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 variable chlorophyll fluorescence forms an important tool for estimating productivity, in which, the chlorophyll 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% (Green, 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°08'E and 9°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 seagrasses. *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 other hand *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 seawater and transported to the laboratory in enriched seawater medium (Walne, 1974) and maintained at ambient temperature.

**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 continuously by a magnetic stirrer. The amount of oxygen evolved was recorded at 25°C and the rate of photosynthesis was expressed as pmol O₂ evolved/gram fresh weight/hour.

**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 (Hamamatsu R 376) placed at 90° to the excitation beam was protected by an interference filter (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...
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stored in a digital oscilloscope (Iwatsu 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 F₀ level, reduces Q₂ totally and reaches to maximum fluorescence level Fₘ. Under this condition all reaction centres were closed. The maximal variable fluorescence of a dark adapted sample Fᵥ is the difference between Fₘ and F₀. Fᵥ indicates the electron transport potential of PSII and quantum yield is the ratio between Fᵥ and Fₘ (Buchel and Wilhelm, 1993).

**FAST KINETICS**

In slow fluorescence, 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 fluorescent 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).

**SLOW KINETICS**

In the present experiment, the fluorescence signal of *G. edulis* and *G. crassa* was correlated with the chlorophyll a content and the Fₚ 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 Fₚ 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 $P_o$ 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 $P_o$ 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 $P_o$ 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 $P_o$ activity declined in all the treated sample on 6th day of observation ranging between 4-75%. At 35 ppt the $P_o$ 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 species of *Gracilaria* on 12th day of observation did not help to increase $P_o$ 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 yield 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 12th day of treatment compared to the corresponding 6th day. The recovery was maximum at 15 ppt salinity (100%) when the $P_Q$ 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 $P_e$ activity reduced to minimum of 0.8 μmol O_2/g fw/h (Fig. 5 & 6).

Fig. 6. Quantum yield of *Gracilariata* spp. at different salinities.

Fig. 5. Quantum yield of *Gracilariata* 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 ratio 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 *Gracilariata* spp ($P/T$ 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; Bisson 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$_a$ 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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References


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