



Light adaptation strategies of tropical marine diatoms

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Abstract

Tropical marine diatoms, *Achnanthes hauckiana* Grun., *Amphora coffeaeformis* (Agardh) Kutz., *Fragilaria pinnata* Ehr., *Synedra tabulata* (Agardh) Kutz., *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle, and *Triceratium dubium* Brightwell were employed in the present investigation to understand their adaptive behaviour when grown under different light intensities under laboratory conditions. Photosynthesis – light intensity curves were drawn with the data generated using oxygen electrode. *T. weissflogii* and *F. pinnata* showed a typical '*Chlorella type*' of adaptation, by changing their chlorophyll content, with low light adapted cells showing a higher P_{max} than high light adapted cells. In *S. tabulata* and *T. dubium*, the 0.5 KLUX and 2 KLUX cells had approximately the same chlorophyll content and their behaviour was similar to the type described as '*Cyclotella type*'. In *A. coffeaeformis* and *A. hauckiana*, 2 and 10 KLUX adapted cells had approximately the same chlorophyll content. However the light saturated rate did not increase as evidenced by the absence of any change both in the P_{max} and in the protein content. Although *A. hauckiana* showed the highest I_k among diatoms, I_k decreased with increasing light intensity contrary to the general behaviour of light adaptation in algae.

Keywords: marine diatoms, light intensity, *Achnanthes hauckiana* Grun., *Amphora coffeaeformis* (Agardh) Kutz., *Fragilaria pinnata* Ehr., *Synedra tabulata* (Agardh) Kutz., *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle, and *Triceratium dubium* Brightwell

Introduction

Diatoms contribute to 25% of the global primary productivity (Scala and Bowler 2001) and are responsible for about 40% of the marine primary productivity (Falkowski *et al.* 1998). Centric diatoms are essentially planktonic microalgae which are found in all open water masses, while pennate are found most of the time in benthic forms, growing on sediments or attached to rocks or macroalgae; and some species can also be found in soil (Lee 1999). Despite their abundance and diversity in nature, few species are cultured in aquaculture or for useful products (Thierry Lebeau and Jean-Michel Robert, 2003). Diatoms store their photosynthetic product and lipid and considered as suitable feedstock for bio-diesel production (Supriya and Ramachandra, 2011). Understanding the biology, adaptive behaviour to environmental factors become necessary for effective handling of large scale algal production systems. The present study is an attempt to understand the adaptation strategies of a few selected tropical marine diatoms when grown under different light intensities at laboratory conditions. Adaptation of these marine diatoms with reference to a variety of nitrogen sources has been already reported (Sivasubramanian and Rao, 1988a and b).

Phytoplankton are subjected to a continuously changing environment and therefore must be able to adapt to short term diel changes, as well as changes that are seasonal and longer. Growth, species composition and production of phytoplankton appear to be related to light quality and intensity, temperature and available nutrients. Many investigations deal with effects of changing light intensity on plants and Oltman's (1892) was perhaps the first. Whitford (1960) concludes that many green algae are high light species, red algae are low light species and diatoms and Chrysophyta are indifferent in light requirements. The rate of photosynthesis is controlled either by the rate of photochemical or by the rate of enzymatic processes (Stemann Nielsen and Jorgensen, 1968). The slope of the initial part of the light-photosynthesis curve is a function of the photochemical part of the photosynthesis and the horizontal part represents the maximum rate of the enzymatic processes. The light intensity at which the initial slope and the horizontal part of the light-photosynthesis curve intersects describes the ratio between the two kinds of processes. The light intensity, introduced as I_k by Talling (1957) is an important means of describing the physiological adjustment of an algal population.

Stemann Nielsen and Jorgensen (1968) reported that *Chlorella pyrenoidosa* adapts to irradiance by changing the pigment content (called *Chlorella type* of adaptation) whereas *Cyclotella meneghiniana* (Jorgensen 1964 a, b) adapts by changing the light – saturated rate without changing the pigment (*Cyclotella type* of adaptation). *Skeletonema costatum* which showed the same rate of photosynthesis per cell at all irradiances at 20 C and 7 C adapts by increasing the enzymes at low temperature (Stemann Nielsen and Jorgensen, 1968), Steemann Nielsen and Hansen (1959) and Jorgensen and Steemann Nielsen (1965)

have advanced the hypothesis that the concentration of enzyme per cell increases when temperature is low. Following these early investigations several investigators have examined experimentally the influence of temperature and irradiance on growth, pigmentation, photosynthesis and respiration of planktonic algae (Brown and Richardson, 1968; Jorgensen, 1969; Hobson, 1974; Cloern, 1977; Jones, 1978; Chan, 1978; Yoder, 1979; Tomas, 1980; Admiraal and Peletier, 1980). Falkowski and Owens (1980) have investigated light-shade adaptation in *Skeletonema costatum* and *Dunaliella tertiolecta*. According to them, in *Skeletonema costatum* light – shade adaptation is characterized primarily by changes in the size and not the number of P₇₀₀ units.

The present paper deals with a comparative study of adaptation of six tropical marine diatoms to light intensity.

Materials and Methods

The diatoms:

Achnanthes hauckiana Grun., *Amphora coffeaeformis* (Agardh) Kutz., *Fragilaria pinnata* Ehr., *Synedra tabulata* (Agardh) Kutz., *Thalassiosira weissflogii* (Grunow) Fryxell and Hasle, and *Triceratium dubium* Brightwell were used in the present study. Table 1 gives information on these isolates. They were made axenic by antibiotic treatment (Droop, 1967) and maintained in Guillard's F/2 medium (Guillard and Ryther, 1962).

Table 1 Diatom cultures used in the present study

Diatoms	Collection site in India	Month and year of isolation
<i>Achnanthes hauckiana</i> Grun.,	Marsh collection, Pitchavaram	March, 1977
<i>Amphora coffeaeformis</i> (Agardh) Kutz.,	Coastal collection rich in organic matter, Karwar	March, 1978
<i>Fragilaria pinnata</i> Ehr.,	Madras Harbour	March, 1978
<i>Synedra tabulata</i> (Agardh) Kutz.,	Moist sand, Mahabalipuram	August, 1979
<i>Thalassiosira weissflogii</i> (Grunow) Fryxell	Backwaters, Ennore	August, 1979
<i>Triceratium dubium</i> Brightwell	Marsh collection, Pitchavaram	March, 1977

Culture conditions and analytical techniques

Cultures were grown at 24±1 C in a thermostatically controlled room and illuminated with cool white fluorescent lamps (Sylvania 40 W) at an intensity of 2000 Lux in a 12:12 light dark regime. For experiments on the effect of irradiation different light intensities ranging from 0.5 to 10 KLUX were employed. Cultures were continuously aerated with sterile, humidified air using an aquarium pump. Growth was measured by counting cells using haemocytometer and calculating division rates. Pigments were determined spectrophotometrically (Strickland and Parsons, 1972). Photosynthetic measurements were made with Clark – type YSI Model 53 Oxygen electrode. Light intensity was measured using a EEL LUX meter. Oxygen exchange rates were calculated from Rawson's nomogram (Welch, 1948).

Experiments and Results

Exponentially grown cultures of the six diatoms were inoculated into F/2 medium and incubated at a light intensity of 2 KLUX and temperature of 24 ± 1 C and cell counts were made on alternate days for 18 days. The results are presented in Table 2.

Table 2 Growth rates of diatoms grown at 2 KLUX light intensity at 24 ± 1° C

Diatoms	Division rates (divisions/day, μ)
<i>Achnanthes hauckiana</i>	0.44
<i>Amphora coffeaeformis</i>	0.80
<i>Fragilaria pinnata</i> Ehr.,	0.97
<i>Synedra tabulata</i>	0.53
<i>Thalassiosira weissflogii</i>	0.73
<i>Triceratium dubium</i>	0.65

F. pinnata exhibited the highest division rate followed by *A. coffeaeformis* and *T. weissflogii*. *A. hauckiana* showed the slowest division rate. Analyses of pigments were made on alternate days up to the 18th day (Fig.1and 2). Chla and Chlc showed steady increase up to 8 days in all diatoms. Carotenoids generally showed increase with age and reached maximum only after the onset of stationary phase. Chla and Chlc in *T. dubium* showed steady increase up to 8 days followed by steep fall on the 10th day and leveled off thereafter. Carotenoids showed dramatic increase on the 10th day and then leveled off. Corresponding to the slow growth rate of *S. tabulata*, pigments increased steadily till the 12th day. Carotenoids peaked only on the 14th day followed by rapid decrease of pigments on the 16th day. This sort of rapid degradation of pigments in stationary phase cultures was not observed in other diatoms which could be attributed to the inhibitory effect of extracellular materials, possibly an autotoxin. In *A. hauckiana*, *A. coffeaeformis*, *F. pinnata* and *T. weissflogii* chlorophyll content increased with age and peaked on the 8th day. Chla decreased after 8 days in these diatoms but in *A. hauckiana* and *F. pinnata* the decrease was not appreciable. Carotenoids increased in *A. coffeaeformis* and *T. weissflogii* up to 8 and 12 days respectively and then leveled off, whereas in *A. hauckiana* and *F. pinnata*, carotenoids increased steadily with age till the 18th day. Ratio of Chlc/Chla did not show any marked variation in these diatoms (Fig. 3). This was calculated specifically because changes in Chlc/Chla ratio was expected during photoadaptation when grown at different light intensities. Rates of photosynthesis expressed in term of cells, for cultures grown at 2 KLUX light intensity and 24±1 C temperature. Rates of photosynthesis peaked on the 8th day in all cultures except S. tabulata where the peak was on the 12th day. Generally photosynthetic curves corresponded to Chla curves. (Fig. 4)

Fig 1 Chla and Chlc composition of diatoms grown in F/2 medium at 2 KLUX light intensity (12:12 light dark regime and 24 ± 1° C)

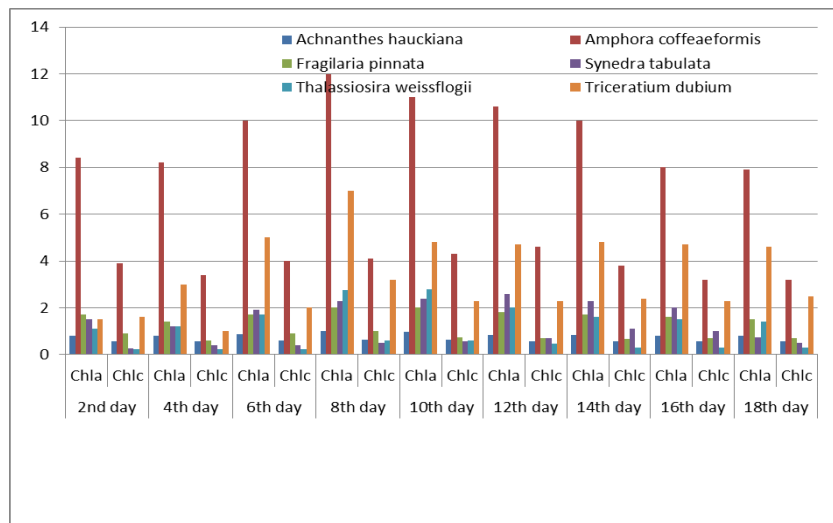


Fig 2. Carotenoids content of diatoms grown in F/2 medium at 2 KLUX light intensity (12:12 light dark regime and $24 \pm 1^\circ \text{C}$)

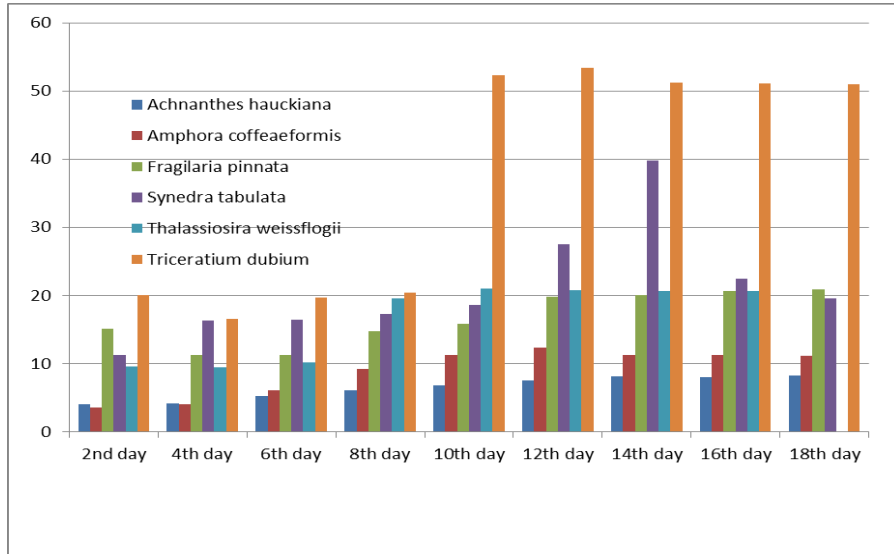


Fig 3. Chlc/Chla ratio of diatoms grown in F/2 medium at 2 KLUX light intensity (12:12 light dark regime and $24 \pm 1^\circ \text{C}$)

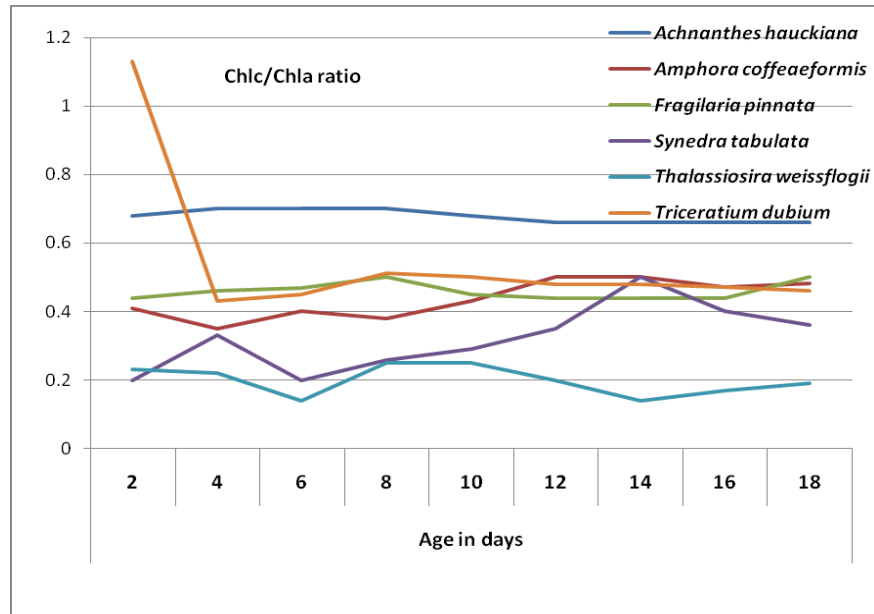
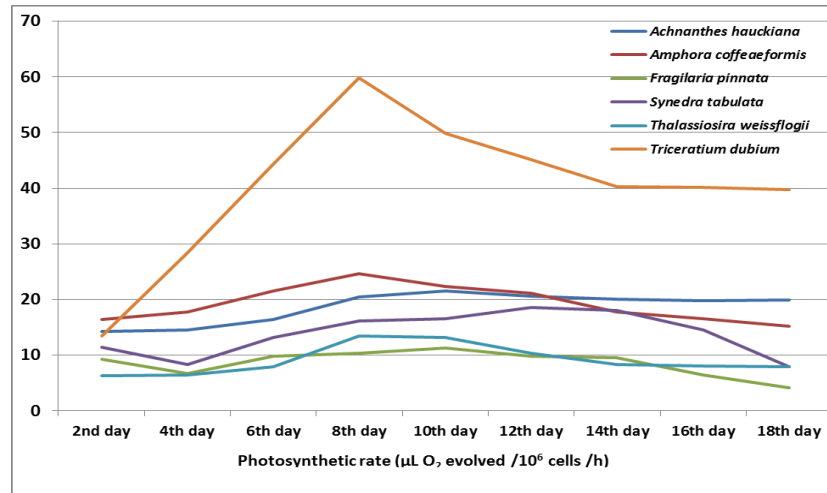


Fig 4. Photosynthetic rates of diatoms grown in F/2 medium at 2 KLUX light intensity and 24 ± 1° C



Growth and pigment composition of diatoms grown under different light intensities

Diatoms were grown in different light intensities ranging from 0.5 KLUX to 10 KLUX, keeping other growth conditions the same as in previous experiments and division rates were calculated (Fig.5). Pigments were analyzed on the 6th day of growth (Fig.6and 7). *T. weissflogii* and *A. coffeaeformis* showed maximum division rate (μ_{max}) at 2 KLUX and 4 KLUX respectively. *S. tabulata* and *F. pinnata* showed μ_{max} at 6 KLUX. *T. dubium* and *A. hauckiana* showed μ_{max} at 8 KLUX and 10 KLUX respectively. Light intensity above 6 KLUX had an inhibitory effect on growth of *S. tabulata*. Optimum light intensity for pigment production by all diatoms was below 2 KLUX. *A. coffeaeformis* had the highest amount of Chla and carotenoids while *T. weissflogii* and *S. tabulata* had the lowest. In *A. coffeaeformis*, *T. weissflogii* and *F. pinnata*, the ratio of Chlc/Chla did not change with increase in light intensity (Table 4). In *T. dubium*, the ratio increased above 6 KLUX whereas in *S. tabulata* and *A. hauckiana* it showed an initial increase up to 4 KLUX followed by a plateau and a steep fall. Protein content of diatoms grown at 0.5, 2 and 8 or 10 KLUX is represented in Table 3. Low light-grown cells showed higher protein content except in *S. tabulata* and *T. dubium*.

Fig 5. Division rates (μ) of diatoms grown in different light intensities

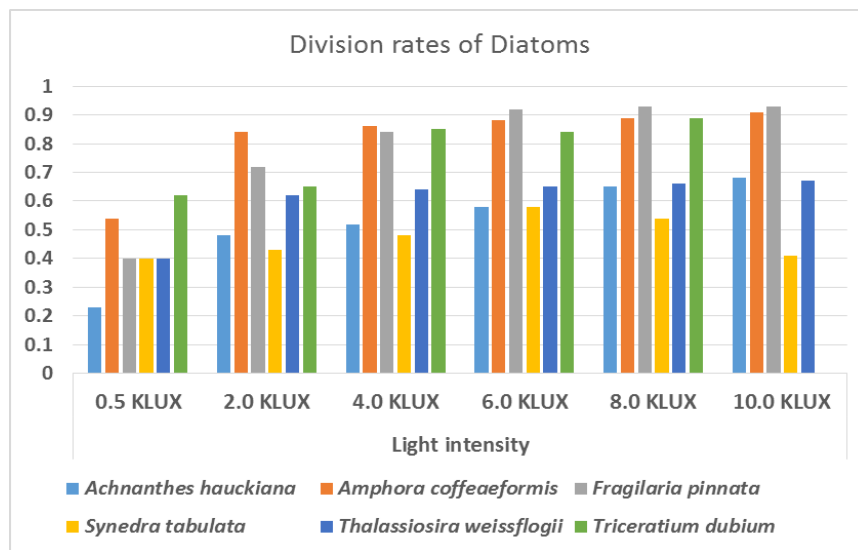


Fig 6. Pigment composition of diatoms grown for 6 days in different light intensities

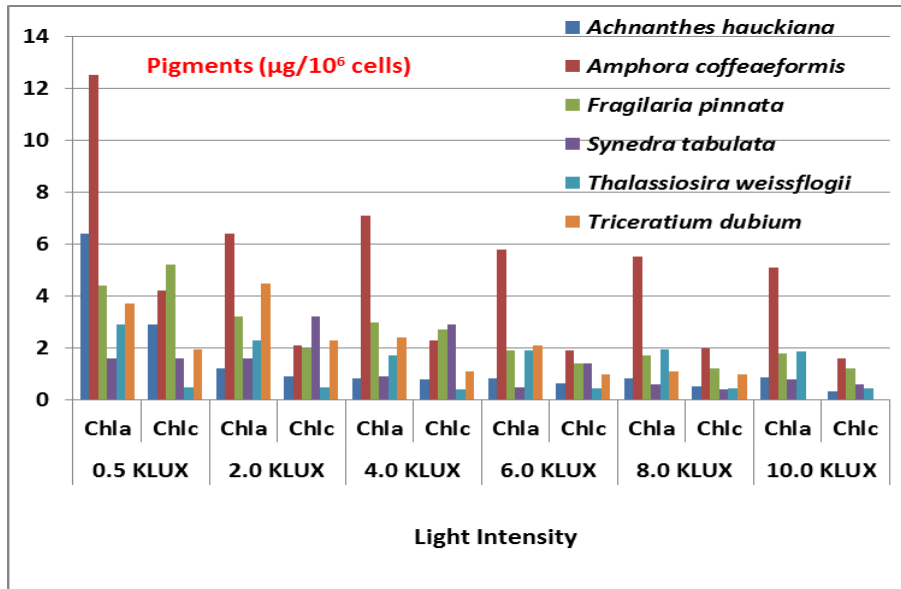


Fig 7. Carotenoids of diatoms grown for 6 days in different light intensities

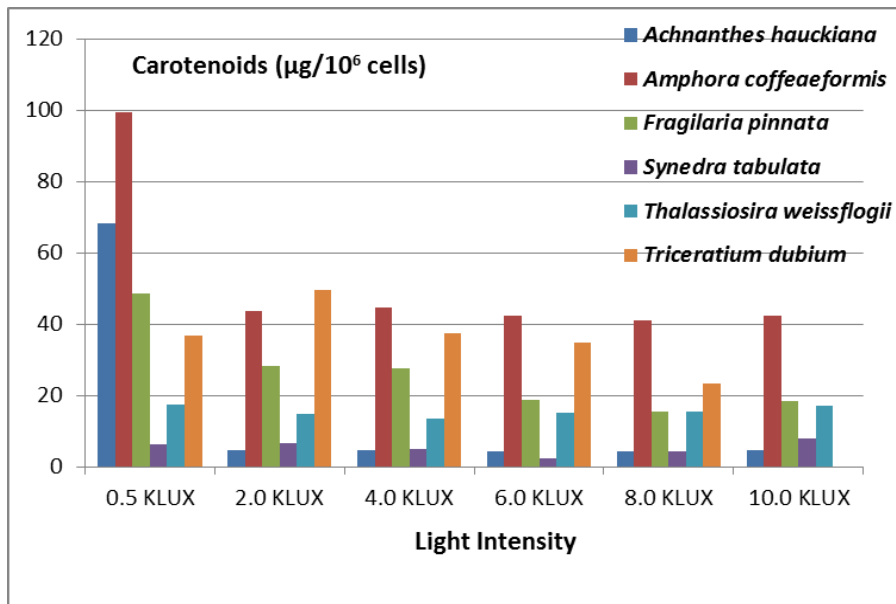


Table 3. Protein content of diatoms grown in different light intensities

Diatoms	Total protein ($\mu\text{g}/10^6$ cells)			
	Light Intensity in KLUX			
	0.5	2	8	10
<i>Achnanthes hauckiana</i>	42	19	-	19
<i>Amphora coffeaeformis</i>	220	88	-	99
<i>Fragilaria pinnata</i>	170	67	-	47
<i>Synedra tabulata</i>	20	27	29	-
<i>Thalassiosira weissflogii</i>	62	51	-	39
<i>Triceratium dubium</i>	180	185	195	-

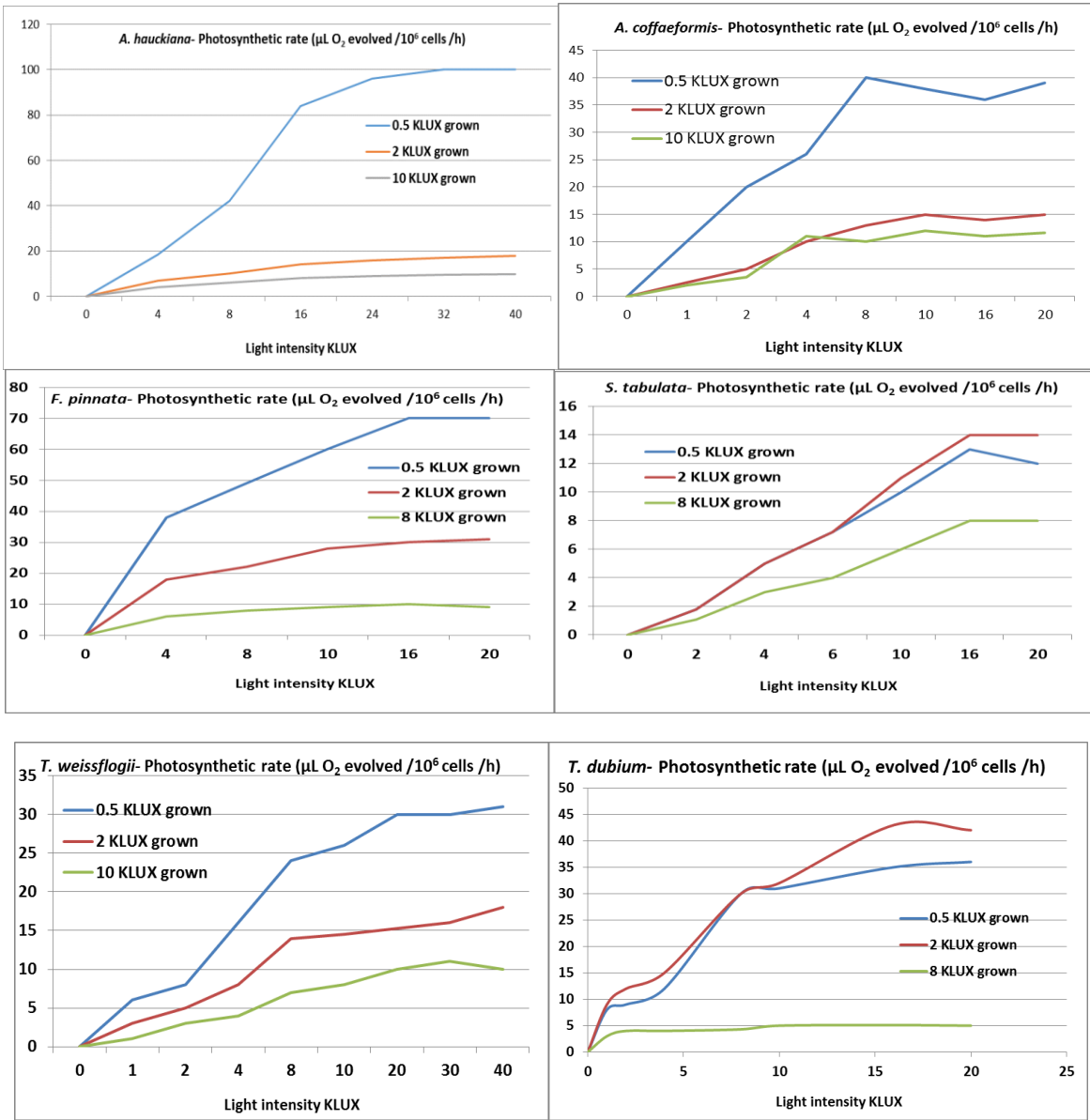
Table 4. Chlc/Chla ratio of diatoms grown for 6 days in different light intensities

Diatoms	Chlc/Chla ratio					
	0.5 KLUX	2.0 KLUX	4.0 KLUX	6.0 KLUX	8.0 KLUX	10.0 KLUX
<i>Achnanthes hauckiana</i>	0.4	0.76	0.75	0.48	0.42	0.41
<i>Amphora coffeaeformis</i>	0.38	0.36	0.38	0.34	0.37	0.35
<i>Fragilaria pinnata</i>	1.1	0.68	0.86	0.65	0.7	0.66
<i>Synedra tabulata</i>	1.0	2.1	3.2	3.3	0.52	0.55
<i>Thalassiosira weissflogii</i>	0.18	0.2	0.22	0.24	0.22	0.23
<i>Triceratium dubium</i>	0.55	0.51	0.5	0.52	0.8	-

Photosynthesis and respiration in diatoms grown under different light intensities

Photosynthetic experiments were carried out under different light intensities ranging from 0.5 KLUX to 40 KLUX in cultures adapted to different light intensities and photosynthesis – light intensity curves (P vs I) were drawn. Rates of photosynthesis were expressed in terms of cell number (Fig. 8) and Chlorophyll a (Fig. 9). It is obvious that the curves showed variations according to the unit applied. Photosynthetic characteristics of diatoms adapted to different light intensities are also given in Table . The initial slope of photosynthesis – light intensity curve for 0.5 KLUX and 2 KLUX grown *T. dubium* cells was similar. Pmax for 2 KLUX adapted cells was higher than 0.5 KLUX adapted cells. The initial slope of 8 KLUX adapted cells was much lower due to almost 4 times lower chlorophyll content than 0.5 KLUX cells. Actual pigment analysis also confirmed this (Fig.6). Due to adaptation, as a general rule, I_K is always higher in diatom cells grown at high light intensities than in cells grown at low intensities. In *T. dubium* , low light adapted cells seemed to have a higher I_K than high light adapted cells (Table 5).

Fig 8. Photosynthesis- Light intensity curves of diatoms adapted to different light intensities (expressed in terms of cell number)



Light saturated rate of photosynthesis for 8 KLUX showed a trend similar to *T. dubium*. The initial slopes of the photosynthesis-light intensity curves of 0.5 and 2.0 KLUX grown cells were similar suggesting that the chlorophyll content per cell had not changed due to light adaptation. This was experimentally verified by actual analysis of cellular pigment content. 2 KLUX adapted cells showed higher light-saturated rate of photosynthesis than 0.5 KLUX cells indicating an adaptation of the photosynthetic enzymatic processes to increased light. This was also evident from the increased total protein content of 2 KLUX adapted cells when compared to 0.5 KLUX cells, which behavior was similar to *T. dubium* under these conditions (Table 3). As in *A. coffeaeformis*, in *A. hauckiana* also cells grown in 2.0 and 10 KLUX had approximately the same chlorophyll content per cell. But there was no increase in the rate of enzymatic process due to increase in light intensity as the light saturated rate did not change. This was also evident from the decrease in protein content in high light adapted cells of these two diatoms.

Fig. 9. Photosynthesis- Light intensity curves of marine diatoms adapted to different light intensities (expressed on the basis of Chlorophyll a)

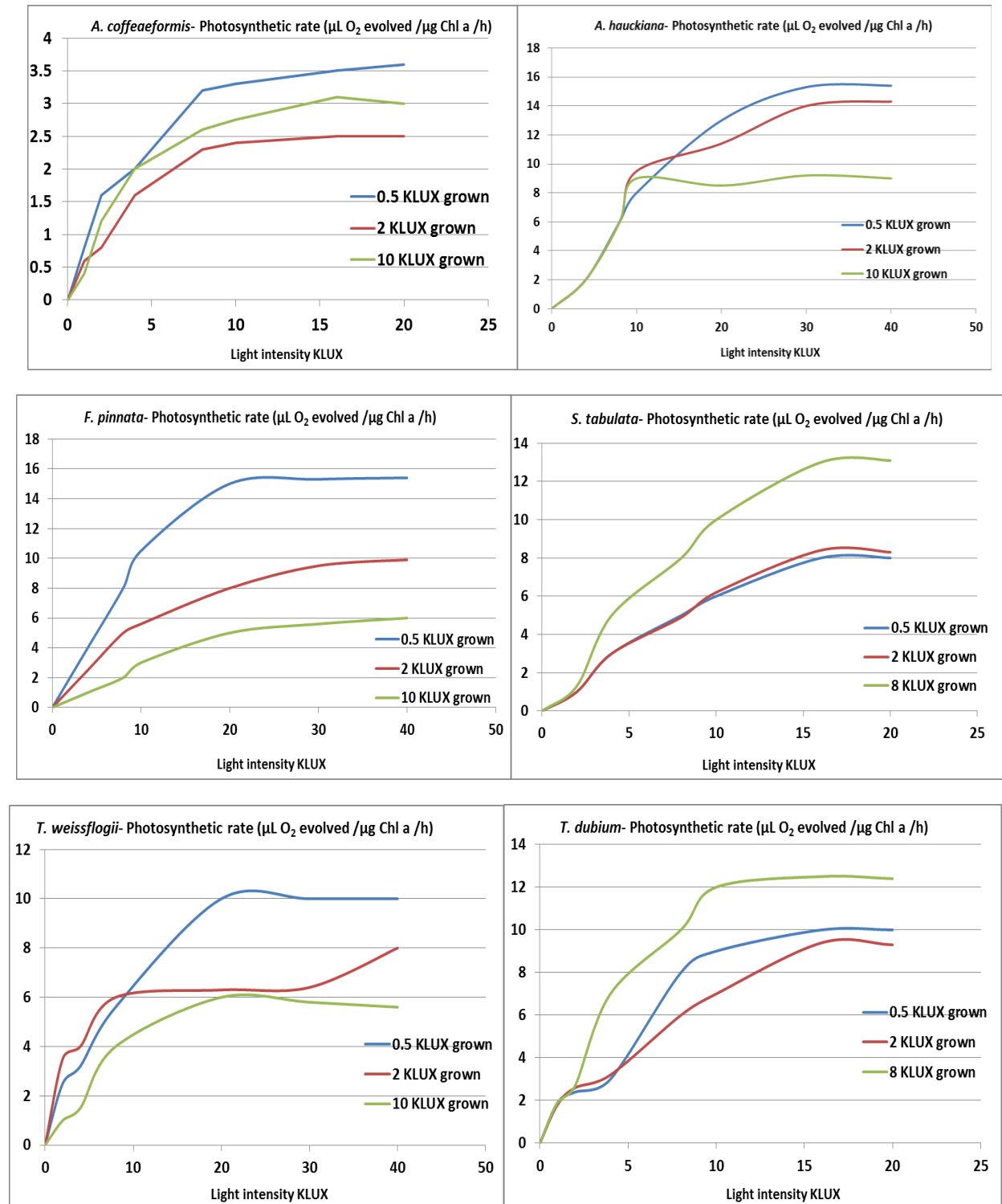


Table. 5. Photosynthetic characteristics of marine diatoms adapted to different light intensities

Diatoms	Light intensity KLUX	Chla $\mu\text{g}/10^6$ cells	*LS 10 KLUX or 8 KLUX in percent of 0.5 KLUX	I_K KLUX	Photosynthetic rate	
					$\mu\text{L O}_2/10^6$ cells/h	$\mu\text{L O}_2/\mu\text{g Chl}$ a/h
<i>Achnanthes hauckiana</i>	0.5	6.3		22.8	101	16.0
	2.0	1.2	8.9	16.8	18	15.0
	10.0	0.9		14.4	9	10.5
<i>Amphora coffeaeformis</i>	0.5	12.5		4.4	40	3.2
	2.0	6.4	35.0	6.2	16	3.1
	10.0	4.5		6.2	14	2.5
<i>Fragilaria pinnata</i>	0.5	4.5		12.0	70	15.5
	2.0	3.0	15.7	16.4	31	10.2
	10.0	1.8		20.0	11	6.2
<i>Synedra tabulata</i>	0.5	1.6		10.8	13	8.4
	2.0	1.6	61.5	11.5	14	8.9
	10.0	0.6		12.0	8	13.0
<i>Thalassiosira weissflogii</i>	0.5	2.9		7.2	30	10.3
	2.0	2.3	38.3	12.0	20	8.5
	10.0	1.8		15.2	11.5	6.5
<i>Triceratium dubium</i>	0.5	3.6		10.0	39	10.7
	2.0	4.5	30.8	12.0	45	10.0
	10.0	1.0		6.5	12	12.5

*LS: Light saturated rate of photosynthesis

Table.6. Respiratory rates of marine diatoms grown under different light intensities

Diatoms	Respiratory rate ($\mu\text{L O}_2/10^6$ cells/h)			
	0.5 KLUX	2 KLUX	8 KLUX	10 KLUX
<i>Achnanthes hauckiana</i>	15.0	2.5	-	4.5
<i>Amphora coffeaeformis</i>	5.0	1.8	-	2.9
<i>Fragilaria pinnata</i>	7.5	7.5	-	4.0
<i>Synedra tabulata</i>	2.8	3.0	1.7	-
<i>Thalassiosira weissflogii</i>	3.5	1.5	-	2.0
<i>Triceratium dubium</i>	6.0	6.6	7.7	-

The initial slopes of photosynthesis-light intensity curves of *T. weissflogii* and *F. pinnata* adapted to different light intensities, were different indicating that these diatoms adapted to increasing light intensity by changing their chlorophyll content; low light grown cells showing the maximum chlorophyll content per cell. Adaptation to increased light intensities was also indicated by the I_K shift which was higher for high light adapted cells. Low light adapted cells showed higher P_{max} than high light adapted cells. Total protein per cell was also very low in high light adapted cells. The behavior of *T. weissflogii* and *F. pinnata* was typical of what is generally referred to as the ***Chlorella type*** of adaptation.

Table 6 shows the respiratory rates of diatoms grown in different light intensities. Generally all diatoms showed higher rates of respiration when grown under low light corresponding to higher rates of photosynthesis. *T. dubium* alone showed a high respiratory rate when grown in high light intensity (8 KLUX).

Discussion

Photosynthesis versus light intensity curves have become more or less fundamental to the study of light adaptation in both natural populations of phytoplankton and laboratory cultures of algae. Cultures of many unicellular algae are known to adapt to irradiance by changing the rates of the enzymatic processes or the photochemical processes (Steemann Nielsen, 1975). Species of *Chlorella* adapt to different light intensities, mainly by varying the pigment content of the cell. The adaptive response of diatoms to changes in light intensity is different. Jorgensen (1964a) observed that cells of the centric diatom *Cyclotella menegheniana*, as also many other diatoms studied, adapt by changing the light saturated rate, which would mean that the content of enzymes active in photosynthesis changes, the light saturated rate being much higher when the diatoms are grown at a strong irradiance than when grown at a weak irradiance. *Skeletonema costatum*, another centric diatom, appears to change the concentration of both pigments and enzymes (Jorgensen and Steemann Nielsen, 1965).

In the present study, *T. weissflogii* and *F. pinnata* showed a typical '***Chlorella type***' of adaptation, by changing their chlorophyll content, with low light adapted cells showing a higher P_{max} than high light adapted cells. Due to adaptation I_K was also high for high light adapted cells. In *S. tabulata* and *T. dubium*, the 0.5 KLUX and 2 KLUX cells had approximately the same chlorophyll content and their behaviour was similar to the type described as '***Cyclotella type***'. The light saturated rate of enzymatic processes as well as the protein content increased in 2 KLUX grown cells as compared to 0.5 KLUX cells. In *A. coffeaeformis* and *A. hauckiana*, 2 and 10 KLUX adapted cells had approximately the same chlorophyll content. However the light saturated rate did not increase as evidenced by the absence of any change both in the P_{max} and in the protein content. Although *A. hauckiana* showed the highest I_K among diatoms, I_K decreased with increasing light intensity contrary to the general behaviour of light adaptation in algae.

Even with the limited number of diatoms studied a great variation in the range of adaptive behaviour was encountered, which included types of light adaptation that are referable as '*Chlorella type*', '*Cyclotella type*' or an intermediate type. The results of the present study are not strictly comparable with results of other studies because of the light: dark regime employed. A 12: 12 light: dark regime was used in the present investigation as against most of the studies in which illumination was continuous. Earlier it was shown that the behaviour of *Skeletonema costatum* (Jorgensen and Steemann Nielsen, 1965), during adaptation to different temperatures, was dependent on whether the light was supplied continuously or intermittently with changes in its duration. Since the diatoms used in this study were derived from an entirely different geographical locations, it is also possible that their response to light was different from those isolated from temperate localities which are seasonally subjected to large fluctuations of both light and temperature. More studies on light and temperature adaptation under different light: dark regimes may be needed for gaining further knowledge on the adaptive behaviour of diatoms.

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