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In vivo fluorescence kinetics of *Gracilaria* spp. subjected to different salinities

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ABSTRACT

Gracilaria species were subjected to different salinities under laboratory conditions and changes in the photosynthetic oxygen evolution and fluorescence kinetics were followed. The plants which were subjected to more or less the normal salinity conditions exhibited low values of variable fluorescence and quantum yield. Prolonged treatment increased the quantum yield but the pigment content and the photosynthetic rate reduced significantly. Among the two species tested, *Gracilaria edulis* was found to be very sensitive to low salinity (15 ppt) and *G. crassa* to higher salinity (45 ppt).

Introduction

The productivity of macrophytes is influenced by multiple environmental factors as well as morphological structure of the plants (Chapin *et al.*, 1987). Several methods such as ^{14}C uptake, oxygen evolution and ATP changes are being adopted to study the productivity. It is also reported that vairable chlorophyll fluorescence forms an important tool for estimating productivity, in which, the cholorophyll molecule under a strong excitation light saturate the photosynthesis and produce a typical oscillatory pattern of fluorescence (Kulandaivelu and Daniell, 1980; Samuelsson and Oquist, 1977).

The salinity of open surface water is generally 34 to 37‰ (Groen, 1980). Seaweeds on open rock surface and tide pools are subjected to frequent salinity fluctuations. Therefore this is an important environmental parameter affecting the distribution, growth, morphology, physiology and chemical composition of marine algae (Haug and Larson, 1958; Kim, 1970; Gessner and Schramm, 1971). The present study is aimed to find out the short term effect of salinity on the physiology of two important Indian agarophytes, *Gracilaria edulis* and *G. crassa,* in a controlled environmental chamber.

Materials and methods

Study site : Tamil Nadu is situated on the southeast coast of India and Mandapam lies between 78^{0}08'E and 9^{0}17'N in between Palk Bay and Gulf of Mannar. Gulf of Mannar is characterised by chains of 21 islands, relatively greater in depth and higher in productivity than Palk Bay. The coast is sparcely rocky and sea bottom is muddy covered by seagrassess. *Gracilaria edulis* and *G. crassa* grow well in the intertidal area of this place. *G. edulis* is

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found attached to the pebbles, rocks and the dead gastropod shells whereas *G. crassa* forms a dense cushion on the muddy bottom. Sometimes it is also found in the rock crevices.

Morphology : Both the species of *Gracilaria* exhibit their morphological identity. *G. edulis* is slender, elongated, cylindrical regularly dichotomously branched and grow to a maximum length of 40-45 cm in India. On the otherhand *G. crassa* is more fleshy, rigid and grows to a maximum size of 8-10 cm.

Sample collection : Both the species of *G. edulis* and *G. crassa* were collected from the intertidal area of Gulf of Mannar near Thonithurai situated 8 km away from Mandapam during low tide in the morning. The plants were thoroughly brushed off epiphytes, cleaned several times in seawater followed by filtered and sterilised seawater and transported to the laboratory in enriched seawater medium (Walne, 1974) and maintained at ambient temperature.

Growth conditions : The plants were kept overnight in growth chamber in a temperature range of 25-28°C and light intensity of 30 $W.m^{-2}$ to overcome the transportation stress. Next day approximately 25 g each of healthy fresh plants were kept in four different glass aquarium tanks having salinities 15, 25, 35 and 45 ppt. The plants were uniformly distributed in each tank having 2 l of filtered, sterilised and enriched seawater. The tanks were kept in the growth chambers and subjected to same temperature and light intensity. The experiment was conducted for 12 days with change of medium on the sixth day. The photosynthetic activity, chlorophyll-a and fluroscence kinetics were measured on 0, 6 and 12th day in plants exposed to four different salinities.

Measurement of photosynthetic activity : The photosynthetic activity was measured using a Hansatech, UK, oxygen electrode. The apical portions of the plants were hung from the top of the cylindrical electrode chamber containing 2 ml of filtered seawater of required salinity. Saturated white light from the slide projector (photophone Ltd., India) was passed through a 10 cm thick water filter. Light intensity at the sample surface was 100 $W.m^{-2}$. The water inside the cylindrical chamber was stirred continously by a magnetic stirrer. The amount of oxygen evolved was recorded at 25°C and the rate of photosynthesis was expressed as µmol O_2 evolved/gram fresh weight/hour.

Estimation of chlorophyll a : The chlorophyll a was extracted from the apical portion of the thallus using 90% acetone and estimated by the method of Jeffery and Humphery (1975).

Fluorescence induction measurement : Prior to the measurement, the plants were adapted to complete darkness for 30 min. A small portion of the thallus was mounted in a plexiglas frame and placed diagonally in a 4.0 ml all side clear glass cuvette and excited with broad band of blue light (400-600 nm, Corning, CS 4-96) at a photon flux density of 100 $W.m^{-2}$. The photomultiplier (Hamamatzu R 376) placed at 90° to the excitation beam was protected by an interference filtre (λ max 690 nm, half band width 12 mm, Schott, Germany). The signal from the photomultiplier was directly displayed either on a recorder (Hitachi, Model 056) or stored in a digital oscilloscope (Itwatsu SS 5802, Japan). The signal was triggered with the help of an electric shutter with an opening time of 10 ms.

Results

The time course of the fluorescence induction kinetics from a dark-adapted sample showed very fast and slow components. Fluorescence changes in a time range of some nanoseconds depend on the process of energy migration within the antennae and to the photosystems. In fast fluorescence, the onset of measuring beam brings the fluorescence to the F_0 level, reduces Q_A totally and reaches to maximum fluorescence level F_m. Under this condition all reaction centres were closed. The maximal variable fluorescence of a dark adapted sample F_v is the difference between F_m and F_0. F_v indicates the electron transport potential of PSII and quantum yield is the ratio between F_v and F_m (Buchel and Wilhelm, 1993) Fig. 1).

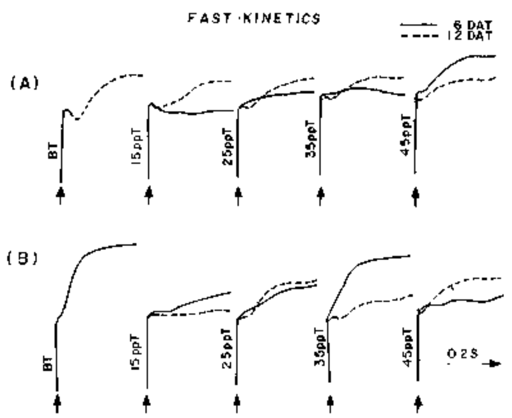

Fig. 1. Fast kinetics of *Gracilaria edulis* (A) and *Gracilaria crassa* (B) exposed to different salinities on 6th and 12th day of treatment (BT express the observation before treatment).

In slow fluroscence, kinetics refer to all changes in the signal from initial level called 'O' to a passing intermediate stage 'I', a dip 'D', peak 'P' and a terminal stage 'T'. Sometimes, the strong reduction of fluroscent signal from P to S is mainly due to the pH gradient across the thylakoid membrane which is yet to be understood but the changes from the peak to terminal stage is the real enzymatic dark reactions (Seaton and Walker, 1990) (Fig. 2).

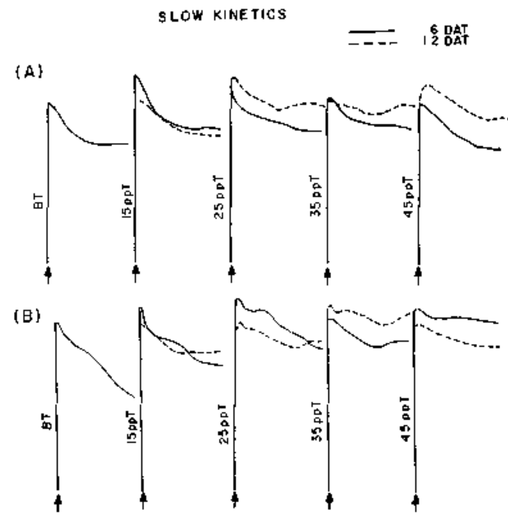

Fig. 2. Slow kinetics of *Gracilaria edulis* (A) and *Gracilaria crassa* (B) exposed to different salinities on 6th and 12th days of treatment (BT express the observation before treatment).

In the present experiment, the fluorescence signal of *G. edulis* and *G. crassa* was correlated with the chlorophyll *a* content and the P_o activity of the particular species. It was observed that *G. edulis* possessed higher photosynthetic activity than *G. crassa* before and after the treatment but there was a general decline in the P_o activity in all the samples of *G. edulis* and *G. crassa* exposed to different salinities. The decline was marginal for the initial 6 days of treatment and became more pronounced on the 12th day. While comparing the P_0 activity of *G. edulis* in different saline conditions, it was observed that maximum decline of photosynthetic activity was noticed at 15 ppt salinity on 6th day of treatment. At 35 ppt salinity the P_0 activity declined marginally by only 6.4% in *G. edulis* on 6th day. The decline was more on 12th day of treatment. The marked decline of the P_0 activity at 35 ppt salinity on prolonged treatment may not account for the stress due to salinity but may be due to some environmental changes in water quality of the culture tank maintained in controlled environmental chamber. In *G. crassa*, similar observations were noticed where the P_0 activity declined in all the treated sample on 6th day of observation ranging between 4-75%. At 35 ppt the P_0 activity declined initially from 4 to 58% after 12 days of treatment (Fig. 3).

The chlorophyll *a* content of *G. edulis* was found to be much higher than *G. crassa* before treatment. Although there was a general decline in the pigment, the chlorophyll content showed an increase of 59% at 35 ppt salinity in *G. edulis* on 6th day of treatment. On 12th day of treatment there was a marked decline in the pigment content in all the treatments. Maximum decline was noticed in *G. edulis* at 15 ppt salinity (66%). At 25 ppt the decline was 34%. In 35 ppt the chlorophyll content was found to be more by 4% in *G. edulis* over 0 day of treatment. In *G. crassa*, the chlorophyll content increased by 3 and 12% respectively at 35 and 25 ppt salinity on 6th day of observation, whereas it declined by 33 and 23% at 15 and 45 ppt salinities. Prolonged treatment showed reduction in chlorophyll content in all the treated samples except at 35 ppt salinity which exhibited further enhancement of chlorophyll by 17%. The increase in chlorophyll content in both the specis of *Gracilaria* on 12th day of observation did not help to increase P_0 activity but there was an increase in quantum yield in *G. edulis* (Fig. 4).

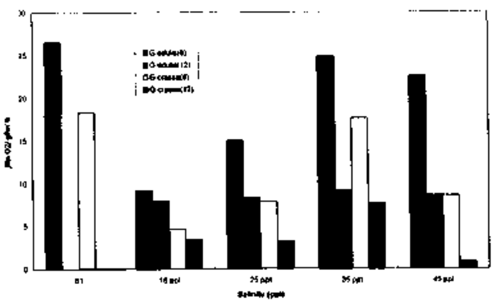

Fig. 3. Photosynthetic activity of *Gracilaria* spp. at different salinities.

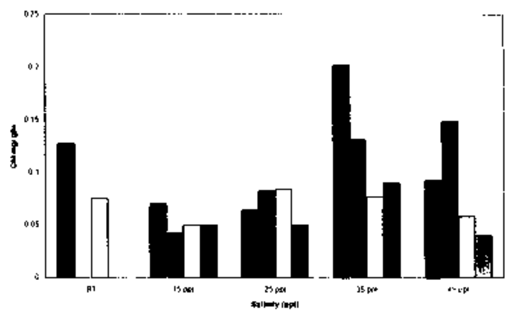

Fig. 4. Chlorophyll content of *Gracilaria* spp. at different salinities.

The fluorescence yield of *G. edulis* and *G. crassa* showed different trend in slow and fast kinetics. In fast kinetics, the variable fluorescence of *G. crassa* was higher than *G. edulis* before treatment whereas the quantum yield was higher in the latter. Overall, the quantum yiled declined from 13 to 73%

in both the species under different treatments. Maximum decline of 73% was noticed in both the species at 15 ppt salinity. It was also observed that there was a marked recovery of quantum yield in all the treated plants of *G. edulis* on 12^{th} day of treatment compared to the corresponding 6^{th} day. The recovery was maximum at 15 ppt salinity (100%) when the P_0 activity was found to be least (8.0 µmol O_2/g fw/h). Similarly in *G. crassa,* the quantum yield showed a recovery of 167% at 45 ppt salinity when the P_0 activity reduced to minimum of 0.8 µmol O_2/g fw/h (Fig. 5 & 6).

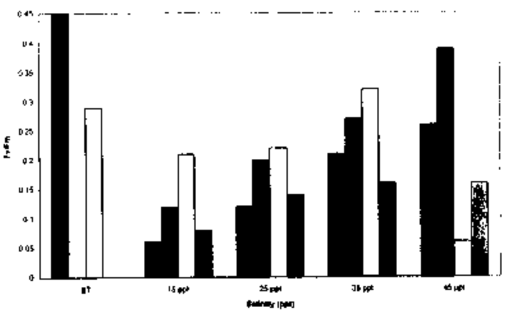

Fig. 5. Quantum yield of *Gracilaria* spp. at different salinities.

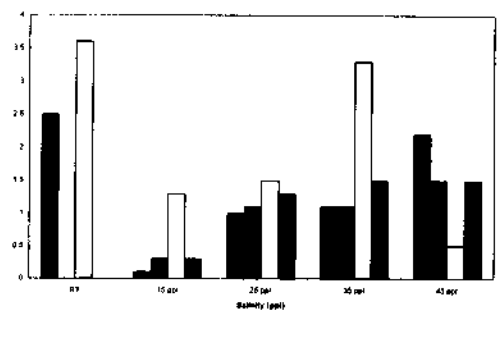

Fig. 6. Variable fluroscence of *Gracilaria* spp. at different salinities.

In slow kinetics, the ratio of peak and terminal value of *G. crassa* was more than *G. edulis.* Upon treatment, *G. edulis* showed an increase in the ratio of P/T at 15, 25 and 45 ppt salinity over 0 day of treatment but declined marginally at 35 ppt. Further treatment reduces the ratio in all treatment but maintained to be high at 15 ppt salinity. In *G. crassa,* similar results were obtained in slow kinetics. The tatio of P/T was more at 15 ppt and declined as the salinity increased. Although, there was a reduction in the ratio of P&T value, it maintained to be high at 15 ppt salinity (Fig. 7).

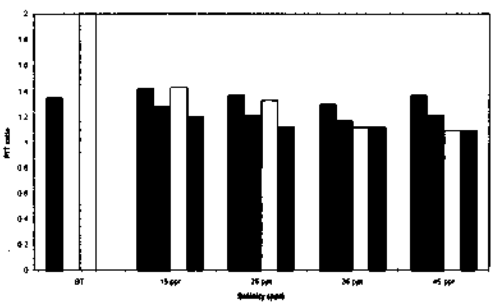

Fig. 7. Slow kinetics of *Gracilaria* sps (P/T ratio at different salinities).

Discussion

Except for Chlorophyta, very few works have been carried out on the fluorescence behaviour of the algae, which point out that they are divergent from the higher plants. The most striking difference between green algae and the other group of algae is the enormous variation in the antenna complex. Red algae possess large extrinsic phycobilisomes mainly connected to PSII whereas a small intrinsic antenna for PSI (Morschel and Schatz, 1988). Due to the large antenna system, red algae possess a very high F_0 value in relation to F_m leading to decreased F_v/F_m ratio (Bose *et al.*, 1988;

Hanelt *et al.,* 1992) as observed in the present experiment. The exact value of F_0 is the decisive factor for the calculation of every quenching parameter. It can be used for evaluation of the absorption cross section of PSII and the core complex in different physiological conditions.

The short and long term effects of salinity on the physiology of intertidal algae have been examined primarily in terms of their physiological accommodation to this stress (Munda and Kremer, 1977; Bission and Kirst, 1979; Reed *et al.,* 1980a; Kirst, 1981; Coudret *et al.,* 1983). It was reported that in red algae, altered salinity resulted in turgor (Reed *et al.,* 1980b). There were reports that photosynthesis and respiration have also been shown to be affected by salinity changes (Kremer, 1979; Coudret *et al.,* 1983). Similar observation was noticed in the present experiment, where prolonged treatment retards the photosynthetic activity to a marked extent at high and low salinity. Even under optimal saline condition, the P_0 activity declined to a marked extent, when maintained in controlled environmental chamber. Besides this the change in the physiological activities of the plant depends on the environmental parameter and on the morphology of the seaweed. It may be presumed here that the marine algae in the natural environment, have no limitation of available dissolved carbon as the thallus is always exposed to the continuous exchange of seawater. However, transferring the plants from their natural habitat to the controlled laboratory conditions may impose severe stress on the organisms (Harder *et al.,* 1998) with the limitation of dissolved carbon, change in pH and deficiency of nutrients, which may finally affect the photosystems. This type of water stress may influence the photosynthetic activity and influence the fluorescence behaviour of the plants.

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