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Oospore shedding in *Sargassum wightii* (Greville) J. Agardh and *Turbinaria conoides* (J. Agardh) Kuetzing at different environmental factors

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ABSTRACT

Experiments were conducted with the receptacles of reproductive plants of *Sargassum wightii* (Greville) J. Agardh and *Turbinaria conoides* (J. Agardh) Kuetzing to study the effect of different environmental factors on the oospore output. The ability to liberate spores and the quantity of spores shed in these two brown algae varied under the environmental factors tested. In general, submerged condition of plants, long day condition at low illuminance, seawater of 30 to 40‰ salinity and 25 to 35°C temperature were found to be suitable for maximum shedding of oospores in these two algin yielding plants.

Introduction

Basically there are two methods in the cultivation of seaweeds-one by vegetative propagation using the fragments from mother plants and the other by reproductive propagation using swarmers and different types of spores. In India, though some work was done on culturing the economically important seaweeds by vegetative propagation, scanty information is available for useful species of marine algae on the settlement and development of spores in the laboratory and their transplantation to the sea for further growth to harvestable size. A high rate of survival of germlings could be achieved with proper care under controlled conditions of the laboratory and transplantation of germlings to the sea could be done successfully for large scale cultivation of seaweeds. Knowledge on environmental factors influencing spore release is very useful not only for cultivation, but also for conservation and management of natural stock of seaweeds.

As scanty information is available on the effect of environmental factors on spore liberation in the brown algae, particularly in the members of Fucales (Umamaheswara Rao and Reddy, 1982; Narasimha Rao, 1989 a, 1989 b; Umamaheswara Rao, 1990; Narasimha Rao and Subbarangaiah, 1991; Ganesan *et. al.*, 1999), studies were made on two commercially important algin yielding brown algae *Sargassum wightii* and *Turbinaria conoides* growing in Mandapam area. Results obtained on the effect of environmental factors such as

exposure to air (desiccation), salinity, temperature, light and photoperiod on oospore output in these two algae are presented in this paper. Data collected on the shape and size of oospores of these two algae and hydrological and environmental parameters from the collection locality are also given.

Materials and Methods

The reproductive plants of *Sargassum wightii* (Greville) J. Agardh growing in the intertidal and subtidal region at Mandapam and Pudumadam and *Turbinaria conoides* (J. Agardh) Kuetzing in the subtidal region at Thonithurai and Kilakkarai were collected during their fruiting season, October to February in 1998 and 1999. They were thoroughly washed in the seawater at the collection sites and transported to the laboratory in plastic bucket containing seawater. Eight to ten healthy and well developed mature receptacles of a species were used for each petridish and for different experiments. The receptacles thus selected for the experiments were cleaned with camel brush and washed thoroughly several times with sterile seawater and placed in petridishes of 5 cm diameter filled with 20 ml of sterile seawater. The experimental sets were illuminated by a fluorescent cool white tube light at 20 $\mu Em^{-2}s^{-1}$ for 8 hours during the day time from 9 AM to 5 PM, except in the case of temperature and light experiments. The oospores liberated were counted daily after 24 hours to study the effect of different enviromental factors on oospore shedding.

The oospores liberated in the petridishes were counted by preparing a spore suspension of known volume and using a plankton counting chamber following the method of Kaliaperumal and Umamaheswara Rao (1982) and Umamaheswara Rao and Kaliaperumal (1983). The degenerating spores were not counted. The average values of two counts and the total volume of spore suspension were taken into account for computing the spore output. When the spore shedding was very less, the counting of spores was made by keeping the petridishes on a transparent grid sheet under monocular microscope. The spore output is finally expressed as number of oospores / receptacle. Ten experiments were conducted for each factor in each species.

For studying the influence of desiccation or exposure to air, the receptacles of *S. wightii* and *T. conoides* were exposed to air in shade and in the sun. The receptacles were blotted using blotting paper to remove the water on their surface before exposing to air. The receptacles were exposed to air in shade upto 2 hours (15 minutes interval upto 1 hour and then $\frac{1}{2}$ hour interval); upto 1 hour in the sun (5, 10, 15, 30, 45 and 60 minutes) and transferred to petridishes containing sterile seawater. Controls (0 minute exposure) were also maintained in all these experiments. During the time of conducting these experiments, the temperature in the shade was 30 ± 2 °C and the relative humidity varied from 48 to 61% and in the open air the temperature was 33 ± 2°C and the relative humidity ranged from 41 to 46‰.

A stock solution of 100‰ salinity was prepared by adding common salt to the seawater collected from the inshore area and sterilizing it. The salinity was determined using a salinometer (Atago Hand Refractometer). The lower grades were prepared from the stock solution by adding requisite quantity of distilled water. The oospore output in the two algae was estimated at 0, 10, 20, 30, 40, 50, 60, 70 and 80‰ salinities.

The influence of 9 different temperatures 0, 10, 15, 20, 25, 30, 35, 40 and 45°C on oospore liberation was studied by maintaining the petridishes with receptacles for 24 hours in a temperature controlled dark incubator or refrigerator.

Light intensity experiments were conducted at 0, 10, 20, 30, 40, 60, 80 and 100 μ $Em^{-2}s^{-1}$. For studying the effect of photoperiod on oospore shedding, petridishes with receptacles were subjected to 0:20, 4:20, 8:16, 12:12, 16:8, 20:4 and 24:0 Light and Dark cycles by keeping them in separate light and dark chambers. These experiments at seven different light and dark regimes were conducted at 20 μ $Em^{-2}s^{-1}$. All the experiments were conducted for a maximum of 7 days depending on the quantity of spore output.

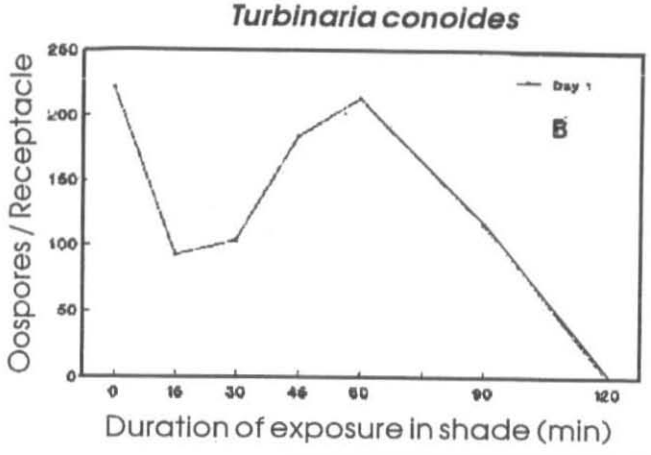
The shape and size of the oospores in both species were studied by taking random samples from the spore suspension in different months of the years. The shape of the spores was noted under monocular microscope and the size was measured using ocular micrometer. Data on atmospheric temperature, seawater temperature, salinity and light intensity were collected from one of the collection sites (Thonithurai) to correlate the results with the environmental conditions existing in the field and to know the optimal conditions required for maximum spore output. The atmospheric and seawater temperature were measured using a thermometer (Jennson Delux) and salinity was determined using a salinometer (Atago Hand Refractometer). The light intensity in the intertidal and subtidal region at the collection locality was measured using an underwater lux meter (EMCON).

Results

Changes observed in the oospore liberation of *S. wightii* and *T. conoides* in controls (0 minute exposure) and at different periods of exposure to air in the shade and in the sun are shown in Fig. 1. These experiments were conducted for a period of 3 days with *S. wightii* and one day with *T. conoides*. These experiments were designed not only to study the effect of exposure during low tides and the resultant desiccation of plants on spore production, but also to know the variations of spore release in shade as well as in areas directly exposed to the sun.

In the receptacles of *S. wightii* exposed to shade (Fig. 1A), maximum oospore shedding was found from the receptacles kept under submerged conditions (0 minute exposure) in all the 3 days and the number of spores liberated declined with increase in the duration of exposure. Spore output was observed on the first day from the receptacles exposed for 15, 30 and 45 minutes duration and on the second day only at 15 and 30 minutes exposure. On the 3rd day there was no liberation of oospores from any of the receptacles exposed to shade for different durations except in control. Liberation of oospores was not observed on the 4th day as the receptacles decayed (Fig. 1A). Data were collected only for one day with *T. conoides* as the receptacles decayed on the second day. The maximum oospore discharge was seen from the receptacles submerged for 24 hours and decline in oospore output was noticed upto 90 minutes exposure, with more number of oospores from the receptacles exposed to 45 and 60 minutes. There was no spore liberation from the receptacles exposed for 120 minutes (Fig. 1B).

Change observed in oospore output were more marked in the receptacles exposed to sun for even short periods of 5, 10 and 15 minutes (Fig. 2). The oospore output in *S. wightii* was found upto 15 minutes exposure on the 1st and 2nd day and upto 10 minutes exposure on the 3rd day. Peak shedding of spores was observed in submerged condition (0 minute exposure) in all the 3 days (Fig. 2A). In *T. conoides*, oospore liberation was noticed upto 30 minutes exposure with slightly more number of spores at 5 minutes exposure than the control (Fig. 2 B).



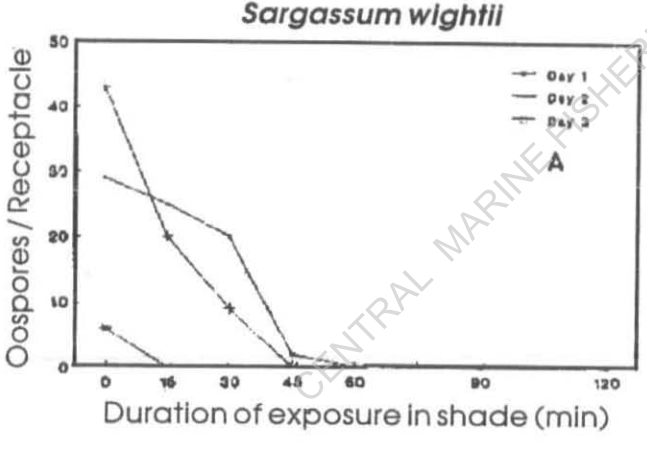
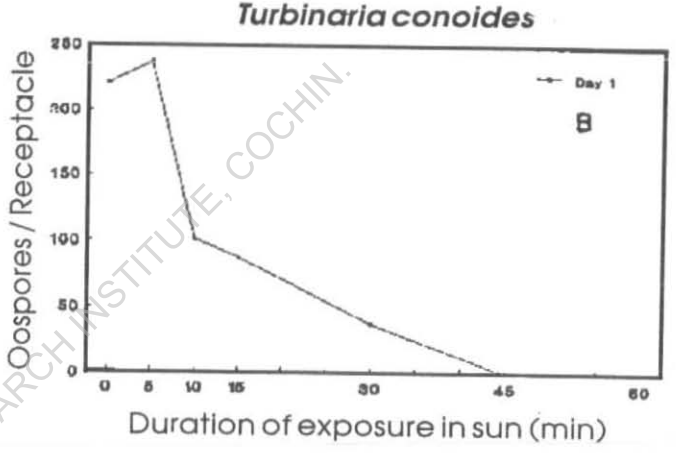


Fig. 1. *Effect of exposure in shade on oospore output : A.* Sargassum wightii; *B.* Turbinaria conoides



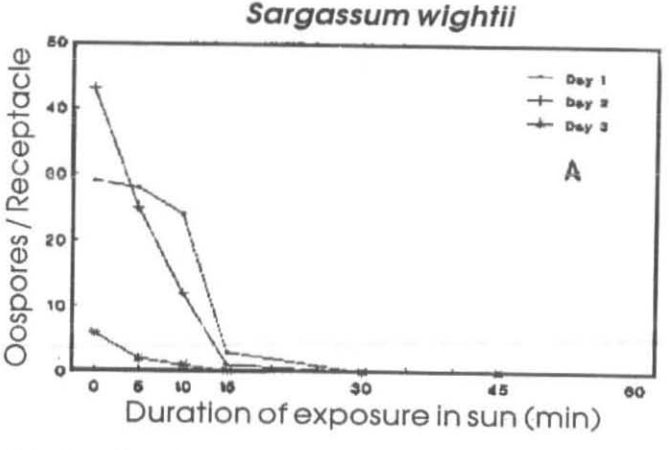


Fig. 2. *Effect of exposure in sun on oospore output : A.* Sargassum wightii; *B.* Turbinaria conoides

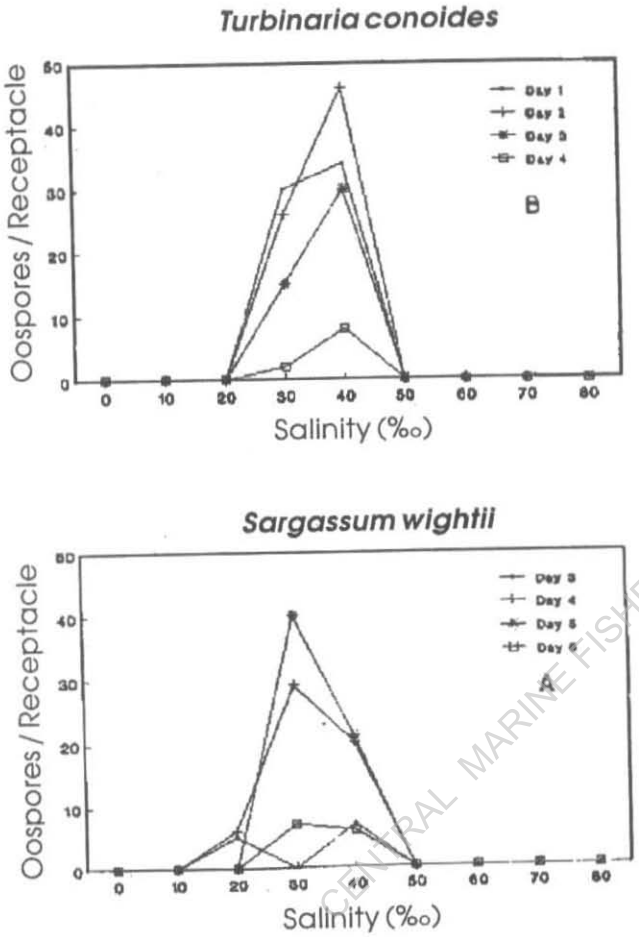


Fig. 3. *Effect of salinity on oospore shedding : A.* Sargassum wightii; *B.* Turbinaria conoides

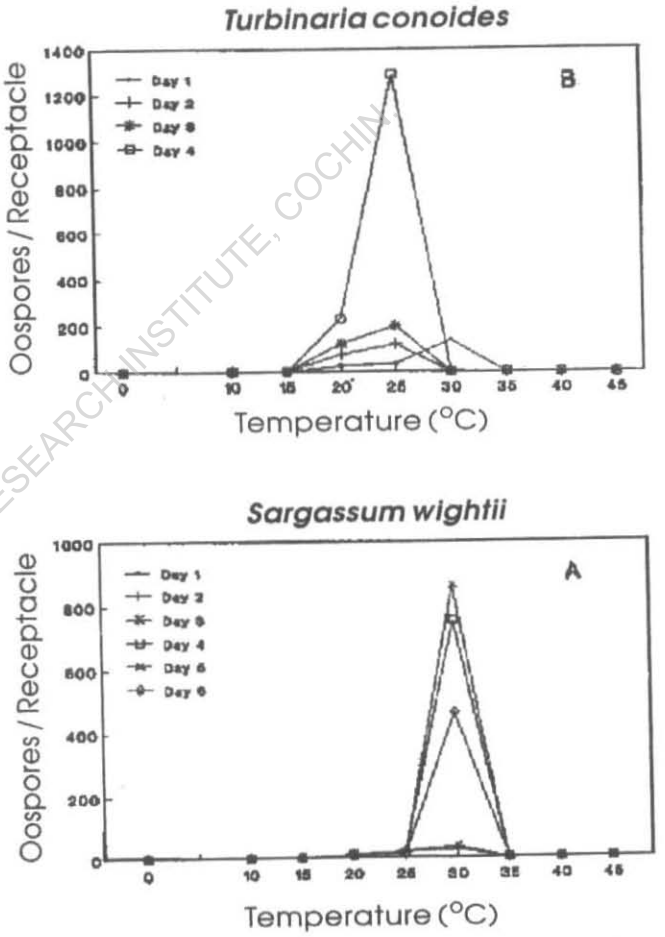
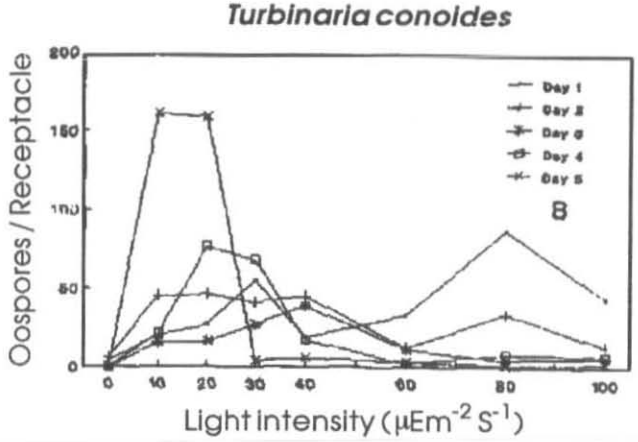
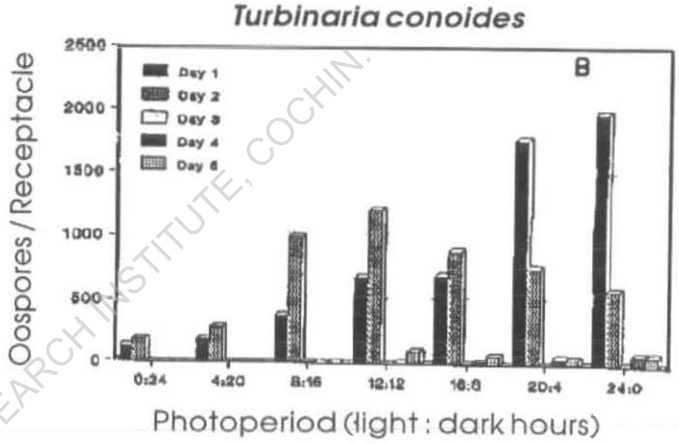
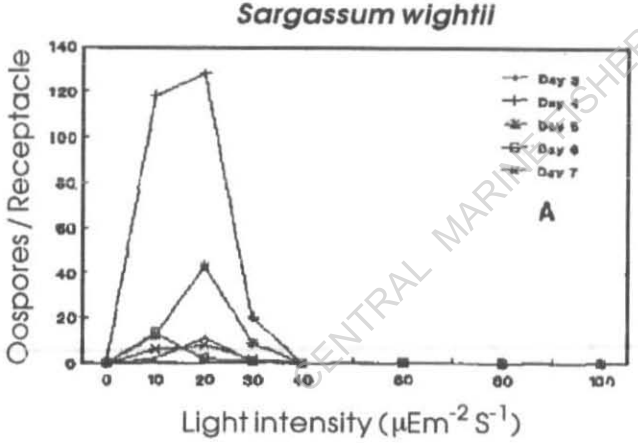


Fig. 4. *Effect of temperature on oospore liberation : A.* Sargassum wightii; *B.* Turbinaria conoides







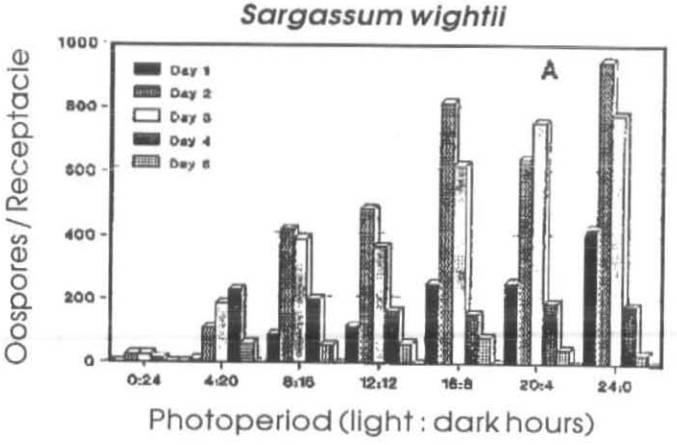


Fig. 5. *Effect of light intensity on oospore shedding : A.* Sargassum wightii; *B.* Turbinaria conoides

Fig. 6. *Effect of photoperiod on oospore shedding : A.* Sargassum wightii; *B.* Turbinaria conoides

Data on the effect of different salinities on oospore output were collected for 4 to 6 days (Fig. 3). In *S. wightii* there was no oospore output for the first 2 days and shedding was observed from 3rd day onwards upto 6th day with peak output at 30‰. Ther was no sporulation on 7th day in any of the salinities due to the degneration of receptacles. Spore output was found at 20-40‰ on 3rd and 4th day and at 30‰ - 40‰ on the 5th and 6th day (Fig. 3A). Shedding was seen in *T. conoides* only at two salinities i.e., at 30 and 40‰, with maximum sheeding at 40‰ in all the four days. The receptacles degenerated after 4 days (Fig. 3B).

Experiments on the output of oospores of *S. wightii* and *T. conoides* at different temperatures were conducted for 4 to 6 days in these two alginophytes (Fig. 4). Spore shedding occurred at different temperature ranges with peak output at a particular temperature in each alga. In *S. wightii*, oospore output was recorded between 20° and 30°C in all the six days with peak liberation at 30°C, (Fig. 4A). In *T. conoides*, shedding of oospores occurred at 20° and 30°C with peak liberation at 30°C on the first day. In all other 3 days sporulation was observed only at 20°C and 25°C with maximum liberation at 20°C (Fig. 4B).

The quantity of oospores liberated for 5-7 days, from *S. wightii* and *T. conoides* in dark (0 light intensity) and at seven different light intensities ranging from 10 to 100 μ Em^2s^1 is shown in Fig. 5. The light intensities at which spore liberation occurred varied in these two species. In *S. wightii* oospore output occurred only from the 3rd day onwards upto 7th day and thereafter the receptacles decayed. During these five days period oospore output was observed at 10, 20 and 30 μ $Em^{-2}s^{-1}$ on 3rd, 4th, 5th and 7th day and on the 6th day at 10, μ Em^2s^1 only (Fig. 5A). In *T. conoides*, oospore output occurred at all the light intensities tested (Fig. 5B) with maximum liberation of oospores at various light intensities during different days. i.e. at 80 μ Em^2s^1 on 1st day, 20 μ Em^2s^1 on 2nd and 4th day, 40 μ Em^2s^1 on the 3rd day and 10 μ Em^2s^1 on 5th day (Fig. 5B).

The effect of different light and dark cycles on oospore shedding in *S. wightii* and *T. conoides* was studied for five days (Fig. 6). The oospore output was observed in *S. wightii* almost in all the five days at seven different photoperiods except at 0:24 LD cycle on the first and second day. The peak output of oospores was recorded at 24:0 LD cycle from 1st to 3rd day, 4:20 LD cycle on the fourth day and 16:8 LD regime on 5th day. The production of oospores was found to be low on the fifth day at all the photoperiods (Fig. 6A). In *T. conoides*, oospore output was seen at all seven photoperiods almost in all the five days. Maximum shedding of oospores was observed at 24:0 LD cycle on the first and fourth day, 12:12 LD cycle on the second and fifth day and 8:16 LD regime on third day. The quantity of oospores liberated was low after the second day at all the photoperiods (Fig. 6B).

In *S. wightii* and *T. conoides*, the oospores were ovoid in shape. The length and breadth of oospores in *S. wightii* were 174-232 μm and 131-160 μm respectively. In *T. conoides*, the length of oospores was 160-189 μm and breadth was 116-145 μm. In general the size of oospores in *S. wightii* was larger than that of *T. conoides*.

Monthly mean data collected at Thonithurai from April 1998 to March 1999 on hydrological and environmental parameters are given in Table 1. The atmospheric temperature varied from 28.3°C in December to 32.6°C in November. The seawater temperature ranged from 27.0°C in January to 32.5°C in October. The salinity of seawater varied from a minimum of 25‰ in

Table 1. *Data collected on hydrological and environmental parameters from the collection locality Thonithurai.*

Month	Atomospheric temperature (°C)	Bottom seawater temperature (°C)	Salinity
April, 1998	32.0	30.0	33.0
May	32.0	30.0	33.0
June	30.8	30.5	30.5
July	31.0	30.5	33.5
August	31.0	29.7	35.0
September	28.0	29.2	34.0
October	30.5	32.5	35.0
November	32.6	31.7	31.5
December	28.3	28.0	28.5
January, 1999	29.0	27.0	25.0
February	29.8	29.0	29.0
March	30.0	30.5	30.0

January to a maximum of 35‰ in August and October. The underwater light intensity during the period of this investigation ranged from 2 μ Em^2s^1 to 408 μ Em^2s^1 in the intertidal and subtidal regions from where the plants were collected.

Discussion

The oospore output declined when the receptacles of *S. wightii* and *T. conoides* were exposed to air and subjected to desiccation indicating that exposure of fruiting plants adversely affected oospore liberation. Submerged condition was found favourable for maximum spore release. Similar observations were made in some members of Fucales viz. *Fucus vesiculosus* (Andersson *et. al.*, 1994), *Sargassum ilicifolium* and *S. vulgare* (Umamaheswara Rao, 1990) and other brown algae such as *Ectocarpus mitchellae* (Narasimha Rao, 1989 a; Narasimha Rao and Subbarangaiah, 1991), *Dictyota dichotoma* (Umamaheswara Rao and Reddy, 1982), *P. boergesenii* (Ganesan *et. al.*, 1999) and *Rosenvingea nhatrangensis* (Narasimha Rao, 1989 b). The adverse effect of exposure was more distinct in the receptacles of these two brown algae exposed in the sun than in the shade because of high temperature and low humidity. In the present study, oospore output occurred from the receptacles exposed to air in shade and sun for longer duration in *T. conoides* than in *S. wightii.* This could be because of the rough texture of the receptacles of *T. conoides* in resisting desiccation (slower dehydration rate) when compared to the receptacles of *S. wightii.*

Peak liberation of oospores was found at 30‰ salinity in *T. conoides* and at 40‰ in *S. wightii.* Similar trend was observed in the peak liberation of spores in Ectocarpus mitchallae (Narasimha Rao, 1989 a, Narasimha Rao and Subbarangiah, 1991), *P. boergesenii* (Ganesan *et. al.*, 1999), *Rosenvingea nhatrangensis* (Narasimha Rao, 1989 b), *Sargassum ilicifolium* and *S. vulgare* (Umamaheswara Rao, 1990). However Baker (1910) and West (1972) suggested that reduced salinity is an important factor causing propagule discharge in *Ascophyllum nodosum* and *Audoniella purpurea.* The optimum salinity range (30-40‰) observed in the present investigation for maximum shedding of oospores in *S. wightii* and *T. conoides* is nearer to the annual range of 25-35‰ salinity recorded in the inshore waters of Mandapam (Table 1). This indicates that the salinity of seawater along the Mandapam coast is suitable for liberation of maximum number of oospores from *S. wightii* and *T. conoides* growing in the interidal and subtidal regions.

The oospore shedding was affected conspicuously at very low and high temperatures tested and the optimal temperature range recorded for maximum liberation of oospores in *S. wightii* and *T. conoides* was 20 to 30°C. This experimental temperature range coincided with the annual

range of seawater temperature recorded in the field (Table 1). The result obtained on this aspect is in conformity with the findings of Umamaheswara Rao and Reddy (1982) in *Dictyota dichotoma* and Umamaheswara Rao (1990) in *Sargassum vulgare* and *S. ilicifolium*. But the gametophytes of *Chorda tomentosum* and *C. filum* reproduced at lower temperatures of 5°C and 5 to 15°C respectively (Novaezek *et. al.,* 1986). Though oospore output was noticed between 20 and 30°C in *S. wightii* and *T. conoides*, more quantity of spores were liberated at the lower temperatures of 20 and 25°C in *T. conoides* when compared with *S. wightii*. This is because of the robust and rigid nature of the receptacles of *T. conoides* to withstand lower temperatures than *S. wightii*.

In *S. wightii* oospore output was found below 30 μ $Em^{-2}s^{-1}$, where as in *T. conoides* oospores released even at 100 μ $Em^{-2}s^{-1}$. This may be due to the fact that the receptacles of *T. conoides* have tough texture than that of *S. wightii*. The light intensities at which peak sporulation occurred in these two brown algae (Fig. 5) can be compared favourably with *Dictyota dichotoma* (Umamaheswara Rao and Reddy, 1982), *P boergesenii* (Ganesan *et. al.,* 1999), *Ectocarpus mitchellae* (Narasimha Rao and Subbarangaiah, 1991) and *Sargassum ilicifolium* and *S. vulgare* (Umamaheswara Rao, 1990). Inhibition of spore emission was reported in *Monostroma* at 10,000 and 20,000 lux (Ohno, 1972). In the present study also at high light intensity of 100 μ $Em^{-2}s^{-1}$ (5000 lux) spore emission was not seen in *S. wightii* eventhough oospores were found on the surface of the receptacles.

In the present investigation long day condition favoured maximum spore shedding in *S. wightii* and *T. conoides*. This is in agreement with the observations made on *Sargassum muticum* (Norton, 1981). But short day condition induced spore liberation in *Padina boergesenii* (Ganesan *et. al.,* 1999).

The oospore size of *S. wightii* and *T. conoides* are larger than that of *Cutleria multifida* (Yamanouchi, 1912) and *Cystoseira* sp. (Krishnamurthy and Mairh, 1967) and more or less similar to that of *Sargassum swartzii* (Chauhan and Krishnamurthy, 1967).

The present study reveals that submerged condition of the algae, light intensity of 10 - 40 μ $Em^{-2}S^{-1}$, long day condition at low illuminance (20 μ $Em^{-2}S^{-1}$), salinity around normal seawater (20-40‰) and water temperature of 20-30°C are favourable for maximum shedding of oospores in *S. wightii* and *T. conoides*. These experimental findings closely agree with the hydrological and environmental conditions existing in the intertidal and subtidal regions of Mandapam coast.

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