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Comparative Study of the Impact of Temperature and Salinity on Growth of Isolated Centric and Pennate Diatoms

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Abstract

The present study was conducted with the objective to compare the impact of temperature and salinity on the growth of centric and pennate diatoms. The phytoplankton samples were collected from Manori Creek, Mumbai during the high tide. Two diatom species, one centric (*Cyclotella* sp.) and one pennate (*Navicula* sp.), were isolated for the experiment on growth. A pure stock culture was maintained for 10 days for both species in F/2 media. Then, the diatoms were exposed to three temperatures (24 °C,27 °C, and 30 °C) and salinities (15 ppt, 25 ppt, and 35 ppt) combinations for a period of 10 days. The initial and final number of cells were counted in the Sedgewick rafter cell. The average daily growth rates (K) were calculated and statistical analysis was done by two-way ANOVA to assess the impact of temperature and salinity on growth of the diatoms. These results indicated that salinity had a more pronounced effect on these diatoms than temperature. *Navicula* sp. showed better growth in varying temperature and salinity conditions. *Cyclotella* sp. showed good growth in lesser salinities indicating that freshwater is more favorable for its growth. This concludes that pennate diatoms are more adapted to changes in environmental conditions and can show good growth even outside their favourable range of temperature and salinities. Therefore, it is economically feasible to prefer pennate diatoms for feedstock in aquaculture practices.

Keywords:
Microalgae, Temperature, Salinity, Growth, Culture, Diatom

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Introduction

Microalgae are considered ecologically very important as they contribute to approximately 50 percent of global photosynthetic activity (Anderson, 1996). They form the basis of the food chain of fishes and other aquatic organisms comprising 70 % of total biomass (Wiessner *et al.*, 1995). At present, fish stocks are declining at a rapid rate due to unsustainable fishing practices in the wild which makes it necessary for culture of microalgae for feedstock to support the fish population (Borowitzka, 1997). Of all the microalgae, diatoms, belonging to class Bacillariophyceae, play a vital role in the ecosystem as they are responsible for around 20–25% of the global primary productivity (Sarthou *et al.*, 2005). They have carved out a distinct place for themselves in the aquaculture sector (Slocombe & Benemann, 2016). Also, they are undeniably the main component of many food webs because they act as fuel for the growth of fishes and animals. The estimation of diatoms' seasonal abundance, variations, and the growth rate has been, and will continue to be, a crucial part of studies in marine science (Kang *et al.*, 2011). Variable growth rates and lipid accumulation of microalgae can be brought on by different culture environmental conditions, such as seasonal fluctuations that result in low and high temperatures (Oliveira *et al.*, 1999; Renaud *et al.*, 2002;

Sheng *et al.,* 2011; Markou *et al.,*., 2012; Wei *et al.,*., 2015; Ippoliti *et al.,*., 2016). The temperature, as well as the salinity responses, are helpful in determining the appropriate environment for boosting the growth of cells in the experimental process (Shubert, 1984; Bonin *et al.,* 1986: Harrison *et al.,* 1993; Rocha *et al.,* 2003; Bano & Siddiqui, 2004), in aquaculture systems (Epifanio *et al.,* 1981; Wikfors *et al.,* 1984; Okauchi & Hirano, 1986; Walsh *et al.,* 1987). Temperature plays a significant role in regulating the biomass and growth of phytoplankton (Eppley, 1972; Lomas & Gilbert, 1999; Paerl & Huisman, 2008). The optimal temperature for a specific strain of algae will have a significant impact on all metabolic activities and the level of productivity that may be attained in a culture (Borowitzka, 2016). The impacts of temperature on microalgae growth are attributed to modifications in cellular metabolic processes and the activity of a crucial enzyme in photosynthesis, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Leggat *et al.,* 2004; Wei *et al.,* 2015). As per Fogg and Thake (1987), the specific growth rate of microalgae decreased when cultivated at high temperatures because of an increase in respiration. Microalgal growth rate significantly declines at growth temperatures above the optimum temperature mainly due to heat stress, which can denature or alter proteins involved in photosynthetic activities (Ratkowsky *et al.,* 1983; Salvucci & Crafts-Brandner, 2004a) or disrupt the functions of enzymes (inactivation, denaturation), which inhibits growth. The rate of photoinhibition, which is known to affect algal growth, may be significantly influenced by temperature. Renaud *et al.,* (2002) found that the growth rate (μ) rises with increasing temperature below the optimum but sharply decreases above it. The effect of temperatures over optimum growth thresholds is considered to be more damaging to the microalgal cells than lower temperatures. Beyond ideal temperatures, the growth rate declines linearly until it more or less abruptly reaches lethal temperatures, depending on the species (Ras *et al.,* 2013). Another significant parameter that influences the metabolic makeup of algal cells is salinity. Algae's growth rate and composition can be altered by exposing them to salinity levels that are lower or higher than their usual (or adapted) levels (Juneja *et al.,*2013). The microalgal species withstanding salinity stress and showing good growth rates are considered to have an advantage over other species (Tonk *et al.,* 2007). Moreover, algal cultures grown on saline medium have a lower possibility of contamination by other species (Zhu *et al.,* 2016). Carr and Wyman (1986) pointed out that several freshwater species are capable of withstanding increased salinities. In a similar way, while many marine forms may endure at lower salinities, they express specific requirements for extra salts for optimum growth (Rippka *et al.,* 1979).

Materials and Methods

Sampling Site

The phytoplankton samples for this experiment were collected from Manori creek located in Mumbai. It lies between 19°11' N 72°47' E and 19°15' N 72°50' E and runs for 10.6 km (approximately) in the province of Maharashtra. Plankton sample was collected by filtering 50 litres of water in a plankton net and brought to the laboratory in a 1L sample bottle for isolation and culture. It should be noted that salinity of water was 0 (zero) ppt during sample collection due to heavy freshwater influx from monsoon rains.

Experiment on growth

Pre-requisites for the experiment

The experiment was conducted in F/2 media which is widely used for brackishwater phytoplankton culture. The media was prepared in filtered and autoclaved natural seawater (950 mL) of salinities 15 ppt, 25 ppt and 35 ppt. Distilled water was added in natural seawater to prepare the seawater of desired salinity. The media was prepared as described in table 1,2 & 3. The final volume was brought to 1.0 L with filtered and autoclaved natural seawater. The media was autoclaved after addition of all components except vitamin solution. Vitamin solution was added after autoclaving as vitamins are heat labile. (Guillard and Ryther, 1962)

For f/2 Trace metal solution, distilled water (950 mL) was taken and the compounds (Table 2) were added. The solution was cloudy initially. 1N NaOH was added to adjust pH to about 4.5 to clear the solution. The final volume was brought to 1.0 L. For vitamin premix, 950 mL dH_2O was taken and following compounds (Table

Table 1: Composition of f/2 medium

Compounds	Stock solution	Quantity	
$NaNO_3$	75 g/L dH_2O	1 mL	Autoclaved separately
$NaH_2PO_4 . H_2O$	5 g/L dH_2O	1 mL	Autoclaved separately
$Na_2SiO_3.9H_2O$	30 g/L dH_2O	1 mL	Autoclaved separately
f/2 Trace Metal Solution	Table 3	1 mL	Filter sterilized
f/2 Vitamin Solution	Table 4	0.5 mL	Filter sterilized

Table 2: Composition of f/2 Trace metal solution

Compounds	Stock solution	Quantity
$FeCl_3.6H_2O$	-	1.3 g
$Na_2EDTA.2H_2O$	-	8.7 g
$CuSO_4.5H_2O$	980 mg / 100 mL dH_2O	1.0 mL
$Na_2MoO_4.2H_2O$	630 mg/ 100 mL dH_2O	1.0 mL
$ZnSO_4.7H_2O$	2.2 g/ 100 mL dH_2O	1.0 mL
$CoCl_2.6H_2O$	1.0 g/ 100 mL dH_2O	1.0 mL
$MnCl_2.4H_2O$	18.0 g/ 100 mL dH_2O	1.0 mL

Table 3: Composition of f/2 vitamin solution

Compounds	Stock Solution	Quantity
Vitamin B_{12}	10 mg / 10 mL dH_2O	1.0 mL
Biotin	10 mg / 10 mL dH_2O	1.0 mL
Thiamine HCl	-	200.0 mg

3) were added. After the addition of all compounds, the final volume was made up to 1.0 L with dH_2O.

After the sample was brought to the laboratory, it was observed under the HUND inverted microscope and Olympus FX 100 microscope for qualitative estimation up to the genus level. Two species were selected for a comparative study of the impact of temperature and salinity on growth. One was centric diatom *Cyclotella* sp. and another was pennate diatom *Navicula* sp. *Cyclotella* sp. and *Navicula* sp. are used as live feed in aquaculture so there is a need to know the optimum environmental conditions for their growth. The selected phytoplankton was isolated under a microscope and then 2 mL of the isolated sample was inoculated into 20 mL f/2 media of different salinities.

Experimental design

The experiment was conducted using the factorial method. Three temperatures 24°C, 27°C and 30°C and three salinities 15 ppt, 25 ppt and 35 ppt were combined for the experiment. The samples were kept in 12 hr light and 12 hr dark (L:D). All other environmental variables were kept constant. The culture aliquots were incubated in the REMI CIS 24 Plus Incubator for 10 days at the above cited temperatures. The initial and final number of cells were counted for each sample. The average daily division rate (K) for the incubation period was calculated from (Smayda, 1969):

$K = \ln(C_t/C_o)(1/t \ln 2)$

where

C_t and C_o are cell concentrations at 10 and 0 d respectively

C_t represents the mean terminal population for the 3 replicates

The most favourable condition for growth was analysed by two-way ANOVA done in the R software.

Results and discussion

Results of two-way ANOVA showed that the effects of temperature and salinity on the growth of *Cyclotella* sp. were not significant. The diatom grew slowly as the temperature increased from 24°C to 30°C. At temperature 24°C, *Cyclotella* sp. showed a slower growth rate at all salinities except at 25 ppt may be due to competition for nutrients by the other species. As the qualitative examination of diatom was upto genus level only, it can also be assumed that different species of genus *Cyclotella* might be present. The variation in growth may be because different species of *Cyclotella* have different temperature and salinity requirements. There was decline in growth rate as the salinity increased at 27°C. At 30°C, a very slow growth rate was ascertained as the salinity increased except at 25 ppt salinity, which again can be due to varied species composition and competition for nutrients. Due to very less growth rate at higher salinities, it can be implied that *Cyclotella sp*. is more adapted to freshwater environment of higher temperatures. Li *et al.,* (2017) also reported good growth of *Cyclotella sp.* at 30°C on experimenting with 15°C, 20°C, 25°C and 30°C in a study without varying salinity which is supported by this work. Also, Roubeix *et al.,* (2008) experimented with various salinities on *Cyclotella sp*. and found out that the species favours intermediate salinities and growth reduces beyond that which is in agreement with the present study.

For *Navicula sp.*, the results revealed that there was a significant impact of salinity on the growth rate. The temperature did not seem to have any effect on the growth rate because there was very little difference between the temperatures opted for the research work, which might not be in a favourable range for the species. *Navicula* sp. showed growth at all salinity treatments. The growth was high at 35 ppt at all the temperatures. This result indicates that *Navicula* sp. prefers a brackishwater ecosystem. Similar observation was reported by Khatoon *et al.,* (2010) on comparing the effects of different salinities on *Navicula sp*. The work of Juneau *et al.,* (2015) confirms the same. Also, this study agrees with McLachlan's (1961) assertion that marine algae are commonly thought to be tolerant and adaptable to a wide range of salinities.

Comparative study between the two diatom species, *Cyclotella* sp. and *Navicula* sp. was done by growth plots. The results showed that *Navicula* sp. had better growth in varying temperature and salinity conditions than *Cyclotella* sp. *Cyclotella* sp. was more suited to higher temperatures and intermediate salinities, whereas *Navicula* sp. showed good growth in higher salinities at all temperatures. Le *et al.,* (2023) had similar observations in his study on diatoms.

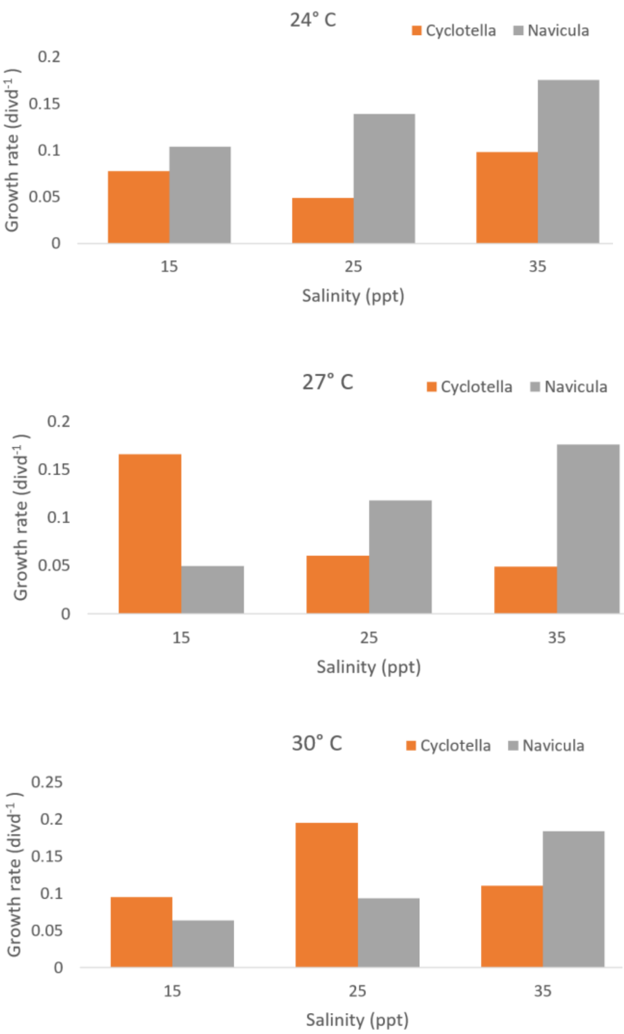

Fig. 1. Comparative average growth rates of *Cyclotella* sp. and *Navicula sp.* at different temerature and salinity concentrations

Conclusion

This study concludes that pennate diatoms are more adapted to change in environmental conditions and can show a good growth even outside their favourable range of temperature and salinities. Therefore, it is advisable to prefer pennate diatoms for feedstock in aquaculture practices which is economically feasible also.

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