

Advances in cultivation of Gelidiales

Michael Friedlander

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Abstract Currently, *Gelidium* and *Pterocladia* (*Gelidiales*) are collected or harvested only from the sea. Despite several attempts to develop a cultivation technology for *Gelidium*, no successful methodology has yet been developed. Initial steps towards developmental efforts in Portugal, Spain, South Africa and Israel have been published. More developments have probably been performed but have not been published. Two different technological concepts have been tested for *Gelidium* cultivation: (1) the attachment of *Gelidium* fragments to concrete cylinders floating in the sea, and (2) free-floating pond cultivation technology. These vegetative cultivation technologies might be partially optimized by controlling physical, chemical and biological growth factors. The pond cultivation technology is the much more controllable option. The effects of all factors are discussed in detail in this review. It seems that the main difficulty with cultivation of *Gelidium* is its low growth rate. The claimed yields of the two technologies are far from being economically attractive at this stage of their development. It seems that in order to introduce *Gelidium* into commercial cultivation, major efforts in genetic improvement through selection or genetic engineering will be required. Only high yield strains will have the potential to compete economically with the present harvesting tradition. However, accumulated experience with genetic improvement of other useful seaweed species suggests that this is possible.

Keywords *Gelidium* · *Pterocladia* · *Gelidiales* · Cultivation

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Introduction

As far as I know there is no current commercial cultivation of *Gelidiales*. Despite several attempts to develop a cultivation technology for *Gelidium* and *Pterocladia*, so far no successful methodology has been developed. Because of the proprietary nature of commercial cultivation, a successful technology may have been developed but has remained unpublished. *Gelidium* and *Pterocladia* (or *Pterocladia*) are currently only collected or harvested, as opposed to other useful seaweeds for which cultivation technology has been developed. The reasons for this situation are discussed in this review, including all important variables affecting *Gelidium* and *Pterocladia* growth. This review will rely mostly on *Gelidium* studies since most of the relevant information in the literature deals with *Gelidium*.

Initial steps

Gelidium has been cultured in Korea since 1989 (Melo 1998) and in China (Fei and Huang 1991). Early cultivation systems for *Gelidium* species have also been described in Spain and Portugal (Salinas 1991; Seoane-Camba 1997; Melo 1998). Cultivation of intertidal *G. pristoides* (Turner) Kützing in the sea was reported from South Africa (Aken et al. 1993), and growth experiments were performed in tanks with *G. crinale* (Turner) Gaillon and *Pterocladia capillacea* (Gmel.) Santelices and Hommersand in Israel (Gal-Or and Israel 2004; Boulus et al. 2007). The ecological studies carried out with *Gelidium* and *Pterocladia* can be reviewed by following the major factors controlling the growth of these seaweeds. Evaluation of these studies may answer the major question of "why is there no mass cultivation of *Gelidium* and *Pterocladia*"?

Technological factors

Two major technologies have been suggested for *Gelidium* cultivation: sea farming and tank culture. Both use *Gelidium* fragments for initiation of the cultures.

Sea culture

In one culture experiment in Galicia (Spain), fragments of *G. sesquipedale* (Clemente) Born. et Thur. were attached to limestone cylinders placed in the sea (Seoane-Camba 1997). This technology has technical, biological and ecological advantages. However, it became clear that non-contaminated seawater and a high level of hydrodynamics are major prerequisites for a *Gelidium* farm because of epiphytes and competitors (Seoane-Camba 1997). Cultivation of intertidal *G. pristoides* in the sea in South Africa was attempted by suspending plants in net bags, or growing on limpet shells on ropes at different depths. However, after significant growth for 2 weeks, the thalli degenerated for unknown reasons (Aken et al. 1993). Systems with *G. rex* Santelices et Abbott hanging in open sea were developed in Chile after reattachment to shells (Rojas et al. 1996).

Tank culture

In Israel, *Gelidium* and *Pterocladia* were grown in outdoor tank culture systems adopted from other seaweed cultivation methods, included the use of 40 L tanks with continuous water flow and aeration (Gal-Or and Israel 2004; Boulus et al. 2007). The advantages are that tank culture allows control of most variables, and is relatively protected from grazers and epiphytes. The disadvantage is its high cost. Other experiments with *Gelidium* free-floating tank cultures in Spain claimed to generate healthy plants in 2–3 months, with a nice deep-red color, but very low growth rates (Salinas 1991a, b; Sosa et al. 2006). Looking at the effects of the major growth factors and at the maximal reported growth rates might improve our understanding of the obstacles to developing mass cultivation.

Physical factors

The major physical factors involved in culture of *Gelidium* and *Pterocladia* are temperature, irradiance and relative seaweed velocity. The optimal combination of these factors might lead to maximal yields. It is possible to study the requirements for these factors from limited experiments conducted in the sea, in tanks or in the laboratory.

Irradiance

G. pulchellum (Turner) Kurtz was cultured under controlled laboratory conditions. Maximum growth rate (10.0% day⁻¹) was obtained at 130–240 μmol photons m⁻² s⁻¹ under continuous light and aeration (Sousa-Pinto et al. 1999). The photosynthetic capacity of *G. sesquipedale* decreased with depth, revealing a 'sun' and 'shade' acclimation pattern (Silva et al. 1998). Photosynthesis is probably limited in *G. canariensis* (Grunow) Seoane-Camba, *G. arbuscula* Bory ex Boergesen and *Pterocladia capillacea* by incident light in their natural habitats (Mercado et al. 2001). *Gelidium sesquipedale* in chemostats showed higher growth rates under blue and red light than under white light of the same photon-fluence rate (40 μmol m⁻² s⁻¹; Carmona et al. 1996). Reattachment of apical portions of *G. sesquipedale* increased under longer days compared to under shorter days (Juanes and Puente 1993). All these studies indicated that irradiance is a significant growth factor, and indicated a positive response to irradiance range. However, summer and winter outdoor experiments conducted in small aerated seawater tanks with *G. crinale* in Israel, showed that light intensity changes were less or non effective, with ambient conditions being almost optimal (Boulus et al. 2007).

Temperature

Temperature was defined as the major seasonal limiting factor for *G. crinale*, and positively affected weekly growth rate. The average daily growth rate was about 6.5% day⁻¹ for the months of active growth (Boulus et al. 2007). The relative abundance of *G. robustum* (Gardn.) Hollenberg et Abbott in Mexico under normal conditions and during La Niña showed a positive correlation with sea surface temperature (Hernandez-Guerrero et al. 2000). Reattachment of apical portions of *G. sesquipedale* in the laboratory increased under a limited range of 16–18°C as compared to 20–22°C (Juanes and Puente 1993). These studies support a strong effect of temperature change on *Gelidium* cultures.

Water movement

A lag period of 3 months showed a positive correlation in situ between relative abundance of *G. robustum* and upwelling index / wind speed (Hernandez-Guerrero et al. 2000). Zones with greater water movement in situ (1.4 m s⁻¹) stimulated the growth of *G. robustum* (1.0% day⁻¹). Growth was directly proportional to water movement under semi-controlled cultivation conditions in Mexico, and treatment with the greatest dynamics demonstrated growth of 3.6% day⁻¹ (Pacheco-Ruiz and Zertuche-Gonzalez 1995).

Thus, all three physical factors—irradiance, temperature and water movement—seem to be major effective growth factors both in the sea and under controlled conditions.

Chemical factors

The major chemical factors are the concentration of inorganic nitrogen and phosphate in seawater, and also the concentration of dissolved inorganic carbon (DIC) in the form of CO_2 or HCO_3^- . Several laboratory and outdoor experiments can supply a basic understanding of these factors.

Nitrogen

Ammonium was a major limiting factor for tank-cultured *G. crinale*, and positively affected growth (Boulus et al. 2007). In outdoor 40 L tank experiments of 4 weeks duration, the growth rate of *Pterocladia capillacea* reached a weekly growth rate of 28.3% in winter and 25.3% in summer with different ammonium regimes (Gal-Or and Israel 2004). Growth of vegetative fragments of *G. sclerophyllum* Taylor was stimulated by high (2–3 mM) nitrogen concentrations in the laboratory (Rodriguez 1996).

Phosphate

The growth rate of *G. robustum* grown in the laboratory generally increased with increasing phosphate concentration up to 21% day^{-1} , obtained at 150 μM (Sousa-Pinto et al. 1996). Branch and rhizoid production of vegetative fragments of *G. sclerophyllum* were stimulated by high P concentrations (100–150 μM ; Rodriguez 1996).

Inorganic carbon

Contradictory evidence has been presented regarding the carbon source of *Gelidiales*. In a pH-stat cultivation system, growth rates of *P. capillacea* were largely unaffected by pH in the range of 6.5–8.5. However, the growth rate decreased significantly above pH 8.5. This suggests that *P. capillacea* can generally take up both the CO_2 and HCO_3^- forms of inorganic carbon (Ci), but has a low ability to use HCO_3^- at alkaline seawater pH (Gal-Or and Israel 2004). Photosynthesis by *G. sesquipedale* was enhanced when the CO_2 concentration was increased by a decrease in pH. Similarly, an increase in pH from 8.1 to 8.7 produced a significant reduction in O_2 evolution rates, indicating that *G. sesquipedale* has a very low capacity to use HCO_3^- (Mercado et al. 1998). No detectable external carbonic anhydrase activity, and high sensitivity to alkaline pH indicated that *G. canariensis*, *G. arbuscula* and *P. capillacea* have a low

capacity for using the external pool of HCO_3^- ; therefore photosynthetic rates in a controlled environment room were dependent on CO_2 availability (Mercado et al. 2001). On the other hand, the weekly outdoor growth rate of *G. crinale* was not significantly affected by addition of a carbon source (CO_2 or NaHCO_3) or by the controlled maintenance of pH 8. This suggests that a daily pH increase up to pH 9 does not decrease the growth rate under these conditions as this species consumes bicarbonate, which is more abundant at high pH (Boulus et al. 2007).

The positive growth effect of nitrogen and phosphate additions to *Gelidium* and *Pterocladia* culture is fully supported; however, the consumption of bicarbonate is not consistent in *Gelidium* and *Pterocladia* cultures.

Biological factors

The major biological factors include strain selection, reproduction methods, seaweed density, epiphytes, and interaction with fish effluents. Only limited information can be found about these factors.

Strain selection

In a single genetic study, backcross-5, red, male-sterile plants of *G. vagum* Okamura grew at a significantly higher rate than plants from other lines, suggesting that male-sterile gametophytes would probably be better candidates for aquaculture than normal wild-type plants of this species (Patwary and Van der Meer 1997).

Reproduction method

Culture of vegetative fragments of different sizes and from different parts of the thallus of *G. sclerophyllum* showed that growth in length and branch production was favored by medial fragments, and rhizoid production was favored by apices (Rodriguez 1996). Research on the culture of *G. rex* was approached from two points of view: growth of thalli from spores, and growth from re-attachment of fragments. Mollusc shells, which are very easy to handle, were used in both systems. The results achieved by these methods showed that the re-attachment technique obtains larger plants in a shorter time (Rojas et al. 1996). Seedlings of *Gelidium* were cultured indoors for 20–30 days in China, and then transferred to the sea, where their recorded growth rate was 3.84% day^{-1} (Pei et al. 1996). Reattachment of apical portions of *G. sesquipedale* was induced in the laboratory under spray cultivation conditions, and occurred mainly at the first stage of development when in contact with the substratum. A probable advantage of sporophytic fronds was defined as compared to gametophytic fragments

(Juanes and Puente 1993). Apices of vegetative *G. sesquipedale* developed rhizoidal clusters during re-attachment to artificial substrate followed by germlings under spray technology in the laboratory, showing higher growth rate than development from sporelings. The germlings were transferred with their substrate to the sea in Spain (Salinas 1991). A new method of plantlet production of *Gelidium* sp. was developed using cell aggregates of apical fragments after a freezing–thawing procedure. It showed a daily growth rate of 13% day⁻¹, and might be an efficient method for generation of mass planting material for cultivation (Titlyanov et al. 2006). In pond culture, only vegetatively propagated, free-floating fragments are utilized.

Density

G. amansii (Lamouroux) Lamouroux plants are able to grow at densities up to 1 kg m⁻² at Izu Peninsula (Japan), this being the most favorable bed. At harvesting, a standing crop of up to 0.2 kg m⁻² is usually left behind, because these plants grow back to 1 kg m⁻² within 2 months of harvesting. Harvesting is carried out by divers three times in a season, reaching an annual yield per square meter of well over 2 kg. *Gelidium* is a perennial with a slow rate of growth. The most reliable technique for propagation is to develop more habitats, but only in favorable areas, by throwing heavy stones into the sea (Shunzo Suto 1971). *Gelidium* species with a density of erect seedlings of 3–19 seedlings cm⁻² were studied in the sea in China. Growth rate of regenerated fragments in the sea was 3.84% day⁻¹ (Pei et al. 1996). In free-floating tank culture, *Gelidium crinale* optimal densities can reach 4 kg m⁻² (M. Friedlander, unpublished observations).

Epiphytes

Although displaying seasonal cycles in abundance, *G. latifolium* (Greville) Bornet et Thuret was colonized by

epiphytes throughout the annual study period in Northern Spain. Distribution of epiphytes on hosts followed a trend of large epiphytes on basal portions of the host, and ephemeral, small-sized epiphytes appearing on the tips of the host thallus (Arrontes 1990). Juvenile plants of *G. rex* in the open sea in Chile suffered from problems of fouling, epiphytes and epibionts caused by bacteria, algae and sessile invertebrates, which resulted in growth inhibition and decay (Rojas et al. 1996). The development of epiphytes was a serious problem in tank culture experiments in Portugal with *G. sesquipedale*, in which fish pond effluents were used (R. Santos, personal communication). Most of the cylinders with attached *G. sesquipedale* in the sea suffered from continuous attacks by epiphytes and epibionts on the cylinders and had to be withdrawn from the culture sites (Seoane-Camba 1997). For *G. amansii* cultured in the sea in Japan, the elimination of harmful weeds such as *Ecklonia* and *Eisenia* is effectively inhibiting epiphytes for 1 or 2 years (Shunzo Suto 1971).

These studies show that sensitivity to epiphytes is a major problem in *Gelidium* culture. This might be partially alleviated by clean seawater resources in tank culture and low nutrient areas in open sea farming.

Interaction with fish effluents

While adapting *G. sesquipedale* to free-floating conditions, fishpond effluents rich in NH₄⁺ has been tried (Salinas 1991). Nitrogen removal experiments using *G. amansii* revealed that it is possible to use macroalgae as biofilters (Liu et al. 2004). In tank culture experiments with *G. sesquipedale*, fish pond effluents were used (R. Santos, personal communication). These studies show that the integration of *Gelidium* culture with fish culture is feasible.

Summarizing the major biological factors shows clearly that reproduction by reattached or free floating fragments is more effective than spore reproduction, and that epiphytes are a major obstacle to *Gelidium* cultivation.

Table 1 Daily growth rate (DGR) of various *Gelidiales*

Species	DGR (%)	Growth conditions	Experiment duration	Reference
<i>G. pulchellum</i>	10.0	Laboratory		Sousa-Pinto et al. 1999
<i>G. robustum</i>	21.0	Laboratory		Sousa-Pinto et al. 1996
<i>G. sclerophyllum</i>	7.0	Laboratory	1 month	Rodriguez 1996
<i>Gelidium</i> sp.	13.2	Laboratory	2 months	Titlyanov et al. 2006
<i>G. sesquipedale</i>	1.9	Chemostats	14 days	Carmona et al. 1996
<i>G. rex</i>	33.3	Laboratory and in situ	2 months	Rojas et al. 1996
<i>Gelidium</i>	3.8	Laboratory and in situ	1 year	Pei et al. 1996
<i>G. amansii</i>	6.7	In situ	2 months	Shunzo Suto 1971
<i>G. sesquipedale</i>	3.0	In situ	6 months	Seoane-Camba 1997
<i>G. robustum</i>	1.0, 3.6	In situ and semi controlled	35 days	Pacheco-Ruiz and Zertuche-Gonzalez 1995
<i>G. crinale</i>	6.5	Outdoor tanks	1 year	Boulus et al. 2007
<i>P. capillacea</i>	28.3	Outdoor tanks	1 month	Gal-Or and Israel 2004

Economic aspects

The following limited information illustrates the conflicting data available regarding economic aspects of *Gelidium* culture. Cultivation of *G. amansii* from spores is not an efficient way of increasing production because the spores take 2 years to grow and reach harvesting size. Branches of plants attached to ropes hung into the sea grow well; however, the costs of plants for seed and the labor force leaves little room for profit. On the other hand, as reported by Shunzo Suto (1971), the expenditure of setting stones for *Gelidium* culture in favorable areas can be recovered within 4 years. Because of the current status of natural coastal stocks of this resource, future applications of this type of culture appears to be more related to the restoration of damaged seaweed beds in Spain rather than to attempts at commercial cultivation, as suggested by Sousa-Pinto et al. (1996). As *G. sesquipedale* is a slow-growing species, it seems impossible to obtain more than one harvest per year in culture under strong water agitation. From these data, it would seem impossible to make *Gelidium* aquaculture profitable in Morocco as suggested by Givernaud and Mouradi (2006).

Table 1 summarizes the growth potential of *Gelidiales* as presented by various authors to date. In short-term experiments, the maximal daily growth rate (DGR) in the laboratory can reach 21%, in the laboratory and in situ 33%, and in tank culture 28%, while an annual outdoor tank experiment reached a DGR of only 6%, and an annual growth experiment in the sea reached only 3.8%.

There is no published economic analysis of *Gelidium* cultivation. However, the above mentioned data lead to a maximal calculated annual yield of *Gelidium* of about 25 kg FW m⁻², which is still far from the break-even point for commercial pond cultivation of agarophytes, which is about 60 kg FW m⁻²year⁻¹ (17% m⁻² day⁻¹) or about 6 kg DW m⁻²year⁻¹. On the other hand the assumed production costs of *Gelidium* with such a yield would be 2–3 US \$ kg DW⁻¹. The production costs were calculated for other seaweed cultures like *Gracilaria*, and might be affected by prices of infrastructure, electricity and labor in each country. The major conclusion is that the annual yield has to be dramatically improved.

Future prospects

Clearly, the major reason for the current lack of commercial cultivation technology for *Gelidium* is the slow growth rate and low yields of the existing species. Therefore, major effort towards the introduction of *Gelidium* into commercial cultivation have to involve genetic improvement through selection, breeding and possibly genetic engineering.

Genetically engineered commercial products are meeting with increased resistance in the marketplace, limiting this technology's utilization. Genetic engineering may not be necessary, considering that many successful high yield seaweed strains that are now commercially produced were selected through more traditional methods. Only high yield strains will have the potential to compete economically with the present harvesting tradition.

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Production, use and fate of Chilean brown seaweeds: re-sources for a sustainable fishery

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Abstract Chile is an important producer of brown seaweeds representing 10% of world supply. Landings of Chilean kelp fluctuated between 40,000 t.year⁻¹ in the early '80s to 250,000 t.year⁻¹ more recently. Commercialized algae come from natural populations and no mass-cultures of involved species have been established. Four species of brown algae are commercially collected in the country: *Lessonia trabeculata*, *L. nigrescens*, *Macrocystis pyrifera* and *M. integrifolia*. Since 2000, the demand of alginate sources and food for cultivated abalones dramatically raised the harvesting of these species. Direct evaluations of *Lessonia* spp. and *Macrocystis* standing-stocks were made along 700 km of coast in northern Chile. *Lessonia* spp. estimated populations exceed 900,000 t, whereas *M. integrifolia* does not exceed 300 t. The insights provided indicate that sustainability of Chilean kelp subjected to intense harvesting would require management programs including the following bio-ecological recommendations: (1) to harvest the entire plant including the holdfast; (2) to harvest plants larger than 20 cm in diameter; (3) to harvest plants sparsely, selecting mayor specimens; (4) rotation of harvesting areas; and (5) for *Macrocystis*, to cut the canopy 1–2 m from the surface. They must be implemented in a National Program of Kelp Management, elaborated by government, scientists, fisherman, and industry.

Keywords Kelp · Chile · *Lessonia* · *Macrocystis* · Abalone

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Introduction

Giant kelps dominate shallow, subtidal rocky-bottom areas in temperate and cold seas to a depth of ca. 40 m (Dayton et al. 1984; Harrold and Pearse 1987; Vásquez 1992; Graham et al. 2007). Many kelp species have economic and social impacts since many local human populations base their economic support on these resources (Shiel and Nelson 1990; Vásquez and Fonck 1993; Stekoll et al. 2006; Rothman et al. 2006; Ugarte et al. 2006). Geographic and local distribution of diverse kelp species in the southeast Pacific is variable: *Lessonia nigrescens* Bory forms intertidal belts along exposed rocky coasts; *Lessonia trabeculata* Villouta & Santelices dominates rocky subtidal environments to a depth of ca. 40 m; *Macrocystis integrifolia* Bory forms shallow kelp beds from the intertidal zone to ca. 15 m in northern latitudes, and is gradually replaced by two species towards southern areas: *Durvillaea antarctica*, which dominates the intertidal zone in wave-exposed areas (Hoffmann and Santelices 1997); and *Macrocystis pyrifera* (Linnaeus) Agardh in both exposed and protected subtidal habitats (Buschmann et al. 2004). While the two mentioned *Lessonia* species have almost continuous distribution along the entire Chilean continental coast, the distribution of *M. integrifolia* is fragmented into patchy populations in northern Chile (Vega et al. 2005), while *M. pyrifera* forms coastal belts from 42°S to Cape Horn, including the fjord zone and the Atlantic coast up to Chubut area, in Argentina. The extension of local populations of *M. pyrifera* from north to south, may vary from 100 m to 100 km. The observed distribution patterns are the result of complex life history strategies and interactions with environmental factors such as spatial and temporal variations in water movement,

nutrient availability, and temperature (Muñoz et al. 2004; Vega et al. 2005; Graham et al. 2007).

Lessonia species are dominant organisms in coastal communities, both in cover and biomass, being the foundation species (sensu Dayton 1972) in the intertidal and shallow subtidal rocky bottom. Several invertebrate and fishes, some of which are of economic interest, use *Lessonia* kelps as habitat, food supply, and area for recruitment (Vásquez 1992; Vásquez et al. 2001), so management of these brown seaweeds is also relevant to conservation of many other coastal fisheries.

In Chile, brown algae, especially those collected on the beaches as result of natural mortality, have been used since the 1960s as raw material for alginic acid extraction, with 90% of this productive activity, which occurs exclusively in Northern Chile between 26° and 32°S (Anuario SERNA-PESCA 1985–2006), supported by both *Lessonia* species. Plants which have been cast ashore are collected by fishermen, dried, bundled and then sold to intermediaries who sell them to the grinding industry. This fishery is currently not regulated since landings are mainly composed of dead plants. Since 1998, extensive harvesting of intertidal and subtidal populations of *Lessonia* spp. in northern Chile, has put fishery authorities, as well as fishermen, industry and scientists, on alert.

This article presents the most relevant information regarding distribution, abundance, productivity, destination and predicted use of commercially important brown algae from Northern Chile. This information brings together all elements needed for planning their sustainable management.

Materials and methods

Since 2004, bio-ecological studies of production and analyses of the socioeconomic aspects of brown algae fisheries in northern Chile have been developed. Their common goals were to elaborate recommendations for organize, and make more efficient, the administration and management of this activity. This study analyzes the availability of biomass from natural populations (2004–2005), the chain of production (2005–2006), and some socioeconomic aspects of this fishery (2004–2006). The study area includes the entire geographical region of this fishery, between 18° and 32°S. Considering that the majority of collection and harvesting, as well as use and destination, occur between 26°–32°S, most of the information comes from this sub-sector of the Chilean coast (Fig. 1).

Collection and harvesting statistics

All data regarding brown algae landings (wet and dry) were obtained from the National Fishery Service, which depends on the Ministry of Economy (<http://www.sernapesca.cl>);

data related to exportation of brown algae (US\$ and tons) as algae meal, for alginic acid extraction, were obtained from the Central Bank of Chile (<http://www.bancocentralchile.cl>).

Distribution and abundance of brown algae

From 2004 to 2005, the distribution (latitudinal and bathymetric) and abundance of *Lessonia trabeculata*, *L. nigrescens* and *Macrocystis integrifolia* were determined along approximately 700 km of coast between 26° and 32°S. In this area, 140 sampling stations were established (Fig. 1), separated by 4.5 linear km, excluding a priori sandy beaches and soft bottom (subtidal) areas.

The basal diameters of the plants' holdfasts were measured in situ, since a significant correlation exists between this dimension and the weight and size of the plants in all three species (Cancino and Santelices 1984; Vásquez and Santelices 1984; Villouta and Santelices 1984, 1986; Vásquez 1992, 1993). Density of plants and type of available substrate were also determined. All evaluations were non-destructive.

***Lessonia trabeculata*:** distribution and abundance of this species was evaluated using 160-m transects, perpendicular to the coastline and located between the intertidal zone and 20-m depth, according the methodology described by Vásquez et al. (2006).

***Lessonia nigrescens*:** distribution and abundance of this species was determined using 10 m² (10×1 m) quadrants. Morphological attributes of adult sporophytes (holdfast diameter, length, number of stipes and presence of reproductive structures), were evaluated using 1-m² quadrants.

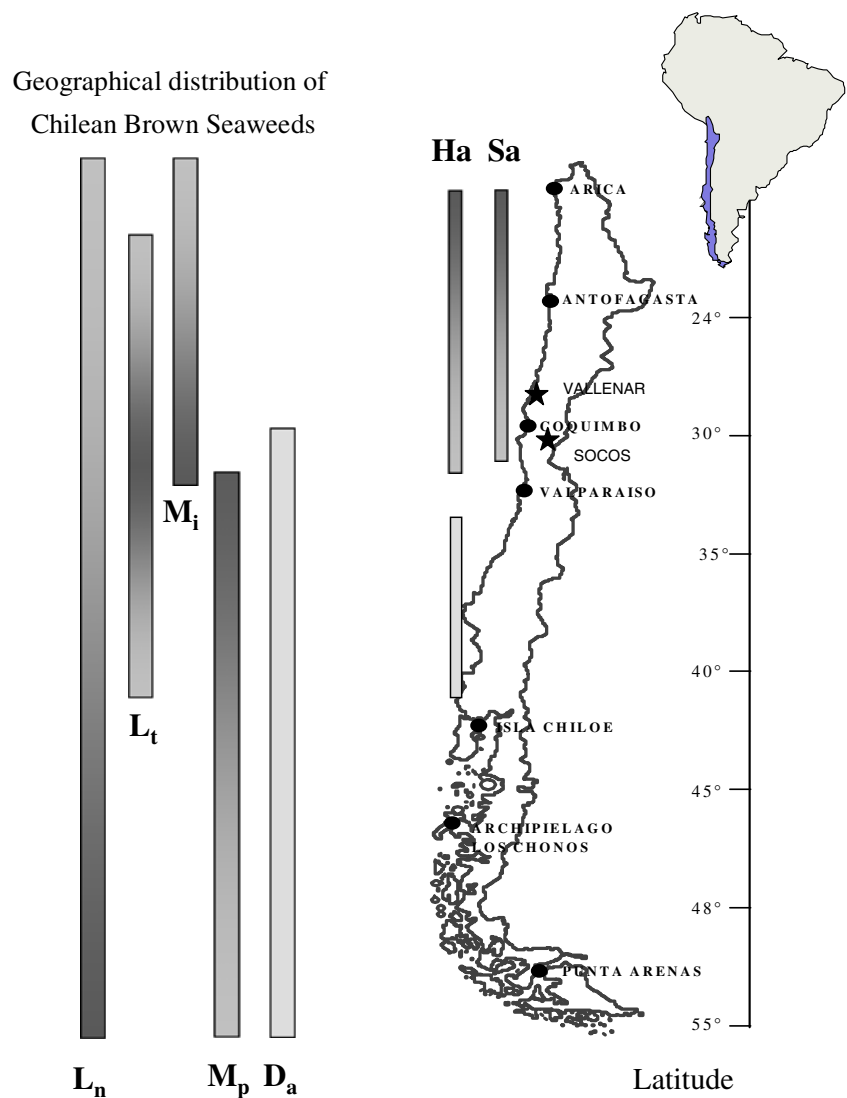
***Macrocystis integrifolia*:** since the fragmented distribution of *M. integrifolia*, 0.25 m² quadrants were randomly thrown within each algae bed (see Vega et al. 2005). When *Macrocystis* extended into the subtidal zone, sampling was done in layers by positioning four 0.25 m² quadrants in different depth ranges (0–2, 2–4, 4–6 >6 m). The limits of each population were georeferenced (GPS-GARMIN 12 Channels) to estimate the total abundance of each bed.

In each sampling area (10 m² for *L. trabeculata*, 1 m² for *L. nigrescens*, and 0.25 m² for *M. integrifolia*) three aspects were evaluated: (1) total number of plants, (2) basal diameter of holdfast, and (3) substrate type. Each sampling area was georeferenced.

Biomass estimation using GIS

The data obtained in the field for each population, and regarding their abundance and distribution, were incorporated into the Geographical Information System (GIS,

Fig. 1 Geographical distribution of brown seaweed of economic interest in Chile, including the study area of its fishery. *L_t* *Lessonia trabeculata*, *L_n* *Lessonia nigrescens*, *M_i* *Macrocystis integrifolia*, *M_p* *Macrocystis pyrifera*, *D_a* *Durvillaea antarctica*, *H_a* Study area, *S_a* Sampling area



ArcView and ArcGIS®). The coordinates of each sampling station and transect were acquired in sextadecimal format (degrees, minutes and seconds) from the Provisional South American spatial reference Datum from 1956 (Psad56).

The geostatistical analysis in the ArcGIS program generated variograms within and between sampled transects (Burrough and McDonnell 1998), and allowed construction of abundance polygons between transects (Klopatek and Francis 1999). An average distribution 160-m width was determined for *L. trabeculata* in agreement with Vásquez (1992) and Vásquez et al. (2006). A 2.5-m-wide belt in the intertidal zone was estimated for *L. nigrescens* (Santelices 1989). The abundance of *M. integrifolia* in each section of the coast was obtained by extrapolating the abundance values determined with quadrants along the depth gradient within the kelp forest (Vega et al. 2005). Standing stock of the three brown algae species was calculated as the sum of the abundance polygons from the entire study area.

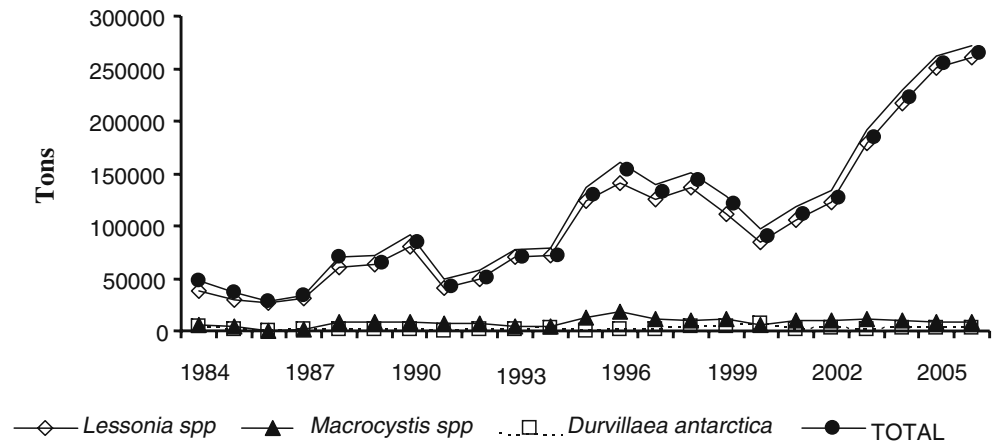
Use and destination

The destination of extracted and collected brown algae from the study area was determined directly by compiling information from brown seaweed processing factories (milling centers) and abalone cultivation centers between 2005 and 2006. In each of these production activities, surveys were taken to evaluate: (1) species processed, (2) their origin, (3) method of collection/harvesting, (4) landed biomass. These same surveys were taken to the fishermen and commercial intermediaries who supply the processing and abalone cultivation centers.

Socioeconomic aspects

Evaluation of some socio-economic aspects of fishermen involved in the kelp collection and harvesting were made with special attention and care. They included determina-

Fig. 2 Annual production (wet biomass) of brown seaweed fishery in Chile (1984–2006)



tion of age, sex, education level, and association to workers unions or consortia.

Results

The brown algae fishery in northern Chile is concentrated between 18° and 32°S. However, the majority of landings are collected or harvested between 26° and 32°S (Fig. 1). In the study area, most of the collection and milling centers are in Vallenar (28°32'S), Ovalle (30°36') and Socos (30°40'). Abalone cultivation centers are located in Caldera (27°06'S) and Coquimbo (29°58') (Fig. 1).

Collection, harvesting and destination of brown algae

During the last 25 years, brown algae landings have fluctuated between 40,000 and 280,000 t·year⁻¹, showing a constant increase since 1999 (Fig. 2). In Chile, *L. nigrescens* and *L. trabeculata* comprise more than 90% of the total production of brown algae, whereas *Macrocystis* sp. and local consumption of *Durvillaea antarctica* contribute only marginally to total landings (Fig. 2). Even if brown algae have an extensive distribution along the southeast Pacific coast (see Fig. 1), their fishery is concentrated between 20° and 32°S (Fig. 3). This is due to the arid climate of the wide Atacama Desert located immediately inland of this region, which allows for rapid drying and consequently a reduction in the productive fees.

Destination of brown algae

The increase in kelp extraction during the last 7 years is explained principally by the significant rise of their utilization as raw material for extraction of alginic acid, both in Chile and outside the country (Fig. 4). A smaller fraction of this increment can be explained by the yield reduction of kelp used for milling. This was consequence of

higher humidity of recently processed plants compared to previous years (Fig. 4). From a different perspective, exports of brown algae during 1997–98, as in other “El Niño” phenomenon, could be related to higher mortalities generated by this large scale oceanographic event. The warming of the ocean surface and decreasing of nutrients concentrations, both associated with “El Niño”, cause high mortalities in kelp populations which are then cast ashore by waves and finally collected by fishermen.

The new and impressively growing abalone cultivation industry in Northern Chile, consumed more than 4,800 t of

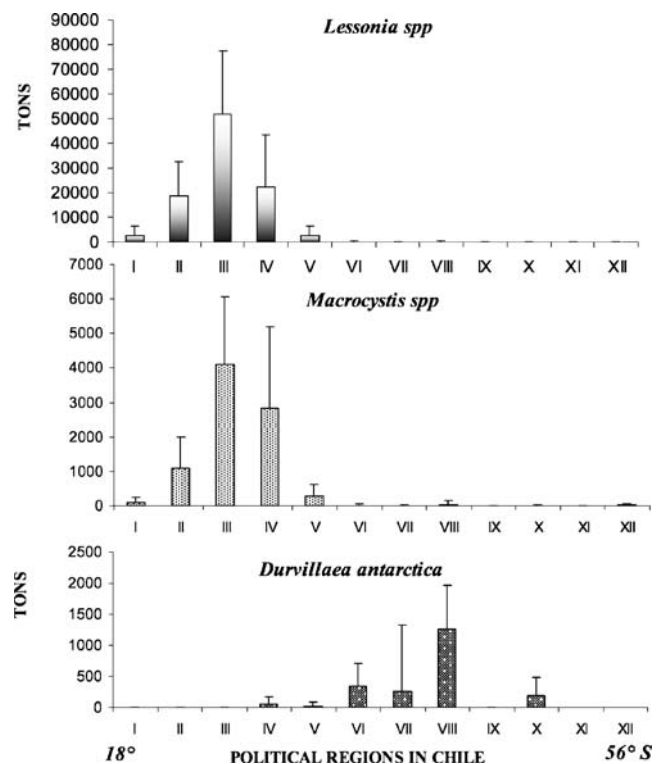


Fig. 3 Average landings (wet biomass) of brown seaweed (1984–2006), along the Chilean coastline: North (Regions I to IV), Central (V to VIII), and South (IX to XII)

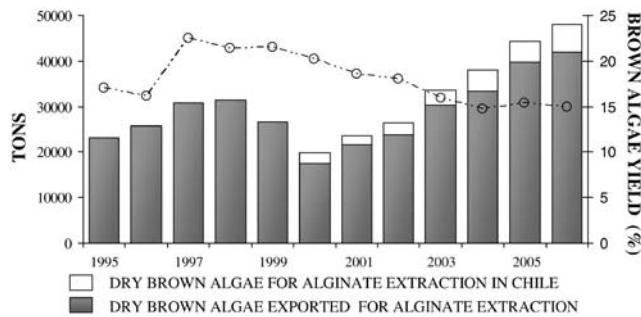


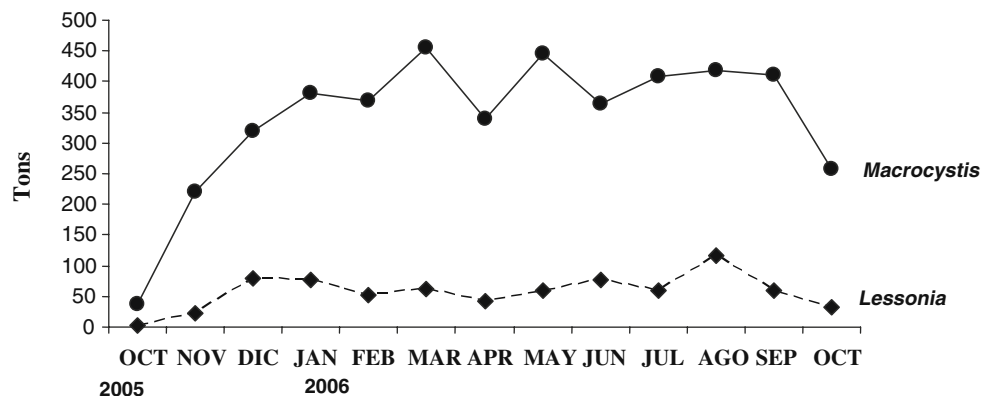
Fig. 4 Annual production (1995–2006) of raw material (dry biomass) for alginate extraction in Chile and overseas. Temporal variability of the percentage of brown seaweed yield (—o—)

fresh algae between 2005 and 2006, principally *Macrocystis* (Fig. 5). However, neither the utilization nor the economic yield of this activity is comparable, until now, to supplying material for alginate extraction (Fig. 6).

Distribution and abundance of brown algae

During 2004–2005, between 26° and 32°S, distribution and abundances of *L. trabeculata*, *L. nigrescens* and *M. integrifolia* were evaluated along more than 700 km of coast (Fig. 7). 140 sampling stations were established separated by 4.5 linear km, excluding a priori sandy beaches and soft bottom subtidal areas because of their unsuitability as a substratum for kelp spore settlement. *Lessonia trabeculata* is found in rocky subtidal habitats between 0 and 30 m depths in the entire study area (Fig. 7). Depending on the extension of the rocky platform into the subtidal zone, local biomasses up to 50,000 wet t were recorded. Abundance polygons indicate a standing stock of approximately 800,000 wet t of *L. trabeculata* in the study area. *Lessonia nigrescens* is distributed in the rocky intertidal zone of the entire study area with local biomass (sampling station) between 50 and 4,000 wet t (Fig. 7). The sum of the abundance polygons constructed with GIS shows a standing stock of more than 100,000 wet t of *L. nigrescens* in the study area. In contrast, *M. integrifolia* shows fragmented distribution within the study area

Fig. 5 Wet biomass of brown seaweeds (*Macrocystis* and *Lessonia*), used as food for abalone culture in northern Chile



(Fig. 7). Local populations are small, with biomasses between 2 and 12 wet t. The sum of local biomass determines a standing stock that does not exceed 200 wet t for the entire study area (Fig. 7).

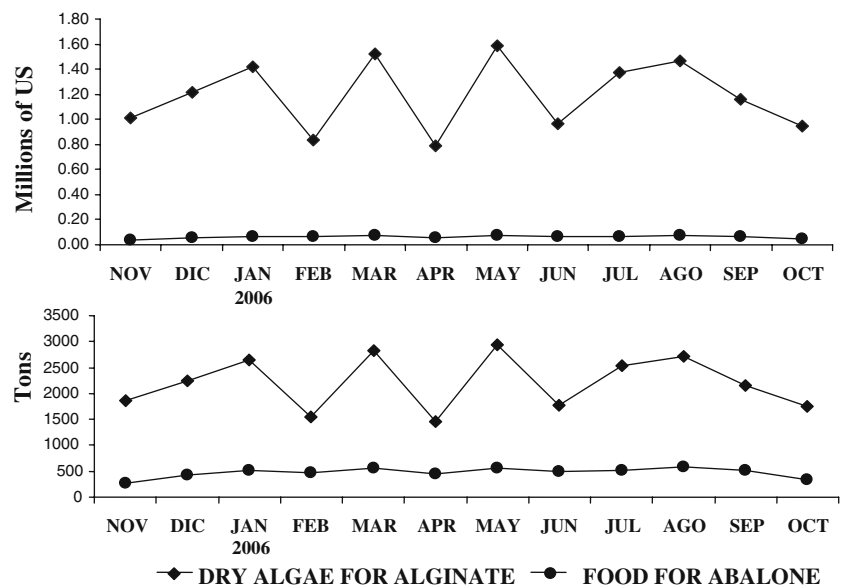
Social indicators

The list of fishermen (<http://www.sernapesca.cl>) shows 7,801 artisanal algae collectors and harvesters in the study area (Table 1). However, only 20% of these individuals showed evidence of permanent collection and harvesting activities during 2005–2006. More than the half of active fishermen (57%) show a preference for working independently, unassociated with consortia or unions; gender analysis shows that 83.4% of active participants are men. The marginality of this activity is reflected in the education of the active fishermen. Almost 70% of participants in this activity have only primary school education, which is generally incomplete. Among the elderly fishermen (>70 years), 4.6% have no formal education, being mostly illiterate. Only 26.2% have secondary education, which in most of cases is incomplete (Table 1).

Discussion and conclusion

Governments have attempted to introduce co-management in coastal waters granting territorial user rights to fishers (TURFs). The rationale behind TURFs is based on a common property approach, which proposes that a well-established rights-based system provides access, withdrawal, and management security for individuals and groups of individuals (Ostrom and Schlager 1996). With such assurances, fishermen would make commitments amongst each other and with the administration, and would develop long-term plans for investing in, and harvesting from, a common-pool resource in a sustainable manner. Accordingly, TURFs change the nature of resource extraction. In this sense, hunting becomes harvesting, a certain degree of predictability is introduced since extraction programs

Fig. 6 Monthly variation of brown seaweed biomass used for alginate extraction, as well as fresh food for abalone culture, and respective incomes (US \$) of both commercial activities



requires at least previous evaluation of target resources, and the skills of the fishermen (as hunters) become less important (Gelcich et al. 2005a).

Since 1997, Chilean Government has adopted co-management policies for the coastline, granting territorial user rights to fishermen (Bernal et al. 1999). Territorial rights related to users into the Chilean Fishery Law, adopted the legal title of Management and Exploitation Areas for Benthic (bottom dwelling) Resources (MEABR). Through this, the Governmental Entity that regulates fisheries assigns defined rights and norms of utilization to syndicates located in defined geographical coastal areas (Gelcich et al. 2005b). By August 2003, 188 exploitation areas (MEABR) had management plans in progress, and 649 were at diverse stages of application procedures (Gelcich et al. 2005b).

To date, between 18°–32°S (see Fig. 1), more than 50% of the coastline is under MEABRs regime, and this area represents the main geographical scenario for kelp fishery in Chile. In this context, fishermen syndicates would apply many of the proposed strategies for brown seaweed conservation in MEABRs. Currently, MEABRs that include brown seaweeds as target species have to harvest according biological and ecological recommendations. In addition, the results of harvesting procedure and the recovery of kelp beds after pruning or removal of individuals must be inserted into each management program, which is updated annually.

In open areas of Northern Chile without an MEABR regime, collection of plants cast ashore is the main activity of fisherman along the coast. Harvesting of *L. nigrescens* is the most difficult to control in terms of regulation enforcement along Chile's extensive coast. In contrast,

due to the location of *Macrocystis*, harvesting is closely related with the proximity of abalone culture centers; the few and small beds of *Macrocystis* near them have been harvested under a co-management plan developed by fisherman jointly with the direct user, the abalone industry.

During the last 50 years, kelp fisheries have been supported by the natural mortality of coastal populations caused by the drag of bottom currents and wave impact on old and/or debilitated plants. The detached biomass is cast ashore by currents and hand-collected by fishermen. In this case, there was no need for administration and management of *L. trabeculata*, *L. nigrescens* and *M. integrifolia*. This feature of brown seaweed fishery was relevant in the past, not only for the conservation of *Lessonia* and *Macrocystis* populations but also for many other associated species with economic interest, such as keyhole limpets, sea urchins, and several fish and mollusc species (Vásquez and Santelices 1984; Vásquez and Vega 2005). Since 2000, extractive pressures from international markets for alginic acid extraction, as well as the emergent need of fresh food for abalone aquaculture, has dramatically raised the harvesting of brown algae. In this context, and considering the foundation species status (sensu Dayton 1972) of Chilean kelp species, any management program must include the whole coastal marine habitat.

The Kelp fishery is also important in other cold and temperate areas around the world, such as Alaska (Stekoll et al. 2006), Southern California, USA, and Baja California, Mexico (Barilotti and Zertuche-González 1990), Norway (Sjotun et al. 1993), South Africa (Anderson et al. 2006; Rothman et al. 2006), New Zealand (Shiel and Nelson 1990), Canada (Ugarte and Sharp 2001; Ugarte et al. 2006), France (Guillaumont et al. 1993), Japan (Terawaki et al.

Table 1 Socio-economic indicators of fisherman of brown seaweeds in Chile

No. of registered fisherman	No. active fisherman	Not-associated fisherman (%)	Female (%)	Male (%)	Education level (%)		
					Uneducated	Elementary	High school
7,801	1,549	57	16.6	83.4	4.6	69.2	26.2

2001), and Ireland (Werner and Kraan 2004). The production and sustainability of involved populations is mostly focused on biological and ecological knowledge, being the management based on a single species resource. In contrast, kelp management based on the ecosystem has been poorly documented.

As in this study, in addition to the bio-ecological features of target species, other management considerations such as maximum exploitation rate, cutting height, gear restriction, effects on associated species, and protected areas have been considered in the sustainability of *Ascophyllum nodosum* in Canada (Ugarte and Sharp 2001) and *Laminaria* spp. in France and Norway (Werner and Kraan 2004). Similarly to *Ascophyllum* studies, universities, government authorities, and industry were involved in the production of a research and monitoring program for the sustainability of economically important kelp including also, social impact. In this context, the most important difference between the kelp fishery in Chile and other countries is the relevance we assigned to the social aspect of this fishery, since it provides the main economic resource for several families under high social risk in Northern Chile.

Location of growth meristems and reproductive structures in different kelp species (Edding et al. 1994; Hoffmann and Santelices 1997) determines that the way of harvesting has different consequences for different species: *Lessonia* has sori on apical fronds and along stipes (Edding et al. 1994; Venegas et al. 1992), whereas sporophylls are basally distributed in *Macrocystis*. The meristems of both genera are apically distributed, and only *Macrocystis* continually produces new stipes from the holdfast. Pruning *Lessonia* inhibits its growth, and regeneration is nonexistent (Vásquez and Santelices 1990; Vásquez 1992; Mendieta 1997), while harvesting of fronds and stipes eliminates gametangia and thus prevents sexual reproduction. In contrast, the regeneration of biomass after harvesting is a rapid and frequent process in *Macrocystis* (Graham et al. 2007); additionally, pruning only removes the canopy of individuals, and does not affect basal sporophylls involved in sexual reproduction.

Brown algae landings have fluctuated between 40,000 and 280,000 t during the last 25 years, with escalating rates since 1999. At present, approximately 300,000 wet t of brown algae are collected or extracted from the coast of

Northern Chile, and *Lessonia* spp. represents more than 90% of this total. This productive activity creates yearly revenues of approximately US\$22 million (<http://www.bancocentralchile.cl>), which comes mainly from supplying raw sources for the alginic acid industry. Chile provides 10% of the world wide sources for alginate extraction, which is used primarily by pharmaceutical and food industries (Avila and Seguel 1993; Vásquez and Fonck 1993; Zemcke-White and Ohno 1999), though high quality alginate production within Chile is a growing activity.

In terms of fisheries statistics, the standardization of humidity content and yield of kelp for industrial processes are required at the national level. This would allow more precise predictions for the industry as functions of the requirements and availability of biomass stock from natural beds.

In Chile at present, the availability of fresh food is the most important constraint for the emerging aquaculture of abalone. In the study area, the estimated requirement of this industry reaches an average of 500 t per month of fresh algae, mainly *Macrocystis*. However, significant consumption of brown algae by cultivated abalone is foreseen. Preliminary studies of conversion rates (Pérez, personal communication) show that the production of 1 t of abalone requires 100 t of fresh algae. In consequence, the projected annual production of cultivated abalone for 2010 is 1,000 t, which would require 100,000 t of brown algae to reach this goal. Considering the low abundance and fragmented distribution of *M. integrifolia* in Northern Chile, this requirement greatly exceeds the production potential of natural populations. Consequently, the sustainability of abalone cultivation depends on the future culture of *Macrocystis*. In this context, successful efforts have been made in Chile to produce *Macrocystis* spp sporophytes using long-line culture systems (Gutierrez et al. 2006; Westermeier et al. 2006). From a different perspective and given the susceptibility of brown algae to interannual oceanographic events like El Niño (Vega et al. 2005; Vásquez et al. 2006), kelp culture would allow independence from unpredictable environmental catastrophic fluctuations and would help the natural recovery of coastal rocky areas after mass mortality events.

In Chile, a second epicenter for the abalone cultivation industry is located at ca. 40° S. At high latitudes, reaching the Pacific coast of Cape Horn and on the Atlantic coast

near Chubut Argentina, *M. pyrifera* forms continuous belts that comprise the most important brown algae reservation in the world (Graham et al. 2007). In spite of this, and contrary to the perennial *M. integrifolia*, *M. pyrifera* populations are annual and therefore minimally available in the winter (Buschmann et al. 2006). For this reason, development of cultures should not be centred exclusively in Northern Chile.

Fishers, of whom only 17% are women, carry out collection and harvesting of brown algae in Chile. In the most active areas located between 18° and 32°S, 7,801 collectors are registered, but only 20% of them permanently participate in collection and harvesting activities. The majority of these fishers are independent, not belonging to any workers union or consortium. The level of education attained by active fishermen reflects the marginality of this work. Almost 70% of the workforce has only elementary education and in most cases incomplete.

The estimated standing stock of *Lessonia* (both species analyzed, more than 1 million t) is enough to sustain the actual brown algae landings. Considering the morphologic and reproductive attributes of *Lessonia*, “how to harvest” seems to be more important than “how much to harvest”. In terms of how to harvest, a few important considerations are relevant: (1) plants should be completely extracted including the holdfast; (2) large plants, with holdfast larger than 20 cm in diameter, should be selected for extraction; (3) harvesting would be sparse leaving a substratum space not larger than 1–2 m between plants. All these recommendations are relatively easy to implement and control in areas with an MEABR regime, even if, in areas with free access, they would be certainly more difficult to carry out. Management Plans would then consider these simple recommendations: (1) to liberate primary substratum for the settlement of juvenile sporophytes, (2) to allow for juvenile recruitment by diminishing competitive interaction between adults and sporelings, (3) to maintain a reproductive stock of adults, while allowing population regeneration, and (4) to prevent overgrazing of plants by benthic herbivores by maintaining a whiplash effect (see Dayton et al. 1984; Vásquez 1992).

In contrast to the situation with *Lessonia*, where how to manage the stock is the most relevant issue, the sustainable fishery of *Macrocystis* should take into account its distribution and abundance, before authorizing the harvesting of natural populations in Northern Chile. This species has a unique genetic heritage due to its restricted and fragmented distribution. It also plays an important ecological role as an engineering (sensu Jones et al. 1994) and foundation species (Graham et al. 2007), because numerous invertebrates and fish depend on its existence (Vásquez and Vega 2005; Graham et al. 2007). Thus, the

small beds of *Macrocystis* in Northern Chile would be under a harvesting program, which must be carried out by local fishermen, funded by the local abalone industry and supervised by fishery authorities.

Currently, some management strategies are being applied in Chile by means of a regionalized system that prohibits the extraction and collection of kelps in open areas (not under MEABR regime) during a certain period of time. Under these restrictions, users are allowed to obtain the resource, as long as they deliver the information required to determine: (1) harvesting and collection effort, (2) locations of collection and harvesting areas, (3) number of fishermen, (4) number intermediate buyers, (5) number and location of milling and abalone cultivation centers, and (6) amount of biomass used by each activity. Using the above information, we collaborated with governmental fishing authorities that organized technical work groups. In these collaborative instances, fishermen, industry representatives, technicians, scientists and administrators work together towards establishing sustainable management strategies that account for the distribution and abundance of brown algae and the requirements of the industries related to these resources.

Biological and ecological knowledge by itself does not guarantee the success of a harvesting program. In practice, social, political and economics aspects (see Vásquez and Westermeier 1993) must be considered for a correct strategy of management of the brown seaweed fishery. Among these, the most relevant are: (1) pressure of national and international markets for raw material, (2) unemployment indexes of coastal workers, which increase seasonal harvesters, (3) control of management recommendation along the extensive coast of northern Chile, and (4) low level of education and incomes of seaweed collectors.

Usually, most management strategies of natural resources start immediately before the imminent collapse or after evident decline of populations subjected to harvesting (e.g., *Gracilaria chilensis*; see Santelices and Doty 1989). This study is an example that illustrates the application of biological and ecological knowledge of target species having regard to the present and future uses of kelp species of economic interest. In this context, concerted actions of government, scientists, fishermen and industry have been taken and should be implemented in time. In the near future, management recommendation will be tested in order to evaluate the stocks of natural populations along Northern Chilean coasts, the production of brown seaweed under culture, and the fate and use of these important benthic marine resources.

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Cultivation of the green alga, *Codium fragile* (Suringar) Hariot, by artificial seed production in Korea

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Abstract *Codium fragile* (Suringar) Hariot is an edible green alga farmed in Korea using seed stock produced from regeneration of isolated utricles and medullary filaments. Experiments were conducted to reveal the optimal conditions for nursery culture and out-growing of *C. fragile*. Sampling and measurement of underwater irradiance were carried out at farms cultivating *C. fragile* at Wando, on the southwestern coast of Korea, from October 2004 to August 2005. Growth of erect thalli and underwater irradiance were measured over a range of depths for three culture stages. During the nursery cultivation stage (Stage I), growth rate was greatest at 0.5 m depth (0.055 ± 0.032 mm day⁻¹), where the average midday irradiance over 60 days was 924 ± 32 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. During the pre-main cultivation stage (Stage II), the greatest growth rate occurred at a depth of 2 m (0.113 ± 0.003 mm day⁻¹) with an average irradiance of 248 ± 116 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. For the main cultivation stage (Stage III) of the alga, thalli achieved the greatest increase in biomass at 1 m depth (7.2 ± 1.0 kg fresh wt m⁻¹). These results suggest that optimal growth at each cultivation stages of *C. fragile* could be controlled by depth of cultivation rope.

Keywords Artificial seed production · Cultivation · *Codium fragile*

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Introduction

The marine green alga, *Codium fragile* (Suringar) Hariot, is widely distributed in temperate areas throughout the world (Silva and Womersley 1956; Yoshida 1998; Trowbridge 1998), and is eaten in Korea, China and Japan (Abbott 1988). In Korea *C. fragile* is an attractive seaweed species for cultivation because it commands a high market value compared to species such as *Porphyra* and *Undaria* (Sohn 1998). The alga is an additive of Kimchi, a traditional fermented vegetable. Small-scale cultivation of *C. fragile* from natural blooming zygotes has been conducted on the southwestern coast of Korea since 1987, but the success of production by natural seeding has varied due to annual variations in environmental conditions. Hwang et al. (2005a) reported that the regeneration capability of *C. fragile* could be a more reliable method for producing seed stock than reliance on wild zygote seeding, and published conditions for artificial seed production and nursery culture (Hwang et al. 2005b).

Codium species have two morphologically different thalli: spongy and filamentous. Spongy thalli contain numerous chloroplasts and an inner structure of intertwined colorless medullary filaments (Arasaki et al. 1956; Borden and Stein 1969; Yoshida 1998). The filamentous thallus consists of fine-branched filaments. Filamentous thalli have been observed in the field (Arasaki et al. 1956; Fletcher et al. 1989; Borden and Stein 1969), and are formed from isolated utricles, medullary filaments, zygotes and parthenogenetic female gametes of the spongy thalli (Borden and Stein 1969; Ramus 1972; Park and Sohn 1992; Yang et al. 1997).

A number of inter-related factors impact on the formation and growth of the spongy thalli, (i.e. irradiance and water movement). Water movement plays an important role

in regulating formation of spongy thalli under laboratory culture condition (Ramus 1972; Park and Sohn 1992). Park and Sohn (1992) reported that the critical irradiance for formation of spongy thalli was over 3,000 lx (ca. 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Nanba et al. (2005) reported that growth of the spongy thalli accelerated under a combination of high irradiance and high water velocity.

While other workers have demonstrated the importance of irradiance levels on controlling life cycle of *C. fragile* in the laboratory, there is no information on optimal irradiance, and consequently optimal culture depth, for the key stages of *C. fragile* cultivation in the field. A full understanding of the relationship between light availability and *C. fragile* light requirements requires site- and species-specific information. We report here the relationships between depth, irradiance, and growth of *C. fragile* during a cultivation cycle at one site.

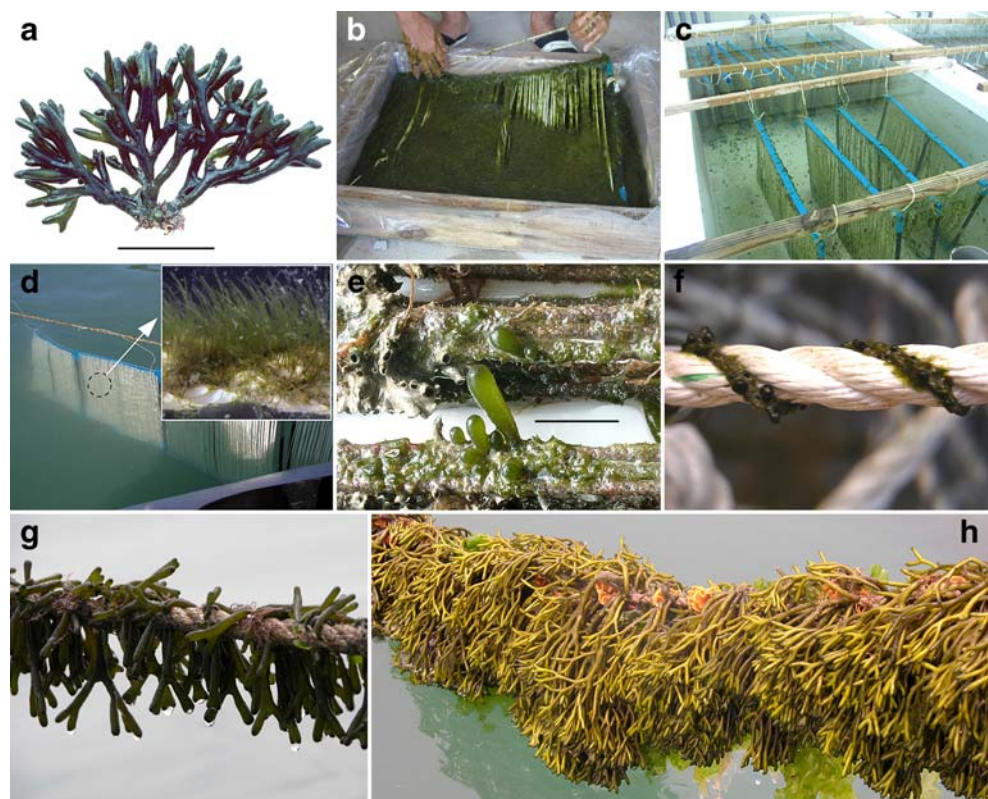
Materials and methods

The *C. fragile* culture experiments were conducted at Wando (34°17'N, 126°42'E) on the southwestern coast of Korea. This is one of the most active seaweed culture areas around the southwestern sea of Korea. Environmental factors such as water temperature, salinity and conductivity, were measured by a logging multi-parameter probe (YSI-

85, YSI Co, Yellow Springs, USA) at the experimental site. The light profile was measured every 7 days at the surface, and at depths of 0.5, 1, 2 and 3 m. Measurements were always taken within 2 h of solar noon, and five replicate measurements were taken at each depth on each occasion. Photosynthetically active radiation (PAR) was measured with a spherical quantum sensor (Li-193 SA; Li-Cor, Lincoln, USA) from October 2004 to August 2005 at the experimental culture ground. Values were recorded on a Li-1400 Data Logger (Li-Cor). The probe was held at each depth for 5 min each and an average reading was taken over that time. Surface PAR measurements were taken between each depth reading at the start and end of the depth profiles at each site. Attenuation coefficients were calculated on each sampling occasion by regression equation of natural logs of light values according to Carruthers et al. (2001).

Artificial seeding and tank culture methods of *C. fragile* were as described by Hwang et al. (2005a, b). Artificial seeding of isolated utricles and medullary filaments were conducted in July 2004. Vegetative thalli (Fig. 1a) were chopped using a hand blender (MR 5550CA, Braun, Barcelona, Spain) until the fragments were less than 5 mm in length. Isolated utricles and medullary filaments seeded onto a seed frame coiled with 100 m seed fiber (Daerim Fiber, Seoul, South Korea) (Fig. 1b). Most of the spongy fragments detached immediately after seeding onto the fibers, but some utricles and medullary filament became

Fig. 1a–h Procedure for artificial seeding and nursery culture of *Codium fragile*. **a** Vegetative thalli. **b** Seeding of the utricles and medullary filaments on seed frame with 100 m coiled seed fiber. **c** Seed frames are held in tank culture for 1 month. **d** Nursery culture of seed frames bearing medullary filaments on the strings in the sea at the different water depths. **e** Regenerated medullary filaments and young erect thalli after 40 days culture in situ. **f** After nursery culture, the seed fiber with young erect attached thalli is spooled around the main culture rope. **g** Young thalli of *C. fragile* after 5 months of culture in May 2005. **h** Fully grown *C. fragile* after 7 months of culture in August 2005. Bars **a** 10 cm, **e** 1 cm



attached to the fibers. The coils of fiber were then moved into a culture tank (Fig. 1c). The cultivation cycle for the alga can be divided into three stages: Stage I, nursery cultivation (Fig. 1d,e; 0–3 mm erect thallus length); Stage II, pre-main cultivation (Fig. 1f, stationary growth period, 0.3–4 cm thallus length); and Stage III, main cultivation (Fig. 1g,h; fast growth period, more than 4 cm thallus length and thalli become buoyant). Out-growing used a horizontal cultivation system modified from Sohn and Kain (1989), where horizontal ropes 100 m long were held at each of the experimental culture depths. For each experiment, a fresh batch of juvenile plants taken from replicate fiber coils from the original medullary filament settlement that had been held under the same environmental conditions (1 m depth) were used.

Seed frames were held in culture tanks for 60 days from July to September 2004 before the outdoor cultivation experiment commenced. From October to December 2004, seed frames of *C. fragile* were moved into the sea for Stage I experiment. For each experimental condition, plants were assessed every 30 days by measuring the maximum length of the medullary filaments or erect thalli using Venier Calipers, and measuring fresh weight on an electrical balance. The Stage II culture experiment ran from December 2004 to May 2005, and Stage III ran from May to August 2005. Water depths used for the Stage I, II and III cultures were 0.5, 1, 2 and 3 m, respectively. For in Stage II and III growth of *C. fragile*, plants were assessed every month by measuring the maximum length of erect thalli, fresh weight and mean number of erect thalli per 1 m of culture rope. One-way ANOVAs were used to test for significant differences in the mean growth rate among depths within each stage, and the mean biomass among

depths. When significant differences were found ($P < 0.01$), Fisher's LSD test was applied *a posteriori* to determine whether treatments varied significantly from each other.

Results

Water temperature varied from 8.0 to 24.8°C during the experimental period (Fig. 2). Maximum water temperature was recorded in September 2004, and the minimum in February 2005. Salinity ranged between 33.2 and 34.6 ppt. Mean water depth was 15–20 m in the culturing area. The mean current in the area was 0.5 ms⁻¹. Underwater irradiance varied according to month and water depth (Fig. 2). Maximum surface irradiance was recorded in August 2005, and the minimum in May 2005.

Growth

During Stage I (nursery cultivation), medullary filaments of *C. fragile* grew densely and developed into erect thalli with a spongy structure (Fig. 3a). Water temperature decreased during the culture period from 20.0 to 13.3°C. After 60 days culture, the mean length of the young erect thalli was greatest for plants held at 0.5 m depth (3.3 ± 1.9 mm, Table 1). There was a significant difference in the mean growth rate among depths in Stage I (ANOVA, $P < 0.01$). Subsequent *a posteriori* testing showed that these plants were significantly larger than at 2 m or 3 m depth (Table 1).

At the beginning of the Stage II cultivation trial, the mean length of *C. fragile* was 2.0 ± 1.1 mm (Fig. 3b, Table 1). During the culture period, the water temperature increased from 8.0 to 13.9°C. At depths of 0.5 m and 1 m,

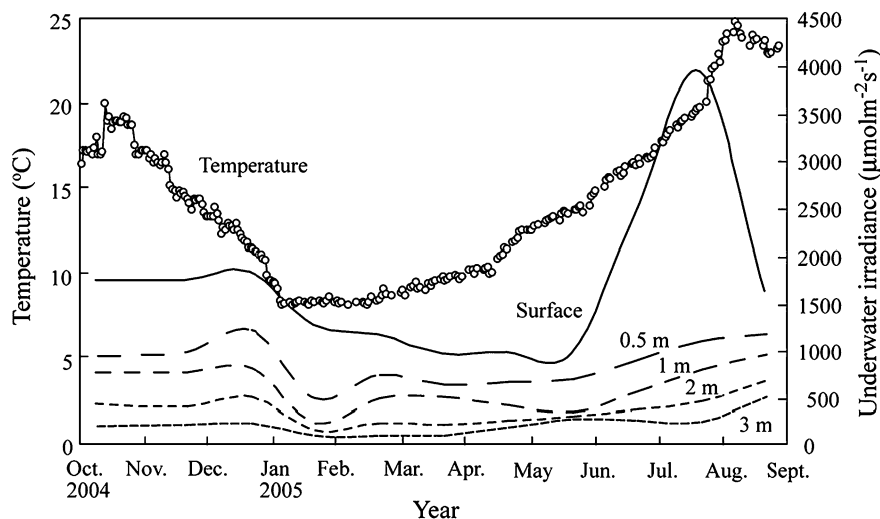


Fig. 2 Fluctuations in water temperature and underwater irradiance during the nursery and main culture periods of *C. fragile*. Irradiance was measured every month between October 2004 and August 2005

in the culture ground at Wando, Korea. Water temperature was recorded at 1 m depth

Fig. 3a–c Growth and development of *C. fragile* at the three stages with different water depth conditions. **a** Stage I (nursery cultivation) conducted from October to December 2004. **b** Stage II (pre-main cultivation) conducted from December to May 2005. **c** Stage III (main cultivation) conducted from May to August 2005

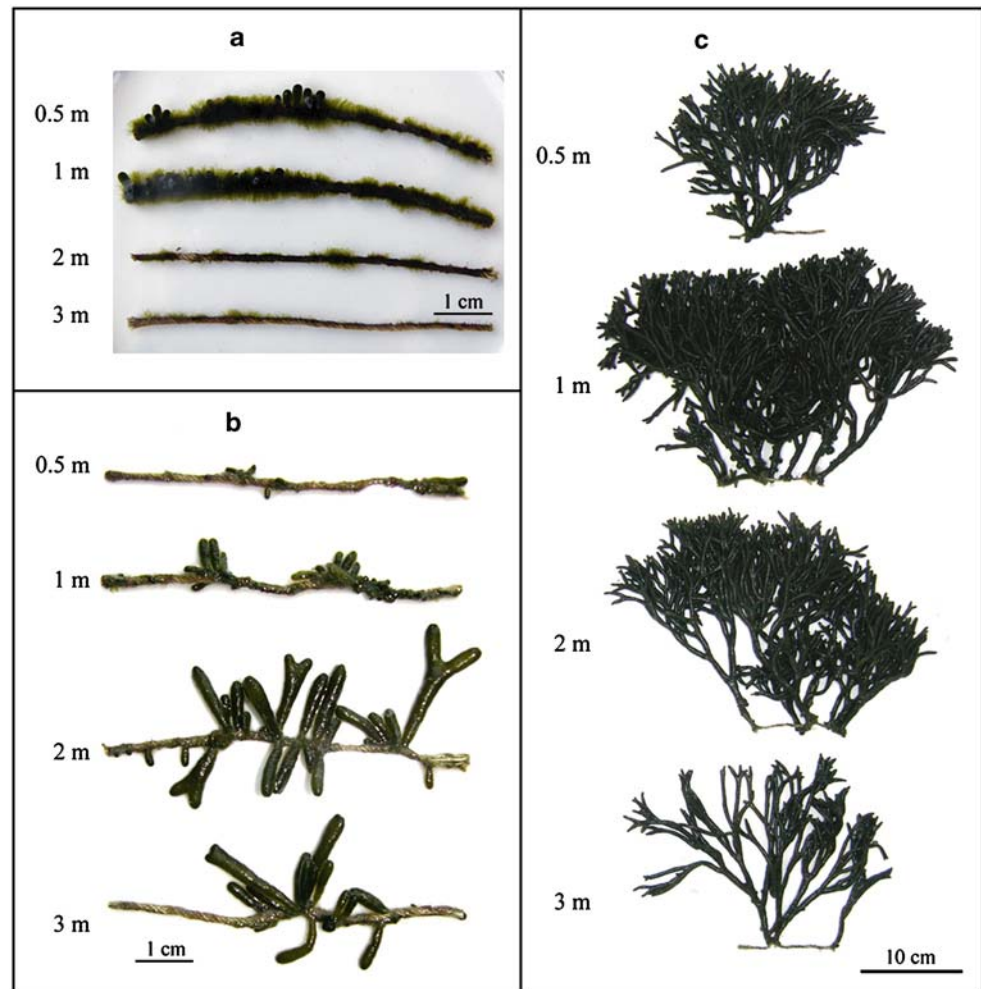


Table 1 Relation between growth rate and depth in *Codium fragile* during the different cultivation periods

Stage	Length growth of erect thalli					Regression of depth vs ln light
	Depth (m)	Initial length (mm)	Final length (mm)	Culture duration (days)	Growth rate (mm day ⁻¹) ^d	
I	0.5	0	3.3±1.9	60	0.055±0.032 ^a	Y=-0.88x+7.48 (r ² =0.96)
	1	0	3.1±1.1	60	0.052±0.018 ^a	
	2	0	1.0±0.3	60	0.017±0.005 ^b	
	3	0	- ^c	60	-	
II	0.5	2.0±1.1	4.7±2.1	150	0.018±0.007 ^a	Y=-0.62x+6.86 (r ² =0.98)
	1	2.0±1.2	5.2±1.1	150	0.021±0.001 ^a	
	2	2.0±1.2	19.0±1.6	150	0.113±0.003 ^b	
	3	2.0±1.1	14.2±0.7	150	0.081±0.003 ^c	
III	0.5	30.2±1.5	142.1±15.3	90	1.24±0.15 ^a	Y=-0.54x+7.27 (r ² =0.91)
	1	30.2±1.3	233.2±18.1	90	2.26±0.19 ^b	
	2	30.2±1.4	198.5±21.6	90	1.87±0.22 ^c	
	3	30.2±1.5	168.4±20.1	90	1.54±0.21 ^a	

^d Values (mean ± s.d. of triplicate groups) in same column having different superscripts are significantly different ($P < 0.01$) according to *a posteriori* Fisher's LSD test

^c No spongy thallus formation

erect thalli were seen to be bleached. A significant difference was found in the mean growth among depths in Stage II (ANOVA, $P < 0.01$). *A posteriori* test revealed that after 150 days culture, the mean length of plants held at 2 m depth was significantly higher (19.0 ± 1.6 mm) than at the other three depths, which did not vary significantly from each other (Table 1).

The largest *C. fragile* plants during the Stage III cultivation trial were observed after 90 days culture in August 2005 amongst plants held at 1 m depth (233.2 ± 18.1 mm, Fig. 3c, Table 1). There was a significant difference in the mean growth rate among depths in Stage III. *A posteriori* showed that the growth rate was significantly higher in 1 m depth than at other depths (Table 1). Water temperature increased during the culture period from 14.5 to 24.8°C. ANOVA showed that significant differences were found in the mean biomass among depths ($P < 0.01$). Subsequently, Fisher's LSD test revealed that there was a significant difference in the mean biomass between all pairwise depths, except for between 0.5 m depth and 3 m depth (Table 2). This result indicates that, during the culture period, the greatest biomass of *C. fragile* was observed at 1 m depth after 90 days. This provided a maximum potential production estimate of 36,110 kg dry wt ha⁻¹.

Relation between underwater irradiance and depth

Underwater irradiance was found to vary with time of day, season and depth. In the first 1 m of the water column, PAR was reduced by around 70% compared to PAR at the surface (Fig. 2). Over the course of the three experiments, the attenuation coefficient varied from 0.54 in the summer to 0.88 in the autumn at the experimental culture ground. The seasonal changes of regression relation between depth vs ln light were reflected in the seasonal nature of each culture stage (Table 2). Growth of *C. fragile* was greater at 0.5–1 m than other depths during Stage I. During this culture stage the mean irradiance was 829 ± 135 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The lowest underwater irradiance mea-

surements were observed during the Stage II cultivation trials. During this trial, mean irradiance at 2 m water depth was 248 ± 116 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and the growth of *C. fragile* was greater at 2 m depth than at other depths. Stage III cultivation occurred during the summer months and, consequently, the highest underwater irradiance values were observed during this stage of the experiment as summarized in Fig. 4.

Discussion

This study has shown that numerous isolated utricles begin to form medullary filaments without a septum immediately after isolation, and forming of filamentous thallus is attributed to the transfer of chloroplasts from the utricles. The utricles rapidly lost their ability to form filamentous thalli after transferring their chloroplasts to the thalli, while the transfer leads the utricles to begin to attach to the substratum by rapidly producing elongated filamentous thalli (Fig. 1d). This study has also shown that the filamentous thalli and utricles of regenerated spongy thalli contain numerous fusiform chloroplasts (Fig. 1e–h). These results suggest that, as mentioned by Nanba et al. (2002), the protoplasm of *Codium* species, which contains numerous chloroplasts, plays an important role in the vegetative reproduction of the isolated utricles and filamentous thalli.

A number of experimental culture studies have revealed that environmental conditions such as temperature, irradiance, water flow, nitrogen source, and salinity are important in controlling the development of *C. fragile* under indoor culture conditions (Ramus 1972; Hanisak 1979; Park and Sohn 1992; Yang et al. 1997). *Codium* thalli are capable of survival and growth over a wide temperature range. In culture, only stationary growth occurs below 12°C. Growth increases as temperature increases up to 24°C. Reduced growth at 30°C is probably due to a sharp increase in respiration at higher temperatures (Hanisak 1979). While *Codium* grows more at warmer temperatures, it can survive

Table 2 Comparison of biomass, production and income of *C. fragile* cultivation in four different water depth conditions

Depth (m)	Biomass (kg fresh wt m ⁻¹) ^a	Biomass (kg dry wt 100 m ⁻¹) ^b	Production (kg dry wt ha ⁻¹) ^c	Estimate income (US\$/ha) ^d	
				<i>Codium</i>	<i>Undaria</i>
0.5	1.58±0.51 ^a	7.9±2.6 ^a	790	9,480	790
1	7.22±1.03 ^b	36.1±5.2 ^b	36,110	433,320	36,110
2	3.43±0.62 ^c	17.2±3.1 ^c	1,720	20,640	1,720
3	1.03±0.31 ^a	5.15±1.6 ^a	515	6,180	515

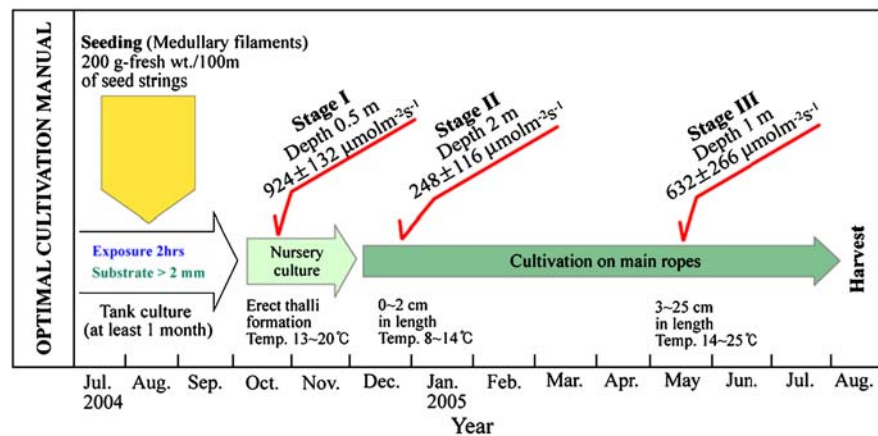
^a Values (mean ± s.d. of triplicate groups) in same column having different superscripts are significantly different ($P < 0.01$) as a result of a *posteriori*

^b Dry yield of *C. fragile* was 5% under 80°C after 24 h

^c Main ropes of cultivation facilities assumed 100 horizontal ropes per 1 m interval from each culture ropes in a 1 ha area

^d Local market price of *C. fragile* and *Undaria pinnatifida* is ca. 12 US\$ and 1 US\$ per kg dry weight, respectively

Fig. 4 Schedule of outdoor cultivation of *C. fragile* showing optimal conditions for different culture stages



temperatures approaching the freezing point of seawater. In this study, the temperature range for the growth of isolated utricles and differentiated thalli was 8.0–24.8°C (Fig. 2). This tolerance to lower temperatures enables *Codium* to survive through the winter in temperate environments. Thus, while the response of *Codium* to temperature is somewhat dependent upon the stage in its life cycle, these stages are adapted to the range of temperatures they experience under natural conditions.

The filamentous thalli of several *Codium* species tolerate a wide range of environmental conditions in their life histories (Borden and Stein 1969; Fletcher et al. 1989). This study showed that isolated utricles have the ability to regenerate young spongy thalli with branched axes, and that their separated axes also have the ability to grow into spongy thalli. The utricles with medullary filaments isolated from various parts of spongy thalli have the ability to form filamentous thalli and attach to the substratum by themselves. Fragments of filamentous thalli grow into full-grown spongy thalli after transfer to the field (Fig. 3). These results suggest that utricles of this species detached by strong waves and grazers in the culture farm can grow into full-grown thalli via filamentous thalli. In an indoor culture experiment, Hwang et al. (2005a) reported that zygotes of *C. fragile* survived at 10–15°C and 10–20 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; however, medullary filaments of *C. fragile* could tolerate a wider range of temperatures (10–25°C) and irradiances (10–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Yang et al. (1997) also observed that filamentous thalli of several *Codium* species grew over a broad range of environmental conditions, while Hanisak (1979) reported that filamentous thalli of *C. fragile* subsp. *tomentosoides* survived and grew in a narrower range of environmental conditions than spongy thalli. During this cultivation experiment, filamentous and spongy thalli of *C. fragile* co-existed on the culture ropes (Fig. 3). The filamentous thalli of *C. fragile* spread and wrapped around culture ropes; thereafter the spongy thalli grew densely. These findings indicate that the formation of

filamentous thalli is an essential prerequisite for the formation of spongy thalli in the life history of *C. fragile*.

Hanisak (1979) reported the submarine irradiance levels for three populations of *Codium* in Rhode Island. Comparing the irradiance required for saturation of growth at various temperatures in culture with that measured in situ, irradiance levels were found to be above saturation during the summer (April–August or September). With one exception, irradiance levels suggested that radiant energy limited growth during the fall months when temperature was still adequate for growth. In this study, the indoor and field investigations showed that the optimum growth light doses of *C. fragile* differ according to growth stage (Table 1). Differences in the response to environmental factors for juvenile and mature stages suggest that this species is capable of altering its physiological activities to meet different environmental demands.

In this study we found that *C. fragile* shows acceptable commercial growth rates when cultured at depths receiving between 22% and 55% of surface irradiance (Table 1). Variation of the optimum light requirement suggests that the different life stages may be sensitive not only to total irradiance but also to spectral composition. These factors may vary between cultivation sites due to the variation in the scattering and light absorption characteristics of the particles and dissolved compounds in the water column (Kenworthy and Fonseca 1996).

Until 2004, fishermen collected naturally blooming zygotes of *C. fragile* and seeded them on cultivation ropes. The success of these natural algal seedings varied annually due to inter-annual variation in environmental conditions. In the case of zygote seeding, the mean biomass has been reported as being normally less than 1 kg fresh wt and the biomass by medullary filament seeding was ca. 7 kg fresh wt per 1 m culture rope under 1 m depth at harvest season, suggesting that the production of *C. fragile* by medullary filament seeding could be higher than that by zygote seeding.

For stable cultivation of this alga, artificial seeding of isolated medullary filament is therefore considered to be the most economic and effective method. The potential production of *C. fragile* has been estimated as 36,110 kg dry wt ha⁻¹ (Table 2). However, to achieve the maximum yield it will be necessary to control the depth at which the plants are cultured. Understanding the appropriate light requirements will assist with site selection and help to expand commercial cultivation of *C. fragile*.

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Distribution and symptoms of epiphyte infection in major carrageenophyte-producing farms

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Abstract High density commercial farming of carrageenophyte *Kappaphycus alvarezii* is often plagued with “ice-ice” disease and epiphyte infection, which eventually leads to reduced production and in some cases collapse of crop. Epiphyte outbreak has been occurring regularly in major carrageenophyte farms in the Philippines, Indonesia, Malaysia and Tanzania. Infected materials from these countries were studied to establish baseline information on the epiphyte’s identity, density, symptoms and secondary infection on the host seaweed. The causative organism was identified as *Neosiphonia apiculata* (Hollenberg) Masuda *et*

Kogame, based on its morphological features. Epiphyte density on host seaweed materials decreased in the following order: the Philippines (88.5 epi cm⁻²), Tanzania (69.0 epi cm⁻²), Indonesia (56.5 epi cm⁻²) and Malaysia (42.0 epi cm⁻²). Initial symptoms were the presence of tiny black spots, indicating the embedded tetrasporeling in seaweed cortex layer. Vegetative form emerged after 2 weeks measuring less than 0.5 mm in length with a density of less than 25.0 epi cm⁻². Upon maturation, infected seaweed takes on a “hairy” appearance with “goose-bumps” like cortical swellings. The epiphyte appears as a solitary plant with multiple secondary rhizoids or as multiple epiphytes appearing from a single cortical opening. At the end of infection, the epiphytes left dark pits on the cortical swelling, and the carrageenophytes are infected by opportunistic bacteria. Bacterial enumeration of healthy and infected seaweed materials showed an increase of more than 300% in total bacterial count on infected materials dominated by *Alteromonas* sp., *Flavobacterium* sp. and *Vibrio* sp.

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Introduction

Red algae genus *Kappaphycus* are farmed as raw material for the extraction of carrageenan, and it is considered an important commercial commodity in the Philippines, Indonesia, Malaysia and Tanzania. More than 90% of the major carrageenophyte farms are located in the coastal waters of these countries (Vairappan *et al.* 2003; Werner *et al.* 2004), with the Philippines producing more than 70%

of the needed raw material. *Kappaphycus* farming is a labour intensive activity and the production is often dependent on culture conditions and disease outbreak. Two significant disease outbreaks often encountered in high density commercial farming are (1) ice-ice disease, and (2) epiphyte infection.

Emergence of “ice-ice” disease was first reported in 1974 in the Philippines by Trono (1974). The involvement of pathogenic microbes and the role of culture conditions were described in detailed by Uyenco et al. (1981) and Largo et al. (1995a, b). Their findings lead to the understanding of how stress induced by culture conditions could trigger the pathogenesis. The causative microbes were identified as *Vibrio-Aeromonas* complex and *Cytophaga-Flavobacterium* complex, and their mode of action was suggested to be based on lytic enzymatic activities of bacterial exudates (Largo et al. 1995a).

On the other hand, epiphytic infection of red filamentous microalgae is not a new phenomenon; it has been known to exist for the last 30 years. An epiphytic outbreak in farmed *Kappaphycus* was reported as early as 1975 by Doty and Alvarez (1975). However, not much interest has been focused on this problem until recently when there has been an increase in epiphyte outbreaks and their impact on production (Critchley et al. 2004; Hurtado et al. 2006; Vairappan 2006). Recent investigation of this problem indicated *Neosiphonia savateri* (Hariot) M.S. Kim et I.K. Lee as the causative filamentous red algae, and revealed some insights of its symptoms and seasonality at culture farms in Sabah, Malaysia (Vairappan 2006). Hurtado et al. (2006) described *Polysiphonia* sp. as the causative organism in the Philippines farms, with a different type of symptoms. The occurrence of epiphyte outbreaks in Malaysia and the Philippines has resulted in a tremendous reduction of biomass production and a decline in carrageenan quality (Vairappan et al. 2007).

Due to the regular outbreaks and economical loss caused by epiphytes, research collaboration was initiated among researchers from the Philippines, Indonesia, Malaysia and Tanzania to gather substantial information on the causative agent in these major seaweed producing countries. Infected seaweed specimens from cultivation farms in the respective countries were preserved, transported and studied at the Laboratory of Natural Products Chemistry, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. This investigation involved identification of the causative organism, infection density, stages of infection and occurrence of secondary bacterial infection after the epiphytes had dropped off. Information on the symptoms of the infected seaweed and its secondary bacterial infection was documented using stereo microscope and scanning electron microscope (SEM) images and microbiological techniques.

Materials and methods

Infected seaweed samples of *Kappaphycus alvarezii*, at various stages of infection, were collected from cultivation farms at Davao, Philippines (6°45'24"N, 125°20'35"E), Tawi-Tawi, Indonesia (4°55'10"N, 120°00'31"E), Semporna, Malaysia (6°02'10"N, 116°01'02"E), and Zanzibar, Tanzania (6°00'08"S, 39°18'21"E). Diseased and healthy seaweeds were collected and analyzed between October 2005 and March 2006. Specimens were cleaned of organic detritus and contaminants, preserved in 10% formalin in seawater and transported to the laboratory in Kota Kinabalu, Sabah, Malaysia. At the laboratory, specimens were further cleaned using a soft tip brush and preserved in freshly prepared formalin (5%) in seawater buffer solution (pH 7.8, salinity 30 ppt).

Epiphyte identification and enumeration

Epiphyte infected specimens were cleaned with a soft brush to remove dirt and other contaminants. Sections of the seaweed were prepared manually using a surgical blade, immersed in seawater and viewed under a stereo microscope (Stemi-2000 CS; Carl Zeiss, Germany). Images were taken using an attached Nikon digital camera. Epiphyte densities were enumerated visually on 1×1 cm² sections of seaweed at 5× magnification. A total of 5 specimens for each location and 10 sections were enumerated for each specimen.

Epiphytes were removed from the host using pith stick and forceps under stereo microscope, and stained with 0.5% (w/v) cotton blue in lactic acid/phenol/glycerol/water [1:1:1:1 (v/v)] solution and mounted in 50% glycerol/seawater on microscope slides. Slides were viewed at 10× and 40× magnifications under a Carl Zeiss compound microscope (Axioskop 40; Carl Zeiss, Germany).

Scanning electron microscope study

Sections of epiphyte infected seaweed were viewed under a stereo microscope, and sections identified for detailed viewing were cropped and fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer solution (salinity: 30 ppt) solution prior to processing. During processing, specimens fixed in 4% glutaraldehyde were taken out of the solution and rinsed twice in distilled water for 10 min per rinse. Sections were then trimmed to dimensions of 5×10 mm. Specimens were post fixed in 1% OsO₄ at 4°C for 2 h, followed by dehydration with graded acetone series. The sections were finally immersed in *t*-butyl alcohol and left for 24 h in a freezer until the solution had completely frozen. The samples were then placed in a freeze dryer (JFD-310 Freeze Drying Device) and the *t*-butyl alcohol was allowed

to sublime completely. Dehydrated specimens were mounted on stubs and coated with 10- to 30-nm layer of gold (JEOL JFC-1600 auto fine coater) before observations were made with a Leica Cambridge S360 electron microscope.

Bacterial enumeration

An area measuring 1 cm² was cut aseptically from both healthy and infected thallus (Sabah specimen), homogenized separately and placed into respective vials containing 10 ml sterile seawater. Serial dilution was carried out to obtain a range of 1×10^{-1} to 1×10^{-5} . Triplicates subsamples of the suspension (100 μ L) were inoculated onto modified Zobell Marine Agar (added 5% healthy seaweed aqueous extract) and spread with a glass rod. Inoculated plates were allowed to dry in a transfer chamber, sealed with parafilm to prevent desiccation, and maintained inverted during incubation at 28°C for 14 days. After this period, bacterial colonies with different morphological characters were counted, isolated, purified and maintained on modified Zobell Marine Agar. Quantitative enumerations of total number of culturable bacteria and bacterial species were recorded. Isolated pure cultures were identified using conventional biochemical and physiological methods as described by Baumann et al. (1972); Kong and Chan (1979); Sawabe et al. (1992, 1998, 2000); Largo et al. (1995a, b) and Vairappan et al. (2001).

Results

Epiphytes were removed from the infected specimens and viewed under a compound microscope at 10 \times and 40 \times magnifications. Filamentous red algae isolated from infected seaweeds from all four countries were similar in morphological features. Detailed observations revealed its thalli as brownish-red, rigid and with percurrent main axes. Its basal attachment system was composed of a primary rhizoid, and later it formed a tuft of rhizoids due to the production of many secondary rhizoids. Primary rhizoids penetrated into basiphyte tissue and well developed thalli also attached themselves to the basiphyte via secondary rhizoids, which were formed from pericentral cells of main axis lower segments and from proximal first-order branches. The epiphyte's main axis became thicker upwards and abruptly tapered at the apices. Each of its vegetative segments consisted of 4 pericentral cells and lacked cortical cells. The most prominent features were its rhizoids, which were cut off from the pericentral cells, the production of lateral-branch initials from successive segments in a spiral arrangement, and a 3-celled carpogonial branches. Its axis also bore vegetative trichoblasts from each segment in a

spiral manner. Relative densities of the epiphytes are shown in Fig. 1, and were in the following order; the Philippines > Tanzania > Indonesia > Malaysia (88.5 > 69.0 > 56.5 > 42.0 epi cm⁻²).

Infection phases

Detailed observation of the epiphyte's infection phases were possible in specimens collected from Semporna, Sabah. Corresponding phases were also noticed in samples collected from the Philippines, Indonesia and Tanzania. Details of this epiphyte's infection phases on *K. alvarezii* are shown in Fig. 2a–f. The first sign of epiphyte infection was observed with the appearance of tiny black spots on the cell layers of the cuticle surface (Fig. 2a), indicating the presence of embedded tetrasporeling between the outer cortex cells. Epiphytes emerge after 2–4 weeks depending on the seawater temperature and salinity conditions, and form a rough surface as shown in Fig. 2b–c. After another 4–6 weeks, the epiphytes mature and are seen as shown in Fig. 2d. The sites where epiphyte entered the basiphyte had protruded to feature a typical “goose-bumps”-like symptom. This seems to be a prominent feature in all the specimens investigated. After about 10 weeks, the epiphytes reached the end of their life cycle and start to drop off from their attachment as shown in Fig. 2e. Their initial sites of attachment turned dark and started to disintegrate as shown in Fig. 2f.

Tissue degradation and secondary infection

Scanning electron microscope images of the tissue degradation and bacterial infection are observed in Fig. 3A–F. Locations where the epiphytes penetrated the basiphyte is a characteristic feature of its infection, featuring a “goose

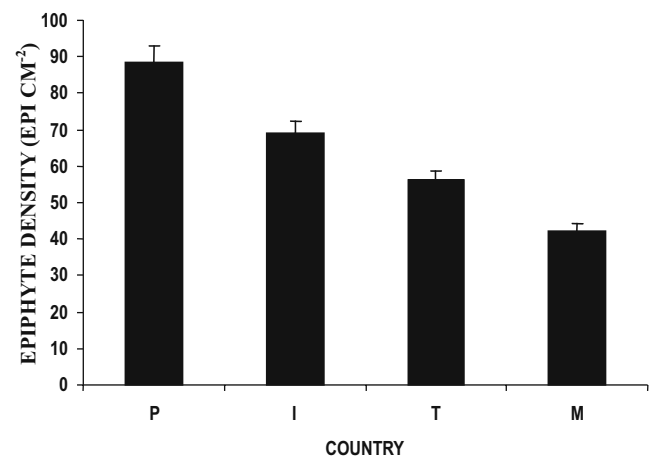
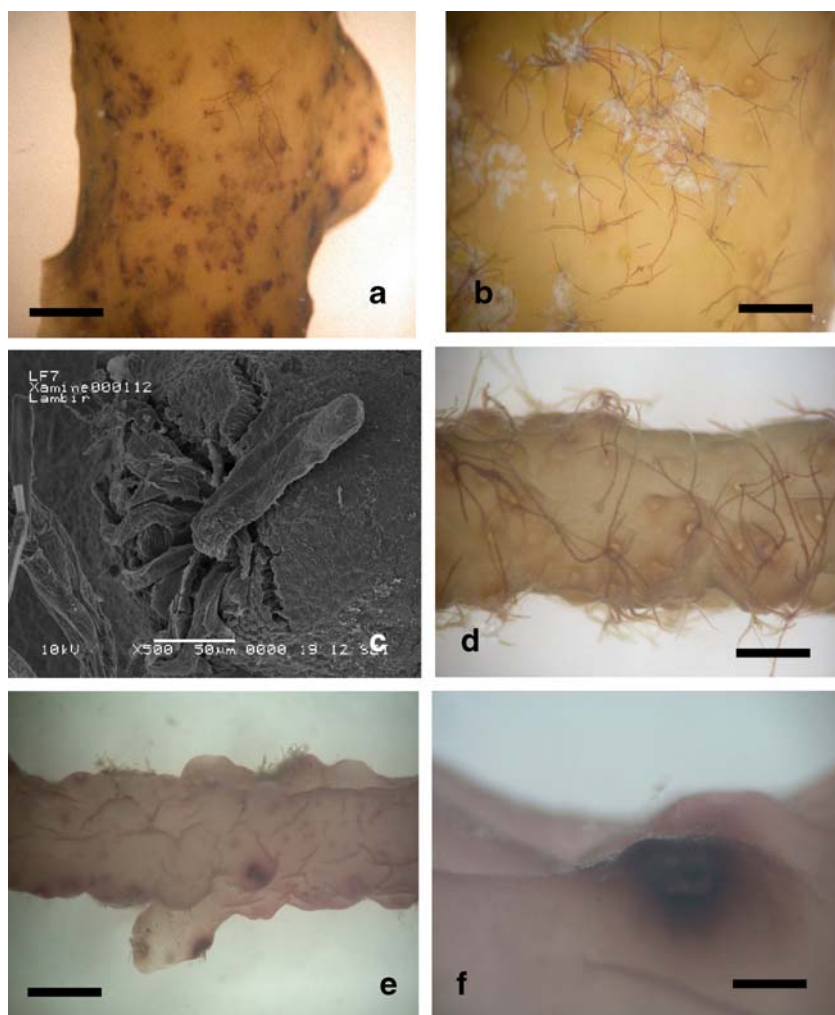


Fig. 1 Epiphyte (*Neosiphonia apiculata*) density (number/cm²) on *Kappaphycus alvarezii* specimens collected from Philippines (P), Indonesia (I), Tanzania (T) and Malaysia (M). Values are means \pm SD for 10 replicates for each specimen

Fig. 2 Phases of epiphyte infection on *Kappaphycus alvarezii* thallus. **a** Host plant with early stage of epiphytes imbedded as tiny black spots in outer cell layers (scale bar=300 μ m). **b** Host plant with epiphyte germ-lings (scale bar=300 μ m). **c** Scanning electron microscope micrographs showing emergence of epiphyte germling from the cortex cell layers. **d** Host plant with mature epiphyte (scale bar=300 μ m). **e** Host thallus with “goose-bump”-like symptoms at the end of the epiphyte infection phase (scale bar=300 μ m). **f** Epiphyte infected “mounts” turn dark with the onset of the secondary bacterial infection (scale bar=300 μ m)



bump”-like swelling and formation of a pit in the middle where its basal primary rhizoid is still loosely attached. Secondary rhizoids and their upper main thalli drop off, leaving multiple “bumps” with a pit in the middle throughout the seaweed’s surface (Fig. 3a). Early tissue degradation could be noticed with the appearance of tiny pores around the tip and sides of the swelling. Then, the remaining rhizoid of the epiphyte started to drop off at this point as shown in Fig. 3b–c. Further progression leads to the disintegration of the “goose bump” walls as shown in Fig. 3d–e. Secondary bacterial infection was observed to set in during this phase as shown by the presence of rod-shaped bacteria in Fig. 3f. The characteristic mount at the location where the epiphyte enters the tissue was completely disintegrated, exposing the host thallus to further attack from grazers and other microbes. The total numbers of culturable bacteria and culturable bacteria species for the healthy and infected seaweed are presented in Table 1. The total number of culturable bacteria from diseased seaweed thalli was more than 300% greater than in the healthy seaweed. A total of five bacterial species (*Alteromonas* sp.,

Aeromonas sp., *Escherichia coli*, *Flavobacterium* sp. and *Vibrio* sp.) were isolated, identified and their relative abundance determined from healthy seaweed. But only three species (*Alteromonas* sp., *Flavobacterium* sp. and *Vibrio* sp.) with much higher abundance were isolated and identified in diseased seaweed thallus as shown in Table 1.

Discussion

Epiphyte outbreaks in farms in all four countries were reported to occur during drastic fluctuations in seawater salinity, temperature and nutrient levels (Hurtado et al. 2006; Vairappan 2006). Newly infected seaweed materials had young epiphytes with multiple turts like secondary rhizoids and vegetative trichoblasts from each segment arranged in a spiral manner. The identity was established based on characteristic features such as rhizoids separated from the parental pericentral cells by a cross wall, its procarp with a 3-celled carpogonial branch, its spermatangial branch arising from the first fork of a fertile trichoblast.