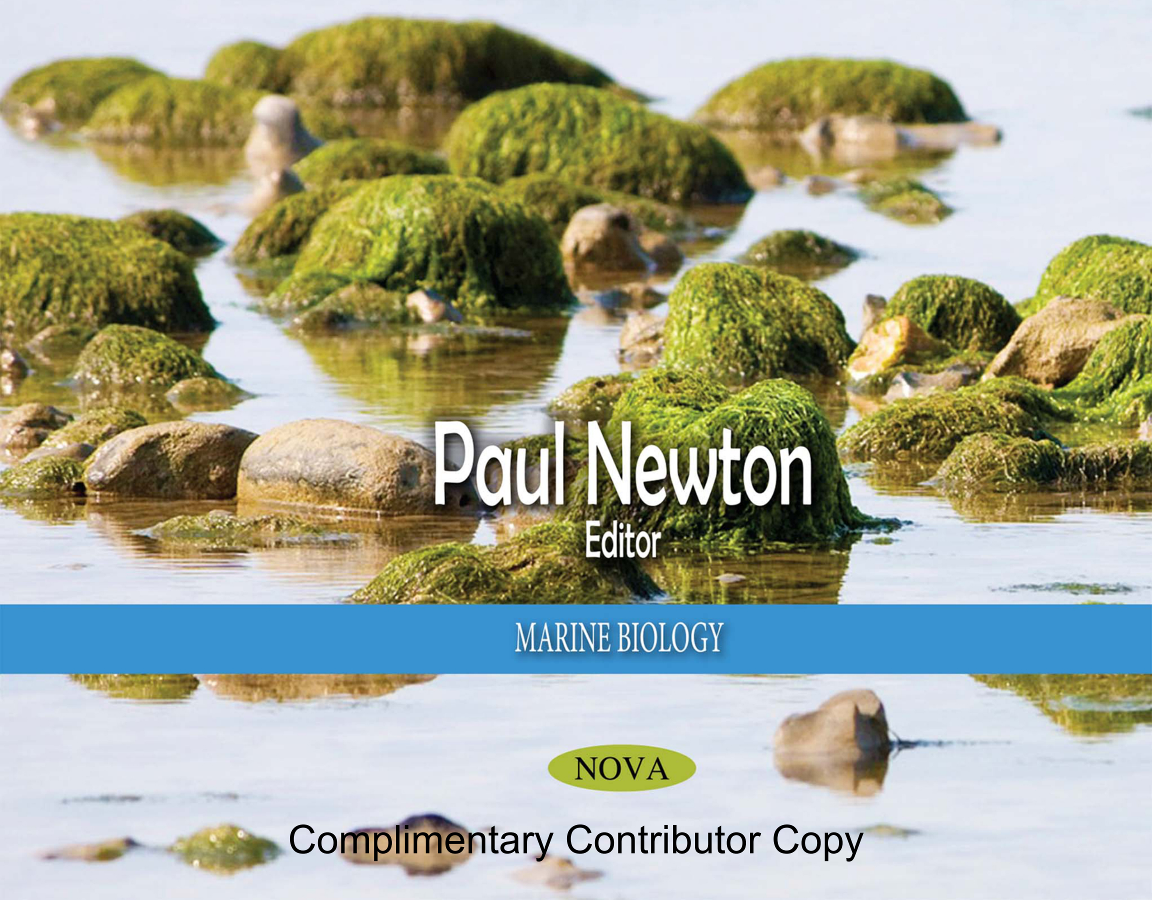
Seaweeds

Biodiversity, Environmental Chemistry and Ecological Impacts



Paul Newton
Editor

MARINE BIOLOGY

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PAUL NEWTON
EDITOR

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NOTICE TO THE READER

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PREFACE

Malaysia has the potential to be a key seaweed production player in the region if proper management and possible interventions take place. Chapter One analyzes the capacity building programs in Malaysia that aim to transform conventional seaweed cultivation techniques to modern seaweed cultivation techniques by applying a scientific approach. Chapter Two studies the marine macroalgae thriving at intertidal rocky shores on the west coast of Portugal, a transition zone where the combined influence of cold waters and warmer waters may favor the development of unique macroalgal communities. In Chapter Three, the authors discuss how problems of environmental deterioration and energy demand could be alleviated by the paradigm shift from fossil to biofuel from marine algae. The chapter elaborates on the unconventional strategies developed for the farming as well as conversion of Ulva rigida to biofuels and biochemicals. Chapter Four discusses the incorporation of macroalgae or macroalgal derived ingredients as a source of both macro-nutrients and micro-nutrients for animal feed production. The biological health benefits of the macroalgal ingredients beyond basic nutrition for the development of functional feed in the aquaculture, the ruminant and the swine sectors are also discussed together with the industrial challenges of its application. To conclude, Chapter Five provides a brief review of seaweed co-culture and its environmental impact on coastal fisheries. First, the current situation and problems facing the coastal fisheries, and the plans to overcome these issues, are discussed. Finally, the positive and negative effects of seaweed culture, role of seaweed co-culture, and the overall environmental impact are addressed.

Chapter 1 - Malaysia has the potential to be a key seaweed production player in the region if proper management and possible interventions take place. The seaweed industry in Malaysia has its own historical background as the first seaweed cultivation activity took place in 1978 in Sabah, East Malaysia. The importance of the seaweed industry has been realised when seaweed was found as an important commodity to be exported to various countries. This brings economic benefits to the country and the development of human resource in this sector. The rural people play an important role in the human resource aspect that produce and cultivate the seaweed within their respective areas. In Malaysia, Sabah is the main state for seaweed cultivation activity because of its natural abundance resources and good climate. Department of Fisheries (DOF) Sabah indicated that seaweed production in Sabah has been increased every year amounting to 32 percent from 2008-2012. This is a good opportunity for seaweed production in Sabah where it has the potential to be developed on a bigger scale. The government of Malaysia has introduced seaweed transformation initiatives through Mini Estate System (MES) and Cluster System (CS) in year 2012 in order to enhance the seaweed industry in adhering to the scientific approach and management. These capacity building programmes' objective is to transform the conventional seaweed cultivation techniques to modern seaweed cultivation techniques by applying scientific approach. In terms of community livelihood activity, the MES and CS systems provide the seaweed cultivators with knowledge and skills in order to transform their conventional seaweed cultivation techniques to new technological approach. This MES and CS systems are still new in terms of practical implications. Therefore, a study to identify the effectiveness of these programmes is needed. Thus, this study is only focused on the development of both programmes by using available secondary data sources and most importantly through the documentation of these programmes. It will benefit the future researchers that are interested in this seaweed studies in Malaysia as well as in other developing countries.

Chapter 2 - This work studied the marine macroalgae thriving at intertidal rocky shores on the west coast of Portugal (Peniche, Central Portugal). Four seashores (Baleal-Norte, Gamboa, Portinho da Areia do Norte and Consolação) located near the borderline between two adjacent types of the Portuguese coast were surveyed, the first 3 inside the northern exposed coast (type A5) and the last shore from the moderately exposed coast at the southwest coast of continental Portugal (type A6). The aim of the study was to contribute to improve knowledge on the marine macroalgal flora of this transition zone, where the combined influence of cold waters (from North Atlantic Ocean) and warmer waters (from subtropical Atlantic Ocean and the Mediterranean Sea) may favour the development of unique macroalgal communities (different arrangement of species). The collected material was housed at herbaria and photographed (records inserted in MACOI – Portuguese Seaweeds Website), and data were compared with historical information, aiming to update the local taxonomic list of marine macroalgae, and to infer about the species evolution along a 50 years' period. Data were also used to assess the ecological quality status of the four sampled sites by applying the methodology MarMAT (Marine Macroalgae Assessment Tool). A total of 87 macroalgal species were identified in the four localities, from which 16% have been not registered 50 years ago in local surveys. Meanwhile, more *taxa* were identified after the initial campaign so no new species were recorded in the present study. Taxonomic composition showed similarities for all sites but sufficient differences were still present and Consolação (A6 typology) appeared separate from the northern shores in the MDS analysis. From the application of MarMAT to present registers and old data, resulted a similar ecological quality status. Although all sites assessed in this study resulted on High quality, MarMAT reported equally high Ecological Quality Ratio for old and some recent data, indicating that sites preserved their excellent quality along the last decades.

Chapter 3 - Problems of environmental deterioration and energy demand could be alleviated by the paradigm shift from fossil to biofuels. Innovative strategies, such as the use of microwave irradiation, sonochemical treatment and solar irradiation were recently developed for the exploitation of biomass for biofuel production. The concept of biomass itself can be understood in an unconventional sense. Apart from terrestrial plant resources, nowadays, seaweed, industrial emissions such as CO_2, and organic remains such as glycogen are being explored as new feedstock for biofuels/chemicals production. The authors' research group in Israel is working on converting biomass (terrestrial and marine) to biofuels (bioethanol) and biochemicals (levulinic acid, furfural, formic acid) using microwave, sonochemical, and hydrothermal methods. Among several types of biomass, marine algae are a promising choice due to several advantages. Marine algae form an abundant and rich source of biomass. Bioethanol production process based on marine algae could be sustainable. A mild sonication-assisted simultaneous saccharification and fermentation (SSF) process for the conversion of *Ulva rigida* to bioethanol in a single step is developed in the current study. Bioethanol is a potential biofuel owing to the similarity of its energy density value (23 MJ/L) to that of gasoline (35 MJ/L). Bioethanol could also be a feedstock for the production of C_2 hydrocarbons. Any progress in the direction of development of a marine-algae-based bioethanol process would open up a new avenue towards sustainable biorefinery. *Ulva rigida*, comprising 37 wt% carbohydrate was used as a feedstock for the SSF process. Initially, saccharification process of *Ulva rigida* (with amylases and cellulases) was carried out under mild sonication conditions (40 kHz, 37°C); 3.1 times higher glucose yield was obtained using sonication of *Ulva rigi*da relative to conventional incubation. The hydrolysate was found to contain glucose exclusively. Subsequently, the SSF process for converting the algae (*Ulva rigida*) to bioethanol in a single step was also accelerated using sonication. The improvement was observed in the total carbohydrate content of the algae using multi-tropic aqua culture. 27-41 times higher specific growth rates were achieved using this approach. Under specific optimal conditions of growth, a starch amount as high as 32 wt% was accumulated. The high-carbohydrate algae were subjected to the sonication- based SSF process. Under optimal process conditions, an ethanol yield as high as 16 wt% was achieved. A unique solar-energy-based continuous flow process for the direct conversion of *Ulva rigida* to bioethanol is outlined. The conversion of macroalgae to the strategically significant chemical, levulinic acid is discussed. In an acid-catalysed hydrothermal process, 12.8 wt% levulinic acid was produced from *Ulva rigida*. The authors therefore elaborate in this chapter on the unconventional strategies developed for the farming as well as conversion of *Ulva rigida* to biofuels and biochemicals.

Chapter 4 - Plant and animal derived products are the main ingredients currently used by the feed industry to produce concentrate feed. There is a need of novel feed ingredients to meet the demand of high quality products by the aquaculture, ruminant and swine production systems, together with the challenge of implementing new sustainable and environmentally friendly processes and ingredients demanded by the modern society. Macroalgae are a large and diverse group of marine organisms that are able to produce a wide range of compounds with unique biological properties. This chapter discusses the incorporation of macroalgae or macroalgal derived ingredients as a source of both macro-nutrients (i.e., proteins, polysaccharides and fatty acids) and micro-nutrients (i.e., minerals and pigments) for animal feed production. The biological health benefits of the macroalgal ingredients beyond basic nutrition for the development of functional feed in the aquaculture, the ruminant and the swine sectors are also discussed together with the industrial challenges of its application.

Chapter 5 - This chapter provides a brief review of seaweed co-culture and its environmental impact on coastal fisheries. First, the current situation and problems facing the coastal fisheries, and the plans to overcome these issues, are discussed. Finally, the positive and negative effects of seaweed culture, role of seaweed co-culture, and the overall environmental impact are addressed. Although worldwide mariculture production has increased steadily, productivity has tended to decline due to continued aquaculture activity in confined areas and natural disasters, such as, typhoons and outbreaks of red tide. In particular, the nutrient loading from unconsumed feed waste results in the deterioration of the water quality and outbreaks of diseases. To overcome these problems, integrated multi-trophic aquaculture has been suggested. In the integrated culture of seaweed and fish, seaweed plays an important role as both a CO_2 sink and biofilter, greatly reducing the environmental impacts on coastal fisheries. The addition of the integrated culture of lugworms to this system could also have further benefits because of its potential for waste recycling and value as bait, which is expected to contribute towards a more sustainable and productive form of aquaculture. Furthermore, the use of cultured seaweed and their waste has been expanded based on the development of useful microbes, reducing their environmental impact.

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Chapter 1

SUSTAINABLE PRODUCTION OF SEAWEED IN MALAYSIA: A REVIEW OF POLICIES AND FUTURE PROSPECTS

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ABSTRACT

Malaysia has the potential to be a key seaweed production player in the region if proper management and possible interventions take place. The seaweed industry in Malaysia has its own historical background as the first seaweed cultivation activity took place in 1978 in Sabah, East Malaysia. The importance of the seaweed industry has been realised when seaweed was found as an important commodity to be exported to various countries. This brings economic benefits to the country and the development of human resource in this sector. The rural people play an important role in the human resource aspect that produce and cultivate the seaweed within their respective areas. In Malaysia, Sabah is the main state for seaweed cultivation activity because of its natural abundance resources and good climate. Department of Fisheries (DOF) Sabah indicated that seaweed production in Sabah has been increased every year amounting to 32 percent from 2008-2012. This is a good opportunity for

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seaweed production in Sabah where it has the potential to be developed on a bigger scale. The government of Malaysia has introduced seaweed transformation initiatives through Mini Estate System (MES) and Cluster System (CS) in year 2012 in order to enhance the seaweed industry in adhering to the scientific approach and management. These capacity building programmes' objective is to transform the conventional seaweed cultivation techniques to modern seaweed cultivation techniques by applying scientific approach. In terms of community livelihood activity, the MES and CS systems provide the seaweed cultivators with knowledge and skills in order to transform their conventional seaweed cultivation techniques to new technological approach. This MES and CS systems are still new in terms of practical implications. Therefore, a study to identify the effectiveness of these programmes is needed. Thus, this study is only focused on the development of both programmes by using available secondary data sources and most importantly through the documentation of these programmes. It will benefit the future researchers that are interested in this seaweed studies in Malaysia as well as in other developing countries.

Keywords: seaweed industry, government-led programmes, development, Sabah

1. INTRODUCTION

It is evident that seaweed industry has brought significant economic revenues to the country as well as improves the standard of living of the coastal communities, for instance, the case of Zanzibar (Msuya, 2006). Seaweed has been considered as important commodity which brings economic benefits to the developing countries that produce seaweed on a large scale such as Indonesia, Malaysia and Philippines. In Malaysia, seaweed industry has been paid attention by the government with intention that it could be able to increase the country revenue through exports. Despite focus on economic benefit alone, seaweed cultivation also has potential to provide side incomes to the fishers those reside in coastal areas of Sabah, Malaysia (Hussin, Yasir, Kunjuraman & Hossin, 2015). Realising on the potential of seaweed industry, the government of Malaysia has been introduced with some possible interventions to scaling up the seaweed industry to ensure the benefits to be shared by all stakeholders. Sabah is the only state in Malaysia that is commercially producing seaweed with focus in four districts on large scale such as Semporna, Lahad Datu, Kudat and Kunak. The coral triangle are of

Philippines, Indonesia and Brunei are the best locations and most appropriate for seaweed farming. With this speciality, seaweed cultivation activities are mainly being focused in Sabah and few small scale in west Malaysia.

However, the seaweed industry in Malaysia needs a transformation in terms of scientific management approach in order to achieve the country's aim. The transformation we meant was the conventional cultivation approach to scientific cultivation approach which considered reliable and efficient for this new era. Since 1970s when seaweed was introduced in Sabah, the conventional approach played a role in its growth and being practised by coastal communities in Sabah. Since then, there were no any initiatives and good methods introduced by any parties in Malaysia. Therefore, this is the right time to consider a new scientific approach in seaweed cultivation activities. There was an argument claimed by Yasir and Ali@Ally (2012) that conventional seaweed cultivation activities brought less productivity and low quality as well as over-reliant towards labour intensive based industry. Thus, the authors urged that new scientific approach in seaweed cultivation is vital in order to boost the growth and production of seaweed which could benefit the country as well as local community. Therefore, several questions arise. First, what is the new scientific approach which could be considered good for seaweed cultivation compared to the conventional methods? Who are the parties involved in developing new scientific approach? Is this new scientific approach fix into people or local community visions despite in fully focus on economic benefits? Is this scientific approach will be proven to increase the seaweed production compared to previous experience? What are the implications of introducing new scientific approach in seaweed cultivation? In the light of the above, the paper proposes that future researchers who are interested in seaweed and development studies would consider the insights of this paper as the main reference for them to carry out the related studies of seaweed in near future.

1.1. Seaweed Cultivation in Malaysia: An Overview

Seaweed cultivation was introduced in Malaysia since year 1978 and cultivated in the island of Semporna called *Karindingan* Island (Yasir & Ali@Ally, 2012). It was a first cultivation farm in Sabah and has been spread to the other island of Semporna as well as few states in west Malaysia. For seaweed cultivation, the East Coast Sabah waters have been identified as an Aquaculture Seaweed Industry Zone due to its resources. What is seaweed?

Seaweed refers to a kind of floating vegetables on top of the sea and cultivated mainly by the coastal communities especially local ethnic groups called 'Bajau'. In native language, seaweed has been called as '*sayur hijau'* or green vegetables. Mohamad, Ahmad, Noh & Saari, (2013) described seaweed as macro-algae which is growing near to the sea and has been classified into three major groups based on its pigmentation of brown (*Phaeophyceae*), red (*Rhodophycea*) or green (*Chloropphyceae*). In year 1989, seaweed species like *Kappaphycus alvarezii* has been commercialised in Sabah after Philippines (1973) and Indonesia (1986). As mentioned earlier, seaweed cultivation activities have been concentrated in four places of Sabah such as Semporna, Kudat, Kunak and Lahad Datu. This is because such areas are suitable for seaweed farm and climate as well as sufficient manpower for cultivation activities. In Asian Pacific region especially in Sabah, seaweed species such as *Euchema Cottonii* and *Kappayachus Alvarezii* are farming as a natural flora. These types of seaweeds grow along the Seaside of Sulu until Celebes Sea, which is located between of Semporna until Zamboanga Island in Philippines (Yasir, 2012). Yasir & Ali@Ally, (2012) stated that Malaysia has became a significant contributor in producing red seaweed species namely *Euchema Cottonii* and *Kappayachus Alvarezii* amounting to 20,000 metric ton of dried seaweed. The revenue are MYR60 million in year 2010. In addition, through this activity, the local community who resides in coastal areas of Sabah enjoyed the economic benefits and 9,000 hectares of seaweed farm is allocated for farming. Other than that, seaweed cultivation activities are mostly being carried out in Kerindingan Island with 15,000 hectares, Bum Bum Island with 3,500 hectares, Sebangkat Island with 7,000 hectares, Sibuan Island with 5,000 hectares and 10,000 hectares in Omadal Island. Due to these characteristics, Malaysia has the potential to be a major seaweed manufacturer and exporter in the region in terms of infrastructure development, manpower, product quality, transfer of technology, industrial support and marketing issues (Kaur & Ang, 2009).

2. The Interventions of Malaysian Government Agencies in Seaweed Production

Realising the seaweed industry has a potential to boost country's economy, the Malaysian government has proactively introduced a few of intervention programmes to enhance the country's seaweed production at par with other producing countries. In Malaysia, seaweed has been considered as one of the new sources of aquaculture commodity since 2006. A government agency called Ministry of Agriculture and Agro-Based Industry of Malaysia have been given the responsibility to deal with seaweed related issues. Seaweed was introduced as important commodity in the Malaysian National Agro-Food Policy (2011-2020) (NAP4) and assumed it could be gain economic benefits to the country. In addition, under the above-mentioned policy, seaweed was identified as one of the high-value commodities under the program of Entry Point Project 3 or EPP3 (Venturing into Commercial Scale Seaweed Farming in Sabah) which was linked with the themes of 'Capitalizing on Malaysia's Competitive Advantage' (Safari, 2015). To ensure the future sustainability of the seaweed industry in Malaysia, the government has introduced various action plans such as establishing Malaysian Seaweed Industrial Development Committee, Strategic Reform Initiatives for establishing quality and standard protocol as well as enhancing research and development activities. Such government-led interventions indicate that seaweed has a big potential to be promoted nationwide as well as considered an important commodity for export purposes. Since seaweed has a potential to boost the country's economic value, the government has realised that without the partnership from the private sectors, the mission could not be achieved. Under the current policy, the government has initiated the partnership from the private sectors in order to increase the productivity of the seaweed industry. Seaweed industrial zone namely Semporna, Kudat, Kunak and Lahad Datu have been targeted to improve the current productivity of seaweed yields and enhance the coordination of quality production of seaweed. For this reason, the private companies have been given the responsible for this matter. On the other hand, these private companies also responsible to monitor and coordinate the seaweed cultivation, improve the quality of seaweeds, manpower arrangement, marketing of seaweeds and the processing of seaweeds into the high-value commodity to be exported. These stakeholders' participation in the seaweed industry in Malaysia could be beneficial for the parties who involved directly as well as the local community who produces or cultivates the seaweeds. In addition, the quality of seaweeds always be an important issue to be tackled by the government. Thus, Research and Development (R&D) is a most important element that should be included in the seaweed related interventions in order to increase the productivity of the seaweeds and safe for consumption. A few public Universities also included to undertake the research on related projects of seaweed cultivation and the suitable methods for cultivation activities. Under the EPP's project, the public Universities such as University Malaysia Sabah (UMS), University Kebangsaan Malaysia (UKM), and University Sains Malaysia (USM) are in the midst of commercialising eight seaweed products which developed based on R&D initiative. In fact, Partnership with government and research institutions has been carried out to develop a Standard Operating Procedure (SOP) for the seaweed cultivation practises (Safari, 2015). Partnership with the multiple organisations are welcomed in seaweed industry and to ensure the sustainability of the industry in the long run. Safari (2015), shared that at present the seaweed production has achieved one metric ton per day and its mission to produce 10 metric tons per day by 2020. Moreover, in order to equip such big amount of seaweed production, Sabah State Government has planned to gazette another 3,000 ha of land for seaweed production. The government mission seems ambitious but with the systematic approach played by the relevant parties are important to achieve this mission.

Number	Responsible agency	Type of agency
1	University Malaysia Sabah (UMS)	Government
2	University Sains Malaysia (USM)	Government
3	University Kebangsaan Malaysia (UKM)	Government
4	University Malaya (UM)	Government
5	Department of Fisheries (DOF)	Government
6	Avanova Group Sdn Bhd	Private
7	Perdana Seaweed Farm	Private
8	*Pertubuhan Peladang Kawasan Semporna*	Semi-government
9	SALS Agriculture	Private
10	NIZH Goodwill Sdn Bhd	Private
11	VC United Sdn Bhd	Private
12	Madesjaya	Private
13	Sebangkat Reef Eco-Plant	Private
14	Ko-Nelayan Negeri Sabah	Semi-government
15	*Kluster Look Butun*	Semi-government
16	*Kluster Gellam-Gellam*	Semi-government
17	*Kluster Koperasi Bajau Laut*	Semi-government
18	*Kluster Kuala Merotai*	Semi-government
19	Permata Sekitar Sdn Bhd	Private
20	Department of Fisheries (DOF) Sabah	Government

Source: Pemandu, 2016.

Figure 1. Partners of seaweed in Malaysia.

2.1. Mini Estate System (MES) and Cluster System (CS) as a New Approach in Seaweed Cultivation in Sabah, East Malaysia

Realising the potential of seaweed as one of the important commodity which could generate economy of the country, the Malaysian government has initiated and introduced new transformation programme called as Seaweed Mini Estate Development. Under the Economic Transformation Programme (ETP), the Malaysian government has identified seaweed as one of the National Key Economic Areas (NKEA). It was placed in one of the 16 Entry Point Project (EPP). Such initiatives have been acknowledged that seaweed was the most important commodity exported by the country. A total of 46 million was allocated to boost the seaweed industry in Malaysia by the government in 2011 (Utusan Borneo, 2011). The seaweed industry in Malaysia especially in Sabah had gained the transformation status (EPP3) when Mini Estate System (MES) has been introduced with the aim to transform the conventional seaweed cultivation to a new scientific approach. The conventional seaweed cultivation refers to those old practices such as tie seaweed with nylon string, and over exposure of seaweed drying under sunlight made the seaweed not healthy and less productive. Such conventional methods of seaweed cultivation need a transformation with the new scientific knowledge like MES. In order to manage the MES programme systematically, University Malaysia Sabah (UMS), a local public university in Sabah has been given the responsibility. Since 2010, the MES programme managed by the UMS and Dr Suhaimi Md. Yasir was the director and leader of this project. The two phases of MES started in 2010 and run concurrently by the UMS. The first phase (2011-2010) focused on the initial development of MES infrastructure as well as appointment of leading companies who have the stake in seaweed cultivation. On the other hand, identification of seaweed cultivation farms also being focused in this phase. Semporna district was identified as suitable place for seaweed production. The second phase (2013-2020) focused on the product development based on seaweeds and marketing issues. It was aimed that MES programme could increase the productivity of seaweed and could produce 150, 000 metric tons of high quality processed seaweeds worth to MYR1.45 billion by the year 2020 (New Strait Times, 2013).

Fully funded by the government, MES using the approach of 'community-based, commercial approach'. MES is a fine concept which is implemented in the seaweed cultivation programme in Semporna, Sabah. There are a few characteristics and definitions of MES. A manual book entitled '*Sistem Mini*

Estet Industri Rumpai Laut Negara' was published by the Department of Fisheries Malaysia clearly mentioned about the background of MES and its significance to the seaweed industry in Malaysia. According to the manual book, MES refers to a better and new approach to the seaweed cultivation. There have been a few important factors considered in the early process of seaweed cultivation activity, namely (1) Holistic management transformation-'poor-man industry to lucrative industry', (2) Science and Technology based mechanism, (3) Reduce labour intensive production, (4) Friendly and sustainable environmental management, (5) seaweed as a new commodity, (6) 'community based, commercial approach', and (7) High quality yields and seaweed related products. There are elements in the MES that could provide the local community and industry manufacturers with the new knowledge and skills in order to embark on the new seaweed cultivation production in Malaysia.

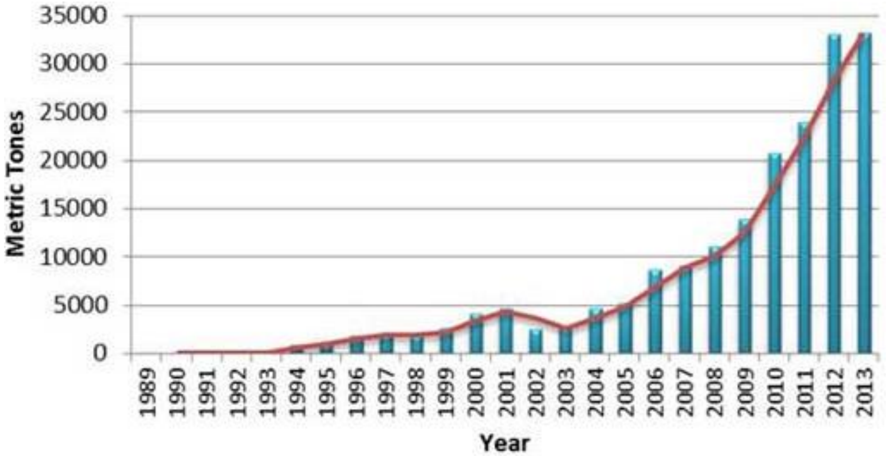
Through the MES, an important part of the seaweed cultivation process is the availability of platform facilities. The platform facilities and accommodation for the seaweed cultivators would enable them to be involved seriously. Previously, a high level of poverty among the fishermen in both islands (Selakan and Bum Bum Islands) has led and forced them to carry out seaweed cultivation activities in a conventional way. Hence, the new technological-based approach have been introduced to assist these people to adapt themselves with the new process of seaweed cultivation. It is hoping for them to enhance their livelihoods. In MES, other facilities that have been introduced including: (1) holistic transformation (an enhancement of physically socio-economic image), (2) management system (previously 80% work on the sea and 20% on seaweed platform, but now 80% work on seaweed platform and 20% on the sea), (3) an integrative management complex, (4) estate management, (5) block management, and (6) a model of entrepreneur development (Yasir, 2012). As a result, the MES has transformed the conventional seaweed cultivation to a new and modern seaweed cultivation management.

According to Yasir (2012), the MES has its own speciality where it had many improvements over the conventional seaweed cultivation methods. For instance, the MES eliminates the use of nylon string to tie seaweeds which has been practised for decades and replaced with the UMS designed eco-friendly Tie-Tie, which is seaweed-based rope. In addition, the MES also introduced organic seaweed fertilizers for high yields and seedling pin tables or commonly known as Casino Tables as well as anchor for systematic and hygiene seaweed cultivation management.

The CS was implemented after the MES. It is considered as a branch of MES because it also focused on the production of a commercial basis rather than the domestic used. However, CS are managed by the Sabah Fisheries Department. Under the CS programme, the community that already engaged in seaweed cultivation activities are considered as participant. The areas selected for cultivation are Bum-Bum Island, Silungan Island and Merotai Island located in the district of Semporna, Sabah, Malaysia.

3. Seaweed Socio-Economic Benefits to Malaysia's Development

In Malaysia, the seaweed production has taken place in Semporna, Sabah in year 1978 and continuously growing until to date. In the initial phases of seaweed cultivation, the seaweed primarily used for coastal communities' daily meals. For coastal communities of Semporna, seaweed cultivation has been the secondary livelihood activity after the fishing activity. Since then, the seaweed cultivation became one of the significant economic activity for the coastal communities in Sabah. In terms of economic benefits, the production of seaweed has been growing gradually after 1989. The seaweed cultivation then started to grow with big scales and spread to the other potential areas of Sabah. According to the Sabah Annual Fisheries Statistic in 2013 showed that the total production of dried seaweed recorded were gradually increased from 1989 to 2001. This is an initial phase of production where it always needs some period of time to increase the efficiency of seaweed cultivation production since year 2001. In year 2002 and 2003, there were a drastic decline in seaweed production. This situation has raised attention for relevant parties to take the necessary actions. After the declination of seaweed production in year 2002 and 2003, the production of seaweed has steadily increased since 2004 until 2013 (refer Figure 2). In terms of value, in 2013, the seaweed production from Sabah amounted to 28 per cent by volume (33, 210 mt) and 3 per cent (MYR198.93 million). This value is based on the total marine aquaculture production. In fact, the seaweed production from Sabah has been recorded to be increased slightly by volume in 2013 to 110.0 metric tons compared to 2012 (Safari, 2015). This is a good revenue where the seaweed production from Sabah has high potential to be developed if serious proactive measures and interventions are taken to enhance this industry run systematically.



Source: Safari, 2015.

Figure 2. Total production of seaweeds in Malaysia (1989-2013).

Figure 3 shows the statistics of Malaysian exports and imports of dried seaweeds from 2009 to 2012. The total amount of Malaysian dried seaweeds exported in the past four years were recorded a slight fluctuation with the volume increasing from 236.94 metric tons in 2009 to 656.63 metric tons in 2010. This shows a steady increasing of quantity of seaweeds exported to other countries. Table 2 also depicts that in 2011, there was a further increment of exported seaweeds amounting to 1,320.70 metric tons with revenue of 3,101,858 US dollars. However, in 2012 the amount of exported seaweeds has dropped to 502.45 metric tons. Obviously, this trend has reflected the value of seaweeds exportation showed in the parallel trend. Table 2 also indicates the statistics of dried seaweeds imported from 2009 to 2012. The amount of dried seaweeds imported have been decreased from 1,058.8 metric tons in 2009 to 668.9 metric tons in 2012. However, the value of imports where it fluctuated with a comparable average of 25 per cent from US$ 5.4 million in 2009 increasing to US$ 6.0 million in 2010 and US$ 7.9 million in 2011 (Safari, 2015). This trend significantly reflected that seaweed industry in Malaysia is fragile in terms of export and import. This trend could be sustained if the stakeholders aware about the marketing strategy and increment of seaweed production. The usage of seaweed for commercial products has raised economic benefits to the country where an element of carrageenan extraction plays an important role for the production. Thus, carrageenan from the seaweeds is valuable and potentially sustained for the seaweed industry in near future.

Year	Export		Import	
	Volume (mt)	Value (US$)	Volume (mt)	Value (US$)
2009	236.94	1,235,767	1,058.8	5,446,238
2010	856.63	1,969,601	917.9	6,033,002
2011	1,320.70	3,101,858	582.6	7,890,801
2012	502.45	931,814	668.9	5,958,578

Source: Safari, 2015.

Figure 3. Exports and Imports of Malaysian dried seaweeds (2009-2012).

4. The Potential Usage of Seaweed in Business Ventures or Commercialisation

At present, the world demand for seaweed production especially the carrageenan. The demand was started during the Second World War where it could be the substitute for animal fat, the most popular colloid for food processing. Generally, the carrageenan was extracted from red seaweeds. In addition, the usage of seaweed specifically the carrageenan was applied in multiple commercial products such as in the food processing, pharmaceutical, animal feed, fertilizers and cosmetic industries (Phang, 2006; Valderrama, Cai, Hishamunda & Ridler, 2013). There are two types of seaweeds in Sabah such as *Euchema Cottonii* and *Kappayachus Alvarezii* were the major sources of carrageenan. Currently, there are three types of carrageenan extraction available for production of materials such as Highly-Refined Carrageenan, Refined Carrageenan and Semi-Refined Carrageenan (Safari, 2015). Such categorisation of carrageenan is widely used by manufacturers to produce products that have commercial value (see Figure 4). In order to produce semi-refined carrageenan, there are two major factories located in Sabah since year 2000 such as Tacara in Morotai, Tawau and Omi-Gel in *Jalan Kemiri* (Kemiri Road), Semporna (Phang, Yeong, Lim, Nor & Gan, 2010).

For the past decades, coastal communities of Sabah used seaweed as daily meals whether as raw or blanched as salads (Phang, 2006). The seaweeds rich with vitamins such as A, C and E and good for health. In addition, it has been also considered as side dish for most of the coastal communities around the Semporna that cultivated seaweed as their full-time or part-time employment. The use of seaweeds as food are well-documented in the literature. There are different types of seaweeds have been used. Types of seaweeds used for food namely Rhodophytes *Gracilaria changii*, *Gracilaria tenuispitata*, *Eucheuma* species, Chlorophytes *Caulerpa lentillifera* and *Caulerpa racemose* are popular (Phang, 2006). Seaweeds are popular among the Chinese communities in Malaysia where they perceived it has traditional medicine value and healthy for consumption. Since seaweeds are largely cultivated in Sabah, the local community especially *Sabahans* are favour for seaweeds to include in their meals. Such popularity of seaweeds as food could sustain the seaweed industry in Sabah. Furthermore, it could become an alternative mechanism for people who prefer healthy lifestyles when it comes to food consumption. Seaweeds are not only famous among the local Malaysian but also among Japanese people. Restaurants and street food courts are now serving seaweeds as food and cook seaweeds in different methods. Among the popular species of seaweeds used in Japanese and Chinese restaurants are the temperate species of *Porphyra* (nori), *Undaria* (wakame), *Laminaria* (kombu) and *Nostoc* (fa' tsoi). In terms of daily food consumption, people can buy dried *Sargassum* and *Turbinaria* in most Chinese medicine shops in Malaysia (Phang, 2006).

Carrageenan products	**Alkali-treated cottonii (ATC) chips**	**Semi-refined carrageenan (SRC)**		**Refined carrageenan (RC)**	
		Non-food-grade SRC	**Food-grade SRC (PES)**	**RC (gel-press)**	**RC (alcohol)**
Major raw materials used (Panlibuton, Porse and Nadela, 2007)	Cottonii			Cottonii or *Gigartina*	Any kind of carrageenan seaweed: cottonii, *spinosum, Gigartina* or *Chondrus*
Processing method (McHugh, 2003)	Seaweed treated in hot alkaline solution of potassium hydroxide to remove water-soluble contents; dried; then:			Carrageenan first extracted into an aqueous solution; then recovered by:	
	chopped into pieces	milled	bleached, milled, and sterilized	gel-press method	alcohol-precipitation method
Main contents (McHugh, 2003)	Kappa + cellulose			Kappa	Any type of carrageenan: kappa/ iota/lambda
Main uses (McHugh, 2003; Panlibuton, Porse and Nadela, 2007; Bixler and Porse, 2011).	Further processed into SRC or RC	Pet food	Low-end product: meat (ham, pre-cooked poultry, fat replacement), dairy (cheese, chocolate milk, etc.), water-based food (water-gel, salad dressing, etc.)		High-end product: toothpaste, cold-soluble dairy products, pharmaceutical products (capsules, etc.)
Major producing regions/countries (Neish, 2008a; Bixler and Porse, 2011).	China, Indonesia and the Philippines			Europe, Americas, China and the Philippines	
Share of total carrageenan production worldwide in 2009 (%), based on the estimation in Bixler and Porse (2011)		10	41	26	23

Source: Adapted from Cai, Hishamunda & Ridler, 2013: 20.

Figure 4. Summary of products based on carrageenan extraction.

5. PROSPECT OF SEAWEED INDUSTRY AND WAY FORWARD

Carrageenan powder is the most important source for used in many commercial products. Therefore, it can boost the country's economy where more innovative products could be developed if the carrageenan production is sustained. The demand of carrageenan was estimated value of 40, 000 metric tons in 2006. It is projected to increase at a rate of 10-15 per cent per annum with an estimated value of US$3.3 billion. With regards to Malaysian development perspectives, the Malaysia's Ministry of Agriculture and Agro-Based Industry targeted for wet seaweed production is expected to grow by 19.7 per cent from 365, 000 metric tons in 2015, tripling to about 900, 000 metric tons in 2020 (Safari, 2015). With this projection, the production of carrageenan are also expected to increase in line with this target. The importance of carrageenan production can be realised through government's interventions. The present government's interventions are seriously focus on the sustainability of the seaweed industry as well as the carrageenan production. It also assumed that the export value of seaweed could be tripled by 2020, from MYR376 million in 2015 to MYR1.4 billion in 2020. In context of food and pharmaceutical industries, the uses of carrageenan are important for the development of various commercial products of related industries. In Malaysia, it is hoped that the production of carrageenan could be enough to supply and fulfil the current demands. In fact, it was estimated that the world population of 8 billion in 2030, thus the overall food consumption is definitely going to increase. In line with this fact, the total production of carrageenan is also expected to increase in near future.

CONCLUSION

Based on the review of Malaysian government policies related to seaweed industry, the study found that the Malaysian government is seriously committed in introducing new interventions in seaweed cultivation in order to enhance the seaweed industry in the country. This is reflected in the form of policy where seaweed production and commercialisation has become an important industry in Malaysia. The government has highlighted seaweed as one of the high-value commodity under the Malaysian National Agro Policy for the year 2011-2020. This is proven that Malaysian seaweed industry has high potential to be developed. It is also could bring economic benefits for the country as well as socio-economic development. Kaur and Ang (2006) believed that Malaysia has the potential to be a major seaweed manufacturer in Southeast Asia region. This vision however, could not be achieved if the proper management and systematic approach take place by the relevant stakeholders. To some extent, such interventions are already in place. But it is too ambitious to claim that the seaweed industry will grow faster and sustain in near future. This is because the seaweed industry is consider as a 'fragile industry' and unpredictable in terms of its implications. Therefore, continuous monitoring efforts from stakeholders are always needed where future proactive measures are still relevant to consider. The sustainability of the seaweed industry is rely on the people hands (in this context is the local people who cultivating seaweed) and industry manufacturers in order to stabilize the production value and the volume of seaweed production. Thus, more efforts especially R&D, prevalent technologies and scientific approach are needed to boost the seaweed industry in the future in Malaysia.

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Chapter 2

THE IDENTIFICATION OF MACROALGAE AND THE ASSESSMENT OF INTERTIDAL ROCKY SHORES' ECOLOGICAL STATUSES IN THE CENTRAL WESTERN COAST OF CONTINENTAL PORTUGAL

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ABSTRACT

This work studied the marine macroalgae thriving at intertidal rocky shores on the west coast of Portugal (Peniche, Central Portugal). Four seashores (Baleal-Norte, Gamboa, Portinho da Areia do Norte and Consolação) located near the borderline between two adjacent types of the Portuguese coast were surveyed, the first 3 inside the northern

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exposed coast (type A5) and the last shore from the moderately exposed coast at the southwest coast of continental Portugal (type A6).

The aim of the study was to contribute to improve knowledge on the marine macroalgal flora of this transition zone, where the combined influence of cold waters (from North Atlantic Ocean) and warmer waters (from subtropical Atlantic Ocean and the Mediterranean Sea) may favour the development of unique macroalgal communities (different arrangement of species). The collected material was housed at herbaria and photographed (records inserted in MACOI – Portuguese Seaweeds Website), and data were compared with historical information, aiming to update the local taxonomic list of marine macroalgae, and to infer about the species evolution along a 50 years' period. Data were also used to assess the ecological quality status of the four sampled sites by applying the methodology MarMAT (Marine Macroalgae Assessment Tool).

A total of 87 macroalgal species were identified in the four localities, from which 16% have been not registered 50 years ago in local surveys. Meanwhile, more *taxa* were identified after the initial campaign so no new species were recorded in the present study. Taxonomic composition showed similarities for all sites but sufficient differences were still present and Consolação (A6 typology) appeared separate from the northern shores in the MDS analysis. From the application of MarMAT to present registers and old data, resulted a similar ecological quality status. Although all sites assessed in this study resulted on High quality, MarMAT reported equally high Ecological Quality Ratio for old and some recent data, indicating that sites preserved their excellent quality along the last decades.

Keywords: western coast of Portugal, Rocky shores, intertidal, marine macroalgae, MarMAT, ecological quality status

INTRODUCTION

The first phycological studies of the Portuguese coast were published by Vandelli in 1788 [in 1] and Correa da Serra in 1796 [2]. In the following years, other studies were carried out by Welwitsch [3], Hauck [4], Palminha [5-8], Mesquita Rodrigues [9, 10], Póvoa dos Reis [11]. However, the most complete study on the Portuguese phycological flora was carried out by Ardré [12-14]. Actually, according to Sousa-Pinto [15], the Portuguese phycological flora did not suffer a significant change in terms of the number of species registered since the study made by Ardré in 1970 [13] in the late sixties, of the last century. Ardré [13] studied, identified and described 404 macroalgae species, from which 246 were Rhodophyta (red algae), 98 Ochrophyta (brown algae) and 60 Chlorophyta (green algae). In 2009, Araújo et al. [16] based on literature references, new records and herbarium data, obtained an updated checklist of the benthic marine algae of the northern coast of Portugal. This checklist includes 346 species, namely 200 Rhodophyta, 70 Ochrophyta, 50 Chlorophyta, and 26 Cyanobacteria. Of these, 33 species were recorded for the first time in this region and 21 were new records for the Portuguese coast. These new records were probably mainly related to the sampling effort, undertaken recently across more localities than in previous works, with a consequent increase of diversity on the habitats surveyed [16]. Recent new floristic records and taxonomic data were published meanwhile by other authors [17-24].

The Portuguese coast is located in a transition region, between the cold waters of the North Atlantic Ocean and warmer waters of subtropical Atlantic Ocean and the Mediterranean Sea, favourable to the development of a unique combination of species that compose the Portuguese macroalgal communities [16, 17]. Southwards there is an increasing number of Rhodophyta species and a simultaneous decrease in Ochrophyta, which enables the division into two groups: algae of the northern zone (between the mouth of the Minho river and the mouth of the Tagus river) and algae of the southern zone (between the mouth of the Tagus river and the Algarve) [25, 26]. Although the intertidal macroalgae in northern Portugal tend to be more similar to those of the central European coast, Brittany and South of British Isles, and the macroalgae of southern Portugal to get included several Mediterranean and African species [15], the global composition of macroalgal communities represents well this transition situation. This is evident when Feldmann [27] and Cheney [28] ratios are compared by geographical region, varying from 2.53 in Britain and Ireland to 3.71 in Moroccan coast, with a higher value registered for the Iberian coasts (3.57 to north Portugal, 4.09 to Basque Country) [16].

Located in the central region of the country, the Baleal-Norte, Gamboa, Portinho da Areia do Norte and Consolação coastal areas also have a great diversity in terms of phycological flora in their mediolittoral rocky shores. The first phycological records of the Baleal-Norte rocky shore were made in 1961 and 1970 by Ardré [12, 13]. Later, inserted in the project MACOI-Portuguese Seaweeds Website [29] (1998 to present), new *taxa* were identified and added to the previously described by Ardré. From a total of 131 species recorded, 20 were Chlorophyta, 29 Ochrophyta and 82 Rhodophyta. The first inventory of Gamboa beach was done by Ardré in 1966 [30] and later in 1970 [13] recording a total of 148 species (21 Chlorophyta, 33 Ochrophyta and 94

Rhodophyta). In Portinho da Areia do Norte and Consolação sites the first records appeared with the project MACOI-Portuguese Seaweeds Website [29], accounting 71 species for the first location (9 Chlorophyta, 18 Ochrophyta and 44 Rhodophyta) and 24 species for the second (5 Chlorophyta, 5 Ochrophyta and 14 Rhodophyta) [12, 13, 29, 30].

Taxonomic composition of macroalgae is important for the structure and functions characterizing marine coastal communities and, consequently, it has influence on the dynamics and changes occurred in coastal environments [31, 32]. Conversely, it is also known that species richness, taxonomic composition and the abundance of certain macroalgal species at intertidal rocky shore communities (proportion of opportunists' coverage in relation to total coverage) may reflect changes resulting from alterations in the water quality. These characteristics allowed the Water Framework Directive (WFD, 2000/60/EC), an European environmental legislation piece, to require the use of marine macroalgae as one of the biological quality elements (BQE) integrated in evaluation schemes developed by European Member States (MS) [31] to detect any significant ecological degradation resulting from anthropogenic activities. The implementation of the international environmental legislation's court forced to a concerted effort between the administration of various MS, regarding the development of environmental and ecological indicators [33]. From this need it was developed in Portugal the Marine Macroalgae Assessment Tool (MarMAT), a methodology created to evaluate the quality of coastal waters based on data collected from intertidal macroalgae communities [34].

Mostly, assessment methods require either a correct identification of macroalgal species present and a deep knowledge of taxonomic lists naturally characterizing the coastal zone under evaluation (i.e., type specific reference conditions) [34, 35]. These aspects are eventually more important in transition zones of the coast, such as the study area here presented, where assessment methods may experience changes in boundary values or in the reference conditions they have to use to compare with but where the availability of data is also low. Due to this, the increase on quality and quantity of information from those sites earns extreme importance for a robust assessment of ecological conditions of rocky shores. In this sense, the present chapter aims to improve the knowhow about the behaviour of intertidal macroalgal communities from a coastal transition zone, namely: (1) to register and update the intertidal phycological flora of four rocky shores from the western coast of Portugal (diversity and photographic records); (2) to analyse the macroalgal community structure, its similarity between study sites, and to compare historical and present taxonomic data; and (3) to assess the Ecological Quality Status (EQS) of coastal water bodies for present and first sampling situations, at the same time that MarMAT robustness is validated for sites located at the borderline of transition zones.

SEASHORES OF THE WESTERN COAST OF PORTUGAL (PENICHE)

Located in the North-East Atlantic (NEA) region of the European coast, the four study sites belong to typology NEA 1/26 of EU coastal waters' category [36]. In terms of national classification, the sites (Baleal-Norte, Gamboa, Portinho da Areia do Norte and Consolação) are displaced on the borderline between two adjacent coastal water types, which are type A5 (exposed coasts, includes the first three sites) and type A6 (moderately exposed coasts, includes the last shore). The classification of Portuguese categories includes also for open coastal waters the sheltered type (A7) on the south part of the country (Algarve), and types A1 to A4 representing transition waters (estuaries) and sheltered coastal waters (coastal lagoons) along all territory [37]. The coast is influenced by a semidiurnal tide, mesotidal (2-4 m amplitude) with prevailing winds from the North/Northwest in the summer months. The sea surface temperature varies between 15 °C and 17 °C and the air between 18 °C and 25 °C during the summer season [38-40].

Inserted on the central region of the west coast of Portugal, the Baleal-Norte, Gamboa, Portinho da Areia do Norte and Consolação shores have characteristics in common, including the fact that they possess mixed substrates, more or less extensive, composed by thin, gold sands and rocky outcrops (Figure 1). However, every location presents a particular physical feature of the coast, given by the arrangement and interactions between the water and these substrates or platforms. Resulting from distinctive abiotic and biotic characteristics, the side by side presence of exposed coasts (A5 coastal waters Portuguese typology: Baleal-Norte, Gamboa and Portinho da Areia do Norte) and moderately exposed ones (A6 coastal waters Portuguese typology: Consolação), promotes the emergence of many species of macroalgae and the consequent enhancement of general diversity in the area.

Figure 1. Location of the studied areas on the Portuguese coast: A) Baleal-Norte (39°22’24.21’’N - 9°20’11.36’’W); B) Gamboa (39°21’51.19’’N - 9°22’19.55’’W); C) Portinho da Areia do Norte (39°22’7.46’’N - 9°22’42.28’’W); D) Consolação (39°19’30.57’’N - 9°21’37.86’’W).

SURVEY OF THE PHYCOLOGICAL FLORA

Seaweed samples were collected between July 2012 and June 2013; maximum possible information was obtained from several visits to sites. The methodology used for harvesting macroalgae in the intertidal level was equal to all sites. The collection of specimens began before the low tide hour (information found at the Hydrographic Institute website) [41] and lasted until after that, making possible the visualization of the lower zone of the intertidal level. The macroalgal species richness was assessed through a random path in the rocks and tide pools area, from the lowest tide level to the highest level in the intertidal. All species found along this course were photographed in place, harvested and stored in labelled plastic bags. In this study was chosen a destructive method, proceeding to the collection of specimens through the uprooting in the case of larger algae and in the case of smaller algae by scraping the surface of the rocks.

The collected material was separated into two groups, the larger algae were conserved in herbarium and the small sized species were transferred into labelled vials and preserved in a solution of formaldehyde at 4% in seawater. The largest specimens were identified into the main taxonomy groups of red, brown and green algae in the laboratory, except when the accurate identification was possible in the field (Cyanobacteria were not included in this study). The screening of small sized algae was done in the laboratory under a Stereo Microscope (Lan binocular optics with digital camera, DCMC 130, 1.3M pixels) and a Light Microscope (Motic BA 310 with camera incorporated) (see Annex I). All collected species were identified and data inserted into MACOI - Portuguese Seaweeds Website [26, 29].

MACROALGAL DIVERSITY AND COMMUNITY STRUCTURE

Along the four intertidal areas were identified in total 87 species of macroalgae. Of these 87 species, 10 belonged to Chlorophyta, 20 to Ochrophyta and 57 to Rhodophyta. Carrier of the highest species richness, the Baleal-Norte beach contained 55 species, than the beaches of Consolação with 53, Portinho da Areia do Norte with 50 and Gamboa with 36 species of macroalgae (Table 1). Of the 55 species of macroalgae referring to the Baleal-Norte beach, 7 belong to Chlorophyta, 12 to Ochrophyta and 36 to Rhodophyta. Of the 53 species from the Consolação beach, 7 belong to Chlorophyta, 13 to Ochrophyta and 33 to Rhodophyta. Of the 50 species from the Portinho da Areia do Norte beach, 7 belong to Chlorophyta, 10 to Ochrophyta and 33 to Rhodophyta. And of the 36 species of Gamboa beach, 5 belonged to Chlorophyta, 14 to Ochrophyta and 17 to Rhodophyta.

In all shores, the number of species belonging to Rhodophyta was the highest, followed by Ochrophyta and Chlorophyta, which permanently had the lowest number of species. Regarding the number of Orders, it was noted that in all sites, the Bryopsidales and Ulvales had the highest number of species from Chlorophyta. On Ochrophyta the Orders that stood out on the Baleal-Norte shore were Dictyotales and Fucales, on Gamboa and Portinho da Areia do Norte were Dictyotales, Fucales and Sphacelariales, and from Consolação were Fucales, Ectocarpales and Sphacelariales. In Rhodophyta, Ceramiales was more abundant in all sites, followed by Corallinales. Apart from these two Orders, on the Portinho da Areia do Norte and Consolação also Gigartinales showed a significantly high number of species. In contrast it was found that Nemaliales, Peyssonneliales, Plocamiales and Rhodymeniales had only a single species each. Similarly, to what was verified individually for seashores, also throughout the species registered on sites was found that Bryopsidales and Ulvales Orders were the most representative of Chlorophyta with 3 and 5 species, respectively. On Ochrophyta, Dictyotales, Ectocarpales and Fucales were the most evident with 5 species each. In Rhodophyta, Ceramiales was the most expressive with 36 species, followed by Gigartinales with 7 species (Figure 2 and 3).

Table 1. List of species identified for the study sites and indication of photograph number

Species	ESG	Opport.	Study sites				RTL
			Baleal-Norte	Gamboa	Portinho da Areia do Norte	Consolação	
Phyllum Chlorophyta							
Order Bryopsidales							
Codium adhaerens (Figure 5)	II			x	X	X	1
Codium tomentosum (Figure 6)	II		x	x	X	X	1
Derbesia tenuissima (Figure 7)			x	x	X		-
Order Cladophorales							
Cladophora prolifera (Figure 8)	II	Yes	x				2
Valonia utricularis (Figure 9)					X	X	-
Order Ulvales							
Ulva clathrata (Figure 12)	II	Yes	x		X	X	3
Ulva compressa (Figure 10)	II	Yes			X	X	3
Ulva rigida (Figure 11A, C)	II	Yes	x	x	X	X	4
Ulva rigida var. *fimbriata* (Figure 11B, D)	II	Yes	x				4
Ulvaria obscura (Figure 13)	II	Yes				X	4
Phyllum Ochrophyta							
Order Dictyotales							
Dictyota dichotoma (Figure 14)	II		x	x	X	X	5

Species	ESG	Opport.	Study sites				RTL
			Baleal-Norte	Gamboa	Portinho da Areia do Norte	Consolação	
Dictyota spiralis (Figure 15)	II		X				5
Dictyopteris polypodioides (Figure 16)	II		x	x	X		6
Padina pavonica (Figure 17)			x	x	X		-
Taonia atomaria (Figure 18)			x	x	X		-
Order Ectocarpales							
*Colpomenia peregrine** (Figure 19A)	II		x	x		X	7
Colpomenia sinuosa (Figure 19B)	II				X		7
Ectocarpus fasciculatus (Figure 20)	II	Yes		x		X	8
Hincksia granulosa (Figure 21A)						x	8
Hincksia hincksiae (Figure 21B)						x	8
Order Fucales							
Bifurcaria bifurcata (Figure 22)	I		x	x		x	9
Cystoseira baccata (Figure 23)	I		x	x			10
Cystoseira tamariscifolia (Figure 24)	I		x	x	X	x	10
Fucus spiralis (Figure 25)	I					x	11
Sargassum vulgare (Figure 26)			x	x	X		-
Order Laminariales							
Laminaria ochroleuca (Figure 27)						x	12
Order Sphacelariales							
Cladostephus spongiosus (Figure 28)	I		x	x	X	x	13
Halopteris scoparia (Figure 29)	II		x	x	X	x	14
Sphacelaria rigidula (Figure 30)	II	Yes		x		x	8
Order Tilopteridales							

Table 1. (Continued)

Species	ESG	Opport.	Study sites				RTL
			Baleal-Norte	Gamboa	Portinho da Areia do Norte	Consolação	
Saccorhiza polyschides (Figure 31)					X	x	15
Phylum Rhodophyta							
Order Bonnemaisoniales							
*Asparagopsis armata** (Figure 32B-D)	II		x	x	X	x	16
Falkenbergia rufolanosa (Figure 32A)	II		x			x	16
Order Ceramiales							
Acrosorium ciliolatum (Figure 33)	II		x	x	X		17
Aglaothamnion pseudobyssoides (Figure 34A, B)	II	Yes				x	18
Aglaothamnion sepositum (Figure 34C, D)	II	Yes	x			x	18
Anotrichium furcellatum (Figure 35)			x				-
Antithamnion densum (Figure 36)	II	Yes			X		18
Apoglossum ruscifolium (Figure 37)	II				X		19
Boergeseniella fruticulosa (Figure 38A, C)	II	Yes	x	x	X	x	18
Boergeseniella thuyoides (Figure 38B, D)	II	Yes	x			x	18
Bornetia secundiflora (Figure 39)	II		x		X	x	20
Callithamnion tetragonum (Figure 40)	II	Yes				x	18
Ceramium ciliatum (Figure 41)	II	Yes	x	x	X	x	18
Ceramium echionotum (Figure 42)	II	Yes			X		18
Gayliella flacida (Figure 43)	II	Yes				x	18
Ceramium pallidum (Figure 44)	II	Yes		x		x	18

Species	ESG	Opport.	Study sites				RTL
			Baleal-Norte	Gamboa	Portinho da Areia do Norte	Consolação	
Ceramium tenuicorne (Figure 45)	II	Yes	X				18
Ceramium virgatum (Figure 46)	II	Yes			X	x	18
Chondria coerulescens (Figure 47)	II		x		X	x	21
Compsothamnion thuyoides (Figure 48)	II	Yes	x			x	18
Cryptopleura ramose (Figure 49)	II					x	17
Crouania attenuata (Figure 50)					X		-
Erythroglossum laciniatum (Figure 51)			x	x			-
Halurus equisetifolius (Figure 52)	II		x				22
Herposiphonia tenella (Figure 53)	II	Yes			X		18
Hypoglossum hypoglossoides (Figure 54)	II		x		X	x	19
Laurencia pyramidalis (Figure 55)	II		x	x	X		23
Leptosiphonia schousboei (Figure 56)	II	Yes	x				18
Lophosiphonia reptabunda (Figure 57)	II	Yes		x	X		18
Nitophyllum punctatum (Figure 58)	II		x			x	24
Ophidocladus simpliciusculus (Figure 59)	II	Yes	x				18
Osmundea pinnatifida (Figure 60)	II					x	23
Pleonosporium borreri (Figure 61)	II	Yes				x	18
Polysiphonia denudata (Figure 62)	II	Yes	x	x	X	x	18
Polysiphonia fucoides (Figure 63)	II	Yes	x			x	18
Xiphosiphonia ardreana (Figure 64)	II	Yes	x				18

Table 1. (Continued)

Species	ESG	Opport.	Study sites				RTL
			Baleal-Norte	Gamboa	Portinho da Areia do Norte	Consolação	
Pterosiphonia complanata (Figure 65)	II		x	x	X	x	25
Xiphosiphonia pennata (Figure 66)	II	Yes	x		X		18
Order Corallinales							
Ellisolandia elongata (Figure 67A)	I		x		X		26
Corallina officinalis (Figure 67B)	I			x	X	x	26
Jania rubens (Figure 68)	I		x	x	X	x	26
Lithophyllum incrustans (Figure 69A)	I		x	x	X	x	27
Lithophyllum byssoides (Figure 69B)	I		x		X	x	27
Mesophyllum lichenoides (Figure 70)	I				X		27
Order Gelidiales							
Gelidium corneum (Figure 71A, B)	I		x				28
Gelidium spinosum (Figure 71C, D)	I		x				28
Order Gigartinales							
Ahnfeltiopsis devoniensis (Figure 72)	II		x		X	x	29
Caulacanthus ustulatus (Figure 73)	II				X	x	30
Chondracanthus acicularis (Figure 74A)	II		x		X	x	31
Chondracanthus teedei (Figure 74B)	II		x		X		32
Dilsea carnosa (Figure 75)	II					x	33
Gigartina pistillata (Figure 76)	II					x	34
Sphaerococcus coronopifolius (Figure 77)	I		x	x	X		35
Order Nemaliales							
Liagora viscida (Figure 78)				x	X		-

Species	ESG	Opport.	Study sites				RTL
			Baleal-Norte	Gamboa	Portinho da Areia do Norte	Consolação	
Order Peyssonneliales							
Peyssonnelia coriacea (Figure 79)	I		X				36
Order Plocamiales							
Plocamium cartilagineum (Figure 80)	I		x	x	X	x	35
Order Rhodymeniales							
Champia parvula (Figure 81)	II				X	x	37

ESG - ecological status groups (I - perennial species; II - annual species); Opport. – opportunistic species; X - species recorded; RTL - different entries in the reduced taxa list (RTL).

The numbers indicate different entry present in the reduced list of species (RTL), since there are several species grouped in some single entry. Thus, the following entries consider more than one possible taxa in RTL (1) *Codium* spp.; (2) *Cladophora* spp.; (3) *Ulva* spp. 'tubular-type'/*Blidingia* spp.; (4) *Ulva* spp. 'sheet-type'/*Ulvaria obscura*/*Prasiola stipitata*; (5) *Dictyota* spp.; (7) *Colpomenia* spp./*Leathesia marina*; (8) filamentous Phaeophyceae; (10) *Cystoseira* spp.; (11) *Fucus* spp.; (12) *Laminaria* spp.; (14) *Halopteris filicina*/*Halopteris scoparia*; (16) *Asparagopsis armata*/*Falkenbergia rufolanosa*; (17) *Acrosorium ciliolatum*/*Callophyllis laciniata*/*Cryptopleura ramosa*; (18) other filamentous Rhodophyta; (19) *Apoglossum ruscifolium*/*Hypoglossum hypoglossoides*; (20) *Bornetia* spp./*Griffithsia* spp.; (21) *Chondria* spp.; (23) *Laurencia* spp./*Osmundea* spp.; (26) erect calcareous species; (27) encrusting calcareous species; (28) Gelidiales; (29) *Ahnfeltiopsis* spp./*Gymnogongrus* spp.; (30) *Catenella caespitosa*/*Caulacanthus ustulatus*; (33) *Dilsea carnosa*/*Schizymenia dubyi*; (35) *Plocamium cartilagineum*/*Sphaerococcus coronopifolius*; (36) *Peyssonnelia* spp.; (37) Champiaceae.

*Exotic species (non-native).

From the above mentioned distribution of taxa was possible to estimate the Cheney ratio - (R + C)/P [28] for all sites. The highest values were achieved by Portinho da Areia do Norte with 4.0, Baleal-Norte was next with 3.58, Consolação follow it with 3.08, and Gamboa was the last with 1.57. Except for the last site, all the others fall inside the ranged by other studies for the European coast [16], which varied from 2.53 to Britain and Ireland, 4.09 to the Basque Coast, 3.57 to North Coast of Portugal, and 3.71 to the Atlantic Coast of Morocco. Concerning the Feldmann ratio - R/P [27], a similar situation was observed, with the highest values registered for Portinho da Areia do Norte with 3.3, next was Baleal-Norte with 3.0, Consolação achieved 2.54, and Gamboa was 1.21. The tendency here (except for Gamboa site) was to be next the values obtained in other studies for the European coasts [16], which varied from 1.87 to Britain and Ireland, 3.31 to the Basque Coast, 2.86 to North Coast of Portugal, and 2.93 to the Atlantic Coast of Morocco.

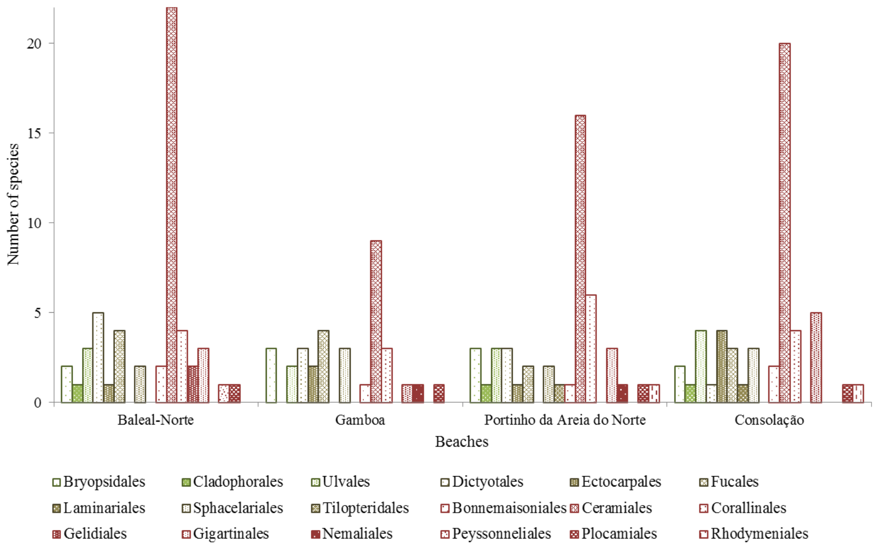

Figure 2. Total number of species per Order on each beach: Baleal-Norte; Gamboa; Portinho da Areia do Norte; and Consolação.

Despite the geographical proximity of study sites, the macroalgal communities were relatively diverse along the sampled space. This was pretty much evident when the community was analysed based on the taxonomic composition present at different sites. Macroalgae presence/absence data were analysed through a non-metric Multidimensional Scaling (n-MDS), with Bray Curtis index as similarity measure (PRIMER 6 + PERMANOVA© software) [42, 43], and produced an ordination of sites through a virtual space where larger distances meant also more differences between pairs of shores. Ordination results showed that, independently of the variation found between the different taxonomic groups, the taxonomic composition present was more similar between Gamboa, Portinho da Areia do Norte and Baleal-Norte, then in comparison to Consolação (Figure 4). The last site was not grouped together with the other three due to the slightly lower affinity of the fourth site existing on taxonomic compositions. Although the number of species registered on Gamboa site was considerably lower, 36 against more than 50 for the other sites, this was not sufficient to displace it from the northern shores' group. Consolação presented the second highest diversity but its list of species may be on the basis of the separation from the rest, with 8 species present in all other three sites except here (*Derbesia tenuissima*, *Dictyopteris polypodioides*, *Padina pavonica*, *Taonia atomaria*, *Sargassum vulgare*, *Acrosorium* *ciliolatum*, *Laurencia pyramidalis*, *Sphaerococcus coronopifolius*) and, in the opposite, 13 taxa being exclusive from this site (*Ulvaria obscura*, *Hincksia granulosa*, *H. hincksiae*, *Fucus spiralis*, *Laminaria ochroleuca*, *Aglaothamnion pseudobyssoides*, *Callithamnion tetragonum*, *Gayliella flacida*, *Cryptopleura ramosa*, *Osmundea pinnatifida*, *Pleonosporium borreri*, *Dilsea carnosa*, *Gigartina pistillata*).

From taxa absent from Consolação, 3 species (*Dictyopteris polypodioides*, *Laurencia pyramidalis*, *Sphaerococcus coronopifolius*) may be considered as Northern-cold species [16] or were not registered in southern locations during the first surveys [12, 13, 30], and from taxa exclusive form this site, eight species (*Ulvaria obscura*, *Fucus spiralis*, *Laminaria ochroleuca*, *Callithamnion tetragonum*, *Cryptopleura ramosa*, *Gigartina pistillata*, *Osmundea pinnatifida*, *Pleonosporium borreri*) are considered Southern-warm species [16] or were not registered for Portuguese northern coast in the first surveys made on the area [12, 13, 30].

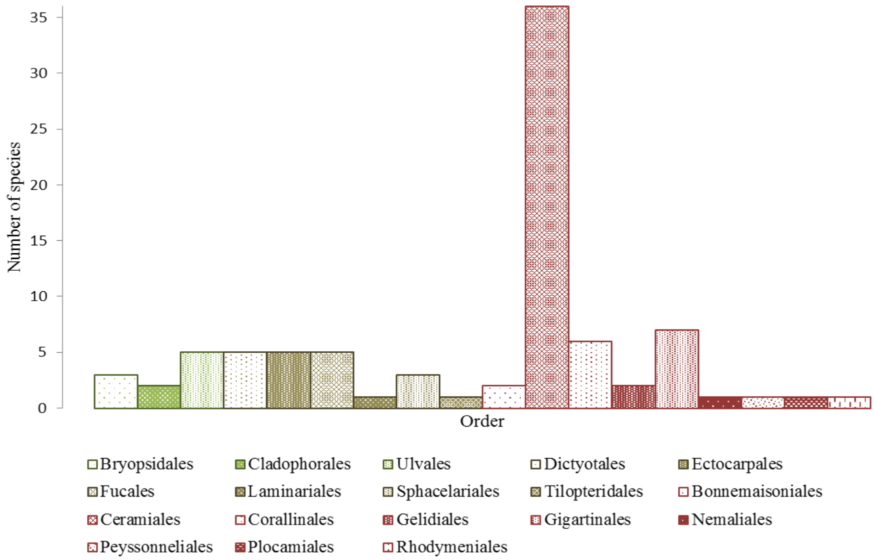

Figure 3. Total number of species per Order in the four beaches of the municipality of Peniche.

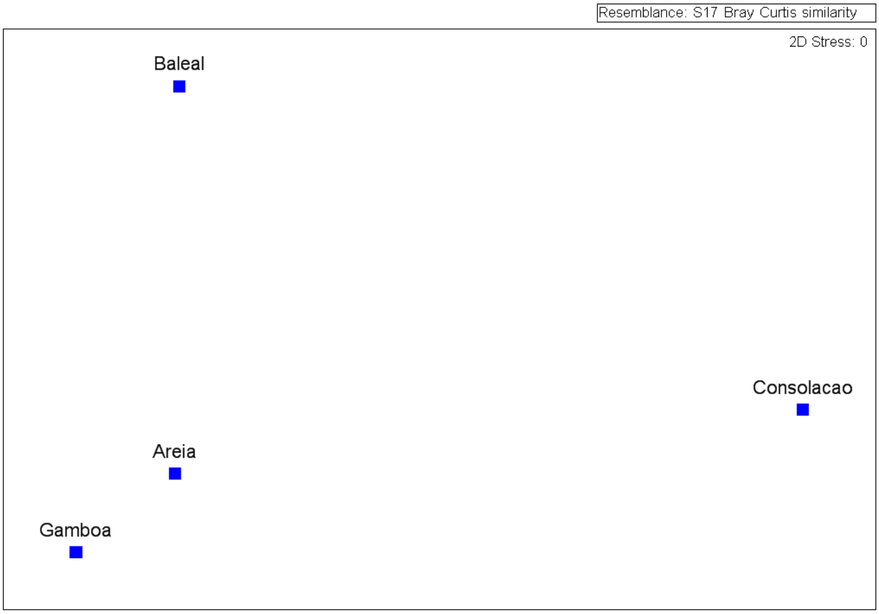

Figure 4. Plot of the four studied rocky shores. Non-metric Multidimensional Scaling (n-MDS), with Bray Curtis index as similarity measure, based on macroalgae presence/absence data.

Comparing the current data, collected in the present survey, with those historically reported in the literature it was found that 84% of the species recorded (73 species) have been already identified 50 years ago by Ardré [12, 13, 16, 30]. The species not identified at that time constitute mainly new species, described more recently, or small sized species that could be easily not seen in the field. These were 14 species (16%) distributed between Ochrophyta and Rhodophyta, respectively with 3 and 11 species. All species from Chlorophyta have been indicated before as present at the surveyed area. From those 14 species, 3 have been registered for northern and southern areas by the Ardré at that time. From the 14 species not present in historical records, 3 species (*Dictyota spiralis, Padina pavonica, Erythroglossum laciniatum*) have been observed by Ardré only at southern sites from the study area, and one species (*Aglaothamnion sepositum*) for northern areas. Additionally, from the 14 species not detected by Ardré but registered in the present survey, these have been meanwhile reported for the local flora listed by recent studies [16, 44].

ASSESSMENT OF THE ECOLOGICAL STATUS OF COASTAL WATER BODIES

The methodology developed in Portugal to assess the ecological quality of rocky shores fulfilling the WFD requirements is known as the Marine Macroalgae Assessment Tool (MarMAT). This tool includes seven different metrics: species richness, proportion of Chlorophyta, number of Rhodophyta, number of opportunists/ESG I (ratio), proportion of opportunists, shore description and coverage of opportunists [34]. The taxonomic composition data (presence/absence of species) registered on site is compared to a Reduced Taxa List (RTL), created for each different water typology based on macroalgae species present under different pressure levels (from naturally undisturbed to heavily impacted conditions). All MarMAT metrics, except the 'Proportion of Opportunists,' are calculated based on RTL structure.

More in detail, the first step is the *in situ* identification of *taxa* and its assignment (or not) into one of the entries in RTL. *Taxa* not assigned are not included in further calculations. Still in the field, is to do the shore description, filing in specific information into an appropriate classification table, and, at last, to calculate the percentage coverage of opportunistic macroalgae in relation to the total species coverage [34]. Species richness, proportion of Chlorophyta, number of Rhodophyta, proportion of opportunists and the ratio of the number of opportunists/Ecological State Group (ESG) I are calculated based on *taxa* in the RTL. The coverage of opportunists is usually estimated from 1-meter sq. photographed quadrats, having in mind the species considered as opportunists in the RTL [34]. The RTL considered in this study was developed by Gaspar et al. [35] for the A5 coastal waters Portuguese typology. The EQS is calculated by attributing scores to each of the MarMAT metrics (Table 2), according to the established in Neto et al. [34]. The metric 'Shore Description' functions as a correcting factor, aiming to reduce the influence of different rocky shores' morphology on the composition and abundance of macroalgae.

Applying the methodology MarMAT to macroalgae data it was possible to assess the Ecological Quality Status (EQS) of the four coastal rocky shores. MarMAT was applied to data collected in the end of June 2013 and using the RTL developed for the Portuguese typology A5. In Table 1 are shown species collected in this survey and the assignments into RTL. The site with the highest species richness was Baleal-Norte with 55 species, however, for the calculation methodology (after assignment to RTL) were recorded 29 *taxa*.

The following site with higher number of species registered was Consolação with 53 species and 31 RTL entries, then Portinho da Areia do Norte with 42 species and 27 entries. The Gamboa site was the one recording the lowest species richness with only 36 species and 19 RTL entries. In all monitoring events, the number of Rhodophyta species was always higher than the number of Ochrophyta and Chlorophyta species. The last registered the lowest number of species for all study sites.

Table 2. Quality class scores attributed to different metrics integrated in MarMAT and the translation of EQR values into Ecological Quality Status (EQS)

Metrics	Bad	Poor	Moderate	Good	High
Species Richness[a]	0-6	7-13	14-20	21-27	28-54
Proportion of Chlorophyta	0.32-1	0.27-0.31	0.21-0.26	0.15-0.20	0-0.14
Number of Rhodophyta	0-3	4-8	9-12	13-17	18-33
Number of opportunists/ESG I	≥1.23	1.01-1.22	0.80-1.00	0.58-0.79	<0.58
Proportion of opportunists	0.59-1	0.47-0.58	0.35-0.46	0.23-0.34	0-0.22
Coverage of opportunists (%)[a]	72-100	59-71	4-58	33-45	0-32
Shore Descriptions	-	15-18	12-14	8-11	1-7
Corresponding Score to Metrics class	0	1	2	3	4
Sum of Scores	0-7	8-14	15-21	22-28	29-36
EQR	0-0.20	0.21-0.40	0.41-0.60	0.61-0.80	0.81-1
EQS	Bad	Poor	Moderate	Good	High

[a]factor of 2, counts twice in the metrics sum of scores calculation.

In Table 3 are discriminated the results and respective scores of different MarMAT metrics, as well as the final quality assessment of studied rocky shores. For the metric shore description Baleal-Norte station presented the highest results, with 11, Portinho da Areia do Norte beach had a score of 10 and Gamboa and Consolação had 9. Regarding the coverage of opportunistic algae, Baleal-Norte showed the lowest value with 21.1%, followed by Consolação and Gamboa stations with 26.3% and 32.6%, respectively. With 45.2% the Portinho da Areia do Norte beach was the one with the highest percentage cover of opportunistic algae. The ecological quality ratios (EQR) of Baleal-Norte, Gamboa, Portinho da Areia do Norte and Consolação were 0.97, 0.81, 0.83 and 0.97, respectively. Despite the EQR differences, results rated all sites with 'High' EQS, which confirms the general elevated quality of sites. Having in mind the macroalgal species registered 50 years ago, during the first intertidal surveys, and assuming that cover of opportunistic macroalgae should have been not worst then nowadays, the calculation of the EQS for 1970 achieved also High status (EQR = 0.97) for the area. From these results, similar to the today's best, it's possible to say that quality of the study area remained in High status since then.

Although Consolação site belongs to A6 the RTL for A5 was the one used. This was due to the geographical proximity of all sites, being also possible to validate the applicability of specific RTLs to sites located near the boundary limits but already outside the typology area.

Table 3. Assessment (MarMAT) results for studied sites (Peniche)

Metrics/Beaches		Baleal-Norte	Gamboa	Portinho da Areia do Norte	Consolação
Results	Shore Description	11	9	10	9
	Species Richness	29	19	27	31
	Number of opportunists/ESG I	0.4	0.4	0.4	0.4
	Proportion of Chlorophyta	0.14	0.11	0.11	0.10
	Number of Rhodophyta	18	9	17	18
	Proportion of opportunists	0.14	0.16	0.11	0.13
	Coverage of the opportunists (%)	21.1	32.6	45.2	26.3
Scores	Shore Description	3	3	3	3
	Species Richness (a)	4 (8)	2 (4)	3 (6)	4 (8)
	Number of opportunists/ESG I	4	4	4	4
	Proportion of Chlorophyta	4	4	4	4
	Number of Rhodophyta	4	2	3	4
	Proportion of opportunists	4	4	4	4
	Coverage of the opportunists (%)[a]	4 (8)	4 (8)	3 (6)	4 (8)
	Sum of the scores	35	29	30	35
	EQR	0.97	0.81	0.83	0.97
	EQS	High	High	High	High

[a] counted twice in the final sum.

CONCLUSION

In this survey, a total of 87 species of macroalgae were identified, from which 10 were Chlorophyta, 20 Ochrophyta and 57 Rhodophyta. The studied seashore with the greatest biodiversity was Baleal-Norte with 55macroalgal species, followed by Consolação with 53 Portinho da Areia do Norte with 50 and Gamboa with 36 species. Among the four sites, it was observed that the biodiversity in terms of taxonomic composition was not too different since a high number of repeated species was found among stations, in particular the large sized ones. Despite that, the taxonomic composition presented was sufficiently different to separate Consolação from the other three sites, which were grouped together in a compact way. The presence of *taxa* simultaneously in all sites constituting the larger group, but not in Consolação, and, by opposition, the exclusive presence of *taxa* only in Consolação, dictated the mentioned relative distribution of sites. The distribution of species through the macroalgae Phyla also places the study sites inside the expected variation for the European coast. The achieved results (except for Gamboa) were perfectly in line with results obtained from studies where the sampling effort was higher [16]. This indicates the survey was balanced and performed in a trustful way, which is important when delicate issues and conclusions are extracted from the collected data (e.g., WFD Ecological Quality Assessment).

Comparing the current data with those historically reported in the literature it is found that about 84% of the species recorded have been already identified 50 years ago by Ardré [12, 13, 16, 30], and all the remain species were meanwhile identified by other researchers so no species were added as a new register by the present survey.

The ecological quality status of Baleal-Norte, Gamboa, Portinho da Areia do Norte and Consolação reached ‘High,’ thus confirming MarMAT’s sensitivity in the assessment of ecological quality of coastal waters. The quality assessment reporting to data collected in 1970 allowed to confirm the high quality of this coastal area, where the High status was achieved for the former survey as well as for the present one.

ACKNOWLEDGMENTS

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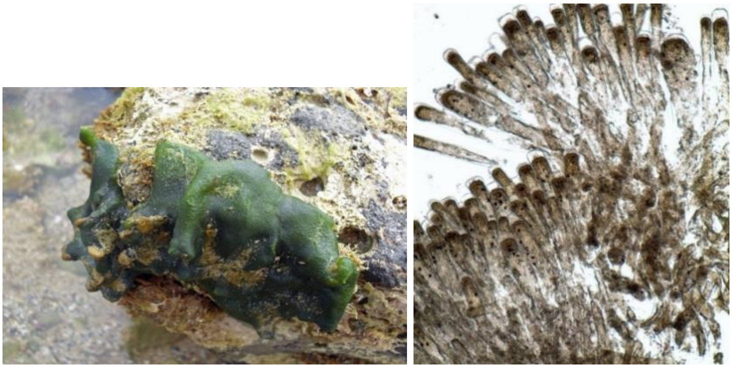
ANNEX I. MACROALGAE BIODIVERSITY IN IMAGES AND THEIR CHARACTERISTICS

CHLOROPHYTA – GREEN ALGAE



A B

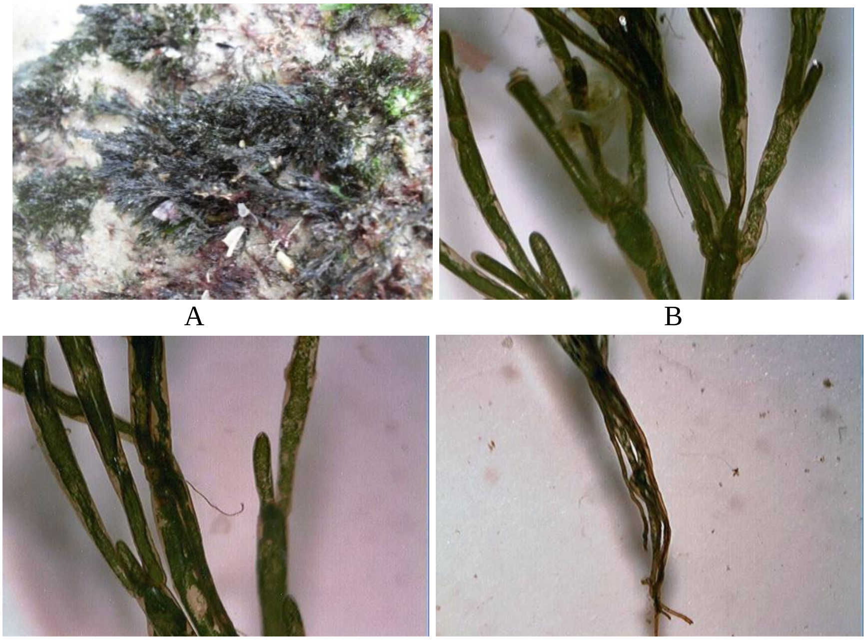
Figure 5. *Codium adhaerens*: (A) Spongy thallus, green light, prostrate, irregularly shaped and presenting with the appearance of a plane carpet firmly fixed to the substrate; (B) cross section of the thallus, showing the narrow and elongated utricles (L.M.100X).

A B

Figure 6. *Codium tomentosum*: (A) A small green alga (up to 30 cm long) with a dichotomously branched, cylindrical frond; the frond is solid and spongy with a felt-like touch; (B) cross section of the thallus, showing the non-mucronate utricles (L.M. 400X).

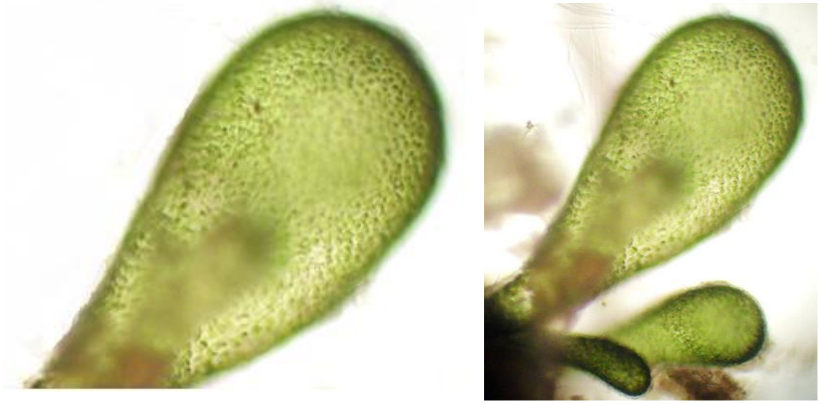
A B

Figure 7. *Derbesia tenuissima*: (A) Thalli bright green, 10-60 mm tall, on rock or on other plants, threadlike, sparsely branched, transverse septa absent; (B) specimens in its natural habitat.



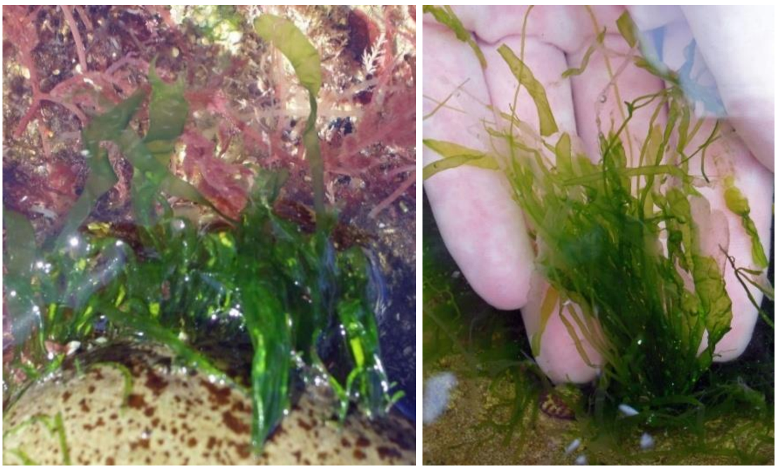
A B C D

Figure 8. *Cladophora prolifera*: (A) Unattached or basally attached coarse filaments that are usually less than 0.5 mm wide and 3-5 cm long; (B) the filaments are formed of a single row of often swollen cells; if attached then by a discoid base or by rhizoidal outgrowths (S.M.15X); (C) ramifications with rounded apexes (S.M.15X); (D) basal part with rhizoids (S.M.15X).



A B

Figure 9. *Valonia utricularis*: (A) Thallus, translucent light- to dark-green, primarily consisting of a large (up to 5 mm thick and 20 mm long) bladder- or cluc- to hose-like "cell," branching at the base rhizoidally. Later due to outgrowths of this cell cylindrical-clavate branches, often contorted and almost gapless densely packed, thus forming intertwined erect stands [32] (L.M. 400X); (B) vesicles forming tufts (L.M. 400X).



A B

Figure 10. *Ulva compressa*: (A-B) This species can have one of two different growth forms: the first is a flat, narrow sheet with ruffled edges; the second form (often referred to as *Enteromorpha compressa*) is a hollow tube of tissue, rounded at the top. In both forms the sheets of tissue are very thin, in fact they are exactly one cell thick; several blades or tubes arise from a common attachment point and can grow up to 200 mm long.

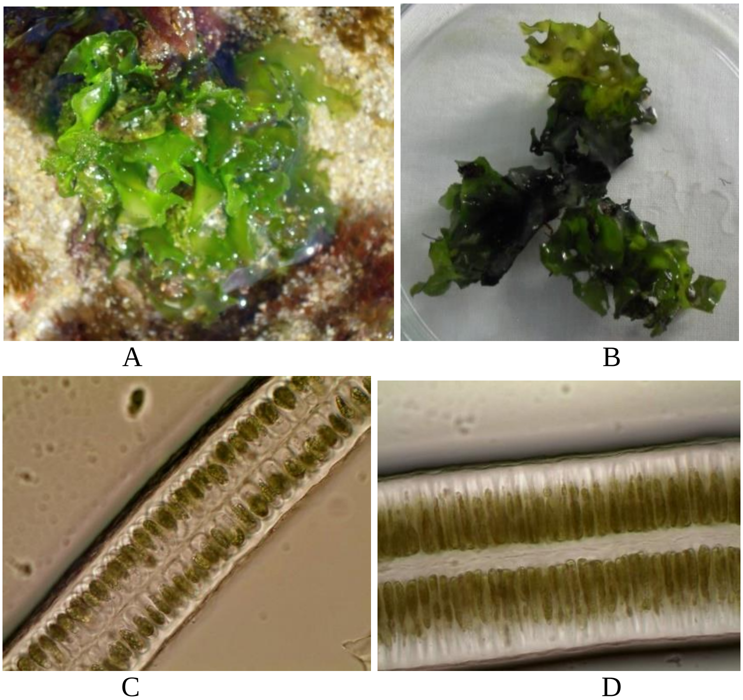


Figure 11. (A) General appearance of *Ulva rigida* thallus; (B) general appearance of *Ulva rigida* var. *fimbriata* thallus; (C) cross section of *U. rigida*'s thallus, in which it is possible to see the rectangular shape of the cells (L.M. 100X); (D) cross section of *U. rigida* var. *fimbriata* thallus, in which it is possible to see the conical shape of the cells (L.M. 400X).

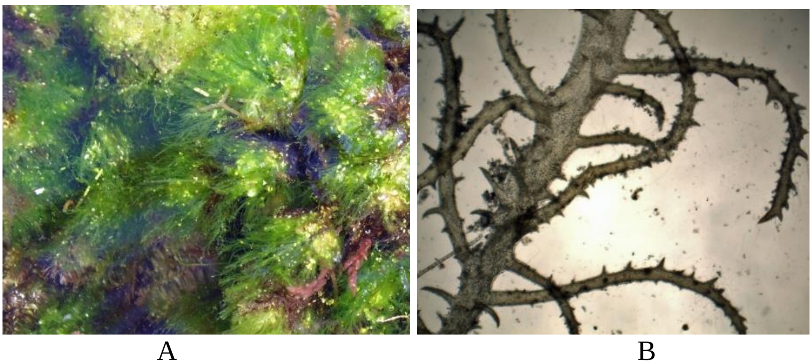
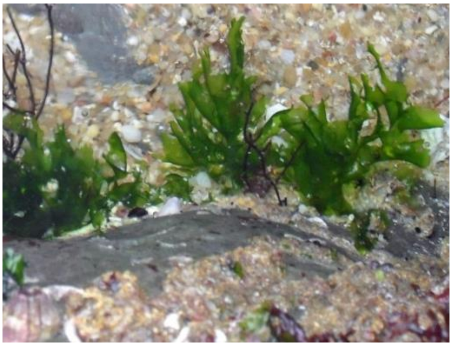


Figure 12. *Ulva clathrata*: (A) This species forms tufts, bright green, composed of branched axes, which can reach several centimeters long (20-30 cm); the main axis and branches are covered with a very characteristic (B) conical branchlets (L.M. 100X).



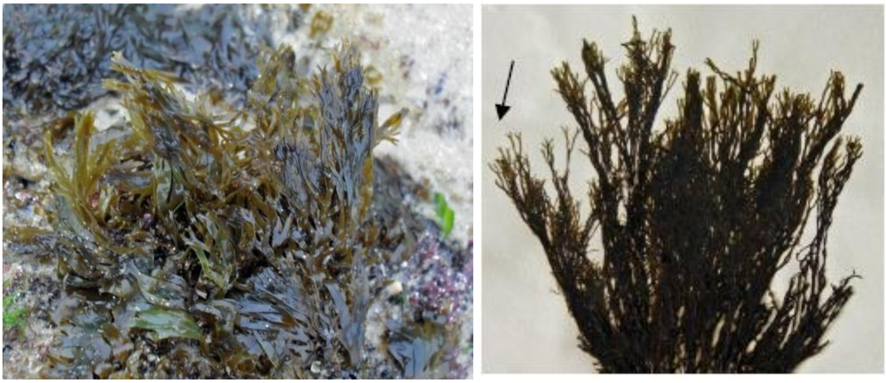
A B

Figure 13. *Ulvaria obscura*: (A) Thallus widely-bladed, monostromatic (B), similar to that of laminar *Ulva*, turning brown on dying (L.M. 100X).

OCHROPHYTA (PHAEOPHYCEAE) – BROWN ALGAE

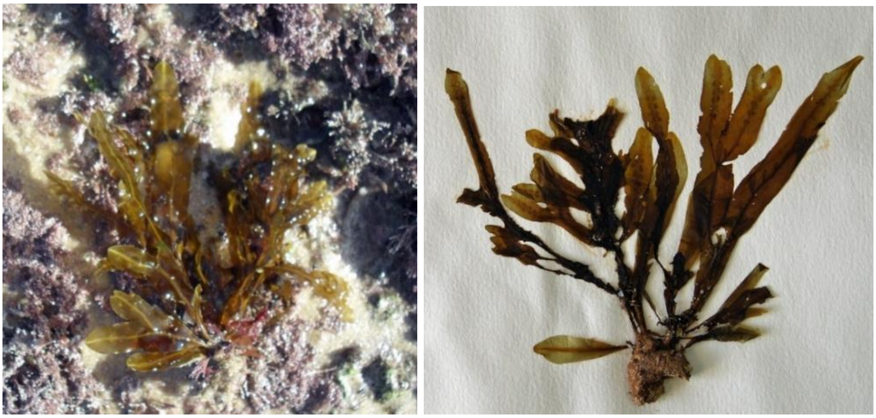
A B

Figure 14. *Dictyota dichotoma*: (A) Thallus flat, homogenous yellow-brown to darker brown, with fairly regular dichotomous branches with parallel sides to 30 cm long, the tips usually bifid; branches 3 to 12 mm wide, membranous, without a mid-rib; (B) fixing disk (arrow).



A B

Figure 15. *Dictyota spiralis*: (A) Thallus leathery, 5-15 cm high, reddish-brown, attached by decumbent stolons; branching dichotomous, often irregular; some segments cease to ramify, others grow closely by 3-4 in tuft; segments near-linear, slightly wider at nodes, basal parts often narrowed; (B) apices broad and blunt (arrow) [30].

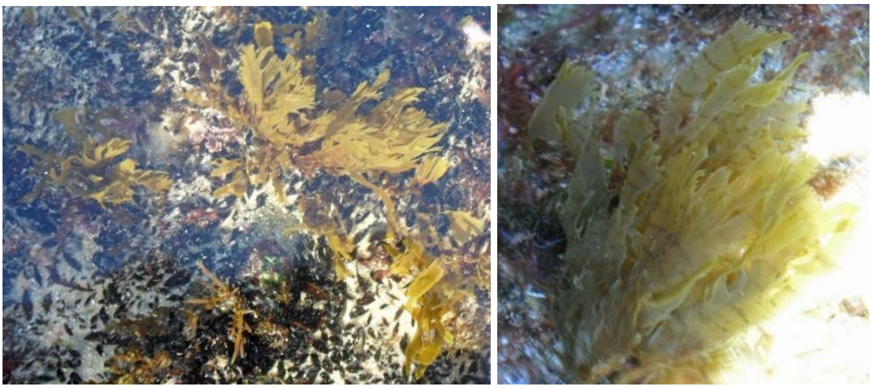


A B

Figure 16. *Dictyopteris polypodioides*: (A) Thallus flat and leaf-like, to 300 mm long and 20-30 mm broad; fronds olive to yellow-brown, translucent, and ± regularly dichotomously forked with a prominent midrib extending to the apices; margins sometimes split to the midrib (B) initially with an unpleasant smell shortly after collection, and degenerating quickly [31].

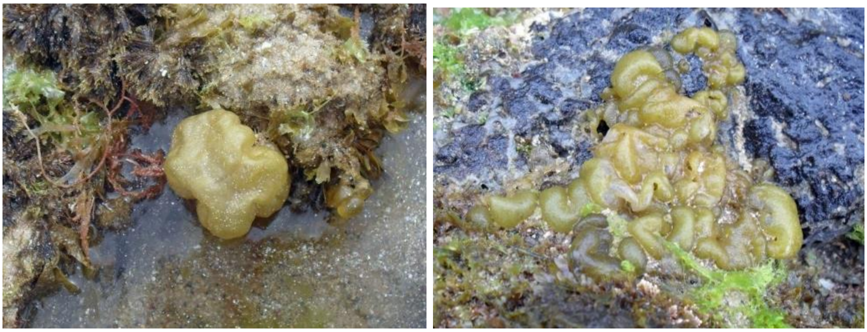
A B

Figure 17. *Padina pavonica*: (A) The frond is thin and leafy, flattish and entire when young, but often concave, or almost funnel shaped in mature specimens, with a laciniate or irregularly lobed margin; (B) the inner (or upper) surface is covered in a thin coating of slime, and the outer (or lower) surface is banded with zones of light brown, dark brown and olive green.



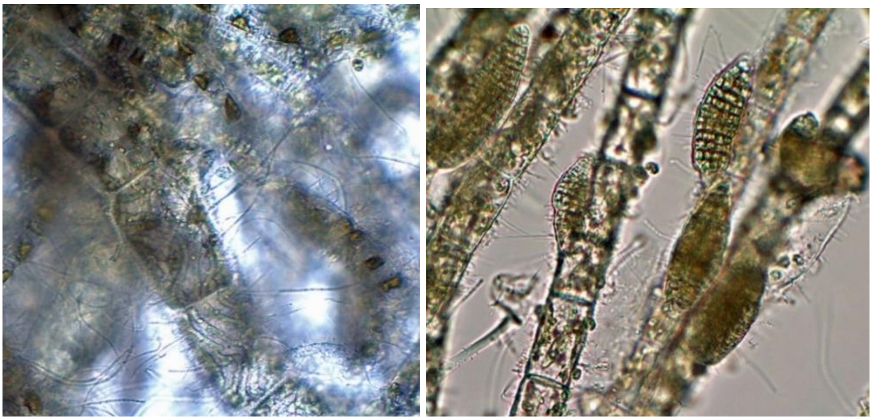
A B

Figure 18. *Taonia atomaria*: (A) Thalli erect, attached by matted, branched rhizoids, up to 30 cm long, complanate, flabellate or lacerate with many elongate, cuneate branches, 0.5-6 cm broad, often tapering to the apex; (B) thallus 2 cells thick near apex increasing to 5-7 cells thick towards the thallus base, not arranged in rows in transverse section.



A B

Figure 19. *Colpomenia peregrina* (exotic species - non-native): (A) Sometimes regularly spherical (B) or more or less irregular outline (*Colpomenia sinuosa*), yellowish-brown color, fixed to the substrate by filamentous rhizoids.



A B

Figure 20. *Ectocarpus fasciculatus*: (A) The thallus consists of profusely branched uniseriate filaments; the cells of the main filaments are taller than broad and have tape-shaped plast (L.M. 400X); (B) lateral branches containing cylindrical plurilocular structures (L.M. 400X).

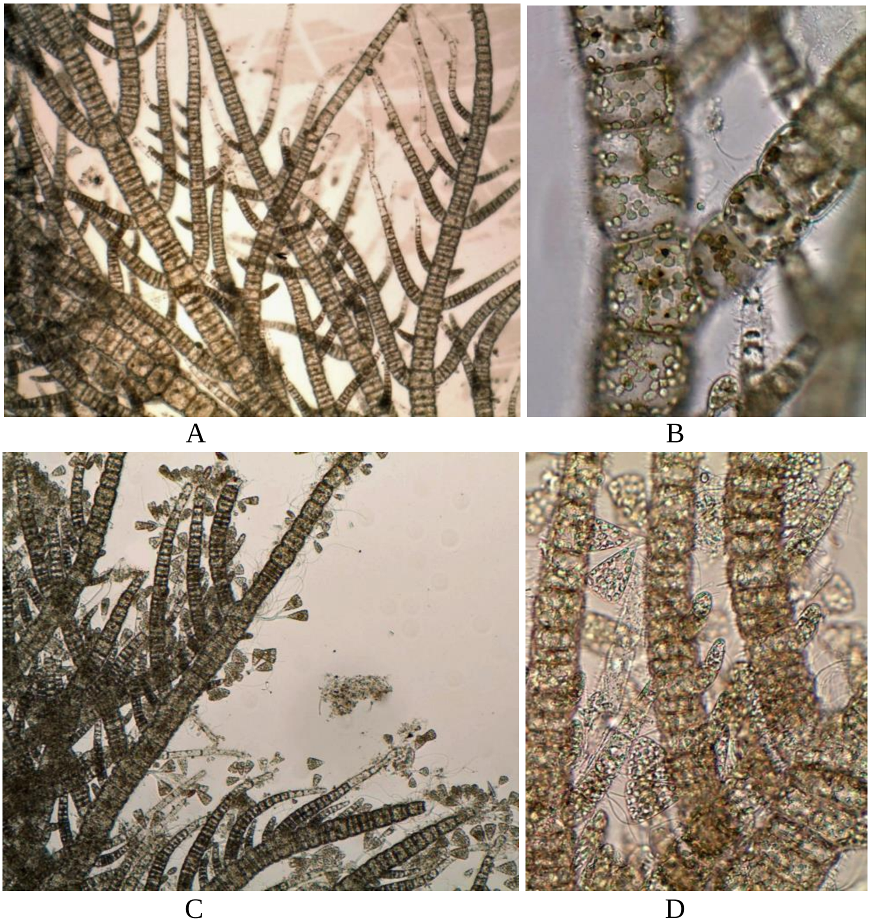
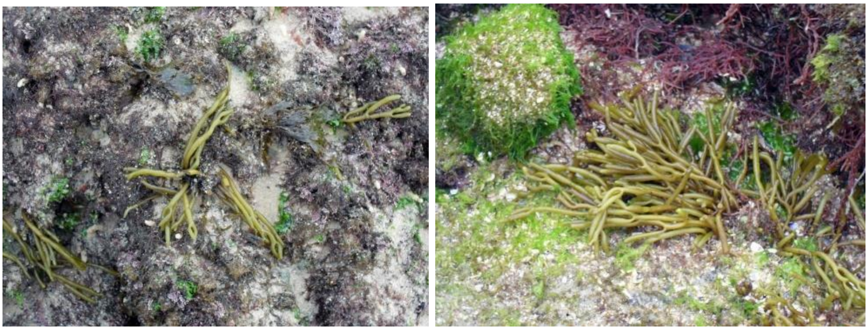


Figure 21. *Hincksia granulosa*: (A) Uniseriate filamentous thalli, erect and with opposing branches that originating several long lateral branches (L.M. 100X); (B) the cells are longer than large, with numerous discoid plasts (L.M. 400X); *Hincksia hincksiae*: (C) The main axes branch out, unilaterally, and support several plurilocular sporangia (L.M. 100X); (D) the cells have many discoid plasts (L.M. 400X).



A B

Figure 22. *Bifurcaria bifurcata*: (A-B) Up to 30 cm in length; olive-yellow in color, but much darker when dry; holdfast expanded and knobbly; frond cylindrical, unbranched near base then branching dichotomously; elongate reproductive bodies present at ends of branches; rounded air bladders sometimes present.

A B

Figure 23. *Cystoseira baccata*: (A) Plants usually solitary, 1 m or more in length, attached by a thick, conical attaching disk; axis simple or branched, up to 1 m in length, flattened, about 1 x 0.4 cm in transverse section; apex smooth and surrounded during periods of active growth by incurred young laterals.; lateral branch systems distichous, alternate, radially symmetrical, profusely branched in a repeatedly pinnate fashion and bearing sparse, filiform, occasionally bifurcate appendages on the branches of higher orders [32]; *Cystoseira nodicaulis:* (B) in water, the thallus has a yellowish-brown coloration.

A B

Figure 24. *Cystoseira tamariscifolia*: (A-B) bushy seaweed, up to 60 cm in length but usually 30-45 cm; it has a cylindrical frond with irregularly branches; olive green in color, almost black when dry; when the plant is seen underwater it has a blue-green iridescence.

A B

Figure 25. *Fucus spiralis*: (A) The fronds are usually easily recognizable by the flattened, twisted, dichotomously branched thallus, lacking bladders, and the large, oval receptacles at the frond tips, each receptacle being surrounded by a narrow rim of vegetative frond. Nevertheless, younger plants are not always so easy to identify, and even mature plants can be confused with other *Fucus* species; (B) the thallus is composed of a blade with midrib and receptacles in the apical zone.

A B

Figure 26. *Sargassum vulgare*: (A-B) Much branched, bushy plants that grow to 50 cm tall and are attached by a discoid holdfast; thalli pseudo-parenchymatous; primary and secondary branches are cylindrical and bear lanceolate foliar branches (4 cm long, 3 mm wide) with serrate margins; bladders are formed on short pedicels [33].

A B

Figure 27. *Laminaria ochroleuca*: (A-B) Young specimen; glossy, yellow-brown kelp that is prevalent along the intertidal zones; this kelp is quite conspicuous as it grows quite large under the right conditions; the maximum length recorded is 4 m long, but this length is rarely attained and occurs only in specific areas; under normal conditions *L. ochroleuca* is more likely to reach a maximum length of about 2 m; *L. ochroleuca* has a large heavy holdfast made up of thick haptera or rhizoids (up to 18 cm in diam.) that supports the plant and anchors (arrow) it to rocks.

A B

Figure 28. *Cladostephus spongiosus*: (A-B) Fairly stiffly branched fronds growing from a crust-like discoid holdfast (arrow), covered with small branchlets arranged in whorls; maximum length usually about 15 cm.

A B

Figure 29. *Halopteris scoparia*: (A) Dark brown algae that forms beautiful fluffy clumps in shallow rocky-bottomed water; growing only up to 15 cm in length, *H. scoparia* has a main axis with alternate plumed (B), S.M. 20X) branches which are more or less fan-shaped when flat, though when buoyed up by water they form inverted cone-shaped tufts with a very delicate appearance due to the many filamentous branches.

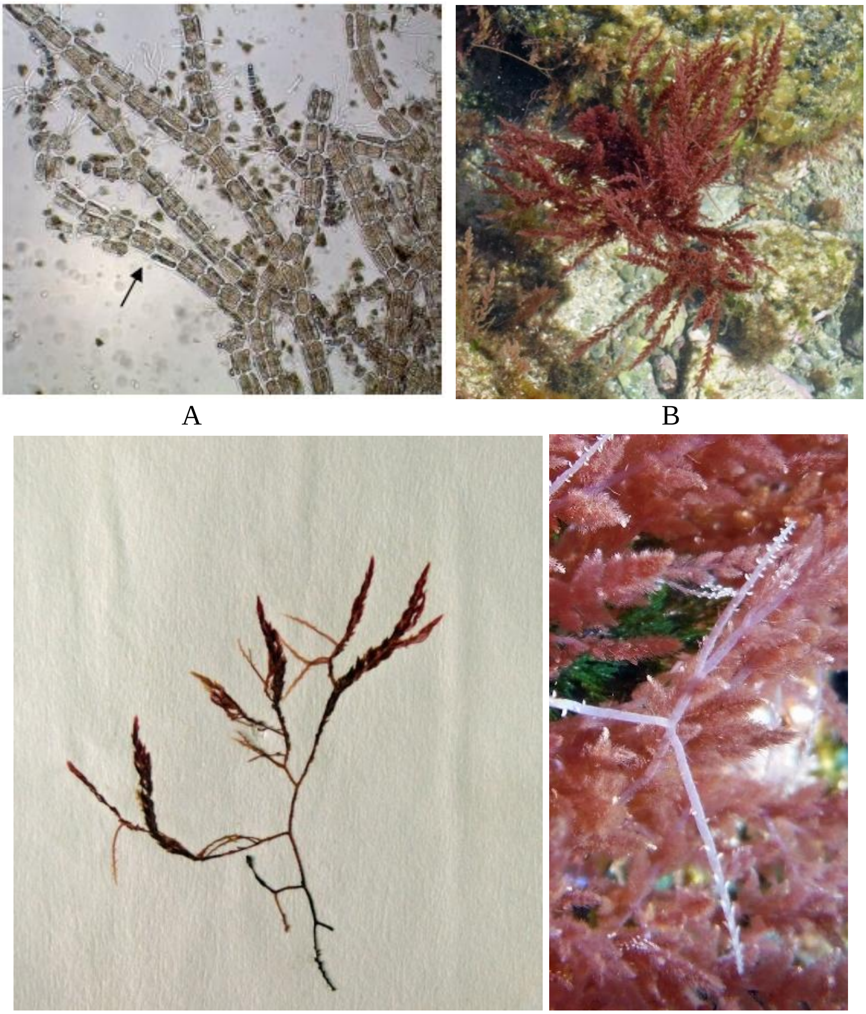
A B

Figure 30. *Sphacelaria rigidula*: (A) Filamentous cushion-like tufts, also occurring as scattered filaments in mixed turf communities, 5-10 mm high, medium to dark brown; branching irregular, sparse (L.M. 40X); (B) the filaments are cylindrical and emerge laterally the propagules, which are also cylindrical and trifurcated (L.M. 100X).

A B

Figura 31. *Saccorhiza polyschides*: (A) Species with a distinctive large warty holdfast and a flattened stipe with a frilly margin (in adult specimens); the stipe is twisted at the base and widens to form a large flat lamina, which is divided into ribbon-like sections; (B) presence of a large bulbous holdfast with warty appearance.

RODOPHYTA – RED ALGAE



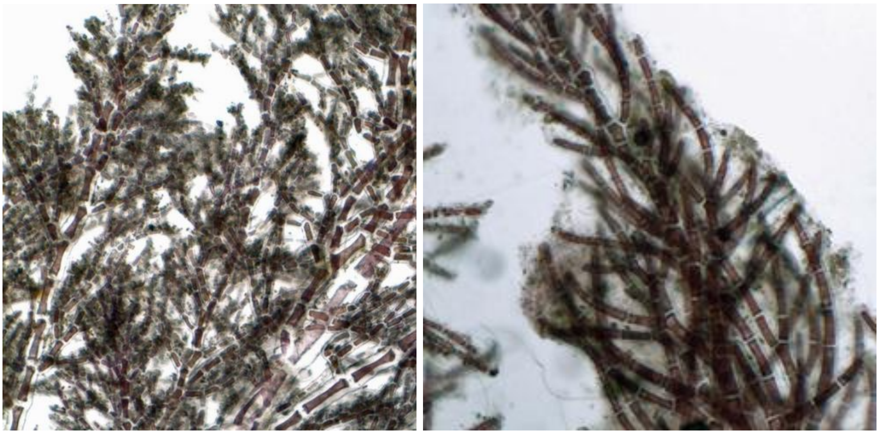
A B

C D

Figure 32. (A) Morphology of the *Falkenbergia rufolanosa* (tetrasporoporic phase of *Asparagopsis armata*) (exotic species – non-native) thallus, where it can be seen that it is composed of slightly branched filamentous axes and the filaments composed of an axial cell and three periaxial cells (L.M. 400X); (B) general appearance of the feathery thallus of *A. armata*; (C-D) spiny, harpoon-shaped (arrow) branches some cm in length.

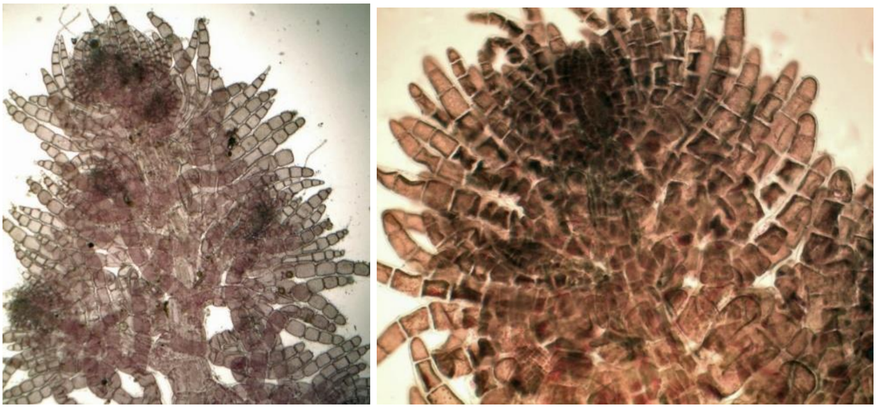
A B

Figure 33. *Acrosorium ciliolatum*: (A) Flattened, membranous, deep-red fronds, 30-200 mm long; frond deeply divided into linear-lanceolate, irregularly branched segments, often terminating in hooks; (B) the frond is traversed by network of microscopic veins, but macroscopic veins are absent (L.M. 100X).



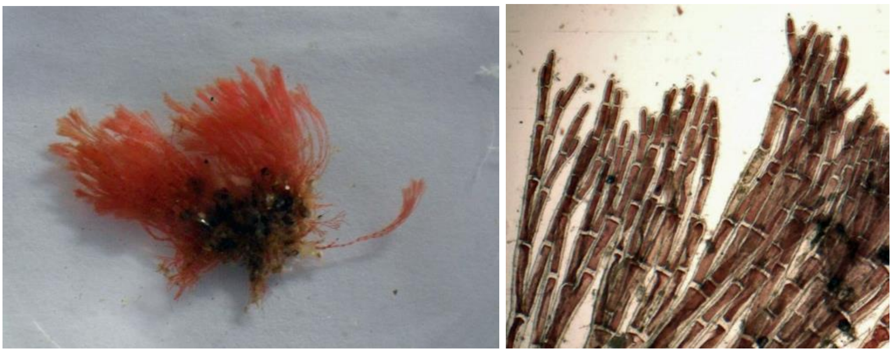
A B

Figure 34. (Continued).



C D

Figure 34. *Aglaothamnion pseudobyssoides*: (A) Uniseriate filaments, uncropped and with alternate ramifications (L.M. 100X); (B) the apical cells of the lateral branches have the rounded ends (L.M. 400X); *Aglaothamnion sepositum*: (C) Thick, corticated, filamentous filament with alternate ramifications (L.M. 40X); (D) the apical cells of the lateral branches assume a false conical shape (L.M. 100X).

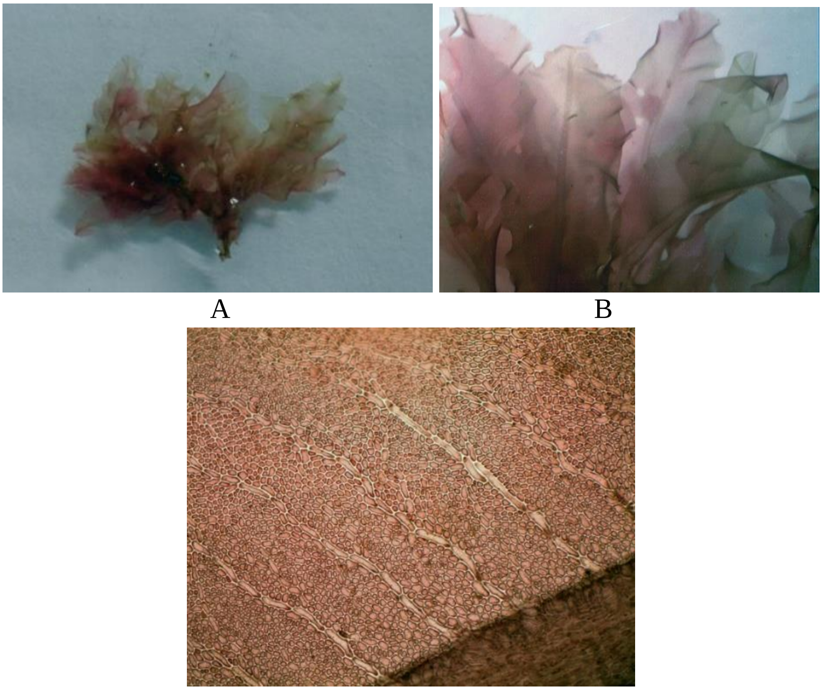


A B

Figure 35. *Anotrichium furcellatum*: (A) Small reddish-pink filamentous tuft; (B) Thin filaments, uniseriate, erect and composed of apical cells with a conical shape (M.O. 40X).

A B

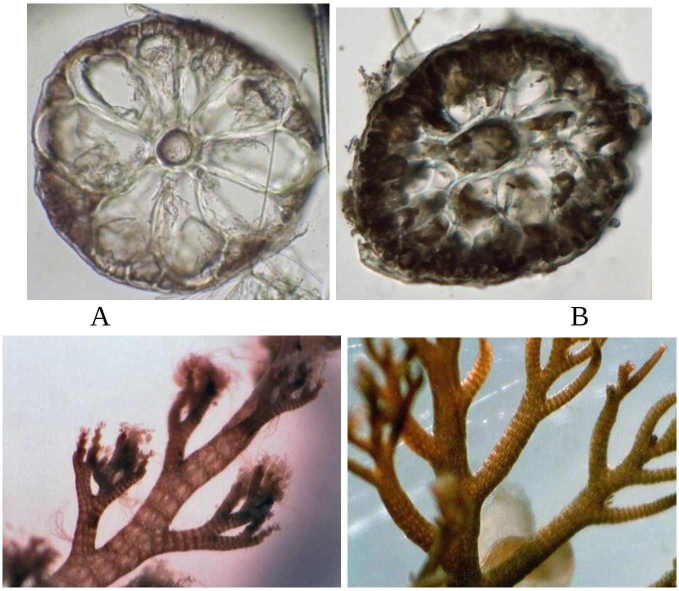
Figure 36. *Antithamnion densum*: A) Uniseriate filamentous thallus with opposing branches (L.M. 100X); (B) the plumed clusters are mostly arranged at the same side (L.M. 100X).



A B

C

Figure 37. *Apoglossum ruscifolium*: (A) Membranous, tufted, bright red fronds, to 100 mm long; (B) frond with conspicuous midrib and wavy margin, to 6 mm wide, repeatedly branched from midrib, apices blunt (S.M.20X); (C) numerous microscopic veins, at wide angle from midrib (L.M. 100X).



A B

C D

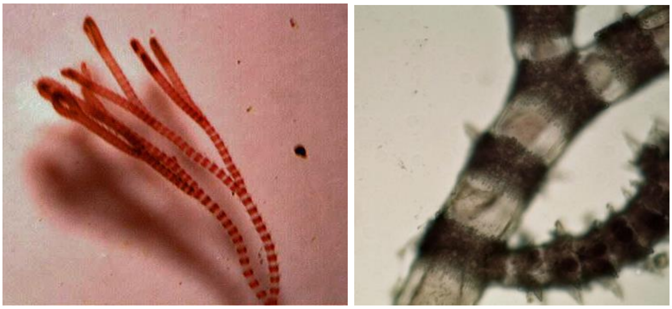
Figure 38. (A) Transverse section of the main axis of *Boergeseniella fruticulosa*, formed by nine periaxial cells, which are surrounded by a reduced cortical cell layer (L.M. 100X); (B) Transverse section of the main axis of *Boergeseniella thuyoides*, formed by ten periaxial cells, which are coated by several layers of cortical cells (L.M. 100X); (C) *Boergeseniella fruticulosa* is composed of thin transverse bands of cortical cells (S.M.35X); (D) *Boergeseniella thuyoides* has transverse bands of thicker cortical cells (S.M.35X).

A B

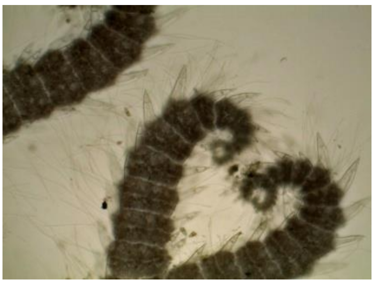
Figure 39. *Bornetia secundiflora*: (A) Dark red in color, firm and rigid when fresh; the thallus is 5-20 cm high when erect, fan-shaped with blunt tips (apices), much branched and tufted, with branches often curved over; the plant has a jelly-like texture; branches are sparse at the base becoming denser towards the apices; (B) each filament consists of a single row of cells that are arranged end-to-end (S.M.20X).

A B

Figure 40. *Callithamnion tetragonum*: (A) Brownish-red fronds, to 50 mm long; uniseriate filaments, corticated below, repeatedly branched with simple, alternate branches (L.M. 40X); (B) ultimate branchlets densely clothed with tufts of alternate ramuli, corymbosely, incurved, attenuate at base and apex (L.M. 100X).

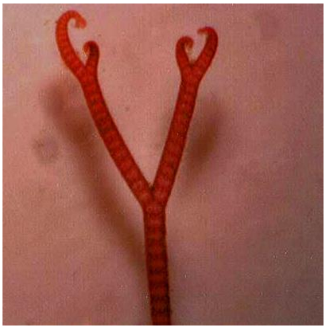


A B



C

Figure 41. *Ceramium ciliatum*: (A) Uniaxial, cylindrical, erect thalli, corticated in transverse bands, in a discontinuous way (S.M.45X); (B) the filaments at the base are comprised of cells wider than tall (between nodes) that are surrounded by transverse bands of cortical cells (nodes) (L.M. 100X); (C) the branches apexes are bifurcated and curved (L.M. 100X).




A B

C

Figure 42. *Ceramium echionotum*: (A) Rough, densely tufted, purplish red fronds, to 150 mm long, repeatedly dichotomously branched, axils wide, apices strongly hooked inwards filaments of almost uniform diameter throughout.(S.M.45X); (B) Articulations corticated at nodes, 3-4 times as long as broad in lower parts, very short distally, with numerous, irregularly distributed, colorless, unicellular, needle-like spines on corticating bands (L.M. 100X); (C) the apexes are wrapped in fork and transverse bands of cortical cells emerge the single-celled spicules (L.M. 100X).


A B

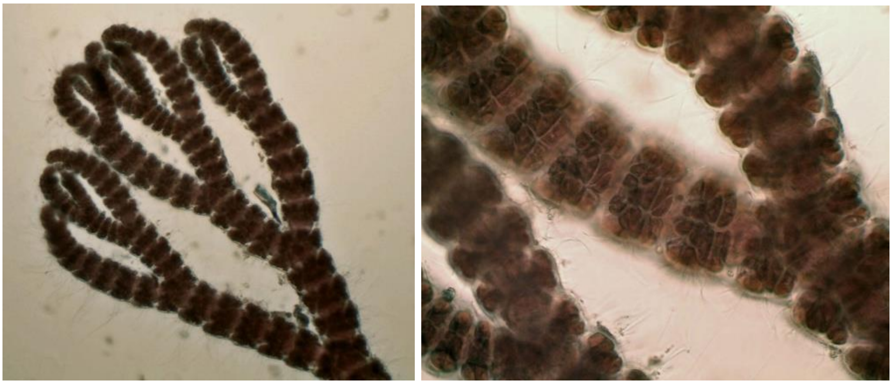
Figure 43. (Continued).

C

Figure 43. *Ceramium pallidum*: (A) Uniaxial, cylindrical, erect thalli, corticated in transverse bands, in a discontinuous way (S.M.40X); (B) the filaments have an alternating branching and are constituted by transverse bands of cortical cells that are intercalated by transparent cells (S.M.35X); (C) bifurcated and slightly curved apexes (S.M.45X).

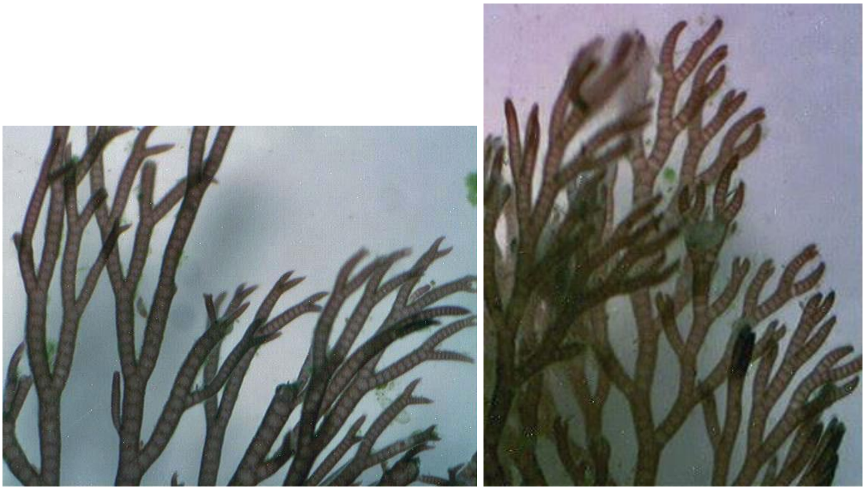
A B

Figure 44. *Ceramium pallidum*: (A) The filaments are composed by transparent cells which assume various shapes and sizes and which are arranged alternately, or not, between the dense transverse bands of cortical cells (L.M. 100X); (B) the branches apexes are bifurcated and curved (L.M. 100X).



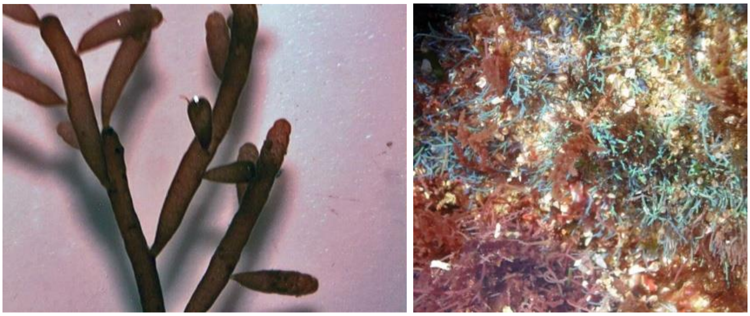
A B

Figure 45. *Ceramium tenuicorne*: (A) Uniaxial, cylindrical, erect and dark red thallus, in which the branches apexes are bifurcated and curved (L.M. 100X); (B) the filaments are composed of transverse bands of low and thick cortical cells (nodes), which are interspersed by small nodes (L.M. 400X).



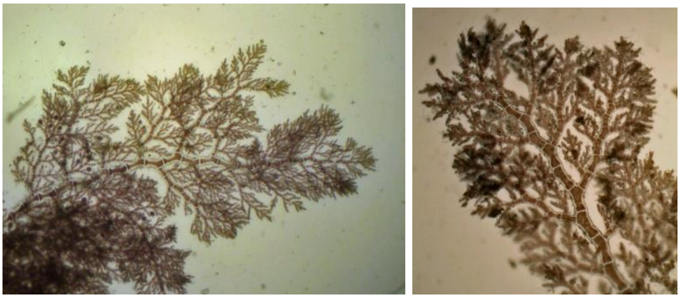
A B

Figure 46. *Ceramium virgatum*: (A) Small red seaweed growing up to 30 cm tall; it has a filamentous frond that is irregularly and dichotomously branched, (S.M.45X); (B) with the branches narrowing towards pincer-like tips (S.M.40X); these little seaweeds are often found growing epiphytically in association with *Fucus* spp., such as *Fucus vesiculosus*.



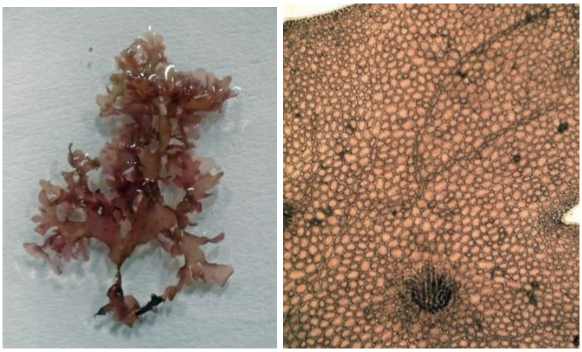
A B

Figure 47. *Chondria coerulescens*: (A) The fronds are flexible and cartilaginous in texture, turning black when dry (S.M.30X); (B) in water this seaweed has bluish or yellowish fronds with blue iridescence.



A B

Figure 48. *Compsothamnion thuyoides*: (A) Uniseriate thallus, erect and with alternate ramifications (L.M. 40X); (B) the lateral branches are, in turn, branched alternately giving rise to branches of higher order which are also branched (L.M. 100X).



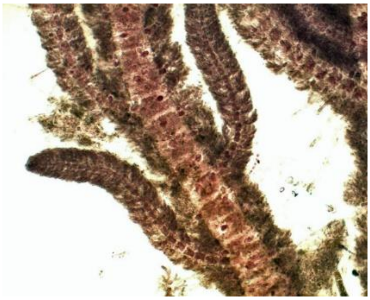
A B

Figure 49. (Continued).

C

Figure 49. *Cryptopleura ramosa*: (A) Thin, membranous, brownish red fronds, to 200 mm long; frond ± dichotomously divided, becoming irregular in upper parts; (B) the blades have microscopic veins that bifurcate and recombine at various points (L.M. 100X); (C) surface view of cystocarp (L.M. 400X).

A



B

C

Figure 50. *Crouania attenuata*: (A) Small pompous tufts of reddish-pink color (L.M. 40X); (B) The tufts are constituted by protruding main axes that ramify of irregular and alternated form, giving lateral branches (L.M. 100X); (C) the lateral branches are formed by quadratic cells that are covered by curved and mucronate ramuli, vertically displaced (L.M. 400X).

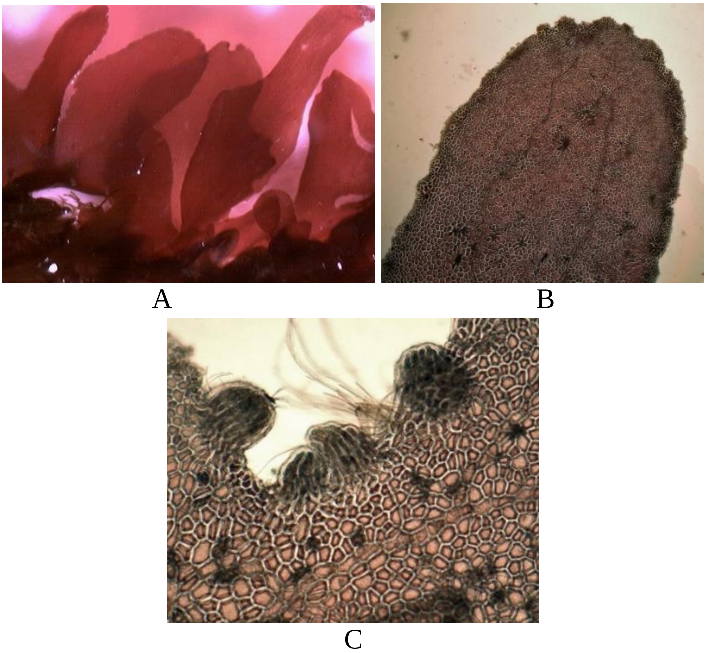


Figure 51. *Erythroglossum laciniatum*: (A) Thallus a short stipe, erect, consisting of one or more blades to 16 cm long and 15 or 20 cm wide, fan-shaped and divided into lobes with pointed apices (S.M.15X); (B) veins macroscopic, also fine veins (M.O. 40X); (C) lateral view of cystocarp on blade edges (L.M. 100X).

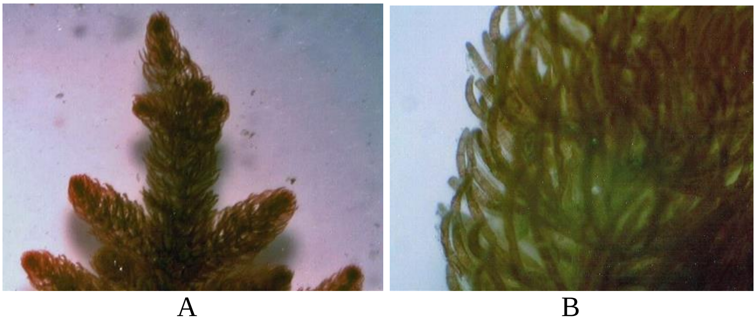
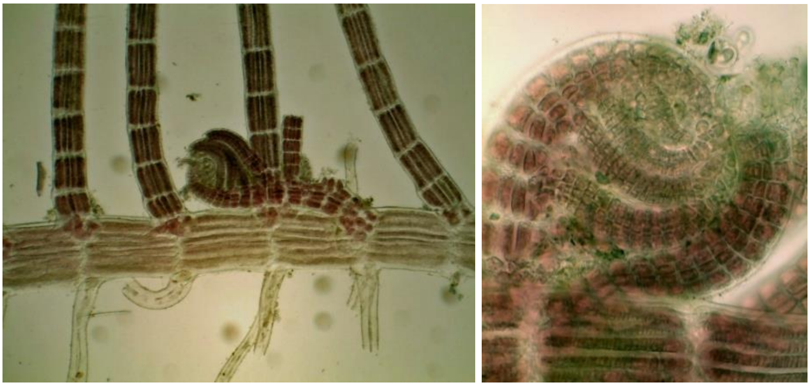
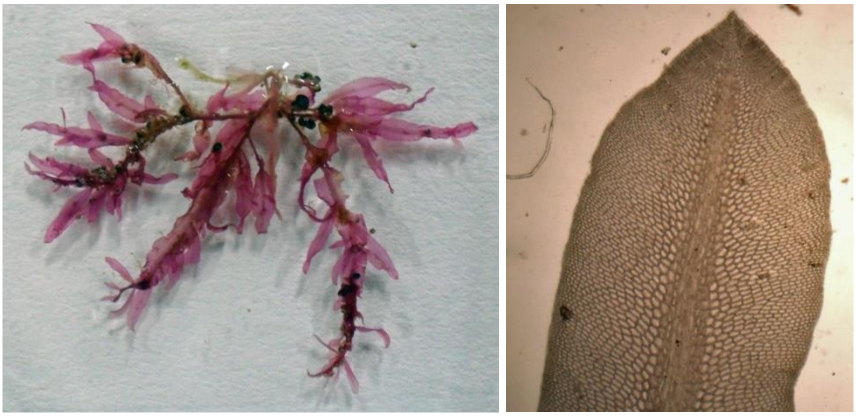


Figure 52. *Halurus equisetifolius*: (A) Erect plants consisting of up to 7 irregularly branched main axes growing 6 to 22.5 cm high and 2 to 3 mm wide, resembling a horsetail fern (S.M.15X); (B) the main axes branch to 4 orders and are clothed (either sparsely or densely) in whorls of between 5 and 8 short incurved branchlets which divide di- to trichotomously; the branchlets are 1.4 to 2.4 mm long and consist of 4 to 7 cells of which the terminal one is mucronate (L.B. 30X).



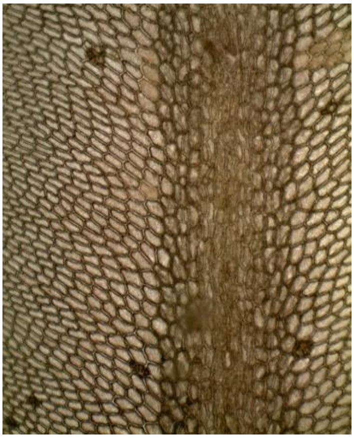
A B

Figure 53. *Herposiphonia tenella*: (A) Plants coarse and brownish red; thalli terete, having a branching pattern of three determinate axes followed by one lateral indeterminate (d/d/d/i pattern); prostate axes 120-150 µm diam., segments 0.8-1.3 diam. long., 7-9 pericentral cells (L.M. 100X); (B) erect determinate axes up to 1 mm in height, 70-80 µm diam., 8-13 segments with 0.81.0 diam. long, 7-8 pericentral cells; apex blunt with an inconspicuous apical cell. Vegetative trichoblasts rudimentary, 1-3 at the tips of determinate axes in successive segments, pseudo-dichotomously divided 24 times (L.M. 400X) [34].



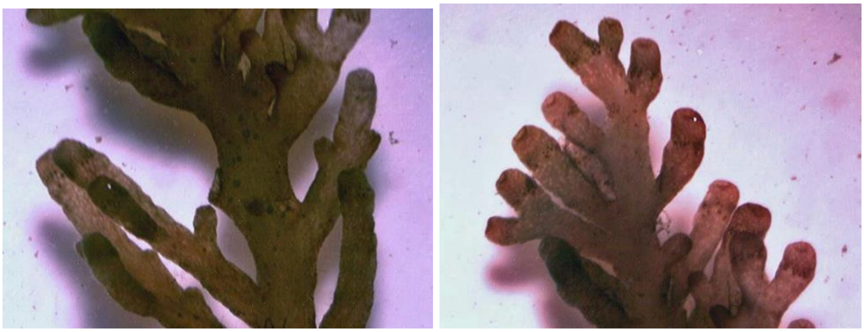
A B

Figure 54. (Continued).



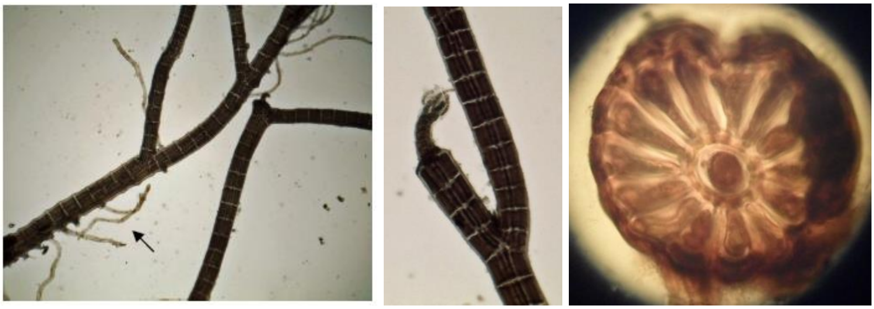
C

Figure 54. *Hypoglossum hypoglossoides*: (A) Membranous, rose-pink fronds, 20-300 mm long, arising from a discoid base; frond linear-lanceolate, with well-marked midrib and thin membranous margins, 1-8 mm wide, repeatedly branched irregularly from midrib (M.O. 40X); (B-C) fronds with pointed apices, margins without microscopic veins, monostromatic except in midribs (M.O. 400X).



A B

Figure 55. *Laurencia pyramidalis*: (A) Globose tufts of brittle, cartilaginous, narrow, cylindrical, reddish brown to yellowish red fronds, 150 mm long, from small discoid base (S.M.15X); (B) axis simple, branches patent, often opposite, spirally arranged, shorter towards apex giving regular pyramidal outline; bifurcated tips and a central hole (S.M.15X).



A B C

Figure 56. *Leptosiphonia schousboei*: (A) Thallus composed by uniaxial axes, corticated and prostrate at the base, attached to the substrate through rhizoids (L.M. 100X); (B) secondary erect axes, polysifonate and presenting apical trichoblasts (L.M. 100X); (C) cross section of the thallus with 14 periaxial cells (L.M. 400X).

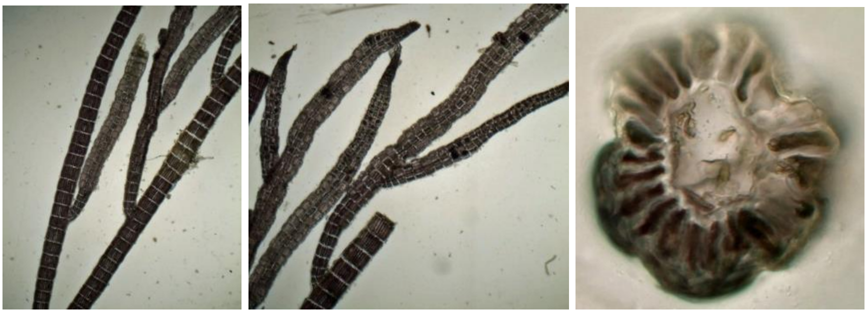
A B

C

Figure 57. *Lophosiphonia reptabunda*: (A) Thallus consisting of prostrated axes, from which depart secondary axes which have a curvature up to the apex (S.M.35X); (B) cross section of the thallus with 19 periaxial cells (L.M. 100X); C) secondary axes with tricoblasts arranged helically (S.M.45X).

A B

Figure 58. *Nitophyllum punctatum*: (A) Delicately membranous, rose-pink fronds with an elongate fan-shaped outline, margins distinctly frilly, to 40 mm or, exceptionally, to 1 m, sessile or shortly stipitate (<2 mm long); (B) frond veinless, undivided or deeply sub-dichotomously divided to the base; apices blunt or rounded, often ribbon-like; gametophyte plants form rounded spots to 5 mm in diameter whilst tetrasporphyte plants form characteristic elongated spots.

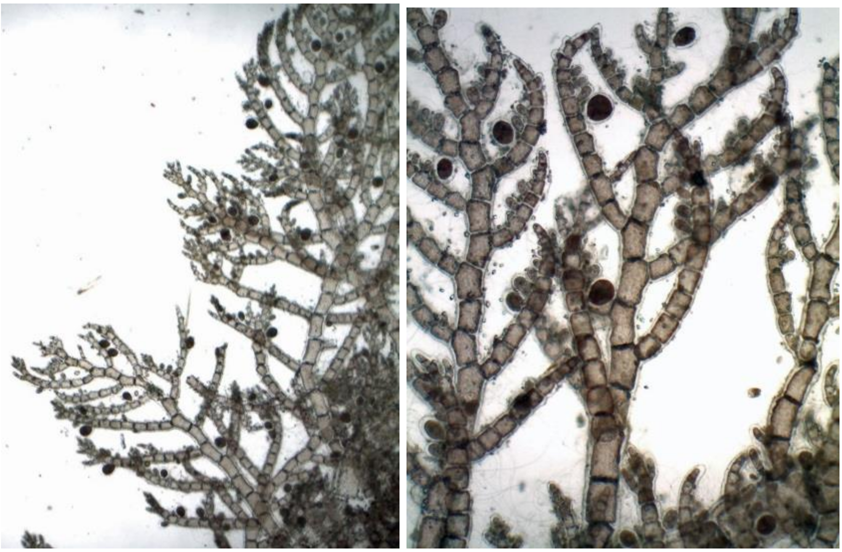


A B C

Figure 59. *Ophidocladus simpliciusculus*: (A) Branches erect simple or branched 1-2 times, with 16-23 periaxial cells (L.M. 40X); (B) from the branches arise tetrasporic secondary axes (L.M. 40X); (C) cross section of the thallus with 23 small periaxial cells surrounding a larger axial cell (M.O. 100X).

A B

Figure 60. *Osmundea pinnatifida*: (A) Cartilaginous, usually markedly compressed, dark purple to pale yellow fronds, to 100 mm or more long, from discoid base; very variable in size and form; main axis usually simple; (B) branching alternate distichous, repeatedly pinnate; ultimate ramuli short, blunt (S.M.15X).



A B

Figure 61. *Pleonosporium borreri*: (A) Erect thallus, filamentous, uncropped and with alternate ramifications (L.M. 40X); (B) the lateral branches also divide alternately giving rise to the cystocarps, which consists of small quadratic cells and rounded apices (L.M. 100X).

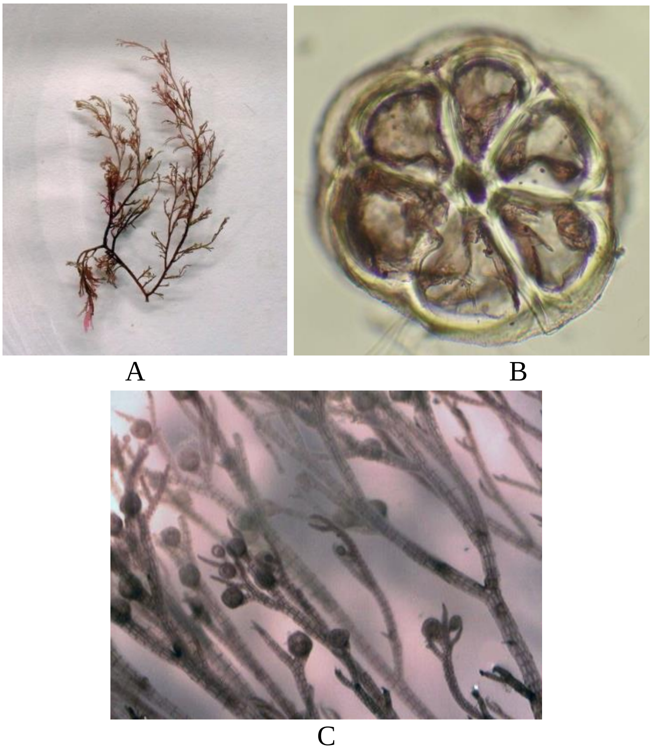
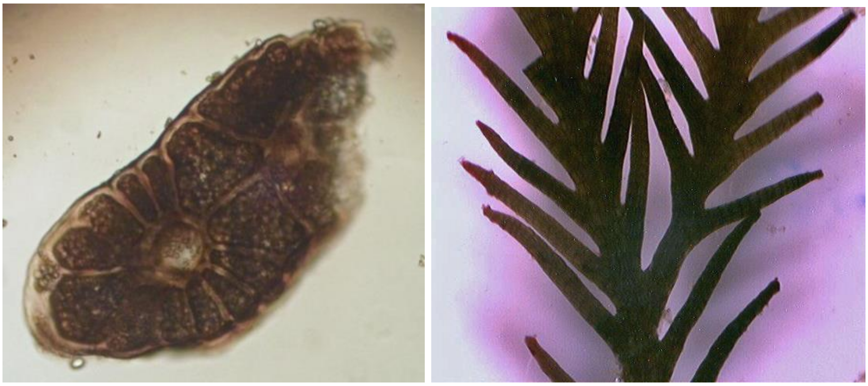


Figure 62. *Polysiphonia denudata*: (A) Small fronds reddish-brown color; (B) transverse section of the thallus with 6 periaxial cells (L.M. 100X); (C) composed thallus of cylindrical, uncrossed axes that branch in dichotomous form giving rise to secondary branches bearing cystocarps (S.M.45X).

Figure 63. (Continued).

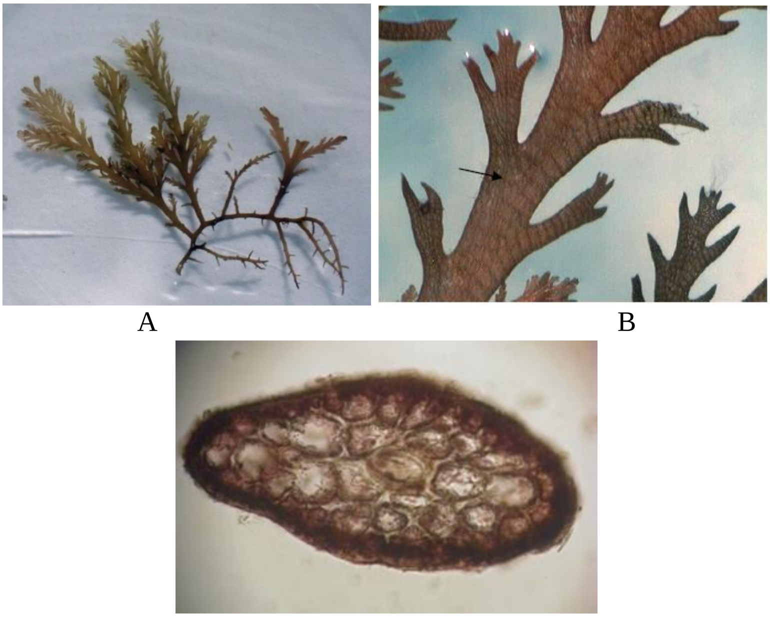
C D

Figure 63. *Polysiphonia fucoides*: (A) Cartilaginous, cylindrical, tufted, brownish purple fronds, to 300 mm long (more usually about 70 mm long), from branched rhizoidal holdfast; (B) branching ± alternate, tripinnate, ramuli with terminal tufts of colorless dichotomous fibrils (L.B. 40X); (C) cross section of the thallus with 19 periaxial cells (L.M. 100X); (D) corticated only at base (S.M.45X).



A B

Figure 64. *Xiphosiphonia ardreana* (formerly *Pterosiphonia ardreana*): (A) Sub-cylindrical thallus composed by 12 periaxial cells (L.M. 100X); (B) the branches are arranged alternately in an almost continuous way and are long and thin (S.M.20X).



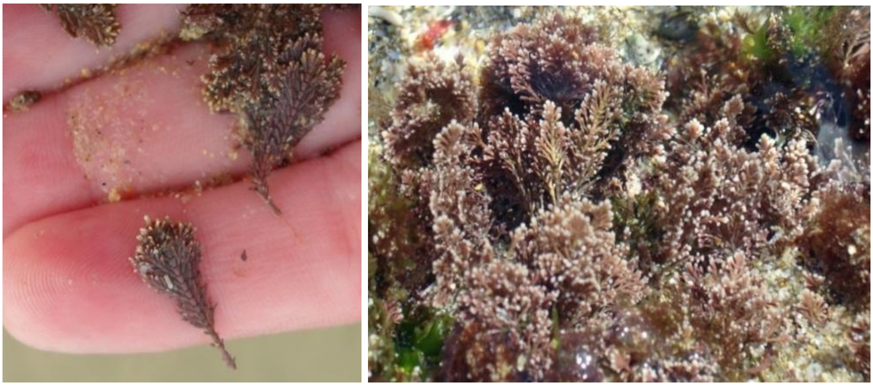
A B

C

Figure 65. *Pterosiphonia complanata*: (A) Thallus with prostate and erect axes, the erect ones flat-compressed and with distichous alternate pinnate branching; (B) brownish-red color, flexible and cartilaginous texture; attached by rhizoids which form discoid adhesive structures; veining visible with a magnifying glass (arrow) (S.M.25X); (C) cross section of main axis with multiple periaxial cells (L.M. 100X).

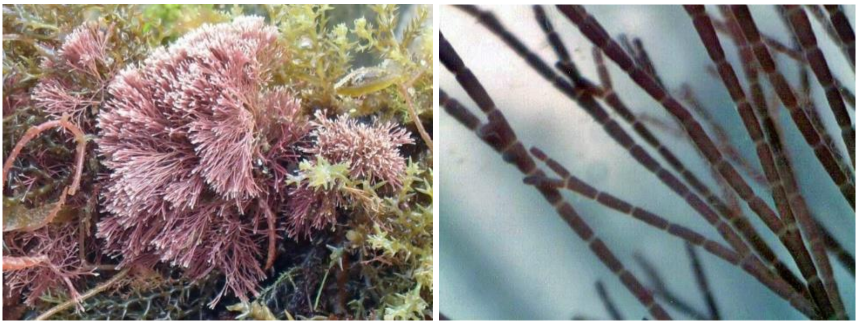
A B

Figure 66. *Xiphosiphonia pennata* (formerly *Pterosiphonia pennata*): (A) Small brownish-red seaweed up to 8 cm high, with a rigid texture; transverse section of the main axis with 9 periaxial cells (L.M. 100X); (B) from the main axis emerge thin and long branches, which are arranged alternately (L.M. 40X).



A B

Figure 67. *Ellisolandia elongata* (formerly *Corallina elongata*): (A) Whitish pink to reddish lilac, calcified, articulated fronds, fish-bone-like arrangement, to 50 mm high, axis compressed, repeatedly pinnate from discoid base; *Corallina officinalis*: (B) whitish-pink to lilac, calcified, articulated fronds, 60-120 mm high, axis cylindrical to compressed, repeatedly pinnate from and expanded discoid base, branching often irregular.

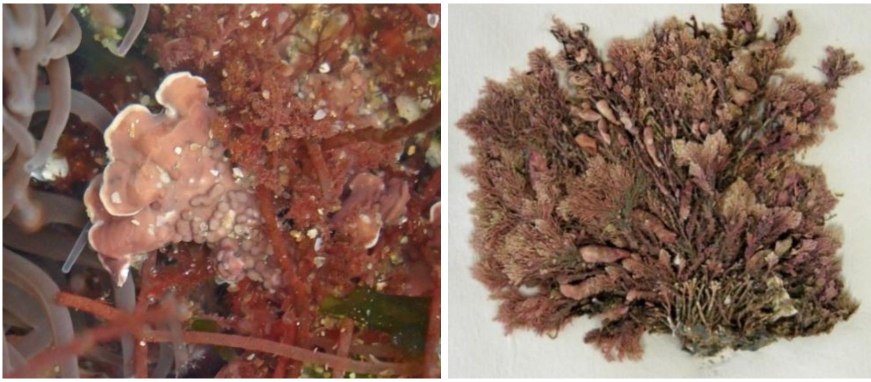


A B

Figure 68. *Jania rubens*: (A) Slender, rose-pink, articulated, calcified fronds, to 50 mm high; repeatedly dichotomously branched, luxuriant specimens secondarily pinnate; fixed by small conical disc, but spreading vegetatively by developing attachment discs from branches in contact with solid substratum; (B) segments cylindrical, to 120 µm diam., those bearing branches somewhat compressed, to 180 µm diam (S.M.20X).

A B

Figure 69. *Lithophyllum incrustans*: (A) Thick, dull chalky, yellowish, pink or lavender calcareous crusts forming irregular concretions, to 40 mm thick, margins ridged where crusts meet; *Lithophyllum byssoides*: (B) calcified and rigid encrusting thallus, strongly adhering to the bedrock; small hemispherical cushions 1 to 2 cm high, white to dark pink; crust covered with upright lamellae 10 mm high and 800 µm wide, anastomosed to each other.



A B

Figure 70. *Mesophyllum lichenoides*: A) Pale to dark purple thin, brittle, leafy calcified fronds, attached at base, margins free, lobed; fronds semicircular, concentrically banded; reproduction takes place in winter and spring in small, wart-like conceptacles; B) Small specimens often epiphytic on *Ellisolandia elongata.*

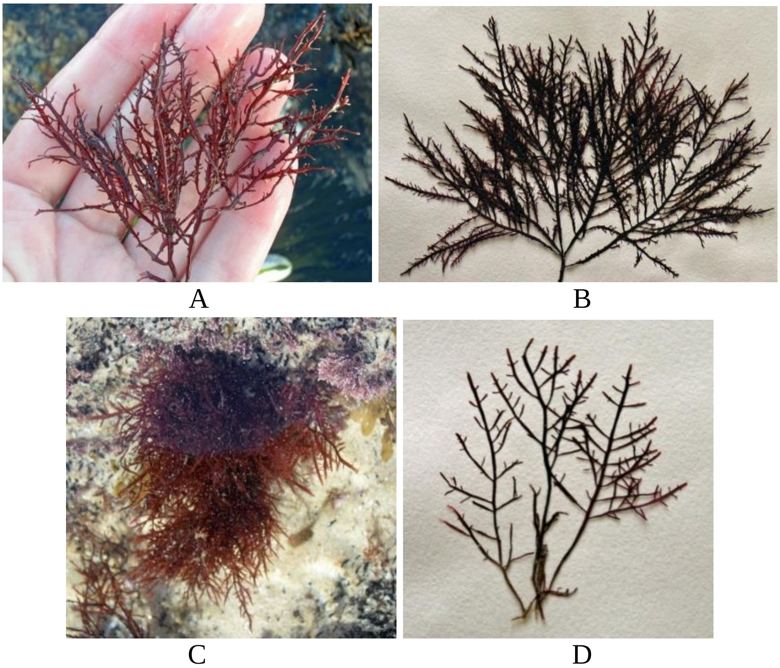


Figure 71. *Gelidium corneum*: (A) Dark red, hard consistency, cartilaginous, and may reach 30 cm in length thalli; (B) branches with obtuse apex and attenuated at the base; this species typically forms dense stands of clumped fronds, often under a Kelp canopy; *Gelidium spinosum*: (C) dark red, hard consistency, cartilaginous, and may reach 30 cm in length thalli. Small alga, cartilaginous, crimson to purplish red, 20-60 mm long; *Pterocladiella capilacea:* (D) main axes distinctly flattened, often narrower at base, ultimate branches short, often opposite, spine-like or spatulate.

Figure 72. *Ahnfeltiopsis devoniensis*: (A) Small red marine alga growing to only several cm in length from a disc-like holdfast; (B) it forms a medium-sized flattened frond with regular dichotomous branching; the branches have parallel sides; the reproductive structures (cystocarps) are internal.

A B

Figure 73. *Caulacanthus ustulatus*: (A) Thalli forming small dense entangled tufts of up to 5 cm, reddish-brown color, which blackens by desiccation, with rough touch; (B) ramifications with acute apices and behaving small triangular-shaped spines (S.M.25X).

A B

Figure 74. *Chondracanthus acicularis*: (A) Dark-red or blackish, sometimes bleached yellow, cartilaginous, cylindrical, erect or re-curved or prostate, branched thalli, with 7 cm long; branches are irregular, curved and sharply pointed; *Chondracanthus teedei*: (B) upper thallus-branches cylindrical, lower ones flattened, dark crimson to black-red; branching repeatedly irregular-pinnate, cartilaginous-firm, lateral branches distant, pointed, the yougest terminal sections thorn-like; discoid base.

Figure 75. *Dilsea carnosa* (drift specimen): Dark red, frequently becoming yellow above, thickest of the foliose red algae in the NE Atlantic, flattened cartilaginous fronds, arising in groups of small, medium and large from a thick, discoid holdfast, obtuse, ovate with tapered base, to 500 mm long, 250 mm broad.

Figure 76. *Gigartina pistillata*: thalli are erect, up to 20 cm tall, dark-red or red-brown, cartilaginous, elastic, dichotomously branched, attached to the substrate through a small disk; presence of external, rounded cystocarps in female gametophyte fronds.

A B

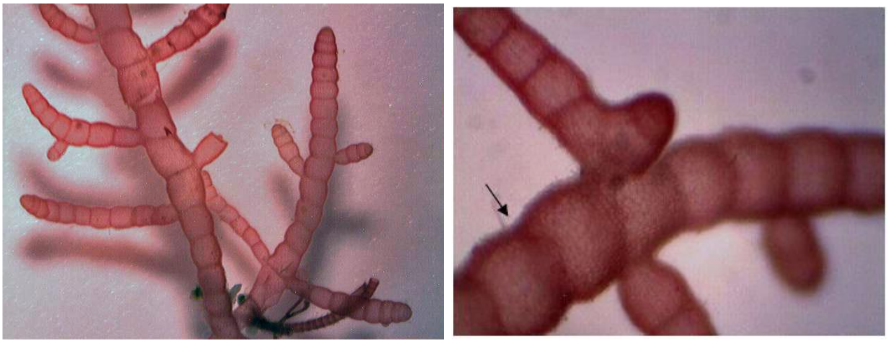
Figure 77. *Sphaerococcus coronopifolius*: (A) Narrow, compressed, two-edged, cartilaginous, scarlet fronds, main axes dark brownish-red, to 300 mm long; (B) branching abundant, distichous, sub-dichotomous or alternate, terminal branchlets acute, fringed with short marginal proliferations.

Figure 78. *Liagora viscida*: Thallus tufted, grey-purple to greenish-white or pink, repeated dense branching at almost the same length, branches terete, tapering towards the top; terminal branches usually spreading as wide-angled bifurcations, moderately calcified, texture flexible-firm [32].

Figure 79. *Peyssonnelia coriacea*: Laminar algae with slightly calcified flat thallus, with wavy margins and subject to the fund by the back with concentric grooves in the margins; its diameter is about 3 cm; color can vary from red to yellow with greenish hues.

A B

Figure 80. *Plocamium cartilagineum*: (A) Narrow, compressed, cartilaginous, bright scarlet fronds, to 300 mm long, tufted, much divided; (B) branching irregularly alternate, pinnules alternately second in twos to fives (arrow), with acute apices, lowest of each set a simple spur, others increasingly strongly pectinate (arrow) (S.M.25X).



A B

Legend: L.M. - Light Microscope; S.M. - Stereo Microscope.

Figure 81. *Champia parvula*: (A) Soft, gelatinous, pinkish red, much-branched fronds, densely matted, with blunt apices, to 100 mm high (S.M.25X); (B) axes segmented, with nodal diaphragms (arrow), segments about as broad as long, filled with a watery mucilage (S.M.45X).

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Chapter 3

POTENTIAL APPLICATIONS OF *ULVA RIGIDA* FOR BIOFUEL AND BIOCHEMICAL PRODUCTION

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ABSTRACT

Problems of environmental deterioration and energy demand could be alleviated by the paradigm shift from fossil to biofuels. Innovative strategies, such as the use of microwave irradiation, sonochemical treatment and solar irradiation were recently developed for the exploitation of biomass for biofuel production. The concept of biomass itself can be understood in an unconventional sense. Apart from terrestrial plant resources, nowadays, seaweed, industrial emissions such as CO_2, and organic remains such as glycogen are being explored as new

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feedstock for biofuels/chemicals production. Our research group in Israel is working on converting biomass (terrestrial and marine) to biofuels (bioethanol) and biochemicals (levulinic acid, furfural, formic acid) using microwave, sonochemical, and hydrothermal methods. Among several types of biomass, marine algae are a promising choice due to several advantages. Marine algae form an abundant and rich source of biomass. Bioethanol production process based on marine algae could be sustainable. A mild sonication-assisted simultaneous saccharification and fermentation (SSF) process for the conversion of *Ulva rigida* to bioethanol in a single step is developed in the current study. Bioethanol is a potential biofuel owing to the similarity of its energy density value (23 MJ/L) to that of gasoline (35 MJ/L). Bioethanol could also be a feedstock for the production of C_2 hydrocarbons. Any progress in the direction of development of a marine-algae-based bioethanol process would open up a new avenue towards sustainable biorefinery. *Ulva rigida*, comprising 37 wt% carbohydrate was used as a feedstock for the SSF process. Initially, saccharification process of *Ulva rigida* (with amylases and cellulases) was carried out under mild sonication conditions (40 kHz, 37°C); 3.1 times higher glucose yield was obtained using sonication of *Ulva rigi*da relative to conventional incubation. The hydrolysate was found to contain glucose exclusively. Subsequently, the SSF process for converting the algae (*Ulva rigida*) to bioethanol in a single step was also accelerated using sonication. The improvement was observed in the total carbohydrate content of the algae using multi-tropic aqua culture. 27-41 times higher specific growth rates were achieved using this approach. Under specific optimal conditions of growth, a starch amount as high as 32 wt% was accumulated. The high-carbohydrate algae were subjected to the sonication- based SSF process. Under optimal process conditions, an ethanol yield as high as 16 wt% was achieved. A unique solar-energy-based continuous flow process for the direct conversion of *Ulva rigida* to bioethanol is outlined. The conversion of macroalgae to the strategically significant chemical, levulinic acid is discussed. In an acid-catalysed hydrothermal process, 12.8 wt% levulinic acid was produced from *Ulva rigida*. We therefore elaborate in this chapter on the unconventional strategies developed for the farming as well as conversion of *Ulva rigida* to biofuels and biochemicals.

Keywords: seaweeds, macroalgae, *Ulva rigida*, bioethanol, SSF, fermentation, sonication, solar energy, levulinic acid, biofuels, biochemicals

1. Introduction

Growing population and increasing standards of living demand additional resources. The emphasis of this chapter is on two major sectors of human well-fare, namely, energy needs and the environment. Petroleum alternatives are an additional resource that would improve the standard of living of human societies. Biomass encompasses a renewable and cost- effective source of petroleum alternatives for fuel and chemical use. The US Department of Energy has listed twelve major chemicals that can be produced from biomass which would act as building blocks for producing a variety of other fuels and chemicals that are used for transportation and materials applications. The term biomass should not be restricted to conventional terrestrial lignocellulosic and algal resources but should be extended to abundant natural resources such as CO_2, another potential carbon feedstock for biorefinery. However, for the sake of brevity, the present compilation is restricted to the exploitation of seaweeds (macroalgae) for the production of biofuels and biochemicals. Oceans make up 70% (2/3rd) of the earth’s surface and offer a wide unused space. Seaweeds possess bioenergy-production potential similar to that of land plants. Thus, cultivating and harvesting huge quantities of seaweed as an energy crop and devising energy-efficient strategies for its conversion to biofuels generate a new alternative energy source that could replace fossil fuels and in turn substantially reduce the CO_2 emissions that lead to global warming and environmental deterioration [1].

Energy insecurity and environmental pollution are the major problems facing mankind in the 21st century. Reduced dependence on fossil-based fuels is the need of the hour. Problems of environmental deterioration and energy demands could be alleviated by the paradigm shift from fossil fuels to biofuels. Innovative methods of activation, such as the use of microwave irradiation, sonochemical treatment and solar irradiation were recently developed for the exploitation of seaweeds for biofuels production. Among several types of biomass, seaweeds (marine algae) are a promising choice owing to several advantages such as (i) absence of lignin which is a hindrance to hydrolysis and fermentation, (ii) absence of food vs fuel conflict, (iii) absence of the requirement of land for growing the biomass and (iv) availability of vast sea shores (2/3 of Earth's surface is ocean). Marine algae forms an abundant and rich source of biomass and biofuels (especially, bioethanol) so that production processes based on marine algae could be sustainable.

Algal biomass is classified into two main groups, namely, microalgae (bluegreen algae, dinoflagellates, bacilloriophyta) and macroalgae (seaweeds like green, brown and red algae). Red algae (*Gelidum*, *Palmaria*, *Poryphyra*) capture light with the reddish protein pigments (phycoerythrins) and as a result have the characteristic reddish colour. Green algae (*Ulva, Codium*) have chlorophyll as the light-absorbing pigments. Brown algae (*Laminaria*, *Fucus, Sargassum*) contain the carotenoid, fucoxanthin, as the dominant light-capturing pigment. Brown and red algae can grow deeper than the green algae. Most seaweeds grow bound to the substrate (rocks near-shore or artificial surfaces) except certain species like *Sargassum* that float freely on the surface of water. Microalgae are a rich source of highly bioactive compounds found in marine resources. Red algal seaweeds are an active metabolite. The conventional use of seaweeds includes human food resources and as a source of gum (phycocollides such as agar agar, alginic acid and carrageenam) [2]. Unconventional applications being explored include biofuels and biochemicals.

Seaweeds are multicellular, macroscopic marine algae classified as non-vascular plants. Global production of seaweeds is more than a million tons (dry weight matter) relative to no more than 20,000 tons of microalgae production. As a result, the cost of macroalgae is nearly ten times lower than that of microalgae. Macroalgae have higher carbohydrate and lower protein and lipid contents compared to microalgae and so the macroalgal seaweeds are an ideal candidate for bioethanol and biochemicals production via the conversion of the carbohydrate component. The carbohydrate content in macroalgae can be up to 80% organic matter (ash-free dry weight) with about 20% protein and 15% lipids. Depending on the method of analysis, growth conditions of the seaweeds and the species under study, the composition varies [3].

1.1. Seaweeds as Biofuel Feedstock

The macroscopic nature of the seaweeds allows simple and low-cost harvesting. Methane, bioethanol and biobutanol are the three common fuels that can be derived from macroalgae. *Ulva species* have relatively high carbohydrate content and can be readily digested to methane gas and subjected to simultaneous scharrification and fermentation (SSF) processes for bioethanol production. As early as 1960's, Howard Wicox suggested the production and exploitation of macroalgae as a solution to the energy crisis and global warming [4]. During the period from 1970-1980, extensive research efforts were devoted in the US to developing open-ocean macroalgae farms as a substitute to natural gas. Despite nearly half a century of research in the area of macroalgae conversion to biofuels, the R&D is still at the initial stage with several practical challenges to be surmounted. The major challenge pertains to the economically competitive sustainable production of the macroalgae. The extension of cultivation systems to deeper, less protected ocean areas without anchoring facilities is a challenge. Even in the cultivation systems near the shore, the production costs are high and major breakthroughs in the cultivation of algal seaweeds are awaited. In spite of these hurdles, beyond doubt, seaweed holds the promise of a sustainable feedstock for food, feed, fuels and chemical production. Motivated by the challenges and rewards, during the last decade, there has been renewed interest in the exploitation of seaweeds for biofuel production with the growing energy demands.

Japan's efforts (Ocean Sunrise Project) were focused on the harvesting of *Sargassum fulvellum* and its conversion to bioethanol in unused maritime areas around Japan [1]. A farming technology was used for *Laminaria* and *Undaria pinnatifida* in both coastal and off-shore areas. The aim of the project is to produce seaweed bioethanol by farming and harvesting *Sargassum horneri*, utilizing the world's sixth largest (4.47 million km^2) areas of the exclusive economic zone (EEZ) and maritime belts of Japan. The research was conducted by Tokyo Fisheries Promotion Foundation. Japan's long-term plan is to proudce 6 million kL of biofuel by 2030. The key to achieving the target lies in farming large quantities of seaweed at low cost.

The UK and Ireland's BioMara joint project aims at the production of sustainable fuels (methane and ethanol) from marine biomass by studying the process feasibility and economics. The objectives of the project comprise of chemical characterization of selected seaweeds (*Laminaria digitata* and byperborea, *Alaria esculenta*, *Saccharina latissima*, *Palmaria palmata* and *Ulva lactuca*), pretreatment (thermal/enzymatic), and fermentation. Utilization of the alginate component of the seaweed by employing alginate lyase for breaking down the alginate matrix exposes the polysaccharide. The action of alginate lyase is similar to hemicellulase. 20% enhancement in ethanol yield was observed upon pretreatment of *Laminaria digitata* with alginate lyase. Successful utilization of alginate component of seaweeds for bioethanol production is projected to enhance the bioethanol yield as high as ~ 400%. German researchers have developed an offshore ring system for farming *Laminaria Saccharina* for food and fuel applications The methodology comprises growing the macroalgae sporophytes in the lab to the appropriate length and then placing them in the ring structures. The ring structural design was found to be stable for offshore farming [5]. China has successfully demonstrated the sustained commercial cultivation of *Laminaria japonica* with yields of ~ 25 t/ha [6]. Similar attempts were made in Korea for the conversion of *Gelidium amansii* to bioethanol [7]; in India for the conversion of *Kappaphycus alvarezii* to bioethanol; in Denmark for the conversion of *Ulva lactuca* to bioethanol [8]. In Israel, Gedanken's group has developed innovative strategies for the farming of carbohydate-rich marine macroalgae *Ulva rigida* and has designed unconventional pathways, based on sonication and solar energy for the single-step conversion of the *Ulva rigida* to bioethanol, which will be descibed in the following sections [9-13].

1.2. Unconventional Strategies for the Farming and Conversion of *Ulva Rigida* to Bioethanol

Bioethanol has relevance to major sectors of human activity such as energy, chemicals, materials and the environment. Bioethanol is a potential biofuel owing to the similarity of its energy density value (23 MJ/L) to that of gasoline (35 MJ/L). Bioethanol production is important not only for transportation applications, but also for its use as feedstock for the production of C_2 hydrocarbons [13]. Fermentation is one of the intrinsic and crucial reactions involved in the conversion of biomass carbohydrate fraction to bioethanol and is usually slow. Glucose fermentation reaction carried out under mild sonication (40 kHz) at 30°C using baker's yeast (*Saccharomyces cerevisiae*) was accelerated by a factor of 2.3. The acceleration of the fermentation process was observed for glucose concentrations as high as 40 wt%. The kinetics of the glucose fermentation reaction was monitered by ^{13}C NMR spectroscopy as well as by measuring the weight loss of the fermentation broth during the course of the reaction. Theoretically, 1 mol of D-glucose yields 2 mol of ethanol and 2 mol of CO_2 during the fermentation process by yeast. The evolution of CO_2 is reflected in the weight decrease which can be correlated to the amount of ethanol produced. However, this method of evaluation is not very accurate as the substrate glucose is not only converted to ethanol and CO_2 but also to the inevitable secondary metabolite glycerol. So another method based on the use of ^{13}C NMR spectroscopy was developed for the evaluation of the kinetics of glucose fermentation. A fairly good correlation between the two methods for the measurement of glucose conversion values and calculation of reaction rate constants of glucose fermentation was observed as depicted in Figure 1.

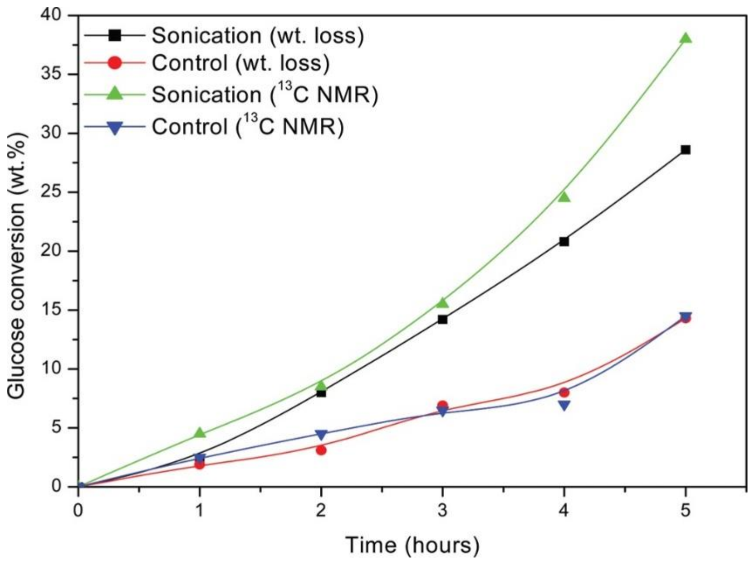
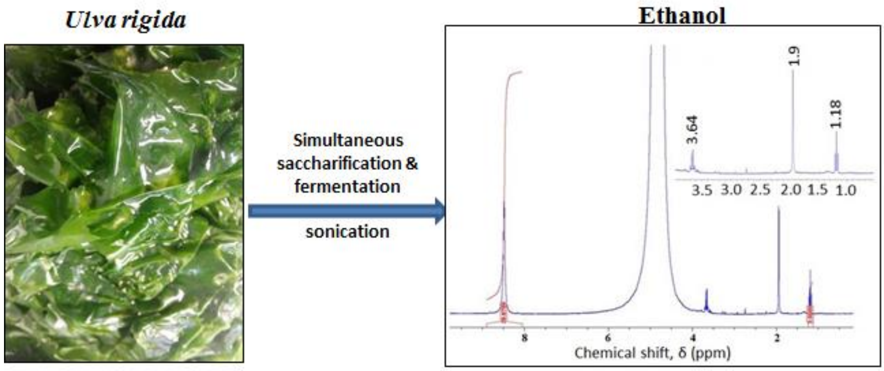


Figure 1. Effect of ultrasound (40 kHz) irradiation on the kinetics of glucose fermentation at 30°C. [Indra Neel Pulidindi, Aharon Gedanken, Rakefet Schwarz and Eleonora Sendersky (2012) Mild sonication accelerates ethanol production by yeast fermentation, Energy and Fuels, 26, 2352-2356]. Adopted by permission of the American Chemical Society.

The glucose conversion values in the fermentation process under mild sonication and conventional stirring at 30°C, measured by the weight decrease of the fermentation broth and by ^{13}C NMR spectra, are shown in Figure 1. The mean rate constant values deduced from weight-loss measurements (13.4×10^{-6} s^{-1}) and ^{13}C NMR analysis (17.3×10^{-6} s^{-1}) are in good agreement. The average of these values is considered as the reaction rate constant for the fermentation of glucose under sonication at 30°C (15.35×10^{-6} s^{-1}) while the corresponding value under conventional stirring at 30°C is 6.67×10^{-6} s^{-1}. The ratio between these values (2.3) is the enhancement factor in the kinetics of glucose fermentation achieved by carrying out the fermentation reaction under sonication.

Extending this methodology for the acceleration of the fermentation process to real biomass (*Ulva rigida*) is a challenge and was attempted successfully [14]. *Ulva rigida* is a common seaweed with the potential for biofuel and biochemical production. Other potential seaweeds reported in literature for bioethanol production include *Gelidium amansi* [15], *Laminaria japonica* [16], *Codium fragile* [17] and *Nizimuddinia zanardini* [18]. The option of seaweeds as feedstock offers several advantages including rapid growth rates of the biomass relative to terrestrial plants: large sea area (70% of earth surface available in principle even though off-shore cultivation is challenging), higher carbohydrate content, higher theoretical ethanol yields, low concentration of crystalline components (like lignin that hinder the action of enzymes and yeast in the hydrolysis and fermentation reactions respectively), no competition with food crops and cultivable land area, serves as a bioremediation crop by lowering eutrophication impact on in-shore waters [19], no requirement of fresh water supply or nutrients [20, 21]. In spite of these advantages, inefficient methods of harvesting, pretreatment, hydrolysis and fermentation processes have resulted in lower ethanol yields from seaweeds, thereby offering wide scope for further research and development. Any progress in the direction of development of a marine algae based bioethanol process would open up a new avenue towards sustainable biorefinery. In view of these aspects Gedanken *et al.* developed a mild sonication-assisted simultaneous saccharification and fermentation (SSF) process for the conversion of *Ulva rigida* to bioethanol [9, 10]. A schematic representation of the sonication based SSF process for the conversion of *Ulva rigida* to bioethanol is shown in Scheme 1.



Scheme 1. Schematic depiction of the conversion of *Ulva rigida* to bioethanol [9].

Ulva rigida, comprising 37 wt% carbohydrate (23.8 wt% cellulose, 7.6 wt% starch, and other components such as ulvan and xylan) was used as a feedstock for the SSF process. Initially, the saccharification process (in the presence of enzymes, amylases and cellulases) of *Ulva rigida* alone was carried out under mild sonication conditions (MRC Clean-01 Ultrasonic cleaner, 40 kHz ultrasound frequency, 120 W ultrasonic power, 37°C) without any prior pretreatment. As a control study, the reaction was studied under identical conditions in an incubator under shaking. The progress of the reaction is monitored by estimating the amount of glucose formed with time (Figure 2). The difference in the glucose yields in the sonication-assisted and control process is more pronounced in the initial period (30 min) of the reaction. A higher (by 3.1 times) yield of glucose is obtained in the sonication-assisted hydrolysis process. The advantage of obtaining higher glucose yield in the hydrolysis process aided by sonication, at any given time, relative to a conventional incubator process under identical conditions is evident from Figure 2. A maximum of 19.6±0.02 wt% glucose is produced in a sonication-based process in 3 h relative to a yield of 16.7±0.27 wt% in an incubation process in 24 h. The accelerated release and improved yield of glucose from *Ulva rigida* upon sonication is due to mechanical and thermal effects.

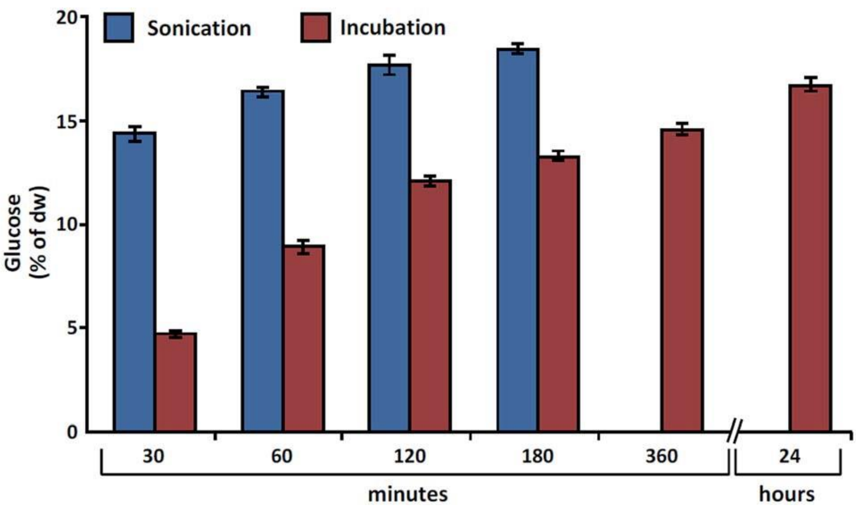


Figure 2. Glucose yields as a function of time in the enzymatic saccharification of *Ulva rigida* under sonication vs incubation at (37°C) [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Single step production of bioethanol from the seaweed *Ulva rigida* using sonication, RSC Advances, 5, 16223-16229]. Adopted with permission from the Royal Society of Chemistry.

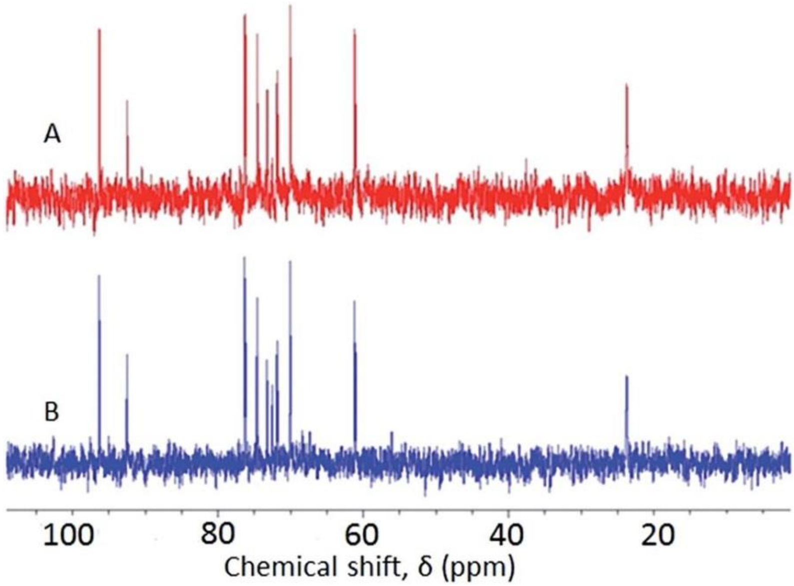


Figure 3. ^{13}C NMR spectra of aliquots from the hydrolysate of *Ulva rigida* produced under sonication (A) and incubation (B) at 37°C at 120 min. [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Single step production of bioethanol from the seaweed *Ulva rigida* using sonication, RSC Advances, 5, 16223-16229]. Adopted with permission from the Royal Society of Chemistry.

The structural rigidity of components of algal biomass is reduced due to sonication. Effective mixing, phase transfer, diffusion of enzymes across the algal cell membranes results in the acceleration of saccharification of *Ulva rigida* during the sonication process [9]. Irrespective of the method of hydrolysis, glucose is produced exclusively as the fermentable sugar. The ^{13}C NMR spectra of the aliquots of the hydrolysate under bath sonication (Figure 3a) and incubation (Figure 3b) at 120 min are shown in Figure 3. Well-resolved intense signals in the range of 60-100 ppm (60.9 (C6), 69.9 (C4), 71.8 (C2β), 73.1 (C2β), 74.5 (C3), 76.2 (C5), 92.4 (C1β) and 96.2 (C1β)) are typical of the α and β isomers of D-glucose. An additional signal at 23.6 ppm is attributed to the carbon nuclei of CH_3COONa used as buffer for the enzymatic hydrolysis of algae.

Further studies were carried out on the single step conversion of *Ulva rigida* (SSF) to bioethanol under sonication. In addition to the algae and enzymes (amylogucosidase, α-amylase, and cellulase), baker's yeast was also added to the reaction medium in glass media bottles with cap (Fisher brand, 100 mL). The progress of the SSF process, under sonication and in an incubator, was monitored by evaluating the amount of ethanol formed at regular intervals of time. The ethanol amount in the analytes was quantified using ^{1}H NMR. The SSF process was also found to be faster in a sonication driven process as shown in Figure 4 [9].

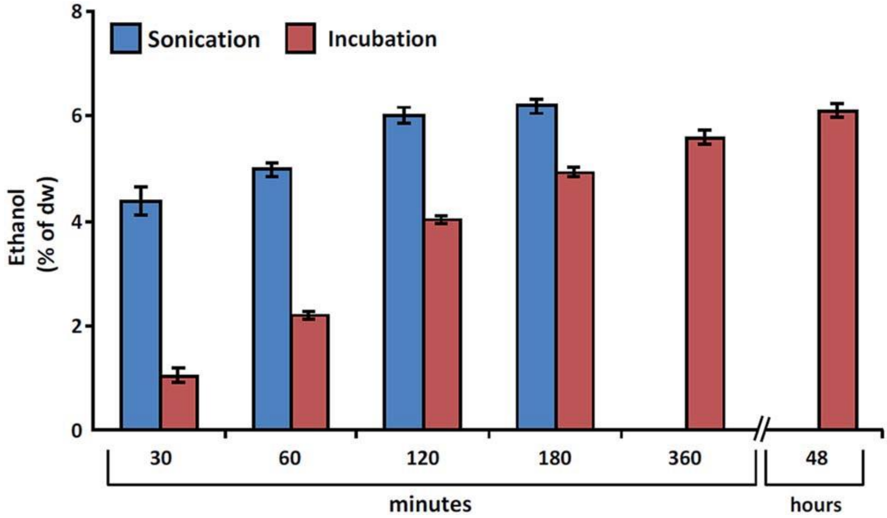


Figure 4. Ethanol yield as a function of time in the enzymatic hydrolysis of *Ulva rigida* under sonication vs incubation at 37°C (replicate no. n = 3; error bars indicate standard deviation, SD). [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Single step production of bioethanol from the seaweed *Ulva rigida* using sonication, RSC Advances, 5, 16223-16229]. Adopted with permission from the Royal Society of Chemistry.

Higher ethanol yields (4.3 ± 0.26 wt%) were observed in a sonication-aided process relative to a process under incubation (1.0 ± 0.13 wt%) in a short duration of 30 min, reaching a maximum value of 6.2 ± 0.13 wt% in 3 h. The 3H (t, 1.18 ppm) and 2H (q, 3.64 ppm) confirm the formation of ethanol from *Ulva rigida* in the SSF product (Figure 5). The signal, 3H (s, 1.9 ppm) corresponds to the buffer (sodium acetate) used. The peak, 1H, s, at 8.5 ppm, is characteristic of the internal standard (HCOOH) used for the quantification of ethanol. Thus an unconventional sonication-based SSF process for the single step conversion of *Ulva rigida* to bioethanol is developed. Compared to a conventional incubation process (4.9 wt% ethanol in 48 h), the current process is faster and the ethanol yield is higher (6.2 wt% ethanol in 3 h) [9].

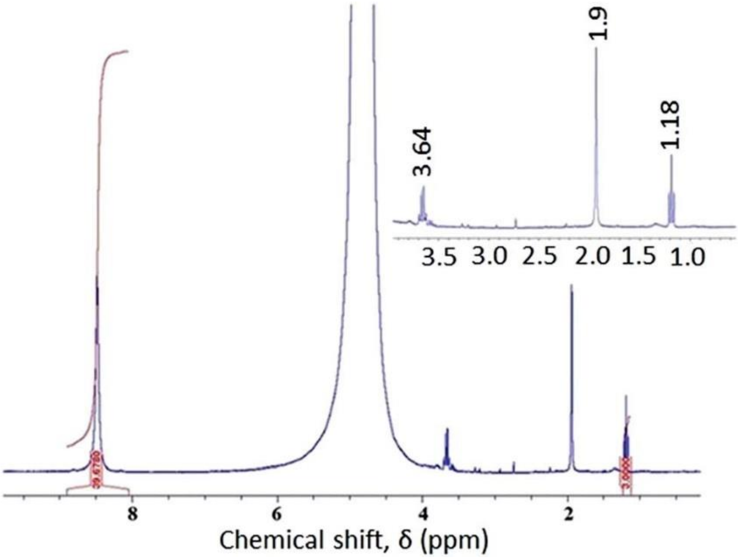


Figure 5. [1]H NMR spectrum of the aliquot of sample collected from the fermentation (SSF) broth under mild sonication at 120 min. [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Single Step Production of Bioethanol from the Seaweed *Ulva rigida* using Sonication, RSC Advances, 5, 16223-16229]. Adopted with permission from the Royal Society of Chemistry.

Table 1. Process efficiency of SSF of *Ulva rigida* in a sonication vs incubation process*[9]

SSF process	Glucose yield (gg^{-1} biomass)	Ethanol yield (gg^{-1} glucose)	Process efficiency (%)
Sonication (t=3 h)	0.196	0.33	64.7
Incubation (t=48 h)	0.173	0.34	66.6

*Reaction conditions: biomass (dry weight) = 1.68 g; distilled water = 40 mL; cellulase = 0.1 g (0.3 units per mg); α-amylase = 40 μL (250 units per mL); amyloglucosidase = 100 mL (300 units per mL); sodium acetate buffer = 40 mL.

Although the SSF process of algae is accelerated upon sonication, the process efficiency (64.7%) remained similar to that of the conventional incubation process (66.6%) (Table 1). Moreover, the bioethanol production process is still a batch process rather than a continuous-flow process with large scope for further improvement.

Typical challenges in the exploitation of seaweed include production in large quantities and also the lower fermentable sugar content available currently. For the commercial-scale production of bioethanol, it is imperative to develop farming strategies resulting in carbohydrate-rich as well as fast growing biomass. Towards attaining this target Gedanken *et al.* developed integrated multi-tropic aquaculture (IMTA) as an alternative farming strategy to the conventional algal mono-culture. The concept of IMTA involves reuse and recycling of internal feed within a culture system minimizing the wastage of resources (nutrients, water and energy). Integrating seaweed farming with aquaculture operations has several advantages. Seaweed turns waste into productive resources and reduces the impact of wastes on the ecosystem. Integration of seaweed cultivation with fed mariculture facilitates recapturing of waste nutrients, leading to increased growth rate, improved fermentable sugar contents in the *Ulva rigida* farmed downstream to fish-culture net pens. So far, the methodology of integrated culture of fish and algae in marine open-waters is focused towards economic and environmental aspects but not towards energy (bioethanol) and chemical (levulinic acid) production which has been the focus of the study of Gedanken's group [10].

The seaweed *Ulva rigida* was co-cultured with fed-fish culture (*Sparus aurata*) in an off-shore fish cage aquaculture complex with the objective of obtaining high-yield carbohydrate- rich biomass. *Ulva rigida* cultivation is carried out in an open sea fish farm (Lev-Yam aqua-culture Ltd) located off the Michmoret coast, Israel. The specific location is shown in the map (Figure 6a).

About 500 g of *Ulva rigida* were placed in nylon net cages (Figure 6e). The cages were attached to buoys and placed at two positions. One site is located within the fish cage surroundings, 15 m downstream to the cages' main water current direction and the control site is located 150 m upstream to the fish cages (Figure 6b). Algal culture cages were placed at a depth of 3 m (Figure 6c). Specific growth rates and the starch content of *Ulva rigida* cultured during Sept/Oct. 2013 (Figure 7 a & c) and during Nov. 2013 (Figure 7 b & d) were shown pictorially in Figure 7. The initial culture or grow-out period is for 14 days and an additional 5 days in a low-nutrient site. Remarkable enhancement in the growth rate of *Ulva rigida* grown under nutrient-rich conditions downstream from the fish cages was observed (Figure 7a & b). The enhancement in the specific growth rates is by a factor of 27 and 41 for the Sept. (Figure 7a) and Nov. (Figure 7b) trials respectively. The availability of inorganic nutrients is the vital parameter facilitating the growth and productivity of seaweed *Ulva rigida*.

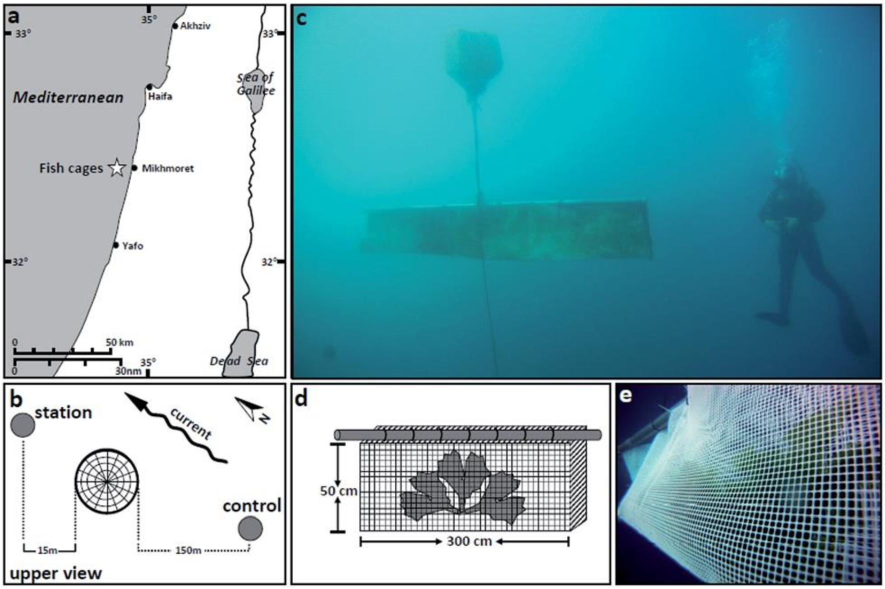


Figure 6. Schematic (a) map of the study area showing the fish farm, (b) scheme of the fish cage and the algal culture cages, (c) algal culture cage suspended at 3 m depth, (d) scheme of an algal culture cage and (e) *Ulva rigida* in culture cage. [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol, RSC Adv 5, 59251-59256]. Adopted with permission from the Royal Society of Chemistry.

In both trials (Sept. Figure 7c and Oct. Figure 7d), the carbohydrate (starch) content was higher at the control site (31.5 wt% of dry weight, Sept. trial) compared to the nutrient- rich site downstream to the cages (24 wt%). This observation is in inverse proportion to the ambient seawater nutrient concentrations. However, the starch contents bounced up and levelled with values similar to those at the control site on culture manipulation for two days at the low-nutrient site. High nutrient concentrations altered the proximate composition in seaweeds and caused a shift to lower levels of starch. Thus, the developed strategy based on IMTA resulted in the accelerated production of *Ulva rigida* with high starch content (31 wt%). The high-carbohydrate *Ulva rigida* was subsequently converted to bioethanol in a sonication-based SSF process.

Under optimized process parameters (enzyme loading, 1 wt%; algal consistency in the broth, 15 wt%, sonication time, 4 h) a high bioethanol yield of 16 wt% is produced (Figure 8).

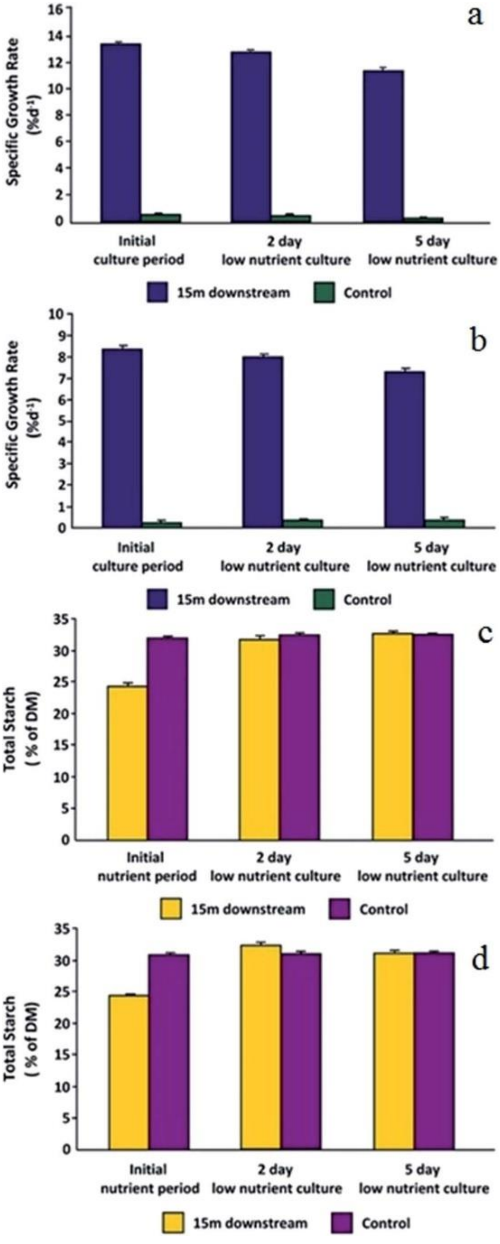


Figure 7. *Ulva rigida* culture and starch content. [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol, RSC Adv 5, 59251-59256]. Adopted with permission from the Royal Society of Chemistry.

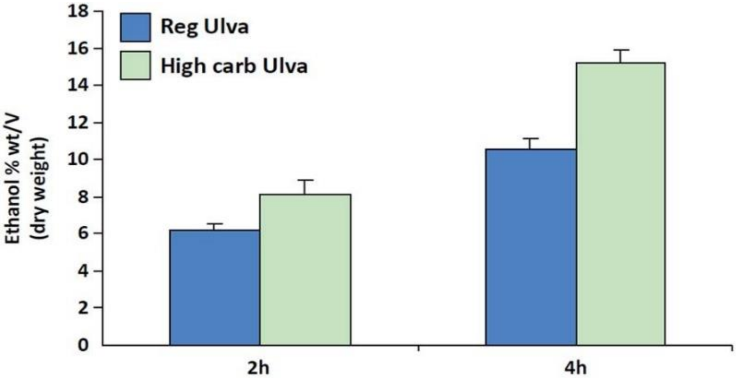


Figure 8. Effect of tailoring the carbohydrate content of *Ulva rigida* on the ethanol yield in the sonication-based SSF process with 15 wt% solid consistency and 1 wt% enzyme loading (replicate no. n = 3; error bars indicate standard deviation, SD). [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol, RSC Adv 5, 59251-59256]. Adopted with permission from the Royal Society of Chemistry.

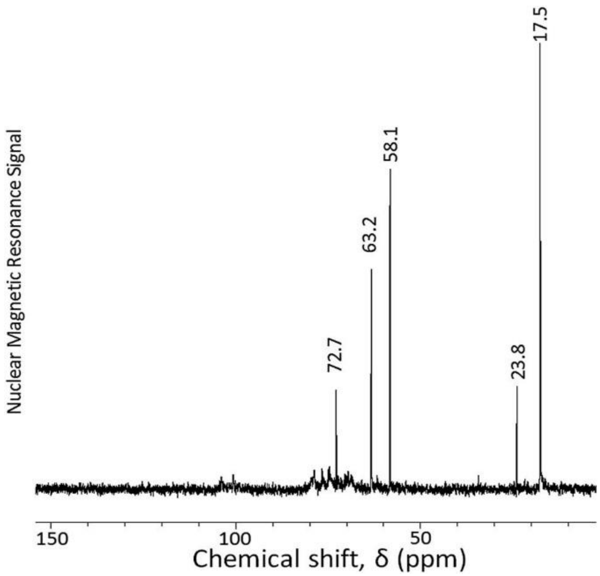


Figure 9. ^{13}C NMR spectrum of aliquot of sample collected from the fermentation (SSF) broth under optimal reaction conditions. [Leor Korzen, Indra Neel Pulidindi, Alvaro Israel, Avigdor Abelson and Aharon Gedanken (2015) Marine integrated culture of carbohydrate rich *Ulva rigida* for enhanced production of bioethanol, RSC Adv 5, 59251-59256]. Adopted with permission from the Royal Society of Chemistry.

Formation of bioethanol from *Ulva rigida* is confirmed by the two characteristic signals at 17.5 and 58.1 ppm typical of ethanol (Figure 9).

Additional signals at 63.2 and 72.7 ppm correspond to the inevitable secondary metabolite glycerol and the signal at 23.8 ppm corresponds to the buffer (CH_3COONa) used in the SSF medium. The absence of signals in the range 60-100 ppm indicates the complete metabolism of glucose by yeast and the effectiveness of the SSF process under sonication. The whole process of farming the high-carbohydrate *Ulva rigida* by the integrated multi-tropic aquaculture (IMTA) and its subsequent conversion to high-concentration bioethanol in a single-step sonication-assisted SSF process is represented in Scheme 2.

Scheme 2. Strategy adopted for the production of starch-rich *Ulva rigida* and its conversion to high-concentration bioethanol [10].

1.3. Continuous-Flow Solar-Energy-Driven SSF Process for the Conversion of *Ulva Rigida* to Bioethanol

The exploitation of solar energy, which is abundant, renewable and environmentally friendly, for the production of bioethanol is an innovative idea. Successful utilization of solar energy for bioethanol production from biomass has the potential to solve the fuel-shortage problem. Utilization of solar energy for the production of bioethanol has never been tested experimentally so far. Gedanken's group was the first to envisage the prospects as well as potential benefits in using solar radiation for biofuel production [22]. Using solar energy for bioethanol production has economic, energy and environmental benefits. The product, bioethanol, has diverse applications apart from being a potential transportation fuel. Bioethanol could act as an economic driver for the upcoming biorefinery industry as it could be a feedstock for alkanes as well as ethers. Moreover, the solar-energy-driven bioethanol process is sustainable owing to the abundance of solar radiation and also because of the wide range of biomass that could serve as feedstock for carbohydrates that are converted to bioethanol in the process of SSF. The feasibility of using the bioethanol produced in a solar-energy-driven process (from glucose and starch as feedstock) was demonstrated for generating electricity from fuel cells, and the performance of the fuel cells was on a par with commercial ethanol of similar concentrations. Such an application is the first of its kind in the literature and can be regarded as a breakthrough and prelude to several other applications (in the field of transportation sector and chemical industries) which are currently unanticipated [12, 13]. The use of this alternate green renewable resource may provide a solution to meet the growing energy demands. The utilization of solar thermal energy for biofuel production has a significant impact on the overall energetics (energy return on energy invested, EROEI) of the process. The exploitation of solar energy for the direct conversion of biomass to bioethanol has not been attempted before and hence the invention has potential for easy adaptation by industry. Moreover, the unique design of the solar reactor (Figure 10) not only accelerates the simultaneous saccharification and fermentation (SSF) process for the conversion of biomass to bioethanol but also facilitates the *in situ* separation of the ethanol formed in the broth by an evaporation-condensation process [11].

A typical continuous-flow process for the solar-aided conversion of an aqueous suspension of *Ulva rigida* to bioethanol consists of feeding the algal solution (500 mL, 5 wt%, prepared by ultrasonication) mixed with specific amounts of enzymes (α-amylase, amyloglucosidase, endo-cellulase, exo-cellulase, and β-glucosidase) into the fermentation chamber of the solar reactor (at a flow rate of 3.9 mL/h) loaded with instant baker's yeast (*Saccharomyces cerevisiae*) covered with activated carbon cloth. In this solar-energy-driven continuous-flow SSF process, the enzymes led to the hydrolysis of starch and cellulose components of the algae to glucose which was further fermented by the yeast and converted to ethanol. When 500 mL *Ulva rigida* feedstock was completely fed to the reactor, DDW was flown through the reactor (with the same flow rate, 3.9 ml/h) in order to convert the residual carbohydrates of the algae to bioethanol. When the DDW was completely fed, additional 500 mL of the feedstock was fed to the reactor to test the reusability of the enzymes and the yeast bed as well as the continuous operability of the system. Again, when the feedstock was completely fed to the reactor, DDW was allowed to flow in order to convert the residual carbohydrate content of the algae to bioethanol. It is important to note that similar bioethanol results were observed when the same experiment was repeated by loading the enzymes and the baker's yeast on activated carbon prior to SSF process.

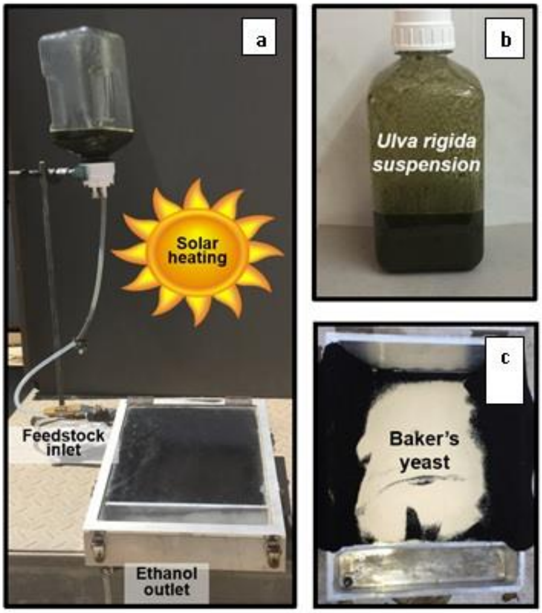


Figure 10. Continuous-flow solar reactor for the single-step conversion of marine macroalgae *Ulva rigida* to bioethanol; (a) complete experimental setup, (b) stable 5 wt% aqueous *Ulva rigida* suspension with enzymes, and (c) fermentation chamber loaded with baker's yeast on activated carbon cloth [11].

1.3.1. Time-on-Stream Studies of Solar-Energy-Driven Bioethanol Production from Continuous-Flow SSF of Ulva Rigida

The continuous-flow SSF process of 5 wt% *Ulva rigida* was monitored for 37 days in the solar reactor (with the same enzymes and the yeast) at 31/24°C average day/night temperature. The fermentation was continuous; most of the evaporation occurred during the day and only a negligible amount occurred at night. The aliquots of products were collected at regular time intervals and quantified for ethanol using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy and high performance liquid chromatography (HPLC). The ethanol yield (deduced from ^{1}H NMR analysis) as a function of time is depicted in Figure 11.

High ethanol yields (84.2 wt% of the theoretical ethanol yield) were observed throughout the SSF process of *Ulva rigida* (7.4 g ethanol in 37 days, 2.64 g ethanol/day/m^2), based on the fermentable sugar content of the biomass. Thus the solar-aided conversion of *Ulva rigida* to bioethanol is highly energy- and atom-efficient.

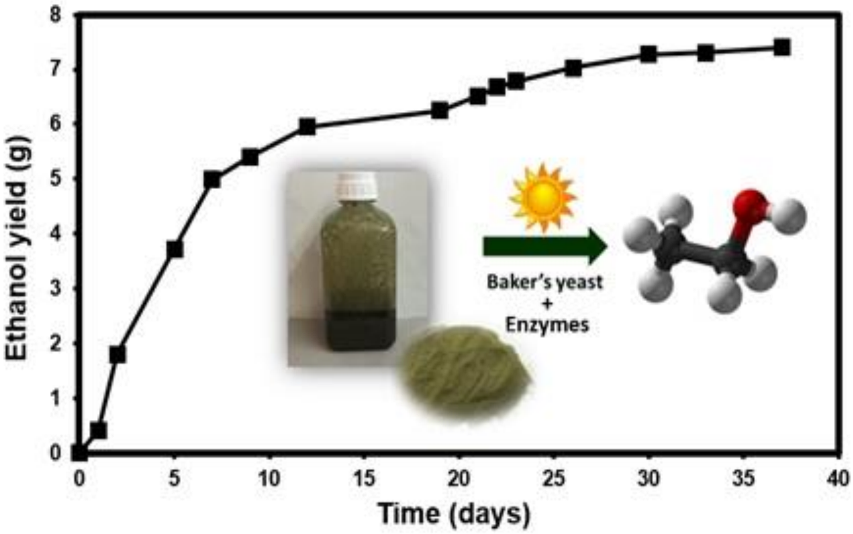
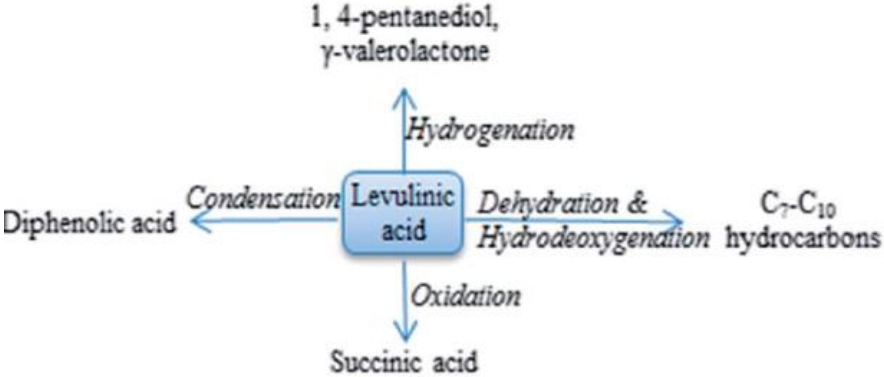


Figure 11. Time-on-stream studies of solar-energy-driven bioethanol production in a continuous-flow SSF process of *Ulva rigida* (5 wt%) ($T_{average}$ day/night: 31/24°C) [11].

1.4. Can Seaweeds Be a Sustainable Feedstock for the Production of Levulinic Acid, a Key Economic Driver for the Biorefinery?

Levulinic acid is a chemical of strategic significance. In fact, production of levulinic acid from cellulose would be more advantageous than converting cellulose to bioethanol. The theoretical yields of levulinic acid and bioethanol from cellulose are 64 [23] and 51 wt% [14] respectively. Not only is the atom efficiency of levulinic acid production process higher than that of the bioethanol production process, but also owing to the multifunctionality of levulinic acid, it could be a building block for several other fuel-grade chemicals such as γ- valerolactone (Scheme 3).

Although the production of levulinic acid has been attempted from a variety of carbohydrate feedstock including direct conversion of widely available agricultural wastes such as *cicer arietinum*, cotton, *pinus radiata*, sugar cane bagasse and rice straw [23, 24], very few attempts have been made for the exploitation of seaweeds as feedstcok for levulinic acid production [25, 26]. Gedanken's group made preliminary studies towards the utilization of the seaweed, *Ulva rigida*, for the production of levulinic acid. An acid (HCl, 3 M) catalyzed hydrothermal process (150°C, 3 h) yielded 12.8 wt% levulinic acid from *Ulva rigida*. However, the process needs to be optimized further with respect to reaction temperature, reaction time, and acid concentration to obtain improved yields of levulinic acid. Using marine algae for levulinic acid production is advantageous and makes the process sustainable. In addition to conventional mineral acid, alternate solid acid catalysts such as (polyoxometallates) need to be developed for the conversion of *Ulva rigida* to levulinic acid.



Scheme 3. Reactivity of levulinic acid making it a key strategic chemical [Amudhavalli Victor, Indra Neel Pulidindi, Aharon Gedanken (2014) Levulinic acid production from *Cicer arietinum*, Cotton, *Pinus radiata* and Sugar cane bagasse, RSC Advances, 4, 44706-4471]. Adopted with permission from the Royal Society of Chemistry.

The design of diverse process pathways for product diversification makes the biorefinery sustainable. Thus strategies should be developed for converting the carbohydate component of seaweeds to various products apart from levulinic acid and bioethanol by devising innovative strategies for improving the yields and reducing the process severity. For instance, in principle, the theoretical limit on the yield of bioethanol from cellulose can be raised from 51 wt% by capturing and reusing the CO_2 evolved in the fermentation process by charging the fermentation broth with cyanobacteria that could be metabolizing CO_2 and synthesizing glycogen which could be further converted to bioethanol. Such efforts need to be made in near future. Likewise, the current commercial levulinic-acid production processes use conventional mineral acid as catalyst. However, the process could be environmentally benign as well as profitable if a reusable solid acid catalyst like activated-carbon-supported heteropoly acids is employed [24]. Development of such pathways requires further research. In addition, the inevitable reaction byproduct of the bioethanol production process, glycerol, could be a valuable feedstock for the production of propane diols which are nearly 20 times more costly and value added [27]. Conversion of glycerol to valuable chemicals is another avenue in biofuel production from seaweed.

CONCLUSION

Typical challenges in the farming and conversion of seaweeds to biofuels and biochemicals are outlined. Solutions, such as the adoption of integrated multi-tropic aquaculture for the effective utilization of resources and production of high carbohydrate (32 wt%) *Ulva rigida* with accelerated growth rates (by a factor of 41), were offered. In addition, unconventional methods, such as ultrasound irradiation and solar energy for the simultaneous saccharification and fermentation (SSF) of seaweeds to bioethanol were demonstrated successfully. The marine seaweed, *Ulva rigida*, is a potential feedstock for the high-yield production of bioethanol (16 wt%) and levulinic acid (12.8 wt%). Solar-energy-driven continuous-flow process for the SSF of *Ulva rigida* to bioethanol is appealing for industrial adaptation owing to its atom and energy efficiency.

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BIOGRAPHICAL SKETCHES

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Patents:

1. A. Gedanken, P. Indra Neel, T. Betina, Solar-aided conversion of marine algae and Biomass to bioethanol, US Provisional Patent Application No 62450107, 25th January 2017.

Book chapters:

1. Betina Tabah, Indra Neel Pulidindi, Venkateswara Rao Chitturi, Leela Mohana Reddy Arava, Aharon Gedanken (2017) Solar-energy driven bioethanol production from carbohydrates for transportation applications, in Solar Energy: Systems, Performance and Recent Developments, Book chapter, Nova Science Publishers, Inc.
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Publications Last 3 Years:

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Honors:

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Publications in the last three years:

1. Tabah, B., Pulidindi, I. N., Chitturi, V. R., Arava, L. M. R., Gedanken, A. Solar-energy driven bioethanol production from carbohydrates for transportation applications. In *Solar Energy and Solar Panels: Systems, Performance and Recent Developments*; Joel G. Carter, Ed.; Energy Science, Engineering and Technology Series; Nova Science Publishers, Inc.: New York, 2017; pp 1-66.
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Education: Mrs. Amudhavalli received her B.Sc. and M.Sc. degrees in Pharmacy from the Tamil Nadu Dr M G R Medical Univeristy. Mrs. Amudhavalli carried out her doctoral studies in the field of renewable energy under the supervision of Prof. Aharon Gedanken and submitted her doctoral thesis titled “Can biomass be a sustainable feedstock for biorefinery?” to the Department of Chemistry, Bar Ilan University, Israel on 22/09/2016.

Address: Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel 5290002.

Professional Appointments:

Mrs. Amudavalli was a doctoral researcher at the department of Chemistry, Bar Ilan University from 01/03/2013 to 22/09/2016. She has six years (2006-2012) of teaching experience as a lecturer at the SRM college of Pharmacy, SRM University, Chennai, India.

Research and Professional Experience:

Environmental deterioration and energy insecurity are the vital challenges society is facing currently. Mrs. Amudhavalli’s research emphasis is on developing alternate chemical and electrochemical energy sources that are renewable and sustainable. Utilization of natural and abundant resources (biomass, agricultural and forest wastes, food wastes, kitchen wastes, municipal wastes, CO_2 and solar energy) for attaining this objective is one of the strategies that she has adopted. Currently, she is developing microwave, sonochemical and solar energy based strategies for the production of biofuels and biochemical, especially from lignin. Also her interest is centered around activation of CO_2 via chemical and photochemical means. Another area of her research focus is to develop cost effective and selective sensors for biomolecules like dopamine that have relevance to health sector. She has 3 peer-reviewed publications to her credit during the last four years of research in the area of renewable energy.

Publications in the Last Three Years:

1. Amudhavalli Victor, Indra Neel Pulidindi, Tae Hyun Kim, Aharon Gedanken, (2016) Design of a selective solid acid catalyst for the

optimization of glucose production from Oryza sativa straw, *RSC Adv*., 6, 31.

2. Amudhavalli Victor, Indra Neel Pulidindi, Aharon Gedanken (2015) Assessment of holocellulose for the production of bioethanol by conserving *Pinus radiata* cones as renewable feedstock, *Journal of Environmental Management*, 162, 215.
3. Amudhavalli Victor, Indra Neel Pulidindi, Aharon Gedanken (2014) Levulinic acid production from *Cicer arietinum*, cotton, *Pinus radiata* and sugarcane bagasse, *RSC Adv*., 4, 44706.

Prof. Alvaro Israel

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Education: Prof. Israel received his M.Sc. and Ph.D. from Tel Aviv University.

Research and Professional experience:

After his post-doctoral research at UCLA, US, he joined the Israel Oceanographic Institute in Haifa, Israel in 1992 where he is currently a senior scientist and Head of Department of Marine Biology. He contributed to the understanding of photosynthesis in seaweeds in response to environmental factors particularly those related to climate change. He lead several national and international projects in marine macroalgae cultivation and biotechnology including development of land- and sea-based cultivation systems. Israel's research interests include carbon fixation and ecology of marine algae, taxonomy of seaweeds applied phycology and renewable energy (biomass conversion to biofuels and biochemicals). Israel has published over 60 papers in peer-reviewed journals.

Publications in the last three years:

1. Korzen L, Indra Neel Pulidindi, Israel A, Abelson A, Gedanken A. (2015) Single step production of bioethanol from the seaweed *Ulva rigida* using sonication, *RSC Advances*, 5, 16223-16229.
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3. Israel A, Gedanken A, Shechter M, Abelson A, Zemach-Shamir S, Korzen L, Krupnik N, Qarri A, Peled Y. (2015). About bioethanol production from marine macroalgae in Israel. *Ecology and Environment*, 6, 211-216.

Prof. Avigdor Abelson

Affiliation: Tel-Aviv University

Education: Prof. Abelson received his M.Sc. and Ph.D. from Tel Aviv University, Israel.

Affiliation: Department of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel

Research and Professional Experience:

1. Restoration Ecology; restoration of marine ecosystems
2. Ecological processes and principles of benthic marine environments, with emphasis on human impact on marine ecosystems
3. Artificial Reefs: Planning, design and implementation
4. Settlement and recruitment of benthic organisms
5. Development and implementation of bio-monitoring methods (from molecular to community levels).
6. Marine Protected Areas (MPAs) and community-based management
7. FADs (Fish Aggregating Devices) and Fishery Management
8. Sustainable aquaculture - Integrated Multi-Trophic Aquaculture (IMTA)
9. Coral reef ecology
10. Marine bioinvasion – Invasion and introduction of exotic marine organisms and their environmental impact on marine communities

Publications in the last three years:

1. Korzen, Leor, et al. "Single step production of bioethanol from the seaweed Ulva rigida using sonication." RSC Advances 5.21 (2015): 16223-16229.
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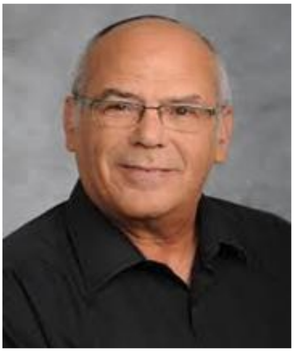

Prof. Aharon Gedanken

Affiliation: Bar-Ilan University

Education: Prof. (Em.) Gedanken received his M.Sc. from Bar-Ilan University and his Ph.D. from Tel Aviv University, Israel.

Address: Department of Chemistry, Bar-Ilan University, Ramat-Gan, Israel 5290002.

Professional Appointments: Emeritus Professor at Bar-Ilan University**.**

Research and Professional Experience: After his post-doctoral research at USC, USA, Prof. (Em.) Gedanken returned to Bar-Ilan University in 1975 as a senior faculty in Chemistry Department. He was a visiting scientist at AT&T Bell Laboratories several times in 1980-1988, and at NIDDK, NIH, USA in the summers of 1989-1991. **In 1999-2001, he was the** chairman of national committee for strategic studies in advanced materials and chemical technologies. In the EC program FP7, he was the Israeli representative to the Nanotechnology, Materials, and Processes (NMP) committee. He was also a partner in 12 EC projects, in which two of them were coordinated by him. From 2012 to 2016, he was a visiting chair professor in Department of Materials Science and Engineering, NCKU, Taiwan. Gedanken's research interests include sonochemistry, surface coating, synthesis of nanomaterials, microwave superheating, synthesis reactions under autogenic pressure at elevated temperatures, fuel cells, renewable energy (biomass conversion to biofuels), carbon materials, sensors, medicinal chemistry, and polymers. Gedanken has published over 725 papers in peer-reviewed journals, has 38 patent applications, and his h-index is 87.

Honors: He is the recipient of 2009 Israel Vacuum Society and 2012 Israel Chemical Society awards of excellence in research.

Publications in the last three years:

1. Graphene-Based "Hot Plate" for the Capture and Destruction of the Herpes Simplex Virus Type 1. A. R. Deokar, A. P. Nagvenkar, I. Kalt, L. Shani, Y. Yeshurun, A. Gedanken, R. Sarid, Bioconj. Chem., DOI: 10.1021/acs.bioconjchem.7b00030, (2017).
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3. Achievement and assessment of direct electron transfer of glucose oxidase in electrochemical biosensing using carbon nanotubes, graphene, and their nanocomposites. J. H. T. Luong, J. D. Glennon, A. Gedanken, S. K. Vashist, Microchimica Acta, 184 (2), 369-388, (2017).
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25. Can r-Graphene Oxide Replace the Noble Metals in SERS Studies: The Detection of Acrylamide. E. Segal, A. Gedanken, Environ. Chem. 13, 58-67, (2016).
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32. Utilization of solar energy for continuous bioethanol production for energy applications. B. Tabah, I. Pulidindi, V. R. Chitturi, L. M. R. Arava, A. Gedanken, RSC Advances 6, 24203-24209, (2016).
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53. Single Step Production of Bioethanol from the Seaweed Ulva rigida using Sonication. L. Korzen, I. Pulidindi, A. Israel, A. Abelson, A. Gedanken, RSC Advances, 5, 16223-16229, (2015).
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Chapter 4

MACROALGAE FOR FUNCTIONAL FEED DEVELOPMENT: APPLICATIONS IN AQUACULTURE, RUMINANT AND SWINE FEED INDUSTRIES

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ABSTRACT

Plant and animal derived products are the main ingredients currently used by the feed industry to produce concentrate feed. There is a need of novel feed ingredients to meet the demand of high quality products by the aquaculture, ruminant and swine production systems, together with the challenge of implementing new sustainable and environmentally friendly

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processes and ingredients demanded by the modern society. Macroalgae are a large and diverse group of marine organisms that are able to produce a wide range of compounds with unique biological properties. This chapter discusses the incorporation of macroalgae or macroalgal derived ingredients as a source of both macro-nutrients (i.e., proteins, polysaccharides and fatty acids) and micro-nutrients (i.e., minerals and pigments) for animal feed production. The biological health benefits of the macroalgal ingredients beyond basic nutrition for the development of functional feed in the aquaculture, the ruminant and the swine sectors are also discussed together with the industrial challenges of its application.

Keywords: macroalgae and seaweed, nutrition, feed, functional feed, aquaculture, ruminant, swine

1. INTRODUCTION

The global human population is projected to grow to 9.6 billion individuals by 2050 and will continue to grow up to 12 billion by 2100 (Gerland et al., 2014). Within this scenario an increase of 70% in animal production and thus 235% more animal feed will be needed to sustain that growth (Herman & Schmidt, 2016). The animal feed industry faces the challenge to meet the demand of high quality products by the aquaculture, ruminant and swine production systems, and implementing new sustainable and environmentally friendly processes and ingredients demanded by the modern society. There is a need to find new alternatives to traditional animal feed ingredients that could compete in the market in terms of nutritional quality (i.e., macro and micro-nutrient composition) and environmentally sustainable (Garcia-Vaquero & Hayes, 2016).

Macroalgae are a diverse group of marine organisms with more than 10,000 different species (Collins et al. 2016). Based on their pigments marine macroalgae are classified as brown (Phaeophyta), red (Rhodophyta) and green macroalgae (Chlorophyta). Marine macroalgae are able to adapt to the changing and extreme marine environmental conditions i.e., salinity, temperature, nutrients, radiation and combination of light and oxygen concentration by producing unique secondary metabolites including proteins, polysaccharides, lipids, pigments and minerals (Collins et al., 2016). Macroalgae are known for their richness in the previously described bioactive molecules with wide variety of biological properties i.e., anti-oxidant, anti-bacterial and anti-tumour amongst others (Holdt & Kraan, 2011). Thus, macroalgae represents great potential for its use in human food and animal feed or for the extraction of biologically active compounds that could be incorporated into the animal's diet.

This chapter discusses the incorporation of macroalgae or macroalgal derived ingredients as a source of both macro-nutrients (i.e., proteins, polysaccharides and polyunsaturated fatty acids) and micro-nutrients (i.e., minerals and pigments) for animal feed production. The nutritional and biological health benefits of the incorporation of macroalgal ingredients for the development of functional feed in aquaculture, ruminant and swine nutrition were discussed together with the advances and challenges for macroalgal incorporation in animal feed.

2. Macroalgae as Source of Macro-Nutrients

Seaweeds have a highly variable composition, with large differences in the final content of both macro- and micro-nutrients depending on multiple factors such as macroalgae species, date of collection and environmental conditions including pollution, water temperature, light intensity and nutrient concentration in water (Mišurcová, 2011).

2.1. Macroalgal Proteins

Macroalgae species and the season of collection are the most common factors affecting both macroalgal protein content and amino acid composition (Joël Fleurence, 1999). The protein content described in brown macroalgae is generally low in comparison with green (10-26%) and red macroalgae species (35-47%) with protein contents comparable to protein-rich foods such as soybean, cereals, eggs and fish (Garcia-Vaquero & Hayes, 2016). Seasonal variations were appreciated in red macroalgae, with higher protein concentrations in the biomass harvested during the winter season (approximately 22%) when compared to the summer period (~ 12%) (Galland-Irmouli et al., 1999).

In addition, most seaweed species are considered a rich source of essential amino acids (Garcia-Vaquero et al., 2016) and acidic amino acids such as aspartic acid and glutamic acid (Fleurence, 2004). The macroalgae contents of amino acids such as threonine, lysine, tryptophan, cysteine, methionine and histidine in macroalgal proteins are higher than those found in terrestrial plants (Fleurence, 1999).

Together with protein, macroalgae also contains large amounts of non-protein nitrogen (i.e., nitrates), resulting in an overestimation of their protein content when analyzed by traditional laboratory methods. Nitrogen to protein conversion factors of 5.38, 4.92 and 5.13 have been proposed for brown, red and green algae respectively as alternatives to the traditional factor of 6.25 (Makkar et al., 2016).

Table 1. Protein contents (% dry weight) in selected macroalgae described in the literature

Algal species	Protein content (% DW)	Reference
Phaeophyta (Brown macroalgae)		
Laminaria spp.	7	Dawczynski, Schubert, and Jahreis (2007)
	4-8	Schiener, Black, Stanley, and Green (2015)
Sargassum spp.	17	Rodrigues et al. (2015)
	12-14	Yu, Zhu, Jiang, Luo, and Hu (2014)
Undaria pinnatifida	17	Taboada, Millan, and Miguez (2013)
	20	Dawczynski et al. (2007)
Himanthalia elongata	5	Cofrades et al. (2010)
	5	Sánchez-Machado, López-Cervantes, López-Hernández, and Paseiro-Losada (2004)
Rhodophyta (Red macroalgae)		
Porphyra spp.	39	Cofrades et al. (2010)
	33	Taboada et al. (2013)
Gracilaria spp.	19	Rohani-Ghadikolaei, Abdulalian, and Ng (2012)
	10	Tabarsa, Rezaei, Ramezanpour, and Waaland (2012)
Chlorophyta (Green macroalgae)		
Ulva spp.	25	Lee, Chang, and Lee (2014)
	7	Satpati and Pal (2011)
Ulva lactula	11	Tabarsa et al. (2012)
	17	Rohani-Ghadikolaei et al. (2012)

Protein is regarded as the most expensive nutrient in animal feed (Rezaei et al., 2013). Thus, the high protein levels described in macroalgae (Table 1) together with its amino acid profile could suggest its incorporation in animal feed as an alternative source of high quality protein

2.2. Macroalgal Polysaccharides

The total polysaccharide concentrations in macroalgae range from 4% to 76% of dry weight with the highest contents described in *Ascophyllum*, *Porphyra* and *Palmaria* spp., in comparison with green macroalgae (Holdt & Kraan, 2011). Differences in polysaccharide content and composition could be appreciated depending on the macroalgae species, parts of the macroalgae sampled and seasonal variations (Skriptsova et al., 2011; Kim, 2012; Men'shova et al., 2012).

Macroalgae contain a number of complex carbohydrates and polysaccharides in variable amounts depending on the macroalgae species. Brown algae contain alginates, sulphated fucose-containing polymers and laminarin; red macroalgae is a rich source of agars, carrageenans, xylans, sulphated galactans and porphyrans; and green algae contain xylans and sulphated galactans (Makkar et al., 2016).

Marine polysaccharides from seaweeds are an integral part of a globally thriving marine-bio industry. Seaweeds are the most abundant source of polysaccharides including (1) alginate, agar, agarose and carrageenan with commercial applications in the biomedical and pharmaceutical industries (Venkatesan et al., 2015) and (2) laminarin and fucoidan with promising potential in food and animal feed. Fucoidan and laminarin, showed a wide range of biological activities such as anti-inflammatory, anti-microbial, anti-coagulant, anti-adhesive, anti-oxidant, anti-viral, anti-peptic, anti-tumour, anti-apoptosis, anti-proliferative and immunostimulatory, in both *in vitro* and *in vivo* model systems (Hahn et al, 2012; Kadam et al., 2015).

2.3. Macroalgal Fatty Acids

Brown macroalgae typically have the highest total lipid content, followed by green and red macroalgae (Gosch et al., 2012). However, there is considerable variation in total lipid between species and also season i.e., levels of 68.9 mg/g dry weight (DW) and 57.5 mg/g DW were described in different species of green macroalgae within the Bryopsidales order (Gosch et al., 2012). Also high lipid contents were described in winter and spring in *Ulva lobate*, *Egregia menziesii* and *Chondracanthus canaliculatus* (Nelson et al., 2002).

In recent years, lipid composition in marine algae has raised considerable interest due to their high content of polyunsaturated fatty acids (PUFA). PUFA contents varied from 34% of the total fatty acids content in *Porphyra* spp. to 74% in *Undaria pinnatifida* (Dawczynski et al., 2007). Macroalgal PUFA include α-linolenic (18:3 n-3), octadecatetraenoic (18:4 n-3), arachidonic (20:4 n-6) and eicosapentaenoic acids (20:5 n-3) (Kendel et al., 2015). I.e., *Ulva* spp. is unique in comparison with plant and fish derived oils due to high levels of octadecatetraenoic acid, as well as offering essential dietary eicosapentaenoic and docosahexaenoic acids, which are generally absent in terrestrial plants (McCauley et al., 2016). PUFA are considered essential nutritional components in humans and animals, playing an important role in the prevention of cardiovascular diseases, osteoarthritis and diabetes. Additional beneficial health properties include anti-microbial, anti-viral, anti-inflammatory and anti-tumour properties (Kendel et al., 2015).

Recent studies showed the future potential of the controlled cultivation of *Ulva* spp. biomass to generate an algal-based oil for human and/or animal nutrition, as part of a biorefinery process for high value products (McCauley et al., 2016).

3. Macroalgae as Source of Micro-Nutrients

3.1. Minerals

The capacity of macroalgae to accumulate metals depends on multiple factors, but it is mainly related to the bioavailability of the metals in the surrounding water, the uptake capacity of the macroalgae (Besada et al., 2009) and the efficient adsorption of metal/organometallic species from seawater (Besada et al., 2009; Romaris-Hortas et al., 2010).

Important essential minerals for human health, such as iodine, copper, selenium and zinc were found in high proportions in macroalgae. *Laminaria* spp. are known to be the best iodine accumulators among all living systems and the accumulation of iodine can be up to 30,000 times larger than in the surrounding environment (Holdt & Kraan, 2011). Copper, selenium and zinc levels described in macroalgae in Norway showed to be safe when applied the macroalgae as food and feed, with higher levels of zinc described in red and brown macroalgae (Duinker et al., 2016). Reported accumulation of heavy metals in seaweed includes arsenic, cadmium, chromium, nickel, vanadium, mercury, lead, cesium-137, and radium-226 (van der Spiege et al., 2013). Large amounts of arsenic in its inorganic form were found in *Sargassum* spp., while the organic arsenic contents ranged from 38 to 75% (Almela et al., 2002). However, in a recent study the health risk due to the toxic elements in seaweed was estimated and the contribution to total element intake of arsenic, cadmium and lead of macroalgae does not appear to pose any threat to the consumers, although the concentrations of these heavy metals should be controlled to protect the consumers' health (Desideri et al., 2016).

3.2. Pigments

There are three different groups of light harvesting and photoprotective pigments in macroalgae named chlorophylls, carotenoids and phycobiliproteins present in different proportions depending on the macroalgae species (Hallerud, 2014).

Chlorophylls are green lipid-soluble photosynthetic pigments found in all macroalgae species, terrestrial plants and cyanobacteria (Holdt & Kraan, 2011). Chlorophyll a is found in all photosynthetic macroalgae, while chlorophyll b and c are found in green and brown macroalgae respectively (Hallerud, 2014). The increasingly restrictive legislation concerning the origin of food preservatives (anti-oxidants and anti-microbials) and the growing demand for natural compounds, has renewed the interest in anti-oxidants such as chlorophylls from natural sources, instead of chemically synthesised molecules (Guedes et al., 2013).

Carotenoids include carotenes and xanthophylls with relative abundance variable depending on the macroalgae species. Green macroalgae species include β-carotene, lutein, violaxanthin, neoxanthin and zeaxanthin, red macroalgae contain mainly α-and β-carotene, lutein and zeaxanthin and brown macroalgae are a rich source of β-carotene, violaxanthin and fucoxanthin (Holdt & Kraan, 2011). Due to their potential health benefits, the incorporation of carotenoids into functional foods or dietary supplements is a major interest of both consumers and the food industry (Salvia-Trujillo et al., 2013). The beneficial effects of carotenoids are thought to be due to their role as anti-oxidants. Other activities include the pro-vitamin A ability of β-carotene (Holdt & Kraan, 2011), the protective role against eye disease showed by lutein and zeaxanthin (Johnson, 2002) and the promising anti-tumour activities of fucoxanthin (Holdt & Kraan, 2011).

Phycobiliproteins include phycoerythrin, phycocyanin, allophycocyanin, and phycoerythrocyanin. Phycoerythrin levels of 12% DW in *Palmaria palmata* and 0.5% in *Gracilaria tikvahiae* (Fleurence, 2004). Phycobiliproteins showed spontaneous fluorescence, property that is used by the biomedical industries in the development of diagnostic techniques such as fluorescent immunoassays (Harnedy & FitzGerald, 2013). Also, phycobiliproteins showed multiple biological activities i.e., anti-oxidant, anti-inflammatory, anti-viral and anti-tumour (Sekar & Chandramohan, 2008) that could be used in the development of functional foods (Langellotti et al., 2013).

4. Macroalgae as Functional Feed

There is an increased interest in the scientific community to discover new functional foods or functional food ingredients. Functional foods were described as foods or dietary components that may provide a health benefit beyond basic nutrition (Wildman et al., 2016). Several functional food ingredients of different chemical nature have been reported to possess anti-oxidant, anti-bacterial, anti-hypertensive, anti-inflammatory and anti-tumour activities (Wildman et al., 2016). Functional foods could help in the prevention or reduce the progression of many chronic diseases, such as cardiovascular disease, cancer and degenerative diseases (Olaiya et al., 2016).

Animal nutrition (i.e., aquaculture, ruminant and swine) has been traditionally evaluated in terms of productive parameters such as animal weight gain or feed utilization (France et al., 2000). Recently, animal nutrition has gained attention as an effective way to produce functional food ingredients with beneficial health effects that could increase the price of animal products in the market (Siró et al., 2008). In example, the use of supplements in cattle (Rey-Crespo et al., 2014; López-Alonso et al., 2016) or swine feeds (Dierick et al., 2009) to increase the contents of essential trace elements in milk or meat.

In animal nutrition, functional feed has not a clear definition to date (Figure 1). Functional feed for pet animals adopt the definition previously described for functional foods, focusing on the additional health benefits that the functional ingredients could provide to the pets such as reduction of risk of obesity, osteoporosis, colon cancer and inflammatory bowel disease (http://www.petfoodindustry.com/articles/2926-advances-in-functional-petfood-ingredients). However, functional feed in production animals such as aquaculture have been modify to incorporate the important economic benefits of the incorporation of the ingredients in animal production. In this sense, functional feeds were described as dietary ingredients that provide growth, health, environmental and economic benefits beyond traditional feeds (Olmos Soto et al., 2015).

Due to the wide variety and biological properties of the compounds discovered in macroalgae, the incorporation of algal biomass or isolated molecules in functional feed formulation could represents a great opportunity in animal nutrition.

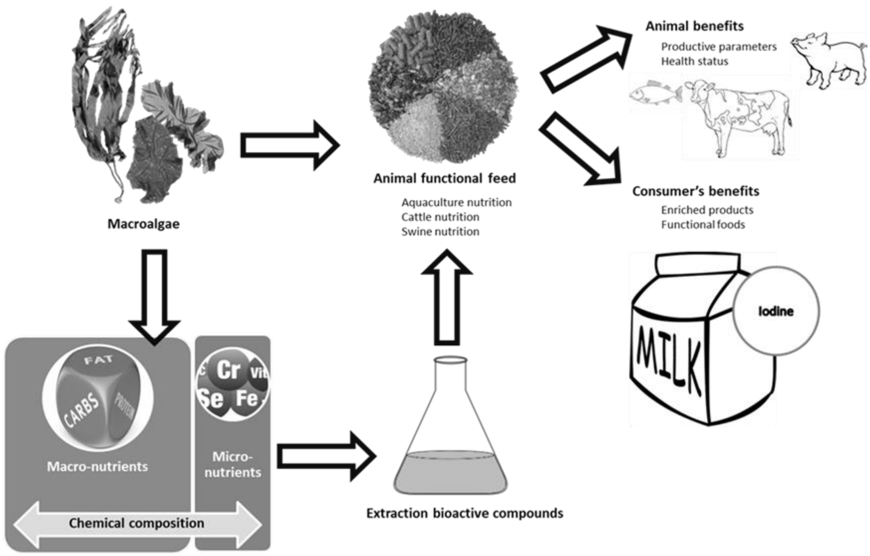

Figure 1. Scheme showing the possibilities for incorporation of macroalgae or macroalgal derived ingredients for the development of functional feeds for animals.

4.1. Aquaculture Nutrition

Aquaculture is the fastest growing sector of the food economy, increasing by more than 10% per year and currently accounts for more than 50% of all shrimp/fish consumed (Olmos Soto et al., 2015). Feeding represents 40-60% of the total production costs in shrimp/fish farming (Olmos Soto et al., 2015) thus, the development of new ingredients or novel feed formulations that could help to reduce the production cost and improved animal health represents a promising field from both scientific and industrial points of view. The use of animal protein sources, such as fish meal in aquaculture feeds is expected to be reduced or completely eliminated as a consequence of increasing economic, environmental and sanitary regulations (Olmos Soto et al., 2015). Macroalgae species with elevated protein content and production rates could be considered as potential novel feed ingredients in aquaculture (Valente et al., 2006).

The partial substitution or inclusion of different percentages of macroalgae into the diet of fish showed promising results improving productive parameters in fish (i.e., growth rates), enhancing animal health (i.e., metabolic rates or response to stress) and increasing certain beneficial compounds in derived animal products (i.e., pigmentation or iodine concentration). *Eucheuma denticulatum* can be efficiently utilized by Japanese flounder juvenile (*Paralichthys olivaceus*) and promote best growth and feed utilization at a level of 3% (Ragaza et al., 2015). The addition of 5% of *Ascophyllum nodosum*, *Porphyra yezoensis* or *Ulva pertusa* to the feed of fingerling red sea bream (*Pagrus major*) increased body weight, feed utilization and muscle protein deposition in comparison with the fish fed a normal diet (Mustafa et al., 1995). *Porphyra dioica* at levels of 10% in rainbow trout's feed showed no negative effects on the growth performance and increased the flesh pigmentation of the fish (Soler-Vila et al., 2009). The inclusion of 5% *Gracilaria* or *Alaria* spp. into the feed of meagre (*Argyrosomus regius*) modulated the metabolic rates and enzymatic responses during a bacterial infection without affecting the growth performance of the fish (Peixoto et al., 2016). Similarly dietary macroalgae supplementation (*Ulva*, *Gracilaria* and *Fucus* spp.) improved the immune and antioxidant responses in European seabass (*Dicentrarchus labrax*) without compromising growth performance of the fish (Peixoto et al., 2016). The inclusion of up to 5% of *Gracilaria vermiculophylla* in diets for rainbow trout (*Oncorhynchus mykiss*) did not affect the growth of the fish. The supplemented fish showed improved flesh quality traits (higher colour intensity and juiciness) and the flesh iodine content on the flesh doubled in comparison with the fish of the control diet (Valente et al., 2015).

Similarly, recent studies used macroalgae in the diet of important aquaculture production systems such as shrimps and molluscs. Commercial feed of marine shrimp (*Litopenaeus vannamei*) could be replaced up to 50% with *Ulva lactuca* as source of protein and lipids without negative effects on the growth performance of shrimps (Pallaoro et al., 2016). The co-culture of juvenile shrimp and green macroalgae *Ulva clathrata* showed increased growth rates, diminished lipids in shrimp carcass and also and higher body carotenoids content in comparison with the animals without co-cultured macroalgae (Cruz-Suárez et al., 2010). In mollusc culture, the culture of macroalgae *Hynea spinella*, *Hynea musciformis* and *Gracilaria cornea* in a biofiltration unit with fishpond waste water effluents was successfully used as feed in juvenile abalone (*Haliotis tuberculata coccinea R.*). The survival and growth rates of juvenile abalone were similar to those raised commercial conditions (Viera et al., 2005).

4.2. Ruminant Nutrition

There is growing interest and evidence of the benefits of using macroalgal biomass in livestock production systems, particularly for ruminants (Machado et al., 2015). The use of extracts from macroalgae *Ascophyllum nodosum* was extensively reported in feed-lot steers (Evans & Critchley, 2014). The potential benefits of the macroalgae extracts include improved carcass characteristics and meat quality (Braden et al., 2007), ruminal organic matter and total tract crude protein digestibility in cattle (Leupp et al., 2005) and color stability and extend beef shelf-life of meat products (Montgomery et al., 2001). Also, the inclusion of 2% of Ascophyllum nodosum extract in feedlot cattle diets showed a reduction in *Escherichia coli* in fecal samples (Braden et al., 2004)

In small ruminants the macroalgae *Laminaria digitata* and *Laminaria hyperborea* biomass could be used as an alternative feed source due to the high organic matter content, digestibility, and rumen dry matter degradability of these macroalgae species (Hansen et al., 2003). The addition of 1% of macroalgal meal to the forage of lambs had no significant influence on relative growth of body components, but it influenced hot carcass weight (Al-Shorepy et al., 2001). Furthermore, the addition of *Ascophyllum nodosum* extract at 2% in the diet of goats improved the anti-oxidant status of the animals exposed to simulated pre-slaughter stress (Kannan et al., 2007).

The use of seaweed to increase mineral content in animal products is currently of interest, especially in relation to increasing the iodine content of foods (Rey-Crespo et al., 2014; López-Alonso et al., 2016). A mixture of three macroalgal species *Ulva rigida*, *Sargassum muticum* and *Sachorhiza polyschides* at 0.5% of the total daily feed intake in organic dairy cattle significantly improved the animals and milk mineral (mainly iodine and selenium) status (Rey-Crespo et al., 2014; López-Alonso et al., 2016). Similarly, the dietary inclusion of *Ascophyllum nodosum* in dairy cows led to an improvement of the iodine content in milk, and to a modification of its microbiota with a positive effect on milk hygiene and transformation (Chaves Lopez et al., 2016).

4.3. Swine Nutrition

Moderate to high amounts of brown macroalgae in the diet may be detrimental to pigs (Makkar et al., 2016) i.e., the inclusion of *Ascophyllum nodosum* at 10% in pigs diets produced weight loss in the animals after several weeks (Jones et al., 1979). However, the incorporation of macroalgal derived polysaccharides, such as laminarin and fucoidan showed promising results used as prebiotics in pigs diets to modulate the microbiota in the digestive tract and immunomodulating properties in pigs (Sweeney et al., 2012; Walsh et al., 2013). *In vivo* studies incorporating laminarin from brown macroalgae in the diet of pigs showed a down-regulation of the expression of inflammatory cytokines in the colon (Sweeney et al., 2012) and mucin gene expression in the ileum and colon (Ryan et al., 2010; Smith et al., 2010). A down-regulation of pro- and anti-inflammatory cytokines was appreciated in the colon of post-weaning pigs supplemented with laminarin and a reduction in *Enterobacteriaceae* counts was appreciated in the animals supplemented with fucoidan (Walsh et al., 2013).

As in the case of ruminants, the use of macroalgae in pigs feed has been proposed to increase iodine concentration in pig meat that could be beneficial for its consumption by deficient population. The organic iodine found in *Laminaria* or *Ascophyllum* spp. is readily metabolized and stored in the muscle, unlike inorganic iodine (Banoch et al., 2010). The addition of 2% of *Ascophyllum nodosum* to the diet of pigs increased the concentration of iodine by 2.7-6.8 in different animal derived products. The consumption of these iodine enriched products could be one safe strategy to help deficient population (Dierick et al., 2009). Similarly, the inclusion of *Enteromorpha* sp. to the diet of pigs did not affect the growth and productive parameters of the animals in comparison with animals supplemented with inorganic salts of copper and zinc. The meat of the animals supplemented with macroalgae showed higher manganese (49%), but also slight increases in iron (13%), copper (12%) and zinc (4%) when compared to the meat of the piglets supplemented with inorganic salts (Michalak et al., 2015).

5. FUTURE PROSPECTS

Crop cultivation induces a significantly high carbon debt and high water consumption, thus, terrestrial biomass seems not to be sustainable at present due to environmental as well as economic impacts (Jung et al., 2013). Macroalgae could be a good alternative as a novel ingredient in both human and animal feed. Overharvesting of wild macroalgae could lead to negative environmental impacts and problems in the sustainability of the supply in the market. Despite this, growth rates of marine macroalgae far exceed those of terrestrial biomass (Kraan, 2013). Also, widely marketed species such as *Ascophyllum nodosum* have shown quick re-generation and growth after being hand harvested in portion only on the top of the fronds allowing annual harvesting from the same beds (Ugarte et al., 2007).

Mariculture could provide a solution for sustainable supply of macroalgae. Cultivation systems include rope farms, tidal flat farms and floating cultivation systems that are currently used successfully in the production of *Laminaria* spp. (offshore), *Ulva* spp. (tidal flat farm) and *Sargassum* spp. (floating cultivation) (Kraan, 2013). Also, the combination of macroalgal culture with other production animals such as fish and molluscs could be a viable commercial alternative (Burg et al., 2013).

As previously commented, the incorporation of macroalgae biomass in animal feed as source of macro-nutrients (i.e., protein) in substitution of traditional ingredients or as a source of micro-nutrients (i.e., mineral or pigments) could be a viable alternative for the development of both functional animal feed and for the fortification of the derived animal products that could be used in the market of functional foods (Figure 1). Also, the extraction of macroalgal compounds (i.e., polysaccharides) for their incorporation into animal feed has shown promising results as pre-biotics improving animal's health.

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Chapter 5

ENVIRONMENTAL IMPACTS OF SEAWEED CO-CULTURE ON COASTAL FISHERIES

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ABSTRACT

This chapter provides a brief review of seaweed co-culture and its environmental impact on coastal fisheries. First, the current situation and problems facing the coastal fisheries, and the plans to overcome these issues, are discussed. Finally, the positive and negative effects of seaweed culture, role of seaweed co-culture, and the overall environmental impact are addressed.

Although worldwide mariculture production has increased steadily, productivity has tended to decline due to continued aquaculture activity in confined areas and natural disasters, such as, typhoons and outbreaks of red tide. In particular, the nutrient loading from unconsumed feed waste results in the deterioration of the water quality and outbreaks of diseases. To overcome these problems, integrated multi-trophic aquaculture has

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been suggested. In the integrated culture of seaweed and fish, seaweed plays an important role as both a CO_2 sink and biofilter, greatly reducing the environmental impacts on coastal fisheries. The addition of the integrated culture of lugworms to this system could also have further benefits because of its potential for waste recycling and value as bait, which is expected to contribute towards a more sustainable and productive form of aquaculture. Furthermore, the use of cultured seaweed and their waste has been expanded based on the development of useful microbes, reducing their environmental impact.

Keywords: coastal fisheries, integrated multi-trophic aquaculture, seaweed co-culture, environmental impact, lugworm

1. COASTAL FISHERIES

1.1. Current Situation and Problems

1.1.1. Global Situation

The growth in global aquaculture has remained strong up to present day. Annual marine aquaculture production has reached 20.1 million tons, and global food fish production has grown almost twelve times in the past three decades (FAO, 2012). By 2020, fish consumption in the developing countries is predicted to increase by 57%, and that in developed countries is predicted to increase by 4% (Delgado et al., 2003). In the developing countries, this estimate is based on the changes in supply and demand for fish protein under rapid population growth, increasing affluence, and urbanization. Total aquaculture production shows an average annual increase of 6-7%, and approximately 90% of this production comes from Asia (Halwart et al., 2007). Although cages were originally used in Asia for holding and transporting fish almost two centuries ago (Pillay and Kutty, 2005), the commercial marine cage culture was pioneered in Norway in the 1970s for salmon farming (Beveridge, 2004). In Asia, the start of cage farming in brackish and inshore waters is relatively recent, and was first adopted in Japan. Cage farming is diverse with a variety of species cultured at different intensities. With no large individual operations, the farming activities form a cluster due to the limited site availability in coastal waters (Halwart et al., 2007).

Apart from traditionally farmed species, such as, amberjacks and snappers, the inshore marine cage farming of groupers and cobia has spread in Southeast Asia. Some cage farming in Asia is still dependent on hatchery stock obtained from wild catch, especially for groupers. China is the largest finfish producer in the region, and its marine fish farming is expected to expand further in line with the rapid economic development (Halwart et al., 2007). Although the future prospects for cage farming appear relatively positive in Asia, it has been suggested that the large-scale, capital-intensive, and vertically-integrated marine cage farming seen in northern Europe and South America are unlikely to occur. Instead, clusters of small cage farming that attain a high efficacy are likely to be seen in the future. Similarly, off-shore cage farming is not expected to become widespread in Asia, due to difficulties in the availability of capital and the hydrography of the surrounding seas. Despite these limitations and constraints, cage farming in Asia will continue to contribute significantly to global aquaculture production, leading the world in total production (Halwart et al., 2007).

Because of the rapid development of aquaculture, fishmeal production cannot sustain the feed demand. In particular, lack of feedstock for fishmeal production has caused a serious problem in aquaculture. This is because the feedstock, such as, sardine, Jack mackerel, and mackerel are also used as food. Therefore, feedstock that is unused at present will have to be newly exploited to balance the demand for aquaculture feed. Due to the imbalance in supply and demand for marine products, increases in the price of marine products often occur through inflation.

1.1.2. Domestic Situation

Fisheries production in Korea has remained at 3,180-3,330 thousand tons since 2010 (Statistics Korea, 2015). Among these fisheries, the production from shallow-sea cultures has steadily increased and reached 1,662 thousand tons in 2015. However, the domestic production of fish aquaculture reached a maximum in 2009 and has decreased since then. The total number of cultured fish was approximately 353,293,000 in 2015. One of the reasons for this trend is that the increase in the production of fish, such as, flounder and rockfish, resulted in a sudden fall in fish price and, as a result, there was intervention in production at aquaculture farms.

In 2014, the total number of households managing fish aquaculture was 1,793, which was 4.0% lower than that in 2013 (Statistics Korea, 2015). This was because of the merging of low competition, small-scale fish aquaculture farms, and the shutdown of others because of red-tide damage. Recently, mass damage of fish culture farms by high water temperature has also occurred due to global climate change. Other factors causing lower production are long-term sequential culture, genetic recessiveness, and high-density culture (iPET, 2010).

Particularly in cage-culture farms, self-pollution resulting from feeding is unavoidable. Furthermore, most culture farm sites located in Korea are concentrated in gently-flowing bays that are vulnerable to pollution. Therefore, lower fishery production is also caused by environmental problems (Halwart et al., 2007).

The multiple uses of coastal waters restrict further development of marine fish aquaculture, and local cage-culture industries have some difficulty in maintaining the initial production levels (Halwart et al., 2007). Another issue in domestic aquaculture is the difficulty of aquaculture farm succession, as the current population of farmers and managers ages. Furthermore, security of fishmeal as aquaculture feed in Korea is mainly dependent on imports. Recently, the imbalance in the supply and demand of fishmeal has increased due to the increase in demand by latecomers, including China, into fish farming. Therefore, it is expected that there will be difficulty in securing a stable supply of fishmeal.

1.1.3. Problems Facing Coastal Fisheries

The development of marine aquaculture has caused some negative impacts, such as, the breakdown of marine habitats, introduction of non-native species, eutrophication, and disease outbreaks. The balance between the risk of marine aquaculture and its benefits has to therefore be considered (Price and Beck-Stimpert, 2014). Another potential impact on aquaculture production is the change in climate caused by global warming, which often results in environmental disasters (De Silva and Soto, 2009). Furthermore, self-pollution from marine net cages is a serious problem confronted by mariculture, clearly showing the importance of environmental management of marine aquaculture (Halwart et al., 2007). The following is a description of factors that are causing problems in coastal fisheries.

1.1.3.1. Environmental Impact

Marine cage culture operations can have an ecological impact on the marine environment and, therefore, on sensitive habitats and biodiversity. Typically, nutrients (mainly C, N, and P) are discharged as excess feed remains or as fish waste, resulting in a series of chemical and biological reactions. In nature, organic waste settling on the ocean floor is consumed by bottom feeding animals or degraded by microbes. Organisms in the sediment take up the nutrients, and promote the diversity of plants and animals. However, if the accumulation of organic waste becomes excessive, the chemical and biological reactions shift to an anaerobic state with little or no available oxygen. Once this phenomenon occurs, it can lead to a decrease in the diversity of the benthic community. Appropriate farm operations in well-flushed areas can help minimize nutrient accumulation, causing the least impact on the benthic community (Price and Beck-Stimpert, 2014). As a result, understanding potential site-specific ecological impacts is necessary prior to initiating farm operations.

1.1.3.2. Diseases

The continued intensification of culture practices has caused an increase in disease outbreaks in marine cage farming (Bondad-Reantaso et al., 2002). Diseases are caused principally by environmental and management effects, nutritional issues, and viral, bacterial, parasitic, and fungal pathogens (Arthur and Ogawa, 1996). The associated viruses include nodavirus, iridoviruses, lymphocystis virus, herpes virus, astro-like virus, and reovirus (Bondad-Reantaso et al., 2002). There is much concern about the increase in the intensification and clustering of marine cage farming in restricted areas. These types of culture practices may lead to outbreaks of major epizootics. In addition, there is a high possibility of trans-boundary movement of broodstock, fry, and fingerlings across cage-farming regions, especially in Asia. However, little attention has been paid to this problem. When such movement occurs, exotic diseases, pests, and invasive alien species can be spread extensively with potential impacts on biodiversity (Halwart et al., 2007).

1.1.3.3. Fish Production

The production of some fish species in cage-farming systems is dependent on the capture of wild juveniles because of the limited availability of hatchery-produced fry and fingerlings of target fish species (Halwart et al., 2007). If sufficient seed fish under high-level hatching skill is secured, this can lead to overproduction exceeding the demand (FAO, 2006; Merican, 2006; Ottolenghi et al., 2004; Rimmer, 2006). Another negative effects on cage farming are the continuous increase in working expenses, such as, feed, oil, and seed, and concerns about the hesitation of fish consumption according to an advance in fish price. The dependency of cage farming systems on feed has increased because feedstock, such as, fishmeal, fish oil, and low-value ‘trash fish’ species, are not sufficiently available as feed (Halwart et al., 2007). If fish feed is not sufficient, it leads to low production that cannot meet the consumer demands. As a substitute for raw fish feed, formulated feed mixture has been developed to provide a stable supply.

1.1.3.4. Social Concerns

Community concerns about the use of shared coastal water bodies for cage farming systems may increase due to problems relating to pollution and the possible displacement by other uses, resulting in the need for consultation with all stakeholders (FAO, 2006). Furthermore, the long-term environmental and ecological sustainability of rearing fish in cage-based farming systems is also of public concern (Goodland, 1997). Therefore, there is an increased need for governmental control of the development of the farming sector under environmental monitoring and implementation of good on-farm management practice (Alston et al., 2006; Boyd, 2005; FAO, 2006).

1.1.3.5. Geographical Effects

The size and strength of cages, and how they respond under in-situ conditions have an effect on fish production in cage-based farming systems (Halwart et al., 2007). For example, when large and robust cages, such as those of Norwegian design, were introduced in Langkawi Island, Malaysia, they were less successful because they were unable to function at full capacity due to the lack of support facilities for such large cages. The regions that have seasonal severe typhoons are not geographically appropriate for cage-farming. Locations, such as the South China Sea, are relatively shallow, and have strong surface and bottom currents but less wave height. Under these geographical features, open-ocean cages must be built to reduce drag rather than to withstand the wave height (Halwart et al., 2007).

1.2. Development Schemes

1.2.1. Existing schemes

1.2.1.1. Environmental Monitoring

Sediment monitoring protocols should include the early detection of excessive nutrient loads, anoxia, and heavy metals, making the implementation of adaptive management possible. Prior to restocking, site planning should be established that includes cage rotation in the event that there is a measurable impact on the sediment. This practice usually takes less than two years, and can provide chemical and biological recovery (Price and Beck-Stimpert, 2014). A way to reduce metabolic waste is the use of formulated feed under good feed management practice. Today some farms install underwater cameras to reduce nutrient discharge to the water column by monitoring feeding and fish activity (Price and Beck-Stimpert, 2014).

1.2.1.2. Feed Management

The use of formulated feed under good feed management practice can help reduce the amount of excess feed discharge. This results in a reduction in the release of heavy metals into the environment because they are less soluble in water compared with fish feed (Price and Beck-Stimpert, 2014). To date, raw fish feed has been used more. In Asian aquaculture, the total amount of trash fish used as feed for marine cage farming was estimated at approximately 53,301 kilotons in 2010 (FAO, 2014). The main reasons for the continued use of trash fish as feed for cage farming are: the farmer’s perception of better performance by trash fish, low price of trash fish compared with formulated feed, the ease of availability, the shortage of suitable formulated feed for all life cycle stages of culture species, and social restraints with the existing livelihood strategies of many coastal fish farmers compared with more organized lot farming (Halwart et al., 2007). However, farmers should be encouraged to use formulated feed to remove the stress of negative environmental impacts by the use of raw fish feed. To reduce the nutrient load on the sediment, high energy feeds with high digestibility should be formulated and used (Halwart et al., 2007).

1.2.1.3. Limitations

Marine cage farms must be placed away from sensitive ecosystems including corals, seagrass, and mangrove habitats (Price and Beck-Stimpert, 2014). In some countries, liquid fisheries waste is disposed of through the municipal sewage system or discharged directly into a waterbody. The receiving waterbody must be able to degrade the biological and chemical constituents present in the waste to have no detrimental effect on the aquatic fauna and flora (Naidoo and Olaniran, 2014; UNICEF and WHO, 2012). Furthermore, the damage on submerged habitats by farm installation must be considered, because they may be sensitive to shading from net pens, cages, and other moored structures. In some cases, many surrounding species of marine organisms can be influenced by the slow leaching of toxicants from copper-based antifoulant coating on nets or cages. Furthermore, the use of chemicals to remove fouling should be avoided. Instead, mechanical scrubbing by divers, or lifting cages or nets out of the water to desiccate fouling organisms is recommended (Price and Beck-Stimpert, 2014). Onsite cleaning may cause an increase in the organic load on the benthos.

1.2.1.4. Farming Site Management

Reducing waste and debris can contribute to the maintenance of water quality at cage farming sites. The timely removal of dead fish will avoid fouling of the water column, impede proliferation of predators, and prevent the spread of disease. During harvest and slaughter, waste can be reduced through careful treatment (Price and Beck-Stimpert, 2014). To prevent disease outbreak, enhanced means for disease resistance or vaccines must be developed (Halwart et al., 2007).

1.2.2. Integrated Multitrophic Aquaculture

The use of integrated multitrophic aquaculture (IMTA) can reduce the impact of nutrient loading on water quality. IMTA is the integrated culture of low-level trophic organisms (shellfish and seaweed) and high-level trophic organisms (fish and prawn). For example, it is common practice in finfish culture to combine production with shellfish (mussels and oysters) and seaweed that filter waste particulates. Lobsters and sea urchins have also been used with some success (Price and Beck-Stimpert, 2014). Therefore, this approach has some potential to mitigate environmental impacts and it simultaneously expands the economic base of the farming operation. As a result, IMTA is an experimentally sustainable marine aquaculture, and in some cases can obtain social acceptance (Price and Beck-Stimpert, 2014). In Figure 1, the simple IMTA model is shown where fish, as the high-level trophic organism, and seaweed, as low-level trophic organism, are cultured together.

1.2.3. Use of Lugworm

Global legal controls exist to protect the marine environment, and this consecutive effort has made aquaculture farms environmentally-friendly. Polychaete worms have the potential to mitigate environmental impact (Brown et al., 2011). The *Nereis* species can efficiently use solid waste (unconsumed feed and fecal material) collected from a marine recirculating system, and is an excellent candidate for integrated aquaculture and waste recycling (Bischoff et al., 2009; García-Alonso et al., 2008; Honda and Kikuchi, 2002). The use of aquatic worms also has great potential in bioremediation of waste deposited on net pens or in fish ponds (Kinoshita et al., 2008; Riise and Roos, 1997). Therefore, their role in the decomposition and mineralization of organics can be critical for recovery in impacted coastal aquaculture sites (Heilskov et al., 2006). Furthermore, their availability at these sites can be used as an indicator of the level of environmental impact by coastal aquaculture operations (Tomasseti and Porrello, 2005).

Lugworm is an important marine resource because of its role in water purification and simultaneous commercial value, mainly as bait (Cho, 2011). The market price of lugworm is twice as much as the prime cost from its culture (Jung, 2014). Therefore, the benefits of lugworm can be applied to maximize the potential of IMTA in terms of productivity and to reduce the level of nutrient loading release into the marine environment. The IMTA models primarily represent co-culture of fish with invertebrates, such as, sea cucumbers and sea urchins. This IMTA model can be modified by considering the main parameters to simulate the growth and nutrient uptake of different species under various environments. Lugworms settle beneath the farm site and perform effectively in recycling the larger organic particles that are produced from the other (feed or non-feed) components in the IMTA system (Aquaculture in Canada, 2013).

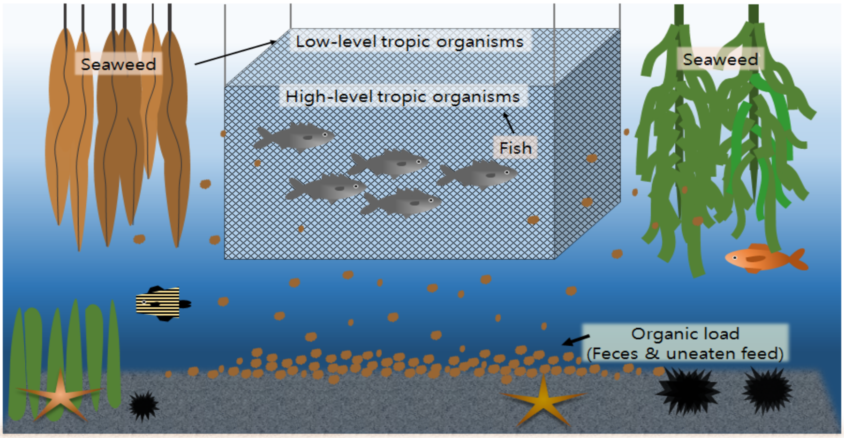

Figure 1. Simple IMTA model showing co-culture of fish and seaweed.

2. SEAWEEDS

2.1. Diversity

Seaweeds are a group of photosynthetic plant-like organisms, or macroalgae, that are macroscopic and multicellular. They are classified into three major groups based on their dominant pigmentation: red (*Rhodophyta*), brown (*Phaeophyta*), and green (*Chlorophyta*). Seaweeds have been traditionally used as food in East Asia. For this reason, about 33 genera of seaweed, mainly red and brown, are commercially harvested and cultivated (McHugh, 2003). Furthermore, close to 500 species in about 100 genera are used locally (Mouritsen, 2013).

The representative red seaweed include *Porphyra tenera*, *Gelidium amansii*, and *Gracilaria verrucosa*, and their carbohydrate content based on dry weight ranges between 40-75% (Suo et al., 1986). Brown seaweed, including *Undaria pinnatifida*, *Laminaria japonica*, *Sargassum fulvellum*, and *Hizikia fusiforme*, contain about 36-60% carbohydrate, while green seaweed, including *Enteromorpha compressa*, *Ulva lactuca*, *Monostroma nitidum*, *Codium fragile*, and *Capsosiphon fulvescens*, contain about 41-53% carbohydrate. The main carbohydrates contained in each seaweed are different: agar, carrageenan, cellulose, mannan and xylan in red seaweed; alginate, laminaran, fucoidan, cellulose, and mannitol in brown seaweed; and cellulose, mannose, xylan, and starch in green seaweed. The content based on dry weight of proteins and lipids are 2-39% and 0-2% (red seaweed), 6-20% and 1-3% (brown seaweed), and 17-23% and 0-1% (green seaweed), respectively.

2.1.1. Current State in Korea

Brown and red seaweed are a good match for the oceanic climate of Korea. The seaweed culture sites in Korea are mainly located on the western side of the south coast, accounting for almost 90% of total seaweed cultivation. The total production of seaweed in 2015 was 1,105,498 metric tons at a value of USD 421,754,469 (Ministry of Oceans and Fisheries, 2015). More specifically, the production of *Pyropia*/*Porphyra*, *Saccharina*/*Laminaria*, *Undaria*, and others were 419,024; 372,311; 283,714; and 30,449 metric tons at a value of USD 269,447,654; USD 67,515,494; USD 58,614,306; and USD 25,177,014, respectively. The production of *Pyropia*/*Porphyra* has increased since the 1980s due to artificial seeding, development of the float culture system, transplantation of new species, and expansion of the culture sites. Its high market price is the result of continuous consumer demand for high-quality products (NOAA, 2015).

2.1.2. Domestic Uses

Koreans have traditionally used raw or dried seaweed for food, and industries have manufactured diverse seaweed products since the 1980s. Recently, fast food-type and diversely packed products have also been developed. Dried laver is the most valuable marine product obtained from a single fishery. Another important product of processed seaweed is salted

Undaria that is manufactured mainly on the southwest coast of Korea. The annual production of *Undaria* has increased steadily due to the increase in the demand for healthy food (NOAA, 2015). At present, about 80% of the total seaweed production is directly consumed by humans. The remaining 20% is used as a source of the phycocolloids used in food, industrial, cosmetic, and medical industries (Browdy et al., 2012; Critchley et al., 2006; Lahaye, 2001; McHugh, 2003; Mouritsen, 2013; Ohno and Critchley, 1993), and as an animal feed additive, fertilizer, water purifier, and probiotic in aquaculture (Abreu et al., 2011; Chopin, 2012; Chopin et al, 2001; Chopin et al, 2012; Fleurence et al., 2012; Kim et al., 2014; Neori et al., 2004; Pereira and Yarish, 2008; Pereira and Yarish, 2010; Rose et al., 2010).

2.2. Seaweed Culture

Seaweed culture is practiced using various culture methods and each method interacts in some way with the environment (Phillips, 1990). Therefore, the nature of the interaction and environmental impact are dependent on the culture method, surface area of the farm, and farming site. Seaweed culture is significantly influenced by environmental factors, including, turbidity, levels of organics and heavy metals, phytoplankton blooms, and temperature and salinity fluctuations (Trono, 1986).

2.2.1. Positive Effects

2.2.1.1. Physical Aspects

There is some potential for large-scale seaweed farms to increase sedimentation of organic matter and to influence coastal water movement, demonstrated by the large areas covered by the *Laminaria japonica* culture in China (Phillips, 1990). Seaweed farms can protect coastal areas from erosion. The large-scale seaweed farms may also provide shelter for other sensitive culture species and systems. For example, mussel or scallop culture species and systems have been protected under *Laminaria japonica* culture zones in China. Furthermore, facilities installed in seaweed culture, such as, rafts, ropes, and anchors, may enhance the production of other marine organisms due to an increase in the substrate surface area. Seaweed culture may also be effectively used to rehabilitate degraded coastal areas.

2.2.1.2. Ecological Aspects

Seaweed culture is an extensive system that depends mainly on a natural nutrient supply. Therefore, seaweed culture has the potential to deplete nutrients present in coastal waters. Lower nutrient levels have been detected in *Laminaria japonica* culture areas (UNDP/FAO, 1989), implying a good correlation between nutrient concentrations and seaweed production (Chung, 1986). The use of supplementary feed in intensive aquaculture systems causes an increase in nutrient levels, and it has a positive effect on seaweed production (NCC, 1989). In some areas placed in a nutrient-depleted state, fertilization is needed for seaweed growth (UNDP/FAO, 1989). The benthic area under seaweed cultures has been used for production of other aquatic animals, such as, abalone and sea cucumber, therefore making the best use of the allotted area (Phillips, 1990). Facilities for seaweed culture also have a significant influence on the productivity of coastal invertebrate and vertebrate populations due to the increased availability of shelter and food (Suo et al., 1986). Furthermore, seaweed culture has been grown in abandoned shrimp ponds, thus making use of wasted resources (Phillips, 1990).

2.2.2. Negative Effects

2.2.2.1. Physical Aspects

The main physical impact of seaweed culture systems is likely the large surface area required. Other factors include site preparation, routine management, and culture facilities. For some species, site preparation involves the removal of rocks or other obstructions, including competitive grasses and predators (Juanich, 1988). Such operations may cause some damage to coastal ecosystems, and in some cases, the loss of species of conservation interest, such as, seagrasses (Pullin, 1989). The routine management of seaweed culture in shallow waters may also cause damage through trampling and accidents. Some areas of seaweed culture are shaded by the culture facilities, and this physical shading may result in changes to the benthic communities and primary production in the water column. Although the influence of seaweed culture on benthic communities has not been studied extensively, a shading problem in large-scale seaweed farming can potentially reduce benthic productivity, especially in shallow inshore areas.

2.2.2.2. Ecological Aspects

Extensive aquaculture as a result of overstocking causes an eventual decrease in aquaculture production because the ‘carrying capacity’ of the environment has been exceeded (Beveridge, 1984). In some locations, over-production has resulted in outbreaks of disease (ICES, 1989), which may be linked to nutrient decline (UNDP/FAO, 1989). To overcome these problems, the balance between the ecological requirements for seaweed culture and the carrying capacity of the environment must be carefully considered. Another concern that has arisen in intensive and semi-intensive aquaculture is the potential impact of chemicals used for the control of disease, predators, and fouling organisms (Santelices and Doty, 1989; North, 1987). Furthermore, the attractive characteristics of seaweed can foster prey-predator interaction between the seaweed, invertebrates, and finfish (North, 1987).

2.3. Seaweed Co-Culture

Seaweed production accounts for approximately 35% of the global mariculture production, but its harvest value represents only 7% of the total value (FAO, 2010). To increase space efficiency and yields, co-culture with alternative or valuable aquaculture species in existing seaweed-culture areas may be necessary. Therefore, co-culture is more efficient than monoculture, especially in limited coastal areas (Beltran-Gutierrez, 2016). Fish cage culture relies on external food supplies, which results in a negative impact on water quality (Fang et al., 2016). Conversely, seaweed culture can reduce nutrient loading from fish aquaculture. Therefore, co-culture of seaweed with other species could provide more profit, and concurrently have ecological benefits (Fang et al., 2016).

2.3.1. Role of IMTA

The implementation of IMTA can be beneficial to an ecosystem. Fish cage culture always produces unconsumed feed waste. This causes nutrient loading on the seabed and results in the release of CO_2 as a greenhouse gas. In this situation, co-culture of seaweed with fish can turn the system into a CO_2 sink through photosynthesis by uptake of nutrients (Tang et al., 2011). In some IMTA systems, co-culture of seaweed with abalones, clams, and sea cucumbers is possible (Tang et al., 2013): seaweeds and phytoplankton proliferate by the uptake of CO_2 and NH_4 from the nutrients present in water. Abalones and clams then grow feeding on the seaweed and phytoplankton, respectively, and produce some detritus. Sea cucumbers finally consume these detritus for their growth. These co-cultured organisms at different levels of the food chain simulate the functioning of natural ecosystems, and this type of a balanced system with recycling nutrients would ultimately provide healthier waters (Aquaculture in Canada, 2013).

Seaweed, including kelp, is able to extract dissolved inorganic nutrients, such as, nitrogen and phosphorus, helping reduce levels of dissolved inorganic nutrients generated in the IMTA system. Therefore, seaweed plays an important role as a bio-filter (Hayash et al., 2008; Neori et al., 2007). To date, *Sacchaarina japonica*, *Gelidium amansii*, and *Codium fragile* can effectively use the nutrients in an IMTA system (Kim et al., 2014). Co-culture of seaweed *Ulva* sp. with Atlantic salmon (*Salmo salar*) and sea urchin (*Paracentrotus lividus*) has also been shown in another IMTA model that considered the environmental conditions on the west coast of Scotland (Lamprianidou et al., 2015). The model simulated the growth, uptake, and release of nitrogen by these organisms to study the nitrogen bioremediation potential of low-level trophic organisms (Lamprianidou et al., 2015). Overall, seaweeds as primary coastal producers can provide nurseries, habitats, and food for aquatic fauna (Ohno, 1993; Watanuki and Yamamoto, 1990).

2.3.2. Association with Lugworm

As a candidate for integrated aquaculture and waste recycling, lugworms can be affiliated with the IMTA system (Bischoff et al., 2009; García-Alonso et al., 2008; Honda and Kikuchi, 2002). They settle beneath the farm site and play an important role to effectively reduce the amount of organic particles produced from the other components in the IMTA system (Aquaculture in Canada, 2013). Therefore, their role is important to remedy impacted coastal aquaculture sites (Heilskov et al., 2006). As a result, they contribute towards a more sustainable and productive form of aquaculture (Aquaculture in Canada, 2013).

For the practical use of lugworms in the IMTA system, at least 2-month old, grown, juvenile lugworms must be used because of high mortality during the larval stage. The normal feed for worms is decaying organic material, such as, seaweed, microalgae, dead animals, and some bacteria (Alyakrinskaya, 2003). In lugworm culture, the early larval stage was fed with marine *Chlorella* and green laver powders, and then feed for adult breeding was fed after the juvenile stage in which the grown larvae infiltrated into sediment (Choi, 2006). Seaweed can then be used as a supplementary feed for lugworms (Harris, 2010). The lugworm species, Tubifex, was cultured using a biofloc system to enhance their survival and growth, because the biofloc system could provide good water-quality by the decomposition of waste loading, including ammonia (Wahyuni et al., 2016).

2.4. Schemes for Diverse Uses

Seaweed is cultivated on the waste resulting from fish culture in IMTA systems, while they are used as ingredients for fish feed, such as, immune-stimulating, feces-binding, and fishmeal-replacing additives (Palstra et al., 2015). The reuse of the seaweed waste generated during their processing has recently gained interest, as the number of people seeking seaweed as a health food increases. Therefore, the security of useful microorganisms for the reuse of seaweed waste is important to reduce the environmental impact of the waste.

2.4.1. Acquisition of Useful Microbes from the Lugworm Viscera

Benthos inhabiting in intertidal zones have been used in the removal of organics and in water purification (Campos et al., 2002; Davidson et al., 2008; Palacios and Timmons, 2001; Vigneswaran et al., 1999). In particular, lugworms, including the *Perinereis* sp., have been used in shrimp culture farms (Fujioka et al., 2007). Suspension feeders, including lugworms, have been studied extensively to understand the nutritional function of suspended bacteria (Gili and Coma, 1998; Orejas et al., 2000; Prieur et al., 1990). Lugworm *Sabella spallanzanii* was able to efficiently accumulate and enrich bacteria from the surroundings (Stabili et al., 2006). This is because the lugworm's viscera can provide an optimum environment for microbial growth (Kathrin et al., 1997). In earthworm viscera, *Brevibacillus agri*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus licheniformis*, and *Brevibacillus parabrevis* were found to be indigenous microbes showing proteolytic and lipolytic activities (Kim et al., 2010). The *Bacillus* species found in lugworm viscera have also synthesized various enzymes, including protease, amylase, and cellulase to degrade diverse organics (Ziaei-Nejad et al., 2006; Verschuere et al., 2000; Ghosh et al., 2002). Furthermore, some strains isolated from lugworms showed antimicrobial activity, indicating that they could be used as a potential source to research bioactive molecules, drugs, and antifouling compounds (Shankar et al., 2015). Among the bacteria, *Bacillus* species found in the marine environment are used as probiotics, because they are able to stimulate the alimentary and immune systems (Gatesoupe, 1999; Ziaei-Nejad et al., 2006). *B. subtilis* is used as live feed by many organisms (Ziaei-Nejad et al., 2006; Verschuere et al., 2000), and some *Bacillus* species have been suggested as alternative antibiotics in shrimp culture farms (Phillips, 1990). In addition, *B. subtilis* (Vaseeharan and Ramasamy, 2003), *B. licheniformis*

(Arena et al., 2006), and *B. pumilus* (Ghosh et al., 2002; Sugita et al., 1998) are able to produce antibiotics, antiviralagent, and digestion-related enzymes, respectively.

2.4.2. Biodegradation of Seaweeds

As a favorite food, various types of seaweed products are sold in Asian countries, in particular. The increase in seaweed consumption results in large generation of seaweed waste. Seaweed waste is composed of carbohydrates, proteins, and lipids (NFRDI, 2009). Through biodegradation, these components can be converted to useful compounds, such as, bioactive substances. To date, many studies have been performed on diverse microbial strains: cellulolytic *Acremonium strictum* (Goldbeck et al., 2013), alginate- and laminarin-degrading *Microbacterium oxydans* (Kim et al., 2013), agar- and carrageen-degrading *Bacillus alcalophilus* (Kang and Kim, 2014), proteolytic *Bacillus pseudofirmus* (Raval et al., 2014), and lipolytic *Aneurinibacillus thermoaerophilus* HZ (Masomian et al., 2013). The biodegraded seaweed waste can also be reused as liquid fertilizer by mixed microbes (Kim et al., 2007; Kim et al, 2010). As useful resources, they can often be applied as fertilizer, fungicides, herbicides, and phycocolloids, such as, alginate, carrageenan, and agar.

2.4.3. Others

Seaweed can be a sink for anthropogenic CO_2 emissions via photosynthesis in the coastal waters of some countries. Therefore, CO_2 removal belts formed by the seaweed culture in coastal waters are expected to be developed together with sustainable seafood production. Furthermore, seaweed is a good resource for the production of reducing sugars, and studies on the use of the produced biomass for biofuel are currently in progress. Seaweed culture can therefore contribute, to some extent, in meeting the global food, feed, and pharmaceutical requirements (Israel et al., 2010).

CONCLUSION

The development of marine aquaculture has caused some negative impacts, such as, the breakdown of marine habitats, self-pollution, disease outbreaks, and environmental disasters, clearly requiring environmental management. To reduce the water-quality impact of nutrient loads sinking to the seabed, an IMTA system has been suggested. Seaweed culture is considered to be an indispensable component of this system because of its role as both CO_2 sink and biofilter. In addition, the introduction of lugworm culture into the established IMTA system is expected to result in more sustainable and productive form of aquaculture. To reduce the environmental impact caused by the seaweed waste, the diversity of microbes associated with lugworms has been studied. This effort for the development of useful microbes has resulted in diverse uses of cultured seaweed and their waste.

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