

## Effect of different temperature regimes on the chlorophyll a concentration in four species of Antarctic macroalgae

V. K. DHARGALKAR

National Institute of Oceanography, Dona Paula, Goa - 403 004, India

### ABSTRACT

Four species of benthic macroalgae belonging to Rhodophyta namely *Palmaria decipiens* (Reinsch) Ricker, *Phyllophora antarctica* A. et. E. S. Gepp, *Porphyra endiviifolium* (A. et. E. S. Gepp.) Chamberlain and *Iridaea cordata* (Turner) Boerg. collected from beneath the sea-ice from the coast of the Vestfold Hills, Antarctica were cultured under different temperature regimes (-4, -1.8, +4, +12, +20°C). The algae were cultured at each of these temperatures and Chlorophyll *a* concentrations of the algae were measured after every 24 and 96 h of exposures. The Chlorophyll *a* concentration in endemic Antarctic macroalgae *P. decipiens* and *P. antarctica* showed peak at 4°C and at 12°C in 24 h respectively, while the cold temperate macroalgae *P. endiviifolium* and *I. cordata* showed peak at 4°C in 96 h and 24 h respectively. Among the four macroalgal species evaluated, a maximum Chlorophyll *a* of 750 mg g<sup>-1</sup> was recorded in *P. endiviifolium*. This alga showed 38% higher Chlorophyll *a* concentration than the control and concentration remained same till the end of the experiment. *I. cordata* and *P. decipiens* recorded a maximum Chlorophyll *a* concentration at 4°C in 24 h and declined thereafter, while in *P. antarctica* Chlorophyll *a* concentration showed an increasing trend with a peak (680 mg g<sup>-1</sup>) at 12°C in 24 h and thereafter, alga started to bleach and degenerated. The experiment indicated that time period was found necessary for optimum Chlorophyll *a* concentration and time required to adapt to each temperature regime.

### Introduction

The geographical distribution of marine benthic macroalgae depend on the temperature regime of the respective habitat and the temperature requirement for their growth, reproduction and survival (van den Hoek, 1982 a, b; Breeman, 1988). Antarctic or cold temperate marine macroalgae showed temperature requirement for their growth between 0 and 10°C and optimum growth was demonstrated in between 0 to 5°C (Wiencke

and Dieck, 1990). The photoperiod may also limit phytogeographic distribution as light : dark cycle is responsible for the induction of different phases of life cycle (Hay, 1981; Gains and Lubchenco, 1982).

Antarctic benthic macroalgae are subjected to numerous factors which limit species diversity and growth. It is likely that metabolic process in these macroalgae are adapted to facilitate growth and reproduction at low temperatures (Thomas and Wiencke, 1991). To survive in such a harsh environment

an alga must have been adapted to survive cycles of freezing and thawing as well as low temperatures. This type of adaptation has resulted in high number of endemic macroalgae that accounts for 35 % of the total marine flora of the West Antarctica (Lunning, 1985).

In the present investigation, time period necessary for optimum Chlorophyll *a* concentration and time required to adapt to each temperature regime in four Antarctic benthic marine macroalgae have been studied.

### Materials and Methods

The macroalgae namely *Palmaria decipiens* (Reinsch) Ricker, *Phyllophora antarctica* A. et. E. S. Gepp. (endemic to Antarctica), *Porphyra endiviifolium* (A.et. E.S. Gepp.) Chamberlain and *Iridaea cordata* (Turner) Boerg, (cold temperate macroalgae) were used for the experiment. The macroalgae were collected with a grab beneath the sea-ice at the ice edge about 2 km from the coast of the Vestfold Hills, Antarctica in 1984. The Vestfold Hills is a rocky area, admeasuring 400 sq km, lies between latitude 68° 22' to 68° 40' S and longitude 77° 49' to 78° 33' E at the eastern side of the Prydz Bay on the coast of Princess Elizabeth Land, Antarctica.

The macroalgae were cultured in Erdscriber media. The soil extract for the media was prepared using the procedure of Gross (1937) with soil collected from the station area. Seawater was filtered through 0.45 m Millipore filter and sterilized at 73 °C. The replicate samples of macroalgae were cultured in 3 l of Erdscriber media, changed weekly (to avoid nutrient depletion) in plastic trays with continuous aeration. The macroalgae were spread at the bottom of the tray to avoid self-shading. Light was provided by 20W cool white fluorescence tubes with 12 : 12 h light and dark cycle and was kept

constant at 14  $\mu\text{E m}^{-2} \text{s}^{-1}$  through out the experiment.

Prior to the beginning of the experiment, macroalgae were acclimatized by exposing to the experimental temperature of  $-1.5 \pm 0.03^\circ\text{C}$  under normal day light for three days. After acclimatization, initial Chlorophyll *a* concentration was estimated for each alga. The algae were then transferred to the culture media and subjected to lower temperature i.e.  $-4^\circ\text{C}$  and then to  $-1.8^\circ\text{C}$  and so on giving time to adapt to respective temperatures. This precaution was taken to avoid damage to macroalgal thalii from sudden transfer to the lowest temperature employed.

The macroalgae were then cultured at different temperature regimes namely - 4, -1.8, + 4, +12 and + 20° C (Fig.1). pH and nutrients were analysed before and after culturing in each temperature regime to ensure that no nutrient depletion had occurred in any of the culture trays ( Srickland and Parsons, 1972). Whenever necessary, nutrients were replenished in the trays during the experiment. The concentration of Chlorophyll *a* was measured after 24 and 96h in each temperature regime. Extraction of Chlorophyll *a* was carried out by homogenizing weighed macroalgal samples in 90% acetone by mortar and pestle and extracted at 4°C for 24 h. The Chlorophyll *a* was analysed following the procedure described by Jeffrey and Humphrey (1975).

### Results and Discussion

The Antarctic coastal waters remained covered with 2 to 3 m thick sea-ice for 9 to 10 months in a year. The temperature of the sea water remained more or less constant between  $-1.8$  to  $2^\circ\text{C}$  for the major part of the year. However, macroalgae from littoral zone are subjected to changes in salinity, temperature and light conditions. The light intensity

varied from total darkness during the winter period to diffused light (i.e. through the sea- ice) in spring to bright 24 h day light during the austral summer (Dhargalkar, 1990).

It has been reported that the Southern Ocean benthic macroalgae are still undergoing a process of adaptation to low temperature (Wiencke and Tom Dieck, 1989). Growth characteristics of several Antarctic macroalgae have indicated that growth optima in the algae are very much restricted to low temperature (Wiencke and Firscher, 1990; Wiencke and Tom Dieck, 1989).

The Chlorophyll *a* concentration increases during the development both within the division cycle of an individual cell in phytoplankton and during the growth and maturation of seaweed thallus (Dring, 1981). In present study, an increase in Chlorophyll *a* concentration was regarded as the measure of growth of the macroalgal (seaweed) thallus.

In this experiment, macroalgae were exposed for a period of 96 h in each temperature regime to study the optimum Chlorophyll *a* concentration and period needed to adapt to the respective temperature. In most of the studies on temperature demand for algal growth and rate of growth have been determined between 24 h and a month (Fortes and Liming, 1980; Novaczek and Breeman, 1990; Tom Dieck and Oliveira, 1993). The different results were obtained when period of exposure was changed because algae will acclimatize to a given temperature and will alter their growth rate during the course of experiment (Wiencke *et al.*, 1994).

The nutrient availability also affects the Chlorophyll *a* concentration when elements such as nitrogen, iron and magnesium are in short supply. In the Antarctic coastal waters, nutrients are in high concentrations and non-limiting for macroalgal growth (Drew and Hasting, 1992; Dhargalkar, 1990). pH values

did not show measurable variation in any of the temperatures and ranged from 7.52 to 8.2.

The endemic macroalgae exhibit very strong adaptation to low temperature and grow up to 5°C (or 10°C) with optimum growth between 0 to 5°C (Wiencke *et al.*, 1994). In the endemic macroalga *P. decipiens*, Chlorophyll *a* concentration started to increase from the beginning of the experiment and a maximum Chlorophyll *a* concentration (115.25 % increase) was recorded in 24 h at 4°C indicating optimum growth of the alga in this temperature regime (Fig 1). The Chlorophyll *a* concentration showed increasing trend from -4°C to 4°C in 24 h and from 96 h onwards in same temperature regime Chlorophyll *a* concentration started to decrease, it however, survived till 12°C and later it started to bleach and degenerate.

Optimum temperature for photosynthesis was 15°C in *Himantothallus*

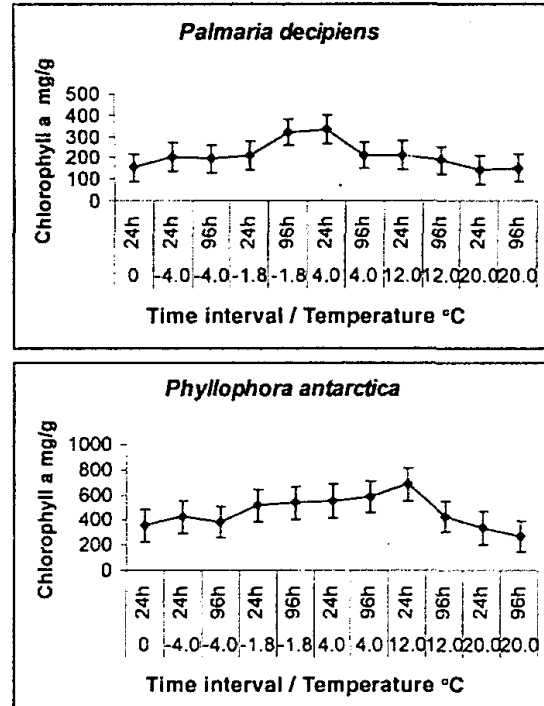


Fig. 1. Chlorophyll concentrations in different regimes in *Palmaria decipiens* and *Phyllophora antarctica*

*grandifolius* (A. et. E.S. Gepp.) Moe et Silva, *Desmeratia anceps* (Montagne) and *Palamria decipiens* which was much higher than the Antarctic water temperature (Drew, 1977). Similarly, Wiencke and Tom Dieck (1989) reported that *P. decipiens* grew up to 10°C, whereas, in the present investigation the temperature requirement was up to 12°C. Significant correlation ( $p < 0.05$ ) was observed between temperature and Chlorophyll *a* concentration. The variation in the temperature requirement of macroalgae can be explained by enzymatic reaction which are temperature dependent resulting into low metabolic rates at low temperature (Drew, 1977).

The distribution of *P. decipiens* is restricted to South of Antarctic Convergence. However, the Northern most occurrence of this species was reported at Macquarie Island. The Northern distribution limit of endemic Antarctic macroalgal species is mainly determined by the macroalgal temperature growth pattern (Wiencke and Tom Diecke, 1989).

The Chlorophyll *a* concentration in *Phyllophora antarctica* was recorded as highest (680.53 mg g<sup>-1</sup>) in 24 h at 12°C showing 90.81% increase in Chlorophyll *a* concentration over the initial value. Later, Chlorophyll *a* concentration in next higher regime started to decrease, sharply. Ohno (1984) recorded optimum temperature for growth of this alga at 3°C in cultured condition while in the natural population from the coast of Lutzou-Holm Bay, optimum temperature for the growth of this alga was 5°C. In the present experiment, maximum temperature for the growth of this alga was 12°C. Significant correlation ( $p < 0.01$ ) was observed between temperature and Chlorophyll *a* concentration in *P. antarctica* indicating algal response to the increasing temperature up to 12°C (Fig. 1). Later,

Chlorophyll *a* concentration started to decrease in the remaining temperature regimes. Alga started losing pigments and degenerated.

The Chlorophyll *a* concentration in *I. cordata* suddenly decreased at -4°C from the initial value and gradually increased in the higher temperature with a peak (476 mg g<sup>-1</sup>) in 24 h at 4°C showing 25.96% increased in Chlorophyll *a* concentration. Later, Chlorophyll *a* concentration in the alga started to decline. From the pattern of Chlorophyll *a* concentration in each temperature regime, it seems that the macroalga required more than 24 h to adapt to the exposed temperature (Fig. 2). Kanwisher (1957) reported that during freezing, salt concentration in the cell greatly increases resulting into the frost plasmolysis showing low Chlorophyll. Eighty percent of the water in the algal thallus freezes

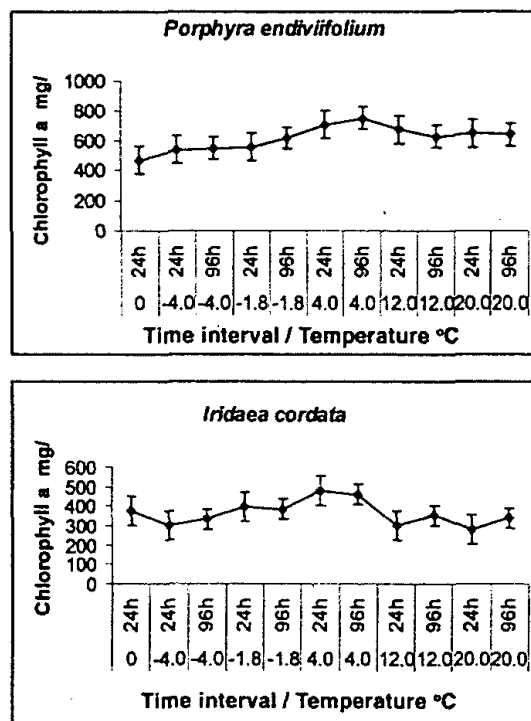


Fig. 2. Chlorophyll concentrations in different regimes in *Porphyra endiviifolium* and *Iridaea cordata*

during freezing and photosynthesize immediately after thawing in *Fucus* plants. Perhaps, this type of mechanism occurs in *I. cordata* where in Chlorophyll *a* concentration increased steadily from freezing temperature of -4 °C to 4 °C. Although this alga is Antarctic cold temperate species, it showed temperature requirement comparable to that of endemic Antarctic species.

In the Antarctic region, *I. cordata* grows at temperature <5 °C. The Northern most distribution of *I. cordata* limited by winter temperature for growth. The development of species with degree of adaptation to the temperature regime South and North of the Antarctic Convergence is also evident in genus *Iridaea*. The Antarctic / cold temperate *I. cordata* grows at the temperature ≤5 °C whereas from South Chile, *I. undulosa* and *I. laminoarioides* grow between 5 and 15°C or 0 to 15°C, respectively. Papenfuss (1964) and Ricker (1987) reported that *I. cordata* from the Northern hemisphere is a different species wherein maximum temperature for growth is 20°C. Despite, this variation in temperature regime over the distributional range in *I. cordata*, there is no study to indicate whether population of this species growing in various temperature regimes differs genetically.

In *P. endiviifolium*, Chlorophyll *a* concentration showed an increase from 24 h in 4°C till 96 h in +4°C, and there after it decreased. However, the Chlorophyll *a* concentration remained higher than the control through out the experiments indicating algal tolerance to the exposed temperature. *Porphyra endiviifolium* showed 34.50 % higher Chlorophyll *a* concentration in all the temperature regimes employed than the initial value (Fig. 2). This showed that alga can tolerate higher temperature range without any detrimental effect to the algal thallus.

Though the optimum Chlorophyll *a* concentration was recorded at 4°C, alga survived till the end of the experiment. The upper survival range in this alga was perhaps higher than the 20°C which could not be assessed in the present experiment as maximum temperature employed was 20°C. The eurythermal algal species such as *Enteromorpha clathrata* (Roth J. Ag.), *Chondria magellanica* Kylin, *Scytosiphon lomentaria* (Lyngbye), *Ectocarpus siliculosus* (Dillwyn) Lyngbye grow between 5 to 15 °C (Wiencke and Tom Dieck, 1990). This temperature requirement was intermediate between cold and warm temperate algae (Norton, 1977; Kaparaun, 1978; Novaczek; 1984). Therefore, it can be said that *P. endiviifolium* is the intermediate species between cold and temperate region. In this study, temperature was changed gradually from low to high at lower range. Lampe (1935) in his experiment transferred *Porphyra lienalis* from 5 to 21 °C and was found badly damaged due to sudden exposure to higher temperature.

In the present experiment, macroalgae were transferred from lower to the higher temperature regime and data presented here indicated temperature requirement in four red macroalgal species for optimum Chlorophyll *a* concentration in controlled conditions. The gamatophytic and sporophytic population of algae exhibit different temperature requirements for growth and survival (Luxoro and Santelices, 1989). This aspect was not taken into account while designing the experiment. However, these two phases could have given more and specific information on the geographical distribution of these macroalgal species (Novaczek, 1984). Algae under study exhibited higher temperature tolerance than the natural habitat where temperature remains below 5 °C. More studies are needed on the physiology of

Antarctic algae to assess this phenotypic adaptation.

The data presented here are not sufficient for the determination of biogeographical distribution of macroalgae. However, it determines the period required by macroalgae for the maximum Chlorophyll a production when exposed to different temperature regimes. Though the period of exposure to each temperature regime was short, it indicated variations in Chlorophyll a concentration from 24 h to 96 h of exposure. Perhaps, longer period of exposure would have given better understanding of the temperature requirement of algae under study. More studies are needed to investigate the adaptative pattern of macroalgae to the Antarctic environment from ecosystem down to the molecular level.

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