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Review Article

The Algal Diversity: Its Conservation Strategies

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Abstract: The term "Biodiversity" refers to the variety and variability among organisms and the ecological complexes in which they occur. Thus, biodiversity can be defined as the totality of genes, species and ecosystem of a region. Sustainable management ensures that the rate of exploitation of biological species by man does not exceed the rate of their regeneration or reproduction. Sustainable management of biodiversity ensure the survival of all the biological species in the world and thereby follows the same trend of nature. So sustainable management can be called a nature-friendly approach. Sustainable management of algal biodiversity promotes the maintenance of genetic diversity that is the basis of any genetic improvement work and indirectly promotes climatic stability and soil productivity. Diverse algalogical species and their gene pool act as the storehouse of valuable genes that can exhibit suitable phenotypes to overcome the biotic as well as abiotic stresses. Sustainable management of algal biodiversity thus holds a great promise in ensuring better life support to our offspring through food, manure and pharamaceutical security. It is the sustainable management alone that can utilize algaldiversity not only to meet our present needs but also future needs.

Keywords: Algaldiversity, Conservation, Sustainable Management

Introduction

"This universe is the creation of the supreme power meant for the benefit of all His creations. Individual species must, therefore, learn to enjoy its benefits by forming a part of the system in close relation with other species. Let not only one species encroach upon the other's rights" (Source: Ishopanishad). Nature is unique in manifestation. We see the beauty of nature in her divese creation of plants, animals and microbes amongest the living domian, apart from the non-living creations of nature. Variation forms the basis of natural biodiversity. In simple worlds, the term biodiversity encloses or encompasses all the species of plants, animals, microbes and the complex ecosystem surrounding them on earth. Biodiversity, in fact, is a complex and balanced network of different species., which are mutually dependent upon each other. We, the human beings, are completely dependent on the biodiversity for the supply of food, fuel, fiber, shelter and medicine. Man thus becomes a component of biodiversity. Biodiversity is a condition for the long-term sustainability of the environment. The term biodiversity is the short form of biological diversity.

Freshwater is the most important natural renewable resource of mankind. It may be regarded as the "pillar of our civilization". The freshwater bodies of Tamilnadu (lotic and lentic types of ecosystems) are the rich source of algal biodiversity. India (8°–37° N and 68°–97° E) is a peninsular country with ca 7500 km long coastline, with an Exclusive

Economic Zone (EEZ) of around 2.5 million km^2 spread into 9 coastal states and 4 UTs. It has a very wide range of coastal ecosystems such as estuaries, lagoons, mangroves, backwaters, salt marshes, rocky coasts, sandy stretches and coral reefs. The Indian coasts consist in nearly on 43% of their total length of sandy beaches, in 11% of rocky with headlands, platforms and cliffs, and in 46% of mud flats and marshy wetlands. The coastline of India shows wide range of variability in its topography, geographical position and provides the great habitats for the enormous diversity of marine macro algae. Marine flora is taxonomically very diverse and fall into mangroves, seagrasses, microalgae and Marine macro algae (seaweeds). Among them marine macro algae play a vital role in the regulation of marine ecosystem. They are exclusively found in marine and range from unicellular organisms to nonvascular filamentous or thalloid plants. Typically, they are epilithic and grow on solid substrata such as rocks, bedrocks, pebbles, molluscs shells and corals. However, they can also be found growing on coastal litters such as plastic ropes, nets and decayed wooden pieces and as epiphytes on other plants like seagrasses and mangroves in shallow, intertidal and subtidal zones and deep waters of sea, even up to a depth of 150 m or up to a depth that can receive more than 0.12% of the photosynthesis light (Markager & Sand-Jensen, 1994).

Marine macro algae belong to the division thallophyta of the sub-kingdom cryptogamae and broadly classified into three classes viz. Chlorophyceae (Green), Phaeophyceae (Brown) and Rhodophyceae (Red), based on the nature of colour, storage of food materials, cell wall and type of photosynthetic pigments (Myslabodski, 2001). In recent years the importance and the economic values of this promising resources has got more momentum throughout the world. At the recent period, a total of 72,500 algal species (including varieties, forma etc.) recorded, of which about 44,000 species scientifically reported and published world-wide (Guiry, 2012). Among them, seaweeds constitute about 11,000 species which include Rhodophyceae with about 7,200 species, followed by Phaeophyceae with 2,000 species and Chlorophyceae with 1,800 species (http://www.seaweed.ie/).

Habits of Marine Macroalgae

Caulerpa racemosa var. *macrophysa* (Sonder ex Kuetz.) W.R.Taylor

Udotea indica A.Gepp & E.Gepp

Padina pavonica (L.) Thivy

Iyengaria stellata (Boergesen) Boergesen

Griffithsia corallinoides (L.) Trevis.

Halymenia floresii (Clemente) C.Agardh

In India, the attention on the algal research was initiated at the end of 19 century. Prof. M.O.P Iyengar (1886-1966), consider as the Father of Indian Algology, formulated the dais for algal research in India and provided a notable publication on seaweeds especially at the east coast of the Krusadai Island in the Gulf of Mannar in 1927. Afterward, Boergesen (1928-1938), played a vital role to explore the algal

diversity of West Coast. He published a series of articles contain more than 150 taxa including 5 new genera and 38 species from the west coast of India, particularly from the coasts of Bombay and Gujarat. Whilst, may of the researchers were also involved to focus on the various aspects of the marine algae from other parts of the country.

Misra (1966) published the first monograph on Indian marine algae named Phaeophyceae of India. that, contains 93 taxa of brown seaweeds. This monograph included 93 species of brown seaweeds, belonging to 33 genera. Chennubhotla (1977) of Central Marine Fisheries Research Institute (CMFRI), Cochin, published a short note on the edible seaweeds in the Indian context. Recent research reports cover the seaweed flora of various states of India (Krishnamurthy and Baluswami, 2010; Palanisamy and Yadav, 2015, 2017; Palanisamy and Yadav 2022a, 2022b).

Oza and Zaidi 2001 from Central Salt and Marine Chemicals Research Institute (CSIR), Bhavnagar published A Revised Checklist of Indian Marine Algae which included 844 seaweed species including forma and varieties from throughout the Indian coasts. Recently, a revised monograph on brown seaweeds i.e. Phaeophyceae of India and Neighbourhood (Volume I & II) have been published by Krishnamurthy and Baluswami (2010) and Krishnamurthy and Ezhili (2013) respectively. On the basis of improved checklist by Rao and Gupta (2015) Indian seaweeds encompasses about 865 taxa belonging to 234 genera and 65 family. Record of 442 species of Rhodophyceae in 138 genera, 212 species of Chlorophyceae in 46 genera and 211 species of Phaeophyceae in 50 genera

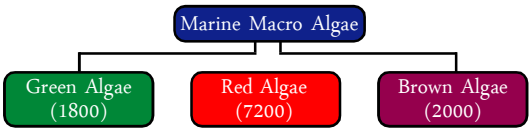


Table. 1. Numerical Data on taxon in worldwide & in India.

Sl. No.	Seaweeds	Worldwide Distribution	In India	Family	Genera
1.	Green (Chlorophyceae)	1800	212	19	46
2.	Red (Rhodophyceae)	7200	442	33	138
3.	Brown (Phaeophyceae)	2000	211	13	50
	Total	11,000	865	65	234

Maps showing rich diversity of seaweeds in India

The term "Biodiversity" refers to the variety and variability among organisms and the ecological complexes in which they occur. Thus, biodiversity can be defined as the totality of genes, species and ecosystem of a region. India is one of the 17 mega diverse countries of the world.

International code of botanical nomenclature has recommended the following suffices for the different categories of algae:

Kingdom	:	Plantae
Sub kingdom	:	Crypto-Games
Division	:	Thallophyta
Phylum	:	Algae
Class	:	Phyceae
Sub Class	:	Phycidae
Order	:	ales
Sub order	:	inales
Family	:	aceae
Sub Family	:	oideae

Origin of biodiversity

Biodiversity found on earth today is the result of 3.5 billion years of evolution. It is the result of two actions - the processes that produce new genotypes, new varieties and new species and the processes that eliminate mutations, variants and species from the system. The actual diversity of the system is

determined by the speed at which new variants originate in relation to the rate at which they are eliminated. The environmental conditions of the site and the range of tolerance of the species determined the site of occurrence of a species. Thus biodiversity varies from place to place. An enormous biodiversity exists on the earth in various habitats of plants and animals.

Table. 2. Elements of Biodiversity (After Heywood and Baste, 1995)

Ecological diversity	Genetic diversity	Organismal diversity
Biomes	Populations	Kingdoms
Bioregions	Individuals	Phyla
Landscapes	Chromosomes	Families
Ecosystems	Genes	Genera
Habitats	Nucleotides	Species
Niches		Subspecies
Populations		Populations
		Individuals

Categories of Biodiversity

Genetic diversity

The diversity in the number and types of genes as well as chromosomes present in different species and the variations in the gene and their alleles in the same species. The genetic variation existing within a species is called genetic diversity.

Species diversity

It refers to the variety of species with a regions. The variety in the number of richness of the species of a region. The number of the species per unit area is called species richness. Number of individuals of different species represent species evenness or species equitability.

Ecological diversity

It is a variety of forms in an ecosystem due to diversity of niches, trophic levels, energy flow, food webs, etc.

a) Alpha diversity (within community diversity) refers to the diversity of organisms *i.e.,* number of species in given community or habitat. It is calculated by the combination of species richness and evenness or equitability.

b) Beta diversity (between community) is diversity which develops due to change in a habitat or community along environmental gradients like altitude, latitude, moisture gradient, etc. The greater the difference or turnover of species between the habitats, the greater is the beta-diversity.

c) Gamma diversity is also called regional diversity. It represents the total richness of species in all the habitats found within a region, geographical area or landscape. When each habitat has a unique biota, gamma diversity is equal to average alpha diversity multiplied by the number of such habitats.

Values of biodiversity

The biodiversity of today is the fruit of billions of years of evolution. It is shaped by natural processes and increasingly by the influence of humans. It forms the web of life of which we (humans) are in integral part and uponwhich we fully depend. It provides a large number of services and goods sustain our lives. Biodiversity has contributed in many ways to the development of human culture. It is the source of food, fibers, fodder manure pharmaceutical, cosmaceutical and neutraceutical products.

Obstacles in algal biodiversity conservation

The main obstacles in biodiversity conservation are briefly discussed below:

1. Scientific knowledge on many species and many ecosystems are meagre. So people do not know which species to conserve and how to conserve.
2. Marine macroalgal resources are undervalued in comparison to mineral resources and other natural resources. The reason for under valuation lies in the fact that it is very difficult to determine the total economic value of the full range of goods and services which biodiversity offers.
3. Biological resources and more particularly the plant genetic resources, as such, are not marketable among people. There is no organized market for genetic resources.
4. Local institutions to conserve and utilize biodiversity are very small in number.
5. There is lack of incentives among common people for promoting biodiversity conservation.

6. Conservation activities are focused too narrowly in government politics of different countries and consequently the fund allocation for conservation of biodiversity is also inadequate.

7. Rich traders and manufactures over exploit some of the valuable biological species and leave the region impoverished with a few species.

8. Lack of political coordination among different countries hampers biodiversity conservation efforts. Sometimes, the exchange of genetic resources between two or more countries is so restricted that the conservation of all the varieties or strains of a species in a particular place is very difficult.

9. Absence of rapid and efficient techniques to identify minor differences among various varieties or strains within a species makes it difficult to conserve the diversity within a species. This difficulty often causes duplication of a particular variety in the conservatory of gene bank that leads to considerable wastage of time, labour and money.

The major threats of algal biodiversity are

1. **Destruction:** The primary threat to the algal biodiversity is the destruction of natural habitats.

2. **Habitat fragmentation:** It limits the dispersal and colonization potential of species and also reduces the foraging ability of animals.

3. **Introduction of new or exotic species:** This process cause a significant loss to the biological communities. The botanical garden, zoo and aquarium, hatchery gene banks which collect and maintain different species of micro and macro algae and their varieties (in plant gene bank) and also various animal breeds (in animal gene bank are covered under *ex situ* conservation strategy.

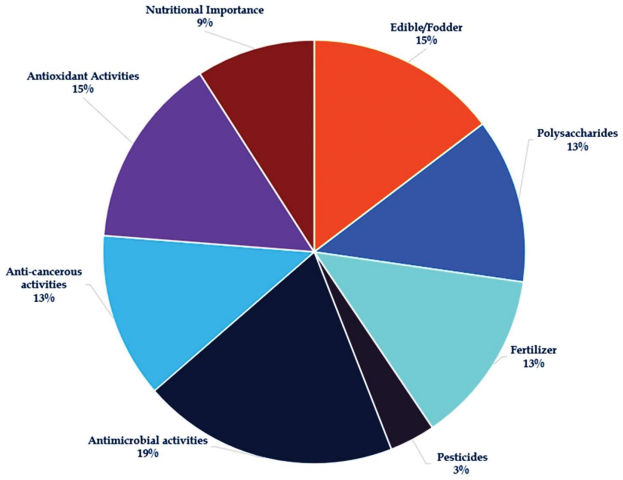
Economic uses of marine macro algae

Marine algae are useful in many ways, they were harvested since BC300 years ago, people practiced using seaweeds as food, fodder and manure. In many nations, seaweeds are commonly used as food and alternative medicine. They serve as the main ingredient in products like wine, cheese, soup, tea, noodles, etc. Additionally, seaweeds have the potential to be rich providers of dietary fibre, vital fatty acids, protein, vitamins, and minerals. The kind and quantity of biologically active substances in seaweeds are influenced by the time of year, the time of harvest, the seaweed's geographic location, and ecological conditions. Polyunsaturated fatty acids, polyphenols, carrageenan, fucoidan, and other bioactive substances have all been explored as potential sources in seaweeds or their extracts. Antioxidant, antimicrobial, antiviral, anticancer, antidiabetic, anti-inflammatory, anti-aging, anti-obesity, and anti-tumor activities have all been demonstrated for these secondary metabolites. Since bioactive components of seaweed are recognized as natural and harmless, they have been employed in the pharmaceutical, medical, and food sectors extensively.

Hydrocolloids are chemicals that gel when exposed to water. They work as stabilisers in biotechnology, coatings and dressings in medicines, gelling agents in food, and constituents in cosmetics. Carrageenan, alginate, and agar all include seaweed hydrocolloids. Carrageenan has drawn a lot of interest in pharmaceutical formulations and possesses a variety of medicinal qualities. Integrating carrageenan and natural polymers (chitosan, starch, cellulose, chitin, and alginate) to produce biodegradable polymers with biomedical uses has the high potential. Due to its special qualities, such as low toxicity, biodegradability, hydrogel formation, prevention

of bacterial infections, and preservation of a moist environment, alginate, a natural polysaccharide, is highly prized for use in wound dressings. In the realm of biomedicine, and microbial media agar is frequently utilized. In modern era most of the coastal villagers harvesting the seaweeds abruptly from the natural beds to meets the industrial requirements.

Economically importance Marine Macroalgal of India



Threats to Marine Macro Algae Biodiversity

Indian is highly sensitive and susceptible to both natural and anthropogenic threats such as Tsunami, coastal erosion, oil spills, influx of industrial effluents and tourism which may directly or indirectly have a negative effect on the coastal environment that ultimately results in the reduction of seaweeds diversity. The deadliest tsunami occurred in 2004 has drastically altered the boundaries of coastline due to heavy erosion which led to the destruction of seaweed habitats. In the monsoon season, many of the low-lying areas particularly in Kerala, Tamilnadan, Andhra Pradesh, Maharashtra and Goa the magnitude of coastal erosion was very high and caused severe damages to the coastal habitats, houses and human settlements. In major coastal habitats affected by disposal of industrial effluents and discharge of domestic sewages. In the coastal regions Oil refineries and gear boats operations were affect the seaweed beds. Generally, lot of plastic debris and disposable materials were dumped along the coast which may lead to disturb the coastal ecosystem and ultimately decline to the seaweed vegetation.

Techniques for the by-products of Marine Macro Algae

India's seaweed industry relies significantly on wild harvesting to produce the phycocolloid. While **Sargassum** spp. and **Turbinaria** spp. are used to produce alginates, species like **Gelidiella acerosa** and **Gracilaria edulis** are collected and used to produce agar. Due to overexploitation, India's natural resources for **Gelidiella acerosa** and **Gracilaria edulis** have been depleted from 2005 to 2016. (Meenakshisundaram *et al.*, 2009). The seaweeds diversity and density are declined due to the climate change. The rising temperature directly influencing the growth of seaweeds worldwide. Also, the pH of ocean is getting acidic due to high CO_2 in atmosphere.

Threats to marine macro algae diversity in India

Sustainable Management of Biodiversity

The term sustainable management has been derived from two words "sustainability" and "management". Sustainability can be defined as a process that meets the current needs of the present generation from a resource base without compromising the prospects of meeting the needs of the future generations. Sustainability implies that the natural resource should be held constant over time despite providing a continuous flow of goods and services. Management refers to the act of managing a resources. Biodiversity is also a natural resource.

With exploding population and rapid loss of biodiversity, sustainable management of biodiversity has assumed very great significant. In simple words, sustainable management of biodiversity for meeting the future needs without causing any harm to biodiversity. We, the human beings, can't help depending on biodiversity that provides us a range of useful goods and services. But dependence does not mean over-exploitation of any species from nature leading to its extinction. So we must continue to exploit biological species for our own benefits keeping the concept of sustainability in our mind. Moreover, just conservation of biodiversity without its proper use in human welfare does not make any sense in modern civilization. Rather rational use of any natural resource is praiseworthy.

It is easy to the prefix sustainable before the terms management, growth, development etc., but it is very difficult to the prefix really meaningful by getting its implications in reality. Sustainable management of biodiversity must have certain basic characteristics that are briefly outlined below:

1. Sustainable management ensures that the rate of exploitation of biological species by man does not exceed the rate of their regeneration or reproduction.
2. It is an eco-friendly approach in biodiversity management it promotes stable ecology
3. It keeps the number of biological species constant over time.
4. Sustainable management does not pose any threat to the survival of any species.
5. Sustainable management does not leave any adverse effect on our environment due to exploitation of different biological species.
6. Sustainable management ensure a continuous flow of goods and services from biodiversity as a natural resource.
7. Sustainable management of biodiversity ensure the survival of all the biological species in the world and thereby follows the same trend of nature. So sustainable management can be called a nature-friendly approach.

8. Sustainable management of biodiversity promotes the maintenance of genetic diversity that is the basis of any genetic improvement work.

9. Sustainable management of biodiversity provides opportunity for research on biological species for human welfare.

10. Sustainable management of biodiversity indirectly promotes climatic stability and soil productivity.

Our life support system are under stress and the loss of the biological resources of our country is increasing (Swaminathan, 1990). Biological resources include both biodiversity across species and genetic resources within each species. Good land for crop husbandry is shrinking, while biotic and abiotic threats to food security are expanding. The answer to these ever increasing threats lies in the sustainable management of all natural resources including biodiversity. Diverse biological species and their gene pool act as the storehouse of valuable genes that can exhibit suitable phenotypes to overcome the biotic as well as abiotic stresses. Sustainable management of biodiversity thus holds a great promise in ensuring better life support to our offspring through food security. It is the sustainable management alone that can utilize biodiversity not only to meet our present needs but also future needs.

Algae are an ancient and extremely diverse group of plantlike organisms, with repres-entatives of the blue-green algae (cyanobacteria) being present for the last 3550 million years (Schopf and Walter, 1982). They range in morphology and size from microscopic picoplanktonic cyanobacteria (<2 mm in diameter) which are prokaryotic and closely resemble other eubacteria, a variety of unicellular, multicellular, filamentous and thalloid forms, to giant kelps that may be up to 60 m long. Their taxonomy is problematic, but it is clear on the basis of both traditional taxonomy and modern molecular techniques that they are polyphyletic (Bold and Wynn, 1985; Cavalier-Smith, 1993). The 'amount' of algal biodiversity, as in other groups of organisms, is largely unknown, however, the advent of molecular biological techniques and improvements in electron microscopy have greatly increased our knowledge-base and assisted in elucidating inter-relationships. Approximately 37,000 species of algae have been recognized/described (Table 3) but estimates of the total number of algal species vary from a relatively conservative 40 000 to >10 000 000 (Hawksworth and Mound, 1991; John, 1994).

Table. 3. Biodiversity of algae.

Algal group	Division	Class Common name	No.[a]
Cyanobacteria	Cyanophyta	Cyanophyceae Blue -green algae	2000
Green algae	Charophyta	Charophyceae Stoneworts	11000
	Chlorophyta	Chlorophyceae	3600
		Ulvophyceae	
	Prasinophyta	Prasinophyceae	120
Chromophyte algae	Bacillariophyta	Bacillariophyceae Diatoms	10000
		Fragilariophyceae	
	Chrysophyta	Chrysophyceae	1000
		Synurophyceae	
	Dictyochophyta	Dictyochophyceae	10
		Pelagophyceae	
	Eustigmatophyta	Eustigmatophyceae	12
	Phaeophyta	Phaeophyceae Brown algae	900
	Prymnesiophyta	Prymnesiophyceae	300
	Raphidophyta	Raphidophyceae	15
	Xanthophyta	Xanthophyceae	600
Red algae	Rhodophyta	Rhodophyceae	4000
Cryptomonads	Cryptophyta	Cryptophyceae	200
Dinoflagellates	Pyrrophyta	Pyrrophyceae	2000
Euglenoids	Euglenophyta	Euglenophyceae	900
Glaucophytes	Glaucophyta	Glaucophyceae	13

Approximate no. species described; data from Norton *et al.* (1996).

From an economic perspective, algae are often considered a nuisance. Those associated with the water industry may think of them as a source of irritation - blocking water filters, causing 'off flavours' etc. - or even a major economic problem when toxic cyanobacteria may result in a reservoir being unusable as a source of drinking water. However, others see the group as a rich source of valuable chemicals or novel

pharmacologically active agents (Glombitza, 1979; Lincoln *et al.,* 1990). Approximately 500 species of algae are used as human food or food products, and about 160 species are considered commercially valuable (Abbott, 1988). Products from algae may have sig-nificant financial value; the Japanese harvest of *Porphyra* (Nori) is worth US$1 billion annually (Mumford and Miura,, 1988) and the annual value of algal polysaccharides, primarily agars and carageanans, is US$500 million (Jensen, 1993). Other commercially important products from algae include: health foods (Lee, 1997); pigments (Cannell, 1990); aquaculture feeds (Borowitzka, 1997) and lipids (Shifrin and Chisholm, 1980). They are also widely used in ecotoxicity testing (OECD, 1984) and may be used to treat waste water (Oswald, 1988). Moreover, algae as a group are responsible for fixing. 40 per cent of the earth's carbon (Bolin *et al,* 1977) and as such are major carbon sinks and also oxygen producers.

Alternative strategies employed to conserve algae

As with other groups of organisms two basic options are available for the long-term conservation of algae: conservation *in situ* in managed or non-managed ecosystems, and *ex situ* conservation. The former has the advantage that the algae will continue to interact with the other biological and physico-chemical factors in their environment and will not vary from 'wild-type' strains. This type of algal conservation occurs in marine parks, or other areas protected from the excesses of man's activities (Phillips, 1998). However, in reality this approach is not appropriate for many organisms. Where access to an algal strain is needed quickly, or an axenic culture is required, *ex situ* maintenance is the only realistic option. Further stimuli to the *ex situ* conservation of living materials have been the Convention on Biodiversity (CBD), specifically Article 9: *ex situ* conservation (UNEP, 1992) and the parallel development of bioprospecting for products with commercial value (Day, 1993).

Roles of genetic resource centres and culture collections

The primary role of an algal culture collection is the same as any other collection of living material that is to be a repository for cultures. In microbial service collections, including algal collections, this role is often associated with other products and services including: provision of authentic specimens for research; material for education; material for bioassay use; aquaculture starter cultures; identification; training; acting as a depository for patent purposes; consultancy; and other commercial applications. All of these require the maintenance of viable, healthy, physiologically and genetically stable cultures. There are more than 11000 strains of algae including representatives of approximately 1600 different species retained in protistan collections around the world (Miyachi *et al.,* 1989), with more than 80 per cent of these being maintained in the six largest algal culture collections (Table 3). These collections provide the scientific community with cultures and their associated information, as well as a variety of other services (see above and Table 4).

Methodological strategies employed to conserve algae

Any conservation methodology adopted should guarantee long-term stability of the morphological, physiological and genetic characteristics of the preserved organism. A variety of methods have been applied to the long-term stabilization/preservation of algae (McLellan *et al.,* 1991; Warren *et al.,* 1997). However, the most commonly used techniques involve the routine serial subculture of the algae under controlled environmental conditions. The alternative approaches, which involve less routine maintenance of the conserved specimens/cultures, all depend on the removal of water and/or altering the cellular physicochemical environment with respect to water activity. These techniques fall into three main categories: drying; freeze-drying and cryopreservation.

Maintenance by serial subculture

Historically algae have been maintained *ex situ* by regular serial subculture (Leeson *et al.,* 1984; Pringsheim, 1946). This continues to be the method of choice for most phycologists, particularly when relatively small numbers of cultures are involved. There is an extensive literature on culture techniques and maintenance conditions (Day and McLellan, 1995; Warren

Table 4. Activities of major* algal culture collection

Name of catalogue	Acronym	Country	No. strains	Latest printed	Available on WWW	Use of Cryopreservation	Patent Deposits	WFCC No.
Algensammlung am Institut fur Botanik	ASIB	Austria	1600	1985	-	-	-	505
American Type Culture Collection	ATCC	USA	200	1993	+	+	+	1
Culture Collection of Algae and Protozoa	CCAP	UK	1700	1995	+	+	+	140/522
Provasoli-Guillard Center for Culture Collection of Marine Phytoplankton	CCMP	USA	1450	1997	+	+/-	-	•2
National Institute for Environmental Studies Collection	NIES	Japan	1000	1997	-	+	-	591
* Sammlung von Algenkulturen	SAG	Germany	1630	1994	-	-	-	192
Culture Collection of Algae at the Univ. of Texas at Austin	UTEX	USA	2100	1993	+	+/-	-	606

[a] Service collections retaining > 1000 algal strains with the addition of the American Type Culture Collection (ATCC).

+Available/currently used.

+/-Under development.

- Not available/not used.

Data from Takishima *et al.* (1989) and Day (1996).

et al., 1997). Medium composition depends both on the requirements of the algae (e.g. diatoms require the inclusion of a silica source) and the preferences of the researcher. Information on medium suitability and full recipes are listed in all major collection catalogues (Andersen *et al.,* 1997; Watanabe and Hiroki, 1997), In general, cultures are maintained under sub-optimal temperature and light regimes (<20°C and <50 mmol photon $m^{-2}s^{-1}$); this maximizes the interval between subcultures and thus minimizes handling/ transfers of the strain. Alternative techniques include the use of medium containing organic carbon for main-taining axenic strains capable of heterotrophic or mixotrophic growth and solidified, rather than liquid medium may be employed to maximize the period between transfer to fresh medium.

Although serial subculture has proven very successful, with some isolates being main-tained for more than 80 years, it is widely recognized as being sub-optimal. It is a labour and consumables intensive process and the continuing increases in costs act as a stimulus to the development of long-term preservation techniques. Furthermore, this technique can potentially lead to selection of a population which may not be representative of the parent culture. In extreme cases this may include changes in important physiological and mor-phological characteristics, for example, irreversible shrinkage of diatoms (Jaworski *et al.,* 1988), loss of spines in *Micractinium pusillum* and alteration of pigment composition in a number of algae (Warren *et al.,* 1997).

Maintenance by storage in liquid medium

Some species of algae, particularly those that may form resistant structures, may be maintained long-term in biphasic medium (Tompkins *et al.,* 1995). This approach has been successfully employed to preserve algal zygotes and cysts for up to 20 years (Coleman, personal communication).

Drying techniques

Some algae are extremely resistant to desiccation and algal cysts/ spores may survive pro-longed exposure to dry conditions and high temperatures (Buzer *et al.,* 1985). Examples of this include air dried soil samples containing *Haematococcus pluvialis* aplanospores that can regenerate fresh cultures after 27 years storage (Leeson *et al.,* 1984) and the cyanobacterium *Nostoc commune* that has been revived from herbaria specimens after 107 years storage (Cameron, 1962).

Drying, generally air drying, may be used successfully for a wide range of cyst forming protists and some strains, e.g. the achlorophylous euglenoid *Polytoma,* are commonly transported as dried material on filter paper (Alexander

et al., 1980; Nerad, 1993). Furthermore, storage of cyanobacterial cultures in dried soil, or non-perfumed cat-litter, has been used by some researchers to maintain 'back-up' cultures for periods of several years (Parker, personal communication). However, drying has not been widely applied as a method of long-term conservation of algae in major service collections. This is primarily due to the low levels of recovery for some organisms and the relatively short shelf-life of stored material (Day *et al.,* 1987).

More recent research using a controlled drying protocol demonstrated that the method has some potential for a number of green algae (Malik, 1993). This study employed equipment which vacuum dried the algae in a suspending medium incorporating protective chemicals including skimmed milk, neutral activated charcoal, mesoinositol or raffmose. This approach may be utilizable for several algae, but it is unlikely to be satisfactory for the long-term preservation of more fragile organisms and, as yet, no long-term data on viability have been published.

Freeze-drying techniques

Lyophilization using conventional freeze-drying equipment and protocols employing 20 per cent (w/v) skimmed milk or 12 per cent (w/v) sucrose as protective agents in the suspending medium has been successfully applied to preserve a range of cyanobacteriaand eukaryotic microalgae (Holm-Hanson, 1973; McGrath *et al.,* 1978). This technique has been regularly used at the American Type Culture Collection (ATCC) for a number of organisms (Daggett and Nerad, 1992). However, levels of post-lyophilization viability may be low, 10^{-2} to less than 10^{-7} per cent of the original level being recorded by McGrath *et al.* (1978). On using this approach at the Culture Collection of Algae and Protozoa (CCAP) low levels or no viability was observed post-rehydration of freeze-drying eukaryotic microalgae. The highest level of viability observed was 1 per cent for *Chlorella emersonii;* however, no viability was detected after storage for one year (Day *et al.,* 1997). It is worth noting that *C. emersonii* has previously been demonstrated to survive up to two years storage using this technique (Day, 1987). In this material viability levels were extremely low, in the range 10^{-4} to less than 10^{-7} per cent of the original level (Day, unpublished data). Freeze-drying cyanobacteria, using the method of Kolkowski and Smith (1995) has proven more successful, with survival of both unicellular and filamentous forms (Day *et al.,* unpublished data). However, where non-axenic isolates were examined, on suspension of the lyophilized samples in fresh medium, the protective agents stimulate bacterial 'blooms' that had a deleterious effect on the recovery of the preserved cyanobacterium. Although this technique may be employed, low levels of viability, problems associated with non-axenic cultures and the possibility of selecting for a tolerant sub-population have dissuaded the major collections from adopting it as a technique to conserve algae.

Cryopreservation techniques

The general theory and principles of Cryopreservation are outlined by Benson. Cryopreservation is the optimal method of long-term storage of algae, where high post-thaw viability can be guaranteed. At ultra-low temperatures (less than -135°C) no further deterioration of stored material can occur and viability levels should be independent of storage duration measured in decades (Grout, 1995). Therefore, assuming there are no perturbations in the storage regime, long-term stability of the frozen specimens can effectively be guaranteed. As yet there are no published reports on the genetic stability, or otherwise, of cryopreserved algae. However, a selection, of the algae originating from different ecological niches, and from different algal Divisions and Classes, were demonstrated to have retained the same levels of post-thaw viability after up to 22 years storage in the CCAP Cryostore (Day *et al.,* 1997). These factors and the signi-ficant savings in costs associated with serial subculture have stimulated all the major collections to consider employing Cryopreservation, with most of them currently using or developing the technique to preserve a proportion of their holdings.

In comparison to drying or freeze-drying, Cryopreservation can result in high levels of post-thaw viability,

with levels in excess of 95 per cent for some members of the Chlorococcales (Morris, 1978). Where levels of viability are high, the possibility of selecting a preservation tolerant sub-population is minimized and also the time required to re-grow a preserved culture to a suitable density for distribution is restricted. At the CCAP, and some of the other major collections, levels of viability in excess of 50 per cent, for non-clonal cultures, are required before cultures are retained only in the cryo-preserved state. However, other collections including the ATCC do not impose arbitrary minimum levels of post-thaw viability (Nerad, personal communication). Currently at the CCAP approximately 35 per cent of the algal strains lodged in the collections are main-tained in a cryopreserved state (Table 5). At least a further 5 per cent, primarily those strains with less than 50 per cent post-thaw viability, are retained both in a cryopreserved state and by serial subculture.

Table 5. Biodiversity of algal strains maintained at CCAP.

Algal group	Division	No. strains maintained	
		Cryopreserved	Total
Cyanobacteria	Cyanophyta	152	228
Green algae	Chlorophyta	433	871
	Prasinophyta	5	118
Chromophyte algae	Bacillariophyta	8	97
	Chrysophyta	0	20
	Eustigmatophyta	15	24
	Phaeophyta	1	3
	Prymnesiophyta	1	34
	Xanthophyta	43	55
Red algae	Rhodophyta	4	79
Cryptomonads	Cryptophyta	1	62
Dinoflagellates	Pyrrhophyta	1	30
Euglenoids	Euglenophyta	30	120
Total		694	1741

Data from Tompkins *et al.* (1995) and Day (1998).

Two-step cooling

Most of the freezing protocols that have been developed utilize a two-step system with controlled/semi-controlled cooling from room temperature to an intermediate holding temperature (-30°C being commonly employed). This allows cryo-dehydration (see Benson, Chapter 6, this volume) of the cells to occur, prior to plunging into liquid nitrogen (-196°C). The frozen material is then stored in either liquid or vapour phase nitrogen in an appropriate liquid nitrogen storage system. Although some organisms can be success-fully cryopreserved and stored at higher subzero temperatures (>-70°C), viability levels rapidly fall on storage (Brown and Day, 1993). Therefore it is necessary to maintain frozen cultures at extremely low temperatures, optimally in liquid nitrogen at -196°C.

The development of most algal cryopreservation protocols has been empirical (Bodas *et al.,* 1995; Morris, 1978). These techniques have been effective in preserving a rel-atively limited taxonomic range of algae and have largely been restricted to morphologically uncomplicated or small species (Table 4). A variety of approaches have been employed to improve post-thaw viability; these may be divided into cooling protocol development and freeze-tolerance improvement. Factors that can be varied to reduce, or prevent, damage on freezing and thawing include: type, concentration and duration of ex-posure to the cryoprotectant (Morris, 1976a); cooling regime employed (Day and Fenwick, 1993); storage temperature/thermal stability of the cryostore; rate of thawing and post-thaw manipulations (Day *et ah,* 1997; Morris, 1976a). Pre- and post-preservation growthconditions of the culture can be altered to increase tolerance to freezing and aid post-thaw recovery; these include: age of culture (Morris, 1978); light intensity (Beaty and Parker, 1992); incubation temperature (Morris, 1976b); osmotic potential of the medium (Canavate and Lubian, 1995); nutrient limitation (Ben-Amotz and Gilboa, 1980) and nutritional mode (Morris *et al.,* 1977). The majority of effective protocols use late log/early station-ary phase cultures; however, the key factor is the 'vigour' of the culture. Cryopreservation of senescent, stressed or damaged cells will invariably result in lower levels of post-thaw survival compared to the same protocol being applied to a healthy 'vigorous' culture of the same algal strain. Full, step-wise descriptions of protocols are available in the literature (see Alexander *et al.,* 1980; Bodas *et al.,.* 1995; Day and DeVille, 1995).

Encapsulation dehydration

Encapsulation in alginate gel followed by dehydration in conjunction with the non-penetrating cryoprotectant sucrose has been used to preserve gametophytes of *Laminaria digitalis* (Vigneron *et al.,* 1997). These had survival levels in the range 25-75 per cent depending on age, sex and stress (Vigneron *et al.,* 1997). This approach has also been employed to preserve a range of microalgae and cyanobacteria (Hirata *et al.,* 1996), The technique was found to be suitable for six of the seven marine algae examined; how-ever, only one freshwater alga, *Chlorella pyrenoidosa,* survived (Hirata *et al.,* 1996). This species is extremely robust and should survive most standard Cryopreservation protocols. An alternative approach employed at the CCAP to preserve *Euglena gracilis* involved encapsulation in calcium alginate and Cryopreservation using a standard two-step protocol. This resulted in high levels of post-thaw viability (Fleck, 1998). The mechanism(s) of the protection afforded by encapsulation are not fully understood; however, it appears to: assist in dehydration of the cells; provide support during the freezing process; protect against freeze-fracture; and possibly provide some protection against free-radical mediated injury by functioning as an exogenous antioxidant (Fleck, 1998).

Mechanisms of cell damage associated with cryopreservation

Successful empirically developed cryopreservation protocols are effective on the basis that they reduce osmotic stress, cold shock and potential damage by intracellular and extracellular ice formation, before and during freezing, and on thawing. Improvements to existing methods and the preservation of a greater diversity of algae require a greater understanding of the mechanisms of the fundamental modes of cell damage during freez-ing and thawing.

Conventional two-step cryopreservation has not proven effective for many of the larger, more morphologically complex, or multicellular algae examined. On employing super-optimal cooling rates, which allow insufficient time for dehydration of the cell, intracellular ice may be formed. In all reported cases in algae, with the exception of *Chlorella prothecoides* (Morris *et al,* 1977), intracellular ice formation (IIP) was lethal. By manipulation of the cooling and cryoprotectant regimes it may be possible to avoid IIP, however, in some algae, e.g. *E. gracilis,* even under optimal conditions a proportion of the cells undergo IIP. The filamentous Chlorophyte *Spirogyra grevilleana* was killed by intracellular ice and gas bubbles on being cooled and frozen using a standard protocol at -10°C min^{-1} (Morris and McGrath, 1981). The filamentous diatom *Fragilaria cortonensis* was lethally injured at fast cooling rates by intracellular ice and at slow rates by freeze-induced hypertonic stress (McLellan, 1989). Furthermore, gametophytes of *Undaria pinnatifida* which were reported to be successfully cryopreserved, only survived four days post-thaw (Arbault *et al,* 1990), indicating that a significant amount of cell damage occurred during the process. In the coenocytic alga *Vaucheria sessilis,* on cooling using a conventional cooling protocol: -1°C min^{-1}Þ -35°CÞLiquid N_2 [5 per cent (w/v) DMSO], lack of cellular compartmentalization allowed the propagation of intracellular ice throughout the thallus, resulting in death of the alga (Fleck *et at.,* 1997).

In addition to the above, significant damage has been observed at the ultrastructural level with physical disruption of cellular organelles and membranes (Fleck, 1998; McLellan, 1989). Other factors causing both lethal and sublethal injuries include: pre-cooling manipulations (e.g. centrifugation), cryoprotectant toxicity and chilling damage (Fleck, 1998). These effects can most readily be detected employing vital staining, measurement of oxygen evolution capacity or gross changes in morphology e.g. flagellar loss (Fleck, 1998). Furthermore, free-radical mediated damage and fluctuations in antioxidant levels have also been implicated in freeze-induced damage in both plant and animal systems (Benson, 1990). Recent studies indicate that this may be an important factor in the apparent freeze-recalcitrance of some algae (Fleck, 1998).

Conservation measurments of marine macroalgae

Natural disasters, coastal erosion, tourism, tourist home constructions on coast beds and over exploitation of natural seaweed resources from natural seaweed beds are threat to seaweed diversity. In order to control the habitat destruction and provide suitable habitats for promoting seaweed vegetation, the following conservation strategies to be followed to conserve the seaweed diversity.

- Artificially laying down of stones and cement blocks in low lying areas to control the coastal erosion and prevent any further damages of natural seaweed beds and coastal areas. The manmade walls on the coast areas accumulate necessary nutrients from sea in due course of time and ultimately provide suitable habitats for luxuriant growth of seaweeds.
- Awareness to the fisherman and coastal villagers regarding the economical importance of seaweeds. Promote the seaweed cultivation in large scale and the seaweed cultivation boon to the coastal villagers and their livelihood.
- Commercially important seaweeds are need to be cultivated to fulfil the industrial demands rather relaying only on natural harvesting. Indigenous species cultivation to be implemented or promoted to the public.
- Natural harvesting of seaweeds has to be monitored and economically important seaweeds to be cultivated in laboratories and introduced in stable coastal areas.
- Natural harvesters have to be given awareness on leaving the lower part of the thallus to regenerate and also on the life cycle of the harvesting seaweed to avoid harvesting during sporulation period.
- Seaweed cultivation also can help mitigate the carbon di-oxide.
- A scientific networking of various research organizations working in this field such as Botanical Survey of India (BSI), Central Marine Fisheries Research Institute (CMFRI), Central Salt and Marine Chemicals Research Institute (CSMCRI), National Institute of Ocean Technology (NIOT), National Institute of Oceanography (NIO), Central Drug Research Institute (CDRI), different Universities etc. should be made for better utilization of this promising natural resources for the welfare of human being.

Conclusion

There are limitations in our current understanding of the modes of cryopreservation-induced damage and specific sites of injury in algae. Future research will invariably involve the use of techniques including flowcytometry, cryomicroscopy and electron microscopy. In parallel, studies on oxidative stress/ injury, other freeze-induced biochemical injuries and the responses of the alga's endogenous protective mechanisms (notably level and composition of antioxidants) to cryopreservation are required.

It is anticipated that elucidation of the key sites of injury will assist in the improvement of existing cryopreservation methodologies and the development of alternative approaches. Areas that present significant challenges include the cryopreservation of large/complex unicellular and multicellular algae. An additional challenge is the improvement of techniques to assess viability. In most published studies, survival has been determined on the basis of post-treatment growth, reaction to vital staining, fluorescence or loss of pigmentation. All of these approaches have shortfalls, regrowth is difficult to assess for non-unicellular algae and other techniques may significantly overestimate post-thaw viability. However, techniques including flowcytometry, measurement of oxygen evolution and response to specific stimuli, e.g. wound healing in *V. sessilis,* may form the basis of alternative rapid viability assays.

In conclusion, although large proportions of the holdings of all the major collections are nominally freeze-recalcitrant, it is probable that if resources were available the majority would be amenable to cryopreservation. The ultimate challenge is to develop approaches that are robust, reliable and result in high levels of post-thaw viability.

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