Chapter 4

Factors Influencing the Growth of Phytoplankton: An Experimental Approach

4.1. Introduction

*In-situ* observations on environmental variables and phytoplankton growth provide only a broad relationship. Experimental studies on the influence of environmental factors on phytoplankton cultures is perhaps the only reliable approach for determining the extent of limitation imposed by different factors. The significance of such experiments lies on the prediction of ecological processes through mathematical models. Thus, the purpose of this study was to conduct laboratory studies undertaken to measure phytoplankton growth under simulated environmental conditions. The factors considered for this study are: (a) salinity (b) light (c) copepod grazing and (d) prey (phytoplankton) size selectivity by copepod.

The conclusion derived in Chapter 3 was that salinity and light are the major physical forces and grazing of phytoplankton by copepod is the major biological factor that limits the growth of phytoplankton in the study region. The present experiment was therefore, designed to measure the
extent to which salinity, light intensity and grazing by copepods control the distribution and growth of phytoplankton in the Cochin backwater.

In estuaries, the mixing creates the well-known estuarine salinity gradient, with seawater near the mouth of the estuary to freshwater near the head of the estuary (Admiraal, 1977; Miller and Kamykowski 1986; Rijstenbil 1989; Kirst 1990; Flameling and Kromkamp 1994; Bisson and Kirst 1995). The salinity gradients influence the phytoplankton growth, because different species have different salinity preferences. Some phytoplankton are freshwater species, others are of marine origin, whereas some others prefer environments that are more saline than others. Phytoplankton generally exhibit a tolerance to a range in the salinity beyond which, they inhibit the growth.

In aquatic environments (sea, estuaries or lakes), the amount of light incident on the surface is rapidly reduced with depth by an exponential function (i.e. not linear). In general, light intensity declines exponentially with depth as described by the Beer-Lambert Equation. The depth of the euphotic zone suitable for photosynthesis is the depth where light energy is reduced to 1% of the intensity (Krik and Oliver 1995). A major interference to the light availability in estuaries is suspended particulate matter (SPM), brought through land runoff which leads to turbidity in the water column. This in, turn attenuates and scatters the light. As the amount of SPM increases, the photic depth decreases (i.e. photic depth above 1% of surface incident light). Light availability in the photic depth influences phytoplankton growth, pigment content and
photosynthetic rate (Yentsch and Ryther 1957). Phytoplankton growth is linearly related to the amount of light intensity or irradiance falling on an individual cell up to a point when no further increase occurs, i.e. saturation (Falkowski and Raven 1997). Photosynthesis vs light can be represented by a P-I curve (Webb et. al., 1974), which is now widely accepted as a useful relationship for examining the photophysiology of phytoplankton (Henley, 1993).

Copepods, the dominant species of mesozooplankton in any aquatic system, (Calbet et. al., 2000; Froneman 2000 and Lo et. al., 2004) play a pivotal role in transferring energy from the primary trophic level to higher trophic levels. (Raymont 1980; Humes 1994). Hence, quantifying the rates of phytoplankton grazing by copepods is essential for understanding the mechanism that regulate phytoplankton populations in any aquatic ecosystems (Morales et. al. 1990; Landry et. al., 1995 a, b; Froneman et. al., 1997; Sautour et. al., 2000).

4.2. Review of Literature

Salinity in an estuary is a dynamic entity regulated by the river discharge, rainfall and tide. Phytoplankton communities are adapted to a certain range of salinity and show complex pattern of distribution along the salinity gradient.

Qasim et. al., (1972 a) reported that tropical phytoplankton species show wide adaptability to changes in salinity.
Desikachary and Rao (1972) studied the salinity preferences of cultured diatoms grouped into euryhaline (tolerate wide range of salinity) and stenohaline (tolerance to very narrow salinity range) species. Any change in salinity is sensitive enough to affect stenohaline phytoplankton species and could alter the phytoplankton community structure into new stable community.

Qasim et al., (1968) reported that Cochin backwater receives maximum solar radiation (500-580 g cal cm\(^{-2}\) d\(^{-1}\)) from January to April and minimum (250-300 g cal cm\(^{-2}\) d\(^{-1}\)) during July and August. The high turbidity prevailing in the backwater greatly reduces light penetration and hence, production of phytoplankton. Qasim et al., (1972 b) studied the effect of solar illumination on phytoplankton using \(^{14}\)C technique and reported that light is never a limiting factor for phytoplankton growth in tropics, but the turbidity due to suspended particulate matter do limit the phytoplankton growth.

Similar kind of studies made elsewhere (Alpine and Cloern, 1988; Lusia and Cantera, 1993; Macedo et al., 2001) have shown that phytoplankton growth is largely controlled by light availability. According to these studies the phytoplankton cells reside in a turbulent medium of an upper photic zone sustains photosynthesis, but the lower aphotic zone does not. Cloern (1987) reported that the photic depth is characteristically shallow in estuaries because of high suspended particulate matter. Hence the mean light exposure of phytoplankton cells and their growth rates are relatively low.
Guillard and Rhyther (1962) studied the growth rate of marine phytoplankton and reported that salinity changes can result in osmotic stress and affect the cellular ionic ratio in phytoplankton. Underwood and Provot (2000) studied the preferences of estuarine diatoms across a range of salinity. Hayatti (2007) studied the effect of salinity on growth and distribution of freshwater diatoms.

Menon et. al., (1971) studied the biomass and faunal composition of the zooplankton in the Cochin backwater. Zooplankton distribution along salinity gradient in Cochin backwater was reported by Nair and Tranter (1971). Haridas et. al., (1973) have studied the salinity, temperature, dissolved oxygen and zooplankton biomass of the backwater from Cochin to Alleppy. Rao et. al., (1975) studied the distribution of zooplankton in space and time in the Cochin backwater. Madhupratap and Haridas (1976) have explained the composition and variations in the abundance of zooplankton of backwater from Cochin to Alleppy. Madhupratap (1978 and 1980) also studied the distribution, community structure and species succession of copepods in the Cochin backwater. Annual variation in zooplankton from a polluted coastal environment was reported by (Haridas et. al., 1980; Madhu et.al., 2007) studied the monsoonal impact on the standing stock and distribution of plankton. Despite these numerous work on copepod ecology, the feeding behavior of copepods on phytoplankton community is not yet studied in the Indian waters. The only study on copepod feeding from the Indian waters is that of (Goes et. al., 1999) where in they have studied the inter-relationship between phytoplankton and copepods. Achuthankutty et. al., (2000), have studied the influence of
salinity on feeding, survival rate, growth and neonate production of cladocera which is a mesozooplankton, but coming under different class.

The effect of phytoplankton size on copepod feeding have been of major concern in a number of recent studies (Hansen et al., 1997; Romam and Gauzens 1997; Gowen et. al., 1999; Head et. al., 1999) made outside the Indian waters.

4.3. Materials and Methodology

4.3.1. Isolation and culture of phytoplankton

Phytoplankton was isolated from the lower estuary Fort Kochi (Stn. 4 described in Chapters 2 & 3) by collecting 3 L of sea water during the spring tide and as brought to the laboratory in ice box. In the laboratory, seawater was filtered through 200 µm bolting silk to remove larger grazers; the filtered seawater was allowed to settle for a minimum five hours. After sedimentation of sea water sample was concentrated to 100 ml and from this 5 ml was added to ten sets of F/2 media prepared in autoclaved seawater. The whole experimental setup was then kept in algal rack provided with ambient light (12 L: 12 D) for a period of 14 days until colour developed inside the flask.

Phytoplankton was isolated from the mixed culture following (a) serial dilution and (b) agar plating method.
(a) Dilution culture method

Serial dilution was followed by the procedure of (Michael et. al., 2004). Dispensed 9 ml sterilized f/2 medium in 5 glass vials of capacity 15 ml, added 1 ml of sample taken from the mixed stock culture into first glass vial and made the dilution $10^{-1}$. From this dispensed 1 ml sample to the second vial and made the dilution $10^{-2}$ and continued the dilution until $10^{-5}$.

(b) Agar plating method

1.5 g of bacterio agar (HiMedia) was added to 1 L of filtered (0.22 μm) estuarine water. The solution was then sterilized in an autoclave for 15 minutes under 150 lb pressure and 120°C temperature. After cooling, the medium was poured into sterilized petri plates and kept for 24 hr. From the concentrated phytoplankton sample, 1 ml of sub sample was streaked on to the agar plate and kept for incubation. These agar plates were then incubated in an algal chamber for 7-8 days providing light and dark period (12 L-12 D hr). Phytoplankton colonies developed on the agar plates were isolated species wise (individual cells) using micro blades under an inverted epifluorescence microscope (Olympus CK IX 51) and transferred to culture tubes and grown as mass mono culture (Michael et. al., 2004).

4.3.2. Phytoplankton growth rate (optimal and varying condition)

Common and major phytoplankton species were isolated using the above techniques (a & b) and were mono cultured. To study the optimum growth rate of phytoplankton, ambient conditions were provided and incubated for 7 to 8 days and growth rate were measured once in a days.
Growth rate studies on varying salinities were carried out by selecting the salinity ranges in the order 0, 5, 10, 15, 20, 25, 30 and 35 which is the salinity range encountered in the study region. These salinities were achieved by diluting the sea water with distilled water and artificial nutrient medium was provided (F/2 medium, HiMedia) to maintain the nutrients level in the cultures till the end of the experiment (Robertson, 2005). The growth rate was calculated

\[ \mu \text{ (d}^{-1}) = \frac{(\ln N_t - N_0)}{t_2 - t_1} \]

Where,

- \( N_t \) = Final density
- \( N_0 \) = Initial density
- \( t_2 \) = Final incubation
- \( t_1 \) = Initial incubation
F/2 Media Preparation Chart

F/2 medium* (Guillard and Ryther 1963)
Updated April 2007

FOUR STOCK SOLUTIONS (1–4)
OBS! For all solutions, use sterilized distilled deionized water!

1. NaNO₃ stock solution
   \[ \text{For 1L} \quad \text{For 0.5L} \]
   \[ \text{NaNO}_3 \quad 75.0 \text{ g} \quad 37.5 \text{ g} \]

2. NaH₂PO₄ stock solution
   \[ \text{For 1L} \quad \text{For 0.5L} \]
   \[ \text{NaH}_2\text{PO}_4 \quad 5.0 \text{ g} \quad 2.5 \text{ g} \]

3. Trace Metals stock solution
   To distilled water add the following:
   \[ \text{For 1L} \quad \text{For 0.5L} \]
   \[ \text{Na}_2\text{EDTA} \quad 4.36 \text{ g} \quad 2.18 \text{ g} \]
   \[ \text{FeCl}_2\cdot6\text{H}_2\text{O} \quad (\text{Ferri Chloride}) \quad 3.15 \text{ g} \quad 1.575 \text{ g} \]
   \[ \text{Primary Metals Stocks (below)} \]
   \[ 1\text{mL of each of the five} \quad 0.5\text{mL of each of five} \]

   Primary Trace Metals stock solutions (make up five separate stocks)
   To the chosen volume of sterile distilled deionized water add the following:

   \[ \text{100mL} \quad \text{50mL} \quad \text{10mL} \]
   \[ \begin{array}{|c|c|c|}
   \hline
   \text{CnSO₄•5H₂O} & 1.0 \text{ g} & 0.50 \text{ g} & 0.10 \text{ g} \\
   \text{ZnSO₄•7H₂O} & 2.2 \text{ g} & 1.10 \text{ g} & 0.22 \text{ g} \\
   \text{CoCl₂•6H₂O} & 1.0 \text{ g} & 0.50 \text{ g} & 0.10 \text{ g} \\
   \text{MgCl₂•6H₂O} & 1.8 \text{ g} & 0.90 \text{ g} & 0.18 \text{ g} \\
   \text{NaMoO₄•2H₂O} & 0.63 \text{ g} & 0.315 \text{ g} & 0.063 \text{ g} \\
   \hline
   \end{array} \]

4. Vitamin Stock solution
   Light sensitive – keep covered in foil!

   \[ \text{For 1.0 L} \quad \text{For 0.5 L} \]
   \[ \text{Biotin} \quad 10.0 \text{ mL of 0.1 mg mL}^{-1} \text{ solution (1mg in 10mL)} \quad 5.0 \text{ mL} \\
   \text{Vitamin B₁₂} \quad 1.0 \text{ mL of 1.0 mg mL}^{-1} \text{ solution (1mg in 1mL)} \quad 0.5 \text{ mL} \\
   \text{Thiamine HCl} \quad 0.2 \text{ g} \quad 0.1 \text{ g} \]

Lastly: Making Final Medium
To 950 mL of 0.22 μM filtered seawater (FSW) add:

<table>
<thead>
<tr>
<th></th>
<th>To make 100 tubes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃ Stock solution</td>
<td>1.0 mL 100 mL</td>
</tr>
<tr>
<td>NaH₂PO₄ Stock Solution</td>
<td>1.0 mL 100 mL</td>
</tr>
<tr>
<td>Trace Metals Stock Solution</td>
<td>1.0 mL 100 mL</td>
</tr>
<tr>
<td>Vitamin Stock Solution</td>
<td>0.5 mL 50 mL</td>
</tr>
</tbody>
</table>

Filter sterilize at 0.22 μM before use and store at 4°C. * Si has been removed from this recipe to reduce the growth of contaminating diatoms.

Tip: Make up a larger batch, just multiply each stock by how many tubes you want to set up. For example, for 100 tubes of 3.5 mL total of F/2 final medium stock, add 100 mL of each of the first three stocks and 50 mL of the Vitamin stock. That gives a total of 350 mL which gives 100 15 mL falcon tubes of 3.5 mL each of F/2 final medium stock, each of which is ready to make up one each of 1.0 L working F/2 media (one tube of 3.5 mL plus 950 mL of filtered seawater.

4.3.3 Photosynthesis-Irradiance (P/I) experiment

Water samples were filtered through 200 μm nylon meshes and dispensed into culture bottles (60 mL) and each culture bottle was spiked
with approximately 1 ml of 5 μ Ci of NaH$^{14}$CO$_3$. The culture bottles were then incubated in light gradient incubator with an external light provided by 1500 W tungsten halogen lamps. Irradiance (ambient condition) was measured using LICOR meter (Bio Spherical Instruments, USA). Attenuation was achieved by neutral density filters and incubated for 2 hr in ambient temperature. Heat produced by the lamp was dissipated using a cold water flow system. Following incubation, samples were filtered in low vacuum ($\leq$250 mm Hg) onto GF/F filter paper (0.7 μm pore size, 25 mm dia) and each filter paper was placed in separate scintillation vial, after fumigating with concentrated HCl. The photosynthetic rate was normalized by dividing with chlorophyll a and expressed in (mg C (mg Chl a)$^{-1}$ h$^{-1}$). P/I curves were plotted based on the equation $[Pm B (1 - \exp (-αI/PmB))]$ (Platt et. al., 1980), using the software ROPE (R Ocean Production Extensions, Version 1.1, Canada 2007).

4.3.4. Estimation of copepod grazing (Gut pigment content)

4.3.4. (a) Copepod Grazing on phytoplankton biomass

Mesozooplankton samples for this study were collected from Cochin backwater (Stn. 4, Fort Kochi, Chapter 2 & 3) during monsoon (2008) and pre monsoon (2009) period using WP net (working plankton net) of mesh size 200 μm and brought to laboratory in ice box. The frozen zooplankton samples were thawed and washed with filtered seawater to remove adhering algae and debris. Copepods (Calanoid) which were the dominant (comprising > 70% of the total mesozooplankton) were carefully sorted and extracted in 5 ml of 90% aqueous acetone maintained at 4º C.
Factors Influencing the Growth of Phytoplankton: An Experimental Approach

in the dark without homogenization in a refrigerator (Atkinson 1996, Hwang et al. 1998, Wong et al. 1998). After extraction overnight, the solution was centrifuged, and the upper clear layer was measured using a Turner Design Model 7200 fluorometer in the laboratory. The extract was then acidified with 0.1ml of 10% HCl and measured again. Due to pheopigment loss during the experiment, all pheopigment values were multiplied by a factor of 1.51 according to (Dagg and Wyman 1983). Gut pigment content was expressed as ng chlorophyll a per individual copepod obtained from the addition of Chl a and pheopigment (pheophorbide expressed as Chl a equivalent) concentrations in the gut. Gut pigment content was calculated using the formula:

\[
\text{ng chlorophyll/copepod} = \frac{K(F_0 - F_a)}{n}
\]

\[
\text{ng pheophytin/copepod} = \frac{K(RF_0 - F_a)}{n}
\]

Where,

\( K \) = machine calibration constant

\( F_0 \) = before acidification

\( F_a \) = after acidification

\( R \) = acidification ratio

\( n \) = number of copepods
4.3.4. (b) Prey-size selectivity of Copepod

From the copepod samples *Acartia tropica* and *Pseudodiaptomous annandalei* were sorted out from the zooplankton sample which was dominant species during monsoon and pre monsoon. They were carefully sorted out and transferred to filtered seawater and starved overnight. Three sets of grazing experiments were conducted in which the increase in total gut pigment (Chl a) was used as a measure of ingestion rates. Experiments were performed with phytoplankton like *Chlorella vulgaris* (6 µm), *Skeletonema costatum* (10 µm), *Nitzschia closterium* (45 µm) and *Coscinodiscus centralis* (105 µm) as prey with different size fractions for studying the size selectivity of copepods.

4.4. Result

4.4.1. Phytoplankton growth at optimal and at varying salinity condition

Growth rate (per day division of the cell) study is one important way of expressing the relative ecological succession of phytoplankton species or strains in adapting to its natural environment or the experimental environment imposed upon it. In an experimental condition there are four main phases of growth (lag, exponential, stationary and death phase). In Plate 4.4.1 only three phases are demonstrated because increase in growth rate occurs only during these three phases. Lag phase is the acclimatization period of phytoplankton to new environment. Once adapted to the conditions, the rate of cell division accelerates and increase in phytoplankton cell number in the culture, this
period is called exponential phase. Cell division rate then slows as light penetration through the culture is limited and also the nutrient. The culture then enters the stationary phase.

![Graph of growth phases of phytoplankton](image)

Plate. 4.4.1 Growth phases of phytoplankton in an experimental condition

To study the growth rate (optimal condition) of phytoplankton in the study region, ten phytoplankton species commonly found in Cochin backwater were isolated and mono cultured following the procedure (4.3.1 a & b). Among them eight were diatoms (*S. costatum, C. calcitrans, C. centralis, B. sinensis, N. closterium, N. distans, P. elongatum and A. coffeaeformis*), and the other two belonged to green algae (*C. vulgaris*) and blue green algae (*Anabaena* sp.). The species belonged to nano (2-20 µm) and micro (20-200 µm) planktonic sizes (Figure.4.4.1 a; Table.)
4.4.1 a). The growth rate of nano and micro phytoplankton studied are the first attempt for the Cochin backwater. The nano phytoplankton growth rate ranged from 0.92 to 2.12 d\(^{-1}\) and micro phytoplankton growth rate range was between 0.56 to 0.85 d\(^{-1}\). Nano phytoplankton showed a faster growth than micro phytoplankton. Nano phytoplankton was contributed by diatoms \((S.\ costatum,\ C.\ calcitrans,\ N.\ closterium,\ N.\ distans,\ P.\ elongatum\ and\ A.\ coffeaefomis)\), green algae \((C.\ vulgaris)\) and blue green algae \((Anabaena\ sp.)\), whereas the micro phytoplankton was mainly the diatoms \((C.\ centralis\ and\ B.\ sinensis)\).

![Growth rate of common phytoplankton isolated from the study region](image-url)

**Figure. 4.4.1 (a) Growth rate of common phytoplankton isolated from the study region**
The salinity preferences of the various phytoplankton species revealed that the optimum salinity required for maximum growth for each phytoplankton species varied in the range 15 - 25 (Figure 4.4.1 b). It was found that half of the species *S. costatum, N. closterium, N. distans, P. elongatum* and *A. coffeaeformis* studied were euryhaline, whereas except *C. calcitrans, C. centralis, C. vulgaris* and *Anabaena* sp. were stenohaline in nature. The optimum salinity preferred by *C. calcitrans*, and *C. centralis* were in a narrow range of 30 – 35, while for *C. vulgaris* and *Anabaena* sp., it was 10 - 20 (Table 4.4.1 b).

### Table 4.4.1 (a) Phytoplankton growth rate (optimal condition) and their size

<table>
<thead>
<tr>
<th>Species</th>
<th>Size (µm)</th>
<th>Growth rate (d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Skeletonema costatum</em></td>
<td>15</td>
<td>1.24 (± .002)</td>
</tr>
<tr>
<td><em>Chaetoceros calcitrans</em></td>
<td>17</td>
<td>0.92 (±0.12)</td>
</tr>
<tr>
<td><em>Coscinodiscus centralis</em></td>
<td>105</td>
<td>0.85 (±0.01)</td>
</tr>
<tr>
<td><em>Biddulphia sinensis</em></td>
<td>92</td>
<td>0.56 (±0.01)</td>
</tr>
<tr>
<td><em>Nitzschia closterium</em></td>
<td>16</td>
<td>1.24 (±0.02)</td>
</tr>
<tr>
<td><em>Navicula distans</em></td>
<td>12</td>
<td>1.02 (±0.02)</td>
</tr>
<tr>
<td><em>Pleurosigma elongatum</em></td>
<td>17</td>
<td>1.02 (±0.1)</td>
</tr>
<tr>
<td><em>Amphora coffeaeformis</em></td>
<td>26</td>
<td>1.52 (±0.02)</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>6</td>
<td>1.26 (±0.03)</td>
</tr>
<tr>
<td><em>Anabaena sp.</em></td>
<td>6</td>
<td>2.12 (±0.05)</td>
</tr>
</tbody>
</table>
Figure. 4.4.1 (b) Salinity preference of various phytoplankton species in Cochin backwater

Table. 4.4.1 (b) Range in salinity showing maximum growth for different unialgal species

<table>
<thead>
<tr>
<th>Phytoplankton species</th>
<th>Optimum Salinity range for maximum growth</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletonema costatum</td>
<td>15-25</td>
<td>Euryhaline (Estuarine)</td>
</tr>
<tr>
<td>Chaetoceros calcitrans</td>
<td>30-35</td>
<td>Stenohaline (Marine)</td>
</tr>
<tr>
<td>Coscinodiscus centralis</td>
<td>30-35</td>
<td>Stenohaline (Marine)</td>
</tr>
<tr>
<td>Biddulphia sinensis</td>
<td>15-30</td>
<td>Euryhaline (Estuarine)</td>
</tr>
<tr>
<td>Nitzschia closterium</td>
<td>20-35</td>
<td>Euryhaline (Estuarine)</td>
</tr>
<tr>
<td>Navicula distans</td>
<td>25-35</td>
<td>Euryhaline (Estuarine)</td>
</tr>
<tr>
<td>Pleurosigma elongatum</td>
<td>25-30</td>
<td>Euryhaline (Estuarine)</td>
</tr>
<tr>
<td>Amphora coffeaeformis</td>
<td>25-35</td>
<td>Euryhaline (Estuarine)</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>10-20</td>
<td>Stenohaline (Brackish)</td>
</tr>
<tr>
<td>Anabaena sp.</td>
<td>10-20</td>
<td>Stenohaline (Brackish)</td>
</tr>
</tbody>
</table>
4.4.2. Influence of light on photosynthetic uptake of phytoplankton

The process of photosynthesis involves the conversion of inorganic carbon into organic carbon with light as the energy source. In plant physiology, the rate of this conversion is called the photosynthetic rate or uptake. Light becomes a limiting factor for photosynthetic uptake of phytoplankton in Cochin backwater during monsoon due to high suspended particulate matter (SPM). Therefore a comparative study was made on photosynthetic uptake of phytoplankton during monsoon (high SPM) and pre monsoon (low SPM). Sampling was done at a single station (Stn. 4, Fort Kochi, Chapter 2 & 3) in both the season at euphotic depth (0.5 m) during spring tide.

The functional response of phytoplankton to available light can be studied through use of the photosynthesis-irradiance (P-I) experiment. Different components of P-I like photosynthetic uptake ($P^B$), saturated photosynthetic uptake ($P^B_m$), photoadaptation ($I_k$) and photosynthetic efficiency ($\alpha^B$) explain about the photophysiology of phytoplankton and their relationship to environmental variables (Plate. 4.4.2). $P^B$ and $P^B_m$ are functions of phytoplankton biomass and species composition. $I_k$ and $\alpha^B$ are functions of specific characteristics of dominant phytoplankton species with respect to their light capturing capacity and environmental variables. Among the environmental variables, salinity and SPM varied considerably during monsoon and pre-monsoon seasons.
Photosynthetic uptake ($P^B$) during monsoon ranged from 2.8 to 6.7 mg C mg Chl$^{-1}$ h$^{-1}$ (av. 4.5 mg C mg Chl$^{-1}$ h$^{-1}$) with maximum during June and minimum during July and August (Figure. 4.4.2 a; Table 4.4.2). High $P^B$ during June can be attributed to the high biomass and relatively low SPM during the period. Low $P^B$ during July and August was due to comparatively high SPM during July (50.0 mg L$^{-1}$) and August (45.3 mg L$^{-1}$) which blemished the quality of light. During pre monsoon $P^B$ ranged from 5.7 to 7.0 mg C mg Chl$^{-1}$ h$^{-1}$ (av. 6.5 mg C mg Chl$^{-1}$ h$^{-1}$). Maximum $P^B$ was recorded during March and minimum February (Figure. 4.4.2 b; Table 4.4.2). The variability in $P^B$ was mainly due to dominant phytoplankton species present. It was observed that maximum diversity was observed during March when compared to April and May even though biomass was
Factors Influencing the Growth of Phytoplankton: An Experimental Approach

high during April (12.8 mg m\(^{-3}\)) and May (13.9 mg m\(^{-3}\)) compared to March (7.2 mg m\(^{-3}\)).

\[P^B_m\] is the saturated photosynthetic uptake which followed the trend of \(P^B\) with maximum range was during pre monsoon (5.5- 6.8 mg C mg Chl\(^{-1}\) h\(^{-1}\)) and minimum during monsoon (2.7-6.2 mg C mg Chl\(^{-1}\) h\(^{-1}\)).

The photo adaptation (\(I_K\)) showed wide variation during monsoon and pre monsoon, the differences in \(I_K\) was mainly due to phytoplankton species diversity (Table. 4.4.2; Figure 4.4.2 d). During monsoon maximum photoadaptation of phytoplankton was during August (111.3 \(\mu\)E m\(^{-2}\) s\(^{-1}\)) and minimum during July (66.7 \(\mu\)E m\(^{-2}\) s\(^{-1}\)) the variations in the \(I_K\) can be attributed to the differences in phytoplankton species. The relative abundance of \(L.\ danicus\) was high during July but was completely absent in August similarly \(T.\ subtilis\) which was abundant during August was completely absent during July. During pre monsoon \(I_K\) was maximum during March (340 \(\mu\)E m\(^{-2}\) s\(^{-1}\)) and minimum during May (131.4 \(\mu\)E m\(^{-2}\) s\(^{-1}\)) this may be probably due to the phytoplankton taxonomical composition differences. Photosynthetic efficiency (\(\alpha^B\)) during monsoon and pre monsoon did not show noticeable differences except during June. This indicates that efficiency of fixing carbon by phytoplankton in the study region is more or less similar.
Figure 4.4.2 (a) Photosynthetic uptake of phytoplankton during monsoon season in the study region
Figure 4.4.2 (b) Photosynthetic uptake of phytoplankton during pre-monsoon season in the study region
Figure 4.4.2 (c) Photosynthetic uptake (average) of phytoplankton during (a) monsoon (b) pre monsoon in the study region
Table 4.4.2 Photosynthetic uptake (P/I) components and related physical and biological parameters in the study region

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Monsoon 2008</th>
<th>Pre monsoon 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>July</td>
</tr>
<tr>
<td>PB</td>
<td>6.7</td>
<td>2.8</td>
</tr>
<tr>
<td>PBm</td>
<td>6.2</td>
<td>2.7</td>
</tr>
<tr>
<td>IK</td>
<td>77.0</td>
<td>66.7</td>
</tr>
<tr>
<td>αB</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Chl. a</td>
<td>7.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Light</td>
<td>852</td>
<td>277</td>
</tr>
<tr>
<td>Salinity</td>
<td>8.5</td>
<td>0.0</td>
</tr>
<tr>
<td>SPM</td>
<td>27.6</td>
<td>50.8</td>
</tr>
</tbody>
</table>

Units

PB and PBm = mg C mg Chl a⁻¹ h⁻¹

IK = µE m⁻² s⁻¹

αB = mg C mg Chl a⁻¹ h⁻¹ µE m⁻² s⁻¹

Light = µE m⁻² s⁻¹

Biomass = mg m⁻³

Suspended particulate matter (SPM) = mg L⁻¹
Figure 4.4.2 (d) Relative abundance (%) of the main phytoplankton species
Photosynthetic uptake is also a function of the dominant phytoplankton species (Figure. 4.4.2 d). Phytoplankton species distribution during the study period showed differences in their relative abundance. During monsoon the dominant species were \textit{S. costatum, T. subtilis, N. distans, N.longa, N. directa} and \textit{N. closterium} whereas during pre monsoon the dominant species were \textit{T. subtilis, C. lorenzianus, C. centralis, N. directa, A. coffeaeformis, R. styliformis} during February and March whereas during April and May more than 80% of the abundance was contributed by \textit{Chaetoceros calcitrans}. The differences in the floral composition played a major role in the in the photosynthetic uptake and light utilization of phytoplankton.

\textbf{4.4.3. Influence of copepod grazing on phytoplankton biomass and their prey size selectivity}

Copepods are the major group in a mesozooplankton sample; more than 80% of the total mesozooplankton is contributed by them. They are efficient grazers of phytoplankton and play an important role as intermediaries for nutrient/energy transformation from primary to tertiary trophic level. So it is essential to study their grazing effect on phytoplankton biomass. Copepod grazing was measured using Chlorophyll \textit{a} (Phytoplankton biomass) as a proxy. Copepod grazing controls phytoplankton biomass and distribution.

Copepod grazing is a major biological factor that was responsible for controlling the phytoplankton biomass in the study region. Time series observations (24 hr) made during monsoon (2008) and pre-monsoon
(2009) seasons revealed that during both periods 90% of the mesozooplankton biomass was contributed by copepods (Figure 4.4.3 a & b). During monsoon, the mesozooplankton biomass ranged from 0.03 ml m\(^{-3}\) (±0.02) to 0.175 ml m\(^{-3}\) (± 0.04) and copepod biomass ranged from 0.025 (± 0.01) to 0.14 ml m\(^{-3}\) (± 0.03). During pre-monsoon, the range in mesozooplankton biomass was between 0.06 (± 0.01) and 0.52 ml m\(^{-3}\) (± 0.15) and that of copepod was between 0.03 (± 0.04) and 0.39 ml m\(^{-3}\) (± 0.18). During both the seasons the mesozooplankton and copepod biomass were found to be high in night hours (9 pm) and early morning (1 am & 5 am). However, the values for the respective time were 2-3 fold higher during pre monsoon as compared to the monsoon.

![Graph](image_url)

**Figure 4.4.3 (a) Time series (24 hr) observation of mesozooplankton and copepod biomass during monsoon in the study region**
Figure 4.4.3 (b) Time series (24 hr) observation of meso zooplankton and copepod biomass during pre monsoon in the study region

Figure 4.4.3 (c) Time series (24 hr) observation of copepod grazing during monsoon and pre monsoon in the study region

Copepod grazing during monsoon and pre monsoon revealed that grazing was relatively high during pre monsoon compared to monsoon. During monsoon the grazing rate ranged from 0.21 to 1.75 ng Chl a copepod$^{-1}$ h$^{-1}$ and during pre monsoon the range was between 0.24 and
3.58 ng Chl a copepod$^{-1}$ h$^{-1}$ (Figure 4.4.3 c). Grazing was high during 9 pm to 5 am and the peak grazing time was 1 am during both monsoon and pre monsoon. Two fold increases in grazing of copepod was observed during night and early morning hours in pre monsoon.

Figure 4.4.3 (d) Prey-size selectivity of Acartia tropica and Psuedodiaptomus annandali

Grazing also indicates the size selectivity of the prey (phytoplankton) by copepods. It was found that Acartia tropica dominated during monsoon and contributed more than 70% of copepod density. The grazing rate of this species decreased as the size of phytoplankton increased. In the case of Psuedodiaptomus annandali which contributed more than 80% of the copepod density during pre monsoon, preferred larger cell size (Fig. 4.4.3 d). Grazing rate of A. tropica ranged from 0.32 to 0.92 ng Chl a copepod$^{-1}$ h$^{-1}$ and the size preference ranged from 6 to 10 µm whereas for P. annandali the grazing rate ranged from 0.49 to 1.0 ng Chl a copepod$^{-1}$ h$^{-1}$ and the size preference ranged from 45 to 105 µm.
The result indicates that *A. tropica* prefers only nano planktonic size while *P. annandalei* can take both nano and micro planktons. It is also observed that *A. tropica* and *P. annandalei* can graze nano planktonic size (10 µm) with more or less same efficiency.

### 4.5 Discussion

#### 4.5.1 Phytoplankton growth at optimal and varying salinity condition

Phytoplankton growth rate study was a first time approach in the Cochin backwater. There are only a few reports available in the Indian waters (Phatarpekar *et. al.*, 2000 and Gireesh *et. al.*, 2008). Similar kind of studies from elsewhere is that of (Raven, 1986; Tang, 1995; Raven and Kubler 2002 and Geraldine *et. al.*, 2005). They obtained maximum growth rates ranging from 0.2 to 3.3 d⁻¹ with an average of 1.5 ± 0.8 d⁻¹ under conditions of saturating light and nutrient sufficiency. In the present observation the phytoplankton growth rate ranged from 0.56 to 2.12 d⁻¹ which is within the range of above reported values.

It was also found that smaller cells exhibited faster growth rate. *N. closterium* and *Anabaena* sp. showed faster growth rate than *C. centralis* and *B. sinensis*. *N. closterium* (16 µm) and *Anabaena* sp. (6 µm) belong to nano planktonic size whereas *C. centralis* (105 µm) and *B. sinensis* (92 µm) are micro planktonic size. The latter two species registered were slower growth rate with a cell division rate of 0.5 and 0.8 d⁻¹ while the former species showed cell division rate of 1.99 and 2.12 d⁻¹. The
reduction in the growth rate with increasing cell size implies that small cells have a distinct advantage over large ones (Raven 1986). Faster growth rate can be related to cell size, which has long been recognized as an important cause of interspecific variability (Kagami and Urabe 2001).

Salinity preference of different phytoplankton species revealed that maximum growth rate was attained in the salinity range 15 to 25 and also most of these species were euryhaline in nature. Observational studies made by (Qasim 1974; Devassy and Bhattathiri 1974; Gopinathan 1975; and Madhu et. al., 2007 and 2010) have reported that maximum biomass and density were observed during low saline period (15-25). Similar kind of observations made in the Mandovi estuary by (Matondkar et. al., 2007) revealed that phytoplankton bloom in the estuary coincides with low saline period (20-25). Patil and Anil (2011) have reported that salinity stratification (17 to 18) favors phytoplankton bloom in the Zuari estuary which incidentally opens to the Arabian Sea where the Mandovi estuary also opens. All these studies points to the fact that salinity is the key controlling factor for phytoplankton growth.

Phytoplankton in the present study region was mostly euryhaline in nature. Of the ten phytoplankton species studied, six were euryhaline (S. costatum, N. closterium, N. distans, P. elongatum, B. sinensis and A. coefffaeformis) and four (C. calcitrans, C. centralis, C. vulgaris and Anabaena sp.) were stenohaline species. C. calcitrans, and C. centralis were marine which could not tolerate salinity less than <25 and C. vulgaris and Anabaena sp. were brackish water in nature and could not survive in
salinity $>20$. A few observational studies made by Devassy and Bhattathiri (1974) and Menon et. al., (2000) have reported that *C. vulgaris* and *Anabaena* sp are fresh/brackish water forms and occur in high density during monsoon period. In the present experiment also, the results were similar in that *C. vulgaris* and *Anabaena* sp showed optimal growth in low salinity. Phytoplankton classified based on their salinity preferences in the Cochin backwater showed that *C. centralis* and some *Chaetoceros* species are purely marine and are stenohaline in nature. These reports substantiate the above results. The observational work made by (Menon et. al., 2000) *B. sinensis* was classified under marine forms which are stenohaline in nature but in the present study it has been experimentally proved that it is an euryhaline form and hence, estuarine in nature.

### 4.5.2 Effect of light on photosynthetic uptake of phytoplankton

Light is an important abiotic factor for photosynthesis that limits phytoplankton growth. The variability in photosynthetic uptake ($P^B$) and saturated photosynthetic uptake ($P^Bm$) was mainly due to the optical property of dominant phytoplankton present during the period. $P^B$, $P^Bm$ and $I_k$ are functions of biomass (Chlorophyll $a$), specific characteristics of the locally dominant phytoplankton species and also changes in environmental conditions. In the present study the environmental conditions during monsoon and pre monsoon were entirely different especially in the case of salinity and SPM. Qasim (1973) while studying biological productivity of the Indian Ocean has reported the assimilation rate (photosynthetic uptake) ranged from 0.6 to 14.0 mg C mg Chl$^{-1}$h$^{-1}$ with
maximum during pre-monsoon and minimum during monsoon. Similar types of observations have been made from other regions (Falkowski, 1981; Cote and Platt, 1983; Falkowski and Raven, 1997) and it has been reported that assimilation rate (P^B and P^B max) was observed during high light intensity. I_K which is frequently used to describe the physiological adjustments of phytoplankton to changing environmental conditions also showed significant variation in the study region. It is also known that seasonal changes in I_K may occur in response to changing photoperiod and species composition (Cote and Platt 1983). In the present study I_K showed wide variation during monsoon and pre monsoon which can be attributed to the characteristics of locally dominant phytoplankton and their optical properties (Macedo et. al., 2001).

4.5.3 Effect of copepod grazing on phytoplankton and their prey size selectivity

The grazing of copepod (Calanoid) revealed that meso zooplankton and copepod biomass was high during pre-monsoon. Biomass was high in the night and early morning during both the seasons. Similar kinds of results have been reported by (Madhupratap and Haridas 1975; Rao et. al., 1975; Madhupratap 1979 and 1987; Haridevi et. al., 2004; Madhu et. al., 2007 and Molly and Krishnan 2009). The abundance of copepod is associated with salinity, because the Cochin backwater becomes an arm of the adjoining sea during pre monsoon season, supporting the entry of marine zooplankton.
Quantitative study on field caught copepod (Calanoid) revealed that grazing (gut pigment content) was high during pre monsoon period as compared to monsoon period (Figure 4.4.3 c). Grazing rate during pre monsoon ranged from 0.24 to 3.58 ng Chl a copepod\(^{-1}\) h\(^{-1}\), whereas during monsoon, it was 0.21 to 1.75 ng Chl a copepod\(^{-1}\) h\(^{-1}\). Earlier studies cited above form the Cochin backwater have not quantified the gut pigment content in copepod. These studies only explained the facts drawn out from filed observations and reported that during pre monsoon season there could be active grazing of copepod on phytoplankton independently or in combination with microzooplankton because the herbivorous copepods are capable of grazing up to 75% of the phytoplankton in a tropical estuary. The present work is a first time attempt to quantify phytoplankton biomass consumed by copepod in the Cochin backwater. Differences of phytoplankton biomass (Chl. a) available in the system and the biomass consumed by the copepod were computed. Phytoplankton biomass in the system ranged from 1.8 to 4.3 mg m\(^{-3}\) during monsoon and 2.1 to 6.6 mg m\(^{-3}\) and the remaining biomass after the grazing of copepod ranged from 1.2 to 4.2 mg m\(^{-3}\) during monsoon and 0.9 to 6.5 mg m\(^{-3}\) during pre monsoon. From the present study it was observed that there was always surplus of food available in the Cochin backwater regardless of season. This is because; the grazing by copepod was much lower than the growth of phytoplankton, as revealed from the growth and grazing experiment. Qasim (1970) estimated the amount of food available in Cochin backwater in terms of carbon. According to him the gross production in the backwater ranged from 270-295 g C/m\(^{2}\)/y (av. 280 g C/m\(^{2}\)/y) while net production, for
days only, is 180-200 g C/m²/y (av. 195 g C/m²/y). The estimated annual consumption by the zooplankton herbivores is only about 30 g C/m². This indicates there is large surplus of basic food in the backwater. The lacking of (Qasim, 1970) work was that they took gross or net production as proxies to measure the grazing pressure of zooplankton on phytoplankton. But these proxies are not the direct measurement of phytoplankton biomass grazed by copepods. Primary productivity is controlled by factors like light and the efficiency of phytoplankton to fix the carbon. But present study overrules these factors since phytoplankton biomass is taken as the proxy to measure the food availability in the system.

The flows of organic matter in pelagic food webs are determined by the food selectivity of the pelagic grazers. Several criteria may be involved in food selection, including prey size, motility, surface characteristics, biochemical composition, electrostatic forces etc. (Poulet and Marsot 1978). Among these criteria, prey size is generally believed to play a major role (Sheldon et.al., 1977; Conover and Huntley, 1980). The present work on prey size selectivity of two copepods (Calanoid) in the Cochin backwater revealed that their grazing rate was more or less similar, but the prey size selectivity was different. The result indicated that *A. tropica* prefers only nano plankton size while *P. annandalei* can graze both nano and micro planktonic prey. Grazing rate of *A. tropica* ranged from 0.32 to 0.92 ng Chl a copepod⁻¹ h⁻¹ whereas for *P. annandalei*, it ranged from 0.49 to 1.0 ng Chl a copepod⁻¹ h⁻¹. Goes *et. al.,* (1999) have obtained the grazing rate to be 1.21 ng Chl copepod⁻¹ h⁻¹ from Indian waters.
Dominance of *A. tropica* has been reported during monsoon season and *P. annendalie* during pre-monsoon season in the Cochin backwater (Madhupratap and Haridas, 1975; Rao *et. al.*, 1975; Madhupratap, 1979). Therefore, the prey (phytoplankton) size selectivity of copepod will be according to the prevailing size of the phytoplankton. According to Støttrup and Jensen (1990) *Acartia* sp. selectively graze on phytoplankton with a size <10 µm. Therefore, copepods can shift their feeding as omnivores when the phytoplankton size becomes too small (mainly pico) for their consumption. On the other hand, copepods prefer to be herbivores when phytoplankton available is of suitable size (mainly micro) (Stoecker and Capuzzo, 1990; Gifford and Dagg, 1991; Foreman, 2002). The prey size selectivity of copepod in the Cochin backwater made during the present study is new information for the Cochin backwater.
Plate. 4.4.3 (a) Laboratory set up for experimental work

Plate. 4.4.3 (a) Copepod grazing experiment set up