EFFECT OF WILD-HARVEST ON A COMMERCIALLY IMPORTANT SEAWEED: A CASE STUDY OF *MAZZAELLA LAMINARIOIDES* (RODOPHYTA, *GIGARTINACEAE*) IN SOUTH-CENTRAL, CHILE

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ABSTRACT

To characterize the uses of seaweed, and investigate the socio-economical and ecological impact of harvest form wild-stocks, I focused on a case-study of *Mazzaella laminarioides* in South-Central Chile. First, I carried out a literature review and ethnobotanical surveys to compile lists of the species of seaweeds used in Chile and in South-Central Chile. I recorded that just 3% of the species of seaweeds present in Continental Chile had traditional and/or contemporary use, and nine of them are currently used for production of polysaccharides. Then I used chemical analyses to test if species used for food and medicine have high nutritional values and antioxidant potential. The results showed high nutritional values and antioxidant content for the species studied, especially for the species *Pyropia columbina*, which presented high values of proteins, minerals and antioxidants.

I also described the international and local context of intensification of seaweed extraction and the socioeconomic changes related to the polysaccharides market in South-Central Chile, I found changes in harvester demography, incorporation of new technologies, and increasing intensity and frequency of harvest.

Then, I used manipulative harvest experiments and chemical analyses to evaluate how environmental factors and harvest affect the antioxidant potential of *Mazzaella laminarioides*; and I found no significant effects of abiotic, biotic predictors, or harvesting, on antioxidant potential. I also recorded a tendency for a negative relationship between temperature and total phenolic compounds.

Lastly, I used a manipulative experiment to test the effects of harvest strategies on population size and reproductive potential of a population of *M. laminarioides* from South-Central Chile. During one harvesting season, I monitored the effects of harvest treatment on variables related to population size and reproductive potential. By the end of the experiment, the treatment that showed a recovery for most of the variables studied, particularly for population size, was hand-pull once. However none of the treatments recovered for total number of reproductive fronds, which can have negative effects on the population at long term. The results of the study suggest that the most sustainable strategy would be pulling fronds by hand, rotating harvesting areas and waiting longer to revisit the same spot. This would provide more and bigger fronds, and reduce the negative impact of harvest on the population.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: INTRODUCTION

Nearly a quarter of the world's human population inhabits coastal areas (Adger *et al.* 2005). Coastal communities are highly exposed to threats from coastal hazards (Lam *et al.* 2016) which have the potential to become disasters with decreases in resilience-driven by human action and environmental changes (Adger *et al.* 2005). Resilience of socio-ecological systems refers to their capacity to cope and adapt to changes (Folke 2006). In contrast, vulnerable systems can have small disturbances with important negative social consequences (Adger 2006). Changes associated with human actions are diverse, and range from development to introduction of invasive species. These changes, to a greater or lesser extent, affect the social, economical and ecological aspects of the coastal system, diminishing their resilience and adaptive capacity.

Coastal peoples have a close relationship with their environment, in some cases depending on their natural resources as a source of livelihood. One of the most important human impacts in marine systems is the removal of large biomass of natural resources, which can result in depletion (Castilla 1999). Overharvesting has direct ecological consequences, but also has immediate and future socio-economical impact on people that depend on these resources, increasing their vulnerability. In this regard, it is important to understand the effect of extraction of natural resources, not just from an ecological point of view, but also putting this into socio-economical context. In marine systems there are numerous studies related to marine resource exploitation, however most of them focus on fisheries (Castilla 1999), while the effect of wild-harvest on seaweeds is not well studied.

In my dissertation, I use a case study of an intertidal red alga, *Mazzaella laminarioides,* in South-Central Chile, to investigate the ecological and socio-economical effects of seaweed harvest for commercial uses. I start by presenting traditional and contemporary uses of numerous seaweeds; I then evaluate their nutritional and medicinal potential, and assess how they can be affected by harvesting and environmental factors. Lastly I characterize the socio-economical conditions of seaweed harvesters, and test the ecological effects of different harvesting strategies.

Context for research

1. Traditional and contemporary uses of seaweeds

Worldwide marine resources are extracted either for human consumption or for sale as raw material. For thousands of years seaweeds have been used for subsistence (Dillehay *et al.* 2008), having cultural and social significance, particularly for coastal and indigenous people (Mösbach 1999; Hart *et al.* 2014). Traditionally, they have been used for food, feed, medicine, and natural dyes among other uses (Guiry & Guiry 2019). Today, the main industrial use of seaweed material corresponds to extraction of the polysaccharides present in their cell walls (Nayar & Bott 2014), followed by production of soil additives, biofuels, fertilizers, food supplements, and pharmaceutical uses (Guiry & Guiry 2019).

Nutritional and medicinal potential

Seaweeds are traditionally used as food and medicine (Hart *et al.* 2014; Hong 2011; Turner 2003), and today, they are recognized for their nutritional and antioxidant content (Hart 2012; Rodriguez-Bernaldo de Quiros *et al.* 2010; Vijayavel & Martinez 2010). Further seaweeds possess bioactive compounds (Marinho-Soriano *et al.* 2006), holding promise for food and pharmaceutical industries as well as for improvement of the diets of coastal people.

Seaweeds growing in the intertidal system are exposed to biotic and abiotic stress, including seasonal and daily variations as immersion and emersion (Flores-Molina *et al.* 2014; Connan *et al.* 2007), desiccation, ultraviolet radiation, variation in nutrient load (Navarro, *et al.* 2014), and interaction with other species (e.g. epiphytes, herbivores, pathogens, competence). These factors have been reported to affect antioxidant activity (Yates & Peckol 1993), species ecology (Mansilla *et al.* 2006), photosynthesis (Vo, *et al.* 2015), and pigment concentration, among others (Davison & Pearson 1996).

To date, there are multiple studies on the antioxidant content and potential of commercially important species (Machu *et al.* 2015; Rodríguez-Bernaldo *et al.* 2010; Guaratini *et al.* 2012; Hart 2012; Yuan *et al.* 2005a, Heo *et al.* 2006; Heo *et al.* 2005); however, few have assessed the effects of environmental factors on the production of antioxidants in the field (Connan *et al.* 2007; Hart 2012; Yates & Peckol 1993). To my knowledge, none have assessed the effects of harvest on the production of these compounds, either in laboratory or in the field.

Seaweed production and polysaccharide industry

By 2015, worldwide commercial production of seaweeds was 30.4 million tones. Ninety five percent of the seaweed material is produced via aquaculture, and the 5% remaining is harvested from wild stocks (Nayar & Bott 2014). For cultured species the leading producers are China, Indonesia, Korea and Philippines; while for wild-harvested species the main producers are Chile, China and Norway (FAO 2018).

Algal polysaccharides - agar, carrageenan or alginates - are structural part of algae cell walls that have gelling and thickening properties when isolated commercially. Because of this characteristic, algal gels are used for a wide range of commercial purposes in food, pharmaceutical and cosmetic industries (Usov *et al.* 2013; Renn 1997). The industry of polysaccharides consumes 62% of the world seaweed production (Nayar & Bott 2014); corresponding to more than one million tons of wet seaweed (wild and cultured) per year (Pereira 2016; McHugh 2003).

Most of the raw material destined to polysaccharide production is used for alginate (460,000 tons/year; Nayar & Bott 2014), which is extracted from wild-harvested brown algae. Carrageenan production is the second in importance and consumes 400,000 tons/year of specific red algae (Nayar & Bott 2014), produced from cultivated and wild harvested species. The main species uses for carrageenan extraction are *Kappaphycus alvarezii* and *Eucheuma denticulatum*, both cultivated in open reef settings in the Philippines and Indonesia; in addition to other species wild-harvested species in South America, such as *Gigartina skottsbergii*, *Sarcothalia crispata* and *Mazzaella laminariodes* harvested in Chile (McHugh 2003). Lastly, agar production uses

125,000 tons of raw material/year, corresponding to two genera of red algae, *Gelidium* and *Gracilaria*, the first one from wild stocks and the second species mostly cultivated.

Prices of the most important species of seaweeds collected for polysaccharides increased greatly in the decade of 1999 to 2009 (Bixler & Porse 2011), related to higher cost of energy, chemicals and transportation, as well as increases in the cost of raw seaweeds (Bixler & Porse 2011). The increase of value of raw material is caused by unpredictable availability of seaweeds and their high demand because of use in new markets such as China, Eastern Europe, and Brazil (Bixler & Porse 2011; McHugh 2003).

While the supplies of some species are limited by natural abundance of the stock, others are limited by harvesting capacity. In the latter case, favorable market conditions have promoted changes towards more intensive harvesting methods. An unregulated intensification of harvest can have long term effects on biological and cultural diversity, affecting: the abundance of the species harvested, their population dynamics (O'Connell-Milne *et al.* 2014; Borras-Chavez *et al.* 2012; Buschmann *et al.* 2001; Santelices & Norambuena 1987), their ecological communities (Vasquez *et al.* 2012; Levitt *et al.* 2002), the socio-economical conditions of people depending on these resources (Rebours *et al.* 2014), and the cultural practices associated to their presence in a particular place (e.g. transference of knowledge, medicinal and food uses, etc; Hart *et al.* 2014; Kamakau 1992).

2. Local case of study: Chile

Chile accounts for 42% of the world wild harvest production (Nayar & Bott 2014), equivalent to 13% of total seaweed world supplies (FAO 2018; Valderrama *et al.* 2013). In 2014, Chilean production reached 413,141 tons of seaweed, with just 5% of this algal material produced from aquaculture (FAO 2018; SERNAPESCA 2015). Today, just one species is grown on a commercial scale, *Gracilaria chilensis* a species that used to be harvested from wild stock until it was overharvested in the 1980s (FAO 2018; Buschmann *et al.* 2008).

Because if Chile's strong dependency on natural stocks, the high volumes of seaweeds needed today for commercial purposes have resulted in the adoption of more intensive harvesting practices, increasing pressure on wild-harvested resources (Buschmann *et al.* 2008).

3. Study subject: ***Mazzaella*** **laminarioides**

Mazzaella laminarioides (Bory de Saint-Vincent) Fredericq is a red alga that belongs to the family *Gigartinaceae* in the phylum Rodophyta. This species provides an interesting opportunity as a case-study to examine the effects of harvest on both antioxidants and population dynamics. *M. laminarioides* is an intertidal species endemic of Chile that is a target of increasing unregulated wild-harvest for carrageenan extraction.

This species possess ecological importance as a dominant element in rocky intertidal communities, growing together with *Ulva sp.*, *Porphyra sp.*, among others (Santelices *et al.* 1981; Jara & Moreno 1984). And it is eaten by herbivores such *Fissurella* limpets and the sea snail *Siphonaria lessonii* (Moreno & Jaramillo 1983; Jara & Moreno 1984).

Regarding its economic significance, *Mazzaella laminarioides* is sold as a species of secondary importance as biomass for extraction of carrageenan. The main species extracted for these purposes in South-Central Chile is *Sarcothalia crispata* (Buschmann *et al.* 2001). *M. laminarioides* is intensively extracted in areas where *Sarcothalia crispata* is absence or its abundance of is low.

This species also represents a good study case to assess nutritional potential, as this alga is traditionally consumed as a food by local indigenous populations but its nutritional potential is overlooked because of its linkage to indigenous foods, which are locally undervalued, and to a common belief that *M. laminarioides* is used for the production of plastic.

4. Research questions and outline

To characterize the uses of seaweed, and investigate the socio-economical and ecological impact of harvest form wild-stocks, I focus on a case- study of *Mazzaella laminarioides* conducted in South-Central Chile. I carried out a literature review, ethnobotanical surveys, participant observation, manipulative experiments and chemical analysis, to address the following questions:

1. What are the historical and contemporary uses of seaweeds in Chile, including subsistence and commercial purposes?

2. Do nutritional values and mineral/ antioxidant contents support their food and medicinal uses?

3. How do environmental factors and harvest affect the antioxidant potential of intertidal seaweeds?

4. What are the effects of different frequencies and methodologies of harvest on population size and reproductive potential of a population of *M. laminarioides* from South-Central Chile?

Specifically, in chapter 2, I compile the species of seaweeds used in Chile, their traditional and contemporary uses, and then evaluate the nutritional and medicinal potential of the species most broadly used for these purposes. Additionally, I describe the international and local context of intensification of seaweed extraction, from small-scale commercial use (and/or subsistence) to large scale wild-harvest; and present the local case of changing socio-economical conditions associated to it in South-Central Chile.

In chapter 3, I use manipulative harvest experiments and chemical analyses to evaluate how environmental factors and harvest affect the antioxidant potential of intertidal seaweeds. I focus on the case of study of *Mazzaella laminarioides.*

In Chapter 4, I use a manipulative experiment to test the effects of different frequencies and methodologies of harvest on population size and reproductive potential of a population of *M. laminarioides* from South-Central Chile.

Finally, in Chapter 5, I summarize the findings from the previous chapters and discuss their implications. I integrate the results of socio-economical and ecological data to provide decision-makers with quantitative and qualitative information to help guide the development of harvest strategies that are more sustainable for *M. laminariodes*. In addition, I summarize the contributions of this research, discuss its limitations and suggest future research needed to understand the long term impact of intensification of harvest on species of seaweeds extracted from wild-stocks.

Chapter 2: ETHNOBOTANY OF CHILEAN SEAWEEDS: TRADITIONAL AND CONTEMPORARY USES OF SEAWEEDS IN SOUTH-CENTRAL CHILE.

2.1 Abstract

I have compiled a list of species of seaweeds used in Chile, their traditional and contemporary uses, and measured the nutritional and medicinal potential of the species most broadly used for these purposes. Using chemical analysis, I test if nutritional values and mineral/ antioxidant contents support their food and medicinal uses, and if they vary between fresh and dry samples and different parts of the alga (when applicable).

In a general comparison of the different species, *Pyropia sp*., a species consumed as food, appears to be the most nutritious type of seaweed, with results of proteins and ash higher than nori. Ash was higher for dry samples of fronds of *Durvillea. antartica* and *Pyropia sp.*; with the highest value for dry fronds of *D. antartica*. The highest total phenolic content (TFC) was found in fresh *Pyropia sp.*, followed by *M. laminarioides*, fresh fronds of *D. antartica*, and fresh stipe of *D. antartica*; while the dry samples showed lower values. The highest antioxidant activity value was found for *Pyropia sp.* (fresh), followed by fresh fronds of *D. antartica*, stipe of *D. antartica*, *M. laminarioides*, and lastly by dry fronds of *D. antartica*. *D. antartica* is the main species used as medicine (specifically the fronds), as ash is used as a proxy of content of minerals; this result is consistent with the medicinal use of this seaweed as a tonic for treating vitamin deficiency.

Finally, in this chapter I describe the international and local context of intensification of seaweed extraction, from small-scale use (commercial and/or subsistence) to large scale wild-harvest, and assess how changes in commercial activity over time have affected the economic and social conditions related to the commercial harvest of *lugas* in South Central Chile. I found that changes in prices, and working conditions, have lead to changes in harvester's demography, increase in accessibility to the natural resources, and incorporation of new (more intensive) technologies, which has resulted in more harvesters, increasing intensity and frequency of harvest.

2.2 Introduction

Worldwide macroalgae are cultivated and wild harvested for a wide variety of uses, which range from traditional to industrial uses. Traditionally, seaweeds have been used for food, feed, medicine, and natural dyes among other uses (Guiry & Guiry 2019). Today macroalgae are used industrially to create biofuels, fertilizers, food supplements, medicines and for the extraction of the polysaccharides present in their cell walls (Guiry & Guiry 2019). Algal polysaccharides - agar, carrageenan and alginates - are used as gelling agents in food, pharmaceuticals, and other products for human consumption (Usov *et al.* 2013; Renn 1997).

In coastal communities the uses of seaweeds as food is deeply rooted in tradition (Mösbach 1999). These uses can be influenced by season, festivities and religious obligations (such as Lent; Pardo *et al.* 2005, Masuda 1988, Ovalle 1969), age, gender (Hart *et al.* 2014; Kamakau 1992), social class, and ethnicity (Masuda 1988). Historically, seaweeds have not only been appreciated by coastal people, but also by inland inhabitants who would trade local supplies for seaweeds and other marine products. This kind of barter improved and diversified the diet of both groups (Mösbach 1999; Masuda 1988). Today the traditional transportation of seaweeds to supply inland inhabitants is still active; however barter has been replaced by the use of money.

Seaweeds are valued as foods because of their high levels of minerals and vitamins combined with low content of fats and non-digestible carbohydrates (Rupérez 2002). Today, they are of commercial interest as ingredients in functional or health-promoting foods (Matos 2013; Sekmokienė *et al.* 2007). One reason for this is their bioactive secondary compounds, which provide them with anticancer, antidiabetic, anti-inflammatory and antibacterial properties (Hart 2014, Maschek *et al.* 2008).

Between their secondary compounds, seaweeds constitute a rich source of antioxidants (Souza *et al.* 2011), one of the defense mechanisms protecting cells from injury and death caused by high levels of reactive oxygen species (ROS). In humans, high levels of ROS can lead to oxidative stress, and have been associated with diseases and disorders such as cancer, stroke, Alzheimer disease, diabetes, etc. (Chew *et al.* 2008). Therefore the presence of antioxidants in seaweeds, gives them antidiabetic and anticancer, as well as other properties (Lee *et al.* 2010).

Because of their high nutritional value, seaweeds are extensively used for improving soil quality, for commercial production of organic fertilizers (Buschmann *et al.* 2008) and feed for animals and aquaculture. In Japan and China *Sargassum* are used in agricultural fields to improve the quality of soil, and in Europe, brown and red algae are commonly used as fertilizers (Matsuda 1988). While some species make good fertilizer, such as kelps of the genus *Lessonia* and *Macrocystis*, others such as *Durvillaea* do not provide good nourishment because of low percentages of nutrients as potassium and nitrogen (Matsuda 1988).

In coastal areas they are used to feed animals. In the North of Europe they complement livestock food when pasture is scarce, being used as feed for sheep, beef cattle and horses and pigs (Matsuda 1986) More recently seaweeds are ground and mixed with grains and hay to improve farm animals' diet (Matsuda 1986). Other recent use of brown algae, as kelp, includes feed on abalone farms (Buschmann *et al.* 2008; Troell *et al.* 2006). In the case of Chile, all farms use brown algae for abalone aquaculture, specifically they use *Lessonia trabeculata*, *Lessonia nigrescens*, and *Macrocystis integrifolia*, or cultivated *Gracilaria chilensis* when other species are scarce (e.g. in winter; Buschmann *et al.* 2008; Flores-Aguilar *et al.* 2007)

The most widespread industrial use of seaweeds is polysaccharide production (Usov *et al.* 2013). Algal polysaccharides (phycocolloids) are a structural part of algae cell walls, and their composition would depend on the taxonomy of the algae. While carrageenan and agars are extracted from red algae (Rhodophyta), alginates are produced by brown algae (Phaeophyceae; Usov *et al.* 2013; Jiao *et al.* 2011). Phycocolloids have commercial importance as gelling and thickening agents, due these properties, they are used for a wide range of commercial purposes in food, pharmaceutical and cosmetic industries (Usov *et al.* 2013). According to Masuda (1986) the commercial production of carrageenan started in 1937 and expanded after World War II, with United States as the main producer (Chapman *et al.* 1980). The commercial use of extracts of brown seaweeds containing alginate started in 1930's (McHugh 2003; Masuda 1986), while agar was discovered in Japan in 1658 (McHugh 2003), with this country having the monopoly until United States, Spain, Morocco and Korea developed their industry, after World War II (Masuda 1986).

By 2015, the world production of seaweeds was 30.4 million tons, with the leader producers being Chile, China, and Norway for species harvested from wild stocks, and China, Indonesia, Korea, and Philippines for cultured species (FAO 2018). With regards to polysaccharides, the production results in more than one million tons of wet seaweed (wild and farmed) per year (Pereira 2016; McHugh 2003). Alginate production equivalent to US$ 213 million comes from wild stocks of brown algae; agar (US$ 132 million) comes from two genera of red algae, *Gelidium* and *Gracilaria*, the first one from wild stocks and the second species mostly cultivated. Carrageenan production reaches US$ 240 million, and comes from diverse sources of red seaweeds. The major part comes from *Kappaphycus alvarezii* and *Eucheuma denticulatum*, both species cultivated in Philippines and Indonesia; and from wild stocks of other species from South America, being the most important *Gigartina skottsbergii*, *Sarcothalia crispata* and *Mazzaella laminariodes,* all of them will harvested in Chile (McHugh 2003).

In the 1980s and 1990s the hydrocolloids market reached a growth of 3-5%, afterwards this growth rate has decreased to growth rates of 1-3% per year Matos 2013; Bixler & Porse 2011). Prices of the most important species of seaweeds collected for hydrocolloids increased greatly in the decade of 1999 to 2009, with the highest increases seen for the brown algae *Lessonia* (171% increase), and for some species of red algae (*Eucheuma cottonii*, *Sarcothalia crispata*, and *Gigartina skottsbergii* with 133, 130 and 114 % increase, respectively; **Table B.1**, Appendix B) . The increase in prices has been related to higher cost of energy, chemicals and transportation added to increases in the cost of raw seaweeds (Bixler & Porse 2011). The increase of value of raw material is caused by unpredictable availability of seaweeds and its high demand caused by the insertion to the market of China, Eastern Europe, and Brazil, among others (Bixler & Porse 2011; McHugh 2003).

Chile is the main producer of seaweeds in South America, accounting for 13% of world supplies (FAO 2018; Valderrama *et al.* 2013). In 2014, Chilean production reached 413,141 tons of seaweed, most of this algal material coming from wild stocks, and just 5% from aquaculture (FAO 2018; SERNAPESCA 2015). Brown algae (such as kelps of the genera *Macrocystis* and *Lessonia)* are predominantly harvested in Northern Chile, while red algae are mainly harvested in the South of the country (Buschmann *et al.* 2008). This dependence on natural stocks confers instability to the market since the natural abundance of the species can be affected by a number of factors, such as climatic conditions (IFOP 2001; Santelices & Norambuena 1987), big swells (Vasquez *et al.* 2008; IFOP 2001; Poblete & Candia 1991), El Nino events (Vasquez *et al.* 2008), earthquakes and tsunamis (Jaramillo *et al.* 2012; Castilla *et al.* 2010) , pests or epiphytes (Buschmann *et al.* 2008; Poblete & Candia 1991; Romo 1986), bans or fishery regulations (Vasquez *et al.* 2008), among others.

The need for bigger volumes of seaweeds related to industrial uses has resulted in larger pressure on wild harvested species and cultivation efforts of a number of seaweeds such as *Gracilaria chilensis, Macrocystis pyrifera, Chondracanthus chamissoi,* and *Callophyllis variegata*, among others (FAO 2018; Buschmann *et al.* 2008). *Gracilaria chilensis* used to be harvested from wild stock, reaching a peak in 1985, followed by a decline due to overharvesting and adverse market conditions (FAO 2018; Buschmann *et al.* 2008). In response to this decline, this species started to be cultivated in the 1980's and today this is the only species grown on a commercial scale (FAO 2018; Valderrama 2014; Buschmann *et al.* 2008).

While the supplies of some species are limited by natural abundance of the stock, others are limited by fishery capacity. In the cases of limitations associated with fishermen or technique capacity for species harvested from wild stock, the favorable market conditions had promoted changes towards more intensive harvesting methods. The changes described above can have long term effects on biological and cultural diversity, affecting directly the population dynamics of some species of seaweeds (Chapter 4), their ecological communities (e.g. the case of kelp forests, *huiro;* Vasquez *et al.* 2012; Levitt *et al.* 2002), the socio-economical conditions of people depending on this resources, and the cultural practices associated to their presence in a particular place (e.g. transference of knowledge, medicinal and food uses, etc).

In this chapter I focus on South Central Chile, which has a long history of traditional uses of seaweeds and is a lead producer of polysaccharides, to address the following questions:

1) What are the historical and contemporary uses of seaweeds, including subsistence and commercial purposes? How has the use of different species changed over time?

2) For species used as food or medicine: (i) what is their nutritional value and do they support the use of these species as food? (ii) Do they have antioxidant potential? (iii) Do mineral/ antioxidant contents support some of their medicinal uses?

3) For species used for polysaccharides (i) what is their value and volume sold nationally and locally in South-Central Chile? (ii) In South Central Chile, who is involved in the harvest, and how (gears and methods used) is harvest carried out? (iii) How have the above changed with the increasing value of seaweeds?

I hypothesized that:

i) Because traditional uses are typically at household scale, commercial and traditional practices will co-ocurr in harvesting locations. Moreover, greater exposure to the resource could encourage their use by more people.

ii) Species of seaweed used as both food and medicine will have high mineral content,

iii) The increase in prices and improvement of working conditions will shift the demographics of people involved in wild harvest, increasing participation of men and young people in an activity that used to be carried out by women, old people and kids; and

iv) The increase in prices will lead to a greater intensity of harvest.

2.3 Methodology

1. Subsistence and commercial uses of seaweeds

To assess which seaweeds are commonly used in South-Central I conducted a literature review. I used information available online, annual fishing statistics, scientific articles, books and missionary chronicles. I focused on species used for food, medicine, fertilizers, feed, and polysaccharides industry. The literature review was conducted in English and Spanish; for the search of material online I used Google, Google scholar, and algaebase (Guiry & Guiry 2019), using as search words the scientific and common names of the species, and terms as: traditional uses of algae/seaweeds, uses of algae, polysaccharides, phycocolloids, seaweed/algal nutrition, *mapuche*, indigenous use of algae/seaweed, among others.

To assess the species used in South-Central Chile, and those that have changed from subsistence to commercial uses, I carried out door to door surveys, direct and participant observations (Alexiades 1996), and informal discussions with key informants (such as middlemen, or seaweed harvesters union's representatives). This part of the study was carried out in January 2014 in the towns of Isla Mocha, North and South; and in January and March 2014 in Isla Santa Maria, North and South. I also conducted informal discussions with key participants, and direct and participant observations, in Matanzas in 2016 and 2017. **Figure 2.1** shows a map of the different locations where I carried out field work.

The door to door surveys were carried out as an explorative approach to establish priorities and have a better understanding of the socio-economical aspects related to the use of seaweeds (subsistence and commercial). The selection of the field sites was based on literature review as sites where seaweeds were important for subsistence and where commercial harvesting of seaweeds is an important source of income

The door to door surveys were conducted with a total of 83 *algueros* (seaweed harvesters; 43 surveys in Isla Mocha and 40 in Santa Maria), they included information about seaweed harvesters, and some questions about the whole household (Appendix B). The participants were interviewed through structured questionnaires to obtain information about their use of seaweeds as food, medicine, and extracted for polysaccharide industry (e.g. volume extracted, harvesting method, etc)

Figure 2.1 Location of field sites in South-Central Chile (Google Earth 2018)

2. Nutritional value and medicinal potential

In order to examine health-food/medicinal potential of some species of seaweeds, I analyzed the nutritional value and the antioxidant content of those species of seaweeds that interviewees most commonly reported to use as food (see **Table 2.3**). Fresh samples were collected in May 2017 along the beach at Matanzas and frozen to transport them to the laboratory. Dry *cochayuyo* was bought to the *algueros* on the same locality, and the dry sample of *luche* was bought in the market in Santiago. *Luche* is a name given to different taxa that look similar, and grow in the same environment (*Pyropia sp.*, *Porphyra sp.*), thus I am not certain of the species (or the precedence) of this sample. However, I considered relevant to include it in the analyses, as it is the type of *luche* found in the markets in non-coastal areas.

To prepare the seaweed extracts, the fresh samples were unfrozen and rinsed with distilled water, then homogenized and finely chopped using a hand blender. In the case of dry samples, they were chopped and storage at room temperature. For the species *Durvillaea antartica*, the leaves and the stipe are consumed in different preparations, and have different common names (*cochayuyo* and *ulte*), thus both parts of the plants were analyzed separately.

a) Nutritional value

To measure proximate composition I analyzed percentage of water, mineral (ash), fat, proteins and carbohydrates as described by the Association of Official Analytical Chemists (**Table 2.1**; AOAC 1990). I carried out the analyses in duplicate using one homogenized sample which contained numerous individuals collected along the beach.

Table 2.1 Summary of some methods used for proximate composition according to AOAC (1990)

Water (moisture)	Air oven at 105°C
Ash	Heating at 525° C
Fat (crude lipid)	Mixed solvent extraction chloroform/methanol
Protein (crude)	Kjeldahl nitrogen, Total N x 6.25
Carbohydrate + fiber	100% – (%protein + %fat + %ash + %water

b) Antioxidant Analyses

Preparation of the Seaweed Extracts

The antioxidant analysis included measures of total phenolic content (TPC) and antioxidant activities (DPPH). To prepare the extracts ten grams of fresh sample, or 5 grams if it was dry, finely chopped were soaked in a mix of 1.5 ml of acetic acid and 48.5 ml of absolute methanol. The samples were soaked overnight in darkness, at room temperature (Rico *et al.* 2012). Afterwards each extract was filtered through filter paper to remove algal particles, and stored in darkness at 4ºC.

Studies have shown differences in results when the same substrates are extracted with water, ethanol, methanol or other solvents, generally finding higher antioxidant activity in water extracts compared to methanol and ethanol (Machu *et al.* 20015; Hwang & Do 2014; Rico *et al.* 2012; Othman *et al.* 2007; Cheung *et al.* 2003; Ismail & Hong 2002). I extracted a first set of samples with water obtaining formation of hydrocolloids for all the samples of *D. antarctica*, and for *M. laminarioides.* The presence of these gels interferes the measurement of absorbance for the chemical analyses explained below, thus I decided to use methanol as solvent for the extractions.

Total Phenolic Contents

The total phenolic content of the extracts was estimated using the Folin-Ciocalteu reagent (Rico *et al.* 2012; Prior *et al.* 2005), where 100 µL of seaweed extract, 4.85 ml of distilled water, and 0.5 ml of Folin-Ciocalteu’s reagent were mixed in a 10 ml volumetric flask. Past three minutes, I added 1.85 ml of 20 % Na_2CO_3, and diluted with distilled water to the mark. After 30 minutes of incubation in darkness at room temperature, absorbance was measured at 765 nm, using a Shimadzu UV-Vis spectrophotometer.

The estimation of phenolic compounds was carried out in triplicate, and averaged. I created a calibration curve of gallic acid and used the regression equation ($y = 0.0008x + 0.0042$, $r^2 =$

0.9937) to express the result of total phenolic compounds as milligrams of gallic acid equivalent (GAE) per 100 gram of dry weight of algae.

Radical Scavenging Activity

Antioxidant activity was measured in terms of free radical scavenging activity (RSA), using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay, following the protocol described by Rico *et al.* (2012). For this assay 100 μL of seaweed extract were mixed with one ml of 0.1mM DPPH methanolic solution. The reduction of DPPH radicals was determined by measuring the decrease in absorbance over 30 minutes, at 515 nm, using a Shimadzu UV-Vis spectrophotometer. The percentage of DPPH radical scavenging activity of the seaweed sample was calculated using the following equation:

$$RSA = \left(1 - \frac{\text{Absorbance in the presence of sample}}{\text{Absorbance in the absence of sample}}\right) \times 100$$

The calculations were carried out in triplicate, and averaged. All the chemical analyses were conducted at the Laboratory of Food Science and Chemical Technology, University of Chile, Santiago, Chile. All data analyses were conducted in Microsoft Excel and R software, package stats (R Core Team 2013).

3. Economic and social aspects of polysaccharide industry

To describe the changes in prices and volumes of extraction of seaweeds related to polysaccharide industry at national scale, I conducted literature review based on annual fishing statistics (SERNAPESCA 2015, 2018), international reports (FAO 2018; Valderrama 2014; McHugh 2003) and scientific reviews of status of algae industry in Chile, seaweed farming, and studies on species used commercially. This search was conducted in English and Spanish; using Google, Google scholar, and the search engine of the University of Hawai‘i. As search words I used the scientific and common names of the species, and terms as: uses of algae, polysaccharides, phycocolloids, algal industry, phycocolloids industry, among others.

To assess changes of prices and volumes at local level, describe socio-economical conditions and changes on frequency and methods used for harvest, I carried out the surveys and observations described above. This included door to door surveys with household information (83 surveys), direct and participant observations, formal discussions with key participants, informal discussions, and direct and participant observations (January 2014, North and South of Isla Mocha; North and South of Isla Santa Maria, January and March 2014; also informal discussions, and direct and participant observations in Matanzas in 2016 and 2017). The door to door surveys included structured questionnaires about economic, social and cultural aspects of commercial extraction of seaweed (Appendix B).

2.4 Results and Discussion

1. Subsistence and commercial uses of seaweeds

a) Past uses of seaweeds

Archeological records

Archeological records of Monte Verde, show prehistoric use of seaweeds in South Chile. Monte Verde is an archeological site located in Southern Chile, Monte Verde II is an upper occupational layer dated between 14,220 and 13,980 years before the present. From this upper layer, Dillehay *et al.* (2008) recovered nine species of marine algae in a sample that corresponded to hearths and braziers of a possible medicinal hut and a residential tent. The species found were: *Durvillaea antarctica*, *Porphyra sp*., *Gracilaria sp*., *Sargassum sp*., *Gigartina sp.; Mazzaella sp., Porphyra columbina*; and fragments most likely belonging to *Sarcothalia crispata* and *Macrocystis pyrifera.* The characteristics of the fragments of seaweeds suggested they have been cooked or dried, probably for transportation or storage.

Indigenous peoples uses

There are old registers of traditional uses of seaweeds by indigenous people, an example is Mösbach, a missionary working with indigenous *mapuche* ('people of the land'), who wrote a book about indigenous botany around 1950, he mentions indigenous names and uses of some seaweeds. The following information is based on Mösbach (1999) and been complemented with additional sources.

Cochayuyo is a quechua word that means 'aquatic weed' (*cocha* means lake, and *yuyo* means weed; Masuda 1986) it refer to the fronds of *Durvillea antartica,* another name is *cochahuasca* 'sea rope'. *Mapuche* call it *Kollof* and *collofe* 'brown tincture', name related to the use of old stipes to dye artifacts with a very dark brown color. Mösbach (1999) describes its harvesting as a rooted tradition of coastal communities. This seaweed is eaten raw or cooked in stew. It has been described to be used as a tonic, as digestive, and anti-scrofula (Mösbach 1999). Additionally *cochayuyo* was used for making balls to play football and *chueca* (also called *palin*, a traditional kind of hockey; Mösbach 1999).

Huiro comes from the quechua word *wiru*, that means 'stalk of tender corn plant'. Mösbach (1999) called two species of the genus *Macrocystis* as *huiro, huiru* or *huildu* (*M. integrifolia, M . angustifolia*). *M.integrifolia* is not edible, however, is used as fertilizer and to treat scrofula due its high iodine content.

Luche is a *mapuche* word that means seaweed (*yerba del mar*; Villagran *et al.* 2003; Pardo *et al.* 2005) and it is used to refer to the species *Porphyra columbina*, however, other species with similar appearance are called the same name by coastal people (e.g. *Ulva lactuca, Porphyra sp, Pyropia sp;* Pardo *et al.* 2005; Masuda 1986; Madlener 1977). *Luche* is used as food, either raw or cooked, and is one of the preferred foods of *lafkenches* (coastal branch of *mapuche* people; Mösbach 1999). As a medicine, it is used to treat inflammations, scrofula and gout (Mösbach 1999).

Luga, *Mazzaella laminarioides, luga cuchara, luga corta* or *yapin* (IFOP 2001) is also called *luga-luga*; these names come from the mapuche word *lúa*, meaning certain edible seaweed (Augusta 1991). The name luga is also given to other species, commonly accompanied by an adjective that indicates some characteristic of the species, e.g. *luga ancha* (broad luga) for *Sarcothalia crispata, luga cuchara* (spoon-like luga) for *M. laminarioides, and luga colorada (luga roja,* red luga) for *Gigartina skottsbergii* (Contreras & Bernales 2014).

Seaweeds were used for barter in the past. Masuda (1986, 1988) registered the exchange of seaweeds, mainly *cochayuyo* for grains and other products. This seaweed was transported in animal driven carts or mules, in trips that took months, from the coast to *inapiremap*u 'territory next to the snow' (in the foothills of the Andes). These practices contributed to improve the diet of inland people, often low in iodine (Pardo *et al.* 2005), but also provide coastal people inland resources, diversifying the diet of both groups (Mösbach 1999; Masuda 1988).

Additionally, Mösbach (1999) observed a differentiation of harvesting activities by gender, while men fished, women and kids collected shellfish and seaweeds at times of low tide.

Presence of seaweeds in legends and mythology

As an important part of Chilean culture, seaweeds are present in some legends, particularly in the Chiloe archipelago, an area well known for its folklore and mythology, located in the South of Chile. The following four legends include seaweeds in their narrative.

The *pincoya*, is a female water spirit that personifies the fertility of the sea. She is beautiful and has long blond hair, in some versions of the story her hair is also made of seaweeds. She rises from the depth of the sea wearing a dress made of *sargazo* (*huiro*, kelp). When she would appear she would dance informing the abundance or deficiency of marine resources: if she faces the mountain while dancing that would mean scarcity, and if she faces the sea it would mean abundance of fish and seafood (Plath 1983).

Two other stories mention *sargazo* mainly related to its size and strength. In the story of the marine horse, this wild and big animal is the embodiment of the waves of the sea, and can just be tamed by sorcerers using reins made of *sargazo.* Another story, the *camahueto,* is a marine goat used by the sorcerers to undertake long trips on his back, to hold to the animal they use *sargazo* ropes because this is the only material that the monster cannot cut (Plath 1983).

Lastly, Plath (1983) mentions some defense power related to two species of seaweeds. The *trauco* is a humanoid creature of small stature that would attract young women to have sexual intercourse with him, making them pregnant. He can produce illness, kill children with his breath and make adults paralytic with his gaze. As a protection *cochayuyo* or *huiro* have to be used to whistle and *sargazo* (or ashes) would be placed in the corner of the houses.

b) Contemporary uses

Ramirez (2010) describes a total of 451 seaweed species for continental Chile, 278 (62 %) of them in the group of red algae (Rhodophyta), 95 species (21% of the total) are brown algae (Phaeophyceae), and 78 species, 17% of the total, belong to the green algae group (Chlorophyta). The literature review showed a total of 13 species, intertidal and subtidal, with some traditional or commercial use. Just one of these species is green algae (*Ulva lactuca*), this species is used as medicine, food, fed and fertilizer (**Table 2.3**). Five species are brown algae, these species are kelps used for extraction of alginate, with the exception of *Durvillea antarctica* which is used for food, medicine and traditionally used for dye. The other seven species are red algae, with five of them used for polysaccharides and two for food: *Pyropia* sp and *Callophyllis variegata.*

Nine species are reported to be used for the extraction of polysaccharides, four species of brown seaweeds belonging to the order Laminariales are used for extraction of alginates, one species of red algae in the order Gracilariales *(Gracilaria chilensis)* is used for extraction of agar, and four species of red algae in the order Gigartinales, are used for carrageenan (**Table 2.2**)

Of the thirteen species with traditional/subsistence uses, five species are used as food, and three used as medicine. Most edible species are used for minerals and vitamins; however *Macrocystis pyrifera (huiro,* giant kelp*)* which is not edible, is also used for its high mineral content. Some species have multiple uses, such as *Durvillea antarctica* (*cochayuyo)* and *M. pyrifera*. *D. Antarctica* is the species most commonly used in Chile for food and medicine (see below). *M. pyrifera* is mainly used as fertilizer, food for abalone and alginate industry, but it is also reported to be used as medicine (see section bellow)

Chile poses a big number of seaweeds growing on its vast coast (451 species; Ramirez 2010). However, the numbers of species used is low. In the literature review conducted, for the whole country I found a total of 13 species that are traditionally used, and nine species extracted for polysaccharides. Additionally, one species (*Mazzaella laminarioides*) have changed its use, passing from being mainly collected for subsistence to now been harvested for industrial purposes.

Table 2.2 Literature review of seaweed species currently and historically used in Chile. H: historical uses, C: current uses, W: harvested from wild stocks, F: farmed or cultivated.

Species[1]	Classification[1]	Common name	Distribution (Regions)[1,2]	Habitat	Regulation	Harvest Type	Medicine	Food	Natural dyes	Fertilizers	Abalone Feed	Animal feed	Agar	Alginates	Carrageenan
Ulva lactuca Linnaeus	Ulvales, Chlorophyta	Lechuga de Mar[2], Green laver[1,6,4] , luche verde, lύa, lύa-lύa, luga-luga[11], luche, Luchi, Sea lettuce, Lettuce laver, Green Laver, Sea Grass, Thin stone brick [1,4]	XV-XII	Intertidal[1,2] and Subtidal[2]		W	C[1,2]	HC[1,2,11]		C[1,2]		C[1]			
Lessonia berteroana Montagne	Laminariales, Phaeophyceae	Huiro negro[8], Chascon Norte[2]	XV-XII	Intertidal and Subtidal[2]	Extractive closure, closed seasons and annual catch quota, free access areas with mandatory brown algae management plans.[8]	W								C[2,8]	
Lessonia spicata (Suhr) Santelices	Laminariales, Phaeophyceae	Huiro negro[8], Chascón Sur[2]	IV-XII	Intertidal and Subtidal[2]	Extractive closure, closed seasons and annual catch quota, free access areas with mandatory brown algae management plans.[8]	W								C[2,8]	

References: [1]Guiry & Guiry 2019, [2]ICYT 2018, [3]McConnaughey 1985, [4]Madlener 1977, [5]Akatsuka 1994, [6]Ohmi, 1968, [7]Avila *et al*. 2012, [8]SUBPESCA 2018, [9]CAPIA 2014, [10]Westermeier *et al.* 1999, [11]Pardo *et al.* 2005, [12]Masuda 1988, [13]Masuda 1986, [14]Mösbach 1999

Table 2.2 (Continued) Literature review of seaweed species currently and historically used in Chile. H: historical uses, C: current uses, W: harvested from wild stocks, F: farmed or cultivated.

Species[1]	**Classification[1]**	**Common name**	**Distribution (Regions)[1,2]**	**Habitat**	**Regulation**	**Harvest Type**	**Medicine**	**Food**	**Natural dyes**	**Fertilizers**	**Abalone Feed**	**Animal feed**	**Agar**	**Alginates**	**Carrageenan**
Lessonia trabeculata Villouta and *Santelices*	Laminariales, Phaeophyceae	Huiro Palo[1,8]	XV-X	Intertidal[2] and Subtidal[1,2]	Extractive closure, closed seasons and annual catch quota, free access areas with mandatory brown algae management plans.[8]	W								C[1,2,8]	
Macrocystis pyrifera (Linnaeus) C.Agardh	Laminariales, Phaeophyceae	Huiro[1,2,8], huiro flotador[2,8], Giant Pacific Kelp[1,2,3], Sea Ivy[1,4], Brown kelp[1,6]	XV-XII	Subtidal[2]	Extractive closure, closed seasons and annual catch quota, free access areas with mandatory brown algae management plans.[8]	W	C[1,2]			C[1]	C[1,2]			C[1,2,8]	

References: [1]Guiry & Guiry 2019, [2]ICYT 2018, [3]McConnaughey 1985, [4]Madlener 1977, [5]Akatsuka 1994, [6]Ohmi, 1968, [7]Avila *et al*. 2012, [8]SUBPESCA 2018, [9]CAPIA 2014, [10]Westermeier *et al.* 1999, [11]Pardo *et al.* 2005, [12]Masuda 1988, [13]Masuda 1986, [14]Mösbach 1999

Table 2.2 (Continued) Literature review of seaweed species currently and historically used in Chile. H: historical uses, C: current uses, W: harvested from wild stocks, F: farmed or cultivated.

Species[1]	Classification[1]	Common name	Distribution (Regions)[1,2]	Habitat	Regulation	Harvest Type	Medicine	Food	Natural dyes	Fertilizers	Abalone Feed	Animal feed	Agar	Alginates	Carrageenan
Durvillaea antarctica (Chamisso) Hariot	Fucales, Phaeophyceae	Cochayuyo[1,2,5,11], Coyofe[1,5,11] ,collofe, collofi, collof, kollof[11] ,miño[11], huilte, ulte[1,5,8], ulti[8], lembo, lemfü[11], Bull kelp[1,6]	IV- XII	Intertidal and Subtidal[2]	Extractive closure, VI and VII regions[8]	W	HC[1]	HC[1,2,9,11]	H[1,14]						
Pyropia sp.	Bangiales Rhodophyta	Luche[2] , luche rojo, luche de playa[11], laver[9], nori[9]	XV-XII	Intertidal [2]		W		HC[1 ,2,9,11]							
Gracilaria chilensis C.J.Bird, McLachlan and *E.C.Oliveira*	Gracilariales, Rhodophyta	Pelillo [2] ogo-nori, chinesse moss	IV-X	Intertidal[1,2] and Subtidal[2]		WF							C[2,8]		
Callophyllis variegata (Bory) Kützing	Gigartinales Rhodophyta	Carola[2,7]	IV-XII	Subtidal[2,7]		F		C[1,2,7,9]							

References: [1]Guiry & Guiry 2019, [2]ICYT 2018, [3]McConnaughey 1985, [4]Madlener 1977, [5]Akatsuka 1994, [6]Ohmi, 1968, [7]Avila *et al*. 2012, [8]SUBPESCA 2018, [9]CAPIA 2014, [10]Westermeier *et al.* 1999, [11]Pardo *et al.* 2005, [12]Masuda 1988, [13]Masuda 1986, [14]Mösbach 1999

Table 2.2 (Continued) Literature review of seaweed species currently and historically used in Chile. H: historical uses, C: current uses, W: harvested from wild stocks, F: farmed or cultivated.

Species[1]	Classification[1]	Common name	Distribution (Regions)[1,2]	Habitat	Regulation	Harvest Type	Medicine	Food	Natural dyes	Fertilizers	Abalone Feed	Animal feed	Agar	Alginates	Carrageenan
Chondracanthus chamissoi (C.Agardh) Kützing	Gigartinales, Rhodophyta	Chicoria de Mar[2]	II-X	Intertidal Subtidal[2]		W		C[1,2,9]							C[2]
Mazzaella laminarioides (Bory) Fredericq	Gigartinales, Rhodophyta	Luga Cuchara[2]	IV-XII	Intertidal [2]		W									C[1,2]
Sarcothalia crispata (Bory) Leister	Gigartinales, Rhodophyta	Luga Negra[2], Luga lija[8]	V-XII	Intertidal and Subtidal[2]	none[8]	W									C[1,2,8]
Gigartina skottsbergii Setchell and *N.L.Gardner*	Gigartinales, Rhodophyta	Luga Roja[2] Cuero de chancho[8]	X-XII	Subtidal[10]	none[8]	W									C[1,2,8]

References: [1]Guiry & Guiry 2019, [2]ICYT 2018, [3]McConnaughey 1985, [4]Madlener 1977, [5]Akatsuka 1994, [6]Ohmi, 1968, [7]Avila *et al*. 2012, [8]SUBPESCA 2018, [9]CAPIA 2014, [10]Westermeier *et al*. 1999, [11]Pardo *et al*. 2005, [12]Masuda 1988, [13]Masuda 1986, [14]Mösbach 1999

Table 2.3 Percentage of participants that reported to use different species of seaweeds, categorized by uses. The values correspond to the percentage of participants using a given species regarding to the total number of interviewees (N=83, the interviews were conducted in Santa Maria and Mocha Island, 2014.

Species	**Classification**	**Common name**	**Food %**	**Medicine %**	**Polysaccharides %**
Pyropia columbina	Bangiales, Rhodophyta	*Luche*	93.98	-	-
Chondracanthus chamissoi	Gigartinales, Rhodophyta	*Chicorea de mar, achicoria*	1.20	-	6.02
Mazzaella laminarioides	Gigartinales, Rhodophyta	*Luga cuchara, luga corta, luga piedra*	10.84	-	51.8
Sarcothalia crispata	Gigartinales, Rhodophyta	*Luga negra, luga ancha, luga larga*	1.20	-	95.2
Durvillaea antarctica	Fucales, Phaeophyceae	*Cochayuyo, colloy, ulte, huilte*	95.18	13.25	-
Macrocystis pyrifera	Laminariales, Phaeophyceae	*Huiro, sargazo*	-	12.05	-

c) Local contemporary uses of seaweed

Household surveys revealed six species currently used for food, medicine and/or commercial harvest for polysaccharide industry (**Table 2.3**). The values correspond to the percentage of participants using a given species regarding to the total number of interviewees (in both locations).

Food

The species used as food are freshly collected and consumed in coastal communities; however they can also be bought in the market. *Pyropia sp.* (*luche*, a type of laver) was reported as a type of seaweed mainly harvested and consumed in winter. To sell it on the market, harvesters usually make a round block of pressed dried seaweed, which needs to be rehydrated and boiled for its use in stews (see

Figure 2.2.A).

Cochayuyo (*D. antartica*) fronds are traditionally used boiled for stew and salads and can be obtained fresh or dried. For the same species of *cochayuyo*, stipes are traditionally called *ulte (huilte;*

Figure 2.2.C*)*, the fresh product is eaten in salads after being boiled and pealed, it is also included in traditional seafood soups, such as *mariscal* and *paila marina.*

Luga cuchara (*M. laminarioides*) was recorded to be consumed fresh, cooked as a soup or as a salad, sometimes boiled and mixed with *luche*. Main consumption registered was raw, at the beach, while harvesting. One person reported to have eaten a*chicorea de mar* as salad, and few mentioned that this species is used for salads in Asia (supported by ICYT 2018; **Table 2.3**). Additionally, one person reported the consumption of *luga negra* by *Mapuche* people, as soup.

In Isla Mocha it was common to see and hear about the consumption of seaweeds by animals; *huiro, ulte*, and *cochayuyo* were eaten by cattle, *huiro* by pigs, and both species of *luga* by sheep and hares. Additionally, a fisherman reported that in long fishing trips, *huiro* is used as ice to keep the corvine fish cold.

Medicinal uses

Among the medicinal uses of seaweeds in this region, *cochayuyo* was reported to be used as a tonic to treat lack of vitamins, due the belief that it possesses high content of vitamins and iodine. It is also used to treat heartburn, stomach ache, and gastric ulcers, to lose weight, to prevent hair loss, to treat cough, diabetes, and regulation of blood pressure. Additionally, this seaweed is used raw for calming the discomfort of teething in infants (

Figure 2.2.D). And in nurslings to treat *quiñe*, also known as oral thrush, an infection caused by the fungus *Candida albicans.*

Macrocystis pyrifera (*huiro*) is used externally, as a bandage in the case of injuries such as sprain, strain and dislocations. The injured limb is wrapped with the seaweed, which helps to immobilize it. The mucilage also helps to treat the injury, so the seaweed is changed when it dries. Additionally, one participant reported to used it as poultice for the same purpose (personal communication).

From the surveys and observations conducted in South-Central Chile, I identified six species with traditional uses, three of those species also harvested for commercial use (*M.laminarioides, Sarcothalia crispata*, and *Chondracanthus chamissoi*). It is notable that the three species with both, traditional and commercial uses, have the lowest occurrence of subsistence use in the area of study.

Figure 2.2 A. Dry Luche and cochayuyo sold in the market in non-coastal areas. B. Dry cochayuyo Transportation, in rural areas, for exchange or sale. C. Fresh ulte and luche, sold in the market in coastal areas (Matanzas). D. Infant chewing cochayuyo for helping with teething

I hypothesized that, since traditional uses are at household scale (in most cases), the change to commercial use should not result in an abandonment of traditional practices, which can be maintained if the species is still available. Moreover, greater exposure to the resource could encourage their use by more people. However, my results suggest that commercial extraction for polysaccharides appears to discourage people from using species as *luga* for food. This could be related to higher income and changing food preferences For example, participants described an unappealing texture of soups cooked with *luga* due to the presence of carrageenan. Also, there is a common belief that *lugas* are used for the production of plastic (personal communication).

Traditionally seaweeds have been used as substitute for meat; currently they are appreciated for their nutritional properties, considered healthy food. In coastal communities they are mostly eaten by adults (mainly women and elderly) because their strong taste is not appreciated by kids and youth. In non-coastal areas, there is an increase in the preference and use of seaweeds, mainly for vegetarian plates, but also promoted for greater exposure to Asian cuisine and due a current trend for the inclusion of this food as a gourmet product. Additionally, in the last decade, the use of seaweeds has been highly promoted as part of governmental initiatives (e.g: CAPIA 2014, ICYT 2014).

For *cochayuyo*, I registered a variety of medicinal uses; many of the participants mentioned the use as tonic and to lose weight. The use of this seaweed for chewing when infants are teething is a common practice in rural areas, but probably was not considered a medicinal use, and consequently not mentioned by a higher number of interviewees.

2. Nutritional value and medicinal potential

a) Nutritional value

The nutritional values of the main species consumed as food are shown in **Table 2.4**. The values for nori were added for comparison of the results with a type of seaweed known for being nutritious. The results show that the percentage of proteins is higher in red seaweeds compared to brown (*D. antarctica)*. The seaweeds analyzed have ranged from 7.8 to 24%, with the highest content of protein in *luche*, followed by *luga cuchara*, *cochayuyo* and lastly *ulte* (stipes of *cochayuyo*). Additionally, dry *luche* seemed to have a higher percentage of protein, compared to the fresh product. This result is also very high when compared to nori (5%).

All the species show very low percentage of fat, with less than 1% for all of them. Carbohydrates and fibers were found to be higher for dry *luche,* followed by *cochayuyo* (dry and fresh, Table 2.4). Ash was higher for the two dry samples (*cochayuyo* and *luche*), with the highest value in dry *cochayuyo* (14.11%). Since ash is used as a proxy of content of vitamins and minerals, this result is consistent with the medicinal use of this seaweed as a tonic for treating vitamin deficiency.

In a general comparison of the different species presented in **Table 2.5**, dry *luche* appears to be the most nutritious type of seaweed, with results of proteins and ash higher than nori.

In the case of fresh *luche*, the percentages I obtained for the analysis sum to > 100%. This is likely related to the measurement of humidity which, in multiple occasions, showed to be highly variable for this type of sample.

Table 2.4 Nutritional content for the main species of seaweeds reported to be used as food in the interviews conducted on 2014 in Santa Maria and Mocha Island. The table shows different species, different parts of the plant consumed, and dry and fresh samples. Results are averages ± standard error. *Nori information from US Department of Agriculture (2018)

Species	**Part of the plant**	**Common name**	**Dry/fresh**	**Water %**	**Mineral, Ash %**	**Fat %**	**Proteins %**	**Carbohydrates and fibers %**
Durvillea antarctica	Fronds	*Cochayuyo*	Fresh	69.84 ± 0.005	5.97 ± 0.03	0.12	11.09 ± 0.01	12.98
Durvillea antarctica	Fronds	*Cochayuyo*	Dry	22.93 ± 0.009	14.11 ± 0.34	0.60	10.78 ± 0.54	51.59
Durvillea antarctica	Stipe	*Ulte, huilte*	Fresh	83.53 ± 0.002	4.33 ± 0.09	0.65	7.83 ± 0.01	3.67
Mazzaella laminarioides	Fronds (whole individual)	*Luga cuchara*	Fresh	74.68 ± 0.003	5.18 ± 0.004	0.12	17.67 ± 0.11	2.35
Pyropia columbina	Fronds (whole individual)	*Luche*	Fresh	83.22 ± 0	2.11 ± 0.05	0.15	20.29 ± 0.33	-
Pyropia sp./ Porphyra sp.	Fronds (whole individual)	*Luche*	Dry	10.69 ± 0.003	12.82 ± 0.14	0.81	24.07 ± 0.06	51.62
Pyropia sp *	Fronds	Nori*	-	85.00	3.77	0.28	5.00	5.40

b) Antioxidant Analyses

The total phenolic content varied between 3.22 mg GAE/ 100 g dry weight for the dry sample of *D. antartica* (*cochayuyo*), to 633 mg GAE/ 100 g dry weight for *P.columbina.* The highest TFC was fresh *P.columbina*, followed by *M. laminarioides*, fresh *cochayuyo* (fronds of *D. antartica*), and fresh *ulte* (stipe of *D. antartica*); while the dry samples showed lower values of TPC (**Table 2.6**).

The highest antioxidant activity value was found for *P.columbina* (fresh), followed by fresh fronds of *D. antartica*, stipe of *D. antartica M. laminarioides*, and lastly by dry fronds of *D. antartica* (**Table 2.5**). I did not measure antioxidant activity for dry *Pyropia sp. / Porphyra* sp., but according to the TPC on this sample, I could expect antioxidant activity to be lower than for the fresh sample.

Table 2.5 Total phenolic content (TPC) and radical scavenging activity (RSA) for the main species of seaweeds reported to be used as food in South -Central Chile. Results are averages ± standard error.

Species	Part of the plant	Common name	Dry/fresh	TPC (mg GAE/100g DW)	RSA (%inhibition)
Durvillea antarctica	Fronds	*Cochayuyo*	Fresh	101.31 ± 4.45	31.56 ± 0.93
Durvillea antarctica	Fronds	*Cochayuyo*	Dry	3.22 ± 1.72	3.162 ± 0.13
Durvillea antarctica	Stipe	*Ulte*	Fresh	140.85 ± 3.35	12.05 ± 0.72
Mazzaella laminarioides	Fronds (whole individual)	*Luga cuchara*	Fresh	176.17 ± 2.79	10.56 ± 0.16
Pyropia columbina	Fronds (whole individual)	*Luche*	Fresh	633.00 ± 11.19	41.74 ± 0.3
Pyropia sp./ Porphyra sp.	Fronds (whole individual)	*Luche*	Dry	56.90 ± 3.01	-

I found a wide range of values for total phenolic content, most of the values fall within the range reported for other algae (see Table C.5, Appendix C). Fresh *P. columbina* has higher values of TPC and antioxidant activity compared to the other species in this study, and results from literature (Table C.5, Appendix C). *D. antartica*, the species reported to be used for medicine, did not have higher total phenolic content compared to *M. laminarioides* and *P. columbina*, however it showed a higher antioxidant activity than *M. laminarioides*, suggesting the presence of compounds (apart from phenols) with antioxidant potential. Other compounds that contribute to radical scavenging capacity of seaweed extracts are sulfated polysaccharides (e.g. carrageenan; Yuan *et al.* 2005b), ascorbic acid, vitamin A (Kumar et al. 2008), pigments (chlorophyll and carotenoids; Indriatmoko *et al.* 2015) and essential oils (Mellouk *et al.* 2017).

Additionally, dry samples had lower total phenolic content compared to the fresh ones. In this case, they are dried out in the sun, with the effect of two factors: heat and light, known to have post-harvesting effects on phenolic content (Manach 2004). Manach (2004) mentions additional factors to consider with regards to antioxidant dietary intake. Storage and cooking have a negative effect on phenols. In the case of dry seaweeds that are storage (for being used in the winter) and boiled before eating, their phenolic content is highly reduced compared to raw and fresh products.

3. Economic and social aspects of polysaccharide industry

a) Economic aspects

Prices and volumes of extraction of seaweeds at Chilean national scale

In Chile in 2002, the market of polysaccharides accounted for landings up to 313,348 dry tons of seaweeds, which increased to 399,704 in 2017 (**Figure 2.3**). The production of seaweeds appears to be increasing; however it is still highly variable and unpredictable. This is explained by the availability of some species that account for most of the Chilean production (genus *Macrocystis* and *Lessonia*, **Figure 2.3**; SERNAPESCA 2018). In this regard, Buschmann (2008) suggests that the decline in landings of brown algae for the period 2006-2007 seen in **Figure 2.4**, (specifically for *Macrocystis* and *Lessonia*), was explained by overharvesting of these resources related to their use as feed in abalone farming.

Prices are highly variable, ranging from USD$25/ton to USD$2040/ton, depending on the species of seaweed, with red algae having higher prices compared to brown algae, such as *Lessonia* and *Macrocystis* (**Figure 2.4**) *Luga roja* (*Gigartina skottsbergii)* and *luga negra*, (*Sarcothalia crispata*) exhibited the highest prices among the period 2011-2013. According to Valderrama *et al.* (2014) price levels tend to be highly variable, regionally and even monthly, and it depends of the buying power and the amount of seaweed available.

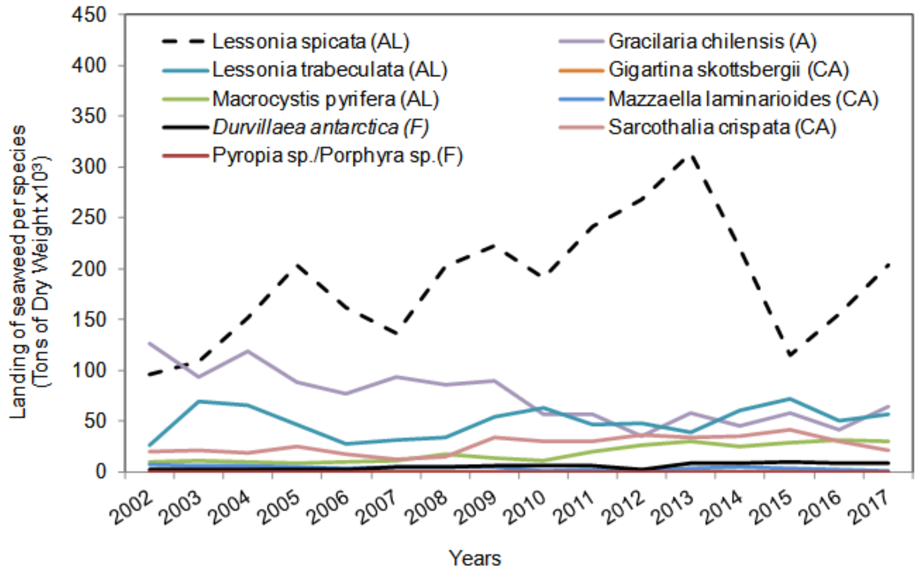


Figure 2.3 Time series of seaweeds extracted for polysaccharide industry from 2002 to 2017 (Data modified from SERNAPESCA 2018). The letters indicate the main uses for the species: AL=alginate, A= agar, CA=carrageenan, F=food.

Prices and volumes of extraction of seaweeds at local scale

In South central Chile, the most important species harvested for polysaccharide was *Sarcothalia crispata* (95% of survey participants). This intertidal species is used as source of carrageenan and is traditionally collected after being washed into the beach. The *lugueros* (harvesters of *luga*) mentioned that the best time to collect was after storms since the action of waves releases more algal material to be washed into the beach.

Mazzaella laminarioides was reported to be harvest by 51.8 % of the participants. This species is also a source of carrageenan, collected in the intertidal zone. This seaweed is pulled by hand, including both reproductive and non-reproductive fronds. In some places such as Isla Mocha and

Isla Santa Maria, this species is of secondary importance compared to luga, while in other regions, such as Matanzas, this is the main species collected for the polysaccharide industry.

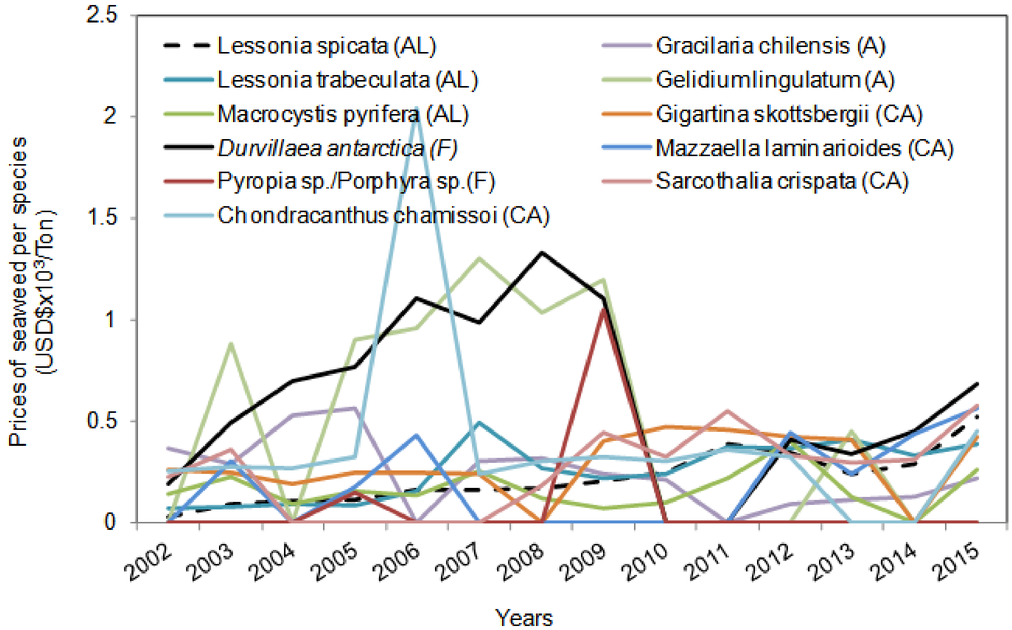


Figure 2.4 Time series of prices of seaweeds for polysaccharide industry from 2002 to 2015 (Data modified from SERNAPESCA 2018). The letters indicate the main uses for the species: AL=alginate, A= agar, CA=carrageenan, F=food.

Some species present in the area of study are not so popular, such as *Chondracanthus chamisoii*, which used to be more important in the past, and *Macrocystis pyrifera*, which is extracted just for subsistence in this area, while in the Northern part of the country, is extracted for polysaccharide industry (production of alginates).

In Isla Santa Maria, they collect an average of *luga negra* of 55 kg of dry seaweed at day per household (**Table 2.6**), with maximums of 150 kg. In Isla Mocha, the average is 116 kg of dry *luga negra* at day, with maximums of 350 kg per household. For *luga cuchara* the average of collection was near to 50 kg of dry seaweed at day, and was similar in both places, with maximums of 100 kg at day. Even though some *lugueros* would prefer to harvest *luga cuchara* versus *luga negra* due an easier processing (do not have to be sieve as *luga negra* that comes with sand), *luga cuchara* is mostly collected when *luga negra* is scarce.

In 2014, the income for collection of *luga negra* was US$ 92/day per household in Isla Mocha, and US$ 55/day in Isla Santa Maria. In this locality, a third species of seaweeds was extracted, *chicoria de mar*, its extraction was just reported by six participants, for whom the extraction of this species of seaweeds meant an income of US$ 19/day for the household (**Table 2.6**)

Prices vary by locality and according to participants there was a rapid increase in prices of *luga negra* over time: comparing 2013 to 2014, prices increased 60% in Isla Mocha, and 150% in Isla Santa Maria. For *luga cuchara* prices increased 60% in Isla Mocha, having the same prices than *luga negra*, and 100% in Isla Santa Maria during the period of just one year (**Table 2.7**). The reason for these changes was mainly attributed to the presence of new middlemen in the locality (more demand; see market chain of carrageenan in Appendix B, **Figure B.1**), but also to a reduced supply due to the effects of the recent earthquake in 2010 which, according to the *lugueros,* affected the availability of seaweeds (personal communication, Jaramillo *et al.* 2012; Vargas *et al.* 2011).

The high demand for seaweed for polysaccharide production, added to local and international factors, spurred an increase in seaweed prices and improved working conditions for *algueros*. An example of change on prices is the change in Isla Santa Maria, from US$0.07 per kg dry weight when collection of seaweeds started, about 40 years ago, to US$1 on 2014 (personal communication). As mentioned in the introduction, the changes in prices can be related to higher demand due changes in the market, and shortage of surplus of seaweed material. The latter reason could be related to climatic factors (e.g. El Nino events; McHugh 2003) or sporadic events as the earthquake of 2010 in the Center and South of Chile. The earthquake affected the availability of subtidal seaweeds due a tsunami event (personal communication; Jaramillo *et al.* 2012; Vargas *et al.* 2011) and exposed the rocks and affected the availability of intertidal species due an important coastal uplift (personal communication; Jaramillo *et al.* 2012; Vargas *et al.* 2011).

Table 2.6 Local production, prices and income from the extraction of seaweeds for polysaccharides at study sites

Seaweed	Locality	Price (USD/kg)	Household production (Kg/day)	Daily Household Income	Average number of people/ household	Daily number of hours worked/ person	$USD/person* hour
Sarcothalia crispata	Isla Mocha	0.8	116	92.8	2.4	2.8	13.8
Sarcothalia crispata	Isla Santa Maria	1	55	55	1.6	4.2	8.2
Mazzaella laminarioides	Isla Mocha	0.8	50	40	2.4	2.8	5.9
Mazzaella laminarioides	Isla Santa Maria	0.6	50	30	1.6	4.2	4.5
Chondracanthus chamissoi	Isla Santa Maria	0.6	32	19.2	1.6	4.2	2.9

Table 2.7 Changes in prices of seaweeds in study sites 2013 and 2014

Seaweed	Locality	2013 (US$ kg-1)	2014 (US$ kg-1)	% Increase
Sarcothalia crispata	Isla Mocha	0.5	0.8	60
Sarcothalia crispata	Isla Santa Maria	0.4	1	150
Mazzaella laminarioides	Isla Mocha	0.5	0.8	60
Mazzaella laminarioides	Isla Santa Maria	0.3	0.6	100

b) Socio-economical conditions of seaweed harvesters

The collection of *luga* occurs in summer, mainly because the weather conditions allow it to dry. *Lugueros* lay the seaweed in the sand and let it dry under the sun for two or three days (**Figure 2.5.A**). When it rains the seaweed cannot be dried and it decompose in contact with fresh water.

In the field I observed very different conditions related to harvesting of seaweeds: people staying in temporary settlements, people staying in their own places and transport to the beach by horse-cart provided by middlemen, and *lugueros* that live close enough to the beach to walk or move by their own means.

Temporary settlements

In Isla Mocha, 51% of the *lugueros* move to live in temporary settlements during the summer (**Figure 2.5.B**). They establish these settlements in the South part of the island, where the beaches are longer and the yield of collection is higher. Also this allows them to go collect twice at day (when the tide changes).


A


B

Figure 2.5 A. Luga negra laid outside of temporary dwellings for sundry. B. Temporary dwelling on Isla Mocha.

The temporary dwellings are light constructions that lack potable water, electricity or sewerage. Water, groceries and electricity generators are brought when the families occupy them. The season in which the dwellings are inhabited starts on Christmas or New years and extends to the beginning of March (student's summer break). In most cases the materials used to build and furnish the dwellings are provided by middlemen, who bring supplies in their boats from the mainland. In these cases, part of the seaweed production goes to pay this debt.

Temporary settlements, related to the extraction of seaweeds are also seen in other parts of the country (e.g. Huasco, in the North of the country). In some cases, these dwellings are established by local people, but they can also be established by people from other places who take advantage of this activity to generate extra income and to pay for their vacations. In the case of Isla Mocha, I recorded three non-local families staying in temporary settlements that harvested seaweed during the summer.

Horse cart transportation

In Isla Santa Maria, middlemen provide horse-carts or trucks to transport people that work for them to the most productive beaches (on the North of the island) and back home daily. This kind of transportation facilitates engagement of more people on this activity, but also discourages the participation of kids and elderly.

Other working conditions

Additionally, in both places, and in Matanzas, I observed people walking to the beach and working for few hours at day, while the tide is low, sometimes taking their kids with them (**Figure 2.6.A**; showed as 'Others' in **Figure 2.7**).

c) Demographics of seaweed harvesters

Age

In Isla Santa Maria people reported that they have been collecting seaweeds for commercial purposes for 40 to 50 years, with many of them starting to collect when they were kids (under 10 years old, 34% of the participants), and teenagers (26%). In Isla Mocha, people started harvesting 40 years ago, when some of them were kids (18%), but most were teenagers or in their 20's (38% and 20%, respectively). Some *algueros* remember going to harvest with their parents when they were kids, after school. Few old people reported that they have not harvested since they were young, and started harvesting again recently, with the purpose of complement their low pensions (personal communication).

I observed elderly of both genders collecting seaweed in all kinds of working conditions (temporary settlements, horse cart transportation, or simply walking from home to the nearest beach; **Figure 2.6.A**). They reported using the money to buy reserves of food and fuel for the winter.

A

B

Figure 2.6 A. Elder collecting *Sarcothalia crispata* in Isla Mocha. B Infants playing and harvesting *Mazzaella laminarioides* in Matanzas

In the temporary settlements of Isla Mocha, the whole family is involved in harvesting practices, in contrast to other situations where mainly adults go to the beach to collect seaweeds (**Figure 2.7**). In contrast, in situations as horse cart transportation, and walk or move by their own means to the beach (showed as 'Others' in **Figure 2.7**), the participation of children was greatly reduced.

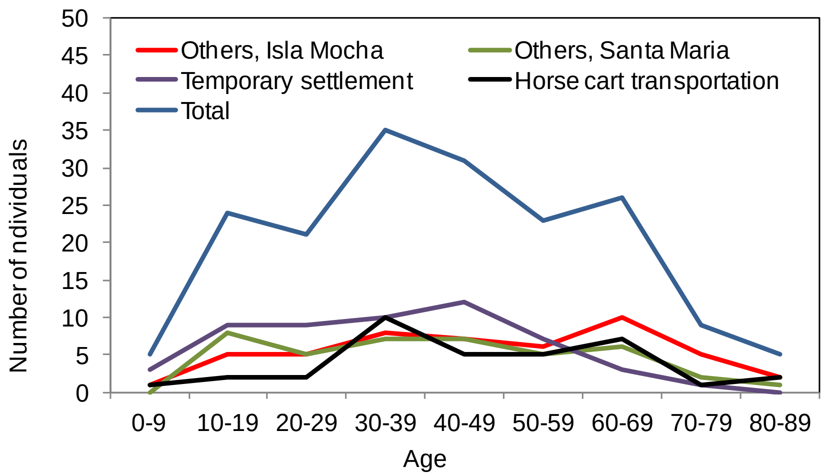


Figure 2.7 Age distribution of individuals participating in harvesting of luga for industrial purposes in different working situations

In temporary dwellings, adults reported using the money from this activity to save for winter and for school; while teenagers reported to use the money to buy clothes, or cell phones, and kids to buy candies.

As a result of increases in prices and better working conditions, the age distributions of people involved in harvesting have changed. The provision of horse carts facilitates greater numbers of people engaging in the activity, but it also discourages the presence of children which could affect transfer of knowledge. In the same location however there are large numbers of teenagers participating in the harvest. This is likely due to the low skill, manual labor nature of the job.

Gender

To be recognized as fishers or harvesters of marine resources, fishermen and seaweed harvesters have to register in the small scale fishers register. The official category of *algueros* according to the National Service of fishery and aquiculture is "fishermen, skin divers and harvesters of benthonic resources". They can be part of different kind of organizations: trade unions, trade associations, cooperatives, indigenous communities, etc (SERNAPESCA 2016). Participation in organizations allows algueros to apply for projects and to areas of management of benthonic resources that work in base to species specific management plans and can include some species of seaweeds (MINECON 1995).

In the 2006 annual statistics report that nationwide there were 58,210 men and 24,941 women registered in the category of "fishermen, skin divers and harvesters of benthonic resources". In the region where I carried out the interviews there were 10,561 (58.5%) registers of men and 7,488 (41.5%) of women (SERNAPESCA 2016). This category includes fishermen and skin divers, and these percentages differ from the results I found for the surveys that focused just on seaweed harvesters.

Seaweed harvesting used to be an activity carried out by women, except in temporary settlements, where the whole family participates. In all the localities visited, I was informed that the unions of *algueros* are mainly constituted by women. Today, with better conditions and higher prices, men also participate in this activity. In the surveys, of the total number of *algueros* reported (189), 54% were women (102) and 46% men (87).

For men harvest of seaweeds provides a secondary source of income in times when fishing is less productive. Both activities are not necessarily mutually exclusive; seaweed harvesting can be carried out at the same time than other activities, such as collection of abalones in the subtidal zone, or limpets and snails in the intertidal zone.

4. Intensity and frequency of harvesting

Gears/ methods used for harvest

Men and women use traditional methods of harvesting for most intertidal species. In the case of subtidal seaweeds, men use more intensive practices. For example, for *Sarcothalia crispata* they skin dive and pull the plant by hand, sometimes extracting the entire individual (personal communication). For *Macrocystis* and *Lessonia trabeculata*, two types of kelp, a method called *barreteo* is employed. *Barreteo* involves extracting the whole seaweed using pickaxes. This method has been reported to have severe environmental impacts and it results in a low rate of recovery, being currently regulated by law (MINECON 2017). *Cochayuyo* is also affected by practices such as *barreteo*, and its harvesting and commercialization is sporadically prohibited in specific places (e.g. ban in the VI and VII Regions for 2018 and 2019, MINECON 2018; Matanzas is located in the VI Region).

While for *S. crispata* changes in intensity of harvesting signify changes in the methodology used to extract the resources, for *M. laminarioides* it means an increase in intensity of harvest. For *S. crispata*, in Isla Santa Maria, most *lugueros* extract the beach wash, but some of them use additional methods including pulling the seaweed by hand when tides are very low (12%), or skin diving and pulling the whole algal body (42%). The latter activity was carried out almost exclusively by men (just one woman reported to practice this activity, personal communication). Additionally, participants mentioned a new dynamic generated between members of same household, while men skin dive and collect entire seaweeds, and the remaining parts of the seaweeds that were loosened are collected by women when washed to the shore.

In Isla Mocha the collection of *S. crispata* was only reported as beach wash. Practices as skin and hookah diving are regulated and by the time of the interviews they were temporally banned due management of abalone (MEABR, management and exploitation areas for benthic resources). This regulation secondarily protects *S. crispata* because both resources share the same habitat.

In the case of *M. laminarioides*, changes are related to the frequency of harvesting. Some participants of the surveys reported that they have reduced the time to revisit the same spot from one month to two weeks, when leaves were just long enough to be pulled by hand (10 cm); they recognize that they would not let the seaweed grow. Some *lugueros* stated that they cut the seaweed “green”, meaning that is still immature. A few participants mentioned that in the past it was practiced to scrap the rocks with knifes when the abundant of this seaweed was too low or the fronds were too short.

The changes on working conditions, better prices, the benefit of independency of work, continuous need of seaweeds for polysaccharide industry, seaweeds being a shared-resource, between other advantages of industrial seaweed harvesting, make this activity attractive for a higher number of people. As I saw in this chapter, this can bring socio- economic and cultural changes.

In contrast, due to the hard conditions of this particular work, none of the participants expressed interest in having their children work as seaweed harvesters in the future. The participants reported having medical conditions associated to the exposure of harvesting seaweed in cold temperatures. Women reported suffering chronic cystitis while men experienced chronic body pain after many years of skin or hookah diving. Males also mentioned the ocean simply felt too cold for them as they got older. However, they were interested in continuing the traditional uses of seaweed as food, linked to their nutritional and healthy properties

The traditional use of seaweeds is generally carried out on a household level scale, which does not represent a big impact for the resource at conservation level. Even commercial extraction of seaweeds for food was not intensive in the past, and did not likely have a big impact. However, the high volumes harvested today due to industrial use, and the adoption of more intensive harvesting practices, can have an ecological effect on the populations of seaweeds harvested over the long term. This topic will be studied in more detail in chapter 4.

Chapter 3: EFFECTS OF HARVESTING AND ENVIRONMENTAL FACTORS ON ANTIOXIDANT PRODUCTION OF MAZZAELLA LAMINARIOIDES

3.1 Abstract

Worldwide seaweeds are cultivated and wild harvested for industrial purposes, however, they can also hold economic importance and cultural value for coastal communities. In terms of nutritional value, some seaweeds have shown high levels of nutrients and antioxidants. Seaweeds growing in the intertidal system are exposed to biotic and abiotic stress, which affects their ecology and physiology, increasing the production of secondary compounds such as antioxidants. Additionally, for some species, harvest constitutes an additional source of stress, however little research has been done on its effect on nutritional value of seaweeds.

The objective of this chapter is to evaluate how environmental factors and harvest, affect the nutritional potential of seaweeds, using a case of study of *Mazzaella laminarioides*, an intertidal seaweed endemic of Chile, increasingly wild harvested for industrial purposes and traditionally consumed as food by local indigenous populations. Its nutritional potential is overlooked due to its linkage to indigenous food and to the common belief that this alga is used for the production of plastic.

Fresh sample of *M. laminarioides* was collected monthly from December 2016 to May 2017, in the intertidal zone of Matanzas beach, in Central-South Chile. I estimated total phenolic content (TPC) using the Folin-Ciocalteu method and the DPPH assay for radical scavenging activity (RSA). For statistical analysis I used linear mixed effect models to test the effect of: harvest, presence of endophytes, phenological stage and temperature, on TPC and RSA.

TPC and RSA values of *M. laminariodes* extracts fall within the range found for other species of seaweeds. Linear mixed effects models showed no significant effect of harvest, presence of endophytes or phenological stage on antioxidant potential of *M. laminarioides*. However, TPC was significantly lower in the warmest month than in the coldest month

While the results of the study do not show effect of harvesting on the antioxidant potential of this species, they can have implications on selection of timing of harvesting for food purposes. Since the environmental factors I measured vary greatly over space and time, it would be recommendable to repeat the study in different locations and record environmental factors at a smaller temporal scale to account for changes at the moment of collecting the samples.

3.2 Introduction

Stress generated from biotic and abiotic factors can trigger changes in plant cells and trigger a cascade of reactions that result in the formation of secondary compounds (Sudha *et al.* 2002) and the production of reactive oxygen species (ROS; Fujita et al. 2006). While primary compounds are essential for the maintenance of plant cells (Kasote *et al.* 2015), secondary compounds are molecules important for the survival and propagation of plants. Among other functions, they act as chemical signals, defense and UV protection (Evert & Eichhorn 2013; Crozier 2008; Connan *et al.*2007).

Reactive oxygen species (or reactive oxygen intermediates, ROIS) are partially reduced forms of oxygen normally generated as part of the aerobic metabolism, whose production can increase under stress conditions (Mittler 2002). Some of the causes of stress that can affect plant homeostasis and augment the production of ROS relate to water availability, extreme temperatures, salt stress, heavy metals, ultraviolet radiation, air pollution, mechanical stress, nutrient deprivation, pathogens, and high light (Mittler 2002).

Since the accumulation of ROS can be detrimental to the cell and cause irreparable damage, its increase is used as signal that triggers defensive responses of plants to the stress (Mittler 2002, Bi & Felton 1995; Cornish & Garbary 2010). Some of these responses are production of enzymes that convert ROS into harmless products, and/ or the production of antioxidants that scavenge them (Bowler *et al.* 1992).

Antioxidants are defined as any substance that prevents oxidation of another component (Hart 2012; Benzie & Wachtel-Galor 2012). In plants, they are mainly represented by the secondary compounds: polyphenols, carotenoids and vitamins (Xu *et al*. 2017; Baiano *et al.* 2016). Phenolic compounds protect plants from ultraviolet radiation through radical scavenging activity. But they can also act as antioxidants, participating on the detoxification of hydrogen peroxide, a ROS produced under stress (Kasote *et al.* 2015).

Environmental stresses that can increase the production of phenols are desiccation, ultraviolet radiation (Hart *et al.* 2014, Pavia & Brock 2000; Connan *et al.* 2007; Kasote *et al.* 2015; Bennett

& Wallsgrove 1994), pathogens (Machu *et al.* 2015; Manach *et al.* 2004; Kasote *et al.* 2015; Connan *et al.* 2007, Bennett & Wallsgrove 1994), herbivory (Kasote *et al.* 2015; Bennett & Wallsgrove 1994) and wounding (Kasote *et al.* 2015; Saltveit 2000).

Plants growing on highly dynamic environments, such as seaweeds in intertidal zones, are expected to exhibit high antioxidant production. Environmental factors including seasonal and daily variations as immersion and emersion (Flores-Molina *et al.* 2014; Connan *et al.* 2007), desiccation, ultraviolet radiation, variation in nutrient load (Navarro *et al.* 2014), and interaction with other species (e.g. epiphytes, herbivores, pathogens, competence, etc); have been reported to affect antioxidant activity, species ecology (Mansilla *et al.* 2006), photosynthesis (Vo *et al.* 2015), and pigment load, among others (Davison & Pearson 1996).

Seaweeds are traditionally used as food and medicine (Hart *et al.* 2014; Hong 2011; Turner 2003). They are also used as raw material for extraction of phycocolloids such as alginates, agar and carrageen. Today they are recognized for their nutritional and antioxidant content (Hart 2012; Rodriguez-Bernaldo de Quiros *et al.* 2010; Vijayavel & Martinez 2010), and presence of bioactive compounds (Marinho-Soriano *et al.* 2006), holding promise for food and pharmaceutical industries as well as for improvement of diet of coastal people.

Yates *et al.* (1993) suggest that species variation in phenolic content is related to a complex interaction of environmental and defense-related factors. There are multiple studies on the antioxidant content and potential of commercially important species (Machu *et al*. 2015; Rodríguez-Bernaldo *et al.* 2010; Guaratini *et al.* 2012; Hart 2012; Yuan *et al.* 2005a; Heo *et al.* 2006; Heo *et al.* 2005); however, few have assessed the effects of environmental factors on the production of antioxidants in the field (Connan *et al.* 2007; Hart 2012; Yates & Peckol 1993). And to my knowledge, none has assessed the effects of harvest on the production of these compounds, either in laboratory or in the field (search-engines: OneSearch and Google scholar)

Phenolic compounds show seasonal and daily variation (Ragan *et al.* 1978; Abdala-Díaz *et al.* 2006). To study the effect of abiotic environmental conditions on antioxidant production over time, I selected two of the many factors that have been reported to affect the production of antioxidants: temperature and ultraviolet radiation. For example, the terrestrial plant *Potentilla fruticosa*, shows a negative correlation between total phenolics and temperature (Liu *et al.* 2016), but opposite *patterns have been found for* three species of brown seaweeds: *Pelvetia canaliculata*, *Ascophyllum nodosum* and *Bifurcaria bifurcate* (Connan 2004). Connan 2004 has proposed that seasonal variation in antioxidant production is species-specific.

In terms of ultraviolet radiation, long term acclimation to UV radiation is positively correlated with the accumulation of phenolic compounds (Perez-Rodriguez *et al.* 1998; Abdala-Díaz 2006). Connan *et al.* (2007) reported differences on acclimation between species of seaweeds growing in the intertidal versus the subtidal zone. Intertidal species are more exposed to daily desiccation and seasonal variations, with higher exposure to solar radiation. In response to this, they accumulate phenolic compounds seasonally (in summer) and daily (before low tide; Connan *et al.* 2007; Connan *et al.* 2004; Pavia & Brock 2000).

Bennet & Wallsgrove (1994) describe how phenolic compounds can act as deterrents for herbivores, fungi, nematodes and bacteria. While some of these organisms can be detrimental for the host, others, known as endophytes, can live in the internal plant tissues without causing any harm to the plant (Petrini 1991). In the last decade, many authors have recognized the importance of production of bioactive compounds by endophytes and indicate the need to study these potential resources (Gouda *et al.* 2016; Hamilton *et al.* 2012). The study of antioxidant production related to presence of endophytes has focused mainly on endophytic fungi associated with plants (Hamilton *et al.* 2012; Huang *et al.* 2007a; Huang *et al.* 2007b; Strobel *et al.* 2003), or with marine algae (Li *et al.* 2017; Flewelling *et al.* 2015; Li *et al.* 2014; Debbab *et al.* 2012; White *et al.* 2010). To my knowledge, the effect of endophytic algae on the production of bioactive compounds production has not been studied for seaweeds.

Another factor that can affect the antioxidant potential of seaweeds is their life stages (Ragan *et al.* 1978). Most red seaweeds present a thriphasic life cycle with gametophytic, carposporophytic and tetrasporophytic stages (Roleda *et al.* 2007). In the case of red algae that produce carrageenan, the composition of these polysaccharides varies across different life stages (Piriz *et al.* 1991; Waaland 1975). Carrageenans present antioxidant activity (De Souza *et al.* 2007), but their potential will depend on their structural composition (Rafiquzzaman *et al.* 2016), which varies as function of species, environmental conditions and life stages (Gómez-Ordóñez *et al.* 2013; Sokolova *et al.* 2011, Van de Velde & De Ruiter 2005Velde *et al.* 2005). Carposporophytes and gametophytes produce κ-carrageenan, while tetrasporophytes produce λ-carrageenan (Piriz *et al.* 1991; Waaland 1975).While K-carrageenan is the most appreciated by its gelling properties (Rafiquzzaman *et al.* 2016; Al-Alawi 2011), λ-carrageenan has been found to have a higher antioxidant activity (De Souza *et al.* 2007).

Lastly, many species of plants and seaweeds are wild harvested. In most cases, seaweeds are pulled by hand, extracting just a portion of the individual. This practice could have similar effects to those of wounding or herbivory, affecting the production of phenolic compounds. Salveit (2000) describes the increase of phenols in response to wounding on lettuce, while other authors have demonstrated the production of phenols on response to herbivory (Kasote *et al.* 2015; Bennett & Wallsgrove 1994; Yates *et al.* 1993). To my knowledge, none have assessed the effects of harvest on the production of these compounds, either in laboratory or in the field.

I used a case of study of *Mazzaella laminarioides* (Bory de Saint-Vincent) Fredericq, a species of rhodophyta endemic to Chile, to examine the effects of environmental factors, harvest on antioxidant potential. This species is distributed across most of the Chilean coast, from Chañaral (29°S) to Punta Arenas (54°S), inhabiting the medium and low intertidal zone (Montecinos 2011; Santelices 1991). *M. laminarioides* is part of the group of carrageenophytes commonly called luga from the indigenous *mapuche* word *lúas*, meaning ‘certain edible seaweed’ (Augusta, 1991), and has been traditionally consumed by *mapuche* people (Montecinos 2011). However, its food potential has been overlooked, and in the last decades it has been increasingly harvested for industrial production of carrageenan (Buschmann *et al.* 2001).

Ogandaga *et al.* (2016) describe two species of endophytes for *M. laminarioides*: the Chlorophyta *Endophyton ramosum* (currently regarded as *Ulvella ramosa*; Sanchez *et al.* 1996; *Endophyton sp* by Correa *et al.* 1994; Faugeron *et al.* 2000) and the cyanobacterium *Pleurocapsa sp.* (Faugeron *et al.* 2000; Correa *et al.* 1994). In this chapter I study the effect of the presence of these two species of endophytes on antioxidant activity of *Mazzaella laminarioides.*

I addressed the following questions:

1) What is the antioxidant potential for *M. laminarioides*?

2) Does the antioxidant potential of the seaweed *M. laminarioides* vary as a function of variation in (i) abiotic factors (temperature, UV radiation), over the harvesting season, (ii) biotic factors (presence or absence of endophytes, phenological stage) and (iii) harvest?

3.3 Methodology

To measure the antioxidant potential of *Mazzaella laminarioides* (Rhodophyta, *Gigartinaceae*) over time, I collected samples monthly during the harvesting season 2016-2017 (December to May). Sampling was carried out in the intertidal zone of Matanzas, a rocky beach located in Central-South Chile, **Figure 3.1**) The plant material was collected from eight permanently marked plots (10 x 10 cm) which were harvested monthly following the traditional harvesting method of pulling the fronds by hand. As a control, samples were collected next to the plots in areas of the rock that did not show signs of harvest. The fresh material was rinsed in fresh seawater and frozen for transportation to the laboratory, where samples were conserved in the freezer at 0°C.

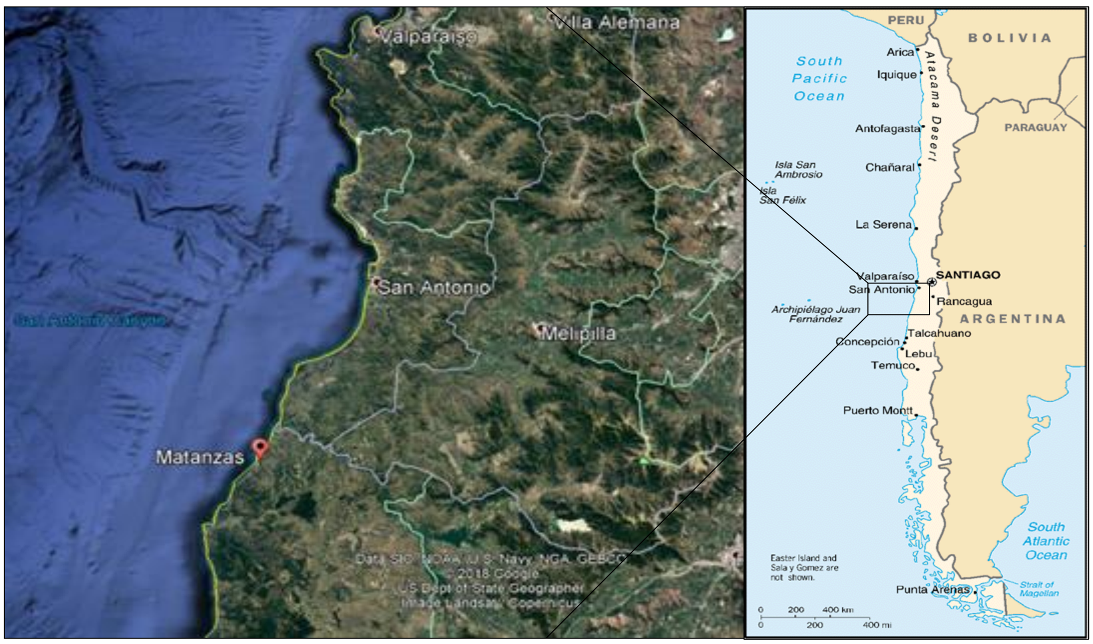


Figure 3.1 Location of field site, Matanzas, Chile (Google Earth 2018)

Data collection

Temperature and ultraviolet radiation values were recorded daily for the study site using information available online by nearby meteorological stations (Dirección metereológica de Chile, for temperature; and Tabla de mareas for UV index). The potentially confounding effect of desiccation was controlled by sampling at low tide the days with lowest tide every month.

To test the effect of phenological stage on antioxidant production, I sorted the fronds of each sample into different categories (carposporophyte, tetrasporophyte, and infertile) and recorded the composition in terms of relative (percentage) weight of each class.

To test the effect of the presence of endophytes: *Endophyton ramosum* and *Pleurocapsa sp*. on antioxidant potential, I separated each sample into categories (presence/absence of both species of endophytes) and determined percentage of weight for each category.

Preparation of the Seaweed Extracts

To prepare the seaweed extracts, the samples were unfrozen and rinsed with distilled water, then homogenized and finely chopped using a hand blender. One gram of seaweed was soaked overnight, in the dark, in a mix of 0.15 ml of acetic acid and 4.85 ml of absolute methanol, at room temperature (Modified from Rico *et al.* 2012). After this each extract was filtered through filter paper to remove algal particles, and stored in darkness at 4ºC.

Studies have shown differences in results when the same substrates are extracted with water, ethanol, methanol or other solvents, with higher antioxidant activity in water extracts compared to methanol and ethanol (Machu *et al.* 20015; Hwang & Do 2014; Rico *et al.* 2012; Othman *et al.* 2007; Cheung *et al.* 2003; Ismail & Hong 2002). However, I decided to carry out the extractions using methanol to avoid the formation of hydrocolloids that occurs for some species of seaweeds when extracted in water (e.g. some species studied in Chapter 2, including *M. laminarioides*). The presence of hydrocolloids complicate the absorbance measurements necessary for the chemical analyses (see below).

Chemical analyses

a) Determination of Total Phenolic Contents

Total phenolic content of the extracts was estimated using the Folin-Ciocalteu reagent (Rico *et al.* 2012; Prior *et al.* 2005), 100 µL of seaweed extract, 4.85 ml of distilled water, and 0.5 ml of Folin-Ciocalteu's reagent were mixed in a 10 ml volumetric flask. After three minutes, I added 1.85 ml of 20 % Na_2CO_3, and diluted with distilled water to the mark. After 30 minutes of incubation in darkness at room temperature, absorbance was measured at 765 nm, using a Shimadzu UV-Vis spectrophotometer.

The estimation of phenolic compounds was carried out in triplicate, and averaged. I made a calibration curve of gallic acid and used the regression equation ($y = 0.0008x + 0.0042$, $r^2 = 0.9937$) to express the result of total phenolic compounds as milligrams of gallic acid equivalent (GAE) per 100 gram of dry weight of algae.

b) Free Radical Scavenging Activity on DPPH

Antioxidant activity was measured in terms of radical scavenging activity (RSA), using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay, following the protocol described by Rico *et al.*. (2012). For this assay 100 µL of seaweed extract were mixed with one ml of 0.1mM DPPH methanolic solution. The reduction of DPPH radicals was determined by measuring the decrease in absorbance over 30 minutes, at 515 nm, using a Shimadzu UV-Vis spectrophotometer. The percentage of DPPH radical scavenging activity of the seaweed sample was calculated using the following equation:

$$RSA = \left(1 - \frac{\text{Absorbance in the presence of sample}}{\text{Absorbance in the absence of sample}}\right) \times 100$$

All the chemical analyses were conducted at the Laboratory of Food Science and Chemical Technology of the University of Chile, in Santiago, Chile.

Data Analysis

UV radiation and temperature were positively correlated (R^2= 0.6757, p<0.001, Appendix C, **Table C.1** and **Figure 3.1**). Additionally, both ultraviolet radiation (R^2= 0.9964, p<0.001, Appendix C, **Table C.2** and **Figure 3.2**), and temperature (R^2= 0.8843, p<0.001, Appendix C, **Table C.3** and **Figure 3.2**) were negatively correlated with months. Based on these result, I decided to use temperature as explanatory variable, and exclude ultraviolet radiation, for further analysis.

I used linear mixed effect models (Zuur *et al.* 2009) to test the effects of abiotic factors (temperature, UV radiation), biotic factors (presence of endophytes, phenological stage), and harvest on antioxidant potential of *Mazzaella laminarioides* I used two response variables: total phenolic content (TPC) and antioxidant activity (radical scavenging activity, RSA). For all models, the fixed explanatory variables included: temperature, percentage of fronds with presence of endophytes (either *Endophyton ramosum* or *Pleurocapsa sp.),* percentage of tetrasporic fronds, percentage of carposporic fronds, and harvest (yes/no). The random effects were the spatial factor replicate (the rock from which the fronds were sampled), and the temporal factor 'month'.

For the analysis of antioxidant activity the response variables and the continuous explanatory variables were log-transformed. Additionally, due to heteroskedasticity of residuals, I included harvest as variance covariate, allowing different variances of the residuals per stratum of the variable harvest (harvested/not harvested; Crawley 2007). To reduce the full models I used a backwards stepwise process. The best model was selected using Akaike Information Criteria (AIC), sequentially dropping the fixed variables with higher p-value (Zuur *et al.* 2009). The analyses were conducted in R software, using lnme package (Pinheiro *et al.* 2011)

To identify specific changes of phenolic content and antioxidant activity over time, I carried out four contrasts: Summer vs. fall months; May (Coldest) vs. other months; May vs. February (Coldest vs. warmest month considered in the study); and December (beginning of harvesting season) vs. other months. The analyses were conducted in R software, using contrast package (Kuhn 2016).

3.4 Results

I found a weak, positive correlation (R2=0.09615, p= 0.006; Appendix C, **Table C.4**) between the two response variables: total phenolic content and antioxidant activity (radical scavenging activity; **Figure 3.2**)

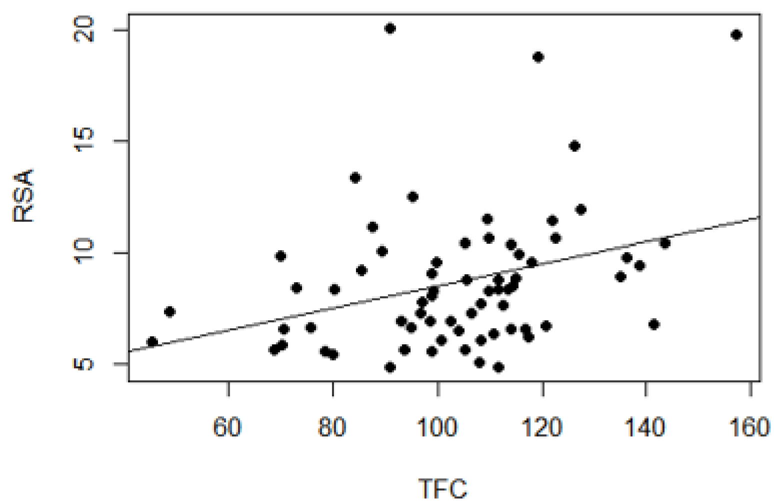


Figure 3.2 Percentage of radical scavenging activity (RSA) versus total phenolic content measured as milligrams of gallic acid equivalent (GAE) per 100 gr of dry weight of algae R2=0.09482, p= 0.006)

a) Effects of environmental factors on total phenolic content of *M. laminarioides*

The total phenolic content of the extracts of *Mazzaella laminariodes* varied between 45 and 157 mg gallic acid equivalent per 100 gram of dry alga. The best fit model included temperature and harvest as explanatory variables (Appendix C, **Table C.6**), with a tendency for temperature to have a negative effect on total phenolic content (**Figure 3.3.A**; **Table 3.1**, T-value=-1.854936, p= 0.0728).

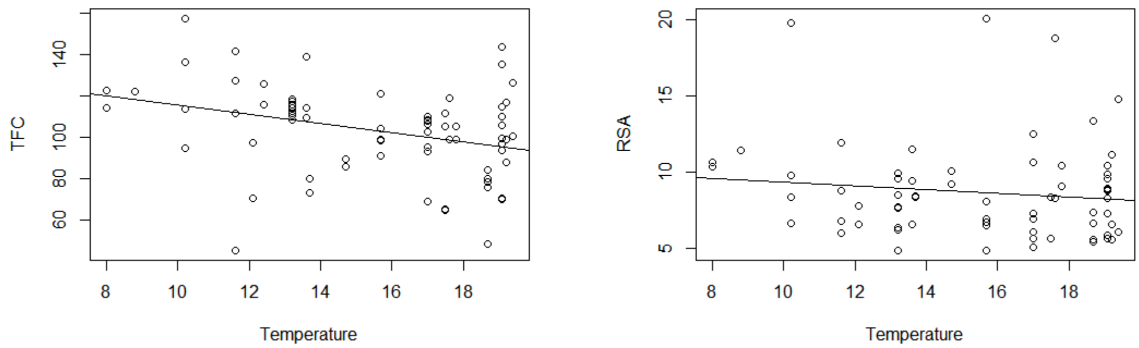
Table 3.1 Effects of environmental factors and harvesting on total phenolic content of Mazzaella laminarioides from the linear mixed-effect model TFCD (Appendix C, **Table C.6**)

Fixed effects	**Value**	**SE**	**DF**	**t-value**	**p-value**
Intercept	136.20788	19.822859	34	6.871253	0.0000
Temperature	-2.29561	1.237569	32	-1.854936	0.0728
Harvest	5.46568	4.205590	34	1.299623	0.2025
Random effects	**SD**				
Month	8.023946				
Replicate (Month)	8.822225				

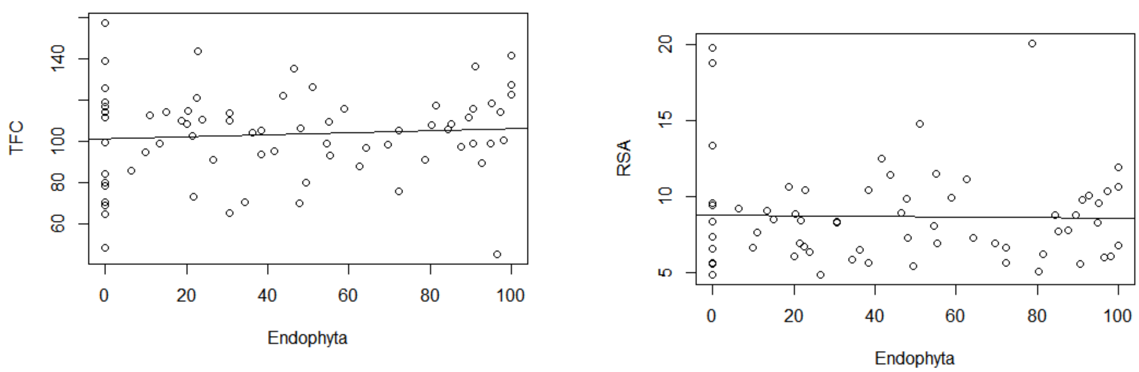
There was a significant difference in total phenolic content between the months of February and May (T-value=3.16674, p= 0.0024; Appendix C, **Table C.7**). The lowest content of phenolic compounds was in February, the warmest month, and the highest content in May, coldest month considered in the study (**Figure 3.4**). The results also suggest higher total phenolic content for the month of May compared to the other months sampled (T-value=1.81401, p=0.0746; Appendix C, **Table C.7**).

Table 3.2 Effects of environmental factors and harvesting on antioxidant activity of Mazzaella laminarioides from a linear mixed-effect model

Fixed effects	**Value**	**SE**	**DF**	**t-value**	**p-value**
Intercept	2.104071	0.03753773	32	56.05217	0.000
Random effects	**SD**				
Month	<0.001				
Replicate (Month)	<0.001				



A



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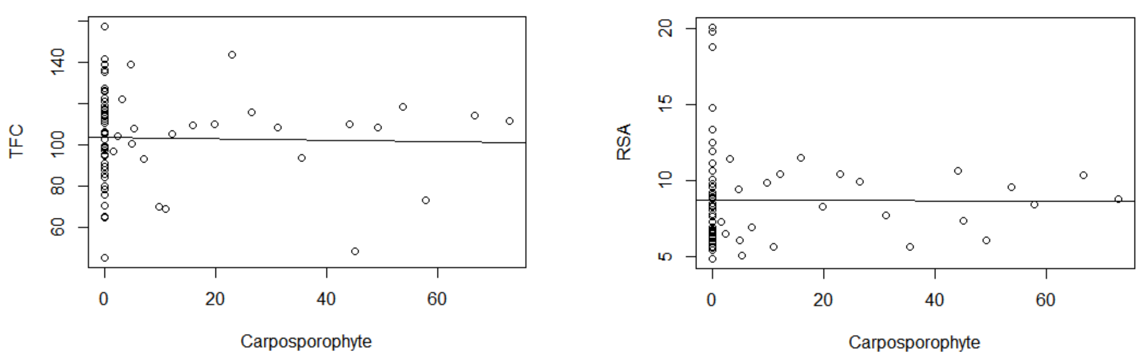
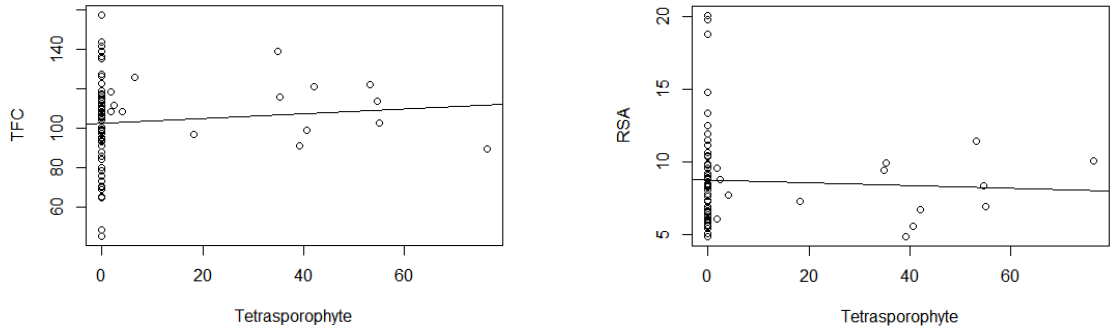


C

Figure 3.3 Effects of environmental factors on total phenolic content (TFC) and radical scavenging activity (RSA). A temperature (°C); B percentage of weight of individuals that present endophytes; C percentage of weight of individuals in carposporophyte life stage; E harvest vs. non harvest



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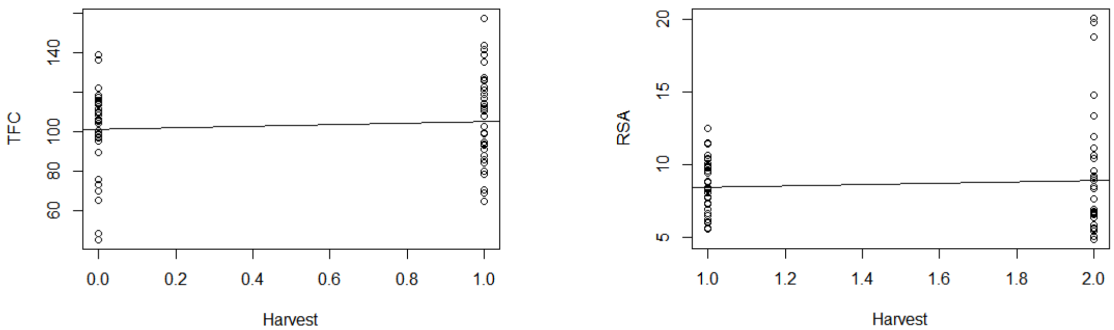


E

Figure 3.3 (Continued) Effects of environmental factors on total phenolic content (TFC) and radical scavenging activity (RSA). A temperature (°C); B percentage of weight of individuals that present endophytes; C percentage of weight of individuals in carposporophyte life stage; D percentage of weight of individuals in tetrasporophyte life stage; E harvest vs. non harvest

b) Effects of environmental factors on antioxidant activity of *M. laminarioides*

The antioxidant activity of *Mazzaella laminarioides* fluctuated between 4.3 and 20.08% of DPPH radical scavenging activity after 30 minutes of reaction. The best fit model was the null model, which did not include any explanatory variable (**Figure 3.3**; **Table 3.2**; Appendix C, **Table C.8**). Contrast analysis of antioxidant activity over time did not show significant differences (**Figure 3.3**; Appendix C, **Table C.9**)

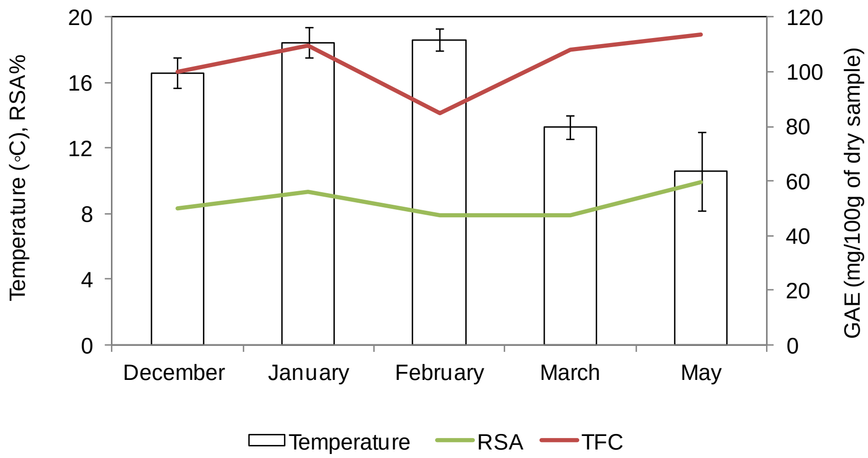


Figure 3.4 Variation of temperature, radical scavenging activity (RSA) and total phenolic content (TFC) during the harvesting season 2016-2017

3.5 Discussion

I tested the effects of abiotic, biotic factors and harvest on two measures of antioxidant production in a species of seaweed culturally and economically important. Consistent with other studies that have measured antioxidant activity and total phenolic content in marine algae, herbs, vegetables and fruits (Rico *et al.* 2015; Devi *et al.* 2011), my results show a positive correlation between both variables (antioxidant activity and total phenolic content). However, the low correlation observed suggest the presence of other compounds apart from phenols that can be accounting for a percentage of the antioxidant activity of *Mazzaella laminariodes*, such as sulfated polysaccharides (e.g. carrageenan; Yuan *et al.* 2005b), ascorbic acid, vitamin A (Kumar et al. 2008), pigments (chlorophyll and carotenoids; Indriatmoko *et al.* 2015) and essential oils (Mellouk *et al.* 2017).

The total phenolic content of the extracts of *M. laminariodes* (45-157 mg GAE/100 g dry weight) falls within the range found for other algae (see Appendix C, Table C.5). Similarly, the percentages of antioxidant activity (4.3 - 20.08% of DPPH radical scavenging activity) are also consistent with the values of other studies shown in Appendix C, Table C.5.

While other foods as tea, wine and cacao are recorded as important sources of polyphenols (Manach *et al.* 2004; Lee *et al.* 2003), it is not possible to compare their values to my findings because the extraction process used different solvents (aqueous versus methanolic extracts). The lack of standard protocols for the preparation of the extracts (different solvents and methodology, processed versus fresh products), and report of data (e.g. results expressed on GAE per serving, fresh or dry weight, etc), difficult the comparison of results between different studies, or different types of samples.

Contrary to my hypotheses, I found no significant effects of abiotic and biotic predictors (temperature, UV radiation, presence of endophytes, phenological stage) and harvest, on antioxidant activity or phenolic content. These results suggest that either the predictors do not have any significant effect on antioxidant production or that their effect is lower than the effects of variation in other environmental factors that I did not consider. Other variables to take into account are variations in time of low tide occurrence and its relation with temperature and ultraviolet radiation (Connan *et al.* 2007), desiccation stress (Flores-Molina *et al.* 2014), exposure of the rocks to sun and to wave action, action of herbivores, etc (Davison & Pearson 1996). A next step could be future research measuring abiotic factors (such as ultraviolet radiation and temperature) on a smaller time scale that can account for changes occurring at the moment of collecting the samples.

Also, in contradiction to my hypothesis, the results suggest a tendency for a negative relationship between temperature and total phenolic compounds ($p<0.07$, **Table 3.1**). The significantly lower values of total phenolic content in February (warmest month) as compared to May (coldest month) could be explained by air temperature. Consequently, the results are opposite to the trend described by Connan (2004), who found maximum values in summer and low values during fall and winter for three species of brown algae.

While further research should be done to confirm the trends found, my results could have implications in terms of optimal timing of harvesting for food, such as collecting this seaweed in winter when it presents highest antioxidant content. Additionally, during winter *luga* is not collected mainly because it is difficult to dry it for polysaccharides industry purposes (see Chapter 2), thus a secondary use as source of antioxidants may be an option for household use and potentially for industrial uses. Since environmental factors vary over time and space, it would be recommendable to repeat the study in different locations.

Chapter 4: ECOLOGICAL EFFECTS OF SEAWEED HARVESTING: A CASE STUDY OF HARVESTING AND POPULATION DYNAMICS OF MAZZAELLA LAMINARIOIDES IN MATANZAS, CHILE

4.1 Abstract

Worldwide seaweeds are cultivated and wild-harvested for industrial purposes, such as biofuels, fertilizers, food supplements, medicines, and for extraction of polysaccharides present in their cell walls. Most seaweeds used industrially are cultivated, however a 5% of them are still harvested from wild stock, with the subsequent pressure and long term effects on their populations. To test the effects of commercial harvest on population size and reproductive potential I use a case study of the alga *Mazzaella laminarioides*, an intertidal endemic of Chile. This species is increasingly wild-harvested for carrageenan production, and the ecological effects of current harvesting practices are unknown.

I evaluate three treatments, simulating current harvest methods and frequencies: (1) pulling fronds by hand monthly, (2) pulling fronds once, at the beginning of the harvest season, and (3) scraping all fronds and holdfasts at the beginning of the season. Using a random block design I established 13 experimental units, containing four plots each, which were subjected to one of the three treatments, or left as a control. Over the course of one harvesting season (December 2016 - May 2017), I monitored the effects of harvest treatment on variables related to population size and to reproductive potential. By the end of the experiment, the treatment that showed a recovery for most of the variables studied was hand-pull once. Plots hand-pulled monthly and scraped plots did not recover for most variables studied. Additionally, none of the treatments recovered for total number of reproductive fronds.

Overall though, the results of the study suggest that the most sustainable strategy would be pulling fronds by hand, rotating harvesting areas and waiting longer than 1 monthto revisit the same spot. This strategy would provide more and bigger fronds, while minimizes the negative impact of harvest on the population, allowing time to these plants to recover and reproduce.

4.2 Introduction

Seaweeds have been used for subsistence for thousands of years (Dillehay *et al.* 2008), and have cultural and social significance, particularly for coastal and indigenous people (Mösbach, 1999; Hart *et al.* 2014). Traditionally, seaweeds have been used for food, feed, medicine, and natural dyes among other uses (Guiry & Guiry 2019). Today they are also used industrially to produce biofuels, fertilizers, food supplements, medicines, and for the extraction of the polysaccharides present in their cell walls (Guiry & Guiry 2019). Additionally, they can make indirect contributions to local livelihoods due their importance in the marine ecosystem, providing food and refuge for fish and other organisms of economic importance (Rebours *et al.* 2014).

Algal polysaccharides - agar, carrageenan and alginates - are a structural part of algae cell walls that have gelling and thickening properties. Because of this characteristic, they are used for a wide range of commercial purposes in food, pharmaceutical and cosmetic industries (Usov *et al.* 2013; Renn 1997). The type of polysaccharides present in seaweeds depends on their taxonomy: carrageenan and agars are extracted from certain genera of red algae (Rhodophyta), while alginates are produced by all brown algae (Phaeophyta; Usov *et al.* 2013; Jiao *et al.* 2011).

By 2015, worldwide commercial production of seaweeds was 30.4 million tones, with the leading producers being China, Indonesia, Korea and Philippines for cultured species; and Chile, China and Norway for species harvested from wild stocks (FAO 2018). Sixty two percent of the world seaweed production is used for polysaccharides (Nayar & Bott 2014); this is equivalent to more than one million tons of wet seaweed (wild and cultured) per year (Pereira 2016; McHugh 2003). Alginate production (US$ 213 million) comes from wild stocks of brown algae; agar (US$ 132 million) comes from two genera of red algae, *Gelidium* and *Gracilaria*, the first one from wild stocks and the second species mostly cultivated. Carrageenan production (US$ 240 million) comes from cultivated and wild species of seaweeds. The main species uses for carrageenan are *Kappaphycus alvarezii* and *Eucheuma denticulatum*, both cultivated in Philippines and Indonesia; in addition to other species wild-harvested in South America, such as *Gigartina skottsbergii*, *Sarcothalia crispata* and *Mazzaella laminariodes* harvested in Chile (McHugh 2003).

Prices of the most important species of seaweeds collected for hydrocolloids increased greatly in the decade of 1999 to 2009, with the highest increases seen for the brown algae *Lessonia* (171% increase) and for some species of red algae (*Eucheuma cottonii, Sarcothalia crispata*, and *Gigartina skottsbergii* with 133, 130, and 114 percent increases, respectively; (**Table B.1**, Appendix B). The increase in prices is related to higher cost of energy, chemicals and transportation, as well as increases in the cost of raw seaweeds (Bixler & Porse 2011). The increase of value of raw material is caused by unpredictable availability of seaweeds and their high demand caused by the insertion to the market of China, Eastern Europe, and Brazil, among others (Bixler & Porse 2011; McHugh 2003).

Chile is the main producer of seaweeds in South America, accounting for 13% of world supplies (FAO 2018; Valderrama *et al.* 2013) and 42 % of the global seaweed wild harvest production (Nayar and Bott, 2014). In 2014, Chilean production reached 413,141 tons of seaweed, with just 5% of this algal material produced by aquaculture (FAO 2018, SERNAPESCA 2015). Brown algae (such as kelps of the genera *Macrocystis* and *Lessonia*) are predominantly harvested in Northern Chile, while red algae are mainly harvested in the South of the country (Buschmann *et al.* 2008). This dependence on natural stocks confers instability to the market since the natural abundance of the species can be affected by a number of factors, such as climatic conditions (IFOP 2001; Santelices & Norambuena 1987), big swells (Vasquez *et al.* 2008, IFOP 2001, Poblete & Candia 1991), El Nino events (Vasquez *et al.* 2008), earthquakes and tsunamis (Jaramillo *et al.* 2012; Castilla *et al.* 2010), plant pests or epiphytes (Buschmann *et al.* 2008, Poblete & Candia 1991; Romo 1986), bans or fishery regulations (Vasquez *et al.* 2008), among others.

While the supplies of some species are limited by natural abundance of the stock, others are limited by harvesting capacity. In the last case, the favorable market conditions have promoted changes towards more intensive harvesting methods. These changes can have long term effects on biological and cultural diversity, affecting: the abundance of some species of seaweeds, the population dynamics (O'Connell-Milne *et al.* 2014; Borras-Chavez *et al.* 2012; Buschmann *et al.* 2001; Santelices & Norambuena 1987), their ecological communities (e.g. the case of kelp forests, *huiro;* Vasquez *et al.* 2012; Levitt *et al.* 2002), the socio-economical conditions of people depending on these resources importance (Rebours *et al.* 2014), and the cultural practices associated to their presence in a particular place (e.g. transference of knowledge, medicinal and food uses, etc; Hart *et al.* 2014; Kamakau 1992).

Many cultures who depend on seaweeds have their own traditional/local methods for harvest developed over time, and informal rules and regulations associated to them, such as who, where and when to harvest (O'Connell-Milne *et al.* 2014; Hart *et al.* 2014; Kamakau 1992; Masuda 1988; Turner 2003). With the rise of commercial harvest these practices have been modified towards more intensive practices, with increase of intensity and frequency.

In seaweeds of commercial importance, most work has focused on describing the biology of the species with the purpose of develop harvesting strategies or cultivation techniques (Vasquez *et al.* 2012; Borras-Chavez *et al.* 2012; Romo *et al.* 2006; Marin *et al.* 2002; Castellanos-González 2003; Buschmann *et al.* 2001; Avila *et al.* 1999; Santelices & Norambuena 1987). However, there are also studies that compare different methods of harvest and some of them have demonstrated that the method of harvesting seaweeds can be more important than how much is extracted (O'Connell-Milne *et al.* 2014; Vasquez *et al.* 2012; Santos 1993).

Additionally, studies such as O'Connell-Milne *et al.* (2014) have compared traditional and modern methods of harvest for red seaweed, showing that the traditional practice of pulling is more sustainable than cutting and scraping. Considering that these results are species specific, it is necessary to do more research on wild harvested species of commercial importance

Study species

Mazzaella laminarioides (Bory de Saint-Vincent) Fredericq, an alga in the family *Gigartinaceae*, division Rodophyta (red algae), is endemic to Chile and holds economic and cultural importance (Montecinos 2011). *Mazzaella laminarioides* is distributed across most of the Chilean coast, from Chañaral (29°S) to Punta Arenas (54°S), inhabiting the intertidal zone (Montecinos 2011; Santelices 1991). Montecinos *et al.* (2012) described three different genetic lineages that correspond to the North, Central-South, and Southern locations. In this study I work with the Central-South type.

This species possess ecological importance in rocky intertidal communities (Santelices 1991), as a dominant element, growing together with *Ulva sp.*, *Porphyra sp.*, among others (Santelices *et al.* 1981; Jara & Moreno 1984). It is also consumed as food by herbivores such *Fissurella* limpets and the sea snail *Siphonaria lessonii* (Moreno & Jaramillo 1983; Jara & Moreno 1984)

M. laminarioides presents a triphasic life cycle with isomorphic alternation of generations, in which coexist male gametophytes, female gametophytes and tetrasporophytes (**Figure 4.1**). After fertilization, the female gametophytes are transformed to cystocarpic fronds, which retain the cystocarps (fertilized gametophytes; **Figure 4.1**). Additionally, this species is procarpic, having the carpogonia (female organs) and auxiliary cells nearby (Martin 1917); in these kinds of algae each cystocarp is the result of one fertilization event (Cole & Sheath 1990). Additionally, *M laminarioides* possesses a perennial holdfast that accounts for vegetative regeneration (Santelices & Norambuena 1987).

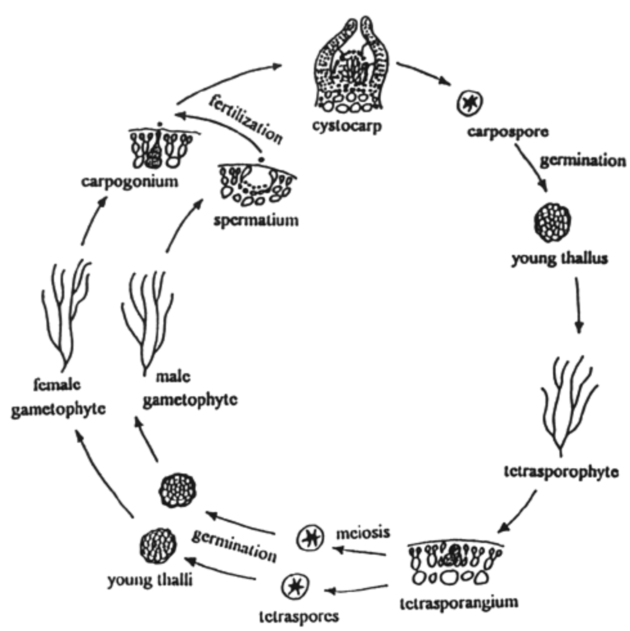


Figure 4.1 Triphasic life cycle found among red algae in Gigartinales (FAO/NACA 1996)

The vernacular name given to this species is *luga cuchara* and it belongs to a diverse group of carrageenophytes commonly called *lugas*, a word derived from the *mapuche* word *lúas,* meaning "edible seaweed" (Augusta 1991). Although this is not a popular species used for food, *M. laminarioides* has been traditionally eaten by indigenous *mapuche* people. Also, there are registers of specimens of the genus *Mazzaella* found in hearths of the archaeological site Monte Verde, suggesting the use of this seaweed for food and medicine 14,220 and 13,980 years before the present (Dillehay *et al.* 2008).

Currently *lugas* are harvested for extraction of carrageenan. The main species of *lugas* exported by Chile are the subtidal seaweeds *Sarcothalia crispata* (*luga negra*) and *Gigartina skottsbergii* (*luga roja*), while *Mazzaella laminarioides* (*luga cuchara*) is sold as a species of secondary importance (Buschmann *et al.* 2001) but highly extracted in the intertidal of areas where *S. crispata* is not abundant, or in periods when the beach-wash of *S. crispata* is low.

To date, there are two studies that assessed the impacts of harvest on *M. laminarioides*: Santelices & Norambuena (1987) and Gomez & Westermeier (1991). The first conducted in South-Central Chile (in the same population than the present study), and the second in Southern Chile. Santelices & Norambuena (1987) analyzed the temporal stock variation, measures of reproduction and gel content, in order to generate a management strategy for *M. laminarioides*. Their results suggest that extending the harvesting season to start extracting right after carpospores release would duplicate the total biomass production. The authors highlight the importance of not to damage the holdfast during harvest, as regeneration of new fronds depends mostly on them, showing faster production of harvestable stock from remnant holdfasts than plots denuded.

Gomez & Westermeier (1991) also emphasize the regeneration of fronds from perennial holdfasts as the main mean of reproduction. They found that the individuals that were pruned at holdfast level showed higher production of fronds and higher growth rates. However, high intensity of harvest (pruning fortnightly) suppresses the productivity of the holdfast after five months. Their management recommendation is to pull the longer fronds and leave the holdfast to ensure the regeneration of the population

Both studies were carried out more than 25 years ago, and no action have been taken to implement this knowledge in the development of a harvesting strategy for this species. For this reason, and an increase in the demand of this resource, it is relevant to reassess the effect of harvest on populations of *M. laminarioides.* Additionally, the present study looks at the effect of current harvest strategies as they are applied by *lugueros.* The past studies looked at the effect of harvest strategies simulating harvest by cutting the fronds, however, in reality, the harvest is carried out by plucking (with extraction or not of the holdfast), and by scraping the rocks.

To understand the current harvest practices, in 2014, I interviewed 83 seaweed harvesters (in four locations of South-Central Chile). The interviews were conducted in a door to door format, and I asked questions related to the whole household. Additionally, I carried out direct and participant observation (in five locations; see chapter 2). The results of interviews and observations showed two common harvesting practices: pulling by hand and scraping the rocks. Although scraping was not observed, a number of participants mentioned that they have used this method when the abundance of *M. laminarioides* was too low, either in the past or in other locations. In terms of frequency, most participants reported waiting between two weeks and one month to revisit the same area, which would allow the algae to grow back to a harvestable size. The harvestable size was reported as fronds longer than 10 cm; however it was observed that fronds were harvested when they were longer than 5 cm.

Chapter 2 examined changes in use of seaweeds from subsistence to commercial use and assessed how changes in commercial activity over time have affected the economic and social conditions related to the commercial harvest of *lugas* in South Central Chile. I also discussed changes in prices, and working conditions, leading to increasing intensity and frequency of harvest. The current chapter evaluates the effects of the intensification of harvest, on population size and reproductive potential, of a population of *M. laminarioides* from South-Central Chile. I address the following questions:

1) What are the effects of two different harvest methods and frequencies on measures of *M. laminariodes* population size: density of holdfasts, density of fronds and density and length of harvestable-sized fronds?

2) What are the effects of two different harvest methods and frequencies on measures of *M. laminariodes* reproductive potential: abundance and length of reproductive fronds, number and density of tetrasporangia and cystocarps?

3) How does time to recovery vary among harvest methods and frequencies, and across measures of growth and reproductive potential? Is this consistent with local practices of harvest?

Based on the above, I discuss which harvesting practices would be more sustainable, allowing the extraction *of M. laminarioides* while minimizing the impact of harvest on the population. I hypothesize that:

i) Comparing harvesting practices, the plots where algae are pulled by hand will have significantly fewer holdfasts, fronds and harvestable fronds than control, but more than scraped. Comparing frequencies, plots hand pulled monthly will have fewer holdfasts, fronds and harvestable fronds than hand pulled once

ii) Plots where algae are pulled by hand will have significantly smaller fronds than algae in control plots, but algae will be bigger than those in scraped plots. Algae in plots that were hand pulled monthly will present smaller fronds than hand pull once.

iii) Algae in harvested plots will present a decrease in abundance and length of reproductive fronds, with scraping having the lowest values, followed by those algae in plots that were pulled by hand monthly, and hand pull once

iv) Algae in harvested plots will present a decrease in total number and density of sporangia, with those plots with scraping will have the lowest values, followed by algae subjected to pull by hand monthly and hand pull once.

v) Fronds subjected to hand pulling will grow large enough to be harvested in one month or less, coinciding with the two week period that *lugueros* wait to revisit a given spot. However, the fronds harvested will not reach reproductive stages during that period of time. Fronds from scraped plots will not reach either harvestable or reproductive size in a period of one month

4.3 Methodology

Study design and data collection

Sampling was carried out in the intertidal zone of Matanzas, a rocky beach located in Central-South Chile (**Figure 4.2**). To measure the effects of harvesting and other environmental factors on the population structure of *Mazzaella laminarioides*, I permanently marked 13 experimental units, and assessed them monthly for the full duration of the harvesting season 2016-2017 (December to May).

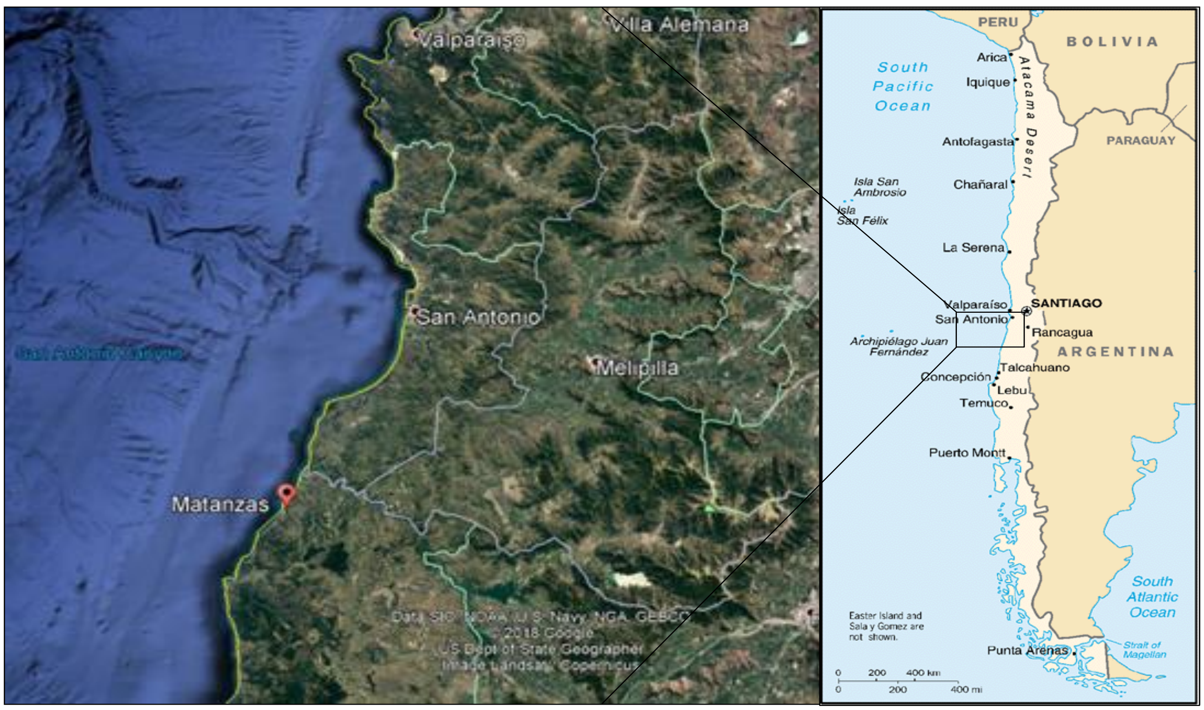


Figure 4.2 Location of field site, Matanzas, Chile (Google Earth 2018)

The experimental units were located in different rocks, separated by 2 meters or more. Each experimental unit was composed by four permanent marked plots (10 x 10 cm). To assess the effect of harvest each plot was exposed to a different combination of two treatments: harvest method and frequency (**Table 4.1**)

Table 4.1 Summary of treatments applied to the four different plots present on each experimental unit

Treatment	Harvesting method	Frequency
Control	Non-harvested	-
Hand pull once	Pulling by hand	Once, at the beginning of harvesting season (December 2016)
Hand pull monthly	Pulling by hand	Once at month
Scraping the holdfasts	Scraping the substrata, (removing fronds and holdfast)	Once, at the beginning of harvesting season (December 2016)

In each plot I recorded total number of holdfasts, total number of fronds, number and length of harvestable fronds. I defined harvestable sized fronds as those that were longer than 5 cm. Although most harvesters reported that the minimum size of fronds harvested is 10 cm, participant observation showed that 5 cm was a more accurate cut-off.

For each plot I photographed the harvestable fronds available (up to 20) and recorded their phenological stage: tetrasporic, cystosporic, or undetermined. The phenological stages were determined by appearance in the field. Undetermined fronds did not present visible cystocarps or tetrasporangia, and could be either infertile or male gametophytes. In order to calculate maximum length, area and density of reproductive structures (tetrasporangia or cystocarps), pictures corresponding to the months of February and May were processed using Image J (Schindelin *et al.* 2012). To calculate the density of reproductive structures (sporangia) per frond, I used the reproductive fronds available per plot (up to10) and I counted the number of structures in a 1x1 cm square placed in the central-upper part of the frond.

Temperature and ultraviolet radiation values were recorded daily for the study area, using information provided online by nearby meteorological stations (Dirección meteorológica de Chile, for temperature, and tabla de mareas for UV index). The potentially confounding effect of desiccation was controlled by sampling at low tide the days with lowest tide every month from December to May, 2017. The month of April could not be monitored due to high surf event during the lowest tide period of the month.

Data Analysis

To test the effects of the harvesting treatments on *Mazzaella laminarioides* populations, I used eight response variables. Four variables related to population growth: density of holdfasts, density of fronds, density and length of harvestable fronds. And four of them related to reproductive potential: abundance of reproductive fronds, length of tetrasporic/ cystocarpic fronds, total number of tetrasporangia/ cystocarps per frond, density of tetrasporangia/ cystocarps.

a) Effects of harvest and frequency on measures of population size

First, I used linear regressions to test for correlations among density of holdfasts, density of fronds, and density of harvestable fronds previous to manipulation of the plots. This corresponds to the density of fronds and holdfasts present in the month of December before applying the harvest treatment (time of exposure =0). However, I did not count the density of harvestable fronds before applying the treatments, consequently, for this parameter t=0 is the density at the start of the experiment (after applying the treatments).

Second, I used linear mixed effect models (Zuur *et al.* 2009) to test the effects of harvesting method and frequency of harvesting on the population size (based on the treatments described in **Table 4.1**). I used density of holdfasts, density of fronds, density and length of harvestable fronds as response variables; the fixed explanatory variables were the initial density of holdfasts/fronds in the plot at the beginning of the experiment, and the factors harvest and time. The variable time refers to the number of months since the beginning of the experiment (month=0 corresponds to December). The random effect was the spatial factor replicate related to the rock from which the fronds were sampled.

The response variables were log-transformed to improve normality. The heteroskedasticity of residuals was addressed including harvest or time as variance covariate for the different models (Appendix D, **Table D.4**). The use of variance covariate allows different variances of the residuals per stratum of the variable (Zuur *et al.* 2009). To reduce the full models I used a backwards stepwise method (Crawley 2007) and the best model was selected using Akaike Information Criteria (Zuur *et al.* 2009). The analyses were conducted in R software, using package stats (R Core Team 2013), and lnme (Pinheiro *et al.* 2011)

b) Effects of harvest and frequency on reproductive potential

To test the effects of harvesting method and frequency of harvest on the reproductive potential I analyzed tetrasporic and cystocarpic fronds separately. The response variables total number of sporangia per frond, length of reproductive fronds, and density of sporangia; were analyzed using linear mixed effect models (Zuur *et al.* 2009) with the response variables log-transformed. The abundance of reproductive fronds was tested using binomial generalized linear mixed-effects models (Zuur *et al.* 2009). The fixed explanatory variables were length, harvest and time, and the random effect was the spatial factor replicate related to the rock from which the fronds were sampled. For length of reproductive fronds the explanatory factors were harvest and time, and the random effect replicate.

For total number of tetrasporangia and cystocarps, length of reproductive fronds and density of sporangia, the variable time just considered the months of February and May. And the analyses of abundance of reproductive fronds considered the months of January, February and May. Models were reduced using backwards stepwise method (Crawley 2007) and the best model was selected using Akaike Information Criteria (Zuur *et al.* 2009). The analyses were conducted in R software, using package stats (R Core Team 2013), lnme (Pinheiro *et al.* 2011), and lme4 (Bates *et al.* 2015).

c) Effects of harvest methods and frequency on recovery

To study the recovery of the response variables at the end of the harvesting season, I assessed difference between treatments and with respect to the control in month 5. For the densities of holdfast, fronds and harvestable fronds, I carried out the following contrast analysis: (i) control vs. hand pull treatments, (ii) hand pull once vs. hand pull monthly, and (iii) control vs. scraping the holdfasts. For length of harvestable fronds I contrasted: (i) control vs. hand pull once, (ii) hand pull once vs. hand pull monthly, and (iii) control vs. scraping the holdfasts. The analyses were conducted in R software, using contrast (Kuhn 2016).

For the variables related to reproductive potential, the number of reproductive fronds in the month of May was too low to do a statistical analysis of the effect of harvest and recovery at the end of the experiment (Appendix D, **Table D.9**- **Table D.10**).

4.4 Results

At the beginning of the experiment (t=0) density of holdfasts and the density of fronds per plot were positively correlated (R^2=0.05727, p= 0.03648; Appendix D, **Figure D.1.A** and **Table D.1**). Initial density of fronds and density of harvestable fronds were not correlated (R^2=0.0617, p=0.1891; Appendix D **Figure D.1.B** and **Table D.2**), nor was the initial density of harvestable fronds and the initial density of holdfasts (R^2=-0.06898, p=0.7609; Appendix D, **Figure D.1.C** and **Table D.3**)

a) Effects of harvest type and frequency on measures of population size

Density of holdfasts

The best fit model included initial density of holdfasts, harvest and time, and did not include interactions between these variables (Appendix D, **Table D.4**). The initial density of holdfasts had a significant positive effect on the density of holdfast per plot after harvesting. The treatment hand pull monthly had a significantly higher density of holdfasts compared to the control, and according to **Figure 4.3.A**, this difference was significant starting at month 3. Additionally, across all treatments, and the control, holdfast density was significantly lower in month 2 (February, **Table 4.2** and **Figure 4.3.A**) than in other months.

Table 4.2 Effects of density of holdfasts pre-harvest ($Holdfast_0$), time and harvesting on density of holdfast per plot

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	0.7304104	0.18161260	157	4.021805	0.0001
Holdfast0	0.6247390	0.09573350	157	6.525814	0.0000
Hand pull once	-0.0162659	0.07528679	157	-0.216053	0.8292
Hand pull monthly	0.2219134	0.07352397	157	3.018246	0.0030
Scraping the holdfasts	-0.0919462	0.10352629	157	-0.888144	0.3758
Month2	-0.2106224	0.07994547	157	-2.634576	0.0093
Month3	0.0043328	0.07944237	157	0.054541	0.9566
Month5	0.0088744	0.08130533	157	0.109149	0.9132
Random effects	**SD**				

Replicate	0.2983673

The contrast analysis at the end of the experiment (month 5=May), showed no significant difference in density of holdfast between treatments and control, or among different treatments (Appendix D, **Table D.5**), however **Figure 4.3**.A shows that the plots harvested had significantly higher density than the control since month 3.

Density of fronds

For density of fronds, the best model included initial density of fronds, harvest and time, with no interaction among the variables (Appendix D, **Table D.4**). Initial density of fronds had a significant positive effect on density of fronds per plot. Throughout the experiment the treatment scraping the holdfasts had a significant negative effect on the density of fronds compared to the control. Similar to density of holdfasts, for pulling treatments and the control, there was lower density of fronds in month 2 (February) than in other months (**Table 4.3**, **Figure 4.3.B**).

For the contrast analysis at month 5 (May), there were significantly fewer fronds in the scraping treatment compared to the control (Appendix D, **Table D.6**).

Table 4.3 Effect of density of fronds pre-harvest ($Fronds_0$), time and harvesting on density of fronds per plot

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	2.4155206	0.8684747	157	2.781337	0.0061
$Fronds_0$	0.6353228	0.1548522	157	4.102769	0.0001
Hand pull once	-0.0559014	0.0889338	157	-0.628574	0.5305
Hand pull monthly	0.0688910	0.1062114	157	0.648622	0.5175
Scraping the holdfasts	-1.4655621	0.2401578	157	-6.102496	0.0000
Month2	-0.2397881	0.1037342	157	-2.311563	0.0221
Month3	-0.1115767	0.1037928	157	-1.074995	0.2840
Month5	0.1383739	0.1053550	157	1.313406	0.1910
Random effects	**SD**				
Replicate	0.3485446				

Density of harvestable fronds

For density of harvestable fronds, the best model included harvest and time, and their interaction (Appendix D, **Table D.1**). The density of harvestable fronds decreased significantly after the first month and continued decreasing over time. Also, all the harvest treatments had significantly fewer harvestable fronds than the control (**Table 4.4**, **Figure 4.3.C**). The decrease in density varied by time and method, hand pulling monthly led to significantly fewer fronds in month 3 (March), and density increased significantly over time for the plots scraped (**Table 4.4**, **Figure 4.3.C**).

Table 4.4 Effects of time and harvesting on density of harvestable fronds (length>5cm) per plot

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	4.330263	0.1775216	148	24.392882	0.0000
Hand pull once	-1.276523	0.3247652	148	-3.930602	0.0001
Hand pull monthly	-1.057784	0.3122545	148	-3.387570	0.0009
Scraping the holdfasts	-4.043670	0.3113319	148	-12.988293	0.0000
Month2	-0.505081	0.2073878	148	-2.435443	0.0161
Month3	-0.921700	0.2104446	148	-4.379775	0.0000
Month5	-1.097912	0.2118048	148	-5.183603	0.0000
Month2:Hand pull once	0.249154	0.4721724	148	0.527676	0.5985
Month2: Hand pull monthly	-0.375055	0.4434330	148	-0.845799	0.3990
Month2: Scraping the holdfasts	1.253915	0.4708963	148	2.662825	0.0086
Month3: Hand pull once	0.516821	0.4556213	148	1.134321	0.2585
Month3:Hand pull monthly	-2.182847	0.4541685	148	-4.806250	0.0000
Month3: Scraping the holdfasts	1.086106	0.4509887	148	2.408278	0.0173
Month5: Hand pull once	0.636903	0.4742244	148	1.343041	0.1813
Month5: Hand pull monthly	-0.638426	0.4551025	148	1.402817	0.1628
Month5:Scraping the holdfasts	1.475385	0.4734638	148	3.116151	0.0022
Random effects	**SD**				
Replicate	0.4884378				

At the end of the experiment (May) the treatment hand pull monthly had significantly lower density than hand pull once, and scraping had significantly fewer harvestable fronds than the control (Appendix D, **Table D.7**).

Length of harvestable fronds

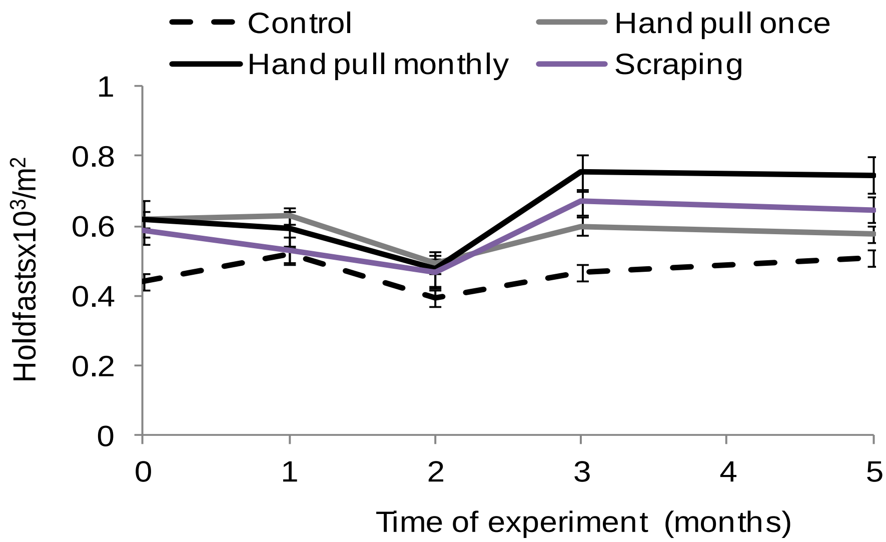
The size of the fronds increased significantly from month 1 to month 2 and 5, doubling their size in a one month period (**Table 4.5**; **Figure 4.3.D**). Fronds are significantly smaller for both hand pull treatments compared to the control and there was a marginally significant difference in frond length between the control and scraping treatments (**Table 4.5**).

Table 4.5 Effects of time and harvesting on the length of harvestable fronds

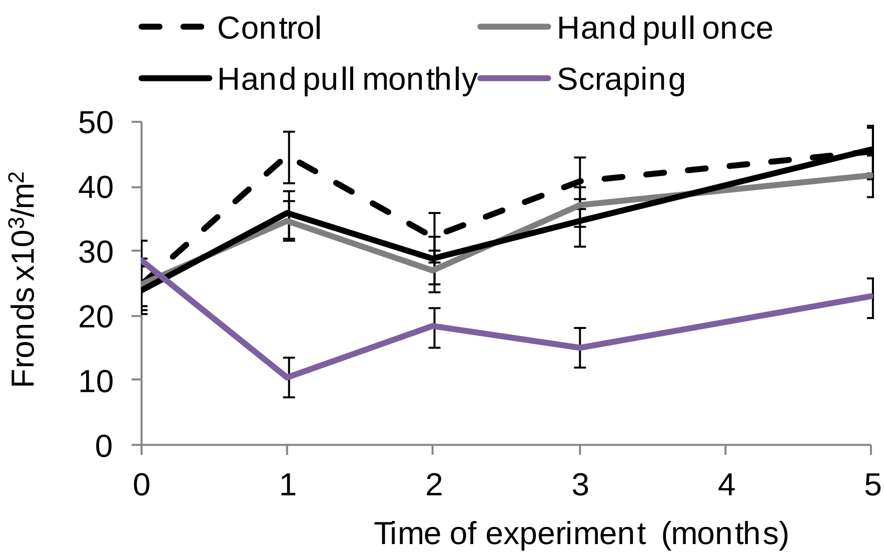
Fixed effects	Value	SE	DF	t-value	p-value
Intercept	2.0446081	0.05982983	742	34.17372	0.0000
Hand pull once	-0.2602149	0.06807158	742	-3.82267	0.0001
Hand pull monthly	-0.5087981	0.08361190	742	-6.08524	0.0000
Scraping the holdfasts	-0.0628552	0.11310565	742	-0.55572	0.5786
Month2	0.9284696	0.06148714	742	15.10022	0.0000
Month5	0.6067256	0.06110433	742	9.92934	0.0000
Month2:Hand pull once	-0.1635662	0.08479632	742	-1.92893	0.0541
Month2:Hand pull monthly	-0.3099520	0.09802332	742	0.00013	0.9999
Month2:Scraping the holdfasts	-0.3099520	0.13450487	742	-2.30439	0.0215
Month5:Hand pull once	0.3256941	0.08594946	742	3.78937	0.0002
Month5:Hand pull monthly	0.2000700	0.10387743	742	1.92602	0.0545
Month5:Scraping the holdfasts	-0.2712032	0.16563718	742	-1.63733	0.1020
Random effects	**SD**				
Replicate	0.3607044				

The treatment hand pull once showed a consistent increase in size over time, while fronds from the plots hand pulled monthly are marginally longer in May (**Table 4.5**; **Figure 4.3.D**), with no significant results for the interaction of this treatment and time. The fronds in the plots scraped were significantly smaller than the control on month 2, and did not vary significantly in month 5 (**Table 4.5**; **Figure 4.3.D**).

At the end of the experiment there was a significant difference for algae in both hand pull treatments, with plots hand pulled monthly having significantly smaller fronds than those in the plots hand-pulled once. Lastly, there was a tendency of the algae in treatment scraping to have smaller fronds than the control, but it was not significant (**Figure 4.3.D**; Appendix D, **Table D.8**).

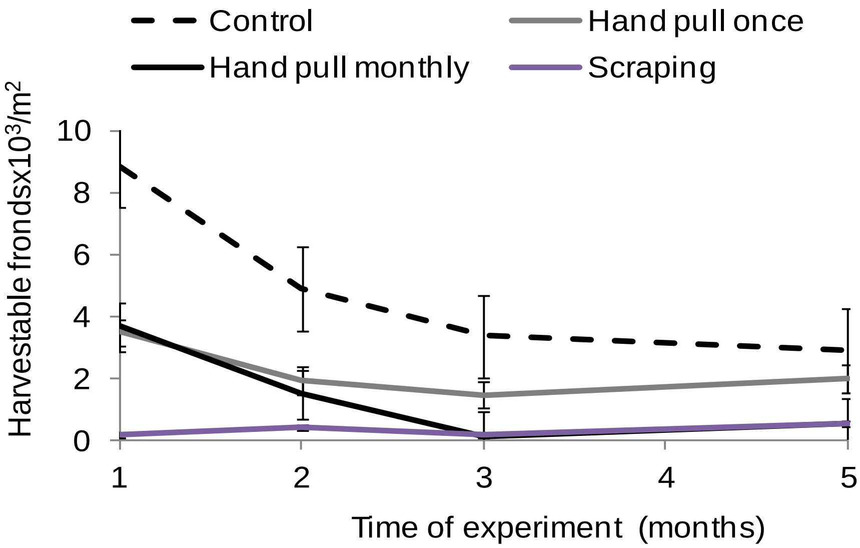


A.



B.

Figure 4.3 Effect of harvest type and time on density of A. holdfasts, B. fronds, C. harvestable fronds (>5cm) and, D. Average length of harvestable fronds. Error bars represent standard error.



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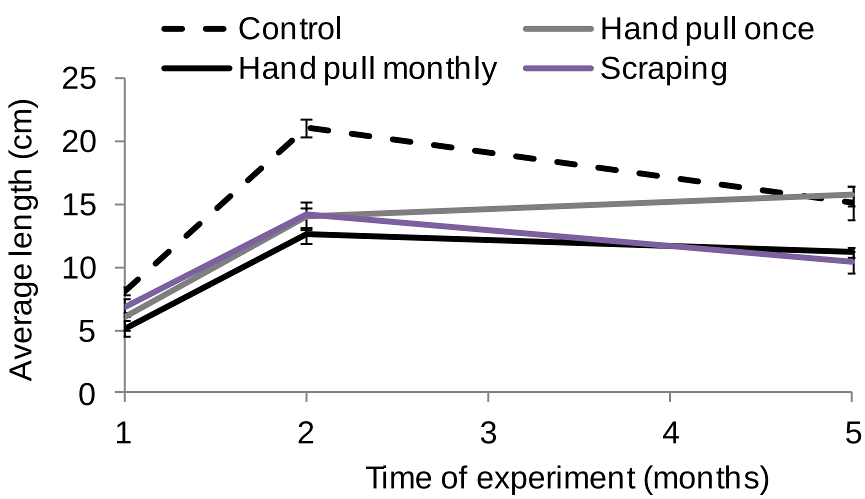


D

Figure 4.3 (Continued) Effect of harvest type and time on density of A. holdfasts, B. fronds, C. harvestable fronds (>5cm) and, D. Average length of harvestable fronds. Error bars represent standard error.

b) Effects of harvest and frequency on reproductive potential

Abundance of reproductive fronds

In general, there were fewer tetrasporic fronds than cystocarpic fronds across the different treatments and over time (**Figure 4.4**; Appendix D, **Table D.9** and **Table D.10**).

The number of reproductive fronds was positively correlated to length of the fronds (**Table 4.6**), and the plots harvested (hand pulled and scraped) had significantly fewer reproductive fronds than the control (**Table 4.6**, **Figure 4.4.A-B**). Additionally, in the month of May the number of reproductive fronds was significantly higher than in January (**Table 4.6**).

In general, the number of reproductive fronds was low; plots scraped presented a total of four reproductive fronds, and harvested monthly presented twelve, for the whole duration of the experiment (Appendix D, **Table D.9** and **Table D.10**). In the month of January reproductive fronds were just recorded in two of the 13 control plots, and in one of the plots pulled by hand once (For total number of reproductive fronds see Appendix D, **Table D.9** and **Table D.10**).

Table 4.6 Effect of density of length, time and harvesting on total number of reproductive fronds (tetrasporic and cystocarpic)

Fixed effects	Value	SE	z-value	Pr(>|z|)
Intercept	-4.15772	0.70330	-5.912	-5.912***
Length	0.16521	0.02158	7.655	1.93e-14 ***
Hand pull once	-1.00440	0.29837	-3.366	0.000762 ***
Hand pull monthly	-1.84092	0.39947	-4.608	4.06e-06 ***
Scraping the holdfasts	-1.76548	0.69729	-2.532	0.011344 *
Month2	-0.18754	0.68052	-0.276	0.782864
Month5	1.64250	0.61478	2.672	0.007547 **

Random effects	SD
Replicate	1.296

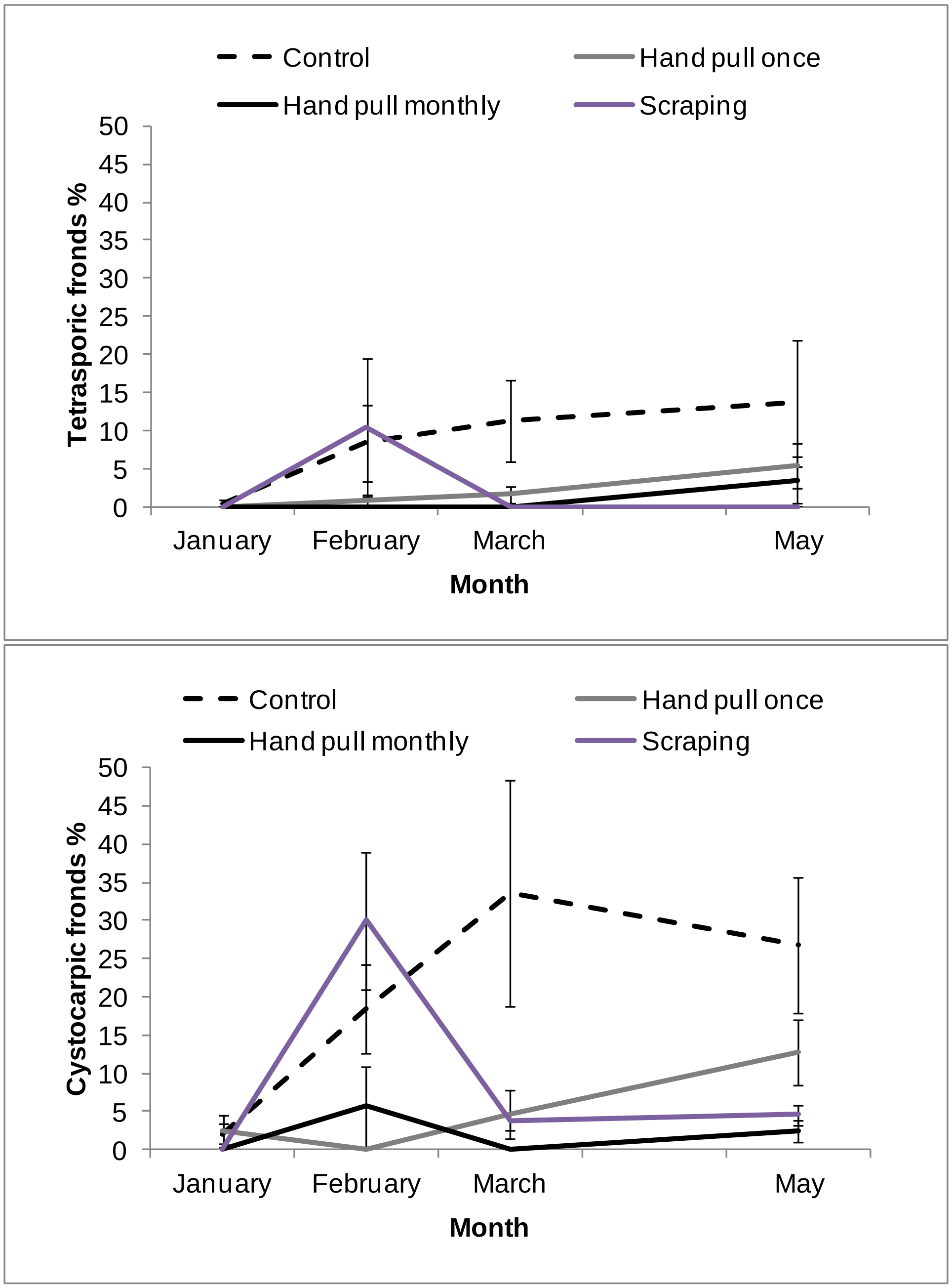


Figure 4.4 Effect of harvest type and time on the percentage of reproductive fronds per plot. A. Tetrasporic fronds, B. Cystocarpic fronds. Error bars represent standard error

Length of reproductive fronds

The model that best explained the variance of the average length of tetrasporic fronds included harvest treatment and time, with May having significantly shorter fronds than February across all treatments (**Table 4.7**; **Figure 4.5.A**). There were significantly smaller fronds in the hand pull monthly plots compared to the control

I did not record tetrasporic fronds for the treatment hand pull monthly in the month of February, or in the plots scraped in May (**Figure 4.5.A**).

Table 4.7 Effects of time and harvesting on the average length of tetrasporic fronds

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	3.170624	0.07133562	133	44.44657	0.0000
Hand pull once	-0.079457	0.07972146	133	-0.99668	0.3207
Hand pull monthly	-0.330103	0.09276354	133	-3.55854	0.0005
Scraping the holdfasts	-0.079923	0.17584667	133	-0.45450	0.6502
Month5	-0.342558	0.05416738	133	-6.32406	0.0000
Random effects	**SD**				
Replicate	0.2877595				

For average size of cystocarpic fronds, the model that best explained the variance just included time, with plots having significantly shorter carposporic fronds in May than February across all harvest treatments (**Table 4.8**; **Figure 4.5.B**). There were no cystocarpic fronds in the plots hand pulled once in February (**Figure 4.5.B**).

Table 4.8 Effect of time on the average length of cystocarpic fronds

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	3.1093987	0.07768946	86	40.02343	0.0000
Month5	-0.4231059	0.06447098	86	-6.56273	0.0000
Random effects	**SD**				
Replicate	0.2814865				

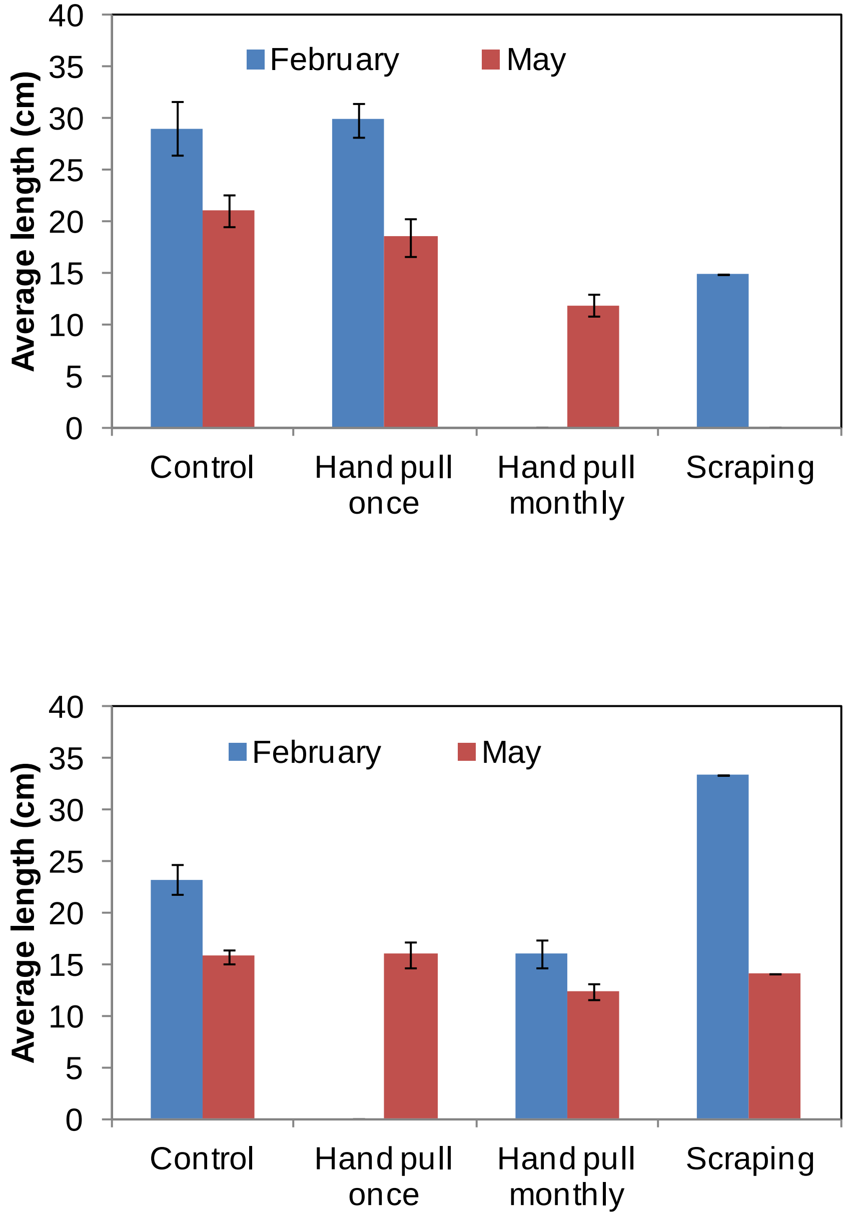


Figure 4.5 Effect of harvest type and time on average length of reproductive frond, A. Tetrasporic fronds, no data collected for February hand-pull monthly, B. Cystocarpic fronds, no data collected for February hand-pull once. Error bars represent standard error.

Total number of sporangia per frond

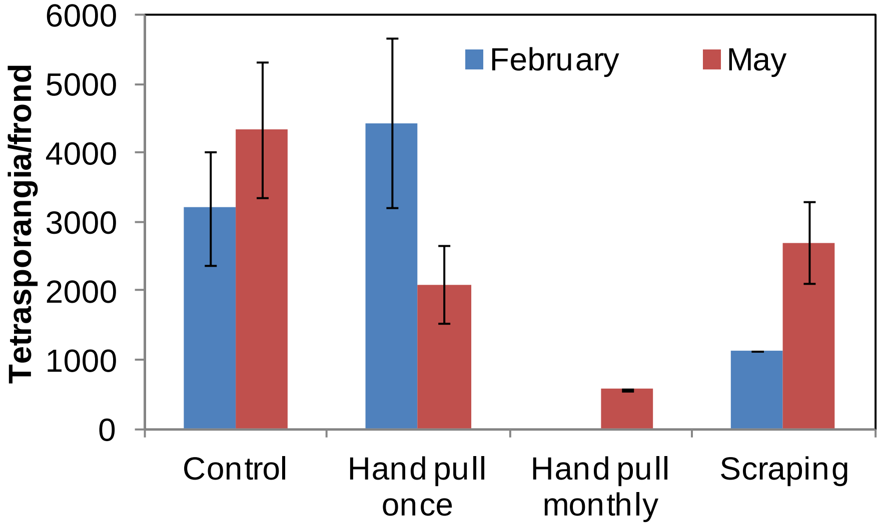
Overall the total number of tretrasporangia was higher than the number of cystocarps per fronds. And, the total number of both tetrasporangia and cystocarps increased significantly with the length of the fronds (**Table 4.9** and **Table 4.10**, **Figure 4.6.A-B**)

Table 4.9 Effect of length and time on the total number of tetrasporangia per frond

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	5.203320	0.5399092	24	9.637398	0.0000
Length	0.086202	0.0176418	24	4.886230	0.0001
Month5	0.474998	0.2854156	24	1.664231	0.1091
Random effects	**SD**				
Replicate	0.627886				

Table 4.10 Effect of length on the total number of cystocarps per frond

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	4.189302	0.3547188	28	11.810206	0.0000
Length	0.124176	0.0173279	28	7.166211	0.0000
Random effects	**SD**				
Replicate	0.7227502				



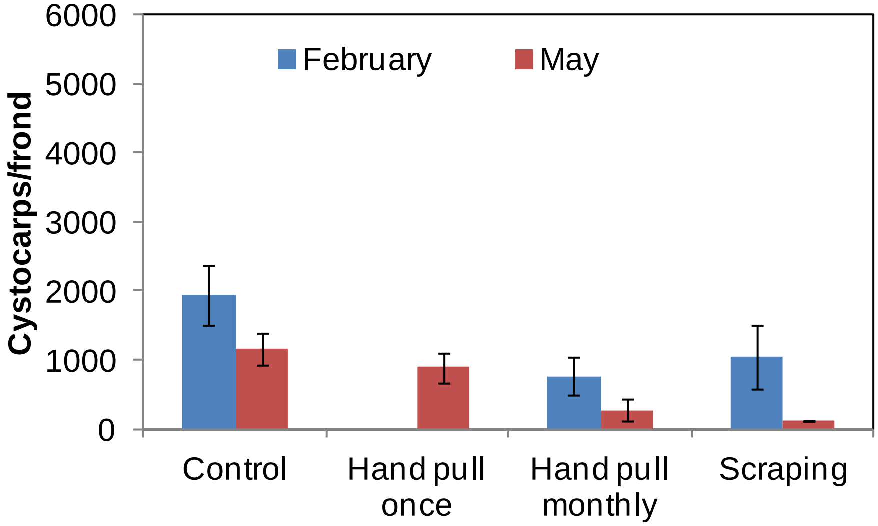


Figure 4.6 Effect of harvest type and time on the total number of sporangia per frond, A. Tetrasporic fronds, no data collected for February hand-pull monthly, B. Cystocarpic fronds, no data collected for February hand-pull once. Error bars represent standard error.

Density of tetrasporangia

The model that best explained variation in the density of tetrasporangia included only the variable time (**Table 4.11**), and the density of tetrasporangia was significantly higher in the month of May than February (**Figure 4.7.A**)

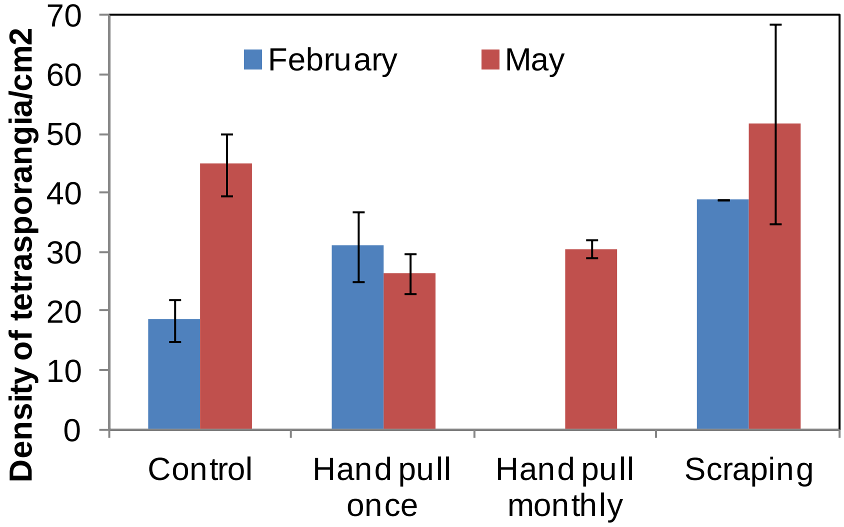
Table 4.11 Effect of time on the density of tetrasporangia

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	2.871244	0.2015210	25	14.247867	0.0000
Month5	0.579405	0.1990579	25	2.910736	0.0075
Random effects	**SD**				
Replicate	0.3591227				

In the case of density of cystocarps, the variance was best explained by harvest treatment, with fronds from the scraped plots having significantly lower density of cystocarps than those in the control plots (**Table 4.12**, **Figure 4.7.B**).

Table 4.12 Effect of harvest on the density of cystocarps

Fixed effects	Value	SE	DF	t-value	p-value
Intercept	2.6082314	0.1743299	26	14.961468	0.0000
Hand pull once	-0.4116754	0.2493768	26	-1.650817	0. 1108
Hand pull monthly	0.1596802	0.2642355	26	0.604310	0.5509
Scraping the holdfasts	-0.7229047	0.3077003	26	-2.349379	0.0267
Random effects	**SD**				
Replicate	0.2877595				



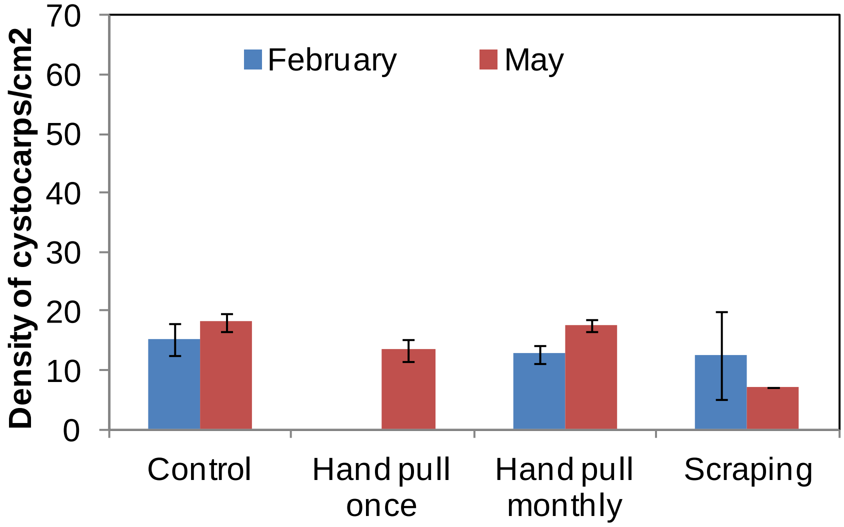


Figure 4.7 Effect of harvest type and time on the density of sporangia, A. Tetrasporic fronds, , no data collected for February hand-pull monthly, B. Cystocarpic fronds, no data collected for February hand-pull once. Error bars represent standard error.

c) Effects of harvest methods and frequency on recovery

The month of May was the end of the harvesting season, and the end of the experiment. By this time the plots harvested recovered for some of the variables measured, but did not recover for others (**Table 4.13**). I used the term recover for the treatments that reached similar levels than the control for a given variable. For reproductive potential there were not enough fronds to test the results with statistic analysis (Appendix D, **Table D.9** and **Table D.10**).

i. Hand pull once recovered for all the variables related to population size, and for most of the reproductive variables. Additionally it had a positive effect on density of holdfasts.
ii. Plots with the treatment "hand pull monthly" had a positive effect on the density of holdfasts, recovered for density of fronds and for density of cystocarps, and did not recover for most of the variables.
iii. Scraped plots recovered for length of cystocarpic fronds, total number of tetrasporangia, and presented a positive effect on density of holdfasts, and density of tetrasporangia.

Additionally the most sensitive variable was the abundance of reproductive fronds, which did not reach the abundance of the control for any of the harvest treatments.

Table 4.13 Summary of effects of harvest treatments on the variable studied at the end of the experiment, +: increase compared to control, -: decrease compared to control, and 0: no significant differences, and NA= absence of fronds. Results of variables of reproductive potential are based on trends shown in graphs, and were not statistically tested

	Control	**Hand pull once**	**Hand pull monthly**	**Scraping**
Population size				
Density of holdfast	0	+	+	+
Density of fronds	0	0	0	-
Density of harvestable fronds	0	0	-	-
Length of harvestable fronds	0	0	-	-
Reproductive potential				
Abundance of reproductive fronds	0	-	-	-
Length of tetrasporic fronds	0	0	-	NA
Length of cystocarpic fronds	0	0	-	0
Total number of tetrasporangia per frond	0	-	-	0
Total number of cystocarps per frond	0	0	-	-
Density of tetrasporangia	0	-	-	+
Density of cystocarps	0	-	0	-

4.5 Discussion

This chapter provides new information about the effects of current harvest strategies, including different methods and frequency, on a population of *Mazzaella laminarioides*. Today the most popular harvesting strategy is the traditional method of "pulling by hand' monthly (or bi-monthly; Chapter 2). Effects of harvesting methods and frequencies on *M. laminarioides* varied across different measures of population size and reproduction. Overall though, the results of this study suggest that the most sustainable harvest strategy would be pulling by hand, rotating harvesting areas and waiting longer to revisit the same spot. This strategy would provide more and bigger fronds for seaweeds harvesters, and minimizes the negative impact of harvest on the population by allowing time to recover and reproduce.

a) Effects of different harvest methods on measures of population size

The density of holdfasts in the control did not show variation over the five month harvesting period (December– May), except for a decrease in month 2 (February). This decrease in the density of holdfasts was observed for all treatments in February. According to the data, this is the warmest month of the harvesting season (Appendix C, **Figure C.1**). Additionally, as recorded in Chapter 3, February presented the lowest presence of endophytes (Appendix C, **Figure C.2**), and lowest phenolic content (**Figure 3.4**). These results could be explained by the exposure of seaweeds to high temperatures, desiccation, and UV radiation during the months of January and February.

Harvesting did not have a negative effect on the density of holdfasts, coinciding with reports on other species of red algae; holdfasts seem to be more tolerant to environmental factors than other portions of the algae (Hayashi *et al.* 2014). Additionally, the density of holdfasts in the control plots was significantly lower than in plots pulled by hand monthly, suggesting that continuous exposure to perturbation can favor the growth of new holdfasts. While scraping implies the removal of all the holdfasts present in a plot, pulling may or may not result in holdfast removal allowing both, vegetative regeneration and spore recruitment in the new space available.

For the scraping treatment, density of holdfast recovered from 0 to the control level just one month after harvest, this suggest establishment of new spores which increases with more availability of substratum (Gomez & Westermeier 1991). These results show differential responses in order to maintain the total number of holdfasts in front of perturbations, from vegetative growth of holdfast to establishment of spores when necessary. The preservation of the density of holdfast is important for the survival of the population, as the regeneration of fronds from holdfasts accounts for most of the reproduction on this species (Santelices & Norambuena 1987; Gomez & Westermeier 1991)

For density of fronds there was an increase in January, followed by a decrease in February. This coincides with records of high growth rate during summer (Gomez & Westermeier 1991), and highest population stock in January, followed by mortality due to desiccation during summer (Santelices & Norambuena 1987). My results could not be compared with standing stocks of the same population in 1984 (Santelices & Norambuena 1987) because of the use of different measures (number of fronds versus dry biomass).

The treatment "scraping the holdfasts", had a significant negative effect on the density of fronds over the duration of the experiment, but the two pulling treatments did not. Because the holdfasts of this alga are perennial, the germination and growth of new holdfasts from spores (for scraping treatments) may require more energy and be slower than the growth of fronds from holdfasts already established such as in the hand-pulling treatments (Santelices & Norambuena 1987).

For harvestable fronds (those longer than 5 cm), there was a drop of density in February, followed by consistent decrease over time, with the control naturally losing 67% of the density of harvestable fronds during the five month period. The decrease in abundance and size of harvestable fronds seen after February can be related to seasonal factors such as exposure to high temperatures and UV radiation during the Summer, higher mortality of bigger fronds (Gomez & Westermeier 1991), and breakage of reproductive fronds that become necrotic or ripped (due perforations) as a result of spore release (Santelices & Norambuena 1987).

For this study I considered harvestable those fronds 5 cm or longer (based on observations). The results show that independently of the treatment the fronds took one month to grow back to this size after the first harvest, and they stayed longer than 5 cm through the duration of the experiment. If I consider 10 cm as the minimum harvestable size (size informed by seaweed harvesters) the fronds took about two months to reach that size. Independent of the type and frequency of harvest, once the fronds reach 10 cm they stayed that size or longer throughout the experiment.

The plots subject to hand pull once recovered to the same length of the control by the end of the experiment (May), and the plots hand pulled monthly and scraped had fewer and smaller fronds than the control during the length of the experiment. In respond to constant perturbation, plots hand-pulled monthly presented stable low density and low growth rate of fronds. The results for the plots scraped can be explained by slower growth rate of holdfast developed from spores, which will result in a slower growth of new fronds.

b) Effects of harvest and time on reproductive potential

Cystocarpic fronds were more abundant than tetrasporic fronds; Santelices & Norambuena (1987), found similar trends for the same population in 1984, while Gomez & Westermeier (1991) registered same proportion of cystocarpic and tetrasporic fronds for a southern population. These differences are expected as proportions of phenological stages are characteristic of different populations.

The phenology observed by other authors coincides with the results of this study; I registered a highest abundance of cystocarpic fronds from March to May, while they observed an increase from March to August for the same population (Santelices & Norambuena 1987). However, Gomez &Westermeier (1991) found a maximum for both, cystocarpic and tetrasporic, fronds in the opposite time of the year for a Southern population (Spring and Summer, i.e. September to March).

For tetrasporic fronds, I observed a consistent increase until May, while Santelices & Norambuena (1987) did not find a clear trend, but observed maximum abundance in August and September, coinciding with Gomez & Westermeier (1991). The differences found are not surprising, as phenological variation is expected between different years and different populations. In the population studied, the period of maturation of the fronds coincides with the harvesting season (maximum abundance of cystocarpic fronds in March, and tetrasporic in May). Harvesting during the production of spores may not affect the number of individuals in the population, but will affect their sexual reproduction. As I mentioned above, the main reproductive strategies of this alga is the regeneration of fronds from perennial holdfasts. However, the maintenance of the population through vegetative regeneration, and the reduction of genetic variation, could affect the population at long term.

The small abundance of reproductive fronds did not allow statistical analysis. The few significant results obtained on the effect of harvesting on reproductive potential were: abundance of reproductive fronds was negatively affected by all harvesting treatments, there were smaller tetrasporic fronds for the treatment hand pull monthly, and there was lower density of cystocarps for plots scraped.

The negative effect of harvest in abundance of reproductive fronds was also observed by Santelices & Norambuena (1987) with individuals harvested monthly remaining vegetative for a 12 month period. *Mazzaella laminarioides* is a procarpic alga; this means that each cystocarp has to be fertilized in an independent event, thus the proximity of male and female fronds is relevant for their reproductive success and a negative effect in density of fronds can also have a negative effect on their probabilities of sexual reproduction (Cole & Sheath 1990).

Although I recorded fewer tetrasporic than cystocaric fronds, tetrasporic fronds were bigger in size. The minimum length recorded for reproductive fronds was 8.7 cm for a cystocarpic frond, and 10.7 cm for tetrasporic fronds, consistent with previous studies that reported the minimum size for reproduction as 10 cm (Gomez & Westermeier 1991; Santelices & Martinez 1997). For fronds to reach the minimum size reported for reproductive fronds (8.7 cm) took more than one month period after been harvested, and the abundance of reproductive fronds did not recover during the 5 month season. In a previous study, *Mazzaella laminarioides* reached reproductive stage between five and six month after pruning (Santelices & Martinez 1997). And, in similar experiments, *Chondrus crispus* recovered reproductive capacity between four months and three years after harvesting simulation (Mathieson & Buns 1975).

Revisiting the hypothesis:

1) My results do not support the first hypothesis. Algae in plots pulled by hand showed different responses for the different variables, not necessarily being lower than control and higher than scraping treatment for all of them. The scraped plots just presented significant differences with hand pulled plots for density of fronds (higher in plots hand pulled than scraped). The hypothesis related to frequency was supported for harvestable fronds; there were lower densities of algae in plots that were hand pull monthly compared to hand pull once.
2) Algae in plots pulled by hand had fronds significantly smaller than control, but the algae were not significantly differently sized than fronds in scraped plots. Algae in plots hand pulled monthly were smaller harvestable fronds than hand pulled once.
3) All treatments had fewer reproductive fronds than controls, with scraping having the lowest number, followed by pull by hand monthly and hand pull once. These results would support the hypothesis; however the differences were not tested statistically because of low number (or absence) of fronds in some treatments. For length of reproductive fronds the results were not clear, except tetrasporic fronds in plots that were hand pulled once were shorter than those in control plots.
4) No clear trends were detected for number or density of sporangia affected by harvesting or frequency, except by algae in scraped plots which had lower total number and density of cystocarps.
5) The results coincide with the perception of *lugueros* that *M. laminarioides* grows back to a harvestable size in less than one month. However, for all treatments, I found fewer and smaller reproductive fronds.

The results of this study suggest that pulling by hand has a lower impact on the variables studied than scraping. Although this depends on the frequency of harvest, hand pull once produces higher number of fronds and bigger harvestable and reproductive fronds than scraping during the whole harvest season. While hand pull monthly does not recover for most variables studied and shows similar results than scraping, especially by the end of the season.

A harvesting strategy that combines pull by hand, switching areas and waiting longer to revisit the same spot would be the most sustainable method. This strategy would produce the highest yields (bigger and higher number of fronds) compared to harvesting the same spot every month, or extracting as much as possible from an area by scraping the substratum. This strategy will also provide better chances of reaching reproductive stages and bigger sizes of fronds with a higher number of reproductive structures. However, the implementation of this strategy will need some type of regulation (formal or local) of the rotation system that guarantees no-harvest during the times of recovery.

Although the experiment allowed us to compare harvest impacts among different methods and frequencies over a single harvesting season, more work needs to be done. For this dissertation, I focused in just one population. Future studies focused on management of this resource should include the effect of harvesting on population dynamics at long term. I had originally proposed to conduct a study using matrix population models and LTREs (life table response experiments; Caswell 2010), to assess the projected effects of different harvesting practices over time. Due presence of coalescence in this species (Vera *et al.* 2008), I proposed to work at ramet level (frond as an individual). Unfortunately the environmental conditions (increasing frequency of high surf events) prevented the retention of tags in the individuals of *M. laminarioides* at either ramet or genet level (holdfast as an individual) long enough to generate the data needed for that kind of analysis. Challenges to keep tags and follow fronds over time have been reported in previous studies (Santelices *et al.* 1999). Future research could involve looking the variables that I measured, monitoring them over multiple years and consider other populations of *M. laminarioides*.

Chapter 5: CONCLUSIONS

My dissertation characterized the uses of seaweeds in Chile, and assesses socio-economic and ecological impacts of their harvest from wild-stock. I focus on a case-study of*: Mazzaella laminarioides* in South-Central Chile.

In this chapter, I synthesize the findings from previous chapters and discuss their implications. I integrate the results of socio-economical and ecological data to guide the development of harvest strategies that are more sustainable for *M. laminariodes*. In addition, I summarize the contributions of this research to scientific literature, discuss its limitations and suggest future research needed to understand the long term impact of intensification of harvest on species of seaweeds extracted from wild-stocks. In addition,

1. Summary of main findings

a) Ethnobotany of Chilean seaweeds: traditional and contemporary uses of seaweeds in South-Central Chile

Using literature review to compile information about species of seaweeds used in Chile, I recorded that 13 of the 451species of seaweeds present in Continental Chile had traditional and/or contemporary uses. At the national scale, five species are used as food, three as medicine and nine species are used for polysaccharides (eight of them are wild-harvested). At local scale in South-Central Chile, five species are used as food, two as medicine (one used externally), and three used for polysaccharides (all of them for extraction of carrageenan). Additionally, for the species *Mazzaella laminarioides*, its use has changed from been consumed as food to now being harvested for extraction of carrageenan.

I used chemical analysis to evaluate the nutritional and medicinal potential of the species most commonly used for food and medicine. I found a high content of proteins for all the samples of seaweeds analyzed, with all of them showing higher protein content than the known nutritious alga, *nori* (*Pyropia sp*). They also had very low percentages of fat, and variable fiber content. The percentage of ashes (proxy of minerals and vitamins) was higher than *nori* for most of the species sampled, and was particularly high for dry *D. antartica*, reported to be used as a tonic due to its high content of vitamins.

The highest values of phenols and antioxidants were found for the red seaweed *Pyropia columbina*,, which was higher in antioxidants than all the species analyzed, and than other species in the literature (see table 3.5, Appendix C).The species *Durvillea antartica,* which is reported to be used for food and medicine, showed the second highest antioxidant activity.

Finally, using ethnobotanical surveys, I assessed the effect of a changing polysaccharide market on the economic and social conditions related to the commercial harvest of *lugas* in South Central Chile. I found that changes in prices and working conditions have lead to changes in the demographic composition of harvesters, increase in accessibility to the resources, and incorporation of new (more intensive) technologies, which has resulted in more harvesters, increasing intensity and frequency of harvest.

b) Effects of harvesting and environmental factors on antioxidant production of *Mazzaella laminarioides*

I used manipulative experiments during one harvest season (December 2016 to May 2017*)* to assess if environmental factors and harvesting can affect the nutritional benefits of seaweeds, more specifically their antioxidant content. I evaluated the effect of environmental factors and harvest on the antioxidant potential of the intertidal species *M. laminarioides*. The study found no significant effects of abiotic and biotic predictors, or harvesting on the antioxidant potential of this species. However, and in contradiction to my hypothesis, our results suggest a tendency for a negative relationship between temperature and total phenolic compounds.

c) Ecological effects of seaweed harvesting: a case study of harvesting and population dynamics of *Mazzaella laminarioides* in Matanzas, Chile

I evaluated the effect of harvesting on a population of *Mazzaella laminarioides* in Matanzas, Chile, using manipulative experiments during one harvest season (December 2016 to May 2017). I found that the effect of harvest methods and frequencies on *M. laminarioides* varied across different measures of population size and reproductive potential. In terms of harvest methods, the results showed that pulling by hand has lower impact on measures of population size and reproductive output than scraping. Although this depends on the frequency of harvest, plots subject to hand pulling once recovered for all measures of population size, and for some of the reproductive variables. While plots pulled by hand monthly did not recover to control levels for most variables studied.

I recorded recovery (or increase) of the density of holdfast in all treatments. Establishment of new spores increased with availability of substratum in plots scraped, while pulling allowed both, vegetative regeneration and spore recruitment in the new space available. These results are important for the survival of the population, since the regeneration of fronds from holdfasts accounts for most of the reproduction on this species (Santelices & Norambuena, 1987; Gomez & Westermeier 1991).

On the other hand, the number of reproductive fronds did not recover to the levels of control plot for any of the treatments. This is consistent with previous research (Santelices & Norambuena 1987). *Mazzaella laminarioides* is a procarpic alga, where each cystocarp has to be fertilized in an independent event, thus the proximity of male and female fronds is relevant for their reproductive success and a negative effect on density of reproductive fronds can also have a negative effect on their probabilities of sexual reproduction (Cole & Sheath, 1990).

Additionally, for the population studied, the period of maturation of the fronds coincides with the harvesting season (maximum abundance of cystocarpic fronds in March, and tetrasporic in May). Harvesting during the maturation of spores may not affect the number of individuals in the population, but will affect their sexual reproduction. The maintenance of the population through vegetative regeneration and the reduction of genetic variability could have negative effects on the population at long term.

2. Contributions

a) Ethnobotany of Chilean seaweeds: traditional and contemporary uses of seaweeds in South-Central Chile

The second chapter of this dissertation provides information related to past and current uses of seaweeds in Chile, and explores the nutritional and medicinal potential of some species. There is a variety of literature, with different approaches, that mention uses of seaweeds by Chilean population. However I did not find any document that explored ethnobotany of Chilean seaweeds, thus this chapter constitutes a first attempt and provides the preliminary data on which a more exhaustive study can be based.

The nutritional analyses showed promising results for the species *Pyropia columbina*, with high content of proteins and antioxidants. This species showed higher antioxidant potential than all the species tested and other species recorded in the literature (Appendix C, **Table C.5**). Due to its high antioxidant potential and the fact that *P. columbina* is an abundant intertidal species, also found in other countries in the South Hemisphere (Guiry & Guiry 2019); this species constitutes an interesting case of study for assessing the effect of environmental factors on antioxidant potential.

On the other hand, my results suggest that *Pyropia columbina* could be further studied as a complement to the diet of coastal people, and as a food supplement or a vegetarian alternative. This species is consumed as food by diverse cultures (Guiry & Guiry 2019), and at local scale is one of the species most commonly eaten in coastal areas of Central and South Chile, however its consumption is not common in non-coastal areas, and has not been promoted as much as *D. antartica*. The information provided in this dissertation can be used as a first step to promote its consumption.

b) Effects of harvesting and environmental factors on antioxidant production of *Mazzaella laminarioides*

As mentioned in chapter three, there are few studies that have assessed the effects of environmental factors on antioxidant potential of algae in the field and, to my knowledge, this is the first study that assessed the effects of harvest on the production of antioxidants. While we found no significant correlations between antioxidant potential and environmental factors, or harvesting, we found a tendency for lower phenolic content with higher temperatures. Further research is needed on this topic. If this relationship is found to be significant and with a large effect size, it can have implications for the selection of timing of harvesting for food purposes, in that it could be preferable to harvest this seaweed in winter, when it has highest antioxidant content.

c) Ecological effects of seaweed harvesting: a case study of harvesting and population dynamics of *Mazzaella laminarioides* in Matanzas, Chile

Chapter four adds new information about the effect of wild-harvest on species of commercial use. One of the novelties of this chapter is the study of current harvest practices, pulling by hand or scraping the seaweeds as applied by seaweed harvesters. This experiment was designed to assess the long term effect of harvest on population dynamics, using matrix population modeling, however it was not possible to use that approach (see below). The study I did provides a case study, which results are limited locally and temporally. As a result, the contributions of this chapter are mostly in terms of recommendations for management at a local scale.

3. Recommendations for management

In Chile, seaweeds are extracted from wild-stock and subject to open access, with just a few species having some kind of regulations on harvest (See chapter 2). In chapter two, I described changes of socio economical conditions influenced by the polysaccharide market: higher demand, better prices of algal material, and improvements of working conditions, which have lead to increasing intensity and frequency of harvest.

For the assessment of the effects of different harvest methods and frequencies, I recorded that pulling by hand is a better alternative than scraping. While scraping implies the removal of all the holdfasts present in a plot, pulling may or may not result in holdfast removal allowing both vegetative regeneration and spore recruitment in the new space available. In terms of frequency of harvest, plots subject to “hand pull once” recovered more quickly than those subject to “hand pull monthly”. The latter showed similar results to the scraping treatment.

Overall, the results of this study suggest that a strategy that combines pulling by hand, switching areas and waiting longer to revisit the same spot would be the most sustainable method. This strategy would produce the highest yields (bigger and higher number of fronds) compared to harvesting the same spot every month, or extracting as much as possible from an area by scraping the substratum. This strategy will also minimize the negative impact of harvest on the population, providing better chances of reaching reproductive stages and bigger sizes of fronds with a higher number of reproductive structures.

The implementation of this kind of strategy would need some type of regulation (formal or local) of the rotation system that guarantees no-harvest during the times of recovery (e.g. regulation of other resources in the MEABR, management and exploitation areas for benthic resources). Additional management measures should include education about the importance of reproductive fronds, time of the year to be avoided (if possible), and minimum size of harvestable fronds.

Because wild harvested seaweeds are a resource with open access, education and outreach are central for the implementation and success of any conservation effort; and should highlight the importance of biodiversity, sustainability, and the role of the focal species at ecological, economical and cultural level.

4. Future research

This study incorporated multiple approaches to assessing the traditional and contemporary uses of seaweeds in Chile. During its development many challenges were faced, opening new possibilities for future studies.

a) Ethnobotany of Chilean seaweeds: traditional and contemporary uses of seaweeds in South-Central Chile

Chapter 2 is an exploratory study on the ethnobotany of seaweeds. Surprisingly I did not find any comprehensive paper on ethnobotany of Chilean seaweeds; thus I plan on use this chapter as the base for a future study.

The results of the nutritional potential of seaweeds showed promising results for one particular species: *Pyropia columbina*, a species easily accessible for coastal people, but not popular between non-coastal people. Because this species is related to *nori* and it is well-known as an edible species, I believe that it has a great potential as a complement of diet. More research needs to be done with other edible (or potentially edible) species. Additionally more research is needed to understand how handling, storage and cooking can affect the nutritional potential of this and others food resources. Antioxidants are particularly sensitive to the processing of food post-harvest (Manach 2004), therefore it is relevant to know the antioxidant content of cooked (or processed) food or more appropriate handling in order to conserve their benefits.

b) Effects of harvesting and environmental factors on antioxidant production of *Mazzaella laminarioides*

Future research that attempts to measure the effect of abiotic factors (such as ultraviolet radiation and temperature) on antioxidant potential will have to consider the variation of low tide occurrences, and measure abiotic factors over a smaller time scale (e.g. hourly) that can account for changes occurring at the moment of collecting the samples.

If future research confirmed that *Mazzaella laminarioides* presents higher levels of antioxidants in winter than in summer, it could guide on the optimal timing of harvesting for food. In addition, during winter *M. laminarioides* is not collected for polysaccharides, mainly because of difficulties in drying it (see Chapter 2), thus a secondary use as source of antioxidants may be an option for household use and potentially for industrial uses. Since environmental factors vary over time and space, it would be recommendable to repeat the study in different locations and sample at different times of the year.

c) Ecological effects of seaweed harvesting: a case study of harvesting and population dynamics of *Mazzaella laminarioides* in Matanzas, Chile

Although my experiment allowed me to compare harvest impacts among different methods and frequencies over a single harvesting season, more work needs to be done. For this dissertation, I focused in just one population. Future studies of this resource should include the effect of harvesting on population dynamics at long term. I had originally proposed to conduct a study using matrix population models and LTREs (life table response experiments; Caswell 2001), to assess the projected effects of different harvesting practices over time, however this was not possible because of the breakage of fronds and rough environmental conditions prevented the retention of tags in the individuals tagged. Future research needs to incorporate another strategy for marking individuals that allows monitoring them over time, or the use of statistical analyses that allow to do long term studies without marking the individuals (e.g. life table analysis). It would be interesting to assess the variables that I measured, monitoring them over multiple years and consider other populations of *M. laminarioides*.

In addition**,** I focused my study on the only species recorded to be harvested for polysaccharides that is exclusively intertidal, hence more exposed to overharvesting. It would be interesting to study the effect of harvesting on subtidal species and look at the effect of different environmental factors and harvesting strategies in the case of species with presence in both, subtidal and intertidal environments.

Appendix A: UNIVERSITY OF HAWAIʻI COMMITTEE ON HUMAN SUBJECTS' RESEARCH EXEMPTION

UNIVERSITY of HAWAIʻI MĀNOA

Office of Research Compliance
Human Studies Program

February 3, 2014

TO: Gioconda Lopez Vargas
Principal Investigator
Botany

FROM: Denise A. Lin-DeShetler, MPH, MA
Director

SUBJECT: CHS #21855- "Ecological and Economic Impact of Harvesting of the Seaweed Luga (Rhodophyta) in Santa Maria and Mocha Island, Chile"

This letter is your record of the Human Studies Program approval of this study as exempt.

On Febuary 3, 2014, the University of Hawai'i (UH) Human Studies Program approved this study as exempt from federal regulations pertaining to the protection of human research participants. The authority for the exemption applicable to your study is documented in the Code of Federal Regulations at 45CFR 46.101(b)(Exempt Category 2).

Exempt studies are subject to the ethical principles articulated in The Belmont Report, found at http://www.hawaii.edu/irb/html/manual/appendices/A/belmont.html.

Exempt studies do not require regular continuing review by the Human Studies Program. However, if you propose to modify your study, you must receive approval from the Human Studies Program prior to implementing any changes. You can submit your proposed changes via email at uhirb@hawaii.edu. (The subject line should read: Exempt Study Modification.) The Human Studies Program may review the exempt status at that time and request an application for approval as non-exempt research.

In order to protect the confidentiality of research participants, we encourage you to destroy private information which can be linked to the identities of individuals as soon as it is reasonable to do so. Signed consent forms, as applicable to your study, should be maintained for at least the duration of your project.

This approval does not expire. However, please notify the Human Studies Program when your study is complete. Upon notification, we will close our files pertaining to your study.

If you have any questions relating to the protection of human research participants, please contact the Human Studies Program at 956-5007 or uhirb@hawaii.edu. We wish you success in carrying out your research project.

1960 East-West Road
Biomedical Sciences Building B104
Honolulu, Hawai'i 96822
Telephone: (808) 956-5007
Fax: (808) 956-8683

An Equal Opportunity/Affirmative Action Institution

1.1 Questionnaire about ethnobotanical information

1. Respondent's Personal Information:

Male/ Female Age_________Years Ethnicity:_____________________________

Main Occupation:____

Location (Name of Village):____

Place of Birth:________________________________Place of raising :_________________________

2. Plants used for food

Local Name	V	A	Use	Plant part used	C	W	I	Season	Location

V=Vascular plant, A=algae; C=cultivated, W= wild, I= imported from mainland

3. Plants used for medicine

Local Name	V	A	Use (Symptoms)	Plant part used	C	W	I	Season	Location

V=Vascular plant, A=algae; C=cultivated, W= wild, I= imported from mainland

4. Plants used for materials (fibers, tools, building, fuel, etc)

Local Name	V	A	Use	Plant part used	C	W	I	Season	Location

V=Vascular plant, A=algae; C=cultivated, W= wild, I= imported from mainland

5. Free Listing: List of main local plants used

Local Name	V	A	Plant part used	C	W	Season

*V=Vascular plant, A=Algae; C=Cultivated, W=Wild

6. Researcher register

Local Name	Botanical name	Photo	GPS point	Herbarium specimen

1.2 Questionnaire for Seaweed Gatherers and Fishermen

1. Respondent's Personal Information:

Male/ Female Age_________Years Ethnicity:______________________________

Main Occupation:____

Former Activity:____ Years in the Activity _________

Group or Union:____

Location (Name of Village):____

Place of Birth:________________________________Place of raise :_________________________

2. Seaweed exploitation:

a. Main activity in fishery season

Gatherer in the shore	Diver
Beginning of working time	Time of sailing
End of working time	Time of arrival
Substrate:	Dive Depth: Substrate:

Fish extracted

Types or species of seaweeds harvested:

Local Name	Size	Reproductive Characteristics (different reproductive stages, visible spores, etc)

b. Gathering method

1) Beach wash
2) Diving
3) Other (specify)

c. How do you harvest the seaweeds? Why do you do it in that way?____
____

What part of the seaweed do you harvest?
Why?____
____

There are differences between species?
Why?____
____

d. Seasonality and Location

Local Name	Location	Season (months)	Best Months

Where are the best locations for seaweed gathering? Why?__________________________________
____

When is the best time to gather seaweeds? Why? There is relationship with the moon or environmental conditions?____
____

What animals eat seaweeds? ____
____

Have you observed some change of this behavior or abundance of these animals over the time?
____
____

e. Seaweed consumption

Do you eat some species of seaweed? Which? Why?_________________________________
____

Does the taste or other characteristics vary with place, timing of harvest, and method of harvest? How?____
____
____

Do you use seaweeds for medicinal purposes? Which? Why? _______________________
____

Do the medicinal characteristics vary with place, timing of harvest, and method of harvest? How?____

f. Sale

Local Name	Dry	Fresh	Middlemen	Union

Name of Middleman:____
Drying or processing plant:____
Company:____
Other:____

g. Volume and Price

Local Name	Volume (kg/day)	Current price ($/kg)	Historical price ($/kg)

h. Household
How many people in the household________________________
Approximate monthly household income____________________
Who in the household works in seaweed gathering?

Age	Relationship	Gender	Gathering method	Seaweed local name

i. Main problems in seaweed extraction:
Low price ___ Difficult to dry ___ Low extraction ___ Difficult commercialization ___

Other:____

j. Characterization of temporary dwellings
Do you stay in a temporary dwelling during the seaweed season?______________________

Who do you share the temporary housing with?

Age	Relationship	Gender

Access to basic needs

Drinking water	At home	Temporary place
Electricity		
Sewage		
Groceries store		
Farmers market		

k. Change in Seaweed gathering after the 2010 earthquake (Just in Isla Santa Maria)

Do you see different seaweeds species currently?____

Which species (local name) have disappeared?____

____

Which species yields have decreased?____

____

Do you gather less seaweed than before the earthquake? YES/NO

Estimate percentage of yield decrease for main species gathered___________________________

Differences in seaweed characteristics after the earthquake_________________________________

____

Differences in prices ____

3. Researcher register: Types or species of seaweeds harvested

Local Name	Botanical name	Photo	GPS point	Herbarium specimen

Appendix B: SUPPLEMENTARY INFORMATON OF TRADITIONAL AND CONTEMPORARY USES OF SEAWEEDS.

Table B.1 Summary of worldwide changes of prices for the most important species of seaweeds, during the period 1999 - 2009 (Modified from Bixler & Porse 2011)

Seaweed	**US$ t^{-1} 1999**	**US$ t^{-1} 2009**	**% increase**
Gracilaria	1,260	1,300	3
Lessonia	350	950	171
Eucheuma cottonii	600	1,400	133
Eucheuma spinosum	350	350	0
Chondrus (Nova Scotia)	1,800	3,400	89
Chondrus (Prince Edward Island)	1,000	1,875	88
Gigartina skottsbergii	1,400	3,000	114
Sarcothalia. crispata	1,000	2,300	130

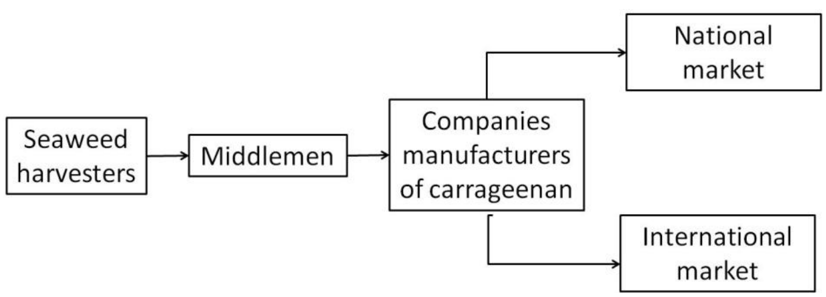


Figure B.1 Carrageenan industry market chain

Appendix C: DETAIL OF RESULTS AND MODELS USED TO ASSESS EFFECTS OF HARVESTING AND ENVIRONMENTAL FACTORS ON ANTIOXIDANT POTENTIAL

3.1 Abiotic factors

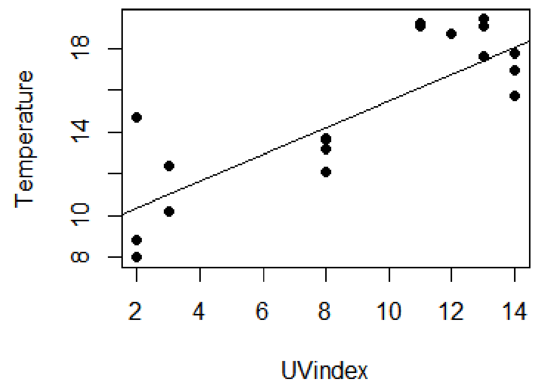


Figure C.1 Temperature versus Ultraviolet radiation (UV index; R2=0.6757, p<0.001)

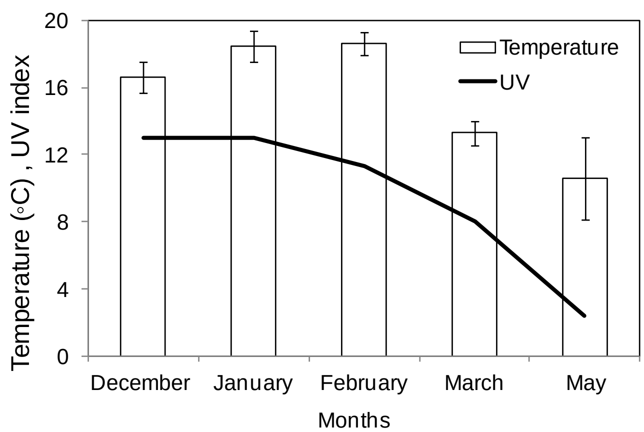


Figure C.2 Variation of temperature and Ultraviolet radiation over time

Table C.1 Linear Model showing the correlation of temperature and UV index

	Estimate	SE	t-value	p-value
Intercept	9.09285	0.60794	14.96	< 2e-16
UVindex	0.63654	0.05671	11.22	2.84e-16

Residual standard error: 1.838 on 59 degrees of freedom
Multiple R-squared: 0.6811, Adjusted R-squared: 0.6757
F-statistic: 126 on 1 and 59 DF, p-value: 2.839e-16

Table C.2 Linear Model showing the correlation of UV index and months

	Estimate	SE	t-value	p-value
Intercept	14.00000	0.06304	222.09	< 2e-16
January	-1.00000	0.09629	-10.38	1.14e-14
February	-2.83333	0.12071	-23.47	< 2e-16
March	-6.00000	0.08915	-67.30	< 2e-16
May	-11.45455	0.09876	-115.98	< 2e-16

Residual standard error: 0.2522 on 56 degrees of freedom
Multiple R-squared: 0.9966, Adjusted R-squared: 0.9964
F-statistic: 4115 on 4 and 56 DF, p-value: < 2.2e-16

Table C.3 Linear Model showing the correlation of temperature and months

	Estimate	SE	t-value	p-value
Intercept	16.6125	0.2732	60.811	< 2e-16
January	2.2875	0.4173	5.482	6.67e-07
February	1.9089	0.3999	4.774	1.00e-05
March	-3.3875	0.3863	-8.768	8.85e-13
May	-5.5325	0.3927	-14.088	< 2e-16

Residual standard error: 1.093 on 68 degrees of freedom
Multiple R-squared: 0.8908, Adjusted R-squared: 0.8843
F-statistic: 138.6 on 4 and 68 DF, p-value: < 2.2e-16

1.2 Biotic factors

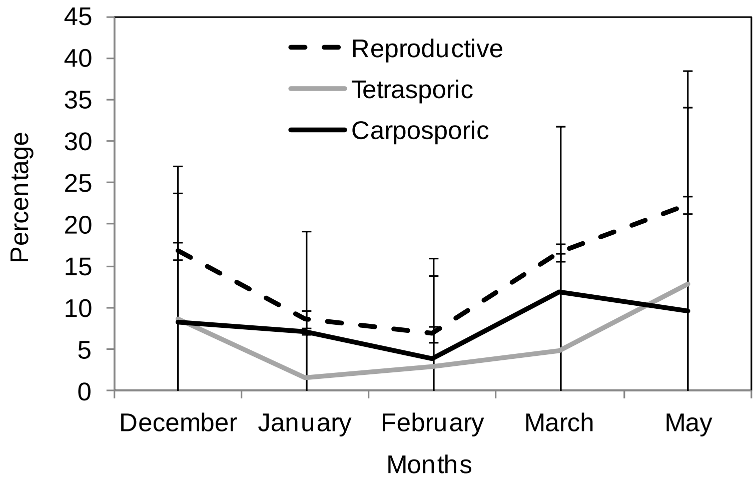


Figure C.3 Temporal variation of percentage of weight for reproductive fronds.

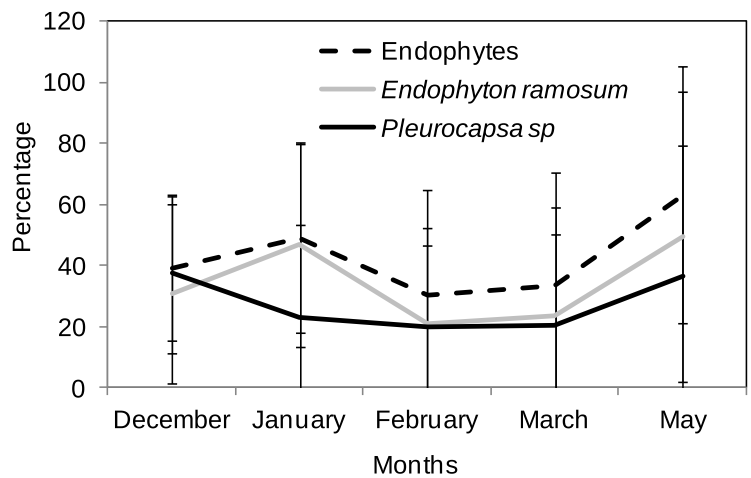


Figure C.4 Temporal variation of percentage of weight of fronds with presence of two species of endophytes, Endophyton ramosum, and Pleurocapsa sp.

3.3 Total phenolic content and antioxidant activity

Table C.4 Linear Model showing the correlation of antioxidant activity and total phenolic content

	Estimate	SE	t-value	p-value
Intercept	84.341	7.078	11.916	< 2e-16
Antioxidant activity	2.189	0.768	2.851	0.00581

Residual standard error: 20.03 on 66 degrees of freedom
Multiple R-squared: 0.1096, Adjusted R-squared: 0.09615
F-statistic: 8.128 on 1 and 66 DF, p-value: 0.005812

Table C.5 Phenolic content (TPC) and radical scavenging activity (RSA) found in methanolic extracts of different species of seaweeds in a variety of studies

Species	Classification	TPC (mg GAE/100g DW)	RSA (%inhibition)	Author
Palmaria palmata	Rhodophyta	0.95	-	Nwosu *et al*. 2011
Ulva lactuca	Chlorophyta	2.86	-	Nwosu *et al.* 2011
Ascophyllum nodosum	Phaeophyceae	14.1	-	Nwosu *et al.* 2011
Durvillea antarctica	Phaeophyceae	101.31	31.56	Chapter 2
Eucheuma kappaphycus	Rhodophyta	150	11.9	Ganesan *et al.* 2008
Mazzaella laminarioides	Rhodophyta	176.17	10.56	Chapter 2
Acanthophora spicifera	Rhodophyta	355	6.91	Ganesan *et al.* 2008
Gracilaria edulis	Rhodophyta	410	5.2	Ganesan *et al*. 2008
Corallina elongata	Rhodophyta	443	37	Rico *et al.* 2012
Pyropia columbina	Rhodophyta	633	41.74	Chapter 2

3.4 Total phenolic

Table C.6 Full and reduced linear mixed-effect models used to analyze effects of environmental factors and harvesting on total phenolic content of Mazzaella laminarioides

Model	Form/R package	Fixed effects	Random effects	Weight	df	AIC
TFCA	lme/nlme	temp+harvest+tetra+carpo+endo	1|month/replicate	-	9	658.0579
TFCB	lme/nlme	temp+harvest+tetra+endo	1|month/replicate	-	8	654.0791
TFCC	lme/nlme	temp+harvest+tetra	1|month/replicate	-	7	649.0445
TFCD	lme/nlme	temp+ harvest	1|month/replicate	-	6	645.215
TFCE	lme/nlme	temp	1|month/replicate	-	5	649.6038
TFCF	lme/nlme	-	1|month/replicate	-	4	652.9084

Table C.7 Linear mixed effects model showing the variation of total phenolic content when contrast different months

	Value	SE	DF	t-value	p-value
Intercept	103.23584	2.610228	61	39.55050	0.0000
Dec vs. all	-1.76769	1.243873	61	-1.42111	0.1604
Jan-Feb vs. Mar-May	0.70218	3.782979	61	0.18562	0.8534
Feb vs. May	24.66882	7.789978	61	3.16674	0.0024
May vs. all	-3.82428	2.108193	61	-1.81401	0.0746

3.5 Antioxidant activity

Table C.8 Full and reduced linear mixed-effect models used to analyze effects of environmental factors and harvesting on antioxidant activity of Mazzaella laminarioides

Model	Form/R package	Fixed effects	Random effects	Weight	df	AIC
RSAA	lme/nlme	temp+harvest+tetra+carpo +endo	1|month/replicate	1 | harvest	10	67.61146
RSAB	lme/nlme	temp+tetra+carpo+endo	1|month/replicate	1 | harvest	9	62.72718
RSAC	lme/nlme	temp +carpo+endo	1|month/replicate	1 | harvest	8	55.01155
RSAD	lme/nlme	temp+endo	1|month/replicate	1 | harvest	7	47.74738
RSAE	lme/nlme	temp	1|month/replicate	1 | harvest	6	40.74075
RSAF	lme/nlme	-	1|month/replicate	1 | harvest	5	40.17659

Table C.9 Linear mixed effects model showing the variation of antioxidant activity when contrast different months

	Value	SE	DF	t-value	p-value
Intercept	2.1057931	0.03850201	56	54.69308	0.0000
Dec vs. all	-0.0064280	0.01647639	56	-0.39014	0.6979
Jan-Feb vs. Mar-May	0.0048723	0.05030886	56	0.09685	0.9232
Feb vs. May	0.1409900	0.10989023	56	1.28301	0.2048
May vs. all	-0.0035268	0.02930407	56	-0.12035	0.9046

Appendix D: DETAIL OF RESULTS AND MODELS USED TO ASSESS ECOLOGICAL EFFECTS OF SEAWEED HARVESTING

Table D.1 Linear model showing the correlation between density of holdfasts and density of fronds, prior to harvest treatment

	Estimate	SE	t-value	p-value
Intercept	3.270956	0.930287	3.516	0.000858
Fronds	0.007529	0.003516	2.141	0.036479

Residual standard error: 2.391 on 58 degrees of freedom
Multiple R-squared: 0.07325, Adjusted R-squared: 0.05727
F-statistic: 4.584 on 1 and 58 DF, p-value: 0.03648

Table D.2 Linear model showing the correlation between density of harvestable fronds after the first treatment was applied and the density of fronds prior to harvest.

	Estimate	SE	t-value	p-value
Intercept	15.85468	10.06982	1.574	0.139
Fronds	0.05286	0.03814	1.386	0.189

Residual standard error: 10.78 on 13 degrees of freedom
Multiple R-squared: 0.1287, Adjusted R-squared: 0.0617
F-statistic: 1.921 on 1 and 13 DF, p-value: 0.1891

Table D.3 Linear Model showing the correlation between density of harvestable fronds after the first treatment was applied and density of holdfasts prior to harvest.

	Estimate	SE	t-value	p-value
Intercept	31.6107	8.1065	3.899	0.00183
Holdfasts	-0.5671	1.8249	-0.311	0.76090

Residual standard error: 11.5 on 13 degrees of freedom
Multiple R-squared: 0.007374, Adjusted R-squared: -0.06898
F-statistic: 0.09658 on 1 and 13 DF, p-value: 0.7609

Table D.4 Linear mixed-effect model used to analyze effects of time and harvesting on: density of holdfasts, fronds, and harvestable fronds (length>5cm) per plot

Model	**Form/R package**	**Fixed effects**	**Random effects**	**Weight**	**df**	**AIC**
Holdfast	lme/nlme	Holdfast0 +harvest+time	1 /replicate	harvest	13	243.84
Fronds	lme/nlme	$Fronds_0$ +harvest+time	1 /replicate	harvest	13	376.97
Harvestable fronds	lme/nlme	Harvest*time	1 /replicate	harvest	21	478.45
Length of harvestable fronds	lme/nlme	Harvest*time	1 /replicate	time	16	693.82

Table D.5 Linear mixed effects model showing the variation of density of holdfasts when contrast different treatments at time 5

	Value	**SE**	**DF**	**t-value**	**p-value**
Intercept	1.8198916	0.07896463	25	23.046921	0.0000
Control vs. both hand pull treatments	0.0192325	0.07896463	25	-1.192504	0.8096
Hand pull once vs. hand pull monthly	0.1377285	0.11337795	25	-1.446914	0.2358
Control vs. scraping the holdfasts	0.0016132	0.14883745	25	0.316515	0.9914

Table D.6 Linear mixed effects model showing the variation of density of fronds when contrast different treatments at time 5

	Value	**SE**	**DF**	**t-value**	**p-value**
Intercept	5.700729	0.1564063	29	36.44820	0.0000
Control vs. both hand pull treatments	0.303555	0.1564063	29	1.94081	0.0621
Hand pull once vs. hand pull monthly	0.057678	0.1125484	29	0.51247	0.6122
Control vs. scraping the holdfasts	-0.985630	0.4334955	29	-2.27368	0.0306

Table D.7 Linear mixed effects model showing the variation of density of harvestable fronds when contrast different treatments at time 5

	Value	SE	DF	t-value	p-value
Intercept	2.0166621	0.1328579	25	15.179086	0.0000
Control vs. both hand pull treatments	0.0853701	0.1328579	25	0.642566	0.5264
Hand pull once vs. hand pull monthly	-0.5111704	0.2283594	25	-2.238447	0.0343
Control vs. scraping the holdfasts	-1.3703165	0.1989074	25	-6.889217	0.0000

Table D.8 Linear mixed effects model showing the variation of length of harvestable fronds when contrast different treatments at time 5

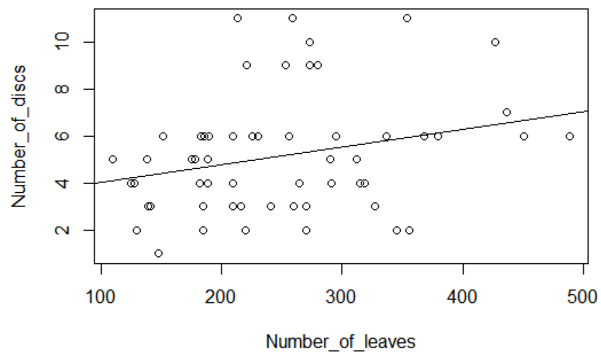
	Value	SE	DF	t-value	p-value
Intercept	13.237783	0.6941872	256	19.069471	0.0000
Control vs. Hand pull once	0.087602	0.9641306	256	0.090861	0.9277
Hand pull once vs. hand pull monthly	-2.419290	0.6896903	256	-3.507791	0.0005
Control vs. scraping the holdfasts	-2.219809	1.2226029	256	-1.815642	0.0706

Table D.9 Total number of Tetrasporic fronds recorded in February and May 2017

	January	February	March	May	Total
Control	1	17	19	21	58
Hand pull once	0	2	3	8	13
Hand pull monthly	0	0	0	2	2
Scraping	0	1	0	0	1
Total	1	20	22	31	74

Table D.10 Total number of Cystocarpic fronds recorded in February and May 2017

	January	February	March	May	Total
Control	13	33	37	37	120
Hand pull once	6	0	11	17	34
Hand pull monthly	0	7	0	3	10
Scraping	0	1	1	1	3
Total	19	41	49	58	167



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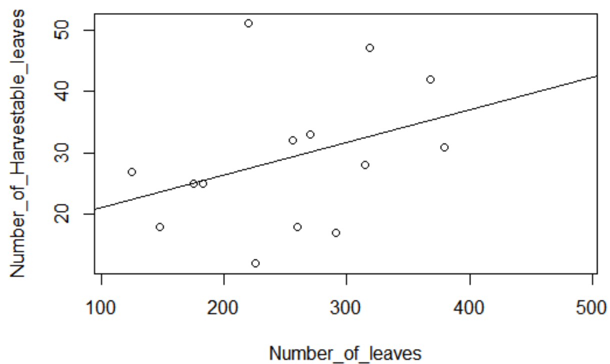

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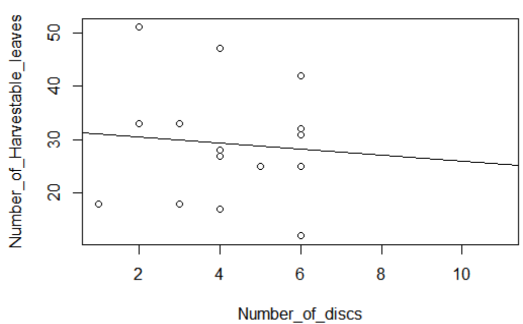

C

Figure D.1 A. Initial number of holdfasts per plot (0.01m2) versus initial number of Fronds (R2=0.05727, p= 0.03648) B. Initial number of harvestable Fronds per plot (0.01m2) versus initial number of Fronds (R2=-0.06898, p= 0.1891) **C.** Initial number of harvestable Fronds per plot ($0.01m^2$) versus initial number of holdfasts (R^2=-0.06898, p=0.7609

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