Temperature, salinity and light duration dependence on the growth and cellular carbon and nitrogen contents of the epiphytic diatom, *Achnanthes longipes* Agardh

Samia K. Mikhail and Wagdy L. Gergis
National Institute of Oceanography and Fisheries, Alexandria, Egypt.
E. mail: Mikhail_samia@yahoo.com

ABSTRACT

Achnanthes longipes Agardh cells were isolated from glass slides submerged in the Eastern Harbour of Alexandria. Addition of D-600 (10 & 25 µM) and EDTA (10^{-3}M) was sufficient to prevent adhesion and aggregation of *A. longipes* cells and get homogenous free cells inocula for culture experiments. The influence of 52 combinations of salinity (5-40 at 5 intervals) and temperature (10^{o}-25^{o}C, at 5^{o}C intervals) under continuous light and 12:12 light/dark cycle on chlorophyll *a* content, the maximum doubling time, cellular carbon and nitrogen of *Achnanthes longipes* was examined under constant light intensity (70-80 µE m^{-2} S^{-1}). No growth was detected at 5 salinity levels with all temperatures tested, and under the combination of 15^{o}C-10 salinity. Maximum Chl. *a* content during the exponential phase was almost the same at the two light regimes (about 1.2 µg ml^{-1}). Based on Chl. *a* variations by days, *A. longipes* showed its maximum growth rate and doubling time (1.18 and 0.82 d^{-1}, respectively) at 20^{o}C-30 salinity (continuous light) and 1.6 and 1.11 d^{-1}, respectively at 20^{o}C-25 salinity (light/dark cycle). A rising in temperature from 15^{o}C to 20^{o}C caused about 1.8 fold increases in the doubling time of Chl. *a* at the two light regimes. The results indicated that Chl. *a* and carbon proceeded in parallel during the exponential phase under the light regimes, and the increasing in carbon content and Chl. *a* content accompanied an increasing in temperature. The carbon content was consistently lower in continuous light cultures than in the corresponding light/dark cultures. No definite trend was found between nitrogen and changed temperature and salinity (continuous light), while nitrogen generally increased with increasing temperature under the light/dark cycle. The maximum carbon/nitrogen was measured at 25^{o}C with salinity range of 35 - 40, it was about two-fold higher at the light/dark (40.03). The carbon: chlorophyll ratio showed a steady increase by days, and varied considerably with changed temperature. However, there was no characteristic relation between temperature, growth rate and carbon: chlorophyll.

Keywords: *Achnanthes longipes,* diatom, salinity, temperature, growth rate

INTRODUCTION

Diatoms adhere to surfaces using apical pads, stalks, tubes, capsules or adhering films (e.g. Hoagland *et al*., 1993; Wustman *et al*., 1997), that provide chemical cues for the settlement of higher organisms such as barnacles and polychaetes resulting in macrofouling (e.g.Lam *et al*., 2005; Patil & Anil, 2005).

Achnanthes longipes Agardh, a metaplanktonic species (a single-cell diatom that grows in colonies or long chain) is a prevalent component of the marine benthic littoral community, often forming dense epilithic or epiphytic populations (Round, 1971; Lange-Bertalot and Krammer 1989). The cosmopolitan occurrence of this diatom in dense populations (e.g. Eker and Kideys, 2000) permanently attach via stalks makes it a significant part of the marine biofouling community on ship hulls (Woods *et al*., 1988), even those coated with antifouling organotin paints (Callow, 1986). *A. longipes* relies on production of highly organized extracellular adhesive biocomposites for cell motility and permanent adhesion to submerged surfaces (Wang *et al*., 1997). Studies on its adhesion focused on factors that affect the production of stalks both in field and experiments (Johnson *et al*., 1995; Wang *et al*., 1997; Lewis *et al*., 2002). *A. longipes* may provide a continuous food source for grazing abalone juveniles (Kawamura *et al*., 1995; Takami *et al*., 2003).

A. longipes was recorded as epiphytic diatom on marine algae and artificial substratum under natural conditions in the Eastern Harbour of Alexandria (Mikhail, 1986), and as free living cells in the phytoplankton community (e.g. Al-Handhal, 1979). The previous studies in the harbour investigated its annual occurrence, and no attention was paid about regulating ecological factors.

In this study, a series of laboratory trials were conducted to determine the optimal culture conditions of *A. longipes* and to assess their effect on its biomass and biochemical composition. Such knowledge is still scarce. The tolerance of *A. longipes* to the present fluctuating culture conditions, including changes in the combination of temperature, salinity and light duration might define its suitability as a food source. Such knowledge about the optimal range for maintaining an adequate food source for growing juveniles maximizes growth and minimizes time and costs associated with the culture of the microalgal species. The study provides some information that will further aid in the development of antifouling techniques, and seeks to obtain robust estimates of the cellular content of carbon and nitrogen in *A. longipes* to help model their role through marine food webs. The physiological responses causing changes in its cellular C:N certainly involve changes in the cellular content of proteins, nucleic acids, carbohydrates, lipids, and polyphosphate (Geider and La Roche, 2002). Very little information is available on the regulation of C:N:Chl. *a* in *A. longipes* grown in culture experiments.

MATERIALS AND METHODS

I- Preparation of free cells inocula

Achnanthes longipes cells were isolated from glass slides submerged for 4 days in the Eastern Harbour of Alexandria. Single cells were taken using drown out Pasteur pipette and transferred through several changes of sterile seawater (Hoshaw and Rosowski, 1973) into ASP-2 medium (Provasoli *et al*., 1957). Two experiments were carried out to overcome the problems of adhesion and aggregation in order to get homogenous inocula of unattached free cells:

1- Equal portion of cell suspension (10 ml) were added to 15-ml beakers containing 22-mm glass microscope slide cover glass and supplemented with $CaCl_2$ at different concentrations (0.25, 0.75 &2.5 mM Ca^{++}). Beakers were incubated at 28ºC under continues light (~70 µE m^{-2} S^{-1}).
Another set was prepared with 10 & 25 µM D-600 (α-Isopropyl-α-[(N-methyl-N-homovertatryl)-γ-aminopropyl]-3,4,5-trimethoxy phenylacetonitrile), incubated for 15 min. before Ca^{++} addition. Triplicate samples were taken every 30-min. for 210 min. The cover glasses were removed from the beakers; viable cells of the suspension and per cover glass were counted after treating with Trypan blue (final concentration, 0.5%).

2- Cells suspension was added to F/2-EDTA medium and F/2 +EDTA (10^{-3}M), and incubated at 28ºC under continues light (~70 µE m^{-2} s^{-1}).

II- Salinity–temperature-growth experiments

Seawater was filtered through a glass fiber filter (Whatman GF/C), autoclaved for 15 min. at 121°C, cooled and stored at 5°C prior to use. The prepared seawater was heated and concentrated to salinity 40, from which a dilution to series (5-35 salinity) was made using distilled water. Growth medium was then prepared by enriching the seawater by the addition of f/2 nutrients (Guillard and Ryther, 1962). The free viable cells resulted from the previous experiment were inoculated in 15x125 mm test tubes containing 8 ml medium. Cultures were established at 10, 15, 20, 25, 30, 35 and 40 salinity and incubated at the different temperatures (10, 15, 20 and 25°C) under continues light and at 15, 20 and 25°C under 12:12 light-dark condition. At all experiments non-axenic cultures were grown at constant light intensity (70-80 µE m^{-2} s^{-1}). At every salinity-temperature combination, a set of test tubes were prepared to cover all samples time. Samples were taken every two days; the entire volume of medium was harvested using a sterile paintbrush to scrape the sides of the tubes and sub-samples of the species suspension were taken for different measurements. Growth results are presented in terms of Chl. *a* content (µg ml^{-1}).

III- Measurements and calculations

Samples for Chl. *a* content were filtered through Whatman GF/C (47 mm) glass fiber filters with addition of 0.01% $MgCO_3$, and Chl. *a* was extracted in 90% acetone and measured according to Lorenzen (1967). Duplicate cultures of Chl. *a* were measured; the mean values of these duplicate measurements were calculated for every experimental condition.

Cultures for organic carbon and nitrogen analysis were filtered onto Whatman GF/C (47 mm) glass fiber filters pre-combusted at 400 ^{o}C for over 12 hr., and then dried in wide mouth vial in a drying oven at 60^{o}C for 36 hrs., and kept drying in desiccators. Carbon analysis was done with elemental analyzer (MT-3, Yanaco, Japan). Cellular carbon and nitrogen are expressed as µg ml^{-1}.

The growth rate (µ) was estimated from Chl. *a* content at two-day intervals in the exponential phase using the formulation of Guillard 1973:

$$\mu = (\ln C_1 - \ln C_0)/ (T_1 - T_0)$$

where C_0 and C_1 representing the mean Chl. *a* content of the duplicate at time T_0 and T_1, respectively. The doubling per day of the Chl. *a* content of the culture was calculated using the following equation:

$$\text{Doubling time (DT)} = \ln 2/\mu$$

RESULTS

Adhesion and aggregation experiments

Generally, when *A. longipes* cells were inoculated, the cells were at first motile, then became sessile and produced a stalk that anchors them to the glass vessel's wall. This stalk continues to be synthesized producing a row of cells stacked one upon the other, became dense aggregated. Some cells eventually detach and begin the cycle again.

The experimental results about adhesion and aggregation of *A. longipes* showed that the number of adhered cells per cover glasses tends to decrease with decreasing Ca^{++} concentration in the media (Table 1), while in the suspension the free cells showed the reverse. The addition of D-600 (10 &25 µM) inhibited adhesion of *A. longipes* cells at all Ca^{++} concentrations (0.25, 0.75 & 2.5 mM). Cells in F/2-EDTA formed many clumps, whereas those in F/2+EDTA generally, remained free.

Table. 1. Time course of adhesion of *A. longipes* grown in ASP-2 medium with different Ca^{++} concentrations (Cells x10^4).

Ca^{++} Concentration	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.	210 min.
0.25 mM Ca^{++}	0.12	0.28	0.4	1.2	1.75	1.8	1.9
0.75 mM Ca^{++}	0.2	0.6	1.4	2.1	3.0	4.2	4.5
2.25 mM Ca^{++}	0.88	1.9	5.8	9.2	17.8	19.5	19.7

Growth as a function of temperature, salinity and light conditions

The growth curves, based on chlorophyll *a* content for the combination of temperature (10-25^{o}C) and salinity (5-40) at continuous light and for the combination of temperature (15-25^{o}C) and salinity (5-40) 12/12 L.D photoperiod are presented in Figs. 1 & 5.

A- Continuous light

The best Chl. *a* values were found at salinity 30 for all temperature. The highest Chl. *a* contents during the exponential phase fluctuated between 0.347 µg ml^{-1} (15^{o}C- 40 salinity, day 6) and 1.232 µg ml^{-1} (20^{o}C – 30 salinity, day 12). Other relatively lower Chl. *a* peaks (about 0.85 µg ml^{-1}) was measured at temperature range 20-25^{o}C and 25-30 salinity. The peak days of Chl. *a* occurred between days 6 and 12, except for 25^{o}C at salinity range 15-30 (day 4).

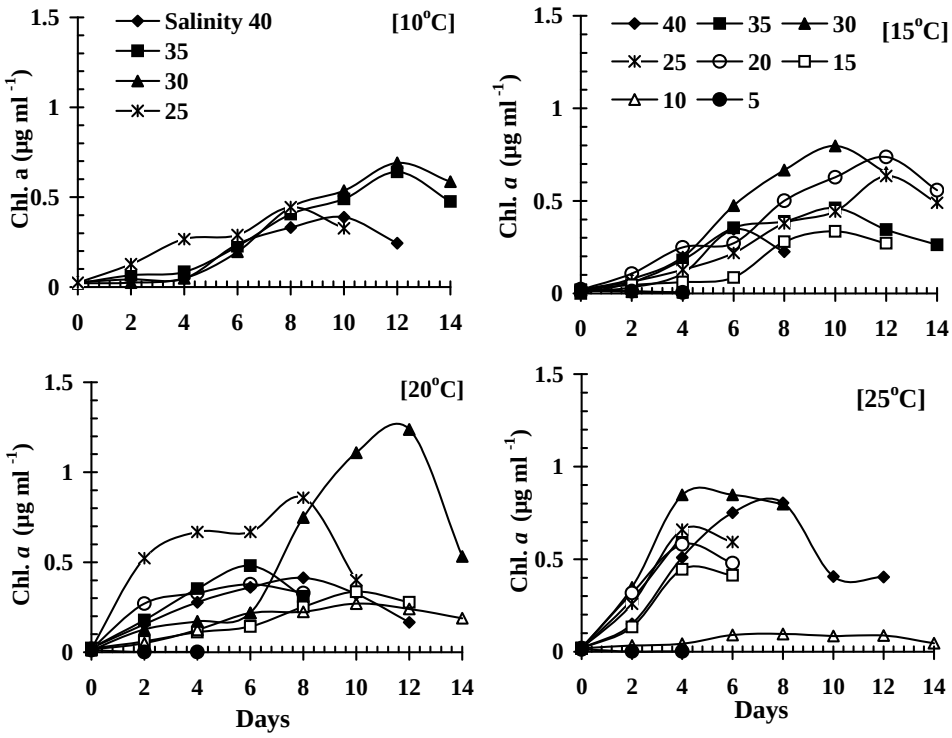

Fig. 1. Chlorophyll *a* concentrations (µg ml^{-1}) as a function of temperature and salinity at continuous light

A. longipes showed its maximum growth rate (1.03 - 1.18 d^{-1}) at 20-25^{o}C and 25 to 35 salinity. No growth was detected at 5 salinity with all temperatures tested, and under the combination of 15^{o}C-10 salinity. The maximum doubling time (DT) as a function of temperature and salinity (Fig. 2) occurred at 20^{o}C-30 salinity (0.82 d^{-1}), while, it was lowest at 20^{o}C-15 salinity (0.22 d^{-1}). An inreverse relationship was found between the maximum DT and salinity range 30-40.

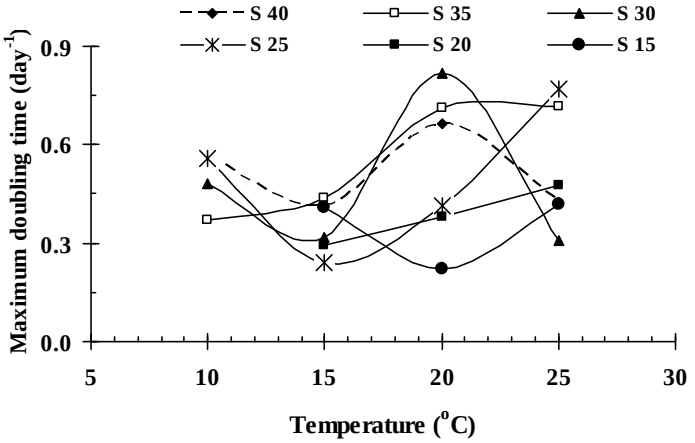

Fig. 2. Maximum doubling time of *A. longipes* as a function of temperature and salinity at continuous light

The carbon and nitrogen contents of *A. longipes* grown under continuous light was measured at 10,15°C (25-40 salinity), and 25°C (30-40 salinity). Generally, neither temperature nor salinity seems influencing the cellular content of C and N. However, C showed increasing trend with salinity at 25°C (Fig. 3). The maximum C value (65.4 µg ml^{-1}) was recorded at 25°C-40 salinity > at 10°C, 30 salinity (44.18 µg ml^{-1}) > at 15°C-30 salinity (40 µg ml^{-1}). The results indicated that Chl. *a* and carbon proceeded in parallel during the exponential phase.

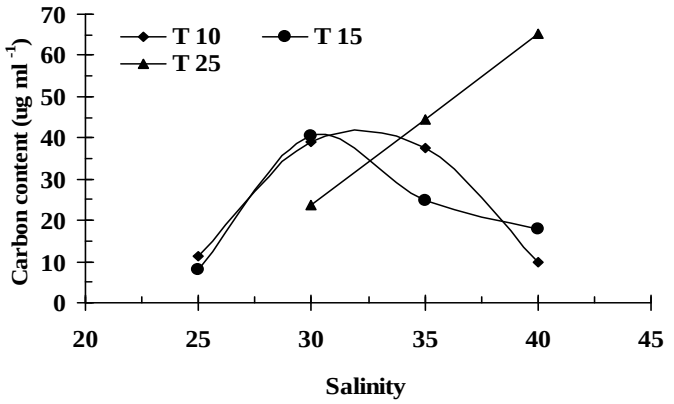

Fig. 3. Carbon content as a function of temperature and salinity at continuous light.

Nitrogen showed an increase with the salinity range of 25-35, then a distinct drop at 10°C-40 salinity (Fig. 4). However, no definite trend was found between N and changed temperature and salinity. The maximum N content (11.54 µg ml^{-1}) was measured at 10°C-35 salinity > at 15°C-30 salinity (8.93 µg ml^{-1}) > at 25°C-35 salinity (5.21 µg ml^{-1}).

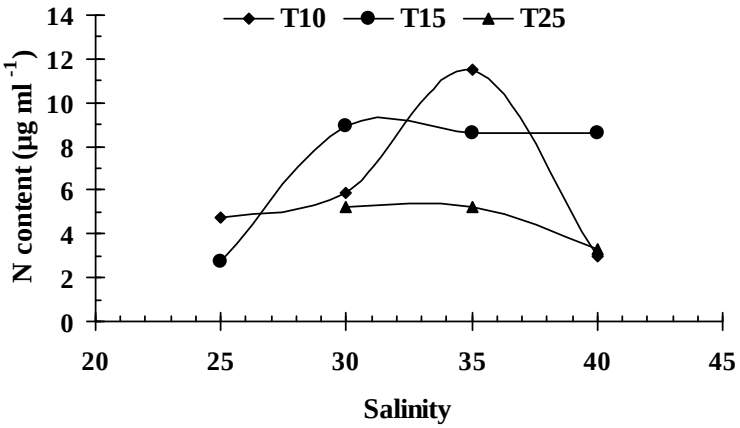

Fig. 4. Nitrogen content as a function of temperature and salinity at continuous light.

The C/N, C/Chl, and N/Chl ratios are given in Table (2). The C/N ratio ranged between 1.6 and 19.7, the maximum at 25°C-40 salinity combination. The ratio showed, generally, increasing trend by days, except at 15°C-30 salinity, when a reverse trend was observed. C/Chl ratios were generally influenced by both temperature and salinity; decreased with temperature at salinity range 25-35 and showed a reverse trend with the higher salinity (40). N/Chl ratio showed a reverse trend with salinity, except for the values at 25°C-all salinities.

B- 12/12 LD photoperiod

The best Chl. *a* values were found at temperature range 20-25 °C with all salinities, with a maximum at 20°C-20 salinity (1.19 µg ml^{-1}). Another high values were recorded with salinity 40 at 15 °C and 20°C (1.035 and 1.188 µg ml^{-1} respectively). The peak days of Chl. *a* occurred between days 10 and 16, except for 20°C-25 salinity (day 8), and at 25 °C-25 salinity (day 4). The time of duration at 40 salinity was longer than that at the continuous light.

The maximum growth rate fluctuated between 0.317 d^{-1} (10°C-25 salinity, day 10), and 1.596 d^{-1} (20°C-25 salinity, day 2). A gradual increasing in maximum growth rate with increased temperature was observed with 20, 30 and 35 salinity. No growth was detected at 5 salinity with all temperature tested, and under the combination of 15°C-10 salinity.

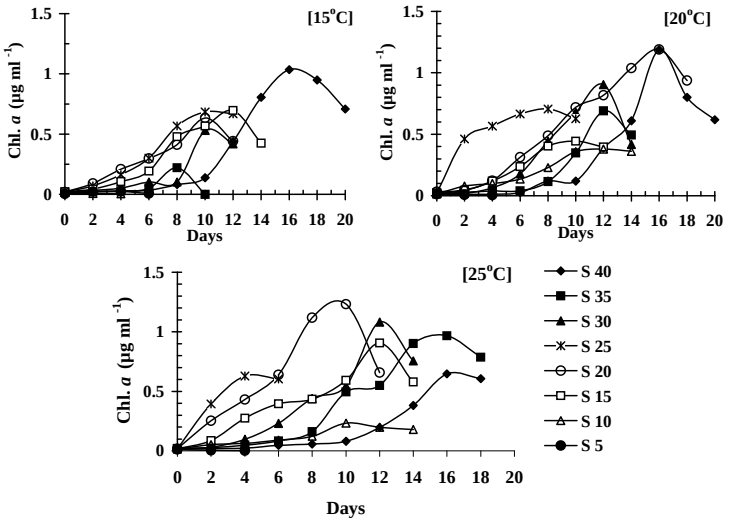

Fig. 5. Chlorophyll *a* concentrations (µg ml^{-1}) as a function of temperature and salinity at 12/12 LD light cycle

The doubling time ranged between 0.22 d^{-1} (25°C- 10 salinity, day 10) and 1.11 d^{-1} (20 °C - 25 salinity, day 2). The species at 20° C exhibited its maximum chlorophyll *a* doubling time at 25 and 40 salinity, while the reverse was seen at 30 and 35 salinity. A steady increase in D.T. with temperature was observed at 15 and 20 salinity (Fig. 6).

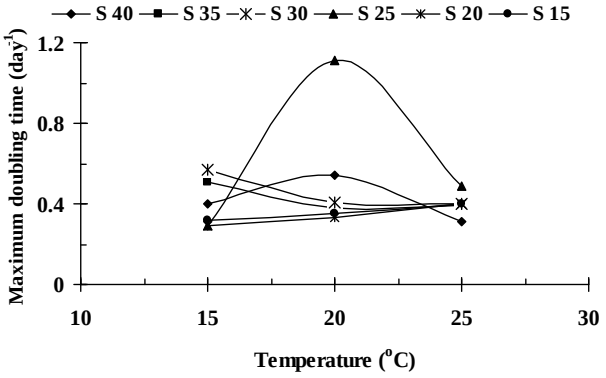

Fig. 6. Maximum doubling time of *A. longipes* as a function of temperature and salinity at 12/12 L.D cycle

The results indicated a parallel relationship between Chl. *a* and carbon contents during the exponential phase, and their coincident peaks as well. *A.*

longipes grew slowly at salinity 40-25°C, at which the highest cellular carbon was measured (72.75 µg ml^{-1}, at day 20, Fig. 7). A relatively high C content was also measured at 25°C-35 salinity (58.81 µg ml^{-1}, day 16).

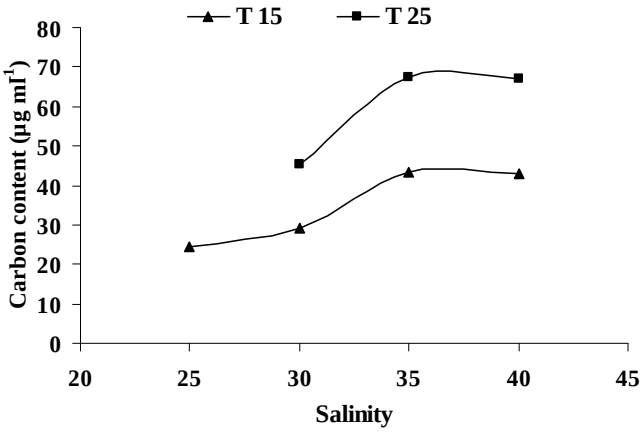

Fig. 7. Carbon content as a function of temperature and salinity at 12/12 L.D cycle

A reverse trend was found between Chl. *a* major peaks and the corresponded N content with all salinity treatments. Generally, N increased with increasing temperature (Fig. 8). At 15°C, the highest value (19.55) was measured at salinity 40 (day 8), while the lowest (0.68, day 4) at salinity 25. Other high values were also measured at 25°C-35 salinity (17.13, day 14), and 25°C-40 salinity (17.71, day 16).

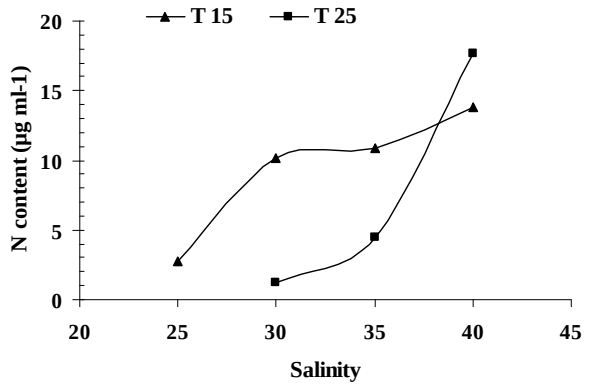

Fig. 8. Nitrogen content as a function of temperature and salinity at 12/12 L.D cycle

C/N ratio showed a significant increase (36.91) at 25°C-30 salinity (day 12), and 40.03 at 25°C-35 salinity (day 10). The C/Chl ratio, generally, exhibited

a steady increasing trend by days, and a positive relation between the values and the increased temperature and salinity, with its highest at 25°C-40 salinity. No specific trend observed between N/Chl and both temperature and salinity. The ratio increased significantly at 25°C-30 salinity, while the reverse was calculated at salinity 40.

Table 2. C/N, C/Chl and N/ Chl ratios of *A. longipes* A. Continuous light and B. 12:12LD photoperiod.

Temp. (°C)	**10**			**15**			**25**		
Salinity	**C/N**	**C/Chl**	**N/Chl**	**C/N**	**C/Chl**	**N/Chl**	**C/N**	**C/Chl**	**N/Chl**
A									
25	2.40	25.69	10.72	2.93	12.59	4.29	-	-	-
30	6.64	56.52	8.51	4.53	50.77	11.20	4.58	24.68	5.39
35	3.25	58.60	18.00	1.60	52.10	32.58	8.58	65.71	7.65
40	3.32	25.13	7.58	2.08	51.38	24.70	19.70	81.34	4.13
B									
25	-	-	-	9.01	20.10	2.23	-	-	-
30	-	-	-	7.47	54.70	19.17	36.91	42.00	1.14
35	-	-	-	7.40	52.16	7.05	15.23	64.17	4.21
40	-	-	-	3.12	41.66	13.36	7.30	103.35	27.37

DISCUSSION

The easy maintenance in culture and presence of remarkable variations in response makes *A. longipes* an especially good model for studying the reproductive and population biology of benthic pennate diatoms (Chepurnov and Mann, 2000; Sabbe *et al*., 2004).

The present experiments confirm the results of Cooksey (1981) that addition was found to be promoted by Ca^{++} and EDTA disaggregates cells (Curtis, 1967). The results proved that 10 & 25 µM D-600 (α-Isopropyl-α- [(N-methyl-N-homovertatryl)-γ-aminopropyl]-3,4,5 trimethoxy phenylacetonitrile) inhibited the adhesion and facilitates getting homogenous free inocula of *A. longipes*. Replacing sulfate in artificial seawater with methionine also resulted in a lack of adhesion (Johnson *et al*., 1995).

Because *A. longipes* (W 75.5±4, L 45.5±5 µm) had considerable ability to acclimate or adapt to growth at the present wide temperature range between 15 and 25°C, it can be classified as an eurythermal diatom, in accordance with DeNicola (1996). The species is also considered an euryhaline form.

The culture conditions, based on Chl. *a* data, modulated the growth in *A. longipes*. The results demonstrated that benthic diatom populations represented by *A. longipes* have the potential to increase its biomass very quickly. Temperature at 20^{o} C and salinity 30 seems to be the optimum for growth, as well as the combination of 20-25^{o}C and 25-30 salinity. Growth was almost ceased at the extremely low temperature and salinity (10^{o}C-5-10 salinity). However, the increasing in temperature to 20-25^{o}C exerted a strong influence on the growth causing a noticeable development in Chl. *a* content by days, particularly at 10 salinity. Lewis *et al*. (2002) reported the growth ability of *A. longipes* to a wide temperature range between 8 to 32°C, with maximum growth at 26°C. A decline in its density with a drop in temperature from 20 to 16^{o}C was observed (Parker *et al*., 2007). At continuous light, cultures grown under 10-15^{o}C&30-40 salinity reached the stationary phase slowly (day 6), and the increased temperature accelerated the development of the stationary phase faster (day 2). Temperature also seems affecting the stalk formation, shorter stalks were seen in the lowest temperature (10^{o}C) in agreement with the results of Lewis *et al.* (2002). At 12/12 LD cycle, the start of the stationary phase exhibited much delaying at 15^{o}C and 30-40 salinity (days 8-10), indicating another impact of light duration on the growth. Despite raising of temperature to 20-25^{o}C, no effect on the start time of the stationary phase was observed at the previously mentioned higher salinity range, but it was obvious at 20-30 salinity (2-4 days). The results are contrary with others (e.g. Nielsen 1992) that many species of phytoplankton actually grow more slowly under continuous light in comparison with a LD cycle, the impact of salinity must be considered (see, Forster *et al*., 2006).

Achnanthes longipes growth rates differed among the tested temperature and salinity combination, ranging as a maximum from 0.15 (10^{o}C-25 salinity) to 0.82 (20^{o}C-30 salinity) doubling time in Chl. *a* concentrations at the continuous light, and between 0.19 (25^{o}C-10 salinity) to 1.11 (20^{o}C-25 salinity) under 12/12 light/dark cycle. The results explained that the maximum growth rate achieved during exponential growth was slightly lower in cultures acclimated to continuous light than that in the 12/12 light/dark cultures. The raised temperature from 15^{o}C to 20^{o}C caused about 1.8 fold increases in the doubling time of Chl. *a* at the two light regime. The present range of the growth rates was in agreement with that reported in literature for benthic diatoms (Underwood and Provot, 2000; Forster & Jezequel-Martin, 2005; Van Der Grinten *et al*., 2005). The results indicate that *A. longipes* cells can grow well under both light conditions and varied temperature levels, which suggests this species is well suited to the changing light conditions (Parker *et al*., 2007).

The carbon content was consistently lower in continuous light cultures than in the corresponding 12:12 LD cultures. The only exception was met at 15^{o}C-40 salinity, when the reverse occurred, which might be affected by the relatively low temperature and the extremely high salinity. The results indicated

that Chl. *a* and cellular carbon proceeded in parallel during the exponential phase under the two light conditions, and carbon and Chl. *a* production were influenced by the increased temperature. The present results for cell composition are largely consistent with previous findings: increasing carbon content and Chl *a* content with increasing temperature have been noted many times before for algae in general (Thompson *et al.* 1992), and diatoms in particular (e.g. Gao *et al.* 2000). There is a large body of literature showing that temperature affects cell composition (Hill *et al*., 1995; Reay *et al*., 1999). The results demonstrated a linear relationship between carbon and Chl. *a* biomass, while, the interfering of complex influences by light, nutrients and temperature caused non-linear relationship between carbon and Chl. *a* of the phytoplankton species in nature (e.g. Armstrong, 2006; Wang *et al*., 2008). It is hard to compare the present Chl:C of *A. longipes* with others grown in cultures since the measurements with phytoplankton grown in culture show that Chl: C is highly variable, ranging from 0.003 (Falkowski *et al*., 1985) to >0.1 (Geider 1987).

C: Chl ratio showed a steady increase by days, and varied considerably with changed temperature. However, there was no unique relation between temperature, growth rate and C:Chl despite this ratio is a sensitive indicator of algal growth rate in the laboratory. (e.g. Cloern *et al*., 1997).

The impact of growth conditions on cellular N content was more variable. The variations in N demonstrated a little effect of temperature under the continuous light. However, increased temperature linked with increased N in 12/12 LD cultures. The results indicated the relative importance of light regimes for the growth of *A. longipes* rather than temperature. Admiraal (1977) obtained maximal growth rates with four species of marine benthic diatoms with a 16-h day length. According to Parker *et al*. (2007) the final cell density of cultured *A. longipes* was more influenced by light than by the temperature levels.

The cellular C:N achieved its highest values at 25^{o}C-30-35 salinity, it was relatively higher under 12/12 LD cycle, indicating the importance of increased temperature and the impact of photoperiod on the growth of *A. longopes*. The finding that C:N ratio increases with increasing temperature is generally supported by other data (e. g. Berges *et al*., 2002**)** but this may also depend on the light regime (Lomas & Glibert, 1999).

ACKNOWLEDGEMENT

The authors would like to express their deep gratitude to Professor M. Terazaki and Professor S. Nishida, of Ocean Research Institute, University of Tokyo for offering the facilities required during the four month of joint study and the training in Japan.

REFERENCES

Admiraal, W. (1977). influence of light and temperature on the growth rate of estuarine benthic diatoms in culture. Mar. Biol., 39:1-9.

Al-Handhal, A. Y. (1979). Systematic and ecological study of phytoplanktonic and benthic diatoms of the Eastern Harbour of Alexandria. M.Sc. Thesis, Alexandria University, 241pp.

Armstrong, R. A. (2006). Optimality-based modeling of nitrogen allocation and photoacclimation in photosynthesis, Deep-Sea Res. Pt. II, 53: 513–531.

Berges, J. A.; Varela, D. E. and Harrison, P. J. (2002). Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom *Thalassiosira pseudonana* (Bacillariophyceae). Mar. Ecol. Prog. Ser., 225: 139–146.

Callow, M. E. (1986). A world-wide survey of slime formation on antifouling paints. *In* Evans, L. V. & Hoagland, K. D. [Eds.] Algal Biofouling. Elsevier, Amsterdam, pp. 1–20.

Chepurnov, V.A. and Mann, D.G. (2000). Variation in the sexual behaviour of *Achnanthes longipes* (Bacillariophyta). III. Progeny of crosses between monoecious and unisexual clones. European Journal of Phycology, 35: 213-223.

Cloern, E. J.; Grenz, C. and Vidergar-Lucas, L. (1997). An empirical model of the phytoplankton chlorophyll: carbon ratio. -the conversion factor between productivity and growth rate, Limnol. Oceanogr., 40(7): 1313-1321.

Cooksey K. E. (1981). Requirement of calcium in adhesion of a fouling diatom to glass. Appl. environ. Microbiol., 41:1378- 1382.

Curtis, A. S. G. (1967). The Cell Surface: It's Molecular Role in Morphogenesis. Logos Press,London, 129 pp.

DeNicola, D. M. (1996). Periphyton responses to temperature at different ecological levels. *In* Stevenson, R. J., Bothwell, M. L. & Lowe, R. L. [Eds.] Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, San Diego, pp. 149–81.

Eker, E. and Kideys, A. E. (2000). Weekly Variations in Phytoplankton Structure of a Harbour in Mersin Bay (northeastern Mediterranean). Turk. J. Bot., 24: 13–24.

Falkowski, P. G.; Dubinsky, Z. and Wyman, K. (1985). Growth-irradiance relationships in phytoplankton. Limnol. Oceanogr., 30: 3 1 1-321.

Forster R. M. and Martin-Jezequel, V. (2005). Photophysiological variability of microphytobenthic diatoms after growth in different types of culture conditions. Phycol., 44:393-402.

Forster, R. M.; Créach1, V.; Sabbe, K.; Vyverman, W. and Stal, L. J. (2006). Biodiversity–ecosystem function relationship in microphytobenthic diatoms of the Westerschelde estuary. Mar. Ecol. Prog. Ser., 311: 191–201.

Gao, Y.; Smith, G. and Alberte, R. (2000). Temperature dependence of nitrate reductase activity in marine phytoplankton: biochemical analysis and ecological implications. J. Phycol., 36:304-313.

Geider, R. J. (1987). Light and temperature dependence of the carbon to chlorophyll *a* ratio in microalgae and cyanobacteria: Implications for physiology and growth of phytoplankton. New Phytol., 106: 1-34.

Geider, R. J. and Roche, J. LA (2002). Redfield revisited: Variability of C:N: P in marine microalgae and its biochemicabasis. Eur. J. Phycol., 37: 1–17.

Guillard, R.R.L. (1973). Culture of Marine Invertebrate Animals. In *Handbook of Phycological Methods*, W. L. Smith and M. H. Chanley (Eds.), pp. 181–194. Plenum Press, N. Y.

Guillard, R. R. L. and Ryther, J. H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and Detonula confervacea (Cleve) Gran. Can. J. Microbiol., 8:229-239.

Hill, W. R.; Ryon, M. G. and Schilling, E. M. (1995). Light limitation in a stream ecosystem: Responses by primary producers and consumers. Ecology, 76:1297–309.

Hoagland, K.D.; Rosowski, J. R.; Gretz, M.R. and Roemer, S.C. (1993). Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. J. Phycol., 29:537-66.

Hoshaw, R. W. and Rosowski, (1973). Handbook of Phycological Methods: Culture methods and growth - Cambridge University Press

Johnson, L.M.; Hoagland, K.D. and Gretz, M.R. (1995). Effects of bromide and iodide on stalk secretion in the biofouling diatom *Achnanthes longipes* (Bacillariophyceae). J. Phycol., 31:401-12.

Kawamura, T.; Saido, T.; Takami, H. and Yamahita, Y. (1995). Dietary value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. J. Exp. Mar. Biol. Ecol. 194:189-199.

Lam, C.; Harder, T. and Qian, P.Y. (2005). Induction of larval settlement in the polychaete *Hydroides elegans* by extracellular polymers of benthic diatoms. Mar. Ecol. Prog. Ser., 286:145-154.

Lange-Bertalot, H. and Krammer, K. (1989). *Achnanthes*, eine Monograph der Gattung: mit Definition der Gattung *Cocconeis* und *Nachtragen* zu den Naviculaceae. Bibliotheca Diatom. Band 18. J. Cramer, Berlin, 393pp.

Lewis, R.J.; Johnson, L.M. and Hoagland, K.D. (2002). Effects of cell density, temperature, and light intensity on growth and stalk production in the biofouling diatom *Achnanthes longipes* (Bacillariophyceae). Journal of Phycology., 38:1125-1131.

Lomas, M.W. and Glibert, P.M. (1999). Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms. Limnol. Oceanogr., 44(3): 556–572.

Lorenzen, C. J. (1967). Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnol. Oceanogr., 12: 343-346.

Mikhail, S. K. (1986). Preliminary investigation of epiphytes growing on some marine algae and artificial substratum in the Eastern Harbour. M.Sc. Thesis, Alexandria University, 178pp.

Nielsen, M. V. (1992). Irradiance and day length effects on growth and chemical composition of *Gyrodinium aureolum* Hulburt in culture. J. Plankton Res., 14: 811–820.

Parker, F.; Davidson, M.;, Freeman, K.; Hair, S. and daume, S. (2007). Investigation of optimal temperature and light conditions for three benthic diatoms and their suitability to commercial scale nursery culture of abalone, J. of Shellfisheries Research, 26(3):751 - 762.

Patil J. S. and Anil, A. C. (2005). Influence of diatom exopolymers and biofilms on metamorphosis in the barnacle *Balanus amphitrite*. Mar. Ecol. Prog. Ser., 301:231-245.

Provasoli, L.; McLaughlin, J. J. A. and Droop, M.R. (1957). The development of artificial media for marine algae. Arch. Mikrobiol., 25:329-428.

Reay, D. S.; Nedwell, D. B.; Priddle, J. and Ellis-Evans, J. C.(1999). Temperature dependence of inorganic nitrogen uptake: reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. Appl. Environ. Microbiol., 65:2577–2584.

Round, F. E. (1971). Benthic marine diatoms. Oceanogr. Mar. Biol.Annu. Rev., 9:83–139.

Sabbe, K.; Chepurnov, V.A.; Vyverman, W. and D.G. Mann (2004). Apomixis in *Achnanthes* (Bacillariophyta); development of a model system for diatom reproductive biology. European Journal of Phycology, 39:327-341.

Takami, H.; Muraoka, D.; Kawamura, T. and Yamashita, Y. (2003). When is the abalone *Haliotis discus hannai* INO 1953 first able to use brown macroalgae? J. Shelfish Res., 22:795-800.

Thompson, P. A.; Guo, M. and Harrison, P. J. (1992). Effects of variation in temperature. I. On the biochemical composition of eight species of marine phytoplankton. J. Phycol., 28:481–488.

Underwood G. J. C. and Provot, L. (2000). Determining the environmental preferences of four estuarine epipelic diatom taxa: growth across a range of salinity, nitrate and ammonium conditions. Eur. J. Phycol., 35:173-182.

Van der Grinten E.; Janssen APHM, De Mutsert K.; Barranguet, C. and Admiraal W. (2005). Temperature- and light-dependent performance of the cyanobacterium *Leptolyngbya foveolarum* and the diatom *Nitzschia perminuta*. Hydrobiol., 548:267-278.

Wang Y.; Lu J-C.; Mollet J-C.; Gretz M.R. and Hoagland K.D. (1997). Extracellular matrix assembly in diatoms (Bacillariophyceae). II. 2,6-Dichlorobenzonitrile inhibition of motility and stalk production in the marine diatom *Achnanthes longipes*. Plant Physiol., 113: 1071–1080.

Wang, X. J.; Behrenfeld, M.; Le Borgne, R.; Murtugudde, R. and Boss, E. (2008). Regulation of phytoplankton carbon to chlorophyll ratio by light,

nutrients and temperature in the equatorial Pacific Ocean: a basin-scale model, Biogeosciences Discuss., 5: 3869–3903.

Woods, D. C.; Fletcher, R. L. and Jones, E. B. G. (1988). Diatom fouling of in-service shipping with particular reference to the influence of hydrodynamic forces. *In* Round, F. E. [Ed.] Proceedings of the Ninth International Diatom Symposium. Biopress, Bristol, pp. 49-59.

Wustman B. A.; Gretz M. R. and Hoagland, K. D. (1997). Extracellular matrix assembly in diatoms (Bacillariophyceae). I. A model of adhesives based on chemical characterization and localization of polysaccharides from the marine diatom *Achnanthes longipes* and other diatoms. Plant Physiol., 113: 1059–1069.