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**“REPRODUCTIVE PROCESSES IN RED ALGAL GENUS *GRACILARIA* AND
IMPACT OF CLIMATE CHANGE”**



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REPRODUCTIVE PROCESSES IN RED ALGAL GENUS *GRACILARIA* AND IMPACT OF CLIMATE CHANGE

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Abstract

The genus *Gracilaria* forms a basis for the multimillion dollar phycocolloid industry. This genus has a tropical origin and represented by about 110 species through out the world. Despite increasing interest in agar as commercial product, relatively little is known about overall reproductive processes in genus *Gracilaria*. The typical life history of *Gracilaria* follows a basic pattern known as ‘*Polysiphonia* type’ which is triphasic in nature, however, various alternating strategies are also known. The ecophysiological differences have been reported in different life cycle stages. The biomass production as well as induction of reproduction has been significantly correlated with seawater temperature. The studies using microsatellite DNA markers have shown that the large-scale and continuous farming practices in *G. chilensis* has significantly modified the important life-history traits as compared to wild populations which could have important consequences on the evolution of the haploid–diploid life cycle. The phase and sex related genetic markers have been identified in *G. lemaneiformis* by RAPD method. The population structure in this genus is determined by the vital rates of spore mortality, recruitments and reproduction associated with different stages of life cycle. The spore production and survival is environmentally controlled in majority of the *Gracilaria* species. However, the spore dispersal has not been affected by the size of spore and haploid spores have better dispersal abilities than diploid ones due to their higher longevity and buoyancy in the water column under *in-vitro* conditions. The performance of non motile spermatia has been evaluated under the field conditions in *G. gracilis* through microsatellite markers, the results have shown that the fertilization success depends on distance traveled by spermatia, male-male competition and female choice. The intrinsic factors such as life cycle and mating system have shown to be responsible for genetic structure within the population in *G. gracilis*. The germination of spore follows the *Dumontia*-type of cell division in many *Gracilaria* species. The germinating spores have shown coalescing nature which is ecology as well as economically important. The over all understanding of the climatically driven environmental changes is much more complex and that include the species tolerance limit towards the particular change, alternative dispersal or reproductive strategy, physiological adaptation, biotic interactions, etc. The effect of such change on reproductive processes of genus *Gracilaria* is mostly unpredicted since results of few laboratory based studies are insignificant to conclude.

1. Introduction

The red algae are eukaryotic autotrophs, of which majority are being reported from marine environment. The marine macroscopic red algae, commonly known as red seaweeds, are distantly related to other two groups namely green and brown seaweeds. The independent evolutionary lineage of red algae is characterized by combination of morpho-anatomical, metabolic and physiological features that includes complex life history patterns (Hawkes, 1990; Coelho et al., 2007). About 5, 800 distinct species have been described in this class (Brodie and Zuccarello, 2008) with few are of immense commercial potential. Some red algal species are edible (Dippakore et al., 2005; Wang et al., 2008) while others are used for the extraction of industrially important phycocolloids such as agar and carrageenan (Zemke-White and Ohno, 1999; Meena et al., 2007). Although, in most of the cases naturally occurring biomass has been harvested for the industrial use, the

cultivation practice is on the rise considerably for the last couple of decades to meet the surging global market demand (Hanisak, 1998; Subba Rao and Mantri, 2006; Ganesan, 2006). In 2004, ca. 4 million wet tones of red seaweeds have been cultivated world wide valuing ca. US\$ 1.9 billion (FAO, 2006).

The development of new cultivation method for many seaweeds relies on the effective control of their reproduction and, hence, the knowledge about their life history patterns and reproductive processes is imminent. The world has witnessed the benefits tremendously from the understanding of reproductive process, when Prof. Drew has discovered the 'conchocelis-phase' in the life-history of red alga *Porphyra umbilicalis* (Drew, 1949). The conchocelis has proven to be another stage in the life history of Bangiales rather than a new species as described earlier. Thus based on the full illustration of the life history of this commercially important genus, the cultivation technique of *Porphyra* has been established. The considerable progress has been made in cultivation of *Porphyra* since then and about an average of 400, 000 tons (wet wt.) *Porphyra* per year is being produced with market value of over US\$ 1500 million (Dippakore et al., 2005). Another example is van den Meer and Tood's (1980) discovery of extremely small female gametophyte of commercial red algal species *Palmaria palmata* (dulse). Field phycologist had found only males and tetrasporophytes, so the plant was believed to have no sexual reproduction (see Bold and Wynne, 1978). The discovery of female gametophyte has helped in development of suitable cultivation procedure for this commercially utilized genus (Gall et al., 2004). Apart from culture and cultivation aspects the knowledge of reproductive patterns and processes has also been essential to understand the ecological process such as early recruitment to determine the organization of the seaweed communities (Santelices and Aedo, 2006; Lamote and Johanson, 2008), dispersion (Norton, 1992) and resource management (Subba Rangaiah, 1984).

The red algal genus *Gracilaria* that forms the raw material for the industrial production of agar, recorded more than 110 species from the tropical shores (Rueness, 2005). The genus *Gracilaria* is characterized by presence of tubular nutritive cells and non superficial spermatia. The annual global harvest of *Gracilaria* has been in excess of 37, 000 dry tons of which ca. one-third accounts for aquaculture (Ye et al., 2006). Despite increasing interest in agar as commercial product, relatively little is known about overall reproductive processes in genus *Gracilaria*. Generally it is assumed that most of the species of *Gracilaria* are characterized by triphasic sexual life history with an alternation of generations. As the case in most of the red algae, the life cycle is diplohaplontic, with haploid gametophytes alternating the diploid sporophytes. However, deviations to this have also been reported. The variation in reproductive success in relation to haploid and diploid stage has also been reported. Studies have also confirmed the differences in the dispersal abilities in haploid and diploid life cycle stages. The different survival strategies under artificial environmental conditions have been reported by both haploid and diploid in *Gracilaria*. It has been shown by using microsatellite DNA markers that the variation in male fertilization success depends on distance traveled by spermatia, male-male competition and female choice. Thus the fertilization is not sperm limited as earlier thought. The knowledge about reproductive biology would not only be helpful for cultivation but also for resource management. The present paper briefly appraises the information on the different reproductive processes in genus *Gracilaria* and the possible effects of changing environment.

2. Life cycle patterns

The sexual life cycle in the seaweeds involves a cyclic alternation between diploid and haploid phases with meiosis mediating the transition from the diploid to the haploid state, while syngamy reconstituting a diploid genome. The typical life history of *Gracilaria* follows a basic pattern known as '*Polysiphonia* type' which is triphasic in nature [Fig. 1]. The gametophyte as well as the sporophyte is morphologically identical and independent, while the gametophytes are dioecious. The haploid male and female gametophytic plants in nature are presumed to be in equal number; however, their identity can be confirmed under the microscope only at maturity. The female gamete is fertilized *in situ* by the single fertilization event. The diploid zygote is then formed involving very complex cytological events. The zygote germinates within the mother tissue forming the distinct new phase called 'carposporophyte'. This stage can easily be identified in the field with

naked eye, as hemispherical, protruding cystocarps are formed all over the surface of female gametophyte. The formation of cystocarp is regarded as a separate phase that grows parasitically on the female gametophyte. The numerous carpospores are produced in the cystocarp by mitotic division, hence genetically identical and diploid. The carpospore production could be compared to that of polyembryony, where embryo derived from bi-parental fusion splits into many genetically identical embryos that are different from the mother (Engel et al., 2004). The each carpospore germinates into a new diploid tetrasporophyte that is morphologically indistinguishable from the gametophytic plants. The tetraspore mother cell upon maturity undergoes meiosis resulting in genetically variable haploid tetraspores. The half of tetraspores would germinate and develop into male and rest half into female gametophytic plants, re-establishing the sequence of cyclic pattern. Numerous field as well as laboratory studies have confirmed the above mentioned life cycle pattern in majority of the *Gracilaria* species (Ogata et al., 1972; Plastino and Oliveira, 1988; Engel et al., 2001, Mantri et al., unpublished). This type of sexual life cycle has been also reported in other Florideophyceae, members including economically important genera such as *Gelidium*, *Chondrus*, *Eucheuma* and *Kappaphycus* (Coelho et al., 2007). In laboratory the life history could be completed within 5 – 12 months of culture, depending upon the species (Kain and Destombe, 1995, Oza and Gorasia, 2001).

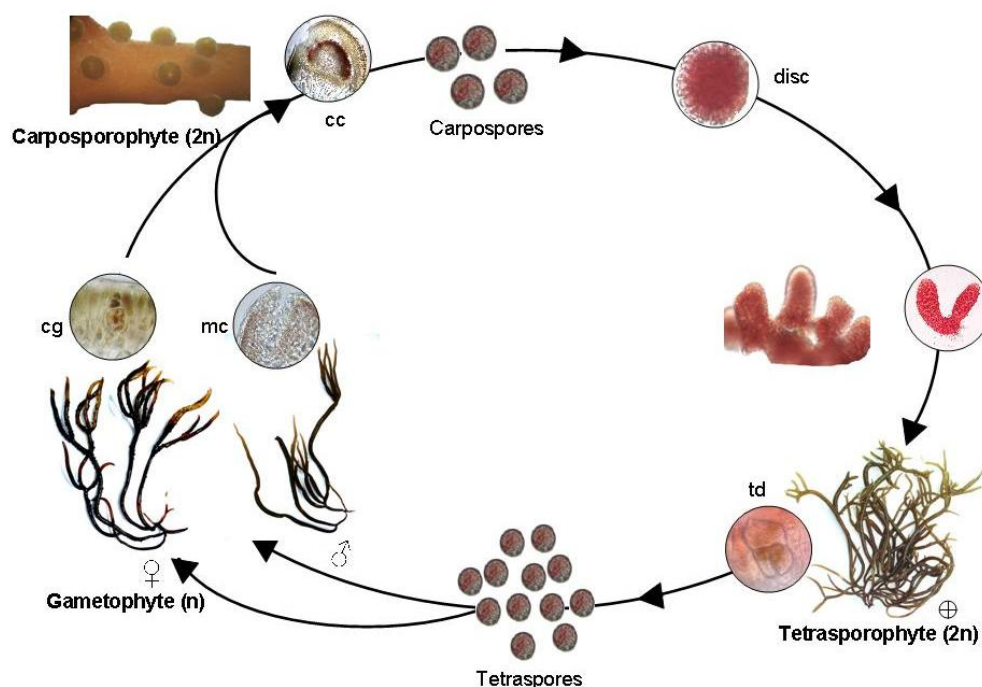


Figure 1: Life cycle of *Gracilaria dura* (C. Agardh) J. Agardh from Indian waters

However, deviations from the typical *Polysiphonia* type of life cycle in *Gracilaria* have been also reported. The *in situ* germination of tetraspores has resulted in formation of very interesting feature called 'mixed-phase' individuals, those exhibit both sporophyte and gametophyte characters in the same single generation. This type of variation has been reported in *G. tikvahia*, *G. debilis* and *G. verrucosa* (Bird et al., 1977; Oliveira and Plastino, 1984; Destombe et al., 1989). This variation effectively allows the population to skip the haploid

microscopic phase of life cycle to some degree. Oliveira and Plastino (1984) reported the aberrant sequence of development in *Gracilaria chilensis*. The *in vitro* culture of carpospore has produced spermatangia at maturity in few cultures instead of tetrasporangia. The authors have further reported the formation of only spermatangia in non-aerated culture of tetraspores; however, the aerated culture of tetraspores produced male and female gametophytes in normal 1:1 ratio. The study inferred that the development of spermatangia on putative female gametophytes, or on putative tetrasporophytes could be due to the different culture conditions under which these have been grown. This study also postulates the environmentally controlled sex determination in red algae. The development of female gametophyte has been reported from the carpospores in *G. edulis* (Krishnamurthy et al., 1969). Monocious condition bearing both male and female structures along with tetrasporangia has also been recorded (Kain and Destombe, 1995). The mitotic recombination and mutation involving male or female repression could be the genetic explanation for such deviation (van der Meer, 1977; van der Meer and Todd, 1977; van der Meer, 1981).

Although reproduction is often linked with sex this is not always the rule. The reproduction involving propagation through asexual spore, specialized vegetative structures etc. can exist either instead of, or in addition to, a sexual cycle. Asexual cycles produce a succession of genetically identical individuals with the same level of ploidy. The vegetative multiplication is known to occur in many natural populations of *Gracilaria* species through fragmentation of the thalli, but only few instances have reported the formation of specialized vegetative structures called propagules in culture (Plastino and Oliveira, 1988; Xing-Hong and Wang, 1993). More recently alternative life cycle pattern has been reported in *G. gracilis* from north-eastern Italy (Polifrone et al., 2006). The formation of spherical agglomerates of cells has been reported that upon release were able to proliferate and develop into new thalli. The propagules have been formed from the germinating carpospore and not from the modified branchlets of the tetrasporophytic plants as reported earlier in *G. chilensis* (Plastino and Oliveira, 1988). Such kind of asexual reproduction could help to ensure the multiplication of species to recover the population growth when it faces sudden environmental change such as elevated temperatures (Polifrone et al., 2006). Zhao et al. (2006) have also reported development of filamentous structures during laboratory culture of carpospore of *G. asiatica*. The development of filamentous fronds is in addition and independent of normal young sporelings. The single cell detached from filament and develops into a new filamentous frond. However, the formation of normal sporeling from filamentous frond is not reported. However, filament formation during carpospore germination could be considered the new asexual pathway in *G. asiatica*. Recently, asexual propagules have also been observed in *G. dura* collected from north-western coast of Indian peninsula (Mantri et al. unpublished). In old stock culture of tetrasporophyte (nearly thirteen to fourteen months old) many thin proliferation arising from the common base have been formed all over the mother thallus. These structures have the capacity to produce the complete mature thallus, if excised from the mother plant. This finding, however, is limited only for laboratory culture. If, these propagules are also present in the natural population of *G. dura*, they would represent a unique means of propagation and distribution.

3. Phenology and differences in life history stages

Phenology is the study of reproductive stages in the life cycle of an organism in accordance to the changes in climatic season. The phenology of species of *Gracilaria* has been studied widely from different geographical areas. At equivalent latitudes the phenologies are remarkably alike (Kain and Destombe, 1995). At higher latitudes the peak growth has been reported in late summer or autumn (Destombe et al., 1988), however, at lower latitudes it takes place in winter as been controlled by monsoon (Kaliaperumal et al., 1986). Only one growth season has been reported in *G. cornea* from Mexico. The biomass production has been significantly correlated with seawater temperature (Orduña-Rojas and Robledo, 2002). The wet season recorded the peak biomass in *G. cornea* followed by cold and dry season with lesser production of plants. Similarly the higher biomass values have been recorded in *G. heteroclada* during winter in Central Philippines (Luhan, 1996). The bimodal growth has been reported in *G. edulis*, *G. arcuata* var. *arcuata*, *G. corticata* var. *cylindrica*, *G.*

verrucosa from India (Umamaheswara Rao, 1973; Kaliaperumal et al., 1986; Oza et al., 1989). Similarly, *G. bursa-pastoris* and *G. gracilis* from Mediterranean lagoon, Thau, France has shown two peaks in the biomass production (Marinho-Soriano et al., 1998).

It is well evident from the literature that induction of reproduction in *Gracilaria* is environmentally controlled (Ye et al., 2006). Hay and Noris (1984) reported that reproduction in subtidal *Gracilaria* species has been associated with the onset of dry season along the Caribbean coast. They have further mentioned the dominance of gametophytic population over tetrasporophytes in three species and *vise-a-versa* in other two species. In most of the perennial red alga, the sporophytic phase in a population dominates the gametangial generation. The maximum occurrence of 65 -33 % has been reported for tetrasporophytic phase in *G. cornea* through out the year from Mexico with only 21-17% carposporophytes and 12% male gametophytes (Orduña-Rojas and Robledo, 2002). The highest proportion of tetrasporophytes in the natural population has also been reported in *G. gracilis* from north-eastern Italy (Polifrone et al., 2006). However, Destombe et al. (1989) reported equal proportion of diploid tetrasporophytes and haploid gametophytes in *G. verrucosa* from Northern France. Recently, the tetrasporophyte predominance in Gelidiales has been attributed to the ecophysiological differences in the phases (Carmona and Santos, 2006). It has been also shown that the diploid tetrasporophytic fronds of species of *Gelidium* have efficient reattaching capacity with more rhizoidal cluster forming ability than gametophytic fronds (Juanes and Puente, 1993). Similar ecophysiological difference has been also reported in few species of *Gracilaria*. The diploid tetrasporophytes of *G. verrucosa* have been shown to be better in growth, survival, tolerance to heavy metal and UV radiations (Destombe et al., 1993). The agar yield of diploid tetrasporophytes has been shown to be higher than haploid female gametophyte in *G. bursa-pastoris* (Marinho-Soriano et al., 1999).

A few species of *Gracilaria* have been cultivated extensively, still very little is known about the relationship between the cultivated and natural propagation. A very little attention has been paid to the possible changes in life-history characters in relation to the culture practice, so also to the causes of using sexual verses asexual propagules for farming. Recently such studies have been undertaken in *G. chilensis*, which has been cultivated extensively in the Chilean water for more than 25 years (Guillemin, et al., 2008). The process of domestication has produced certain voluntary or involuntary selection for superior growth performance. The microsatellite DNA markers have been used to study the changes in genetic diversity and life-history traits associated with farming. The results suggested that genetic diversity has been reduced due to continuous clonal propagation. The predominance of diploid individuals in farm suggested that farming practices had significantly modified the important life-history traits as compared to wild populations. The recruitment of individuals resulting from sexual reproduction is very infrequent in farmed individuals, which strongly supports the fact that the sexual life cycle is not competed under farmed condition. The dominance of diploid individuals associated with farming practices may have important consequences on the evolution of the haploid–diploid life cycle. The spread of selected genotype at local scale has been reported and attributed to the large scale and continuous farming of specific genotype.

4. Distribution, development of reproductive structures and sex and phase linked molecular markers

The reproductive structures in *Gracilaria* are distributed randomly through out the surface. Few studies have shown that basal portion of main axis represent highest number of both tetrasporangia and cystocarps (Garza-Sanchez et al., 2000). In *G. lemaneiformis* it has been observed that the distribution of tetraspores on the first generation branches was significantly higher than the subsequent generation branches. The first generation branches possess 80 % of the total tetraspores while remaining 20% have been produced on second and third generation branches (Ye et al., 2006). Similarly the germination rate in terms of percentage survival depends on from where they have been released. The spore death rate has been considerably lower (14%) for the tetraspores originated from first generation branches than the subsequent order (54%) in *G. lemaneiformis* (Ye

et al., 2006). Although such study has not been carried out for the other species of *Gracilaria* the more research in this aspect is desirable to attribute the causes for such discrepancy.

The information related to development of reproductive structures as a whole in genus *Gracilaria* is still fragmentary, but it has been worked out fairly well in few species (Greig-Smith, 1954; Oza, 1976; Ryan and Nelson, 1991; Bouzon et al., 2000). The female gametes in *Gracilaria* are stationary, called 'carpogonia'. These are produced profusely all along the surface of female gametophyte at maturity. The male gametes as in case of other red algae are non-motile, called 'spermatia'. The spermatia are produced in specialized structure called spermatangial cavity. The arrangement of spermatangial cavity is one of the characteristics of paramount importance, as has been used for delineating the genera as well as species of gracilarioid alga. The carpogonium branch has been formed on the supporting cells and has cortical origin. The presence of true axillary cell in certain taxa is doubtful. The fusion of two to many vegetative cells to form a 'fusion-cell' has been reported. The gonimoblast has been developed from the fusion cells. The spermatangia have been produced in sori that can be of two types the simple or confluent cavity or patches in shallow depression. The five types of spermatangial cavities have been reported in *Gracilaria*, namely *corda* type, *symmetrica* type, *textorii* type, *verrucosa* type and *henriquesiana* type (Kain and Destombe, 1995). The location of spore mother cell has been reported in the outer cortex in *G. corticata* (Oza, 1976). The male gametogenesis has been studied in *G. caudata* and *G. mammillaris* (Bouzon et al., 2000). The electron microscopic examination revealed that the spermatangial mother cells differ from surrounding vegetative cells by having poorly developed chloroplasts and numerous plastoglobuli. The endoplasmic reticulum has been concentrated at the cell periphery, contributing to the formation of the spermatangial vesicle. The each spermatium has the cell wall with two layers, but becomes necked upon its release. The tetraspore mother cell has been differentiated in the cortical cell in *Gracilaria corticata* (Oza, 1976). Morphologically this is the end cell of the lateral system. During the development the cell accumulates the reserve food enlarges and becomes densely pigmented. The spermatium divides and forms four tetraspores which are either cruciately or cunately arranged.

It is very difficult in *Gracilaria* to identify the sexuality before maturity due to the isomorphic nature of the life cycle. The phase and sex related genetic markers have been identified in *G. lemaneiformis* through amplification of genomic DNA by RAPD method (Xiang et al., 1998).

5. Spore and gamete production, viability and fertilization

The population structure is determined by the vital rates of spore mortality, recruitments and reproduction associated with different stages of life cycle. In *Gracilaria*, the recruitment and the relative frequencies of the gametophyte stage depend on the vital rates of the tetrasporophyte stage, and *vice-a-versa*. However, the dominance of one generation of the life history in certain species could be explained by phase-specific differences in above mentioned factors. However, it has been discovered that in perennial red alga *Gracilaria gracilis* the survival of the gametophyte and tetrasporophyte stages is more important for population persistence and growth than for the fertility aspects (Engel et al. 2004). This indicates that the survival of adults is much more important for population dynamics than is reproductive success. The large numbers of studies have been carried out to estimate the spore output and the factors that control the spore release in *Gracilaria* (Kain and Destombe, 1995). A few studies have also dealt with the gamete production and their survival. The spore shedding experiments conducted in the laboratory have resulted in very interesting finding pertaining to reproductive strategies in *Gracilaria*. It is generally considered that the presence of fertile plants in the natural population guarantee the spore release and their subsequent recruitment. The spore shedding can be more seasonally confined as shown in many species of *Gracilaria* (Kaliaperumal et al., 1986; Mal and Subbaramaiah, 1990). However, some of the species such as *G. corticata* from Mandapam coast, India showed the spore shedding through out the year (Umamaheswara Rao, 1976). The carpospores have advantage over tetraspores as released as a mass in mucilaginous sac, which ensures rapid and maximum spore settlement (Polifrone et al., 2006). The maximum spore shedding has been reported within the first three days in many of the *Gracilaria* species under laboratory conditions (Chennubhotla et al., 1986; Oza and Krishnamurthy, 1968;

Shyam Sundar et al., 1991). Nevertheless, prolonged spore discharge lasting for about twenty five days has been reported in *G. corticata* (Joseph and Krishnamurthy, 1977) and for thirty days in *G. edulis* (Rama Rao and Thomas, 1974) and *G. verrucosa* (Lefebvre et al., 1987). The marked diurnal variation has been reported in spore shedding pattern of different *Gracilaria* species with peak output at different times. *G. corticata* and *G. sjoestedtii* reported maximum carpospore as well as tetraspore production during night and the lengthened darkness has enhanced the spore production (Umamaheswara Rao, 1976, Umamaheswara Rao and Subbarangaiah, 1981; Chennubhotla et al., 1986). In contrast, in *G. textorii* peak spore shedding has been reported at the end of the day (Subbarangaiah, 1984). Umamaheswara Rao and Subbarangaiah (1981) have further reported that the timing of spore shedding has been affected by temperature alone but not irradiance, desiccation and salinity. The difference in the carpospore and tetraspore size has been reported by many workers. It has been suggested that there is a positive correlation between spore size and their sedimentation rate. The spore dispersal depends primarily on their viability and longevity in the suspended state. Destombe et al. (1992) showed that in *G. verrucosa* the spore dispersal has not been affected by the size of spore. Further, they have reported that the haploid spores have better dispersal abilities than diploid ones due to their higher longevity and buoyancy in the water column under *in-vitro* conditions.

The stationary female gamete in *Gracilaria* fuses with spermatia. The gametic fusion involved complex chain of events described elsewhere in this chapter. The spermatia of *G. verrucosa* are effective for less than five hours and have the dispersion range of 80 meters (Destombe et al., 1990). The spermatia viability has been thus considered the prime cause of fertilization limitation in *Gracilaria*. The performance of non motile spermatia has been evaluated under the field conditions in *G. gracilis* through microsatellite markers. Interestingly the results showed that the cystocarp yield has not been sperm limited. It has been further inferred from the observations that the variation in male fertilization success depends on the various factors such as distance traveled by spermatia, male-male competition and female choice (Engel et al., 1999).

The intrinsic factors such as life cycle and mating system are though to be responsible for genetic structure within the population. Such factors also govern the abilities of dispersal between different populations. Engel et. al. (2004) evaluated the consequences of the haploid – diploid life history and intertidal rocky shore landscape on a fine scale genetic structure in *G. gracilis* using seven polymorphic microsatellite loci. The reproduction in *G. gracilis* occurs in allogamous manner. Within single population no significant difference has been observed in allele frequencies, gene diversities and mean number of alleles between haploid and diploid. Although within-population allele frequencies have been similar between haploid and diploid samples, the overall genetic difference among haploid samples has been more than twice that of diploid. The weak but significant population differentiation has been detected in both haploids and diploids that varied with landscape features and not with geographic distance.

6. Spore germination and coalescence

The spores (both carpospores and tetraspores) in *Gracilaria* that have been attached to the substratum immediately undergo germination without any resting stage. The germination of spore follows the *Dumontia*-type of cell division (Oza and Krishnamurthy, 1967; Oza, 1975; Orduna-Rojas and Robledo, 1999; Polifrone et al., 2006, Mantri et al. unpublished). In *G. gracilis*, *G. corticata*, *G. cornea* the first division of spore took place in transverse plane to form two celled sporeling (Oza, 1975; Orduña-Rojas and Robledo, 1999; Polifrone et al., 2006). In *G. corticata* slightly oblique division took place in carpospore to form three celled stage, whereas in tetraspore, two resultant cells under went second division in an oblique plane with respect to the first to form four celled germling. In *G. gracilis* the first two divisions have been taken place without expanding the cell volume (Polifrone et al., 2006). After the four celled stage, each cell in *G. corticata* divides transversely to the first median plane forming eight celled primary disc. The cells of the primary disc are arranged in two superimposed tiers of cells. Further the peripheral cells of the primary disc divide periclinally to form the arched shape dome forming at the center. The establishment of the apical cell takes place at the summit, while the initiation of the rhizoids takes place in the lower half of the sporeling from the outer most

cells that are in direct contact of the substratum (Oza, 1975). The similar pattern of spore germination and development as disc, holdfast stage and apical dome formation has also been reported in *G. changii* (Yeong et al., 2008).

The germinating spores which are in close proximity tend to fuse and form the irregular shaped tissue mass in natural as well as under laboratory conditions from which the chimeric plant develops. This phenomenon is termed as spore coalescence and has been first reported in *G. verrucosa* (Jones, 1956). The spore coalescence produces more and larger shoots developed than isolated spore. The viability in terms of survival increases many fold in coalescing spores. In majority of the cases, coalesced spore mass is a mosaic of spore derivatives and thus exhibit the significant differences in growth and other phenotypic characters. In *G. chilensis* bicolor individuals have been produced due to spore coalescence (Santelices, et al., 1996). The chimeric hold fast produced the red as well as green cells, so also the upright chimeric fronds. The random amplified polymorphic DNA analysis further confirmed the existence of two genetically different phenotypes combined due to coalescence of spores. The coalescence of spores under natural conditions forms the basal crust which could withstand the adverse environmental conditions due to its microscopic nature that is physiologically better adapted than the adult thallus. The coalescing macroalgae are thus ecologically important members of intertidal and shallow subtidal communities. Since the number of upright shoot formation is increased due to the spore coalescence, up to certain extent it governs the population structure. In general the spore coalescence increases the size of the sporelings, thereby reducing further probability of sporeling mortality.

7. Global climate change and its predicted effects on reproductive processes in *Gracilaria*

The human population has precipitated irreversible changes in the biosphere since the advent of industrial era. The increasing deposition of pollutants and high levels of green house gases have elevated the global temperature. In addition to these the habitat disturbance by anthropogenic activities also caused substantial change in both the terrestrial and aquatic ecosystems. Thus the climate change is ranking high in scientific and public agendas. The ecological processes in few groups of marine organisms have been alarmingly altered due to global change in the environmental parameters (Gibbons and Richardson, 2009; Wanless et al., 2008; Broitman et al., 2008; Edwards and Richardson, 2004). The certain seaweeds such as members of non-geniculate coralline algae provide excellent material to assess their response to changing climatic conditions due to the intact fossil deposits available for the study. However, there are too few data to allow any confident statements on the effects of global climate change on the different biological parameters in the seaweeds, including reproduction. It has been generally assumed that the global warming would cause a pole-ward shift in the distributional boundaries of species with an associated replacement of cold-water species by warm-water species. There are interestingly no concise and confirmatory evidences in support of above statement available for any of the groups of marine organisms. However, the critical role of temperature in determining algal species distributions has been evident on much smaller spatial scales. The recent one such study has revealed that the rise in seawater temperature by 3.5°C, induced by the thermal outfall of a power-generating station, over 10 years along 2 km of rocky coastline in California has resulted in significant community-wide changes in 150 marine species including algae. The communities have been greatly altered in cascading responses to changes in abundance of several key taxa, particularly habitat-forming subtidal kelps and intertidal foliose red algae. The temperature-sensitive algae have been decreased greatly in abundance (Schiel et al., 2004). The fragmentary information is also available to correlate the potential environmental induced changes on the algal distribution, dispersal and establishment (Coleman et al., 2005; Edwards and Richardson 2004, Smayda et al. 2004; Sagarin et al. 1999). The study has been conducted along the North Sea coast to understand the possible effects of predicted climate change in the seaweed flora. The results have demonstrated that *Gracilaria verrucosa* which been the conspicuous members of the macro-algal flora of North Sea until the middle of the twentieth century, has been completely disappeared along with few other seaweed species by 1997 (Ducrottoy,

1999). The author has related this change to the altered environmental parameters such as increased levels of nutrients and oxygen depletion.

Having the tropical origin, genus *Gracilaria* would be capable of reproducing over a wide range of temperatures. The adult plants in *Gracilaria* have been found to be more sensitive to the elevated temperatures. The Mediterranean population of *G. gracilis* has shown the rapid decline in number of macroscopic thalli at the end of May when the water temperature reaches to its peak (25 – 28°C). Similarly *G. dura* population from northwestern peninsular India vanishes at the end of June when the seawater temperature reaches to its maximum (above 30°C) Mantri et al (unpublished). However, interestingly a fall in temperature (below 10°C) induces the reproduction in *G. gracilis* (Polifrone et al., 2006). However, the expected rise of average 2°C in global temperature may or may not induce the sporulation in *G. gracilis*. However, correlating reproductive phenology and temperature has been a difficult task because of our incomplete knowledge about how temperature influences reproductive maturity and at what stage of development. Temperature has been also the important factor that controls the spore shedding in many of the tropical species of *Gracilaria*. It has been shown that higher and lower temperatures than the normal would either preponed or postponed the timings of diurnal periodicity of the spores in *G. corticata*, *G. textorri* and *G. sjoestedtii* (Subba Rangaiah, 1985). In *G. edulis* about 3180 carpospores cystocarp⁻¹ day⁻¹ have been produced at 28.5°C in March, however, in May when the seawater temperature reaches to 32.5 the carpospore yield has been only 1696 spores cystocarp⁻¹ day⁻¹ at Gulf of Mannar, India (Mal and Subbaramaiah, 1990). The 4°C increase in seawater temperature has significantly reduced the carpospore output in this species by half of the average output. Garza-Sanchez et al. (2000) also reported that carpospore as well as tetraspore release and attachment in *G. pacifica* are temperature and irradiance dependent. The maximum tetraspore release has been achieved at 24°C and 140 µmole quanta m⁻² s⁻¹ in winter as well as autumn. However, the maximum carpospore release has been achieved at moderate temperature 20°C and 60 µmole quanta m⁻² s⁻¹. The higher temperature of 24°C and higher irradiance of 140 µmole quanta m⁻² s⁻¹ has drastically reduced the spore output in *G. pacifica*.

The spore survival in *G. pacifica* has been studied in spring, summer, autumn and winter. In all the season the moderate temperature of 20°C and moderate irradiance of 60 µmole quanta m⁻² s⁻¹ has shown higher percentage survival. In *G. lemaneiformis* it has been observed that the tetraspore survival rate remains at the same level of about 85% with the temperature variance from 10 – 30°C. However, the higher temperatures have resulted in significantly lower growth of the spore disc (Ye et al., 2006).

Ozone layer in the stratosphere provides the protection to all the living organisms on the Earth from harmful ultraviolet radiation (UVR). The human-induced ozone loss has resulted in elevated levels of UVR reaching the Earth's surface in recent decades due to the thinning of stratospheric ozone layer. In spite of the fact that the worldwide production of ozone depleting chemicals has already been reduced by 95%, the environmental disturbances are expected to persist for about the next half a century. Since UVR can penetrate water column they can cause a range of deleterious effects on aquatic organisms, with early life stages at particular risk (reviewed by Hader et al., 2007). UVR have been found to affect marine macroalgae by affecting key physiological processes including photosynthesis, enzyme metabolism DNA lesions etc. (reviewed by Franklin and Foster, 1997; Xue et al., 2005). However, the effect of UVR on reproductive process in seaweeds is not frequent in literature. Roleda et al. (2004) have studied the effect of PAR and UVBR on early life stages of *Mastocarpus stellatus* and *Chondrus crispus*. The germination and photosynthesis of the low light adapted carpospores of both species has been inhibited with increase in PAR. The carpospore viability in *C. crispus* has been found to be sensitive to UVR. Similarly, the effect of UVB radiation on early developmental stages, spore survival and embryo growth has been studied in *Mazzaella laminarioides*, *Gigartina skottsberdii*, *Macrocystis pyrifera* (Navarro et al., 2007). The survival and growth has been found to be affected in these seaweeds due to UVB. It is been observed that the percentage inhibition of sporulation in cystocarpic plants of *G. corticata* increased with increasing period of exposure to UV radiation. The maximum inhibition of sporulation of about 55% has been observed when the cystocarpic plant has been subjected to 60 min exposure to UVB (CSMCRI, unpublished results). Therefore, it could be concluded that the UVR may affect the reproductive process and recruitment in *Gracilaria* species.

The global climate change has increased the levels of atmospheric carbon di oxide in last few decades. The abundant growth of *Gracilaria* has been reported in the intertidal area of some topical countries. The few species of *Gracilaria* with high growth rate could be also considered as the important CO₂ sink, although such studies have not yet carried out. The genus *Gracilaria* has complex life history and varying reproductive strategies. The studies have proved beyond doubt that the differences do exist in different life cycle stages at physiological, ecological as well as molecular level. The juvenile stages such as spores, germling disc and early growth forms are of great importance as such stages have the essential role to play in dispersal and subsequent recruitment. The survival of these transitional life history stages is the most critical phase leading to the successful formation of a benthic population. The spermatia viability in *Gracilaria* is highly limited; this could be easily vulnerable to climatic changes. Considering the small size and simple cellular organization of these reproductive as well as early life history stages, any kind of climatic change may exert stress which ultimately would affect the biology of the species. However, the response of particular species towards change has been often very subtle and cannot be assessed simply from the results of few studies that subjected different life stages to a range of variable environmental conditions. The each species has its separate and unique strategy to combat the changing environmental conditions. Moreover, all the climatic changes are not sudden but take several years, and thus species might adjust to such changes up to certain extent by altering the physiological needs. The *G. salicornia* populations collected from Japan and Thailand have shown resilience against the changes in temperature, salinity and irradiance under *in-vitro* conditions (Phooprong et al., 2007). The different response in photosynthesis and respiration ability towards the short term change in irradiance, salinity and temperature has been recoded. The population from Thailand has shown the adaptability to high irradiance and temperature, however, the one from Japan has shown adaptability to the low submarine irradiance under *in-vitro* conditions. The same species has been intentionally introduced in Hawaii, in the 1970s for experimental aquaculture purpose (Smith et al., 2004). It took thirty years for this species to evident its invasive tendencies. This phenomenon has shown that the species might respond differently to the environmental change over the years by climatically adapting. The over all understanding of the climatically driven environmental changes is much more complex and that include the species tolerance limit towards the particular change, alternative dispersal or reproductive strategy, physiological adaptation, biotic interactions, etc. The effect of varying environmental conditions on reproductive processes of genus *Gracilaria* is largely unpredicted since the information pertaining to the effects is limited.

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9. References

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