89.95± 2.76	90.25±4.64 ***@@@	81.91±4.03 @	69.87±3.7	68.86±4.09
C+N	96.09±2.13	92.12± 2.71 *@@	88.23±4.39 **@££	75.04±2.41 $£££	68.64±1.59	68.91±4.32
C+D	93.62±2.91 *	93.56± 2.21 *@@	87.0±4.72	78.89±2.76	71.70±4.01 **	72.43± 3.93 ***@
D+N	92.46±3.32 ***@	91.34± 3.89 ** @@@	83.69±3.71 @	83.21±3.57 **@@ ΨΨΨλ	71.83±3.45 **	71.70±3.79 ***@

Table 5.6. Survival rate of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to C
$^{@@@}p<0.001$, $^{@@}p<0.01$, $^{@}p<0.05$, when compared to I+C
$^{\$}p<0.05$ when compared to I+N
££££$p<0.001$, ££$p<0.01$, when compared to I+D
ΨΨΨ$p<0.001$, when compared to C+N
λ$p<0.05$ when compared to C+D





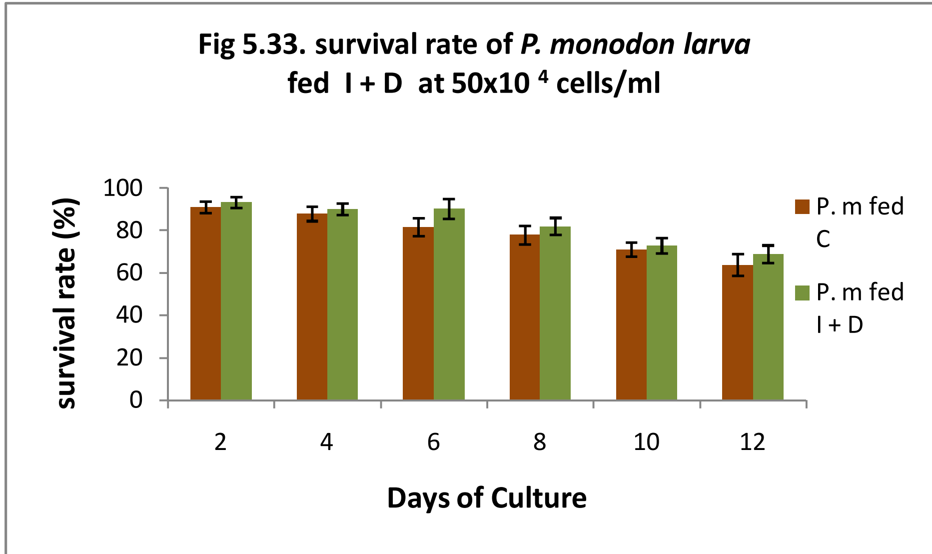


Fig 5.33. survival rate of P. monodon larva
fed I + D at 50x10 4 cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + D

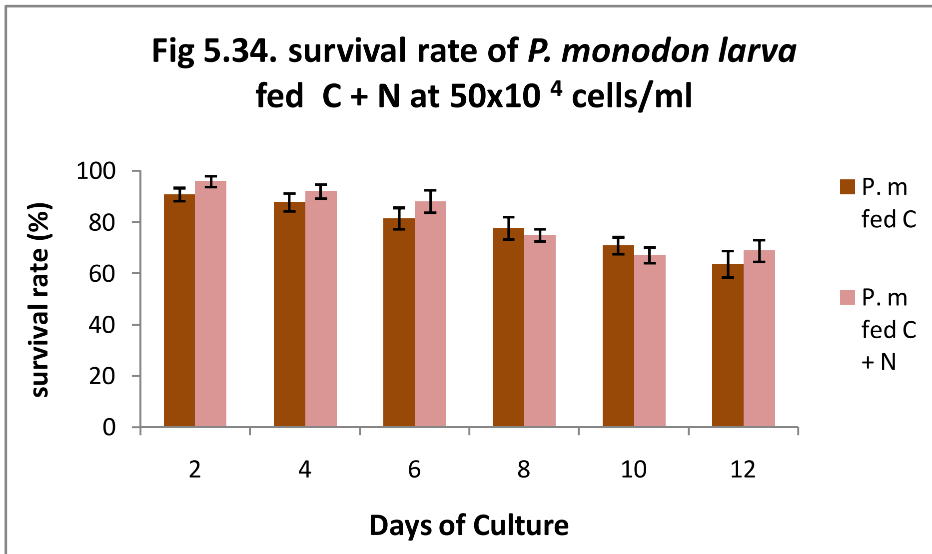


Fig 5.34. survival rate of P. monodon larva
fed C + N at 50x10 4 cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + N

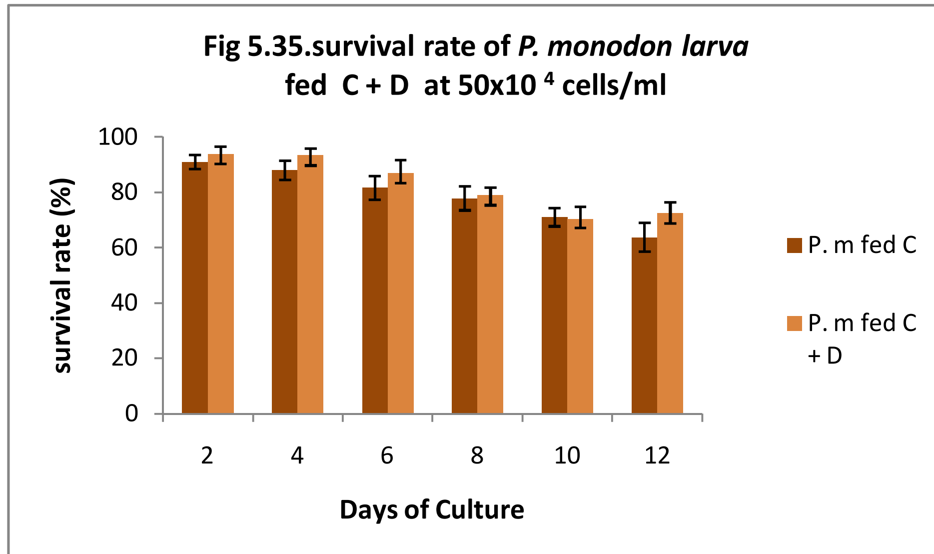


Fig 5.35.survival rate of *P. monodon larva* fed C + D at 50x10 4 cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + D

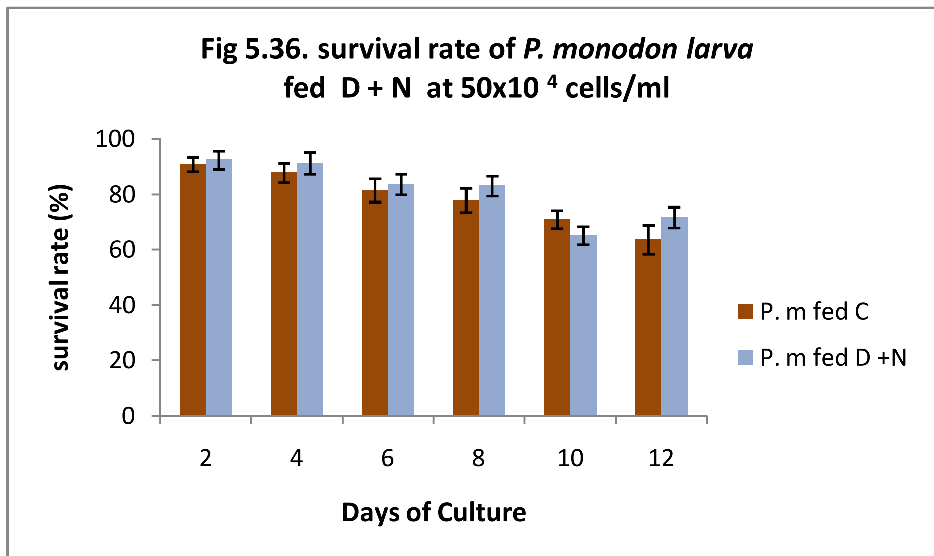


Fig 5.36. survival rate of *P. monodon larva* fed D + N at 50x10 4 cells/ml
survival rate (%)
100
80
60
40
20
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

From the above results it could be inferred that the P.m larvae fed with the micro algae at cell concentration $50x10^4$ showed better survival and increment in growth. Hence for the algal cell concentration of $50x\ 10^4$ further analysis of developmental index of the larvae indicating the stages of development, ingestion rate of algal cells by the P.m larvae, weight of the larvae, percentage increment in length and weight of the larvae specific growth rate and the water quality parameters were discussed.

5.3. Developmental index of *P. monodon* larvae

5.3.1. Effect of mixed-algal diet ($50x10^4$ cells/ ml) on developmental index of *P. monodon* larvae

Table 5.7 summarizes about developmental index of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $25x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to *C* fed Pm larvae throughout the 12 days of study, a significant increase was observed in the developmental index of Pm larvae fed with *I+C* only on 10th day ($p<0.05$). A significant increase was observed in the developmental index of Pm larvae fed with *I+N* on 2nd day 4th day, 6th day ($p<0.05$), and a significant increase was observed in the developmental index of Pm larvae fed with *I+D* on 2nd day ($p<0.05$), 6th day and 10th day ($p<0.001$) with *C+N* on 4th day ($p<0.05$), 6th day ($p<0.01$). A significant increase was observed in the developmental index of Pm larvae fed with *C+D*, on 2nd day 4th day, 10th day ($p<0.05$), and on 12th day ($p<0.001$) and). A significant increase was observed in the developmental index of Pm larvae fed with *D +N* on all days ($p<0.001$).

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the developmental index of Pm larvae fed with *I+N* and *C+N* on 4th day ($p<0.01$) and 6th day ($p<0.05$). A significant increase was observed in the developmental index of P.m larvae fed with *I+D* on 6th day ($p<0.001$), and with *C+D* on 4th day ($p<0.01$) and 12th day ($p<0.05$). A significant increase was observed in the developmental index of P.m larvae fed with *D +N* on 2nd day ($p<0.05$), 4th day ($p<0.001$), 6th day ($p<0.05$), 8th day ($p<0.01$) and 12th day ($p<0.05$).

A significant increase was observed in the developmental index of P.m larvae fed with *C+N* and *C+D*, on 4th day ($p<0.05$) and 12th day ($p<0.001$). A significant increase was observed in the developmental index of Pm larvae fed with *D +N* on 4th day 6th day, 8th day, ($p<0.05$) and 10th day ($p<0.01$) when compared with that of Pm larvae fed with *I+N*

A significant increase was observed in the developmental index of P.m larvae fed with *C+D* on 10th day ($p<0.05$) and 12th day ($p<0.001$) and also with *D +N* on 8th day, 10th day and 12th day ($p<0.01$) when compared with that of *I+D*.

A significant increase was observed in the developmental index of P.m larvae fed with *C+D* on 8th day ($p<0.001$). A significant increase was observed in the developmental index of Pm larvae fed with *D +N* on 8th day ($p<0.05$) 10th day ($p<0.05$) and 12th day ($p<0.001$) when compared with that of *C+N*.

Exp. Day	2	4	6	8	10	12
C	0.96±0.11	1.86±0.11	2.94±0.08	3.96±0.13	4.84±0.05	5.96±0.11
I + C	1.0±0.15	1.84±0.11	2.92±0.08	3.98±0.08	4.96±0.11	6.12±0.08
I + N	1.08±0.08	1.98±0.13	3.02±0.08	4.02±0.08	5.04±0.13 *	6.16±0.11
I + D	1.3±0.15 **@@$	2.02±0.08	3.06±0.08	4.08±0.08	5.12±0.08 *	6.06±0.08
C + N	1.28±0.08 **@@$	2.1±0.07 *@	3.14±0.05 *@	4.08±0.13	5.12±0.16 **	6.22±0.13 *
C + D	1.36±0.11 ***@@$	2.16±0.15 **@@	3.16±0.11 *@	4.3±0.15 **@@$	5.26±0.16 ***@@@$	6.38±0.13 ***@@@$££
D +N	1.32±0.14 **@@$	2.24±0.20 **@@$	3.26±0.18 ***@@$£	4.28±0.20 **@$	5.36±0.11 ***@@@$$£ λ	6.38±0.19 ***@$££

Table 5.7. Developmental index of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 5 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to C
@@@p<0.001, @@p<0.01, @p<0.05, when compared to I+C
$$ p<0.01, $p<0.05 when compared to I+N
££p<0.01, £p<0.05 when compared to I+D
λ p<0.05 when compared to C+D.

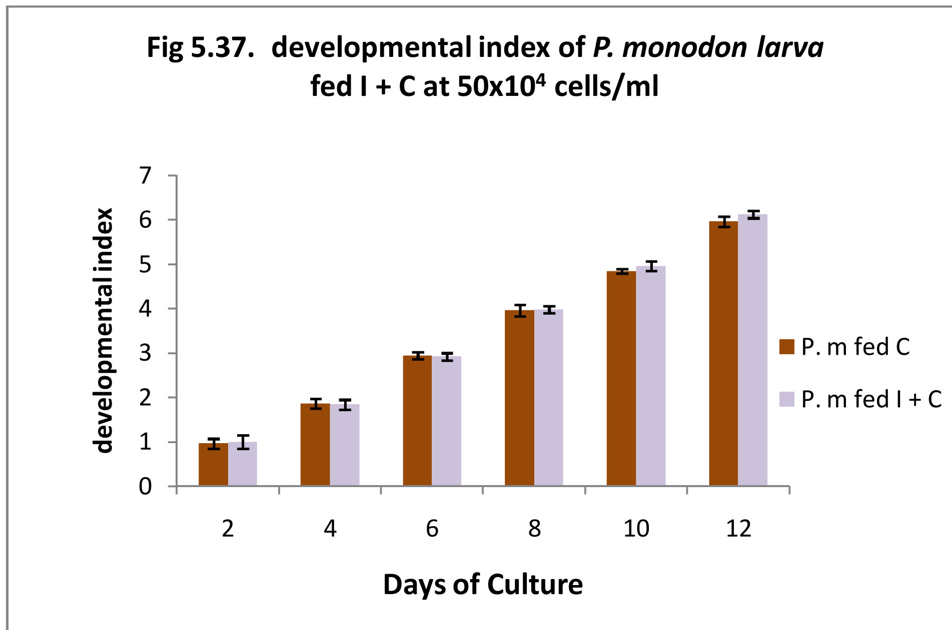


Fig 5.37. developmental index of P. monodon larva
fed I + C at 50x10⁴ cells/ml
developmental index
0
1
2
3
4
5
6
7
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + C

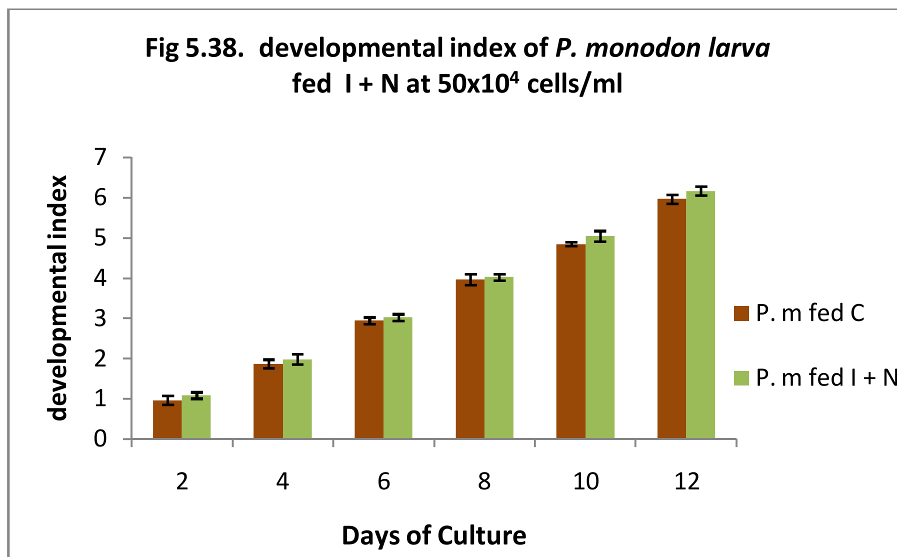


Fig 5.38. developmental index of P. monodon larva
fed I + N at 50x10⁴ cells/ml
developmental index
0
1
2
3
4
5
6
7
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + N

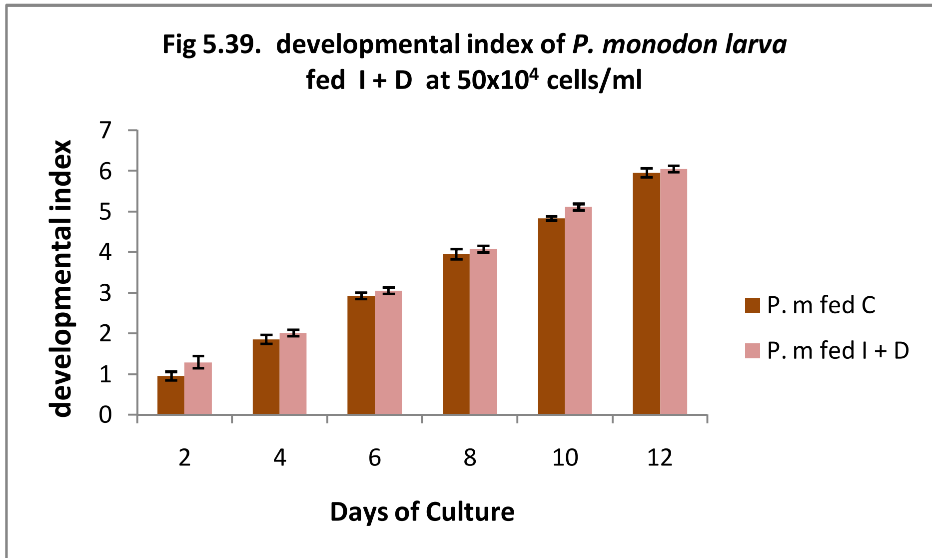


Fig 5.39. developmental index of P. monodon larva
fed I + D at 50x10⁴ cells/ml
developmental index
0
1
2
3
4
5
6
7
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + D

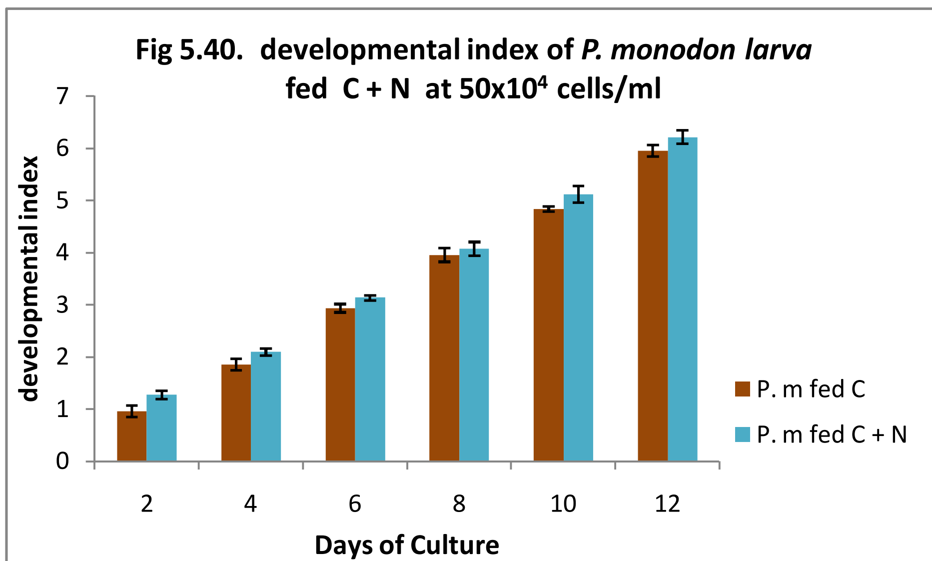


Fig 5.40. developmental index of P. monodon larva
fed C + N at 50x10⁴ cells/ml
developmental index
0
1
2
3
4
5
6
7
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + N

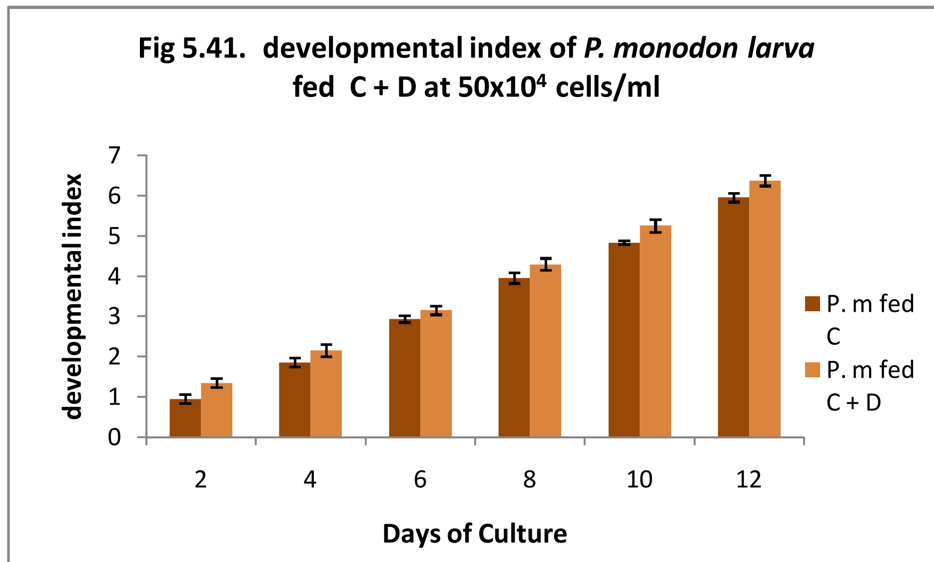


Fig 5.41. developmental index of P. monodon larva
fed C + D at 50x10⁴ cells/ml
developmental index
7
6
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + D

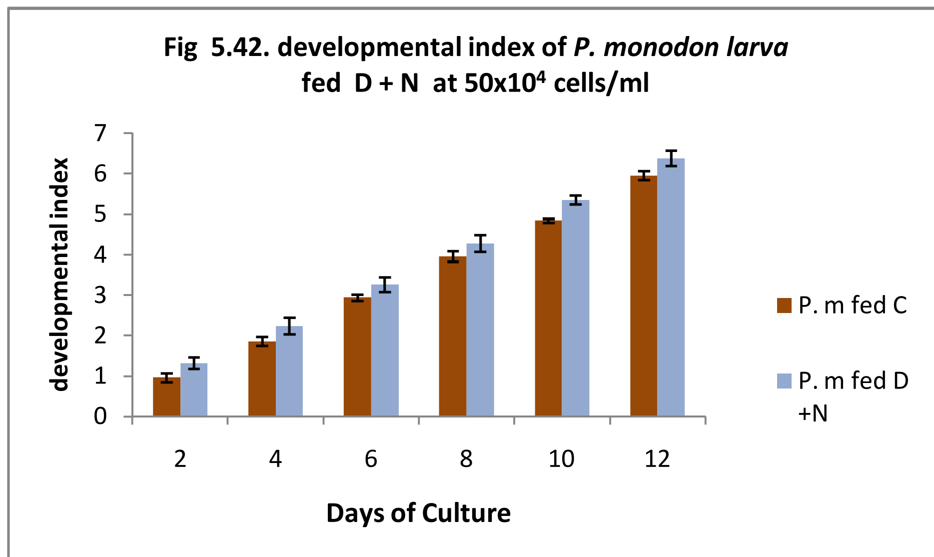


Fig 5.42. developmental index of P. monodon larva
fed D + N at 50x10⁴ cells/ml
developmental index
7
6
5
4
3
2
1
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.4. Ingestion rate of *P. monodon* larvae

5.4.1. Effect of mixed-algal diet ($50x10^4$ cells/ ml) on ingestion rate of *P. monodon* larvae

Table 5.8 summarizes about ingestion rates of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $50x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to *C* fed P.m larvae throughout the 12 days of study, a significant increase was observed in the ingestion rate of Pm larvae fed with *I+C* only on 10th day ($p<0.05$). A significant increase was observed in the ingestion rate of P.m larvae fed with *I+N* on 2nd day 4th day, 6th day ($p<0.05$), and with *I+D* on 2nd day ($p<0.05$), 6th day and 10th day ($p<0.001$). A significant increase was observed in the ingestion rate of P.m larvae fed with *C+N* on 4th day ($p<0.05$), 6th day ($p<0.01$) and with *C+D*, on 2nd day 4th day, 10th day ($p<0.05$), and on 12th day ($p<0.001$). A significant increase was observed in the ingestion rate of P.m larvae fed and with *D +N* on all days ($p<0.001$).

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the ingestion rate of P.m larvae fed with *I+N* and *C+N* on 4th day($p<0.01$) and 6th day ($p<0.05$). A significant increase was observed in the ingestion rate of P.m larvae fed with *I+D* on 6th day ($p<0.001$), and with *C+D* on 4th day ($p<0.01$) and 12th day ($p<0.05$). A significant increase was observed in the ingestion rate of P.m larvae fed with *D +N* on 2nd day ($p<0.05$), 4th day ($p<0.001$), 6th day ($p<0.05$), 8th day ($p<0.01$) and 12th day ($p<0.05$).

A significant increase was observed in the ingestion rate of P.m larvae fed with *C+N* and *C+D*, on 4th day ($p<0.05$) and 12th day ($p<0.001$). A significant increase was observed in the ingestion rate of P.m larvae fed with *D +N* on 4th day 6th day, 8th day, ($p<0.05$) and 10th day ($p<0.01$) when compared with that of P.m larvae fed with *I+N*

A significant increase was observed in the ingestion rate of P.m larvae fed with *C+D* on 10th day ($p<0.05$) and 12th day ($p<0.001$) and also on P.m larvae fed with *D +N* on 8th day, 10th day and 12th day ($p<0.01$) when compared with that of *I+D.*

A significant increase was observed in the ingestion rate of P.m larvae fed with *C+D* on 8th day ($p<0.001$). A significant increase was observed in the ingestion rate of P.m larvae fed with *D +N* on 8th day ($p<0.05$) 10th day ($p<0.05$) and 12th day ($p<0.001$) when compared with that of *C+N.*

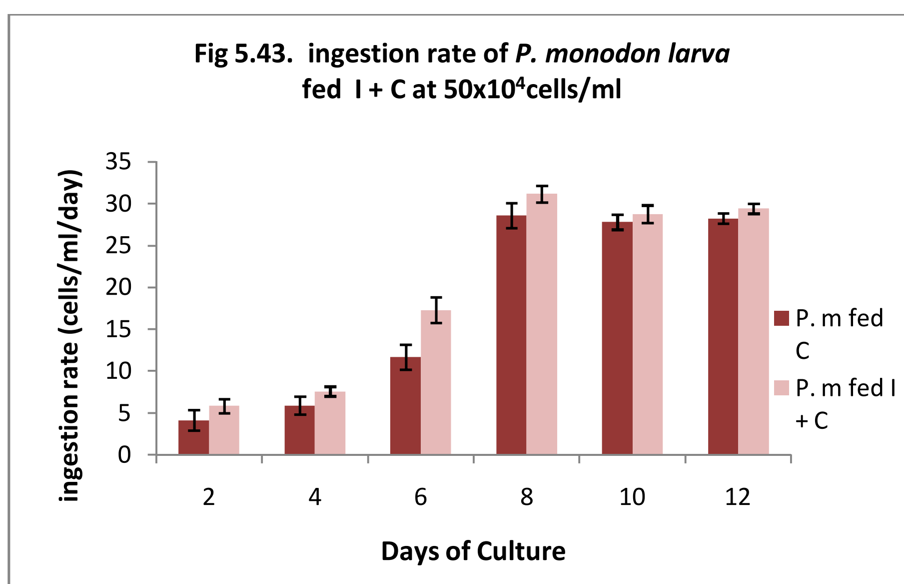
Exp. Day	2	4	6	8	10	12
C	4.14±1.21	5.91±1.05	11.66±1.49	28.60±1.49	27.8±0.90	28.22±0.60
I+C	5.85±0.84 ***	7.58±0.57 ***	17.29±1.53 ***	31.15±0.98 ***	28.74±1.06 *	29.4±0.60 ***
I+N	7.39±0.26 ***@@	9.88±0.39 ***@@@	18.89±1.19 ***	31.00±0.70 ***	30.67±0.54 ***@@@	30.48±0.48 ***@
I+D	6.38±0.55 ***	9.16±0.20 ***@@@	17.61±1.48 ***	31.96±0.49 ***	31.53±0.75 ***@@@	30.92±0.25 ***@@
C+ N	7.15±0.35 ***@	9.55±0.20 ***@@@	20.28±0.99 ***@@$$	33.79±0.26 ***@@@$$	32.97±0.46 ***@@@$$$	32.92±0.52 ***@@@$$$
C+ D	6.04±0.52 ***$Ψ	8.20±0.92 ***$$$ΨΨ	20.85±0.73 ***@@$$	33.98±0.40 ***@@@$$	34.27±0.42 ***@@@$$$ ΨΨ	33.79±1.11 ***@@@$$$
D +N	7.68±0.29 ***@@ λλ	10.17±0.43 ***@@@ λλλ	22.32±1.36 ***@@@$$$Ψ	34.46±0.49 ***@@@$$$	35.04±0.44 ***@@@$$$ ΨΨΨ	34.36±0.92 ***@@@$$$ ΨΨ

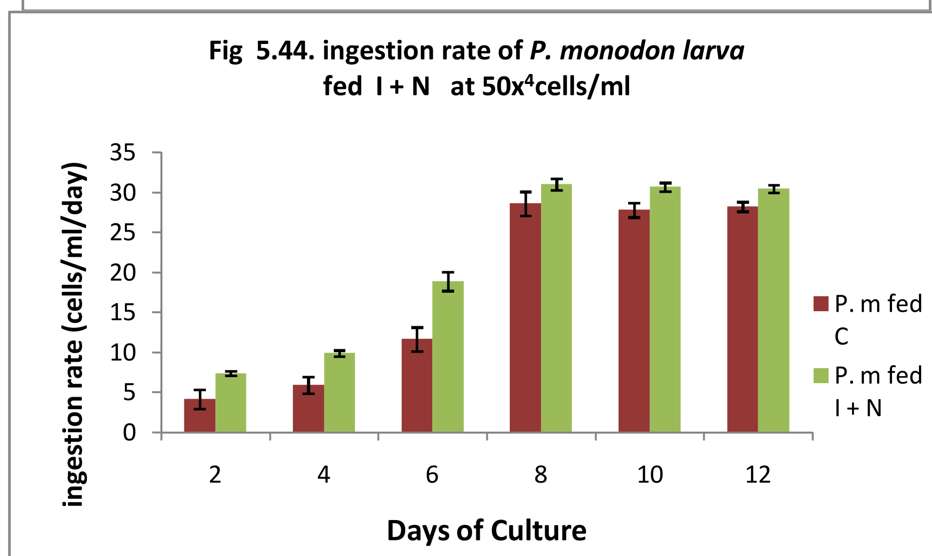
Table 5.8. Ingestion rate of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 5 in each group.

ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, *$p<0.05$ when compared to C
@@@$p<0.001$, @@$p<0.01$, @$p<0.05$, when compared to I+C
$$$$p<0.001$, $$$p<0.01$, $$p<0.05$ when compared to I+N
££££p<0.001$, ££$p<0.01$, £$p<0.05$ when compared to I+D
ΨΨΨ$p<0.001$, ΨΨ$p<0.01$, Ψ$p<0.05$, when compared to C+N
λλλ $p<0.001$, λλ$p<0.01$, when compared to C+D





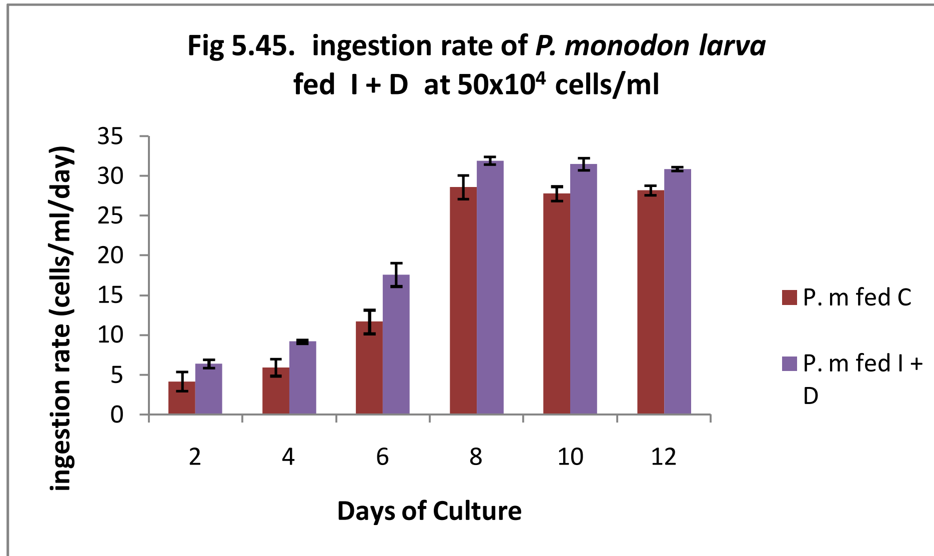


Fig 5.45. ingestion rate of P. monodon larva
fed I + D at 50x10⁴ cells/ml
ingestion rate (cells/ml/day)
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed I + D

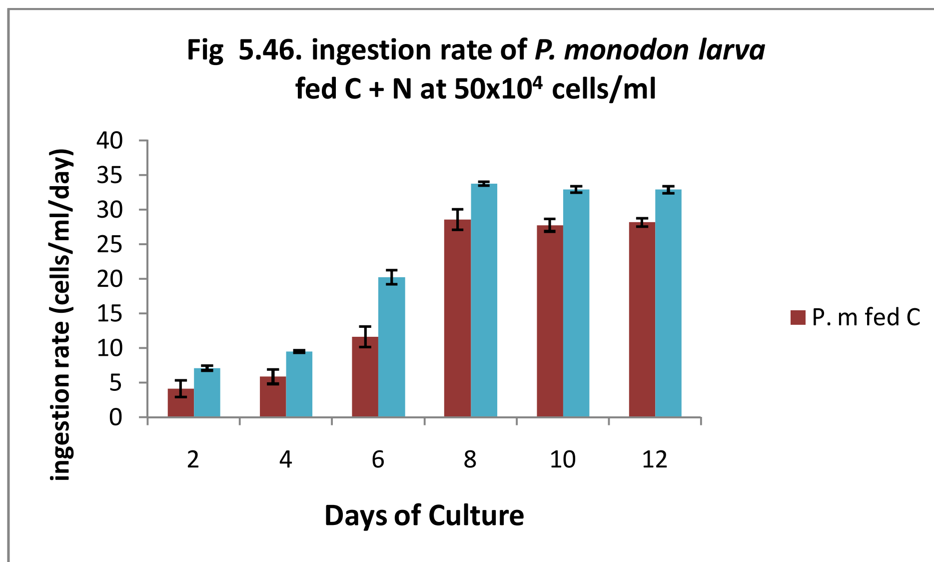


Fig 5.46. ingestion rate of P. monodon larva
fed C + N at 50x10⁴ cells/ml
ingestion rate (cells/ml/day)
40
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days of Culture
P. m fed C

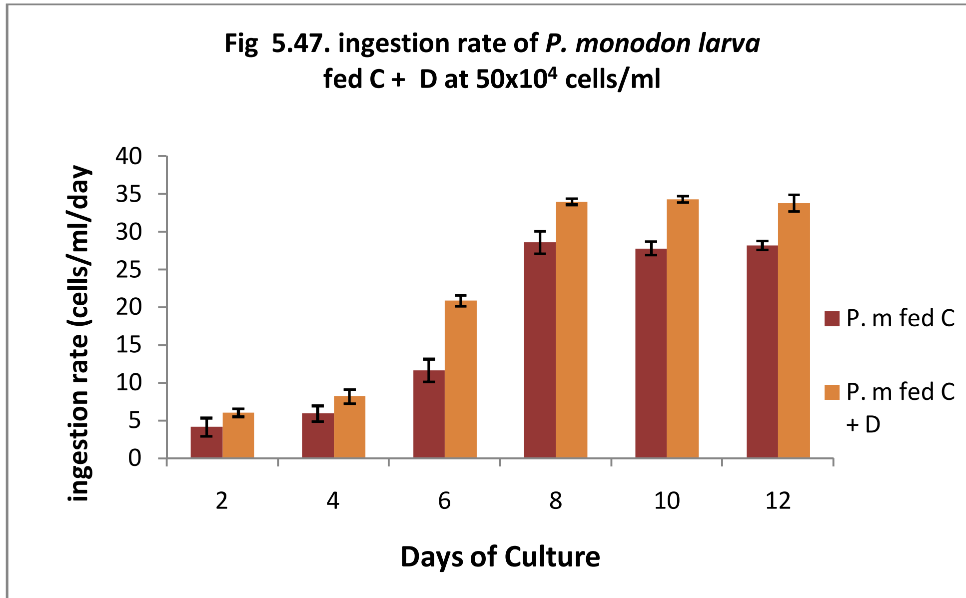


Fig 5.47. ingestion rate of *P. monodon larva* fed C + D at 50x10⁴ cells/ml
ingestion rate (cells/ml/day
40
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed C + D

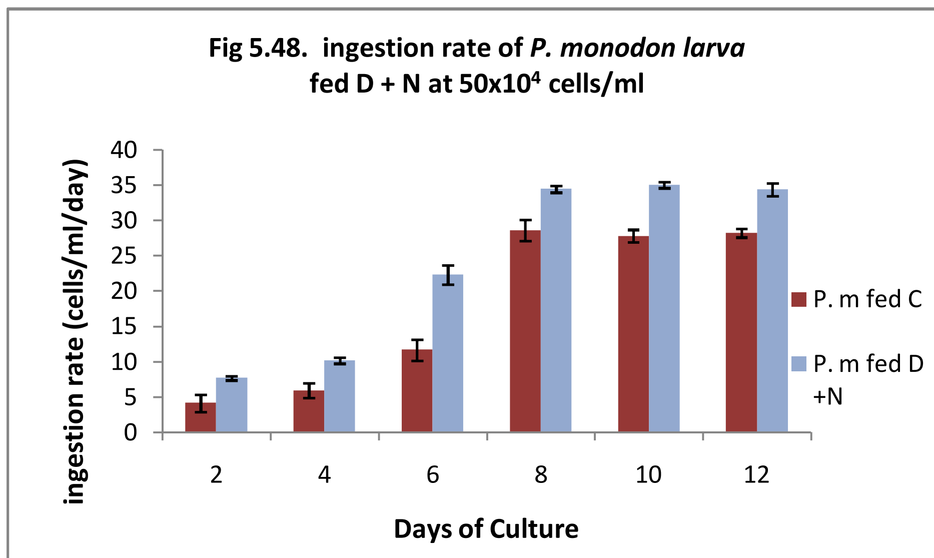


Fig 5.48. ingestion rate of *P. monodon larva* fed D + N at 50x10⁴ cells/ml
ingestion rate (cells/ml/day)
40
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.5. Weight gain of *P. monodon* larvae

5.5.1. Effect of mixed-algal diet ($50x10^4$ cells/ ml) on weight of *P. monodon* larvae

Table 5.9 summarizes about weight gain of the rearing experiments of Penaeus monodon larvae fed with C, I+C, I+N, I+D, C+N, C+D, D +N at the cell concentration of $50x10^4$ cells/ ml starting from first protozoea stage (PZ-1). No significant changes was observed with the Z3 stages of the larvae whereas a significant increase in the weight gain was observed in the of P.m larvae fed with D +N ($p<0.01$) and C+D ($p<0.05$), when compare to P.m larvae fed with that of C throughout the 12 days of study.

	Larval stages	
	Z3	**M3**
Algae		
C	20.688±1.746	63.506±1.529
I + C	21.684±0.9240	66.440±1.806
I + N	20.538±0.6095	65.886±2.552
I + D	20.612±1.121	67.364±2.956
C + N	20.842±0.6539	66.996±1.323
C + D	21.138±1.311	68.356±1.694*
D +N	21.166±1.308	69.452±3.243**

Table 5.9. Weight of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

**p<0.01, *p<0.05 when compared to Chaetoceros

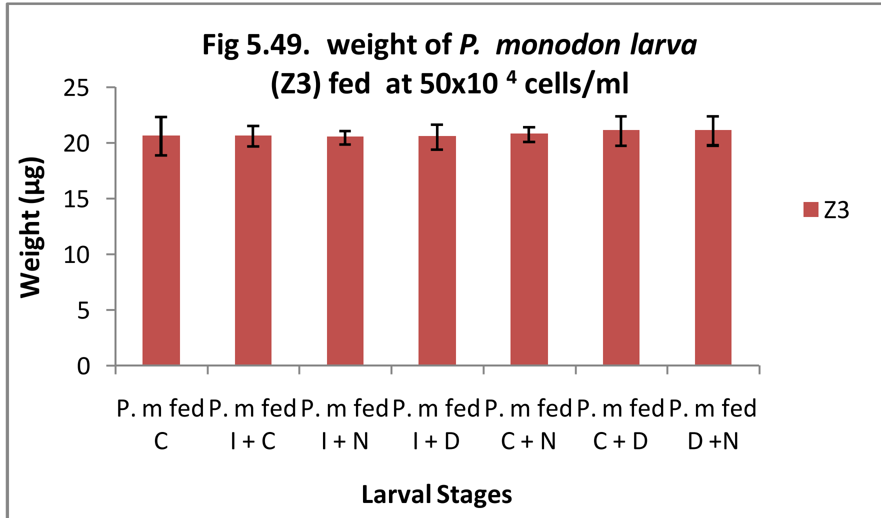


Fig 5.49. weight of P. monodon larva
(Z3) fed at 50x10 4 cells/ml
Weight (µg)
25
20
15
10
5
0
Z3
P. m fed C
P. m fed I + C
P. m fed I + N
P. m fed I + D
P. m fed C + N
P. m fed C + D
P. m fed D +N
Larval Stages

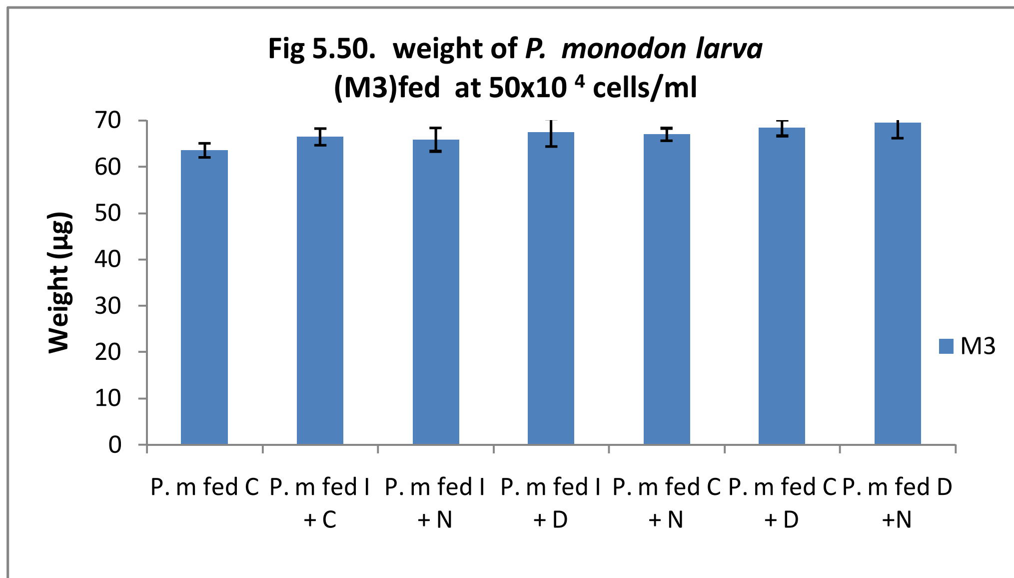


Fig 5.50. weight of P. monodon larva
(M3)fed at 50x10 4 cells/ml
Weight (µg)
70
60
50
40
30
20
10
0
M3
P. m fed C
P. m fed I + C
P. m fed I + N
P. m fed I + D
P. m fed C + N
P. m fed C + D
P. m fed D +N

5.6. Percentage increment on length of *P. monodon* larvae

5.6.1. Effect of mixed-algal diet ($50x10^4$ cells/ ml) on percentage increment on growth (length) of *P. monodon* larvae

Table 5.10 summarizes about percentage increment on growth (length) of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $25x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to P.m larvae fed with *C* throughout the 12 days of study, a significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *I+C* on 4th day, 6th day ($p<0.05$), 8th day($p<0.001$), 10th day, and 12th day ($p<0.01$). A significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *I+N* on 2nd day ($p<0.05$), 4th day, 6th day ($p<0.01$), 8th day, 10th day ($p<0.001$) and on 12th day ($p<0.01$). A significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *I+D, C+N* and *C+D*, on 2nd day($p<0.01$), and all other days ($p<0.001$), and with *D +N* on all days ($p<0.001$).

When compared to P.m larvae fed with *I+C*, a significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *I+D* and *C+N* on 2nd day ($p<0.05$). A significant increase was observed in the percentage increment on growth (length) of P.m larvae fed and with *C+D* on 2nd day and 4th day ($p<0.05$) 6th day, 8th day, 10th day ($p<0.01$), and on 12th day ($p<0.001$). A significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *D +N* on 2nd day 4th day, 6th day, 8th day, ($p<0.01$) 10th day, and 12th day ($p<0.001$).

A significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *C+N* and *C+D*, on 12th day ($p<0.001$) with *D +N* on 4th day 6th day, 8th day, ($p<0.05$) and 10th day ($p<0.01$) and on 12th day ($p<0.001$) when compared with that of P.m larvae fed with *I+N*.

A significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *C+D* on 10th day ($p<0.05$) and 12th day ($p<0.001$) and also with *D +N* on 8th day ($p<0.01$), 10th day and 12th day ($p<0.001$) when compared with that of *I+D*.

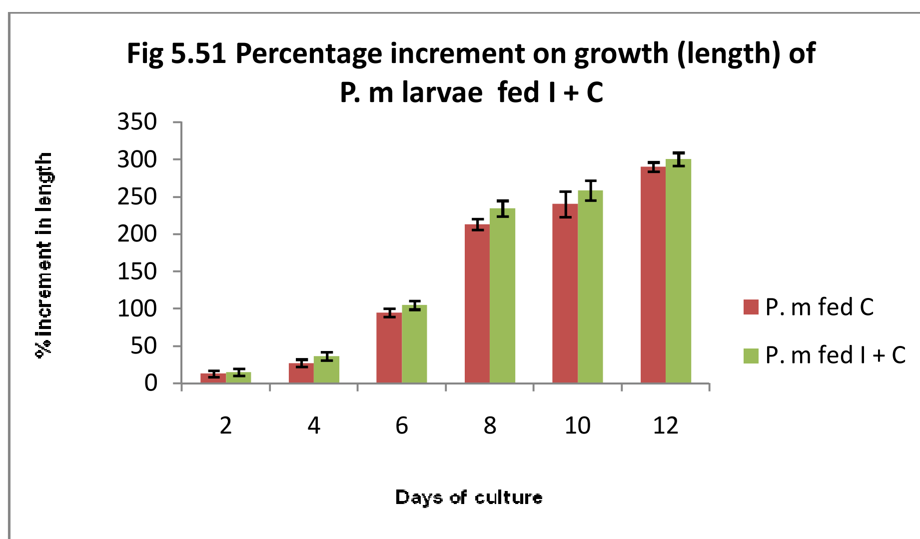
A significant increase was observed in the percentage increment on growth (length) of P.m larvae fed with *C+D* on 12th day ($p<0.001$). A significant increase was observed in the length of P.m larvae fed with *D +N* on 8th day ($p<0.05$) 10th day ($p<0.05$) and 12th day ($p<0.001$) when compared with that of *C+N*.

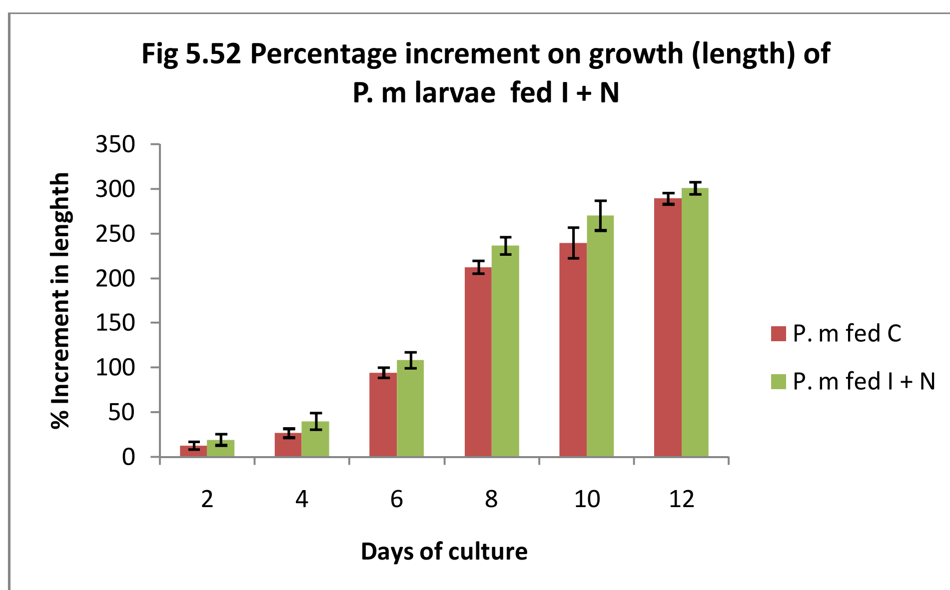
Exp. Day	2	4	6	8	10	12
C	12.762± 4.21	26.857± 5.01	94.667± 5.64	212.86± 7.07	239.81±17.33	289.62±6.4
I+C	14.952± 4.77	36.19± 5.66 *	104.48± 5.75 *	233.9± 10.61 ***	258.57±13.31 **	300.1± 8.8 **
I+N	19.429± 6.17 *	40.095± 9.32 **	108.48± 8.98 **	236.67± 9.94 ***	270.57±16.72 ***	301.05±6.8 **
I+D	22.571± 6.79 **@	44.667± 9.46 ***	111.62± 11.28 ***	235.24± 11.95 ***	266.95±12.03 ***	308.1±7.7 ***
C+N	23.81±6.38 **@	44.571± 4.13 ***	112.1± 9.65 ***	238.0± 14.46 ***	271.9± 15.18 ***	314.57±12.9 *** @@@$$$
C+D	22.0± 6.51 **@	48.19± 9.92 ***@	118.38± 8.91 ***@@	246.67± 8.31 ***@	283.81±13.17 *** @@£	331.24± 3.9 *** @@@$$$ £££ΨΨΨ
D+N	25.143± 7.08 ***@@	51.048± 11.68 ***@@$	121.14± 11.59 ***@@$	250.48± 4.28 *** @@$££Ψ	294.1± 8.79 *** @@@$$ £££ΨΨ	335.9± 4.44 *** @@@$$$ £££ΨΨΨ

Table 5.10. Percentage increment on growth (Length) of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to C
@@@p<0.001, @@p<0.01, @p<0.05, when compared to I+C
$$$p<0.001, $$p<0.01, $p<0.05 when compared to I+N
£££p<0.001, ££p<0.01, £p<0.05 when compared to I+D
ΨΨΨp<0.001, ΨΨp<0.01, Ψp<0.05, when compared to C+N





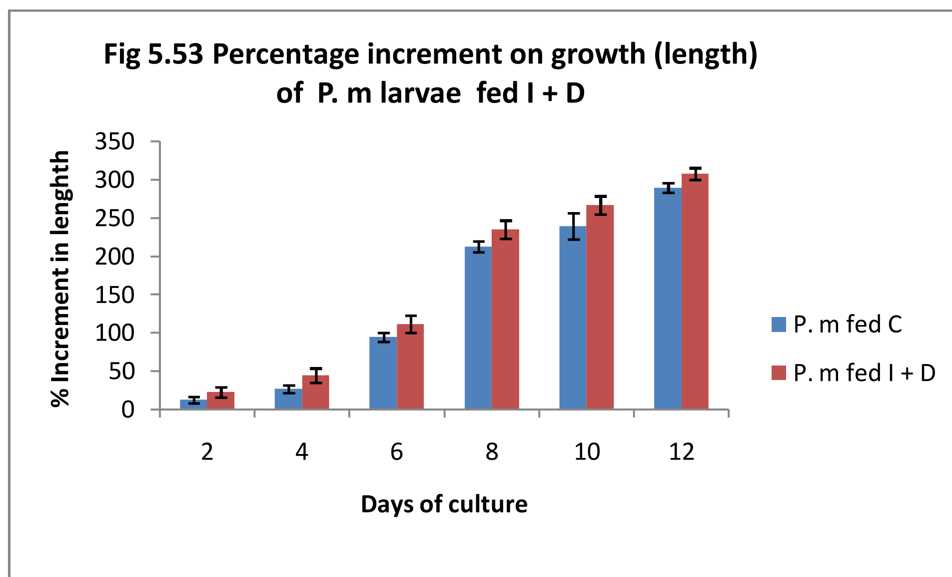


Fig 5.53 Percentage increment on growth (length) of P. m larvae fed I + D
% Increment in lenghth
350
300
250
200
150
100
50
0
2
4
6
8
10
12
Days of culture
P. m fed C
P. m fed I + D

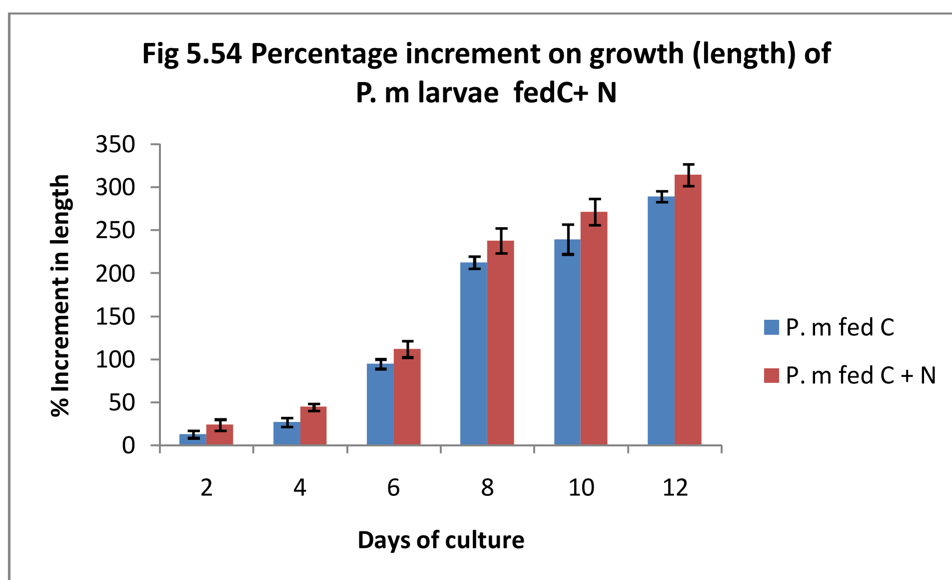


Fig 5.54 Percentage increment on growth (length) of P. m larvae fedC+ N
% Increment in length
350
300
250
200
150
100
50
0
2
4
6
8
10
12
Days of culture
P. m fed C
P. m fed C + N

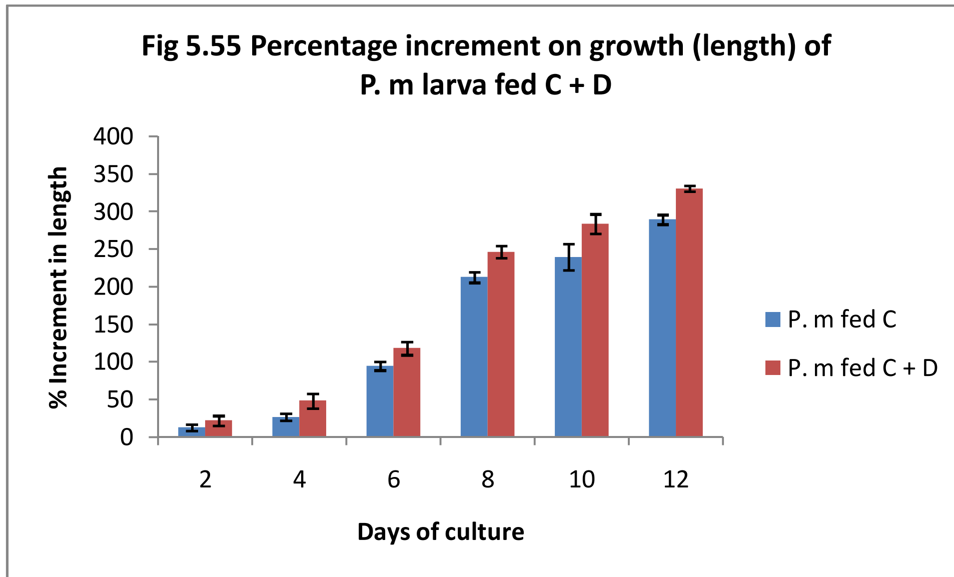


Fig 5.55 Percentage increment on growth (length) of
P. m larva fed C + D
% Increment in length
400
350
300
250
200
150
100
50
0
2
4
6
8
10
12
Days of culture
P. m fed C
P. m fed C + D

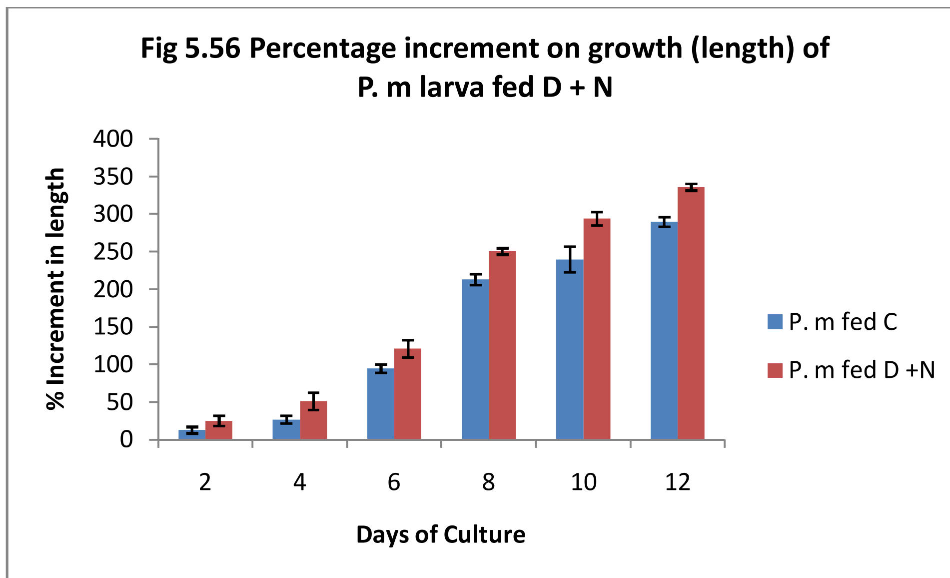


Fig 5.56 Percentage increment on growth (length) of
P. m larva fed D + N
% Increment in length
400
350
300
250
200
150
100
50
0
2
4
6
8
10
12
Days of Culture
P. m fed C
P. m fed D +N

5.7. Growth rate (length) of *P. monodon* larvae

5.7.1. Effect of mixed-algal diet ($50x10^4$ cells/ ml) growth rate (length) of *P. monodon* larvae

Table 5.11 summarizes about growth rate (length) of the rearing experiments of *Penaeus monodon* larvae fed with *C, I+C, I+N, I+D, C+N, C+D, D +N* at the cell concentration of $25x10^4$ cells/ ml starting from first protozoea stage (PZ-1). When compared to *C* fed P.m larvae throughout the 12 days of study, a significant increase was observed in the growth rate (length) of Pm larvae fed with *I+C* on 4th day ($p<0.01$), 6th day ($p<0.05$), 8th day($p<0.001$), 10th day, and 12th day ($p<0.01$). A significant increase was observed in the growth rate (length) of Pm larvae fed with *I+N* on 2nd day ($p<0.05$), 4th day, 6th day ($p<0.01$), 8th day, 10th day ($p<0.001$) and on 12th day ($p<0.01$). A significant increase was observed in the growth rate (length) of P.m larvae fed with *I+D, C+N* and *C+D*, on 2nd day($p<0.01$), and all other days ($p<0.001$), and with *D +N* on all days ($p<0.001$)

when compared to P.m larvae fed with *I+C*, a significant increase was observed in the growth rate (length) of P.m larvae fed with *I+D* and *C+N* on 2nd day ($p<0.05$), and with *C+D* on 2nd day and 4th day ($p<0.05$) 6th day, 8th day, 10th day ($p<0.01$), and on 12th day ($p<0.001$). A significant increase was observed in the growth rate (length) of Pm larvae fed with *D +N* on 2nd day 4th day, 6th day, 8th day, ($p<0.01$) 10th day, and 12th day ($p<0.001$).

A significant increase was observed in the growth rate (length) of P.m larvae fed with *C+N* and *C+D*, on 12th day ($p<0.001$) with *D +N* on 4th day 6th day, 8th day, ($p<0.05$) and 10th day ($p<0.01$) and on 12th day ($p<0.001$) when compared with that of P.m larvae fed with *I+N.*

A significant increase was observed in the growth rate (length) of P.m larvae fed with *C+D* on 10th day ($p<0.05$) and 12th day ($p<0.001$) and also with *D +N* on 8th day ($p<0.01$), 10th day and 12th day ($p<0.001$) when compared with that of *I+D*.

A significant increase was observed in the growth rate (length) of P.m larvae fed with *C+D* on 12th day ($p<0.001$). A significant increase was observed in the growth rate (length) of P.m larvae fed with *D +N* on 8th day ($p<0.05$) 10th day ($p<0.05$) and 12th day ($p<0.001$) when compared with that of *C+N*.

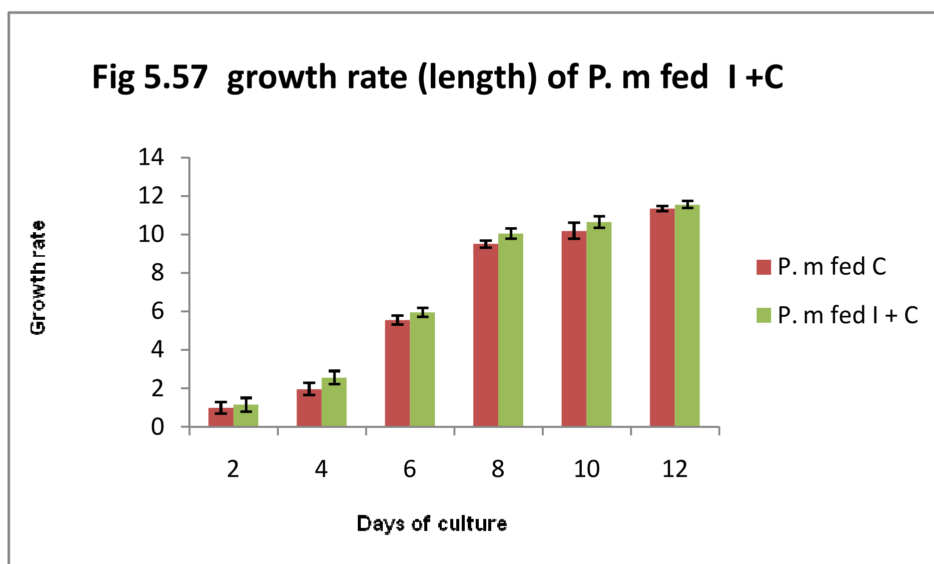
Exp. Day	**2**	**4**	**6**	**8**	**10**	**12**
C	0.995±0.31	1.977±.32	5.548±0.24	9.503±0.18	10.184±0.42	11.332±0.13
I+C	1.155±0.35	2.567±0.34 **	5.958±0.23 *	10.043± 0.26 ***	10.636±0.30 **	11.553±0.18 **
I+N	1.469±0.44 *	2.792±0.57 **	6.115±0.36 **	10.113± 0.24 ***	10.908±0.37 ***	11.573±0.13 **
I+D	1.684±0.47 **@	3.061±0.55 ***	6.236±0.43 ***	10.076±0.3 ***	10.83±0.26 ***	11.718±0.15 ***
C+N	1.769±0.44 **@	3.069±0.24 ***	6.258±0.37 ***	10.142± 0.36 ***	10.939±0.33 ***	11.847±0.25 *** @@@$$$
C+D	1.646±0.45 **@	3.26±0.57 ***@	6.503±0.34 ***@@	10.358±0.2 ***	11.204±0.28 ***@@	12.179±0.07 *** @@@$$$ £££ΨΨΨ
D+N	1.857±0.48 ***@@	3.414±0.64 ***@@$	6.603±0.43 ***@@$	10.45± 0.10*** @@$£ Ψ	11.427± 0.18*** @@@$$ £££ΨΨ	12.268± 0.08*** @@@$$$ £££ΨΨΨ

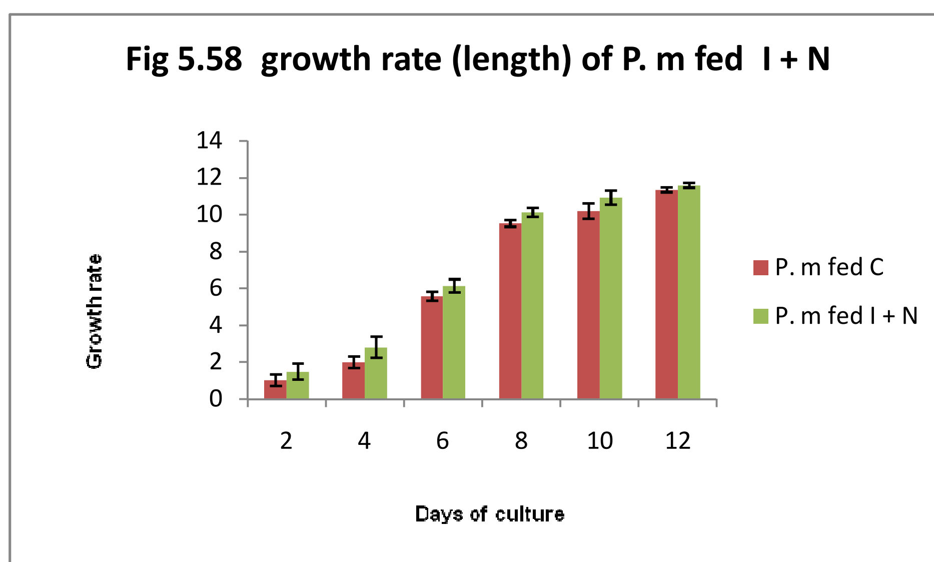
Table 5.11. Growth rate (Length) of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group.

ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$, *$p<0.05$ when compared to *Chaetoceros*
@@@$p<0.001$, @@$p<0.01$, @$p<0.05$, when compared to I+C
$$$p<0.001, $$p<0.01, $p<0.05 when compared to I+N
£££$p<0.001$, ££$p<0.01$, £$p<0.05$ when compared to I+D
ΨΨΨ$p<0.001$, ΨΨ$p<0.01$, Ψ$p<0.05$, when compared to C+N





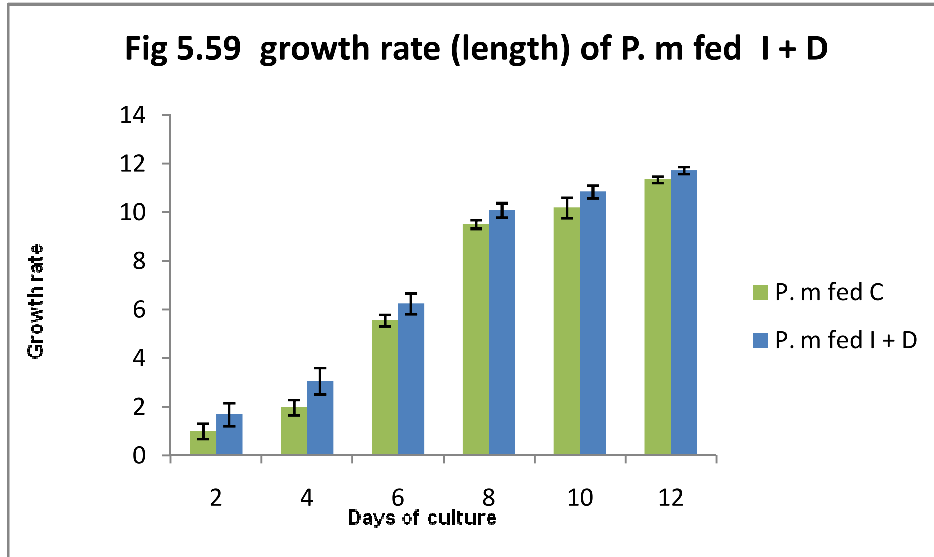


Fig 5.59 growth rate (length) of P. m fed I + D
14
12
10
8
6
4
2
0
Growth rate
2
4
6
8
10
12
Days of culture
P. m fed C
P. m fed I + D

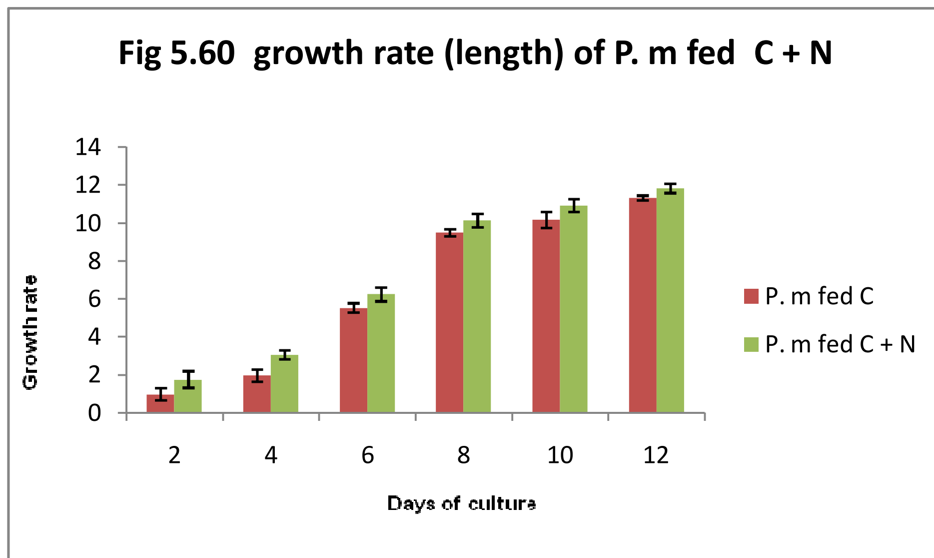


Fig 5.60 growth rate (length) of P. m fed C + N
14
12
10
8
6
4
2
0
Growth rate
2
4
6
8
10
12
Days of culture
P. m fed C
P. m fed C + N

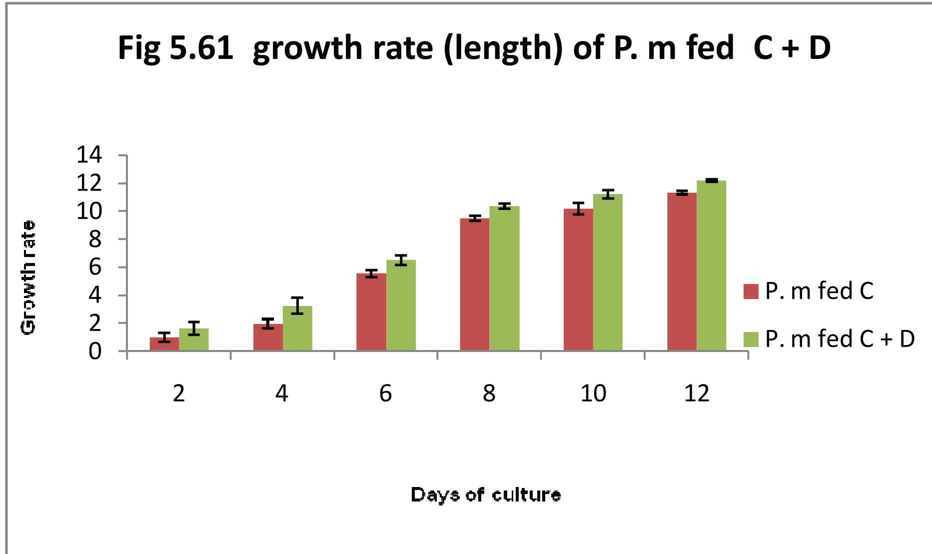


Fig 5.61 growth rate (length) of P. m fed C + D
Growth rate
14
12
10
8
6
4
2
0
2
4
6
8
10
12
P. m fed C
P. m fed C + D
Days of culture

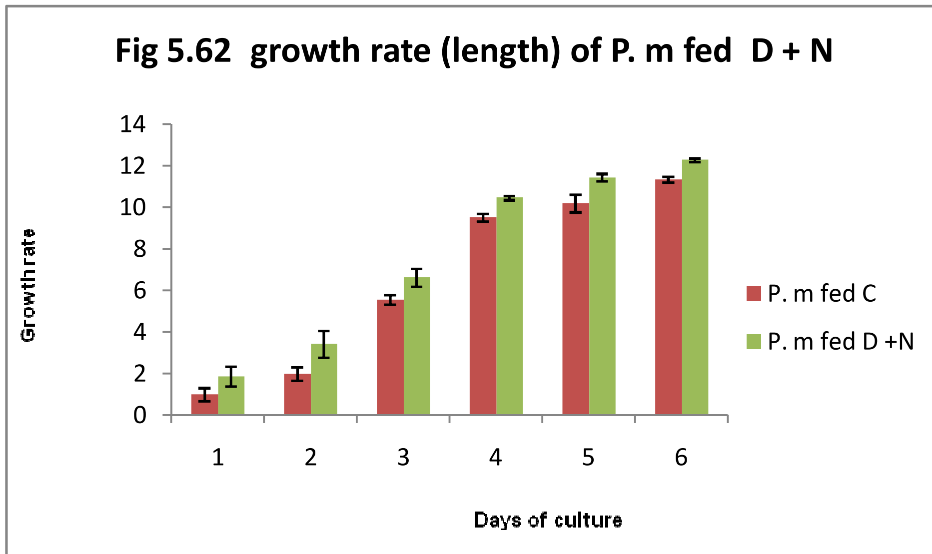


Fig 5.62 growth rate (length) of P. m fed D + N
Growth rate
14
12
10
8
6
4
2
0
1
2
3
4
5
6
P. m fed C
P. m fed D +N
Days of culture

5.8. Percentage increment weight of *P. monodon* larvae

5.12. Effect of mixed-algal diet ($50x10^4$ cells/ ml) on percentage increment (weight) of *P. monodon* larvae

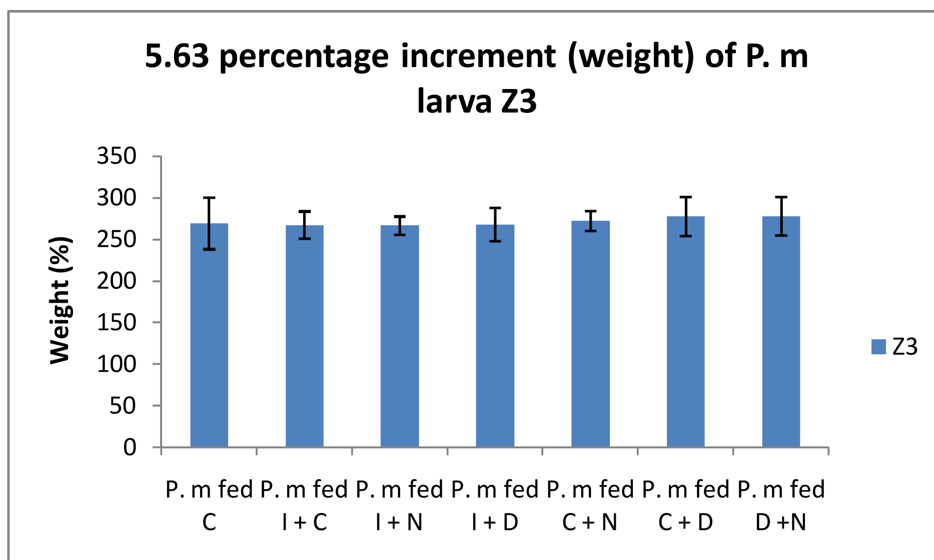
Table 5.12 summarizes about on percentage increment (weight)of the rearing experiments of P. m larvae fed with C, I+C, I+N, I+D, C+N, C+D, D +N at the cell concentration of $50x10^4$ cells/ ml starting from first protozoea stage (PZ-1). No significant changes was observed with the Z3 stages of the larvae whereas a significant increase in the weight gain was observed in the of P.m larvae fed with D +N ($p<0.01$) and C+D ($p<0.05$), when compare with that of P.m larvae fed with C throughout the 12 days of study.

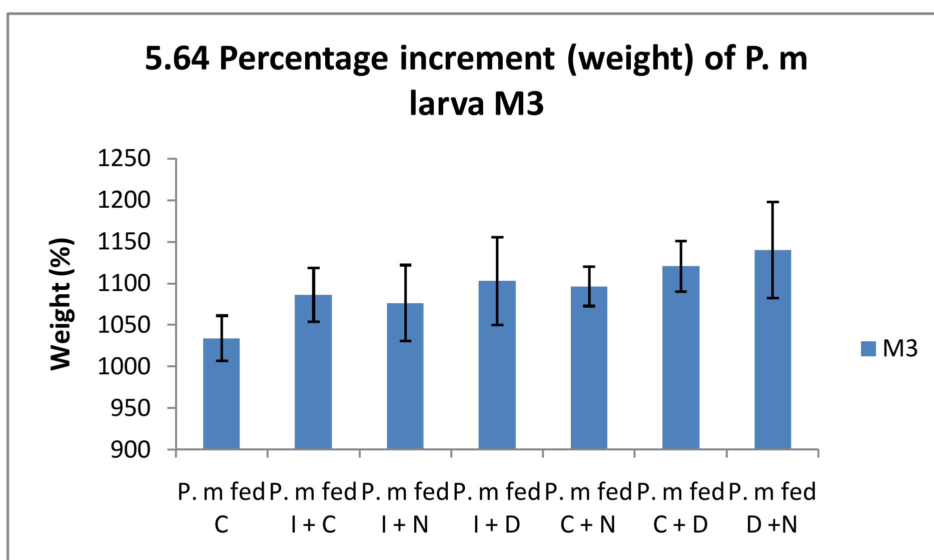
	Larval stages	
Algae	**Z3**	**M3**
C	269.43±31.18	1034.0 ±27.30
I + C	287.21±16.5	1086.4±32.25
I + N	266.75±10.88	1076.5±45.57
I + D	268.07±20.02	1102.9±52.78
C + N	272.18±11.67	1096.4±23.62
C + D	277.46±23.4	1120.6±30.25*
D +N	277.96±23.34	1140.2±57.91**

Table 5.12. Percentage increment on growth (weight) of *P. monodon* larvae at cell conc. ($50x10^4$ cells/ ml)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

**p<0.01, *p<0.05 when compared to *Chaetoceros*





5.9. Growth rate (weight) of *P. monodon* larvae

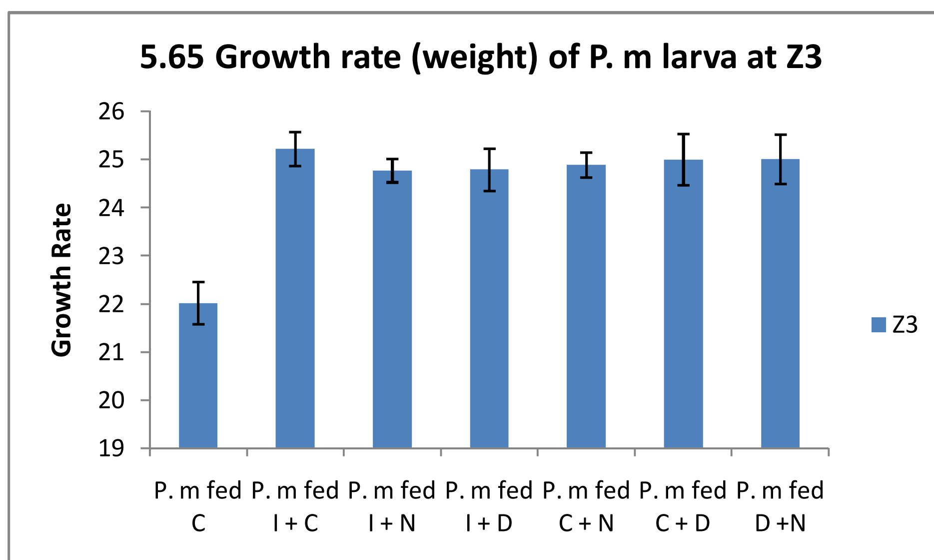
5.9.1. Effect of mixed-algal diet (50x 10^4 cells/ ml) on growth rate (weight) of *P. monodon* larvae

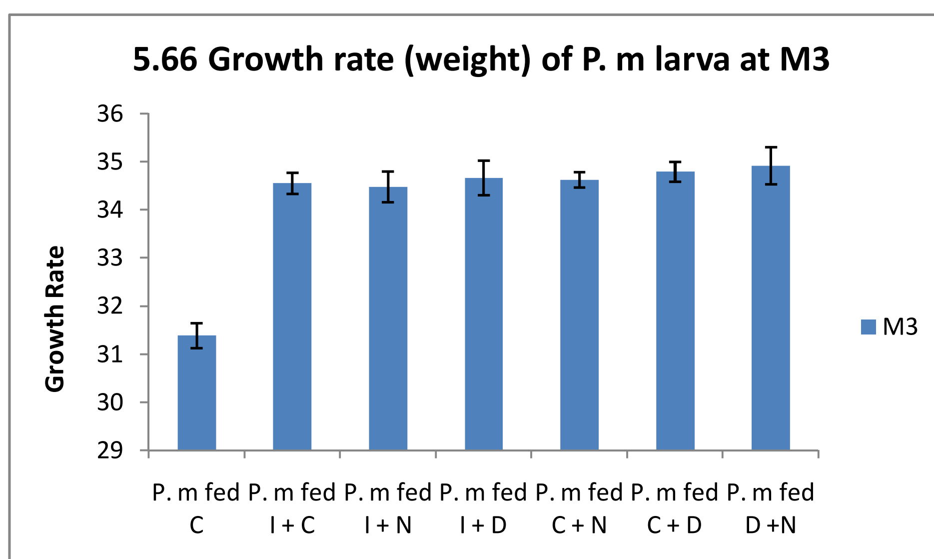
Table5.13 summarizes about the growth rate (weight) of the rearing experiments of P.m larvae fed with C, I+C, I+N, I+D, C+N, C+D, D +N at the cell concentration of 50x0^4 cells/ ml starting from first protozoea stage (PZ-1). The changes in growth rate (weight) were not significant in the Z3 stages and M3 stages of the larvae fed with C, I+C, I+N, I+D, C+N, C+D, D +N.

	Larval stages	
	Z3	**M3**
Algae		
C	22.026±6.44	31.394±6.26
I + C	25.225±0.35	34.56±0.22
I + N	24.776±0.24	34.488±0.32
I + D	24.799±0.44	34.671±0.36
C + N	24.898±0.26	34.631±0.16
C + D	25.006±0.53	34.797±0.21
D +N	25.017±0.51	34.925±0.387

Table 5.13. Growth rate (weight) of *P. monodon* larva at cell conc. (50x 10^4 cells/ ml).

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.





5.10. Water quality parameters

5.10.1. Water quality parameters of *P. monodon larva* fed with mixed algal diets at cell concentration (50x10^4 cells/ ml)

The water quality parameters like temperature, pH, salinity and dissolved oxygen were maintained throughout the experimental condition. Water temperature was in the range of 25^{oc} -27^{oc} (26±1^{oc}) and salinity (30–32ppt), and dissolved oxygen (DO) concentration (6.30–6.85 mg L−1) and the tank water pH (7.66–8.5). Total ammonia nitrogen (NH_3 optimum <0.1 ppm NH_3), NO_2N, NO_3N and PO_4 were less than 1mg/l.

DOC*	Temp (⁰C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.9±.05	31.3±0.6	8.3±0.2	7.1±0.3	2.22±0.08	2.65±0.07	1.12±0.09	3.69±0.06
4	26.8±0.4	31.2±0.4	7.7±0.4	7.1±0.5	3.05±0.03	3.76±0.05	1.14±0.03	2.06±0.07
6	26.7±0.5	31.5±0.5	7.9±0.6	7±0.7	2.49±0.06	3.66±0.08	1.16±0.08	2.68±0.08
8	26.1±0.7	30.8±0.3	7.8±0.4	6.7±0.3	3.11±0.03	4.16±0.04	1.16±0.05	4.16±0.04
10	25.8±0.5	30.8±0.6	8.1±0.7	6.6±0.4	2.90±0.07	2.00±0.07	1.16±0.08	2.34±0.06
12	25.9±0.6	31.8±0.4	7.9±0.5	6.5±0.7	2.82±0.08	2.80±0.09	1.12±0.07	3.06±0.06

Table 5.14. Water quality parameters of *P. monodon* larvae fed with C

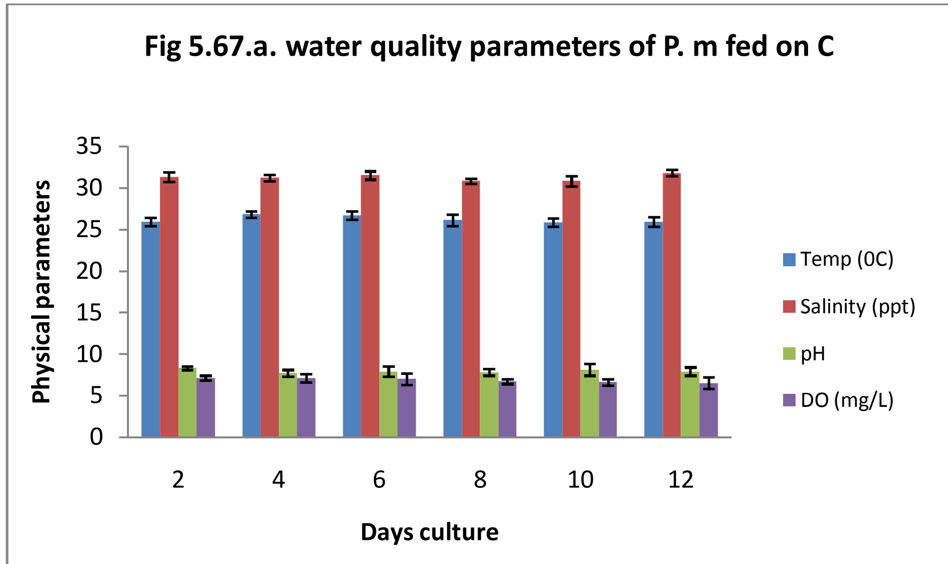


Fig 5.67.a. water quality parameters of P. m fed on C
Physical parameters
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days culture
Temp (0C)
Salinity (ppt)
pH
DO (mg/L)

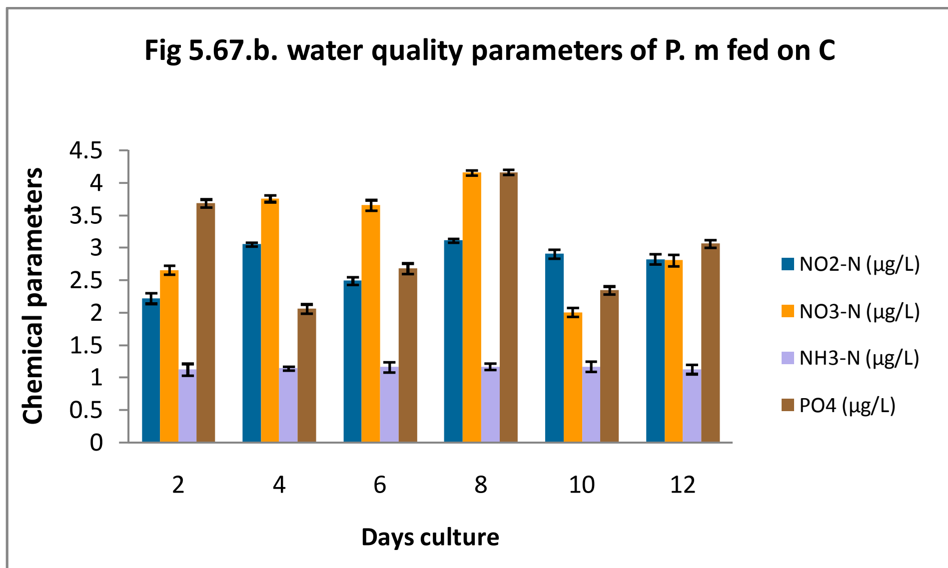
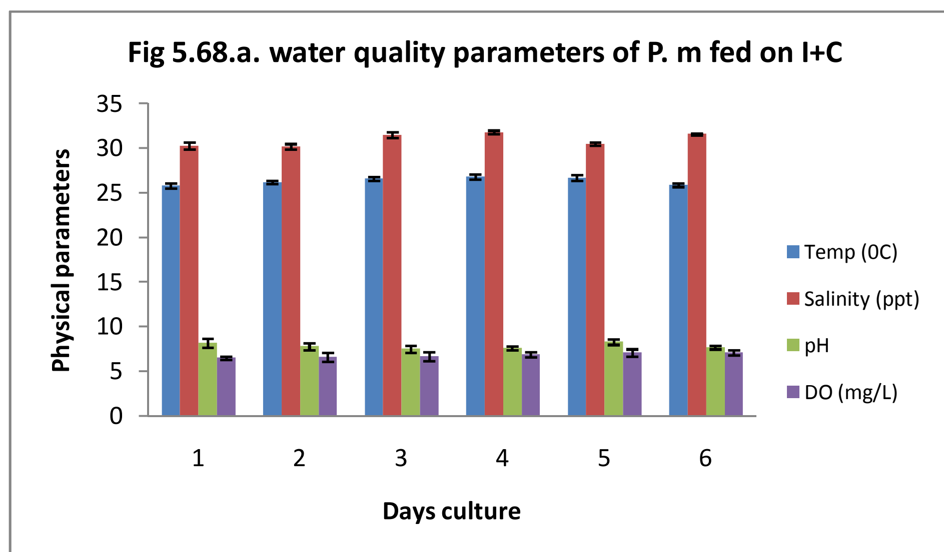
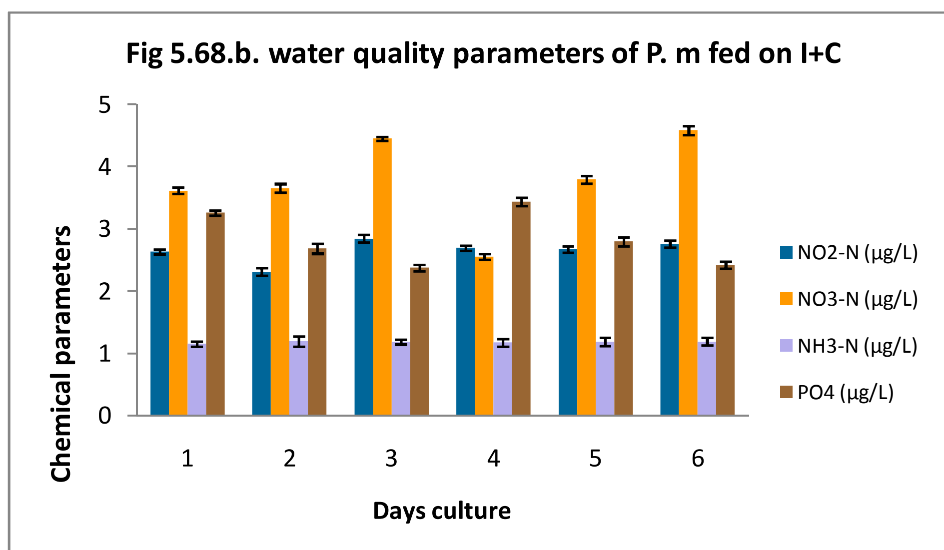


Fig 5.67.b. water quality parameters of P. m fed on C
Chemical parameters
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days culture
NO2-N (µg/L)
NO3-N (µg/L)
NH3-N (µg/L)
PO4 (µg/L)

DOC	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.8±0.3	30.3±0.4	8.2±0.5	6.5±0.2	2.63±0.04	3.61±0.05	1.15±0.04	3.26±0.04
4	26.2±0.2	30.2±0.3	7.8±0.4	6.6±0.5	2.31±0.06	3.65±0.07	1.19±0.08	2.68±0.08
6	26.6±0.2	31.5±0.3	7.5±0.4	6.7±0.5	2.84±0.06	4.45±0.03	1.19±0.04	2.38±0.05
8	26.8±0.3	31.8±0.2	7.6±0.2	6.9±0.3	2.69±0.04	2.55±0.05	1.17±0.06	3.44±0.07
10	26.7±0.3	30.5±0.2	8.3±0.3	7.1±0.4	2.67±0.05	3.79±0.06	1.19±0.07	2.79±0.07
12	25.9±0.2	31.6±0.1	7.7±0.2	7.1±0.3	2.76±0.06	4.58±0.07	1.19±0.06	2.42±0.06

Table 5.15. Water quality parameters of *P. monodon* larvaefed with I+C





DOC*	Temp (°C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.7±0.3	31.3±0.6	8.1±0.5	7.1±0.6	2.42±0.07	2.28±0.07	1.18±0.06	2.42±0.07
4	26.2±0.4	31.2±0.5	7.5±0.5	6.8±0.6	2.85±0.06	3.75±0.07	1.18±0.08	2.15±0.09
6	26.1±0.5	31.5±0.6	7.9±0.7	6.7±0.6	3.53±0.07	3.24±0.08	1.19±0.08	2.26±0.08
8	25.9±0.4	30.8±0.5	7.6±0.6	6.4±0.6	2.82±0.06	2.65±0.07	1.14±0.04	2.62±0.04
10	25.8±0.1	30.8±0.2	8.3±0.2	6.5±0.3	2.70±0.04	2.36±0.05	1.19±0.06	2.10±0.06
12	25.7±0.4	31.8±0.3	7.8±0.4	7.2±0.4	1.49±0.04	1.75±0.05	1.14±0.06	2.29±0.05

Table 5.16. Water quality parameters of *P. monodon* larvae fed with I+N

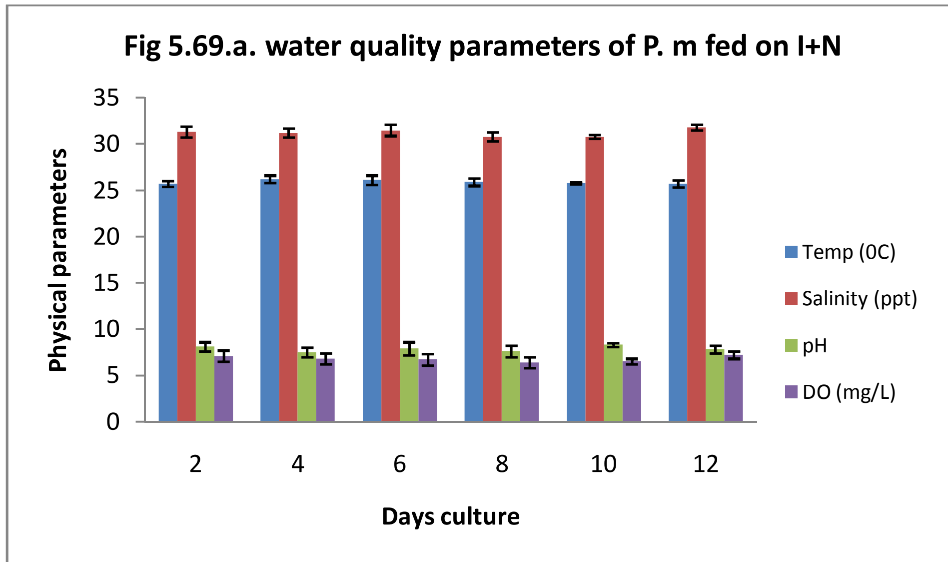


Fig 5.69.a. water quality parameters of P. m fed on I+N
Physical parameters
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days culture
Temp (0C)
Salinity (ppt)
pH
DO (mg/L)

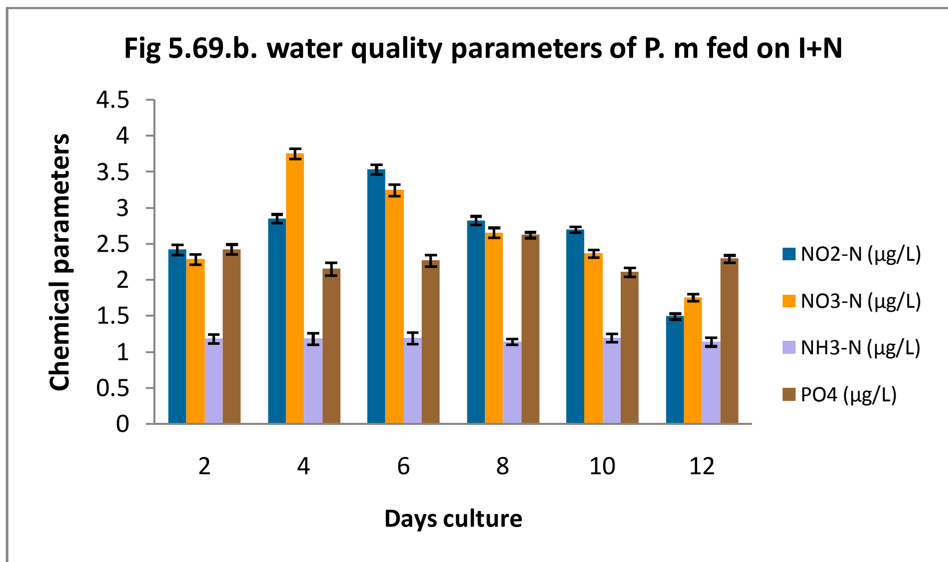
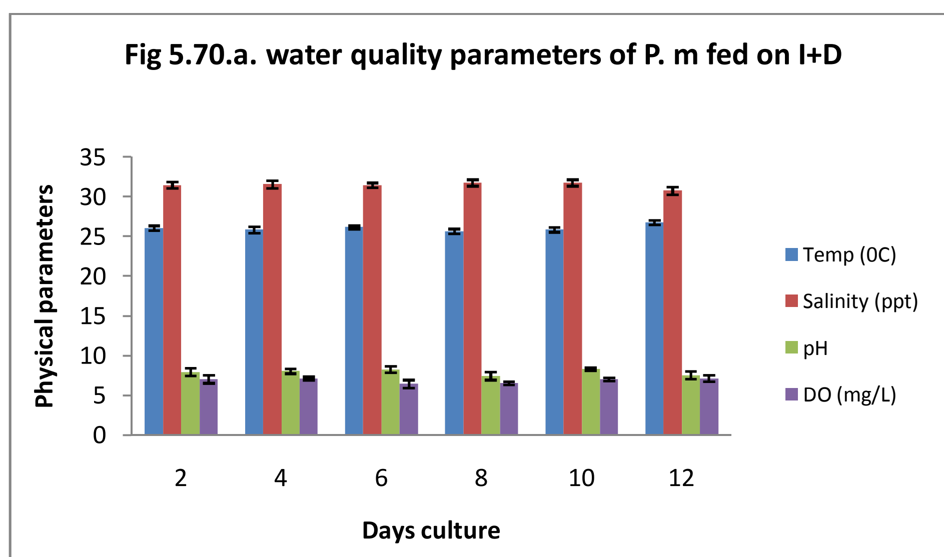
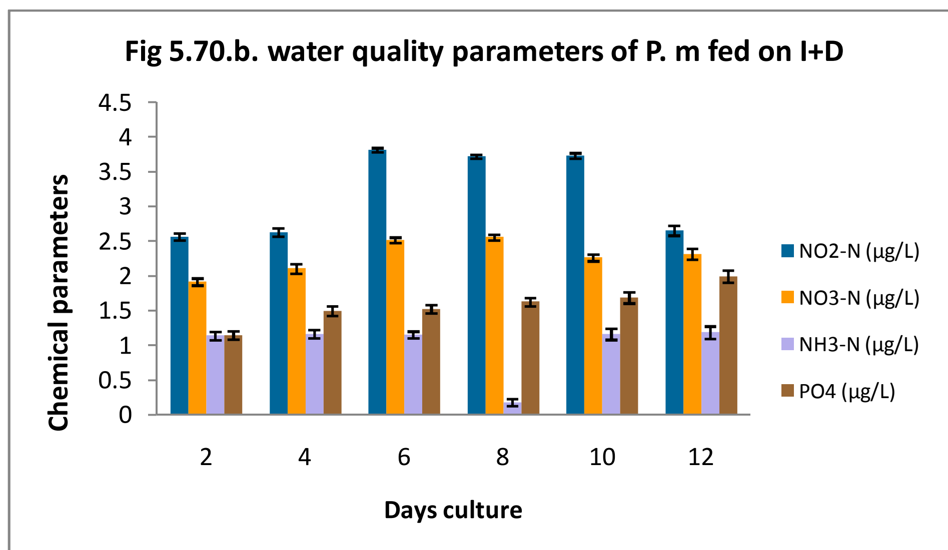


Fig 5.69.b. water quality parameters of P. m fed on I+N
Chemical parameters
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days culture
NO2-N (µg/L)
NO3-N (µg/L)
NH3-N (µg/L)
PO4 (µg/L)

DOC*	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	26.1±0.3	31.5±0.4	8±0.5	7.1±0.5	2.56±0.05	1.91±0.05	1.14±0.06	1.14±0.06
4	25.9±0.4	31.6±0.5	8.1±0.3	7.2±0.2	2.63±0.06	2.10±0.07	1.16±0.06	1.49±0.07
6	26.2±0.2	31.5±0.3	8.3±0.4	6.5±0.5	3.81±0.03	2.51±0.04	1.15±0.05	1.52±0.06
8	25.7±0.3	31.8±0.4	7.5±0.5	6.6±0.2	3.72±0.03	2.55±0.04	0.17±0.05	1.63±0.06
10	25.9±0.3	31.8±0.4	8.4±0.2	7.1±0.2	3.73±0.04	2.26±0.05	1.16±0.08	1.68±0.08
12	26.8±0.3	30.8±0.5	7.6±0.5	7.2±0.4	2.65±0.07	2.31±0.08	1.18±0.09	1.99±0.09

Table 5.17. Water quality parameters of *P. monodon* larvae fed with I+D





DOC*	Temp (⁰C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.9±0.2	31.3±0.3	8.5±0.4	6.5±0.5	2.78±0.06	2.35±0.07	1.14±0.08	1.48±0.02
4	26.5±0.2	31.2±0.3	7.5±0.4	6.6±0.5	3.55±0.03	1.85±0.04	1.12±0.05	1.91±0.06
6	26.3±0.3	30.5±0.4	8±0.4	6.4±0.5	2.53±0.06	2.05±0.05	1.13±0.06	2.43±0.07
8	26.8±0.2	30.5±0.3	7.9±0.2	6.7±0.2	3.43±0.03	1.75±0.04	1.13±0.05	2.15±0.06
10	26.4±0.2	30.6±0.2	8.2±0.3	6.8±0.3	3.41±0.04	2.25±0.05	1.13±0.06	1.81±0.06
12	26.5±0.2	30.8±0.2	8.5±0.2	6.7±0.3	2.49±0.04	1.90±0.03	1.13±0.04	1.67±0.05

5.18. Water quality parameters of *P. monodon* larvae fed with C+N

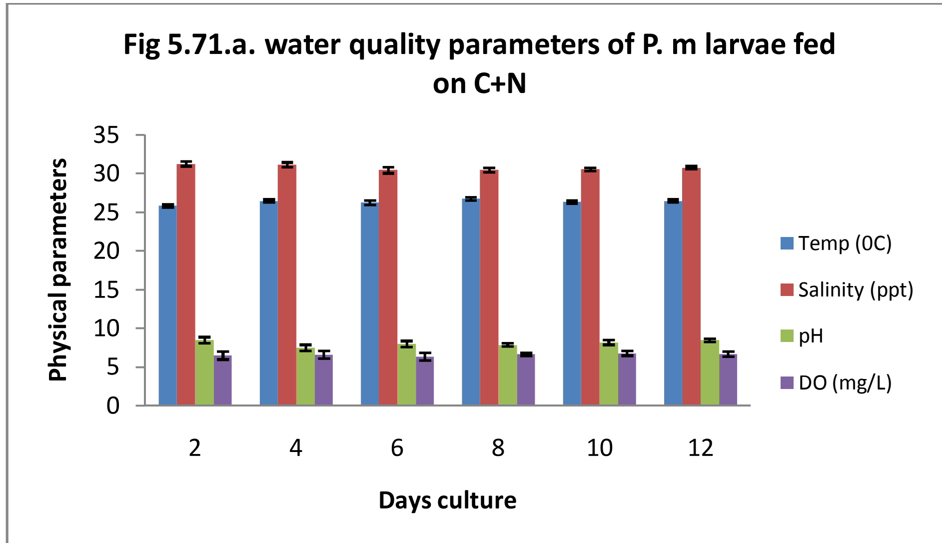


Fig 5.71.a. water quality parameters of P. m larvae fed on C+N
Physical parameters
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days culture
Temp (0C)
Salinity (ppt)
pH
DO (mg/L)

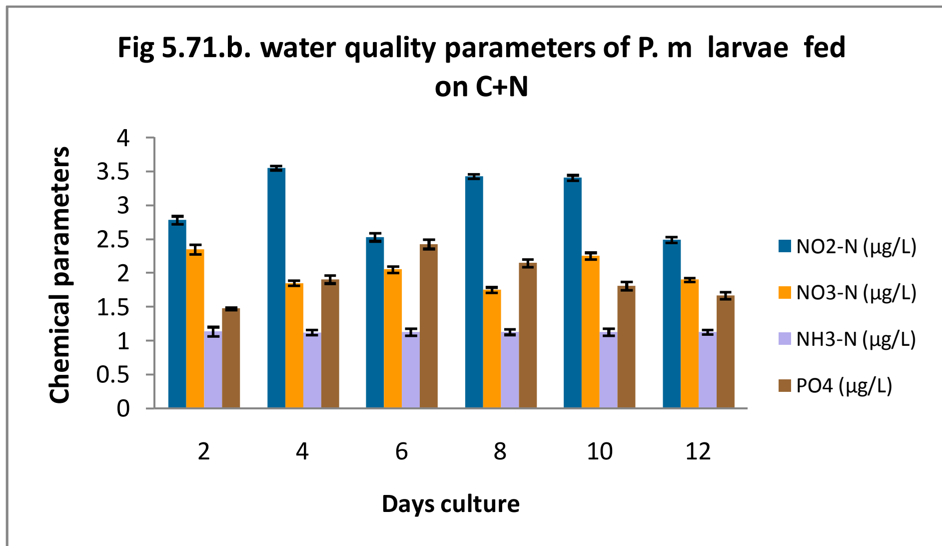
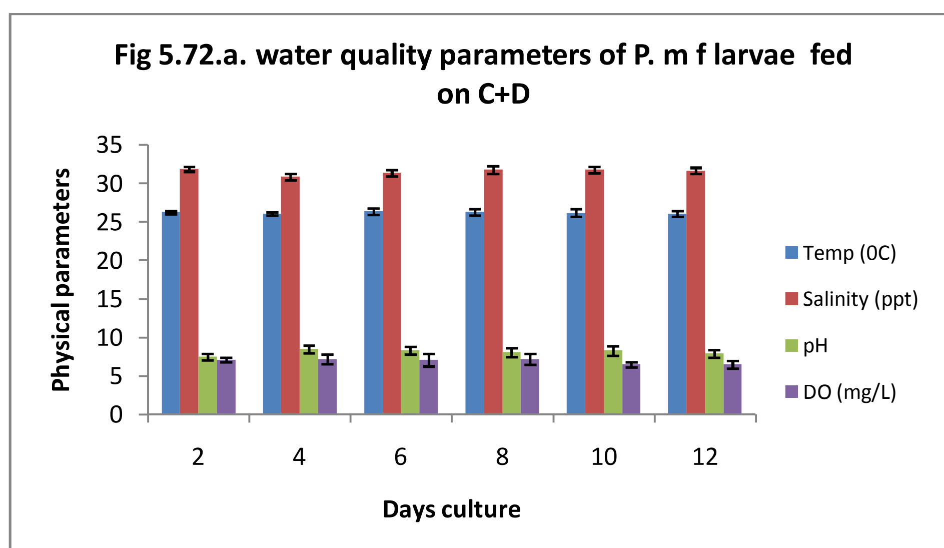
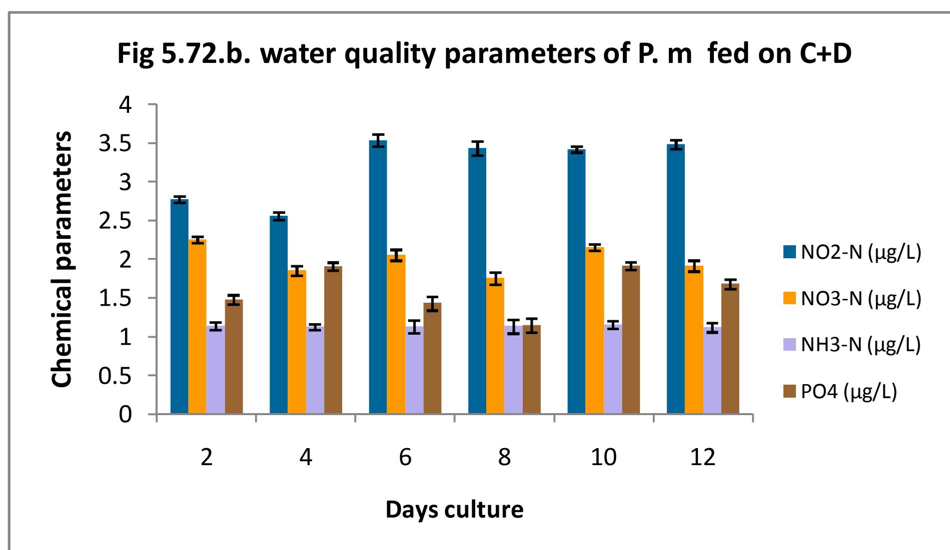


Fig 5.71.b. water quality parameters of P. m larvae fed on C+N
Chemical parameters
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days culture
NO2-N (µg/L)
NO3-N (µg/L)
NH3-N (µg/L)
PO4 (µg/L)

DOC*	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	26.3±0.2	31.9±0.3	7.5±0.4	7.1±0.3	2.77±0.04	2.25±0.04	1.14±0.05	1.48±0.06
4	26.1±0.2	30.9±0.4	8.5±0.5	7.2±0.6	2.55±0.05	1.85±0.06	1.12±0.04	1.91±0.05
6	26.4±0.4	31.4±0.4	8.3±0.5	7.1±0.8	3.53±0.08	2.05±0.07	1.13±0.08	1.43±0.09
8	26.3±0.4	31.8±0.5	8.1±0.6	7.2±0.7	3.43±0.09	1.75±0.08	1.13±0.09	1.15±0.09
10	26.2±0.5	31.8±0.4	8.3±0.6	6.5±0.3	3.42±0.04	2.15±0.04	1.15±0.05	1.91±0.05
12	26.1±0.4	31.7±0.4	7.9±0.5	6.5±0.5	3.48±0.06	1.91±0.07	1.12±0.06	1.68±0.06

5.19. Water quality parameters of *P. monodon* larvae fed with C+D





DOC*	Temp (^{0}C)	Salinity (ppt)	pH	DO (mg/L)	NO_2-N (µg/L)	NO_3-N (µg/L)	NH_3-N (µg/L)	PO_4 (µg/L)
2	25.3±0.4	31.3±0.4	7.9±0.5	6.8±0.6	2.70±0.07	2.15±0.06	1.13±0.07	1.96±0.07
4	26.6±0.3	31.2±0.2	8±0.3	6.9±0.4	3.72±0.05	2.30±0.06	1.13±0.07	1.86±0.07
6	26.9±0.4	31.5±0.3	8.1±0.4	7.1±0.5	3.58±0.06	1.95±0.07	1.13±0.08	1.67±0.09
8	26.8±0.2	31.5±0.7	8.2±0.4	7.2±0.5	3.49±0.06	1.85±0.07	1.13±0.07	1.62±0.04
10	26.7±0.2	31.9±0.3	8.3±0.4	6.4±0.5	3.53±0.05	1.95±0.06	1.13±0.07	2.00±0.07
12	26.6±0.3	31.5±0.4	8.4±0.4	6.5±0.5	3.74±0.06	2.40±0.07	1.13±0.08	2.24±0.06

5.20. Water quality parameters of *P. monodon* larvae fed with D+N

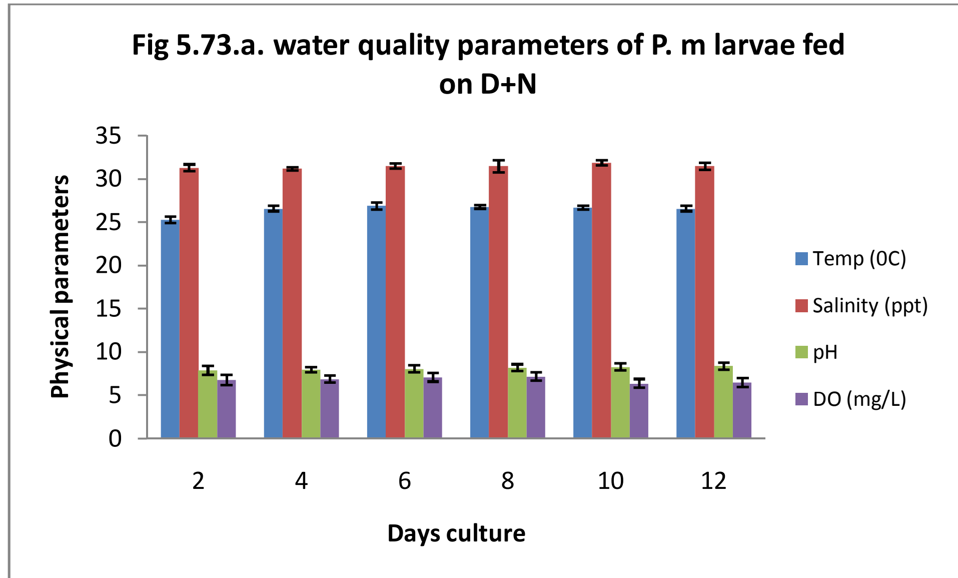


Fig 5.73.a. water quality parameters of P. m larvae fed on D+N
Physical parameters
35
30
25
20
15
10
5
0
2
4
6
8
10
12
Days culture
Temp (0C)
Salinity (ppt)
pH
DO (mg/L)

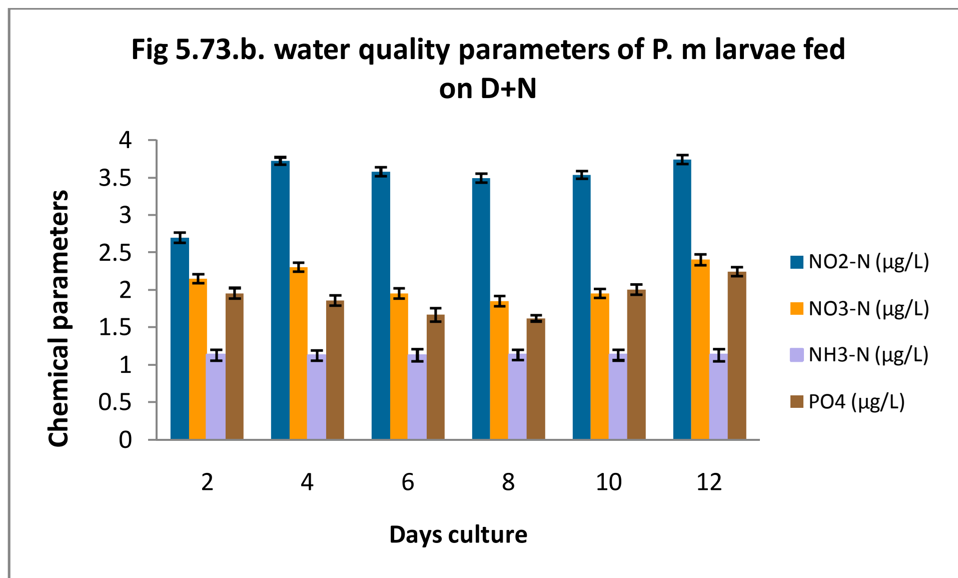


Fig 5.73.b. water quality parameters of P. m larvae fed on D+N
Chemical parameters
4
3.5
3
2.5
2
1.5
1
0.5
0
2
4
6
8
10
12
Days culture
NO2-N (µg/L)
NO3-N (µg/L)
NH3-N (µg/L)
PO4 (µg/L)

DISCUSSION

From the present study it was seen that the pm larvae fed with that of mixture of algal diets performed well. Hence, we consider the differences in larvae response to be a consequence of the nutritional composition of the diet. Feed mixtures are generally recommended because they produce better results than when a particular feed is used alone, as mixtures contain a complementary blend of nutrients, such as amino acids, that meet or exceed nutritional requirements (Webster and Lovell, 1991).

Even though the use of a combination of several microalgae as a diet for shrimp larvae is recommended, monoalgal diets have been widely used for commercial culture. The biochemical composition of each microalgae species varies, hence the use of monoalgal diets could produce a shortage of essential nutrients needed for the adequate development of penaeid shrimp (Yúfera and Lubián1984).

The study of Kurmaly *et al* 1989 showed that prawn larvae (*Penaeus* spp.) do as well, or better, on a diet of *Chaetoceros muelleri* alone than on a diet of either of the other three algae. However, their survival and development may be better on a mixed diet of *C. muelleri* and *Tetraselmis suecica.* The survival of larvae fed *C. muelleri* or the mixed diet was always higher than that of larvae fed *T. suecica, T-iso* or *Dunaliella tertiolecta* alone. Similar results was seen in the present study that the mixed algae fed *P. monodon* had better developmental rate with *P. monodon* larvae fed with *D. salina* which was found to be very efficient in combination with *N. salina* as well as that of *C. calcitrans*. (table 6)

The development of larvae fed *C. muelleri* or the mixed diet was always at least as fast as those fed *T. suecica*, and always faster than that of larvae fed the other diets. The poorest algal diet in terms of survival and development of the larvae was *Dunaliella tertiolecta*, which had previously been found to be inadequate for *Penaeus monodon* larvae. In the present study *D. salina* was found to be very efficient in combination with *N. salina* as well as that of *C. calcitrans* although the better results was obtained with the combination that of *N. salina* and *D. salina*. The combinations of algal diets was seem to produce greater length (table1,2,3) but the survival rate was showing greater variation (table4,5,6).

The low cell concentrations of either single algal feeds or mixed algal diets do not produce satisfactory larval growth and survival in *F. indicus*. The flagellate *T. chuii* and diatom *S. costatum* fed in unmixed form at 10-20 cells μL^{-1} did not enable survival further than the PZ1/PZ2 stages. However, when the mixed algal (*T. chuii/ S. costatum*) diet was given at 20 cells μL^{-1}, 28% survival was obtained in the M1 stage. In the present study at 50x 10^4 cell/ml conc. the survival rate of *P. monodon* fed larvae with *D. salina* was found to be very efficient in combination with *N. salina* and *C. calcitrans* although the higher survival rate of 71.3% was obtained with the combination of *N. salina* and *D. salina*. (table 6)

Emmerson (1980) reports very good survival (96%) and normal larval development using *T. weissflogii* (10.7 µm in diameter) at 7 cells μL^{-1} in the culture of *P. indicus*, but at a stocking density of only 35 larvae L^{-1} in large (70^{-L}) culture vessels at 26˚C. It appears that the higher larval stocking density (150 larvae L^{-1}) used in the study presented here produces a grazing demand which cannot be met at low cell densities.

Amjad and Jones 1992 reports total mortality in *P. monodon* larvae at a cell density of 10 cells μL^{-1} and low survival at 20 cells μL^{-1} (*T. chuii/R. reticulata*) in similar culture conditions to those described here. Aquacop 1983 suggests that a cell density of 100 cells μL^{-1} of mixed algae is required at high larval stocking densities (100-120 larvae L^{-1}), whereas Galgani and Aquacop 1988 recommend 30-40 cells μL^{-1} algae at a larval stocking density of 100 L^{-1} during the protozeal culture of *F. indicus*.

Kurmaly *et al* 1989 reported that cell density was increased from 30 to 40 and then 50 cells μL^{-1}, the larval survival and growth of *F. indicus* progressively increased. This indicates that a low algal-cell density cannot provide sufficient nutrients or energy at 100 larvae L^{-1} for *F. indicus*. From the present study it was seen that *P. monodon* PZ1 larvae at a stocking density of 150 larvae/ litre had ingested upto about 35 $x10^{4}$cells per day for optimal survival and growth. In the experiments described, the ingestion rate has reached upto $35x10^{4}$ cells /ml per day at a concentration of $50x10^{4}$ cells /ml. Hence, levels below these are inadequate for larval growth, survival and development (table8).

Low survival and growth obtained during the protozoeal stages affected the subsequent results at metamorphosis. The evaluation of algae as live feeds for penaied larvae is generally based on the selection of species that sustain the maximum growth, survival and development. From the previous studies it is seen that, of the unicellular algal species tested, the diatom *S. costatum* promotes better larval growth, survival and development throughout all larval stages than the flagellate *T. chuii*. It is known that *S. costatum* is one of the most suitable live diets for penaied larvae during the protozoeal stages and is therefore commoly used in

hatcheries. However, it has been observed that S. *costatum* at high cell concentrations (70-80 cells μL^{-1}) causes mechanical fouling that may hamper the feeding and respiration of penaeid larvae. Liao *et al* 1993 report that the exclusive use of *S. costatum* may be harmful to penaeid larvae if the alga is harvested in or after the stationary growth phase.

The different growth and survival responses of penaeid larvae to the algal feeds may be due to variations in the nutritive value, cell size, digestibility or chemical composition of the algal species used. In this study, it is unlikely that the cell size of the algal feeds was inappropriate for the larvae (see Table 1). The nutritive value of microalgae may vary greatly even within the same species, depending on culture conditions and the time of harvest.

The species selected for the present study the *C. calcitrans* had a cell size of 4-6 µm and *I. galbana* had a cell size of 3-5 µm and *D. salina* had a cell size of 4-5 µm and the *N. salina* was the smallest had a cell size of only 1-2 µm. It might be due to this that during the mixed algal combinations *N. salina* and *D. salina* shown to be the better algal diet as it provides sufficient nutrition during the protozoea and mysis stages. During the protozoea stage the larvae may ingest the smaller size particle and during the mysis it may prefer the larger algae.(table 3)

In this study, the combination of *D. salina and N. sallina* consistently produced significantly superior larval growth and survival compared to single algal feeds ($P<0.05$) in both the first and second experiments. Hence, these results confirm that using mixed algal species helps to ensure good results. The present study also shows that pm larvae

can be reared upto M3 stage with 70% survival at a cell concentration of $50x10^4$ cell/ml (table6).

Combinations of centrifuged concentrates are far superior diets for both larval and juvenile oysters compared with single algal diets (Heasman *et al* 2000). Therefore combinations of flocculated concentrates of different species may prove to be better diets for prawn larvae than single species, as is the case with combinations of two species of fresh algae (D'Souza and and Kelly 2000). Combinations of fresh and concentrated algae in different proportions may also be effective for prawn larvae, while reducing reliance on fresh algal culture.

The pm larvae fed with C+D and D+N showed a significant increase in weight gain when compared to that of C even though the differences in weight gain with all other combinations were not significant (table9).

The differences in the composition of fatty acids in the algal diets seemed to be the factor most likely to explain the differences in larval survival and development. The gross lipid and carbohydrate compositions of the algal diets did not explain the observed differences in growth of the larvae nor did they correspond to the gross composition of the larvae. Although protein levels were not presented here due to problems with the assay those that were measured in this study were similar (30 to 40% dry wt) for the different species of algae; always high, and presumably of very similar amino acid composition (Brown 2002). Previous studies have also found that the gross composition of algae alone could not explain differences in the survival and growth of prawn and bivalve larvae.

The ingestion rates of Pm larvae at cell concentration $50x10^4$ cell/ml shows that when compared to C all other combinations shed significant increase on all other days but the maximum ingestion rate was obtained with the M2 stage of D fed Pm larva on the 10th day (table8).

The above results shows that the P. m larvae fed the combinations of D+N provide better growth, survival and IR. As both of the algae provide sufficient requirement of nutrients in the stages of larvae as N is better during the protozoeal stages and D is better during the Mysis stages.

Chapter- 6

BIOCHEMICAL COMPOSITION OF P. MONODON LARVAE

Nauplii shift from endogenous feeding to herbivorous protozoeae, which become omnivorous at PZ III. A change from a planktonic to a benthic existence takes place during the first postlarval substages. In protozoea, swimming and feeding are virtually continuous, and the relative ingestion rate increases through each substage, reaching its maximum during larval development in PZ III. The retention time of food in the gut is lower than in mysis and postlarva, whereas feces production is high. Therefore, knowledge of the optimum level of protein and the protein-sparing effects of non-protein nutrients such as lipids or carbohydrates would be effective in reducing feed costs and water pollution. The types and levels of these nutrients in the diet have been shown to affect the growth. Hence an attempt was done to evaluate whether the gross lipid and carbohydrate compositions of the algal diets do not explain the observed differences in growth of the larvae nor did they correspond to the gross composition of the larvae.

6.1. Biochemical composition of *P. monodon* larvae fed mono-algae at Z3 stage

Table 6.1 summarizes about Biochemical composition of mono-algae fed *P. monodon* larvae at Z3 stage. When compared to *C*, a significant increase was observed in the protein of *D* ($p<0.001$) and *N* ($p<0.001$) but no significant change ($p>0.05$) was observed in the protein of *I*. When compared to *I* a significant increase was observed in the protein of *D* ($p<0.001$) and *N* ($p<0.001$). When compared to *D* No significant change ($p>0.05$) was observed in the protein of *N*.

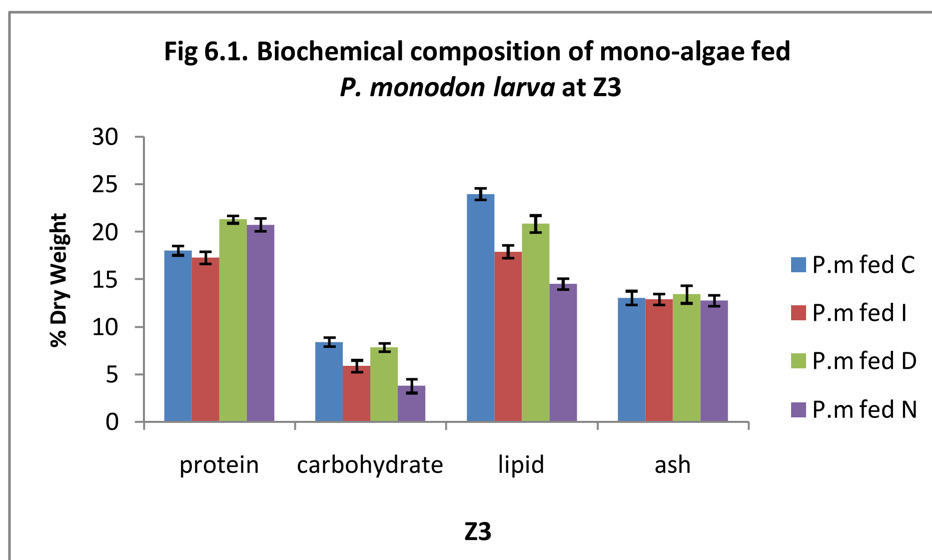
When compared to *C*, a significant decrease was observed in the carbohydrate of *I* ($p<0.001$) and *N* ($p<0.001$) but no significant change ($p>0.05$) was observed in the protein of *D*. When compared to *I* a significant increase was observed in the carbohydrate of *D* ($p<0.001$) and a significant decrease was observed in the carbohydrate of *N* ($p<0.001$). When compared to *D* significant change ($p<0.001$) was observed in the carbohydrate of *N*.

Significant change ($p<0.001$) was observed in the lipid of the algae when compared to each other. High value of lipid was seen in C and then seen in D. No significant change ($p>0.05$) was observed in the Ash of the algae when compared to each other.

	Algae			
	Protein	Carbohydrate	Lipid	Ash
P.m fed C	18.08±0.486	8.44±0.63	24.02±0.396	13.08±0.676
P.m fed I	17.32±0.471	5.92±0.614 ***	17.94±0.427 ***	12.96±0.723
P.m fed D	21.36±0.602 ***@@@	7.88±0.668 @@@	20.88±0.887 ***@@	13.46±0.585
P.m fed N	20.78±0.736 ***@@@	3.82±0.576 ***@@@$$$	14.56±0.931 ***@@@$$$	12.8±0.556

Table 6.1. Biochemical composition of *P. monodon* larvae fed mono-algae at Z3 stage(% dry weight)
Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, when compared to C
@@@p<0.001, when compared to I
$$$p<0.001, when compared to D



6.2. Biochemical composition of *P. monodon* larvae fed mono algae at M3 stage

Table 6.2 summarizes about Biochemical composition of mono-algae fed *P. monodon* larvae at M3 stage. When compared to *C*, a significant increase was observed in the protein of *D* ($p<0.001$) and *N* ($p<0.001$) but No significant change ($p>0.05$) was observed in the protein of *I*. When compared to *I* a significant increase was observed in the protein of *D* ($p<0.001$) and *N* ($p<0.001$). When compared to *D* No significant change ($p>0.05$) was observed in the protein of *N*.

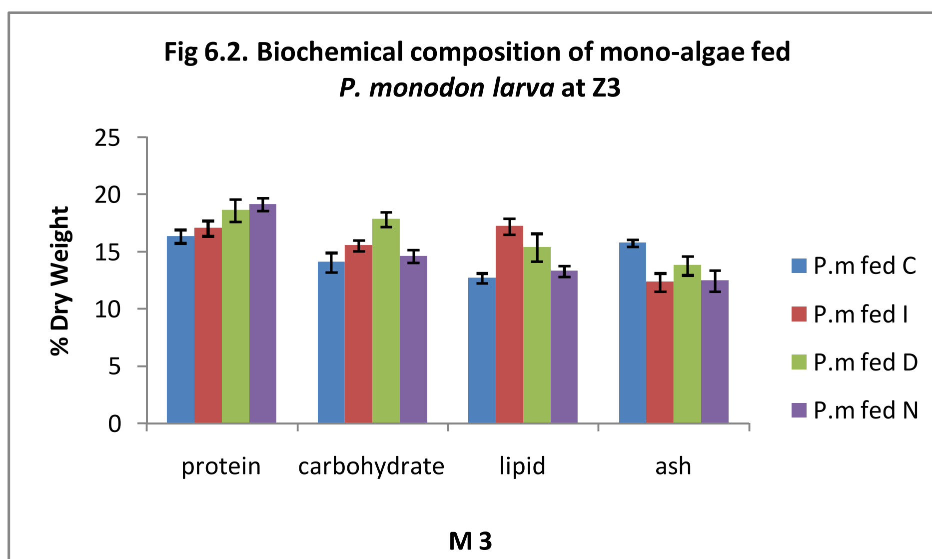
When compared to *C*, a significant increase was observed in the carbohydrate of *I* ($p<0.001$) and *D* ($p<0.001$) but No significant change ($p>0.05$) was observed in the carbohydrate of *N*. When compared to *I* a significant increase was observed in the carbohydrate of *D* ($p<0.001$) and *N* ($p<0.05$). When compared to *D* significant change ($p<0.001$) was observed in the carbohydrate of *N*. Significant change ($p<0.001$) was observed in the lipid of the algae when compared to each other The lipid value was seen to be higher in I and D. But no significant change ($p>0.05$) was observed in the lipid of *N* when compared with that of *C*. No significant change ($p>0.05$) was observed in the Ash of the *D* and *N* when compared to *I* and *D*. When compared to *C*, a significant change was observed in the ash of *I* ($p<0.001$) *D* ($p<0.05$) and *N* ($p<0.001$).

Biochemical composition				
Algae	**Protein**	**Carbohydrate**	**Lipid**	**Ash**
P.m fed C	16.38±0.589	14.1±0.674	12.74±0.983	15.78±1.11
P.m fed I	17.08±0.858	15.56±0.472 ***	17.24±0.646 ***	12.376±1.106 ***
P.m fed D	18.66±0.427 ***@@@	17.86±0.709 ***@@@	15.42±1.21 ***@@	13.83±0.807 *
P.m fed N	19.18±0.303 ***@@@	14.64±0.789 @$$$	13.32±0.834 @@@$$	12.504±1.16 ***

Table 6.2. Biochemical composition of *P. monodon* larvae fed mono algae at M3 stage (% dry weight)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, *$p<0.05$ when compared to C
@@@$p<0.001$, @@$p<0.01$, @$p<0.05$, when compared to I
$$$$p<0.001$, when compared to D



6.3. Biochemical composition of *P. monodon* larvae fed mixed algae at Z3 stage

Table 6.3 summarizes about Biochemical composition of mixed-algae fed *P. monodon larva* at Z3 stage. The protein values were seen to be higher in combinations of I+D, C+N, C+D, and D+N. When compared to *C*, a significant change was observed in the protein of I+D (p<0.001), C+N (p<0.001), C+D (p<0.001), D +N (p<0.001) but No significant change (p>0.05) was observed in the protein of *I*+C and I+N. When compared to *I+C* a significant change was observed in the protein of I+D (p<0.001), C+N (p<0.001), C+D (p<0.001), D +N (p<0.001) but No significant change (p>0.05) was observed in the protein of I+N. When compared to *I+N* a significant change was observed in the protein of I+D (p<0.001), C+N (p<0.001), C+D (p<0.001), D +N (p<0.001). When compared to *I+D* no significant change was observed in the protein of C+N (p>0.05), C+D (p>0.05), D +N (p>0.05). When compared to *C+D* no significant change was observed in the protein of D +N (p>0.05).

The lipid value was seen to be higher in combinations of C, I+N, I+D, C+N. Low lipid value was seen in C+D and D+N. When compared to *C*, a significant change was observed in the lipid content of *I*+C (p<0.01), C+D (p<0.001), D +N (p<0.001) but No significant change (p>0.05) was observed in the lipid content of I+N, I+D and C+N. When compared to *I+C* a significant change was observed in the lipid content of C+D (p<0.01), D +N (p<0.05) but no significant change (p>0.05) was observed in the lipid content of I+N, I+D and C+N. When compared to *I+N* no significant change (p>0.05) was observed in the lipid content of I+D, C+N, C+D (p<0.001), D +N (p<0.01). When compared to *I+D* no significant change was observed in the lipid content of C+N (p>0.05), but a significant change

was observed in the lipid content of C+D ($p<0.001$), D +N ($p<0.001$). When compared to *C+D* no significant change was observed in the lipid content of D +N ($p>0.05$).

When compared to no significant change ($p>0.05$) was observed in the carbohydrate content of C, *I*+C, I+N, I+D, C+N, C+D, D +N.

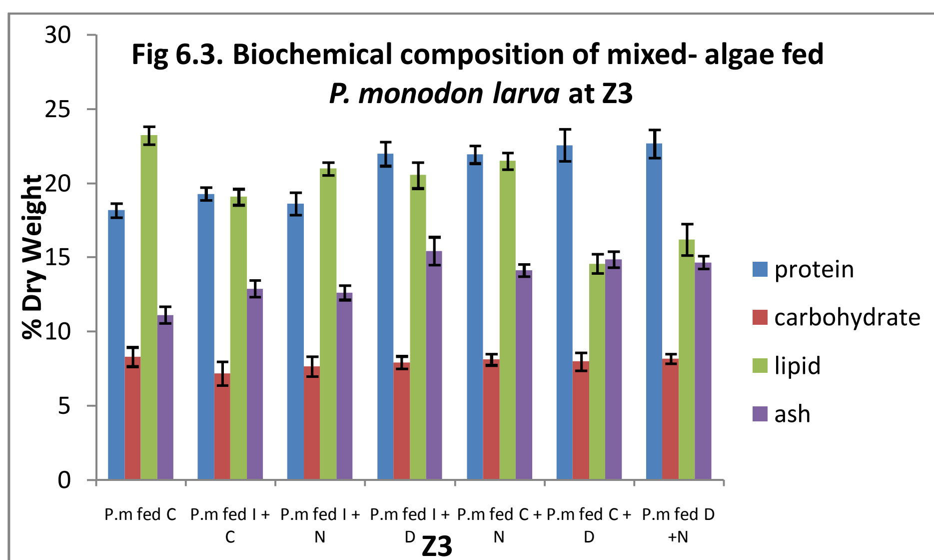
Ash content was seen to be high in combinations of I+D,C+N,C+D,D+N. When compared to *C*, a significant change ($p<0.001$) was observed in the ash content of *I*+C, I+N, I+D, C+N, C+D and D +N. When compared to *I+C* no significant change ($p>0.05$) was observed in the ash content of I+N. but a significant change was observed in the ash content of I+D ($p<0.001$), C+N ($p<0.01$), C+D ($p<0.001$), D +N ($p<0.001$). When compared to *I+N* a significant change was observed in the ash content of I+D ($p<0.001$), C+N ($p<0.001$), C+D ($p<0.001$), D +N ($p<0.001$). When compared to *I+D* no significant change was observed in the ash content C+D ($p>0.05$), D +N ($p>0.05$). But a significant change was observed in the ash content of C+N ($p<0.01$). When compared to *C+D* no significant change was observed in the ash of D +N ($p>0.05$).

Algae	Biochemical composition			
	Protein	Carbohydrate	Lipid	Ash
P.m fed C	18.18±0.465	8.32±0.649	23.22±0.614	11.14±0.555
P.m fed I + C	19.28±0.432	7.2±0.787	19.08±0.54 **	12.906±0.560 ***
P.m fed I + N	18.634±0.758	7.68±0.672	20.98±4.39	12.646±0.485 ***
P.m fed I + D	21.98±0.81 ***@@@$$$	7.94±0.421	20.54±0.879	15.45± 0.934 ***$$$
P.m fed C + N	21.94±0.594 ***@@@$$$	8.14±0.378	21.5±0.552	14.15±0.406 ***@@ $$$
P.m fed C + D	22.56±1.08 ***@@@$$$	8.0±0.595	14.588±0.637 ***@@$$$ £££ΨΨΨ	14.876±0.546 ***@@@ $$$££
P.m fed D +N	22.67±0.956 ***@@@$$$	8.2±0.324	16.208±1.057 ***@$$ ££ΨΨΨ	14.676±0.424 ***@@@$$$

Table 6.3. Biochemical composition of *P. monodon* larvae fed mixed algae at Z3 stage(% dry weight)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, when compared to C
@@@p<0.001, @@p<0.01, @p<0.05, when compared to I+C
$$$p<0.001, $$p<0.01, $p<0.05 when compared to I+N
£££p<0.001, ££p<0.01 when compared to I+D
ΨΨΨp<0.001, when compared to C+N



6.4. Biochemical composition of *P. monodon* larvae fed mixed algae at M3 stage

Table 6.4 summarizes about Biochemical composition of mixed-algae fed *P. monodon larva* at M3 stage. At M3 the protein value was seen to be lower than Z3 and the carbohydrate was seen to be higher than the Z3 stage. The ash content didn't show much significant variation. The protein value was seen to be higher in C+Dand D+N. The carbohydrate value was seen to be higher in I+D and C+N almost similar to their protein values. The lipid was seen to be higher in C,I+C, I+N.

When compared to *C*, a significant change was observed in the protein of I+D ($p<0.001$), C+N ($p<0.001$), C+D ($p<0.001$), D +N ($p<0.001$) but no significant change ($p>0.05$) was observed in the protein of *I*+C and I+N. When compared to *I+C* a significant change was observed in the protein of I+D ($p<0.001$), C+N ($p<0.001$), C+D ($p<0.001$), D +N ($p<0.001$) but no significant change ($p>0.05$) was observed in the protein of I+N. When compared to *I+N* a significant change was observed in the protein of

I+D ($p<0.01$), C+N ($p<0.01$), C+D ($p<0.001$), D +N ($p<0.001$). When compared to *I+D* no significant change was observed in the protein of C+N ($p>0.05$), but a significant change was observed in the protein of C+D ($p<0.001$), D +N ($p<0.001$). When compared to *C+N* a significant change ($p<0.001$) was observed in the protein content of C+D and D+N. When compared to *C+D* no significant change was observed in the protein of D +N ($p>0.05$).

When compared to *C*, a significant change ($p<0.001$) was observed in the carbohydrate content of I+D, C+N, C+D, D +N and I+N ($p<0.01$) but no significant change ($p>0.05$) was observed in the carbohydrate content of *I*+C. When compared to *I+C* a significant change ($p<0.001$) was observed in the carbohydrate content I+N, I+D, C+N, C+D, D +N. When compared to *I+N* a significant change ($p<0.001$) was observed in the carbohydrate content of I+D, C+N, C+D and D +N. When compared to *I+D* no significant change was observed in the carbohydrate content of C+N and D +N ($p>0.05$), but a significant change was observed in the carbohydrate content of C+D ($p<0.01$). When compared to *C+N* a significant change ($p<0.001$) was observed in the carbohydrate content of C+D and D+N. When compared to *C+D* no significant change was observed in the carbohydrate content of D +N ($p>0.05$).

When compared to *C*, a significant change ($p<0.001$) was observed in the lipid content of *I*+C, I+N, I+D, C+D, C+N and D +N ($p<0.001$). When compared to *I+C* a significant change was observed in the lipid content of I+N ($p<0.01$), I+D, C+D, C+N and D +N ($p<0.001$). When compared to *I+N* a significant change was observed in the lipid content of I+D, C+N, C+D and D +N ($p<0.001$). When compared to *I+D* no

significant change was observed in the lipid content of C+N and D +N ($p>0.05$) but a significant change was observed in the lipid content of C+D ($p<0.05$). When compared to *C+N* a significant change was observed in the lipid content of C+D ($p<0.05$) but no significant change was observed in the lipid content of D +N ($p>0.05$).When compared to *C+D* no significant change was observed in the lipid content of D +N ($p>0.05$).

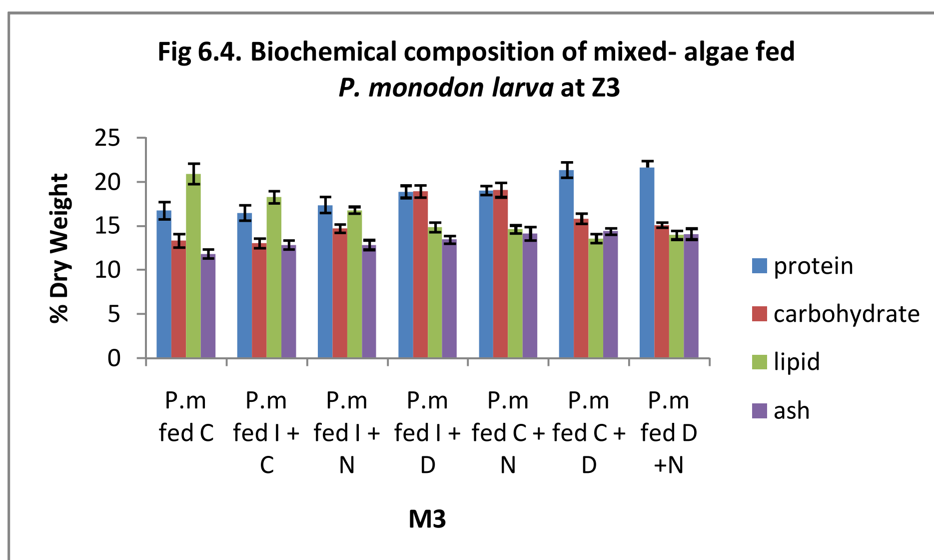
When compared to *C*, a significant change ($p<0.001$) was observed in the ash content of *I*+C ($p<0.01$), I+N ($p<0.05$), I+D, C+N, C+D and D +N. When compared to *I+C* no significant change ($p>0.05$) was observed in the ash content of I+N and I+D. but a significant change ($p<0.01$)was observed in the ash content of C+N, C+D, D +N. When compared to *I+N* a significant change ($p<0.001$)was observed in the ash content of C+N, C+D and D +N, but no significant change ($p>0.05$) was observed in the ash content of I+D. When compared to *I+D* no significant change was observed in the ash content of C+N, C+D and D +N ($p>0.05$). When compared to *C+D* and C+N no significant change was observed in the ash of D +N ($p>0.05$).

	Biochemical composition			
Algae	**Protein**	**Carbohydrate**	**Lipid**	**Ash**
P.m fed C	16.74± 0.976	13.32± 0.766	20.92±1.152	11.844±0.498
P.m fed I + C	16.48± 0.87	13.04± 0.555	18.28±0.668 ***	12.856±0.533**
P.m fed I + N	17.38± 0.906	14.7± 0.484 **@@@	16.8± 0.393 ***@@	12.856± 0.548 *
P.m fed I + D	18.9± 0.689 ***@@@$$	18.94± 0.712 ***@@@$$$	14.84±0.541 ***@@@$$$	13.45±0.441 ***
P.m fed C + N	19.02± 0.507 ***@@@$$ ΨΨΨ	19.1± 0.824 ***@@@ $$$ΨΨΨ	14.62±0.460 ***@@@$$$	14.116± 0.756 ***@@$$ ££
P.m fed C + D	21.38± 0.858 ***@@@$$$ £££ΨΨΨ	15.84± 0.577 ***@@@$ £££ΨΨΨ	13.58±0.486 ***@@@$$$ £££Ψ	14.396± 0.382 ***@@$$
P.m fed D +N	21.64±0.766 ***@@@ $$$£££	15.12± 0.309 *** @@@£££	13.96±0.487 ***@@@ $$$££	14.096± 0.620 ***@@$$

Table 6.4. Biochemical composition of *P. monodon* larvae fed mixed algae at M3 stage (% dry weight)

Values are mean ± SD of 4-5 separate experiments; n = 10 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, *p<0.05 when compared to C
@@@p<0.001, @@p<0.01, when compared to I+C
$$$p<0.001, $$p<0.01 when compared to I+N
£££p<0.001, ££p<0.01, when compared to I+D
ΨΨΨp<0.001, Ψp<0.05, when compared to C+N



DISCUSSION

Feed use by crustaceans is dependent, among other things, on feed size, digestibility, nutrient biodisponibility and essential nutrients (Kurmaly *et al* 1989; Webster *et al* 1994). Hence, the differences in larvae response to be a consequence of the nutritional composition of the diet have to be considered. Feed mixtures are generally recommended because they produce better results than when a particular feed is used alone, as mixtures contain a complementary blend of nutrients, such as amino acids, that meet or exceed nutritional requirements. For our diets, increased substitution of *C. muelleri* by SPM resulted in higher protein being offered to larvae, while lipids and energy diminished (Table 3). The chemical score shows that histidine and lysine may become limiting at substitution levels over 50%. Shuli and Baoqing (1992) used *C. muelleri* and *Spirulina sp*. to feed larvae of *Penaeus orientalis* and found that, regardless of the protein content, feeds produced poorer results when used alone, rather than in combination.

The protein values of *Farfantepenaeus paulensis* decreased to a minimum in Protozoea Stages I±III and M I, and thereafter gradually increased until postlarva stage; this is similar to observations of the larval development of *Marsupenaeus japonicus* and *Litopenaeus setiferus* (Lovett and Felder 1990). The reduced protein content in protozoea coincides with a low RNA: DNA ratio, indicating a decreased cellular multiplication rate and consequently less protein synthesis.

In late larval stages, thoracic appendices are now more specialized, enabling a better manipulation and selection of food particles (Jones *et al* 1992) , leading to the ingestion of more digestible parts of food. The retention time of food in the gut is longer (Jones *et al* 1992), and the development of the gastric mill may contribute to the processing of food during early post- larval stages (Lovett and Felder 1989). The morphological and behavioral changes in mysis and early postlarvae may compensate for the lower digestive -enzyme activity when the transition to the benthic life begins. The reduced metabolic activity, food uptake, and assimilation capacity of early postlarval stages accompany the search for a suitable new habitat after migration into inshore, brackish, nursery grounds.

The biomass consumed by *Litopenaeus vannamei* larvae differed depending on the algae used for feeding, even though all the species tested were ingested by the larvae. Of all the microalgae used in this experiment, the diatoms produced the highest consumable biomass. This demonstrates the advantage of using diatom species as food for larval development of shrimp and other marine organisms (Simon1978).

Achieving optimal algal composition for bivalve feed which produces favorable traits has been the aim of extensive nutritional studies for many aquaculture species (Brown and Robert, 2002). However, the often unreliable and time consuming production of microalgae is presently a major issue in bivalve hatcheries. Experiments performed in the study of Pettersen *et al* 2010 were carried out to improve hatchery efficiency by evaluating the nutritional importance of *C. calcitrans*, the culture of which has proven to be a negative impact on overall hatchery efficiency and management costs. This study clearly shows that the algal composition of the diet can significantly affect the performance of hatchery reared blue mussel larvae.

The visual scoring of lipid content was highly variable and somewhat subjective and thus caution should be used in the interpretation of such data. Further validation of this technique is required to determine if it reflects real differences in lipid content in larvae. Nevertheless, relative individual fatty acids of the larvae differed significantly between treatments, and are likely to reflect the fatty acid composition of the algae species being preferentially consumed. As such, the predominant influence of diatoms on fatty acid composition of the larvae tissue clearly suggests the existence of selective utilization of *C. calcitrans*, followed by *C. muelleri*. In contrast, the fatty acid composition of *I. galbana* and *P. lutheri* appear to make little contribution towards the final larval body composition (low R2 values), which suggests that flagellates are not actively consumed, at least when diatoms are also provided, under these experimental conditions. This conclusion is consistent with a previous report on *M. edulis* adults where diatoms were selectively digested in the gut compared with flagellates. However iin the present study the fattyacids and

aminoacids of the larvae is not given due to the technical difficulties experienced during the assay.

Fatty acid groups detected in the larvae tissue were also found to show a significant correlation with larval survival, where mortality proportionally increased with MUFA and decreased with higher SFA. This was unexpected, given a paucity of information in research to support the importance of SFA and MUFA for bivalve larvae survival. The physiological importance of these fatty acids, where the MUFA products formed from the desaturation of some SFA possess lower melting temperatures. Hence conversion from SFA to MUFA products can alter the viscosity of the cell membranes and, hence, cellular activities, which may have caused detrimental effects on larval survival.

It is suggested that the ratio between n-3 LC-PUFA/n-6 LC-PUFA in microalgae that plays a significant role in fulfilling the dietary requirements of viable bivalve larvae, rather than their individual fatty acids, as demonstrated by Rico-Villa *et al* (2006) with imbalances in EFA producing poor settlement. Consequently, the present findings have further highlighted the importance of determining a correct ratio of dietary components, such as DHA, EPA and ARA, to identifying a suitable mixed microalgae species diet.

The protein allowances in shrimp diets, like fin fishes, are appreciably higher than those in the diets of terrestrial warm-blooded animals. The optimal protein level for *P. monodon* has been studied mostly with juveniles (between 0.5 to 1.8 g) using various types of protein (Table 1). In general, the optimal level is around 40% of the diet based on weight

gain and feed conversion. In one study with adult shrimp, the optimal protein level in the diet of broodstock *P. monodon* was suggested to be between 50 and 55% (Millamena *et al* 1986). No information is available as to the requirements of young adults. Protein requirements of finfish are suggested to decrease as fish approach maturity. The reported estimates of protein requirements must be carefully examined because the requirements are dependent on quality (essential amino acid profile and digestibility) of dietary protein, age, and physiological state of crustaceans. Protein requirements of shrimp may be affected by the environment. *P. monodon* grow significantly faster in brackish water than in seawater (Deshimaru *et al* 1985).

The optimal dietary protein level (40%) of juvenile *P. monodon* reared in seawater was found to be lower than that (44%) of the shrimp reared at 16 ppt (Shiau and Peng 1992). These salinity effects may be caused by the differential utilization of dietary protein as an energy source or protein digestibility when the shrimp are raised at various salinities. *P. monodon* acclimated at low salinity showed higher ammonium-N excretion than those acclimated at high salinity (Lei *et al* 1983), indicating that shrimp raised in low salinity are prone to use protein, not lipid, as an energy source. Salinity has been reported to affect protein digestibility of the diets but the mechanism is not clear. Little information exists to show the effects of water temperature. Protein requirements for finfish are unaffected by water temperature. Various proteins from animal and plant sources have been tested for their dietary effectiveness in *P. monodon*. Pascual *et al* 1983 found no significant differences in weight gains of juvenile *P. monodon* fed diets with varying levels (15 to 55%) of defatted soybean meal when the shrimp were raised in net cages set on the bottom of an earthen pond where natural foods were available.

Simple carbohydrates are considered to be inferior to complex carbohydrates in promoting growth in many shrimp, including *P. monodon* (Chen, 1998). Dietary lipid is utilized to satisfy the need for essential fatty acids, other lipid-soluble compounds, and energy. Among the required fat-soluble compounds in the diets of *P. monodon*, polyunsaturated fatty acids, phospholipids, and sterols have received the most attention. Sheen and Liao 1993 found that weight gains of juvenile *P. monodon* fed isoenergetic and isonitrogenous diets containing between 4 and 11.3% of a mixture of cod liver oil and corn oil are significantly higher than those containing 0 or 2% oil mixture. They suggested that *P. monodon*, like other penaeid species, does not require a specific level of dietary lipid if requirements for essential lipid-soluble components are satisfied. Little is known as to the essential fatty acid requirements of *P. monodon*. From the growth results of various lipid studies the essential fatty acid requirements of *P. monodon* are similar to or lower than those of *P. japonicus*. *P. japonicus* was suggested to require n-3 highly unsaturated fatty acid (HUFA) such as 20:5n-3 and 22:6n-3 at a combined level of 0.5 to 1% of diet for optimal growth (Kanazawa et al., 1979).

Growth experiments of early postlarval *P. monodon* indicated the requirement for n-3 HUFA to be no less than 0.5 to 1% of diet (Chen and Tsai, 1986). Postlarval *P. monodon* grew well on an *Artemia* diet low in n-3 HUFA content, and excessive n-3 HUFA was not beneficial to the growth of the postlarvae. Their requirements in *P. monodon* have been well studied. Pascual (1986) reported that weight gain of *P. monodon* significantly increased as the level of crude soy lecithin was increased from 0 to 2% regardless of lipid source. Chen (1993) fed juvenile *P. monodon* with test diets containing three levels of cholesterol (0 to 1% of diet) in

combination with four levels of purified phosphatidylcholine (0 to 5%). The shrimp attained optimal growth when the diets contained 0.5% cholesterol or 1.25% phosphatidylcholine. The optimal dietary level for phospholipids was suggested to be 2.5% when crude lecithin is used. Sheen and Liao (1993) found no difference inweight gain in *P. monodon* fed diets containing between 0.2 and 0.8% cholesterol and suggested that 1% addition may have an adverse or toxic effect on growth.

Although the energy contents in the diets are best presented as digestible energy in addressing the energy need little information is available for the digestible energy in feedstuffs for *P. monodon*. Despite the rapid advancement in many aspects of shrimp nutrition, mineral requirements of *P. monodon* have received little attention.

Methods to manufacture the diets and to effectively feed the animals have received more attention in larval *P. monodon* nutrition than the nutrients in the diets. Studies examining the relationships between spawner nutrition, egg quality, and larval growth and survival, especially in terms of lipid requirement have also attracted attention (Millamena, 1980). Despite all the progress in recent years, knowledge of the nutritional requirements of *P. monodon* still lags behind industry needs. A more thorough understanding of nutritional requirements is needed to optimize a cost effective and environment- friendly feed formulation for *P. monodon* and other shrimp species.

Chapter- 7

BIOCHEMICAL COMPOSITION OF MICRO ALGAE

Nauplii shift from endogenous feeding to herbivorous protozoeae, Shrimp nutrition is very complex because the nutritional requirements of shrimp change with each stage of the life cycle. Throughout their life cycle, shrimp exhibit various modes of feeding. As young larvae zoea and mysis, they are planktivorous, filtering microscopic algae and other suspended materials out of the water. As older larvae they are primarily predators consuming largely animal protein sources (e.g. Artemia). Thus, shrimp feeds must be specifically formulated for different stages of the life cycle. Although the nutrient source may vary, certain nutrients are required by all growing animals for normal growth and maintenance. These are known as essential or indispensable nutrients like essential amino acids, carbohydrates which can be derived from various feed ingredients, stored and released via several metabolic processes, in addition dietary lipids and lipid stores can serve as energy sources. Finally, there are essential fatty acids (components of lipids), vitamins and minerals.

Because the biochemical composition of each micro algae species varies, the use of mono algal diets could produce a shortage of essential nutrients needed for the adequate development of penaeid shrimp. Protein, which is required for growth and maintenance, is an expensive component in a diet. Excessive protein levels will increase feed cost and nitrogenous

waste. Therefore, knowledge of the optimum level of protein and the protein-sparing effects of non-protein nutrients such as lipids or carbohydrates would be effective in reducing feed costs and water pollution. Shrimp, like other crustaceans, entirely depend on their dietary supply of carotenoid. The major carotenoid found in crustacean tissues and responsible for typical color of *P. monodon.*

7.1. Biochemical composition of algae

The protein was found to be significantly higher in D when compared to all other algae. The next high value was seen in N and then in I. The C had the low protein value. The carbohydrate was found to be significantly higher in N, when compared to all the other algae the values were almost same. The lipid was found to be higher in D and then almost same in C and N. The ash content was found to be higher in C and then N and then almost same in I and D.

Biochemical composition / Algae	Protein	Carbohydrate	Lipid	Ash
Chaetoceros	24.425±1.491	12.863±1.721	14.53±0.751	16.525±0.857
Isochyrsis	33.578±1.779 ***	13.5±0.6164	11.585±0.588 ***	12.65±1.34 **
Dunaliella	45.5± 2.192 ***@@@	12.525±0.7136	25.575±0.899 ***@@@	12.75±1.173 ***
Nanochloropsis	37.368±2.663 ***@$$$	22.75±2.493 ***@@@$$$	15.733±0.908 @@@$$$	15.575±0.899 @@$$

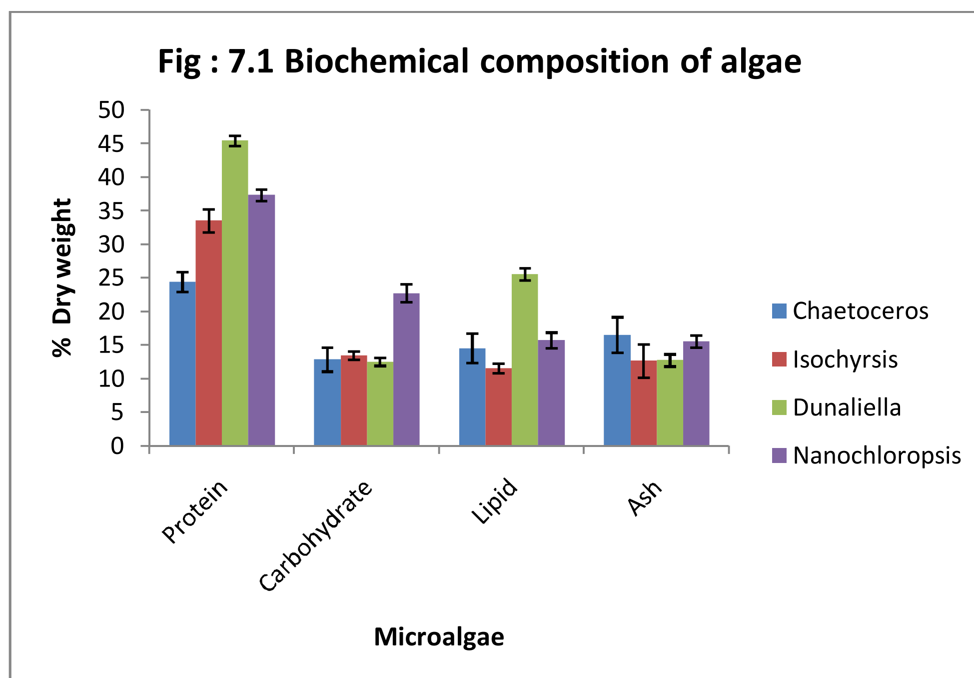
Table 7.1. Biochemical composition of algae(% dry weight)

Values are mean ± SD of 3-4 separate experiments; n = 4-5 in each group. ANOVA followed by Students-Newman-Keuls Test.

$^{***}p<0.001$, $^{**}p<0.01$, when compared to *Chaetoceros*

$^{@@@}p<0.001$, when compared to *Isochyrsis*

$^{\$\$\$}p<0.001$, when compared to *Dunaliella*



7.2. Amino acid composition of algae

The essential amino acids Threonine high in N, Arginine and Valine high in C Histidine was seen to be high in N, Isoleucine high in D and N, Methionine was seen to be high in C, Phenylalanine high in I and N, Leucine was seen to be high in N, Lysine was seen to be high in C, Tryptophan was seen to be high in N.

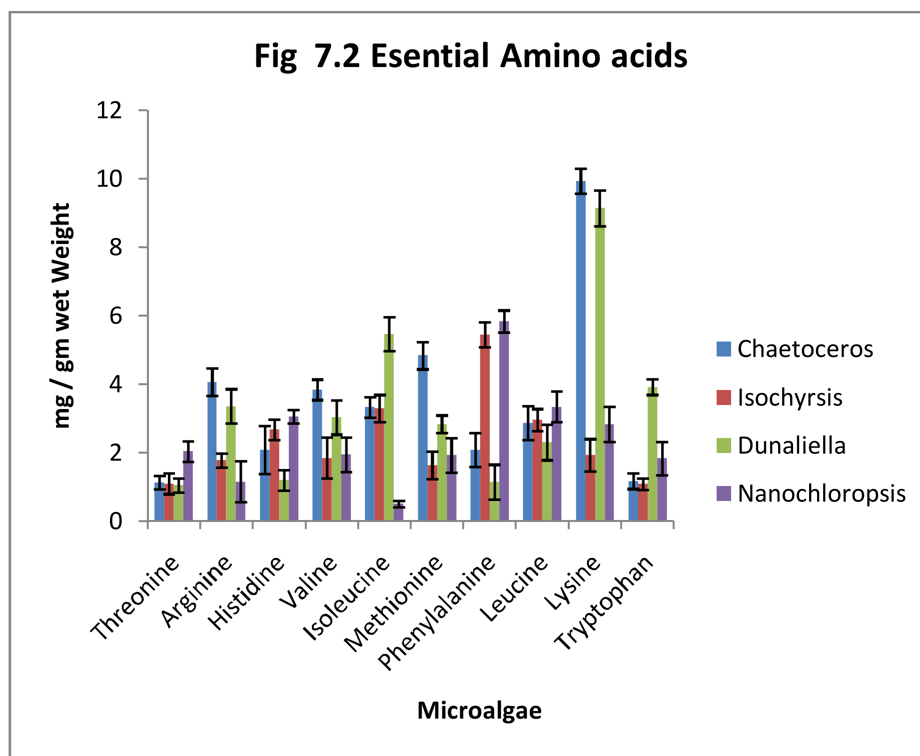
The non essential aminoacids Glycine high in C and N, Cysteine high in C and D, Tyrosine high in C and I, Aspartic acid same in in C, I, D and nil in N, Serine high in C and N, Glutamine high in D, Asparginine high in C, and then in D and N, Proline high in C and then in D, Glutamic acid high in C and then D, Alanine was seen to be high in C and N.

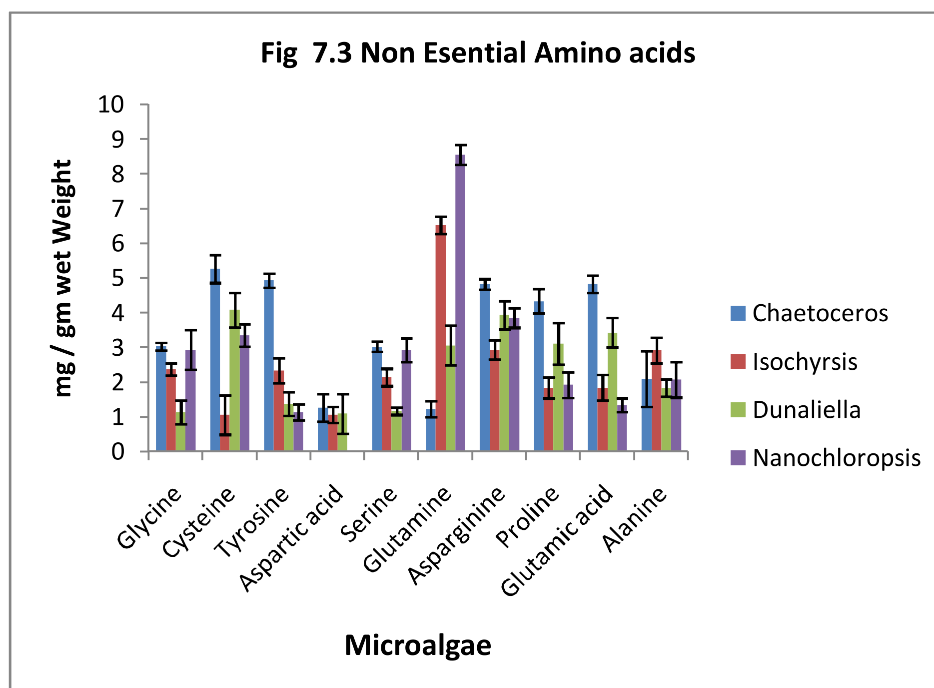
Algae Amino acid	*Chaetoceros*	*Isochyrsis*	*Dunaliella*	*Nanochloropsis*
Threonine	1.13± 0.2	1.09± 0.3***	1.04± 0. 2***@@@	2.04± 0.3***@@@$$$
Arginine	4.06± 0.4	1.77± 0.2***	3.36±0.5***@@@	1.15± 0.6***@@@$$$
Histidine	2.08± 0.7	2.67± 0.3***	1.19± 0.3***@@@	3.05± 0.2***@@@$$$
Valine	3.84± 0.3	1.84± 0.6***	3.03± 0.5***@@@	1.94± 0.5***@@@$$$
Isoleucine	3.33±0.3	3.29± 0.4	5.47± 0.5***@@@	5.00± 0.5***@@@
Methionine	4.84±0.4	1.63± 0.4***	2.83± .26***@@@	1.92± 0.5***@@@$$$
Phenylalanine	2.08±0.5	5.45± 0. 36*	1.14± .51***@@@	5.84± 0.32***@@@$$$
Leucine	2.87± 0.5	2.96± 0.32*	0.23± .52***@@@	3.34± 0.45***@@@$$$
Lysine	9.94± 0.36	1.93± 0.47***	9.15± .52***@@@	2.83± 0.51***@@@$$$
Tryptophan	1.17± 0.23	1.08± 0.17**	3.92± .23***@@@	1.83± 0.49***@@@$$$
Glycine	3.04± 0.11	2.38± 0.17***	1.15± .34***@@@	2.94± 0.57**@@@$$$
Cysteine	5.27±0.4	1.07± 0.57***	4.09± 0.5***@@@	3.36± 0.32***@@@$$$
Tyrosine	4.94±0.02	2.35± 0.36***	1.38± .34***@@@	1.15± 0.23***@@@$$$
Aspartic acid	1.27± 0.4	1.07± 0.23***	1.10± 0.57***	0.000***@@@$$$
Serine	3.03± 0.15	2.15± 0.25***	1.18± .11***@@@	2.94± 0.34**@@@$$$
Glutamine	1.23±0. 23	6.53±0.25***	3.07±0.57**@@@	8.56± 0.28***$$$
Asparginine	4.83±0.152	2.94±0.28***	3.94± 0. 4***@@@	3.86± 0.28***@@@$$$
Proline	4.34±0.35***	1.85±0.3***	3.12± 0.6***@@@	1.93± 0.37***@$$$
Glutamic acid	4.83±0.25	1.85±0.37***	3.44±0.43***@@@	1.35±0. 0@@@$$$
Alanine	2.1±0.8	2.93±0. 37***	1.84±0.25***@@@	2.08±0.51***@@@$$$

Table 7.2. Amino acid composition of algae (mg/g wet wt)

Values are mean ± SD of 3-4 separate experiments; n = 4-5 in each group. ANOVA followed by Students-Newman-Keuls Test.

***p<0.001, **p<0.01, when compared to *Chaetoceros*
@@@p<0.001, @ p<0.05, when compared to *Isochyrsis*
$$$p<0.001, when compared to *Dunaliella*





7.3. Fatty acid composition of algae

The major fattyacids were analysed. Palmitic acid margaric acid Stearic acid Oleic acid Linolenic acid α - Linolenic acid allwere seen to be higher in *Dunaliella salina.* The EPA was found to be higher in N and then in D. The DHA was found to be higher in C and then almost same in I and D and nil in N

Algae / Fatty acid	*Chaetoceros*	*Isochyrsis*	*Dunaliella*	*Nanochloropsis*
Palmitic acid	6.43±0. 25	4.46±0. 45***	1.04±0.41***$^{@@@}$	3.5±0.45***$^{@\$\$\$}$
margaric acid	0.000	153±0. 25***	0.000$^{@@@}$	1.76±0.23***$^{\$\$\$}$
Stearic acid	3.56±0. 3	5.66±0.2***	2.12±0.15***$^{@@@}$	6.6±0.15***$^{@@@\$\$\$}$
Oleic acid	1.26±0. 2	7.53±0. 35**	4.35±0.4***	756±0.35***$^{@@@\$\$\$}$
Linolenic acid	5.33±0. 35	633±0. 32**	605±0.4***	6.43±0.11***$^{@@@\$\$\$}$
α - Linolenic acid	8.66±0. 32	8.33±0. 32	7.85±0.32***$^{@@@}$	8.3±0.2$^{\$\$\$}$
EPA	0.000	2.4±0. 45	8.96±0.49***$^{@@@}$	1.197±0.57***$^{@@@\$\$\$}$
DHA	13.3±0.7	6.68±0.15***	6.61±0.63	0.000***$^{@@@\$\$\$}$

Table 7.3. Fatty acid composition of algae(mg/g wet wt)

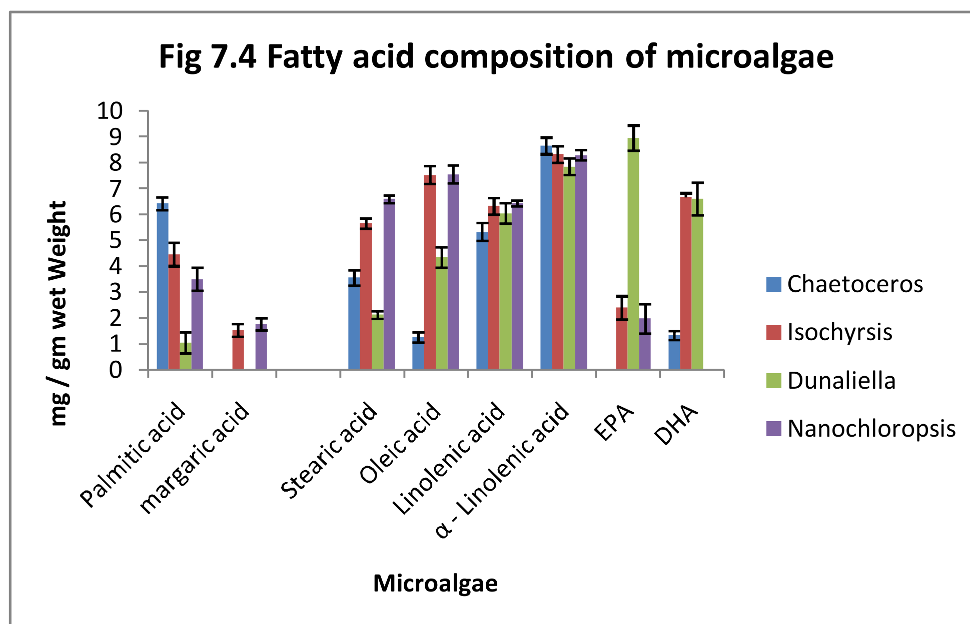
Values are mean ± SD of 3-4 separate experiments; n = 4-5 in each group.

ANOVA followed by Students-Newman-Keuls Test.

***$p<0.001$, **$p<0.01$, when compared to *Chaetoceros*

$^{@@@}p<0.001$, $^{@@}p<0.01$, when compared to *Isochyrsis*

$^{\$\$\$}p<0.001$, when compared to *Dunaliella*



7.4. Correlations Analysis - Protein

The protein values shows +ve correlation with C, C+N, C + D, D + N

Algae	Z3	M3
Chaetoceros	0.999	0.950
Isochyrsis	-0.968	0.282
Dunaliella	-0.039	-0.852
Nanochloropsis	-0.652	-0.318
C	-0.431	0.748
I + C	-0.326	-0.783
I + N	-0.910	0.043
I + D	0.144	-0.942
C + N	0.909	0.504
C + D	0.108	-0.912
D + N	0.362	0.576

Table 7.4. Correlations Analysis - Protein

7.5. Correlations Analysis - Carbohydrate

The Carbohydrate values shows +ve correlation with C, D, N, C + D, D + N

Algae	Z3	M3
Chaetoceros	0.144	-0.152
Isochyrsis	0.107	-0.208
Dunaliella	0.425	0.777
Nanochloropsis	0.857	0.820
C	-0.160	-0.802
I + C	0.072	-0.746
I + N	0.465	0.401
I + D	-0.463	-0.321
C + N	-0.071	-0.075
C + D	0.578	0.816
D + N	0.778	0.323

Table 7.5. Correlations Analysis – Carbohydrate

7.6. Correlations Analysis - Lipid

The lipid values shows +ve correlation with C, I, D, C + D, D + N, -ve correlation with N, I+D, C+N,

Algae	Z3	M3
Chaetoceros	0.939	0.416
Isochyrsis	0.353	0.822
Dunaliella	0.650	0.919
Nanochloropsis	-0.934	-0.318
C	0.118	0.157
I + C	-0.215	-0.407
I + N	-0.420	-0.015
I + D	0.895	-0.938
C + N	-0.668	-0.765
C + D	0.731	0.545
D + N	0.366	0.164

Table 7.6. Correlations Analysis - Lipid

7.7. Correlations Analysis - Ash

Algae	Z3	M3
Chaetoceros	-0.765	-0.056
Isochyrsis	-0.768	-0.800
Dunaliella	0.231	-0.862
Nanochloropsis	-0.798	-0.110
C	0.895	-0.934
I + C	-0.018	-0.698
I + N	0.613	-0.236
I + D	-0.442	-0.527
C + N	-0.563	-0.091
C + D	0.277	-0.637
D + N	0.717	0.066

Table 7.7. Correlations Analysis - Ash

7.8 Pigments of the Algae

pigments	A	B	C	D
Beta carotene	++	+	+	+
Chlorophyll a	+++	+++	++	+++
Chlorophyll b	-	-	-	+
Chlorophyll c	-	++	++	-
Lutein	+	-	+	++
Xanthophylls	++	++	++	+++
Fucoxanthin	-	+++	+++	-
Violaxanthin	+	-	+++	+++
Neoxanthin	-	-	+++	+++
+-Faint, ++-medium, +++-High				

A- *Nannochloropsis*
B- *Isochrysis*
C- *Chaetoceros*
D- *Dunaliella*

DISCUSSION

With respect to the nutritional value of some species of algae of interest for aquaculture, Fernandez-Reiriz *et al* (1989) have shown that flagellates have a better nutritional value than diatoms as a result of the higher concentration of protein and polyunsaturated fatty acids (n-3 PUFA). Considering aspects, size and nutritional quality, the greater need for diatoms in comparison with flagellates becomes clear in the case of *P. setiferus* larvae. Since diatoms are small they can be consumed from the first phases of larval development (PZ1 and PZ2) onwards, while the nutritional deficiencies are compensated by ingesting large quantities. Thus diatoms would determine development, growth and survival of the larvae as a result of their availability during the first larval phases, rather than of their nutritional value.

It has been reported that protein is an essential nutrient for prawns (Andrews *et al* 1972; Venkataramiah *et al* 1975). Protein, which is required for growth and maintenance, is an expensive component in a diet. Excessive protein levels will increase feed cost and nitrogenous waste. Therefore, knowledge of the optimum level of protein and the protein-sparing effects of non-protein nutrients such as lipids or carbohydrates would be effective in reducing feed costs and water pollution. Carbohydrate is the most economical dietary energy source (cost/kcal). There is little information on the carbohydrate nutrition of prawns (New 1990; Kanazawa 1985). The types and levels of carbohydrate in the diet have been shown to affect the growth of *P. japonicus* (Deshimaru and Yone 1978; Abdel *et al* 1979), *P. aztecus* (Andrews *et al* 1972) and *P. duorarum*.

Various carotenoid pigments have been tested in the diets of crustaceans to enhance the pigmentation of the animals in order to increase market value. Crustaceans cannot synthesize carotenoids *de novo* but are able to transform dietary pigments into endogenous forms. Carotenoids such as astaxanthin, canthaxanthin, and b-carotene as well as pigment-containing algal meal or oils are the major sources of pigments. The body color of *P. monodon* is the result of interaction between blue carotenoproteins and red astaxanthin esters.

Shrimp, like other crustaceans, entirely depend on their dietary supply of carotenoid. The difference between juveniles and larvae could be attributed to the developmental stage of the shrimp or the type of diet (microparticulate diets were used in the study by Teshima *et al* 1983). The inability of juvenile or adult shrimp to utilize crystalline amino acids in the diet have complicated the study of essential amino acid requirements.

Astaxanthin is present more in the muscular epidermis than in the exoskeleton. The content of astaxanthin as carotenoproteins in the epidermis is constant regardless of body color, whereas the content of astaxanthin esters, especially monoester, is higher in the epidermis of well-pigmented shrimp than that of pale ones. Highest increase of carotenoid content in exoskeleton was observed when *P. monodon* were fed *Spirulina* meal, when b-carotene, *Spirulina*, *Phaffia,* or krill oil were compared. The major carotenoid in *Spirulina,* zeaxanthin, was suggested to be rapidly converted to astaxanthin by *P. monodon*. Purified astaxanthin was found to be more effective in enhancing the body color of *P. japonicus* than b-carotene and a microalgal (*Dunaliella salina*) meal (Chien and Chih 2004). The comparative effectiveness of purified carotenoids in *P. monodon* is yet

to be determined. Larval rearing with microparticulate diets is widely practiced. The nutritional requirements of larval *P. monodon* are yet to be defined. Their status has been briefly reviewed by Chen 1993.

The major carotenoid found in crustacean tissues and responsible for typical color of *P. monodon* is astaxanthin. Nutritional deficiency with respect to carotenoids has been suggested as the cause of blue disease in farmed *P. monodon* although *P. monodon* can convert canthaxanthin to asthaxanthin, the efficiency of conversion was only 2-3 to 1 or lower. Distinction of color was developed in 15 days of feeding a diet containing 50-75 ppm astaxanthin.

The differences in the composition of fatty acids in the algal diets seemed to be the factor most likely to explain the differences in larval survival and development. The gross lipid and carbohydrate compositions of the algal diets did not explain the observed differences in growth of the larvae nor did they correspond to the gross composition of the larvae. The protein levels were not presented due to problems with the assay (D' Souza 1998).In the present study the protein that were measured in this study were 24 to 45% dry wt for the different species of algae(Brown *et al* 1997).

Kanazawa *et al* 1979 have shown the absence of *de novo* synthesis of linoleic (18:2n-6), linolenic (18:3n-3), eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids from 1 4C acetate or 1 4C palmitic acid in *P. monodon.* This suggests that *P. monodon* require some of these fatty acids as essential nutrients. Catacutan (1991) reported that 2.6% dietary n-3 HUFA enhanced growth of juvenile *P.monodon,* but levels of 18:2n-6 higher than 5% have a negative effect. Kontara (1986) found that *P.*

monodon larvae which received *Artemia* enriched with n-3 HUFA had higher growth and survival rate than shrimp larvae fed *Artemia* nauplii of the same strain. Catacutan (1991) and Millamena (1989) indicated that n-3 HUFA was an essential fatty acid for *P.* monodon and the requirement was estimated (from 15.03% n-3 HUFA in a 12.14% dietary lipid) to be 1.8-2.6% of the diet.

Quantitative requirements of several essential amino acids for *P. monodon* have been revealed. Studies utilizing microencapsulated L-arginine indicated a requirement of 2.5 g/100 g diet (5.5 g/100 g protein) to achieve optimal growth by juvenile *P. monodon* (Chen *et al* 1992). By adjusting dietary pH to neutrality and increasing meal frequency to five times per day, Liou and Yang (1994) successfully incorporated crystalline methionine and other amino acids in the diet of juvenile *P. monodon* and estimated the methionine (+cystine) requirement to be 1.4 g/100 g diet (4.0 g/100 g protein). Millamena *et al* 1993 showed that the threonine requirement for postlarval *P. monodon* growth is be 1.4 g/100 g diet (3.5 g/100 g protein) using diets containing a pure amino acid mixture. The amino acids were neutralized and coated with carboxylmethylcellulose and feed pellets were coated with carrageenan. Using the same technique they found the requirements of *P. monodon* for arginine, lysine, methionine and valine to be 5.5, 5.55, 2.2, and 3.75 g/100 g protein, respectively.

The requirements for 10 essential amino acids by *P. monodon* have been estimated based on the empirically determined arginine requirement (5.5 g/100 g protein) and the muscle amino acid profile of juvenile *P. monodon* (Table 2). Growth of postlarval *P. monodon* fed diets containing varied levels of protein and corn starch (10 to 40%) was not affected by

starch content (Bages and Sloane, 1981). On the other hand, when dietary sugar ranging between 10 and 30% was evaluated, a level of 20% resulted in the best weight gain of *P. monodon,* whereas the 30% level gave the lowest weight gain (Alava and Pascual, 1987). There was no differential growth among *P. monodon* juveniles fed straight wheat flour, first-grade clear wheat flour, or second-grade clear wheat flour at a level of 35% (Shiau *et al* 1991).

The *T. suecica* component may improve the mixed diet because it contains high proportions of 18:2 (n-6) and 18:3 (n-3). A mixture of *C. muelleri* and T-*iso* might also provide a diet encouraging high survival and fast development because of the presence of 18:2 (n-6), 18:3 (n-3) and 22:6 (n-3) in the T-iso cells. These latter two species are part of the standard Aquacop protocol for rearing *Penaeus vannamei* larvae, but there are no survival and growth comparisons to other species of algae to confirm their superiority as a diet (Aquacop1983).

The species belonging to the genus *Chaetoceros* have an adequate biochemical composition of essential polyunsaturated fatty acids, mainly omega 3 highly unsaturated fattyacids (D'Souza and Loneragan 1999), required for the growth of several aquatic organisms. The species *C. gracilis* and *Chaetoceros* sp. G1 have high levels of lipids (>12%), mainly from polyunsaturated fatty acids. Although *C. calcitrans* demonstrated the second best results as a diet for *P. monodon* larvae in this study, *Chaetoceros* produced a survival rate which was statistically different.

It appears that concentrating and storing *C. calcitrans* reduces its nutritional properties for prawn larvae. However centrifuged *C. calcitrans* stored for 6–7weeks and fed to oyster larvae apparently performs as well as its fresh counter part in terms of survival of oyster larvae but with as light decline in growth rate. Survival alone is not a sensitive measure of the nutritional value of diets for prawn larvae (D'Souza and Kelly 2000).

Combinations of centrifuged concentrates are far superior diets for both larval and juvenile oysters compared with single algal diets (Heasman *et al* 2000). Therefore combinations of flocculated concentrates of different species may prove to be better diets for prawn larvae than single species, as is the case with combinations of two species of fresh algae (D'Souza and Loneragan1999). Combinations of fresh and concentrated algae in different proportions may also be effective for prawn larvae, while reducing reliance on fresh algal culture.

The macronutrient content and fatty-acid profile of the algal species used in this study are summarised by Kurmaly *et al* 1989. The quantities of these macronutrients in all the algal species are within the range required for penaeid larvae. It is not always correct to give the nutritional contents of algae as the only reason for poor larval performance with algal feeds. Rodriguez *et al* 1994, fed *P. japonicus* mysis larvae on *Chaetoceros gracilis* containing only 7% protein and separately on *Artemia* with a much higher protein content, they obtained no significant differences in the growth, survival, protein or lipid content of the postlarvae. Hence, rearing penaeid larvae on more than one algal species with a wide diversity of macronutrients and micronutrients, such as vitamins, has a better chance of meeting nutritional requirements.

Nearly all microalgae biomass was reported to be rich in polyunsaturated fatty acids (PUFA) and could be an important source of essential FA for aquatic animals (Becker, 1994; Olvera-Novoa et al., 1998). However, in the study conducted by Ju *et al* 2009, the two algae samples of *Nannochloropsis* and a diatom had no nutritional advantage in terms of FA composition, compared with the control diet.

According to the amino acid requirements of the species, the authors consider that this was a consequence of lysine, arginine and methionine being limiting in both feeds. Thus, optimal FL and DI responses can be explained in terms of the nutrient balance achieved with a particular diet. The benefit of using dietary mixtures that exceed nutritional requirements should be understood as nutrients in excess have also a high energetic cost (Tacon, 1990).

The fatty acid composition of *P. pinguis* measured in the study (Haesman *et al* 2000., Ponnis *et al* 2008) was similar to that reported by Volkman *et al* (1989), while higher saturated and monounsaturated contents and lower polyunsaturated fatty acid levels (with particular reference to EPA and DHA) were reported for the same species by McCausland et al. (1999). Apart from a lower content of 16:1n-7 and higher concentrations of 18:4n-3 EPA and DHA reported here, the fatty acid profile of R. salina corresponds to that reported for the same species by Volkman *et al* 1991.

DHA levels in algal tissue samples were found to be relatively low for both *C. calcitrans* and *C. muelleri*, consistent with levels reported by Delaunay *et al* (1993) and Martinez *et al* (1998). However, *C. calcitrans*

contained significantly higher levels of EPA (Whyte *et al* 1990). A distinct correlation between bodily DHA content and survival shows the importance of DHA for larval health, which is demonstrated by an observed increase from treatments rich in *C. calcitrans* and lower in *C. muelleri*. Similar results in the sea scallop, where a diet which included *C. muelleri* produced moderate larval survival (compared with diets excluding the diatom), and which was suggested to be the result of a dietary deficiency in DHA.

Although several studies on the nutritional value of microalgae and basic nutritional requirements of reared molluscs have been carried out, their relationships remain poorly defined. The food value of a given microalga depends on both the mollusc species and growth stage considered (Brown *et al* 1997; Knauer and Southgate, 1999; Muller *et a*l 2003). For example, in the present study, the low food value of *R. salina* for *C. gigas* and *P. maximu*s larvae has been demonstrated. However, this species has been reported to be of interest when used as food for *Pecten margaritifera* larvae and for *C. gigas* juveniles (Brown *et al* 1998).

For both larvae and juveniles, diets containing *C. calcitrans* (live or concentrated) were usually amongst the better-performing diets. Brown and Robert (2002) found that the control oysters had good growth and survival and these were not improved by supplementing with any of the *T. Isochrysis* live or concentrated diets. The good food value of *C. calcitrans* as a single species for *Ostrea edulis* spat has been reported by Laing and Millican (1986) who considered that this species is a complete or almost complete food for juvenile flat oysters. Such high nutritional performances

with mollusc larvae including *C. gigas* have been related by Robert *et al* (1989) and Utting and Millican (1997).

Cellular carbohydrates vary quantitatively according to culture conditions (Brown *et al* 1998). Carbohydrates are the first products of photosynthesis in all algae (Calvin-Benson cycle) and provide the precursors for all cell components. The principal storage polysaccharide in the Eustigmatophyceae (including *N. salina*) is β-glucan.

The major monosaturated fatty acids were C16:1 in *P. tricornutum, Synechococcus sp*. and *Tribonema sp*., and C18:1 in the others. The abundance of PUFAs showed pronounced variation between algal species and classes. The highest amount of EPA was found in *N. salina* (11.9 mg g^{-1}), followed by *D. salina* (8.9 mg g^{-1}), while DHA was abundant only in *C. calcitrans* (13.3 mg g^{-1}) (Table 7.4). In general, our data were in good accordance with results from the literature. The results shown here were also representative of the growth and composition of the same algae cultured in earlier series.

Senthil *et al* 2011 studied the proximate composition analyses of crude protein, lipid and carbohydrate showed a linear increase in concentration in the *Artemia* biomass which was directly proportional with hours of enrichment and increase in concentration of enrichment media. Devi *et al* 2012 showed the nutrients such as protein, carbohydrate, lipid present in *Nannochloropsis salina, Chlorella marina* and *A. platensis* were transferred to *Artemia* through the enrichment process.

The diatoms differed in their levels of protein, carbohydrate and ash, but the lipid content of all species was similar. *C. fusiformis* and *L.*

annulata contained more carbohydrate and ash, but less protein, than the other species. Ash was a major component (> 10%) in all species except the tropical *Skeletonema.* High ash content is characteristic of diatom species with heavily silicified cell walls (Parsons *et al.,* 1961; Whyte, 1987). The nutritional value of the diatoms may differ because of their gross composition, but nutritional value also depends on the requirements of the feeding animal. High-protein (45.5%) (table 7.1) in *D. salina* produced higher growth rates in *P. monodon* larvae high protein (<20%). The dietary ratio of carbohydrate: protein may not be so critical for crustaceans. Low-protein, high-carbohydrate artificial diets resulted in growth and survival of prawn larvae *(Penaeus japonicus)* equal to that of high-protein, low-carbohydrate diets (Teshima and Kanazawa, 1984). Differences in the gross composition of the algae may therefore be nutritionally significant to prawn larvae from the present study.

Many of the algal proteins performing specific functions in the cell and common to all species are strongly conserved in their amino-acid composition across taxa. The nutritional quality of protein depends on the proportion and availability of its constituent amino acids. Ten amino acids are considered essential for most marine animals. Although the availability of the diatom amino acids to the animal was not studied, the proportions of these diatom acids were very similar to those of abalone and prawn tissue (Table 3), suggesting that the protein quality of the diatoms may be high for these animals.

Diatoms are a good source of the two essential polyunsaturated fatty acids, *20:5(n-3)* (5 to 30% of total fatty acids) and 22:6(n-3) (0.3 to 6%) (Volkman *et al,* 1989, Dunstan *et al,* 1994). They vary in sugar

composition (e.g. 21 to 82% glucose in their polysaccharide; Brown, 1991) and are rich in the vitamins, ascorbic acid, riboflavin and thiamin (Brown and Miller, 1992; Brown and Farmer, 1994). They therefore constitute an excellent food source for feeding animals. Changes in the lipid composition and maximization of the polyunsaturated fatty acid content of three microalgae grown in mass culture.

Because the biochemical composition of each microalgae species varies, the use of monoalgal diets could produce a shortage of essential nutrients needed for the adequate development of penaeid shrimp (Yúfera and Lubián1990). Even though the use of a combination of several microalgae as a diet for shrimp larvae is recommended, monoalgal diets have been widely used for commercial culture due to reduced contamination risks and costs, as well as easy manipulation and feeding regimes.

Chapter- 8

SUMMARY AND CONCLUSIONS

From the larval rearing experiment, the *Dunaliella salina* was found to be a suitable food organism for the black tiger shrimp, *Penaeus monodon.* The survival rate and growth performance was found to be high in those larvae which fed on *Dunaliella salina* and the next was *Chaetoceros calcitrans. Nannochloropsis salina* was found to be a suitable food during the protozoeal stages but not during the late mysis stages. It might be due to the small size of the algae which may not be preferable for the mysis which has wider mouth gape than that of protozoea. In this experiment the growth and survival rate of the prawn larvae fed on mixed algae (*Dunaliella salina* + *Nannocholopsis salina*) was high compared with that of monospecific algal trials.

It is seen that the differences in larval performance between algal diets were due to the differences in size of the algal cells. Increasing the proportion of *D. salina* was found to improve the diet, since *D. salina* alone also performs well. The pm larvae fed with C+D and D+N showed a significant increase in weight gain when compared to that of C even though the differences in weight gain with all other combinations were not significant.

Although the ingestion rate was found to be increasing with increasing larval stages, it was found to be declined after M1 stage. It was

found that the relative ingestion rate increases through each substage, reaching its maximum during larval development in PZ III. To avoid food waste, under feeding, and water fouling, optimum feeding response to a particular food during the particular larval stage must be known. The application of food levels based on the ingestion rates for each larval stage is an effective strategy if larval growth, development and survival are consequently maximized. From the above results it could be inferred that the *Pm* larvae fed with the micro algae at cell concentration $50x10^4$ showed better survival and increment in growth.

From the present study it was seen that the *P. monodon* larvae fed with that of mixture of algal diets performed well. Hence, we consider the differences in larvae response to be a consequence of the nutritional composition of the diet. Feed mixtures are generally recommended because they produce better results than when a particular feed is used alone, as mixtures contain a complementary blend of nutrients, such as amino acids, that meet or exceed nutritional requirements.

The differences in the composition of fatty acids in the algal diets seemed to be the factor most likely to explain the differences in larval survival and development. The gross lipid and carbohydrate compositions of the algal diets did not explain the observed differences in growth of the larvae nor did they correspond to the gross composition of the larvae. Although the amino acid and fatty acid composition of the larvae levels were not presented here due to problems with the assay.

The biochemical composition of each microalgae species varies, hence the use of monoalgal diets could produce a shortage of essential

nutrients needed for the adequate development of penaeid shrimp. The mixed algae fed *P. monodon* had better developmental rate with *P. monodon* larvae fed with *D. salina* which was found to be very efficient in combination with *N. salina* as well as that of *C. calcitrans*. Higher survival rate of 71.3% was obtained with the combination of *N. salina* and *D. salina*.

Even though the use of a combination of several microalgae as a diet for shrimp larvae is recommended, monoalgal diets have been widely used for commercial culture due to reduced contamination risks and costs, as well as easy manipulation and feeding regimes. In that sense *D. salina* can be recommended as a suitable diet for *P. monodon* larvae in hatchery rearing.

The future prospects of the study can be done by the enrichment of the algae and its response on the larvae, the digestive enzyme activity, gut histopathology and the feed efficiency of the larvae can be evaluated in response to various algal diets, amino acid and fatty acid profile of the larvae and the response of the larvae to varying salinity conditions can be evaluated.

Chapter- 9

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