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The Status of Kelp Exploitation and Marine Agronomy, with Emphasis on *Macrocystis pyrifera*, in Chile

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Abstract

Kelp cultivation started in Japan, China and Korea, mainly for human consumption; new applications are still expanding. In Chile, three 'wild' *Lessonia* species and *Macrocystis pyrifera* are under a strong and increasing pressure of exploitation mainly for alginate production and as a source of feed for abalone. Regulatory restrictions for kelp

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© 2014 Elsevier Ltd. All rights reserved. 161 exploitation and the increased demand for biomass provided a positive environment for the installation of a kelp farming industry. Pilot-production studies demonstrated that 200 tonnes (fresh)/ha/year can be achieved and genetic diversity and breeding studies suggested that this volume could be increased. Kelp disease research is a necessary condition for securing the future development of this industry, as are environmental studies on the impacts of large-scale aquaculture. Beyond the positive bioremediation, ecosystem service effects that kelp farming can provide, especially in a region such as in southern Chile, where intensive salmon and mussel cultivation occurs. Life Cycle Assessment suggests that the energy returns on investment in kelp farming are positive, but more detailed data are still required.

6.1 INTRODUCTION

The cultivation of brown seaweeds started about 300 years ago in Japan, China and Korea, mainly for human consumption (e.g. Hanisak, 1998; Kaneko, 1999; Tseng, 1987). More recently, the use of natural stocks of kelp expanded to the majority of temperate regions, where such species occur naturally. This geographic expansion was driven by an increased demand for brown seaweed biomass for the extraction of alginate (Bixler & Porse, 2011), and potentially by interests in the development of new uses (e.g. alginate to increase lithium batteries' performance: Kovalenko et al., 2011; as 'health' ingredient of food products: Prabhasankar et al., 2009; or extracts for agronomic applications: Craigie, 2011). Traditional and new applications are increasing the pressures on natural stocks worldwide, including on the Chilean coast (e.g. Ugarte & Sharp, 2012; Vásquez, 2008; Vega, Broitman, & Vásquez, 2014). During the last decade, abalone farming in Chile grew significantly, from 73 tonnes in 2001, to 840 tonnes in 2011 (Sernapesca, 2013). The expanding abalone industry alone demanded high volumes seaweed; 3.6–8.9g of kelp per day are required to produce a 1g increase in abalone weight (Greenier & Takekawa, 1992). Harvesting to meet this demand has already caused some deterioration of natural kelp populations (Vásquez, Piaget, & Vega, 2012) and considering the increased biomass demand associated to new applications that has actually taken place, the farming of Macrocystis pyrifera (L.) C. Agardh is being developed in Chile (Buschmann, Varela, & Hernández-González, 2008).

In addition, Chile is one of the largest mariculture producers of the western world, producing 361,000 tonnes of products in 1998 and over 870,000 tonnes in 2010 (Buschmann et al., 2013). Salmonid culture accounts for the majority of Chilean aquaculture production, and it grew rapidly since the 1980s, with Chile becoming the worlds' second largest producer

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of farmed salmon, after Norway (Buschmann et al., 2013). In Chile, rapid growth and concentration of aquaculture activities within enclosed areas have led to concerns regarding potential environmental impacts (Buschmann et al., 2009). To mitigate the effects associated with dissolved inorganic and suspended organic emissions of the high-trophic level organisms, Integrated Multi-Trophic Aquaculture (IMTA) models were developed (see reviews by Chopin et al., 2001; Neori et al., 2004; Troell et al., 2003). The initiation of IMTA research in Chile began in the late 1980s using land-based systems (Buschmann et al., 1994; Buschmann, Troell, Kautsky, & Kautsky, 1996). The first published research of IMTA within an open-water context was by Troell et al. (1997). The red alga Gracilaria chilensis Bird, McLachlan, & Oliveira was found to have increased growth rates (20-40%) when cultured in proximity to trout and Atlantic salmon grow-out sites. Buschmann, Varela, Hernández-González, and Huovinen (2008) demonstrated that both G. chilensis and M. pyrifera were suitable candidates for bioremediation within Chilean waters, and the co-culture of these two different species has been proposed as a means of increasing biomass productivity, therefore maximising the potential for bioremediation. However, due to inadequate regulations and the lack of financial incentives for seaweed aquaculture (Buschmann et al., 2013), industrial cultivation of kelp has only been carried out at experimental and pilot-scale (Correa, et al., in press; Gutiérrez et al., 2006; Macchiavello, Araya, & Bulboa, 2010; Westermeier, Patiño, Piel, Maier, & Müller, 2006), and official data from Chilean aquaculture indicated a total production of only 1 tonne (wet weight) in 2010 (Sernapesca, 2013).

Despite a research demonstrating the operational feasibility and advantages of integration, purposely constructed IMTA systems are as yet to be developed within Chile. Economic considerations remain a challenge to the adoption of IMTA technology by the salmon industry. To attract investors, pilot demonstrations are required to show how profitable IMTA approaches are, as has been shown in Canada (Ridler et al., 2007). In the case of *M. pyrifera*, this encouraged efforts to develop novel products in order to add value and stimulate demand (Buschmann, Varela, Hernández-González, & Huovinen, 2008; Gutiérrez et al., 2006). Furthermore, current research is investigating the efficacy of bioethanol production from cultured *M. pyrifera*. It has been recently demonstrated that the carbohydrates of giant kelp can be fermented and transformed into bioethanol (Wargacki et al., 2012). The variety of possible end-uses of *M. pyrifera* provides interesting opportunities for research on different aspects of kelp biology for improving sustainability beyond pilot-biomass production studies.

A considerable amount of information on *M. pyrifera* cultivation already exists (see as an example North, Gerard, & Kuwabara, 1982). However, additional biological data are required to make successful commercial cultivation a viable option. It is especially important to acquire knowledge of the differences in environmental requirements and the complex morphological and reproductive variability between differing populations of the giant kelp (see Graham, Vásquez, & Buschmann, 2007). The anticipated market driving forces mentioned above, and the potential impacts of cultivation upon natural populations and the wider environment, and the limited biological knowledge necessary to produce a high quality product, all present areas of important consideration. Although there have been recent reviews on seaweed aquaculture in Chile (e.g. Buschmann, Varela, & Hernández-González, 2008), there is a need for an in-depth analysis of new perspectives, challenges and considerations, in light of the additional kelp research and development that has taken place in recent years. This paper reviews recent literature on kelp fisheries and aquaculture production in Chile. It also presents new information gathered during the development of a 21-ha pilot cultivation site for *M. pyrifera*, as well as from associated research activities regarding kelp genetics and diseases, and environmental impact analysis using Life Cycle Assessment (LCA). In addition, an economic analysis is performed, which takes into account different production scales and algal price scenarios.

6.2 KELP FISHERIES IN CHILE

Harvesting and collection from natural populations of Chilean brown seaweeds, mainly for alginate extraction, represents 10% of the world supply; with landings of up to 300,000 dry tonnes per year, the industry was valued at US\$ 60 million (Vásquez et al., 2012). Until 2000, the Chilean brown seaweed fishery was mainly sustained by natural mortality, whereby artisanal fishermen collected all individuals, which had been cast on the shore. Since then, driven mainly by international demands for raw material, three kelp species of economic importance (*Lessonia nigrescens*,¹ *Lessonia trabeculata* Villouta & Santelices and *M. pyrifera*) have been intensively harvested, from coastal areas between 18° and 42° S (Vásquez et al., 2012;Vega et al., 2014) (Figure 6.1).

Despite the fact that Lessonia and Macrocystis are distributed along the entire Chilean continental coast (Hoffmann & Santelices, 1997),

¹ Lessonia nigrescens was recently split in two cyptic species: Lessonia berteroana Montagne and L. spicata (Suhr) Santelices (González et al., 2012).

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Figure 6.1 Kelp landings in the north of Chile: *Lessonia trabeculata* (A) and *Macrocystis pyrifera* (B). (See the colour plate.)

their collection and extraction is concentrated between 18° and 32° S (Vásquez, 2008). The geographical location and the desert climatic conditions of northern Chile allow for a significant cost reduction in the drying of raw material and, consequently, in the total processing cost. In the

last decade, exploitation of brown seaweeds of economic importance in northern Chile was carried out using best practices, agreed upon by consensus between fishermen, industry, government and scientists (Vásquez, 2008; Vásquez et al., 2012). These practices focused on the selective harvest of sporophytes to allow for the maintenance of a reproductive stock, facilitating recruitment and minimising the impacts of grazing, benthic herbivores. Recently, Vega et al. (2014) introduced several ecological indicators to monitor the sustainability of kelp species. These indicators are related to density, biomass, recruitment and the population size structure, all used for comparisons of coastal areas with different regimens of harvesting administration (e.g. areas with co-management practices, versus open access areas, or marine protected areas without human intervention). Harvesting strategies for Lessonia (e.g. Santelices, 1982; Vega et al., 2014) and Macrocystis (e.g. Westermeier et al., 2012) were developed to ensure the sustainability of harvesting practices. Ecological indicators, including demographic variables, should reinforce the co-management administration of kelp resources (see Castilla, Campo, & Bustamante, 2007; Gelcich, Godoy, & Castilla, 2009). This would facilitate a viable harvesting administration system along the entire Chilean coast, allowing for the sustainability of a brown seaweed fishery. Moreover, ecological indicators, together with other harvesting parameters, could be useful for justification of the application of other administration strategies, such as quotas, bans or access restrictions for fishermen.

Various Lessonia species represents more than 70% of the total brown seaweed landings, which go as raw material to the alginate industry. On the other hand, at present, the availability of fresh M. pyrifera, as a feed input, is one of the most important constraints in the emerging abalone aquaculture industry. Between 18° and 32°S, M. pyrifera has low abundance and a fragmented distribution (Vásquez, 2008). The demands for kelp for abalone cultivation in Chile greatly exceeds the production potential of the natural kelp populations. At high latitudes, south of 40°S, a second epicenter for abalone cultivation is located; here M. pyrifera forms continuous belts that comprise the most important reserve of brown algae in the world (Graham et al., 2007). However, in contrast to the perennial populations in northern Chile, *M. pyrifera* in southern locations is annual and therefore minimally available in winter (Buschmann, Moreno, Vásquez, & Hernández-González, 2006). Considering this natural constraint, the feasibility of abalone cultivation depends on the future culture of this kelp species and should not be centred exclusively within Northern Chile.

Kelp beds along the Chilean coast present a relevant ecological, economical, and social value. Vásquez et al. (2014) estimated that kelp beds along the northern coast of Chile, alone, had a value of US\$ 540 million. The study indicated that kelp fisheries accounted for 75% of this value, and that the environmental services kelps provided for CO_2 capture represented only 9% of that total value. For these reasons, to secure the sustainability of Chilean kelp fisheries, it is highly relevant to introduce conservation targets and a higher demand for the utilisation of biomass should be entertained using re-population techniques or by the development of large-scale, intensive kelp aquaculture.

6.3 THE DEVELOPMENT OF KELP AQUACULTURE

Considering the large-scale kelp fisheries, which exploit the natural beds in Chile, and also the susceptibility of brown algae to oceanographic events such as El Niño (Martínez, Cárdenas, & Pinto, 2003; Vásquez, Vega, & Buschmann, 2006; Vega, Vázquez, & Buschmann, 2005), kelp farming appears as to be a logical developmental initiative. Kelp sporophyte production, under laboratory conditions, as well as in the natural environment, can help the natural recovery of coastal rocky areas after mass mortality events. More importantly, it could allow for the development of a sustainable biomass resource for a variety of applications. However, some unknown biological features of kelps, such as its life history strategies and responses under culture conditions, are still constraints for large-scale cultivation. The technological development of large scale kelp faming in oriental countries has demonstrated that this activity is technically and economically feasible. However, due to the different market conditions and cost structures of mariculture sectors in the Western countries, kelp farming is still underdeveloped. Presently, there are several kelp research and pilot farming projects, worldwide (e.g. Buck & Buchholz, 2004; Kraan, 2013; Peteiro, Salinas, Freire, & Fuertes, 2006). However, various characteristics of M. pyrifera (i.e. its unique morphology, genetic and reproductive factors, and regrowth capacity) necessitate the development of new culture technologies and farming strategies, within the limits set by environmental regulations, if the farming of this species is to be successful.

In general, two different aquaculture production approaches have been tested in Chile. In one method, free-floating cultivation of the sexual phase, producing unattached, floating sporophytes, which are grown in tanks for several months, after which individual juvenile kelps can be attached manually to floating long-line ropes in the sea (Westermeier et al., 2006). The second method is closer to those applied in East Asian regions (see Sahoo & Yarish, 2005), which allow the gametophytes to settle on strings installed in tanks. The gametophyte and juvenile sporophytes develop attached to artificial substrata (e.g. ropes), and after 30–40 days are installed in sea cultivation sites (Gutiérrez et al., 2006; Macchiavello et al., 2010).

By using the second approach, a 4 ha, suspended pilot-farm of *M. pyrifera* was established in southern Chile. The biomass was cultured on 100-m long-lines, and a yield of 41 kg wet weight/m of culture line per year was achieved (Correa et al., in press). As this pilot trial allowed for the manipulation of some cultivation variables, such as kelp density, we can predict that a final production of up to 200 fresh tonnes/ha/year could be obtained in a suspended culture system. Higher production values were obtained by Westermeier, Patiño, Müller, and Müller (2010), Westermeier, Murúa, Patiño, and Müller (2011) on an experimental scale, using isolated, individual plants, produced through controlled sexual crosses. These results suggested that through genetic selection and breeding programs, the production values indicated previously can still be improved upon.

Under farmed conditions, the protein content of cultivated *M. pyrifera* reached a value of 9% dry weight, which was significantly higher than that of tested, wild populations (Gutiérrez et al., in press). If the kelp farm was situated near salmon cages, with higher nitrogen availability, the protein content could increase to more than 13% of dry biomass (Buschmann, Varela, Hernández-González, & Huovinen, 2008). This is highly relevant to the value of the kelp biomass as abalone feed. At this stage of development, we can conclude that farmed production can be increased further if a breeding program is developed and if the abalone food quality can be improved by placing suspended *M. pyrifera* cultivation systems in proximity to salmonid cages sites, in southern Chile. By doing so, production could be increased, while also performing bioremediation services through the removal of fish nutrient emissions (Buschmann, Varela, Hernández-González, & Huovinen, 2008).

Using the above results and the 4-ha pilot-scale cost information, it was possible to calculate the economic profitability of a *M. pyrifera* farm, located in Chile, when the crop was used as a feed input, for abalone cultivation (Correa et al., in press). The main costs for a 10 ha farm are given in Figure 6.2. The culture installation represented the highest cost, at 44% of the total. Other significant costs were personnel, purchase of the aquaculture



Figure 6.2 Capital and operational costs and revenues values in US\$ estimated for a culture system estimated from a 4 ha kelp farm established in southern Chile.

license and equipment required (e.g. boats, harvesting platforms; Correa et al., in press). The economic analysis shows that the farm size and crop price were important aspects to be considered in order to achieve economic profitability (Correa et al., in press). The results indicated that only a culture system greater than 30 ha was economically viable, based on the prevailing price of US\$ 78/tonne of *M. pyrifera*. These results demonstrated that *M. pyrifera* farming could be commercially viable in southern Chile. If goods, with added value were to be developed (e.g. human food; Gutiérrez et al., 2006), the commercial interest in this economic activity would increase.

6.4 KELP GENETICS AND STRAIN SELECTION

There are relatively few published studies regarding kelp breeding and genetic improvement, most of which are focused on *Saccharina japonica* (for a review see Robinson, Winberg, & Kirkendale, 2013). In China and neighbouring countries, where the species is extensively cultivated, the main strategy has been to perform repetitive breeding generations of a few inbred lines (Robinson et al., 2013). This has led either to improved biomass production, or the increase of specific traits/molecules. To date, knowledge of kelp genetics in Chile is focused on evolutionary and ecological topics: (1) global phylogeography and parapatric speciation; (2) regional phylogeography and post-glacial recolonisation; and (3) local genetic diversity and structure following natural and human perturbations. The first published studies were aimed at understanding the effects of natural (i.e. ENSO, Martínez et al., 2003) and anthropogenic disruptions (i.e. copper mining wastes, Faugeron, Martínez, Correa, & Billot, 2005) on the spatial

and temporal dynamics of the kelp L. nigrescens in northern Chile. Both studies characterised population genetic structure, within and between continuous stands, separated by the impacted areas. They showed that the disruption of an otherwise continuous distribution of this kelp coincided with increased genetic differentiation between stands occurring northward and southwards from the impacted area. These authors demonstrated that gene flow occurred mainly between populations separated by short distances (i.e. a few km at most) as a result of their relatively short dispersal capacity. Indeed, after the 1982/83 ENSO event, even after 20 years L. nigrescens had not recolonised more than 50 km of coastline. This is a slow rate considering that the event killed extensive kelps beds along 600 km of coastline, leaving only sparse relic populations in upwelling centres (Martínez et al., 2003). These results were further confirmed by phylogeographic studies embracing most of the species distribution (Tellier, Meynard, Correa, Faugeron, & Valero, 2009). Based upon nuclear, mitochondrial and plastidial markers, a low genetic diversity was observed in each sampled locality, and shared only with neighbouring populations. In addition, strong genetic divergence was observed in the 30° S biogeographic transition zone described by Camus (2001) and Thiel et al. (2007). This genetic divergence corresponded to a speciation process (Tellier, Tapia, Faugeron, Destombe, & Valero, 2011), as a result of both the reduced dispersal and the ecological divergence (López-Cristoffanini, Tellier, Otaiza, Correa, & Contreras-Porcia, 2013; Oppliger et al., 2012, 2011). Overall, these results showed that specific traits (in this case, traits related to temperature and desiccation tolerance) could evolve in response to pressures of natural selection in wild populations.

Genetic studies in *M. pyrifera*, along the coast of Chile, focused on global phylogeography. As for *L. nigrescens*, Macaya and Zuccarello (2010) showed poor local (i.e. intrapopulation) genetic diversity and a strong genetic differentiation between the populations of *M. pyrifera*, and/or between regions along the Chilean coast north of the 42° S. This was surprising for a species capable of long-distance dispersal through rafting (Macaya et al., 2005; Thiel & Haye, 2006), and again suggested that local, rather than global, processes were shaping the genetic diversity predominantly because of restricted dispersal. On a global scale, the reduced genetic diversity was emphasised by the relatively recent arrival of the genus to the southern hemisphere, as a product of *trans*-tropical dispersal,

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from the northern hemisphere, during late Pleistocene (see Coyer, Smith, & Andersen, 2001; Macaya & Zuccarello, 2010). The situation is different south of 42° S where post-glacial colonisation led to the presence of a single haplotype in the region of the Magellan Strait (Macaya & Zuccarello, 2010). There, the importance of long-distance dispersal has been a determining factor in shaping the genetic diversity of such a large geographic region. Indeed, this single haplotype is shared with other sub-Antarctic regions, including south New Zealand, a pattern very similar to the one reported for the bull kelp, *Durvillaea antarctica* (Fraser, Nikula, Spencer, & Waters, 2009). However, in persistent populations, for which equilibrium is reached between the respective influence of gene flow, genetic drift and mutation, it seemed that restricted dispersal of spores was the dominant process shaping local and regional genetic diversity (see Alberto et al., 2010, 2011). This was a seemingly common pattern in most kelp species (see Valero et al., 2011).

One interesting feature of the phylogeographic studies was that they reported sharing haplotypes between all known morphotypes, previously considered as different species (e.g. Macrocystis integrifolia, Macrocystis angustifolia and Macrocystis laevis sensu Demes, Graham, & Suskiewicz, 2009), which suggested that they shared a very recent ancestor. However, this result is still under discussion, as suggested by Astorga, Hernández, Valenzuela, Avaria-Llautureo, and Westermeier (2012) who used nuclear markers (ITS-2) and representatives of Macrocystis from Canada, USA, South Africa, Australia and Chile. The authors observed a strong phylogenetic signal of morphological differences, which indicates that historical processes associated with diversification may have determined the current differentiation of the morphotype. Recent evidence of heterosis in the giant kelp (Westermeier et al., 2010, 2011) was an additional indication that phenotypic differentiation existed between genetically differentiated populations in Chile, and that local diversity could be re-arranged by controlled crosses into new genotypes, producing valuable phenotypes (Westermeier et al., 2011). This contrasted strongly with the paradigm of a high phenotypic plasticity underlying Macrocystis diversity, as suggested by Graham et al. (2007) and Demes et al. (2009). In addition to morphological plasticity, plastic phenotypic responses in terms of physiological and life history strategies were observed in different coastal habitats (Buschmann et al., 2006, 2014, 2004). Therefore, the relative roles of plasticity and selection in shaping kelp phenotypes are still a debate that requires dedicated scientific attention.

Future prospects for the establishment of a breeding program in Macrocystis should involve a detailed analysis of the phenotypic (morphology, chemical composition and other relevant production traits) differentiation between natural populations, together with an estimation of the genetic contribution (i.e. heritability) to trait values. It is important to pursue research on natural populations because the domestication process of this species is incipient, and up to now, most algal production has been based on spores obtained from natural stands. On the other hand, genetic interaction of cultivated and natural stocks will require attention in order to avoid potential genetic modifications of natural stocks through gene flow from modified cultivars, when cultivated outdoors, which has also been suggested for algae such as G. chilensis (Guillemin et al., 2008). Finally, kelp life cycle management is an issue that requires special attention for its potential to use parthenogenesis to produce pure genetic lines (i.e. fully homozygous genotypes) in a single generation, as suggested by recent studies showing that parthenogenetic sporophytes can produce single-sexed (e.g. 100% females) gametophytic progenies (Shan, Pang, & Gao, 2013). Pure genetic lines are essential for the quantitative genetics of specific traits (e.g. QTL analysis) and the definition of genetic markers to assist breeding strategies.

6.5 DISEASES

Information on the pathogens and diseases of brown algal kelps, of the order of Laminariales, is limited (Andrews, 1976; Correa, 1996; Gachon, Sime-Ngando, Strittmatter, Chambouvet, & Kim, 2010; Ishikawa & Saga, 1989; Potin, Bouarab, Salaun, Pohnert, & Kloareg, 2002; Wang et al., 2008). In natural populations, kelps are plagued by prokaryotes (Goecke, Labes, Wiese, & Imhoff, 2010; Michelou, Caporaso, Knight, & Palumbi, 2013), viruses (Easton, Lewis, & Pearson, 1997) and eukaryotic colonisers such as fungi and oomycetes (Li, Zhang, Tang, & Wang, 2010; Schatz, 1984). Other microscopic filamentous algae (Eggert, Peters, & Küpper, 2010; Potin, 2012) and invertebrates (Wahl & Mark, 1999) may also affect kelps. In the last 10 years, the effects of disease on natural marine communities have become increasingly apparent, mainly due to human impacts, via pollution or climatic change. For example, recent studies demonstrate how increased temperatures can negatively affect the chemical defences that ultimately result in diseases of natural algal populations (Harder, Campbell, Egan, & Steinberg, 2012).

Reports of major pathogen outbreaks in kelp natural populations are rare. However, Ecklonia radiata, the dominant habitat-forming seaweed in temperate Australian and New Zealand waters, was reported to experience high rates of mortality (Lachnit et al., 2013), which was attributed to a combination of environmental stress and viral infections, leading to discolouration and bleaching. Laboratory inoculation experiments with the extracted virii of 'sick', infected tissue resulted in cross-infection of healthy tissue of E. radiata. The frequency of putative disease morphotypes, and the relative abundance of virii in E. radiata around Sydney, appeared to be higher at sites that were impacted by untreated, wastewater discharge (Lachnit et al., 2013). This was the second reported case of virii found in Ecklonia (Easton et al., 1997). The absence of reports of virii in other kelp species is, most likely, due to lack of research, rather than to the absence of viral infections (Dodds, 1979). Previous virus and virus-like particle discoveries in algae have occurred during microscopic investigations of the ultrastructural aspects of algal life histories, rather than an investigation of a disease (van Etten, Lane, & Meints, 1991).

During the last 50 years in Asia, the rapid development of intensive mariculture has led to an increase in the number of diseases that attack commercially cultivated kombu (Saccharina (Laminaria) japonica). Disease symptoms were described affecting both sporelings during cultivation in nurseries and maturing sporophytes in the grow-out stages on long-line cultures (Wang et al., 2008). Among the pathologies recorded in kombu, several studies showed marine bacteria as causative agents. In China, South Korea and Japan, various bacterial pathogens (e.g. Pseudoalteromonas, Alteromonas) caused rot/hole disease of the kelp blades and significant loss of biomass (Ezura, Yamamoto, & Kimura, 1988; Yumoto, Ezura, & Kimura, 1989). Recently, by using phenotypic features and molecular identification, Pseudoalteromonas bacteriolytica was verified to be correlated with red-spot disease of S. japonica (Sawabe et al., 1998). In addition, another marine bacterium, Pseudoalteromonas elyakovii was considered to be associated with the spotwounded fronds of S. japonica (Sawabe et al., 2000). In the Eastern Pacific, kelp-associated bacteria were recently reinvestigated using molecular ecology methods (Hengst, Andrade, González, & Correa, 2010), including M. pyrifera beds in California (Michelou et al., 2013). However, no bacterial disease has yet been described in Chilean kelps.

Although infections by oomycetes do occur in kelps, these eukaryotic pathogens have not yet been described as pathogens of maricultured kelps (Strittmatter, Gachon, & Küpper, 2009). However, they were shown in natural populations to invade the kelp stipe and frond and severely perturb its growth. Kelp endophytes were recently reviewed by Eggert et al. (2010). Some of these endophytes caused only minor changes in their hosts, such as tissue darkening, whereas others are known to produce either degradative losses or tumor-like lesions, accompanied by severe deformation of the thalli (Apt, 1988; Peters & Schaffelke, 1996). In Europe, epidemiological studies of kelp populations demonstrated that endophytic infections may affect 100% of the host population (Andrews, 1976; Ellertsdottir & Peters, 1997; Lein, Sjoetun, & Wakili, 1991; Peters & Schaffelke, 1996). Such endophytes have been found in the South-East Pacific Ocean and Antarctic regions. Along the Pacific coasts of South America, M. pyrifera is typically infected by Laminariocolax macrocystis (Burkhardt & Peters, 1998), initially described as Streblonema macrocystis (Peters, 1991). Recent results showed that galls, affecting natural populations of L. nigrescens were associated with infection by a filamentous brown algal endophyte, of the genus Laminariocolax (Thomas et al., 2009). Similar patterns of a few filamentous algae growing as endophytes in Antarctic subtidal habitats has been found, except within the thalli of the larger chemically defended macroalgae (Peters, 2003).

During a 21-ha pilot suspended farming experience of M. pyrifera, installed in the Chiloé region of southern Chile (42° 29'27"S; 73° 18'28"W), no deformative or tissue destructive diseases were found to affect any of the biomass production, over a 3-year period. Nevertheless, some preliminary symptoms were found (see Figure 6.3). These symptoms never attained a prevalence above 10%, of the culture population and no potential associated pathogens were found. Compared to the progress in genetics and breeding and ecology of farmed kelps in Chile, the study of pathogens and diseases is still in its infancy. Considering the potential negative impacts and economic losses of diseases in farmed Asian kelps, there is an urgent need to study the causative agents/virulence factors, modes of transmission, genetic basis of virulence, and resistance and environmental factors that lead to symptoms in cultivated kelps. These investigations will help to avoid major mistakes and will lead to the application of biosecurity and epidemiological programs designed to mitigate the risk factors for disease emergence in kelps. Other outputs may also lead to the development of guidelines for the selection of disease-resistant kelp varieties

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Figure 6.3 Kelp disease symptoms found in kelp cultivated in a 21 ha farm located in southern Chile. Protuberance of the lamina: (A) Superficial view of protuberance on the lamina; (B) Cross-section of the protuberance; (C) Opening of the medulla due to protuberance ((Med) = medulla and (C)= cortex). White spot on the lamina: (D) Superficial view of white spot; (E) and (F) Cross-section showing presence of bacteria. Green spot on the lamina: (G) and (H) Superficial view of green spot; (I) Cross-section of lamina showing decompression of the cells of the meristoderm, breakdown and loss of cortical cells. Plication of the lamina: (J) Superficial view of the frond; (K) and (L) Cross-section of twisted showing overgrowth of the meristodermatic area. (See the colour plate.)

and promote the exploration and interest of host immune-stimulation in land-based aquaculture facilities. It must be pointed out that as the scale of commercial cultivation increases, disease risks will most likely become more relevant and therefore kelp disease studies will require more urgent attention in the near future.

6.6 ENVIRONMENTAL IMPLICATIONS OF LARGE-SCALE AQUACULTURE

Seaweed farming is often considered as the least environmentally damaging form of aquaculture (Folke, Kautsky, Berg, Jansson, & Troell, 1998): it requires little or no input of fertilizers, fresh water resources, or medicines, and does not cause any major physical alterations of the environment (Bryceson, 2002; Johnstone & Ólafsson, 1995). However, awareness is developing regarding the environmental side effects, which may occur as a consequence of large-scale farming, occupying extensive marine areas (e.g. Zemke-White & Smith, 2006). By altering the habitat and the involuntary spread of cultivars of algae to adjacent areas, seaweed farming appears to affect many components of natural communities, e.g. bacteria (Johnstone & Ólafsson, 1995), meiofauna (Ólafsson, Johnstone, & Ndaro, 1995), benthic macrofauna (Eklöf, de la Torre, Adelsköld, Jiddawi, & Kautsky, 2005), fish (Bergman, Svensson, & Öhman, 2001) and corals (Russell, 1983). The installation of large beds of seaweed can contribute to complex modifications of local species interactions, which are not yet well understood (Buschmann, Correa, Westermeier, Hernández-González, & Norambuena, 2001). For example, Eucheuma denticulatum (Burman) Collins & Harvey and Kappaphycus alvarezii (Doty) have been farmed in shallow lagoons for the polysaccharide, carrageenan, using the 'off-bottom' method where algal thalli, tied to strings stretched between wooden sticks, driven into the sea bottom, are harvested every 2-3 months. In the mid-1990s, farms covered a total area of approximately 1000 ha (Ólafsson et al., 1995). If left unmanaged, such a development could, in the worst case, contribute to large-scale ecosystem changes (e.g. extensive seagrass loss, or spread of farmed algae to adjacent coral reefs), with implications for the production of several ecological goods and services (Eklöf, Henriksson, & Kautsky, 2006; Eklöf et al., 2005). Another study showed that kelp farming in Sungou Bay in China has low benthic environment impacts, but the results also showed that the impacts in summer and autumn were likely to be greater than in the winter and spring (Zhang, Hansen, Fang, Wang, & Jiang, 2009). As environmental impacts of open ocean seaweed cultivation are still disputed to some degree (see e.g. Ask, 2001), there remains a clear need for experimental studies in order to validate environmental effects of large-scale seaweed aquaculture systems (Trono, 1990; Zemke-White & Smith, 2006).

The environmental benefits of recycling inorganic nutrients and preventing eutrophication conditions through seaweed farming have been

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reviewed by various authors (e.g. Buschmann, Troell, & Kautsky, 2001; Chopin et al., 2001; Troell, Rönnbäck, Halling, Kautsky, & Buschmann, 1999). In the case of kelp farming, several studies point out that these large brown algae can be efficient removers of nitrogen inputs from fish aquaculture, in different regions of the world (e.g. Ahn, Petrell, & Harrison, 1998; Petrell & Alie, 1996), as also with the case of Chile (Buschmann, Varela, Hernández-González, & Huovinen, 2008). However, large-scale kelp cultivation could have unknown impacts, as a consequence of biomass losses, dissolved polysaccharide losses or an undesirably high nutrient uptake with consequences to local primary productivity. As these aspects are not well understood, they require attention. Preliminary environmental studies at the 21 ha pilot-farm of M. pyrifera, installed in southern Chile (42° 29'27"S; 73° 18'28"W) indicated that no benthic modifications were found, even after 3 years of cultivation (Figure 6.3). Few algal fronds have been found on the bottom under the kelp farm. The organic matter under the culture site also did not show a significant trend of increase over time, always under 2% (Figure 6.4). In



Figure 6.4 Underwater bottom images under the 21 ha kelp farm (42° 29'27"S; 73° 18'28"W). Images correspond to transects made at: (A) summer 2012, (B) winter 2012, (C) spring 2012, (D) summer 2013. (See the colour plate.)



Figure 6.5 Mean (\pm 1SD) total organic matter (%) in sediment under a 21 ha kelp farm and a control site 650 m from the kelp farming area in southern Chile (42° 29'27"S; 73° 18'28"W).

addition seasonally repeated measurements over a 2 year period have shown that nitrogen were not affected and slight increases of oxygen levels could be measured, at the outflow of the farm during summer. As this pilot-farm for kelp was installed at a depth of 30–40 m, light attenuation was considered not to be an issue. However, if the scale of cultivation were to be increased further studies should be taken. It may also be necessary to include a study of the genetic impacts of farmed beds of kelp may have on natural stocks, especially if a breeding and genetic selection program is considered. In the case of the red seaweed *G. chilensis*, losses in genetic diversity, in natural population processes, have been observed. This could be avoided in kelps through actions, such as maintaining the diversity of the species in a germplasm (Guillemin et al., 2008) (Figure 6.5).

6.7 LIFE-CYCLE ASSESSMENT (LCA) OF KELP AQUACULTURE

Environmental impacts are not only confined to local areas, aquaculture production systems also contribute to regional and global scale impacts (e.g. climate change or regional coastal eutrophication) (Liu et al., 2013; Pelletier et al., 2007; Pelletier & Tyedmers, 2008; Pelletier et al., 2009). Life Cycle Assessment (LCA) is a tool used to investigate and analyse the impacts of at these scales. When performing LCA, the material and energy inputs and outputs associated with the studied product system, are quantified. The contributions of these material and energy flows to a variety of environmental impacts are then modelled. In 2010, the global production of macroalgae was approximately 18.1 million tonnes, more than 98.9% of which was seaweed (FAO, 2012). Despite this level of production, among the published LCAs of aquaculture, macroalgal systems have been the focus of relatively few (e.g. Aresta, Dibenedetto, & Baeberio, 2005; Fry, Joyce, & Aumônier, 2012; Langlois et al., 2012). This information, combined with a renewed interest in product development and integrated fish/seaweed systems, presents a context in which LCA can provide some useful insights.

Some authors link a reduction of surface water carbon levels, at largescale seaweed cultivation sites, to the sequestration of atmospheric CO₂ (Hughes et al., 2012; Tang, Zhang, & Fang, 2011). Even if this is realistic, expanding the focus of assessment beyond the cultivation phase will likely show such a perspective to be limited, as the crops' end use will involve emissions. For example, carbon emissions will be released during the production and subsequent combustion of kelp-derived biofuel. Similarly, when considering the potential of kelp cultivation to remove nutrients released into coastal waters by anthropogenic sources, life-cycle thinking should be applied. In this case, nutrients extracted by kelp cultivation may be transferred to other ecosystem compartments during its utilisation (e.g. agronomic nutrient products entering into freshwater compartments after application; feeding of cultured abalone resulting in some nutrients being released back into the ocean). Thus, it can be seen that LCA is useful for highlighting potential cases of shifting environmental problems. LCA methodology can also be used to compare the environmental performance of products (Gorrée et al., 2002; ISO, 2006a, 2006b). In the case of biofuels, it has been suggested that macroalgae cultivation is a means of producing feedstock that avoids the fertiliser inputs and competition for land-use with food-crop systems, that is associated with terrestrial biofuel feedstock production (Wargacki et al., 2012; Wei, Quarterman, & Jin, 2013). LCA provides a framework, with which such considerations, as well as others, can be explored; a low contribution to one environmental impact category may be offset by contributions to others. In order to provide meaningful comparisons, it is important that equivalent units of comparison are selected for each product (Gorrée et al., 2002; ISO, 2006a, 2006b). For example, in the case of biofuel, this unit may be 'the provision of one MJ of energy'. However, various, complex challenges will be faced when making these comparisons, which requires expert application of LCA methodology (see Wardenaar et al., 2012).

Partnership trials using the cultivated kelp crop for abalone feed and as a feedstock for the production of bioethanol in a purpose built commercial scale refinery are among the advances being made by the Centro i-mar of

the Universidad de Los Lagos towards the development of M. pyrifera cultivation. These activities, including other advances described in this paper, present various stages of an experimental product value chain for which analyses using LCA are highly appropriate. The first stage of the assessment showed that the infrastructure is an important contributor to the life cycle impacts attributed to the seed production and cultivation stages. Thus, an increase in crop yield would reduce these impacts, and infrastructure alternatives should be explored. These outcomes also agree with other publications based on hypothetical and small-scale cultivation (Fry et al., 2012; Langlois et al., 2012) and are also in accordance with kelp farming and a hypothetical biofuel production in Denmark, which shows that the kelp production phase for biogas and bioethanol was the most energy intensive processing step (50-57%; Alvarado-Morales et al., 2013). The next stage of the LCA will compare the life cycle impacts of the different kelp products. The bioethanol refinery is an opportunity of particular interest. LCA will be used to calculate the cumulative energy demand of all the stages involved in the production of the crop and bioethanol, including the production and transport of energy and infrastructure inputs. This should provide a good indication of the potential for this bioethanol product to contribute positively to energy supplies within Chile. In a previous study, estimations of energy return on investment (EROI) for the hypothetical production of bioethanol using Saccharina latissima as a feedstock in Canada, produced an EROI of 1.78, but this value varied greatly as a factor of methods and assumptions (Philippsen, 2010).

6.8 CONCLUSIONS

The exploitation of Chilean natural kelp populations in central-northern Chile has reached maximum levels and an increase in biomass production seems achievable only if farming activities are implemented. Pilot-scale cultivation studies of *M. pyrifera* demonstrated that maximum fresh biomass production yields of 200 tonnes/ha/year could be achieved and might be improved incrementally through a breeding programme. However, kelp disease research is a necessity in order to secure the development of kelp aquaculture. Also relevant are some environmental impacts of large-scale aquaculture, which may arise beyond the positive bioremediation aspects that kelp farming can provide in a region such as in southern Chile, already experiencing the effects of intensive salmon and mussels production. Initial Life Cycle Assessment suggested that the energy returns on investment from kelp farming are positive, and continued research is investigating the impacts of the full macroalgae farming value chain.

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