ECOLOGICAL AND CHEMICAL RESPONSES OF MACROCYSTIS SPP
(PHAEOPHYCEAE) UNDER PRIMITIVE HARVESTING METHODS.
THE PURSUIT OF A SUSTAINABLE METHOD
OF HARVESTING IN N. CHILE

A Thesis
Presented to the
Faculty of
San Diego State University

In Partial Fulfillment
of the Requirements for the Degree
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in
Biology

by
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The Undersigned Faculty Committee Approves the
Thesis of Renato Francisco Borras Chavez:

Ecological and Chemical Responses of Macrocystis spp (phaeophyceae) Under
Primitive Harvesting Methods. The Pursuit of a Sustainable Method of
Harvesting in N. Chile

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Approval Date
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by

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DEDICATION

"The family or social unit occupying such a place" is how the dictionary defines the word "home" well, my personal definition is "a transportable feeling where you place your valuable people for the rest of your life".

There are 7 people that I would like to mention in this dedication.

Sandra Chavez and Miguel Borras G: my parents, who cried with me when loneliness took place over and gently whispered in my ear "calm down, we will always be with you".

Jandy, Migue and Toti: my siblings, who were there always supporting and encouraging my ideas of "everything is possible", "fight for freedom" and most important than everything "be happy".

Paz Francisca Oyarzun "fran": my great lost in this process... who left me perhaps to soon but taught me how peaceful can be that moment, and that physical distance does not mean anything if your love is still back in home. Thanks for illuminate now every night of my life.

Paula Ayerdi: my dear friend...you were born from nature, you live purely for her and you felt sleep in her arms. Te extraño Polilla...

I would also like to dedicate this thesis to all that people in "home", family, negros, Pete and friends that were standing right next to me every day giving me their strength but mostly laughing, dancing and living with me.

My endless love will always be with you.
ABSTRACT OF THE THESIS


by

Renato Francisco Borras Chavez
Master of Science in Biology
San Diego State University, 2011

Kelp harvesting and its ecological impacts on Chilean near shore communities have been well documented throughout the last twenty years. However, regulation of this industry is fairly recent and extraction methods that maintain resilience of kelp forests have never been designed. Therefore, it is imperative to develop sustainable ways of kelp extraction, especially for species such as *Macrocystis pyrifera*, due to their economical importance and fragile situation in northern Chile. In addition, chemical variation of the primary products obtained by this industry under repetitive harvesting of kelp populations has never been examined and it is not known if the quality of these products is reduced through time. To investigate this, two experimental harvesting treatments of natural *M. pyrifera* populations in Chile were tested in order to identify an alternative extraction method that is less destructive than the one currently used - where all adult individuals in the population are removed by hand. Since *M. pyrifera* grows primarily on boulders throughout northern Chile, all individuals on a boulder were hereafter considered as an algal unit. The two harvesting treatments tested were (1) half of the kelp biomass per algal unit was removed, and (2) all of the biomass per algal unit was removed from half of the algal units. In addition, a negative control where all the biomass was removed from all the algal units, and a positive control were no harvesting was performed were also established. Understory algal composition, herbivore densities, growth of new stipes, and recruitment of new *M. pyrifera* were monitored weekly in each treatment for two and a half months in summer 2009. Results suggest that removing only half of the biomass per algal unit was the most sustainable way of harvesting; no significant differences in understory algal community were observed, there was a constant growth of new stipes in each algal unit, physical abrasion (i.e. “whiplash effect”) by the remaining stipes reduced herbivore presence on the boulders, and recruitment of new *M. pyrifera* sporophytes was maintained. In addition, variation of alginate quality and percentage of major macromolecules (proteins, lipids, crude fiber, carbohydrates and calories) under short-term harvesting and long-term harvesting pressure were analyzed to examine if there was a significant effect of repeated harvesting on these properties. No significant differences in these compounds were found over either a short-term (3.5 months) or after long-term (approximately 15 years) repetitive harvesting, suggesting that repetitive harvesting does not negatively impact the final products. Consequently, my results recommend the extraction of half of the biomass per algal unit within an area to obtain an ecologically sustainable industry focused on northern Chilean *M. pyrifera* populations, and
with a harvesting frequency that depends exclusively on the time required for recovery of new stipes.
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ECOLOGICAL RESPONSES OF *MACROCYSTIS PYRIFERA* UNDER THREE HARVESTING TREATMENTS IN NORTHERN CHILE

**ABSTRACT**

Kelp harvesting in Chile has been well documented throughout the last two decades. Nevertheless, information on how harvesting impacts ecological patterns such as recruitment, competition, grazing and trophic interactions have rarely been incorporated into the design of harvesting methods. The kelp harvesting industry in Chile is managed by local fishermen and is not part of an organized national industry. As a result, current harvesting practices are not sustainable and many kelp species are near extinction in northern Chile, highlighting the need for educating kelp harvesters within the local fisherman communities. Further, for species such as *Macrocystis pyrifera* that are under increasing demand as food for abalone aquaculture, the need for sustainable harvesting practices is increasingly important. Here, experiments designed to provide alternative, sustainable kelp harvesting methods were carried out on the coast of Chile, and their effects on ecological processes were assessed over a two-month period.

Two methods of harvesting *M. pyrifera* were compared, taking into consideration biotic and abiotic factors affecting the harvested populations. These methods were similar to the current method used by fishermen, which involves removal of all kelp individuals within the harvest area. The methods used here involve removing the same amount of biomass from a population but differ in how this biomass is selected for harvesting, and subsequently result in differences in the availability of open space, physical disturbance, and incidence of light.
reaching the benthos. Treatments were evaluated over a two-month period with an expectation of rapid recovery of harvested populations. Kelp-herbivore interactions, understory algal biodiversity, recruitment of new sporophytes, and growth of the remaining kelp individuals were measured in the field. Results indicate that relative to removing either all of the individuals from the harvest area (i.e. the method currently used by local fishermen) or all of the stipes from half of the individuals in the harvest area, removing only half of the stipes from all the individuals in a population reduces herbivore densities through physical abrasion by the remaining blades (i.e. the “whiplash effect”), enhances recruitment of individuals by maintaining sporophyll abundances in close proximity to the available substrate, promotes rapid growth of new stipes on the remaining individuals, and is consequently far superior for the sustainability of this industry.

**INTRODUCTION**

Biogenic primary producers can play a vital role within their respective ecosystems. Species of macroalgae in the order Laminariales are able to form large and conspicuous underwater stands known as "kelp forests" and are distributed in temperate to subarctic coastal environments around the world, with several species in the Pacific Ocean (North 1971; Kain 1982; Dayton 1985a, 1985b). Kelp forests create habitat structure and provide food for many benthic subtidal and intertidal organisms. Kelps also play an important role as nurseries for innumerable marine species (North 1971; Dayton 1985a; Santelices 1989; see also the review, Graham et al. 2007). Consequently, maintaining the integrity of these forests is crucial to proper ecosystem function in these coastal environments.

One of the most conspicuous kelp species in the eastern Pacific Ocean is the giant kelp, *Macrocystis pyrifera* (Linnaeus) C. Agardh. Although several species in the genus
Macrocystis have been described, recent studies have argued that they are all ecomorphs of the same species whose dissimilar morphologies are the result of adaptation and phenotypic plasticity in response to different environments (Demes et al. 2009; Macaya and Zuccarello 2010). *M. pyrifera* is a cosmopolitan species that can reach up to 30 meters in length. (North 1971; Dayton 1985a). When it reaches the surface it continues to grow, generating a considerable amount of biomass that remains floating at the surface; this portion is called the "canopy". As with other kelps, this species undergoes a heteromorphic alternation of generations between macroscopic and microscopic stages. The sporophytes (the macroscopic stage that makes up the kelp bed) release billions of spores from specialized blades called sporophylls. After a short (<72 hr) dispersal period, the spores settle on the benthos and give rise to male and female microscopic life stages (gametophytes). Male gametophytes release sperm that fertilize female gametophytes and the resulting zygote develops into the macroscopic sporophyte (Neushul 1963; North 1971; Santelices 1990a). Following this, growth and survival of the sporophytes is regulated primarily by abiotic factors such as the incidence of light, variation in temperature, the amount of nutrients (nitrogen), and hydrodynamic conditions (Gerard 1982, 1984; Gerard and North 1984; North and Zimmerman 1984; Zimmerman and Kremer 1984; Casas-Valdez et al. 2003; Buschmann et al. 2004; Matson and Edwards 2007).

During the last three centuries, communities around the world have become economically reliant on kelp harvesting for the extraction of alginates (McCleneghan and Houk 1985; Foster and Barilotti 1990; Schiel and Nelson 1990; Vasquez 1995; see the review Kirkman and Kendrick 1997; Rothman et al. 2006). The word “alginate” comes from the salts and derivatives of alginic acid, a polysaccharide found as a structural component in
the cell walls that upon extraction, is used for its properties of stabilizing, thickening, film-forming, and antiviral activity (Kelco 1976; Skjåk-bræk and Martinsen 1991a, 1991b; Reyes-Tisnado et al. 2005; Yabur et al. 2007). The harvesting of *M. pyriforma* in California, USA has been historically one of the most sustainable industries in the world (Frey 1971; Kelco 1976). Beginning with the production of potash for making explosives during World War I, this vast and consolidated industry exploited kelp forests along the southern California coast for more than 30 years. To sustain this industry, a solid management plan was constructed elaborating methods of extraction according to the ecological impact on the kelp forest ecosystems (Barilotti and McPeak 1985; Roland 1985; Bodkin 1988; Barilotti and Zertuche-Gonzalez 1990). Several methods of harvesting *M. pyriforma* in southern California have been developed since 1911, with the most modern methods employing kelp-harvesting ships that cut the canopies with reciprocating blades mounted at the base of a conveyor system. Modern harvesters (used since the 1970’s) have a conveyor system called drapers mounted on the stern of a vessel that assist with the process. When the harvester arrives at the kelp bed, drapers are lowered into the water to a depth of 1 meter. A bow propeller pushes the vessel (stern first) through the water pushing large cutting racks through the kelp bed. As a result, these harvesters can collect as much as 550 metric tons per day (Frey 1971; Kelco 1976). Ecological studies on the effects of this method of harvesting have shown some important effects such as temporal decreases in fish assemblages (Bodkin 1988) and temporary destruction of canopy habitat (McClenneghan and Houk 1985), though no persistent effects on benthic communities have been shown (Barilotti and McPeak 1985; Barilotti and Zertuche-Gonzalez 1990; Foster and Barilotti 1990).
Chile is also considered to have one of the most productive kelp forest coastlines, accounting for 10% of the worldwide raw material supply for alginate extraction (Vasquez 2008). Romo et al. (1984) found that techniques for kelp harvesting developed for *M. pyrifera* in California were also effective and sustainable in Chilean kelp forest ecosystems despite the large geographic difference. The extraction of the canopy portion (floating portion to 1.5 meters below surface) was found to have negligible impacts on kelps or their associated ecosystems (see also Santelices and Ojeda 1984) and similar mechanical techniques are being considered to develop this industry in the southern regions (Filun pers comm). However, harvesting of kelp forests in Chile happens entirely along a relatively small portion of coastline (between 26° and 32° South) (Vasquez 2008) and since the ecomorphs (Demes et al. 2009) collected in this industry are different from Californian species, techniques of extraction need to be targeted at the local populations.

In northern Chile, communities of fishermen conduct kelp harvesting and are exclusively dependent upon this resource, which integrates socio-economic concerns (Vasquez and Santelices 1990; Vasquez and Westermeier 1993; Vasquez 2008). Vasquez and Westermeier (1993) highlighted the limitations of this fishery with regards to the lack of education, the increase of the international demand, and a low level of regulation, potentially playing a large role in the collapse of natural beds. Moreover, the last few El Niño Southern Oscillation events, especially the one in 1997-1998, depleted *M. pyrifera* populations and other kelps throughout large portions of this region (Vasquez and Vega 2004; Vega et al. 2005; Vasquez et al. 2006). Consequently, in 2005, the status of the remaining natural beds forced the Chilean government to ban *Lessonia* spp and *M. pyrifera* extraction for an 18-month period; this restriction had never been implemented for harvesting of algal species in
the past. As a result, and due to the effort of algal population biologists (e.g. Vega et al. 2005), some populations of *M. pyrifera* have recovered and harvesting as resumed. Therefore, governmental monitoring of the fisherman communities and the development of management plans for these resources, and a sustainable harvesting method for these resources are sorely needed (Vasquez 2008).

More than 90% of the kelp extracted for alginate extraction in Chile is comprised of two species of *Lessonia*, *L. nigrens* and *L. trabeculata*. However, the impressive growth of abalone aquaculture since 2000 has created a demand for fresh kelp as food of approximately 4,800 tons per year (Anuario Estadistico de Pesca 1985-2006), primarily *M. pyrifera* (Vasquez 2008). Beach-cast gathering, a method commonly used in other countries, is generally used for species that are targeted for alginate extraction (Kirkman and Kendrick 1997; Casas-Valdez et al. 2003) and is not appropriate for *M. pyrifera*. Rather, in northern Chile, *M. pyrifera* is extracted by hand from a boat, shore or by using SCUBA. Often this removal technique includes collecting the entire individual, including the reproductive sporophylls. Using these methods, the ecological effects of harvesting are analogous to the effects of severe physical or biological disturbances such as those caused by storms, which remove the entire individuals. However, the distribution of reproductive structures within the individuals, the presence of herbivores, and the distribution of the kelp populations are factors that vary with each species and ecomorph of kelp (Vasquez 1995), and a single method will not have the same effect on all species. Thus, the method in northern Chile to extract *M. pyrifera* is considered primitive and does not address any ecological concerns. Furthermore, the unique phenotypic plasticity shown by *M. pyrifera* (Demes et al. 2009; Macaya and Zuccarello 2010) makes general regulation of harvesting difficult for this species.
since response to harvesting methods may differ from one place to another. In the north of Chile, *M. pyrifera* grows mostly in shallow subtidal depths (2 to 11m) (Vega et al. 2005) in protected, semi-exposed or exposed waters (Vasquez 2008). Population ecology, biodiversity and ecological responses to harvesting have been well studied in protected and semi-exposed areas (Vasquez 1995; Vega et al. 2005; Graham et al. 2007; Villegas et al. 2008). However, exposed shallow waters that are accessible for fisherman have not been well studied and fragmented populations of this macroalgae have grown abundantly in these areas.

The following chapter represents the first approach for a sustainable harvesting technique of *M. pyrifera* in northern Chile. Following the studies of Vasquez (2008), this is the first description of how to best harvest these populations considering the increasing demand for abalone aquaculture (Flores-Aguilar 2007; Vasquez 2008; Buschmann et al. 2008) and its potential use for alginate extraction in the future. It is important to evaluate the ecological effect of specifics methods that are used to yield a sustainable harvesting method.
MATERIALS AND METHODS

SITE DESCRIPTION

Experiments were conducted in the National Humboldt Penguin Reserve near the town of Punta Choros, a fishing community located about a 100 km northward La Serena city, Chile (Figure 1). Experiments were conducted at two sites "Memo Ruz" and "Ventana" (29°14′ 44.32″ S; 71°27′ 59.27″ W and 29°12′ 32.95″ S; 71°29′ 00.84″ W, respectively) during late Austral summer and beginning of fall 2009 (February, March and April). Populations are highly exposed to wave action and distributed in shallow waters reaching a maximum depth of 3 meters at high tide. The Memo Ruz (MR) kelp bed was 15 m x 20 m in size, and Ventana (V) was 10 m x 20 m. Harvesting of *M. pyrifera* is no longer active at these sites, however this is one of the few places with several fragmented and exposed populations that are relatively accessible.

![Figure 1. Study sites in the Humboldt Penguin National Reserve, Chile. 1 = Memo Ruz, and 2 = Ventana. Sites were separated from one another by 4.42 km.](image-url)
**Harvesting Treatments**

Two experimental treatments were designed according to the morphology, growth and distribution of the *M. pyrifera* ecomorph at these sites. The original method used by fishermen to remove kelp (pulling them by hand) was not modified since the high exposure to wave action and the lack of resources for more sophisticate equipment does not allow another methodology and any severe changes in the methodology may cause the non-utilization of this new procedure in the future. It is important to note that unlike California *M. pyrifera* populations, populations in northern Chile grow mostly on boulders in shallow water, in highly wave-exposed sites, and occur in very small patches. Thus, subtidal continuous monitoring is extremely complicated since it involves diving in 1 to 3 meters depth within the surf zone. This fact in addition to the fragile situation of northern populations (that almost reach local extinction in 2005) forced us to design the experiments at a small spatial scale.

Continuous wave exposure and overlapping of holdfasts on each boulder made it impossible to identify each sporophyte (individual), so we used “algal units” (AU) defined as a group of stipes growing on a single boulder in place of “individuals”. Four circular areas of four meters diameter and separated by 10 meters were set up at each site. These consisted of two experimental harvesting treatments and two control treatments; one negative control where all kelp was cleared from all the boulders (-C), and one positive control where the kelps were left un-manipulated (+C) (Figures 2 and 3). All AU were identified within each experimental treatment and tagged for future monitoring. In the first treatment (T1), half of all the stipes were removed from each AU in the area, while in the second treatment (T2), all of the stipes were removed from each of half of
Figure 2. Experimental and control treatments established at each site. Treatments consisted of circular areas of 4 m diameter that were separated from each other by 10 m. All algal units within each treatment were tagged.

Figure 3. Experimental harvesting treatments. +C = Positive control (unharvested area); T1 = Treatment one (all stipes were removed in half of each AU in the area); T2 = Treatment two (half of all AU present in the area were randomly selected and completely cleared of all stipes). –C = Negative control (All AU in the area were cleared of all stipes).

the AU (Figure 3). Both experimental treatments (T1 and T2) removed approximately the same amount of biomass per area but left a different distribution of the remaining stipes. All altered AU (n= 6 to 10 per treatment) were tagged with an ID number for future identification.
To examine the effects of harvest method on stipe density (growth), recruitment of new sporophytes, herbivory and benthic algal diversity, AU were monitored weekly for 9 weeks at Memo Ruz (MR) and 8 weeks at Ventana (V) using SCUBA. Stipes were counted on each AU 1 m above the holdfast. Recruitment was assessed by counting the number of new sporophytes on each AU. A “recruit” was defined as any sporophyte with three or fewer fronds according to Druehl and Wheeler (1986). Once counted, recruits were tagged with cable-ties and a plastic number, following which only new recruits were counted each week during the 9-week experimental period. A survey was conducted during the first week of the study in order to identify major herbivores present on the AU. The density of only the most abundant species, the gastropod, *Tegula tridentata*, which comprised more than 90% of all herbivores was measured weekly in five 0.25 m² quadrats randomly placed on each AU. Lastly, the abundance of benthic algae was also monitored on each AU during the course of the study. However, unlike places such as California, understory algae are not common in northern Chile and therefore responses of the understory algal community were expected to be minimal (Santelices and Ojeda 1984; Buschmann et al. 2006). Therefore, percent cover of understory algae was measured only during the first and last week of the study. To do this, five 0.25 m² quadrats were placed randomly within each treatment and percentage cover of macroalgae other than *M. pyrifera* was recorded.

**Statistical Analysis**

Univariate statistical analyses were done using Systat version 12, and all multivariate analyses were done using Primer 6. Data was tested for normality and equal variances prior to testing. Analyses over time and between treatments and controls for stipes density and herbivore abundance were done with separate repeated measures ANOVAs for each site. I
chose to keep the analyses for each site separate because I wanted to examine how each site responded independently. Pairwise comparisons were performed when significant differences among treatments were found. Differences in kelp recruitment among treatments were tested using a three-way PERMANOVA, since a considerable amount of zeros were found, making ANOVA inappropriate. The PERMANOVA in contrast is a distribution-free test and thus, variables like this can be analyzed. In these analyses, harvesting treatments, time and sites (MR and V) were fixed factors, and again pairwise comparisons tests were performed when significant differences were found. Analyses of changes in percent cover of understory algal species between the initial and final concentration was done using a two-way PERMANOVA (site and treatment as factors) and when there was significant difference a pairwise comparison post-hoc analysis was done.
RESULTS

Experimental harvest treatments and positive control (T1, T2 and +C) presented similar variation in the number of stipes over the experiment at both sites, each showing slight increases in stipe number until week 5 (Figures 4A and 4B). Variability through time was significant at both sites MR (p = 0.05) and V (p = 0.027), and pairwise post-hoc analyses revealed that this was mainly driven by changes that occurred between weeks 5 and 6, and 6 and 7 for MR site (p < 0.001), and is consistent with the variation in the amount of stipes per algal unit expected after an unusually strong summer storm that occurred in week number 5. Further, when all the stipes were removed from the harvest area (-C), no new stipes appeared on any of the AU (Figures 4A and 4B), indicating that the current method of harvesting used by the fishermen was not sustainable in a short-term period.

Recruitment of new sporophytes varied significantly among harvesting treatments (p = 0.002), but not between sites (p = 0.396; Table 1). Further, the relative differences between treatments also did not vary significantly between the two study sites (treatment x site interaction p = 0.621). Specifically, at MR, recruitment of new sporophytes was only observed in T1 and T2; where half of the stipes was removed from each AU and where all the stipes were removed from half of the AU, respectively but no recruitment was observed in either the positive or negative controls (Figure 5A). At V, recruitment was observed in T1 and in the unmanipulated (+C) control site, but not in either of the other two treatments (Figures 5A and 5B). No significant differences in average number of recruits were found between experimental treatments at V (T1 and T2; p = 0.058), but
Figure 4. Experimental responses under different harvesting methods through time. A = Average number of stipes per AU at Memo Ruz; B = Average number of stipes per AU through time at Ventana 4C. Average *Tegula tridentata* per 25cm² per site at Memo Ruz site. 4D = Average *Tegula tridentata* per 25cm² at Ventana site.

Table 1. Two-Way PERMANOVA Table with Factors Sites and Treatments for Variable Accumulative Recruitment per Algal Unit

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</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>383.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference at 0.05 significance level
Figure 5. Initial and final concentration of *M. pyrifera* recruitment per treatment. 5A Memo Ruz site. 5B Ventana site.

T1 was significantly different than both controls (-C and +C, p = 0.007 and 0.026, respectively) (Table 2). Taken together, no recruits were observed in the harvest areas where all the stipes were removed from all the boulders (i.e. current harvesting methods), the greatest recruitment was observed in the harvest areas where half of the stipes were removed from all the boulders (T1), AND Intermediate BUT spatially variable recruitment was observed on the other two treatments.

**Table 2. Pair-Wise Test, Term Treatments for Accumulative Recruitment**

<table>
<thead>
<tr>
<th>Groups</th>
<th>t</th>
<th>P (Perm)</th>
<th>Unique perms</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1, T2</td>
<td>2.076</td>
<td>0.058</td>
<td>914</td>
</tr>
<tr>
<td>T1, -C</td>
<td>2.784</td>
<td>0.007*</td>
<td>843</td>
</tr>
<tr>
<td>T1, +C</td>
<td>2.316</td>
<td>0.026*</td>
<td>917</td>
</tr>
<tr>
<td>T2, -C</td>
<td>1.898</td>
<td>0.085</td>
<td>6</td>
</tr>
<tr>
<td>T2, +C</td>
<td>0.933</td>
<td>0.377</td>
<td>18</td>
</tr>
</tbody>
</table>

* Significant difference at 0.05 significance level

Surveys conducted during the first week of the study revealed three potential herbivores that might graze on *M. pyrifera* in northern Chile (two gastropods, *Tegula*
tridentata and Tegula atra, and the echinoid Tetraxygus niger). However, only T. tridentata was consistently present in the experimental areas (more than 3% cover per m²) and thus considered further. An increase in the number of T. tridentata was observed in both experimental treatments and the controls, however it was considerably higher in -C at both sites (MR and V) where pieces of holdfasts and space were more available for herbivory. (Figures 4C and 4D (p. 14), Figure 6). Significant differences were found between treatments through time in both sites (treatment X time interaction, p < 0.05 for both sites). These differences may be attributed to the available space over the boulders and the pieces of holdfast and stipes remaining in the boulders that serve as food for the gastropod. Interestingly, gastropod densities were in –C and T2 where either all the stipes were removed from all the boulders or all of the stipes were removed from half of the AU, likely due to the fact that both treatments involved removing all stipes from the boulders, thus creating bare space and removing existing fronds that physically scour the boulder via a “whiplash effect” (Figures 4A and 4B, p. 14). T1 showed the lowest gastropod abundance during the experimental period (Figures 4C and 4D, p. 14).

Unlike patterns observed in California, understory algal communities are not abundant in Chile, with only a few species of brown algae are commonly found. In this study, only two individuals of one subtidal kelp (Lessonia trabeculata) were found in T1, treatment where half of the stipes per AU were removed. Some turfs of the orders Gelidiales, Corallinales and small patches of Halopteris spp were also found (Figures 7A and 7B), but no significant differences were observed between sites (p = 0.144) or times (p = 0.174). However, species composition was different between treatments (p = 0.002). This significant variation due to the harvesting process is likely not biologically
Figure 6. *Tegula tridentata* feeding upon remains of *M. pyrifera* on cleared boulders.
Figure 7. (A) Algal biodiversity composition at Memo Ruz for both experimental treatments and controls. (B) Algal biodiversity composition at Ventana site for both experimental treatments and controls.
important since composition of the understory algal community was varying by only a very small amount (see Y axes of Figures 7A and 7B, pp. 19-20) and no trend related to the harvesting process was identified. Further, interactions between all variables (treatments, sites and time) were all not significant (p = 0.71), again suggesting that harvesting did not impact biodiversity differently in the treatments through time (see also Table 3). Post-hoc analysis at each time level revealed significant variation in the initial concentration between treatments (Table 4) however no significant variation was found at the end of the experimental period between treatments (Table 5) showing similar composition of the understory algal community and no significant effect of the treatment used.

Table 3. 3-Way PERMANOVA Table. Abundance of Understory Algal Biodiversity with Factors Time, Site and Treatment

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Pseudo-F</th>
<th>p (Perm)</th>
<th>Unique perms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti</td>
<td>1</td>
<td>4.09</td>
<td>4.09</td>
<td>1.59</td>
<td>0.174</td>
<td>999</td>
</tr>
<tr>
<td>Si</td>
<td>1</td>
<td>4.4</td>
<td>4.4</td>
<td>1.71</td>
<td>0.144</td>
<td>999</td>
</tr>
<tr>
<td>Tr</td>
<td>3</td>
<td>28.56</td>
<td>9.52</td>
<td>3.7</td>
<td><strong>0.002</strong></td>
<td>999</td>
</tr>
<tr>
<td>TixSi</td>
<td>1</td>
<td>1.68</td>
<td>1.68</td>
<td>0.65</td>
<td>0.603</td>
<td>999</td>
</tr>
<tr>
<td>TixTr</td>
<td>3</td>
<td>17.75</td>
<td>5.92</td>
<td>2.3</td>
<td><strong>0.012</strong></td>
<td>998</td>
</tr>
<tr>
<td>SixTr</td>
<td>3</td>
<td>7.34</td>
<td>2.45</td>
<td>0.95</td>
<td>0.509</td>
<td>999</td>
</tr>
<tr>
<td>TixSixTr</td>
<td>3</td>
<td>5.52</td>
<td>1.84</td>
<td>0.72</td>
<td>0.71</td>
<td>997</td>
</tr>
<tr>
<td>Res</td>
<td>64</td>
<td>164.66</td>
<td>2.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>234.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference at 0.05 significance level

Ti: Time; Tr: Treatment; Si: Site
Table 4. Pair-Wise for Level "Initial Concentrations of Biodiversity" between Treatments of Factor "Time"

<table>
<thead>
<tr>
<th>Groups</th>
<th>t</th>
<th>p (perm)</th>
<th>Unique Perms</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1, T2</td>
<td>1.47</td>
<td>0.061</td>
<td>999</td>
</tr>
<tr>
<td>T1, -C</td>
<td>2.68</td>
<td>0.001</td>
<td>998</td>
</tr>
<tr>
<td>T1, +C</td>
<td>4.43</td>
<td>0.001</td>
<td>999</td>
</tr>
<tr>
<td>T2, -C</td>
<td>1.97</td>
<td>0.007</td>
<td>999</td>
</tr>
<tr>
<td>T2, +C</td>
<td>3.2</td>
<td>0.001</td>
<td>999</td>
</tr>
<tr>
<td>+C, - C</td>
<td>0.97</td>
<td>0.455</td>
<td>998</td>
</tr>
</tbody>
</table>

Table 5. Pair-Wise for Level "Final Concentration of Biodiversity" between Treatment of Factor "Time"

<table>
<thead>
<tr>
<th>Groups</th>
<th>t</th>
<th>p (perm)</th>
<th>Unique Perms</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1, T2</td>
<td>0.6</td>
<td>0.825</td>
<td>999</td>
</tr>
<tr>
<td>T1, -C</td>
<td>0.47</td>
<td>0.958</td>
<td>998</td>
</tr>
<tr>
<td>T1, +C</td>
<td>1.06</td>
<td>0.352</td>
<td>999</td>
</tr>
<tr>
<td>T2, -C</td>
<td>0.83</td>
<td>0.593</td>
<td>999</td>
</tr>
<tr>
<td>T2, +C</td>
<td>0.82</td>
<td>0.6</td>
<td>999</td>
</tr>
<tr>
<td>+C, - C</td>
<td>1.33</td>
<td>0.14</td>
<td>998</td>
</tr>
</tbody>
</table>
DISCUSSION

Ecological consequences of kelp harvesting have been discussed for more than twenty years (McCleneghan and Houk 1985; Foster and Barilotti 1990; Schiel and Nelson 1990; Vasquez 1995; see review Kirkman and Kendrick 1997; Rothman et al. 2006) and most studies have shown little to no significant impacts to the ecosystem relative to those expected from natural meteorological and biological disturbances (Santelices and Ojeda 1984). In many places like Chile, the effects of specific harvesting techniques and their possible effects on ecological patterns such as competition, herbivory and recruitment has not been considered in the management of targeted species despite the potential importance. Impacts of these methods may differ from those of the better-known methods and thus it is important to redesign harvesting methods taking into consideration ecological and demographic factors for each species.

In northern Chile, *M. pyrifera* grow on boulders and according to this study, harvesting of only half of the stipes per boulder showed considerably more benefits to the population than other possible methods or the methods currently used by local harvesters where all the stipes are removed from all the boulders. Experimental treatments where half of the stipes per boulder were removed and all of the stipes on half of the boulders were removed were designed according to the actual method of harvesting these populations (i.e. pulling the stipes from the surface). Biomass removal was approximately the same for both treatments and a significant difference between treatments in the number of stipes through time was found. In contrast, within each treatment no significant variation was noticed through time or between sites suggesting a relatively
consistent rate of stipe production on each algal unit when there were remaining stipes on the boulder. The spread of individuals by vegetative growth is the most likely alternative of recovery. This type of growth has been supported when natural (Lobban 1978a, 1978b) and artificial (Druehl and Kemp 1982) fragmentation occurs. The lack of new stipes growing in boulders where all the stipes were removed suggests no recovery over a short-term time period and could explain also the decline of population in the last years after severe storm and harvesting pressure (Vega 2005; Vega et al. 2005).

In addition to vegetative growth, populations of kelp recover from disturbances via recruitment of new individuals. These arise from zoospores that are released from sporophylls on nearby adults, settle on the benthos and undergo gametogenesis. Consequently, recruitment of *M. pyrifera* can be limited to short distances from the remaining sporophytes. However, recruitment of *M. pyrifera* was not expected since experiments were conducted in summer, when recruitment is lowest, and even though *M. pyrifera* in northern Chile contains sporophylls year-round, the production of spores is very low in this season (Buschmann et al. 2004). The recruitment that occurred over the experimental time may have come from zoospores that were able to settle prior to the experiment, or from sporophytes that were so small they were not observed at the beginning of the experiment. For *M. pyrifera* presence of “seed banks”, and the possible delay development of gametophytes (Carney and Edwards 2010) may explain growth of new individuals in such a short period of time, however further experiments need to be conducted in order to see if this occurs in northern Chile. Regardless, my data indicates that removing all of the stipes from the boulders limits recruitment of new sporophytes,
presumably due to limited zoospore settlement, but removing only half of the stipes from a boulder results in the greatest recruitment.

Understory biodiversity is known to have a strong relationship with the foundational species that construct the three-dimensional habitat where they occur (Hughes 2010). No significant variation has been shown for algal understory in *M. pyriforma* for populations in southern Chile after canopy removal (Santelices and Ojeda 1984) and results from this study seem to reaffirm this pattern. The decrease of Corallinales, Gelidiales and *Halopteris* spp in both experimental treatments was negligible. An alternative explanation is that with less *M. pyriforma* stipes covering that benthos, species were being exposed to wave action and whiplash effect that was not present in the past diminishing the concentration of these species, an effect described with other kelp species in the world (Kennelly 1989; Taylor and Schiel 2005; Hughes 2010) and in Chile (Santelices 1990b). However, the opposite response observed in Ventana for corallines and Gelidiales (Figure 7B, p. 20) suggesting that this variation is based on local temporal conditions and/or stochastic processes. Further investigation needs to be conducted to conclusively identify physical abrasion as being responsible for this variation in the algal community specially since significant variation of the biodiversity was based on changes between very low percentages cover.

Macrograzers can be a significant biological factor controlling the abundance and distribution of benthic macroalgae (see review by Lawrence 1975, Dayton 1985b; Schiel and Foster 1986; Johnson and Mann 1988 and review by Vasquez and Buschmann 1997). For instance, sea urchins have a major role in shaping the structure of many kelp communities around the world (see also Mann 1977; Estes et al. 1978). However in
Chile, sea urchin communities do not appear to have as much of a role in shaping *Macrocystis* communities (Castilla and Moreno 1982; Vasquez et al. 1984; Dayton 1985b). In northern Chile, sea urchins control deeper kelp communities (*L. trabeculata; Villouta et Santelices*) (Vasquez 1989, 1992, 1993) whereas *Tegula tridentata* may be far more important in shallow *M. pyrifera* communities (Fariña et al. 2005; Villegas et al. 2008). According to my data *T. tridentata* responds differently to different harvesting processes, increasing in areas where all the kelp stipes have been removed from the boulders, but remaining lower on boulders where some or all of the stipes remain. This suggests that removing only half of the stipes per boulder may reduce grazing pressures by reducing herbivore abundances. This likely occurred due to the physical abrasion caused by the remaining stipes during periods of stronger wave action. This “whiplash effect” may be important in clearing gastropods from the boulders thereby preventing, them from to dominated the available space. This process has been described as a natural indirect defense strategy against herbivory of sea urchins communities in North America (Vasquez and McPeak 1998; Konar 2000), as artificial mechanisms for kelp restoration (Vasquez and McPeak, 1998) and also fairly described in Chile in species such as *Lessonia trabeculata* (Vasquez 1992; see also Vasquez and Buschmann 1997) and other countries (Taylor and Schiel 2005). Where all of the stipes were removed from half of the AU (T2), gastropods were more abundant than T1 where half of the stipes remained on each boulder, thus promoting this whiplash effect. This hypothesis is also supported by the higher amount of gastropods found in the -C where no stipes were left in the area (See Figures 4C and 4D. p. 14) and no whiplash effect was clearing the boulders or impeding settlement of gastropods. Furthermore, this crucial physical interaction may enhance
recruitment and vegetative growth *M. pyrifera* while act synergistically with the correct harvesting method (T1).

Finally, while fisherman communities that are extracting *M. pyrifera* are smaller than others communities extracting species such as *Lessonia* spp., there will likely be an increase in harvesting of this species with the increasing demand as food for abalone aquaculture (Flores-Aguilar et al. 2007), the so-called “socio-ecological” aspects (Estevez et al. 2010) that promote sustainable industry upon species extracted need to be incorporated. It is the perfect time to make this information available to fishing communities through educational programs. If kelp populations can recover, and the harvesting community can implement these methods a sustainable industry of this resource will rise. Consequently, I strongly recommend removing half of the stipes from each boulder (T1) as a sustainable method of harvesting *M. pyrifera* in northern Chile as it results in the greatest rates of kelp recruitment and stipe growth, and lowest abundances of grazers, especially compared to methods currently used.
CHEMICAL RESPONSES OF *MACROCYSTIS PYRIFERA* UNDER SHORT AND LONG-TERM HARVESTING

**ABSTRACT**

Kelp harvesting is a profitable business in several places around the world. Although variation in chemical composition of species such as *Macrocystis pyrifera* caused by repetitive harvesting has never been examined, the quality of the final products may be reduced due to the physiological stress caused by this process. To investigate this, repetitive harvesting was performed on a population of *M. pyrifera* at the Point Loma kelp forest to identify responses in tissue chemistry throughout the summer 2010. The effects of repetitive harvesting on alginate quality, yield, viscosity and compressive strength were measured. Although no overall differences in these metrics were observed between individuals that were harvested repeatedly versus those that were not harvested repeatedly, variation of all three variables was significant through time, likely due to variability in oceanographic conditions. In addition, variability in tissue proteins, carbohydrates, lipids and ash content were analyzed to identify possible changes in kelp nutritional content under repeated harvesting. Similar to alginate quality, these results show significant variation through time for each variable but no significant differences between harvested and unharvested individuals was observed, suggesting no major changes in the content of these compounds during a short-term (three month) period. In addition, tissue samples were taken from Chilean *M. pyrifera* populations that had never been harvested and populations that have been repeatedly harvested for more than 20
years to evaluate if there is an effect of repetitive harvesting on chemical composition in the long term. For these samples, analyses of carbohydrates and crude fiber were added. Similar to the short-term experimental populations, no significant differences were found between harvested and unharvested populations for any of the variables measured, suggesting no effects of repeated harvesting over a long-term period. Overall, these results suggest that repetitive harvesting does not have any significant effects on alginate quality or nutritive content.

**INTRODUCTION**

Variability in chemical composition in kelp species has been studied to identify how populations respond to changes in ocean nutrient availability (Zimmerman and Kremer 1984, 1986; Gerard 1997). In addition, chemical composition in kelps has been studied for economical interests, such identifying mechanisms that control growth (Brain et al. 1973; Skjåk-Bræk and Martinsen 1991b see also review of Bradley 1991), using kelp products in biomedical applications (Hong and Hien 2004; Osbourn and Lanzotti 2009), and extracting phycocolloids for industrial purposes (Rodriguez-Montesinos and Hernandez-Carmona 1991; Arvizu et al. 2007). It is within this latter field of study, where we find studies on alginates, the primary phycocolloid extracted from kelp. This copolymer is a structural polysaccharide extracted from the cell walls that has been abundantly studied for its commercial properties such as stabilizers, film formers, thickeners, mechanical strength and its viscosity (Martinsen et al. 1989; Skjåk-Bræk and Martinsen 1991a, 1991b). Its use has been extended to many fields such as medicine, microbiology and food industries (King, 1983; Margaritis and Merchant, 1984; Shymali et al. 1984; Yabur et al. 2007). These structural properties are also important for the
species since they are the most abundant components of the cell wall, representing 14 to 80% of the dry matter (Smidsrød and Draget 1996) and providing mechanical properties to the algae (McHugh 1987). Despite this, understanding of how the harvesting this resource impacts the quality of these products remains sorely lacking.

Great variability of alginate quality and yield in kelps is usually caused by oceanographic factors such as temperature, coastal currents and wave exposure (Haug and Jensen 1956; Percival and McDowel 1967; Espinoza-Avalos and Rodriguez-Garza 1986; Rodriguez-Montesinos and Hernandez-Carmona 1991). In contrast, anthropogenic effects such as harvesting on these properties has not been studied, and even though harvesting is comparable to the ecological effects of storm removal (Vasquez and Santelices 1990), the chemical variation of these populations may be quite different following repetitive harvesting that removes only a portion of each individual but at multiple times relative to single storm events that remove the entire individual. Consequently, a lower quality of the final product extracted from the kelp tissues would reduce the demand and/or monetary price of the product, and taking into consideration that in countries such as Chile this industry is performed by fisherman communities, the social impact would be of great concern (Vasquez and Santelices 1990; Vasquez and Westermeier 1993; Vasquez 2008).

In Chile, harvesting kelps for alginate extraction occurs exclusively between 26° and 32°S, Northern Chile (Anuario Estadistico de Pesca 1985–2006). More than 90% of the total alginate extraction occurs from Lessonia trabeculata (Villouta and Santelices) and L. nigrescens (Bory) (Vasquez 2008), and less than 10% is of the total is from Macrocystis pyrifera. However, the use of M. pyrifera in abalone aquaculture in Chile is
increasingly important due to its nutritional content. It has been described as an important source of fiber that helps with the digestive process (Lahaye 1991) and also as a good source of protein and carbohydrates. However, like alginate quality, variation in these chemical compounds has never been analyzed after periods of frequent harvesting. This may be of significant importance considering almost 100% of the total fresh algae that was consumed in Chilean abalone aquaculture between 2005 and 2006 was *M. pyrifera* and estimates suggest that in order to produce 1,000 tons of abalone in 2010, 100,000 tons of fresh *M. pyrifera* are required (Vasquez 2008). Based on these estimates, the amount of *M. pyrifera* extracted for future abalone aquaculture must increase and could deplete natural populations throughout northern Chile. The goal of this study is to determine if repetitive harvesting (as recommended in Chapter One of this thesis), has significant effects on alginate quality or as the amount of protein, lipids, fiber and carbohydrates extracted, over either short-term or long-term periods.
MATERIAL AND METHODS

STUDY SITES

This study was conducted in two locations along the Chilean and Californian coasts. To examine the impacts of long-term repetitive harvesting on *M. pyrifera* tissue chemical content, two different regions in northern Chile were identified for study. Populations within the Humboldt National Penguin Reserve (see Figure 1, p. 8) were identified and considered has never been harvested (UH), while populations at Chasco Bay, approximately 50 km northward from the Humboldt Penguin National Reserve, were identified and considered as having been harvested repeatedly for more than 20 years. Within each location, three sites were chosen for study; Humboldt Penguin National Reserve (Site one - 27° 41’ 0.812” S, 71° 02’ 19.77” W; Site two - 27° 40’ 07.31” S, 71° 01’ 17.30” W; Site three - 27° 40’ 04.88” S, 70° 57’ 05.13” W), and Chasco Bay (Site one -29° 14’ 44.32” S, 71° 27’ 59.27” W; Site two - 29° 13’ 32.22” S, 71° 27’ 54.51” W; Site three - 29° 12’ 32.95’’ S, 71° 29’ 0.84’’ W). At each site, tissue samples of blades and stipes of 30 randomly selected *M. pyrifera* individuals were collected and mixed to obtain a representation of the population in each site. In addition, samples from the Point Loma kelp forest in California, USA (Figure 8) were used as an outgroup to compare geographical variation in alginate parameters as well as nutritional value, and to provide context for the short term experiments described below.

Short-term harvesting effects on tissue chemical composition were examined at the Point Loma kelp forest, California, USA (32° 40’ 57.6” N and 117° 15’ 58.5” W)
(Figure 8). Forty-five *M. pyrifera* individuals were tagged underwater and half of their stipes were removed by cutting them from the base of the holdfast every other week for three and a half months. After the first harvesting bout, growth of new stipes was evident. These new stipes were again harvested every other week. Stipes and blades of at least 30 individuals were collected and mixed every two weeks to obtain a representation of the entire population. A second site within the kelp forest was randomly chosen on each harvest day and identified as a control, where the same number of stipes and blades were collected from individuals that had not been harvested prior.
**ALGINATE**

All kelp tissue samples from the short-term (California) and long-term (Chile) harvesting experiments were sun-dried and ground to a particle size of 20 mesh. 200g per sample were taken to La Paz, México for laboratory analysis. There, alginates were extracted by the method of Arvizu-Higuera et al. (2002). All the analyses were done in triplicate and the following description represent the process per each replicate. 20 g per sample were hydrated with 180 ml of formaldehyde at 0.1% overnight. Samples were rinsed with distilled water, placed in beakers with 300ml of distilled water and then washed with HCl 1N, reducing the pH to 4.0 while stirring the samples with a magnetic stirrer for 15 minutes. Samples were rinsed and placed with 250 ml of distilled water for every 10 g (500 ml per beaker). Then, the extraction was done using Na₂CO₃ by placing the samples into a water bath at 80°C with high torque overhead stirrers for 2 hours maintaining the pH at 10.0. The remaining paste was diluted by filtration using a low-pressure vacuum pump adding also 220 g of diatomaceous earth to facilitate the process. Finally the clarified solution was precipitated doubling the volume with ethanol at 77%. The obtained alginate fibers were placed in petri dishes and dried at 50°C for 12 hours.

Alginate yield was obtained calculating the weight difference between the initial samples and final products, and the extracted alginate weight per sample then expressed as percentage/dry weight. Alginate viscosity was measured according to Rodriguez-Montesinos and Hernandez-Carmona (2008) in 1% (w/v) solution with a viscosimeter LVT Brookfield, Brookfield, MA at 22°C at 60rpm and adding 0.5% sodium hexametaphosphate as calcium sequester. Compressive strength of the resulting alginate
gel was prepared with, a 1% sodium solution in 8 x 4.5 cm dialysis tubes. A 10% calcium chloride solution was prepared to convert the sodium alginate to calcium alginate by dialysis leaving the tubes immersed overnight. Tubes were carefully cut in half and compressive strength was measured with a texturometer TA.XT plus, programmed to perform a penetration of 2 cm for 5 seconds.

**PROXIMAL ANALYSIS**

Proximal analysis of dry samples was performed at CIBNOR (Center of Biological Investigations of the North) Mexico. Tissue moisture was calculated by weight difference between wet and dried (at 105 °C for 4 hours) samples. Ash was also a weight difference by calcinations of the samples at 600°C for 5 hours. Crude protein was obtained by using the Micro-Kjeldahl method where the amount of total nitrogen content is determined and then multiplied by 6.25 to obtain the amount of crude protein. Ether extractable (lipid content) was determined by the ether-extraction method using a Soxtec-Avanti, Tecator apparatus. A successive hydrolysis (acid/alkali) was performed to extract and determine crude fiber. Energy expressed in calories was obtained by calorimetry. Finally, the nitrogen-free extract (carbohydrate fraction of the total sample) was obtained by subtraction between the total percentage dry and the measures of the other compounds (% protein, % ash, % lipids and % crude fiber). All these methods were obtained from the association of official Agriculture chemists (Association of Official Agricultural Chemists [AOAC] 1995).
**STATISTICAL ANALYSIS**

All multivariate analyses were performed using PRIMER 6.1.11. For the short-term repetitive harvesting experiments conducted in San Diego, differences in kelp tissue moisture, lipid content, crude fiber, crude protein, carbohydrates, energy, compressive strength, alginate yield and alginate viscosity were assessed between harvested and unharvested (control) treatments using a PERMANOVA, with time as a random factor and harvesting treatment (harvested vs. unharvested) as a fixed factor. When there was significant variation, a SIMPER analyses was conducted to identify the percent contribution of each variable to the differences. For the long-term harvesting experiments in Chile, a nested PERMANOVA was conducted with sites nested within treatment (Harvested or unharvested) and the same variables as the short-term experiment were analyzed.
RESULTS

SHORT-TERM REPETITIVE HARVESTING

Overall, no significant differences in tissue chemistry were found between harvested and unharvested treatments ($p = 0.206$, see also Table 6), but high temporal variation ($p=0.001$) in these compounds was observed. Significant differences in tissue chemistry between harvested and unharvested populations in the Point Loma Kelp forest were observed through time (interaction $p = 0.001$) Figures 9 and 10). A SIMPER analysis was performed to identify the contribution of each variable to the total daily variation. Most of the variability was contributed by the variables that explain alginate quality (from day 1 to 15 alginate yield = 36.7%; from day 30 to 60 Alginate viscosity = 34.12% and from day 60 to 90 alginate viscosity and strength contributed with 61.90% of the total variation). Out of all the variables of the proximal analysis only protein had a high contribution from the day 1 to 15 = 32% and from 15 to 30 = 34.73%. However the value obtained in the day number 15 at the harvested treatment was a methodological error consistent with the contribution to the significant difference mainly given by this value (see Table 7 and Figure 9C). Temperature was constant throughout the experimental period (average Temperature of 11.5 °C) indicating it was likely not responsible for the observed temporal variation.

LONG-TERM REPETITIVE HARVESTING

Overall, significant variation in these variables was observed among sites in each study location ($p = 0.001$; Table 8; Figures 11 and 12). However, no significant variation
Table 6. PERMANOVA Table for Short-Term Experiments in San Diego with Control and Experimental Sites as Treatments (Fixed Factor) and Time as Random Factor

<table>
<thead>
<tr>
<th>Sources</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Pseudo-F</th>
<th>p (Perm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (Tr)</td>
<td>1</td>
<td>14.228</td>
<td>14.228</td>
<td>1.5249</td>
<td>0.206</td>
</tr>
<tr>
<td>Time (Ti)</td>
<td>4</td>
<td>109.43</td>
<td>27.358</td>
<td>42.033</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Tr x Ti</td>
<td>4</td>
<td>37.322</td>
<td>9.3306</td>
<td>14.336</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Res</td>
<td>20</td>
<td>13.017</td>
<td>0.65087</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>174</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference at 0.05 significance level
Figure 9. Proximal Analysis Responses for harvested and unharvested *M. pyrifera* through time under short-term harvesting in the Point Loma Kelp Forest: A = Variation of lipid content, B = Variation of the energy content expressed in calories/g, C = Variation of Protein content, D = Variation of ash content.
Figure 10. Alginate quality in individuals exposed to repeated harvesting and unharvested through time under short-term harvesting in the Point Loma Kelp Forest: A = Alginate viscosity. B = Alginate Compressive Strength of gel produced. C = Alginate yield.
Table 7. Variables Contributing with High Percentage to the Significant Variation Found between Days

<table>
<thead>
<tr>
<th>Days</th>
<th>Variable</th>
<th>Contribution %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&amp;15</td>
<td>Yield</td>
<td>36.71</td>
</tr>
<tr>
<td></td>
<td>Proteins</td>
<td>32.26</td>
</tr>
<tr>
<td></td>
<td>Energy</td>
<td>12.96</td>
</tr>
<tr>
<td></td>
<td>Lipids</td>
<td>12.69</td>
</tr>
<tr>
<td>15&amp;30</td>
<td>Proteins</td>
<td>34.73</td>
</tr>
<tr>
<td></td>
<td>Viscosity</td>
<td>17.17</td>
</tr>
<tr>
<td></td>
<td>Lipids</td>
<td>14.96</td>
</tr>
<tr>
<td></td>
<td>Yield</td>
<td>13.33</td>
</tr>
<tr>
<td></td>
<td>Strength</td>
<td>12.54</td>
</tr>
<tr>
<td>30&amp;60</td>
<td>Viscosity</td>
<td>34.12</td>
</tr>
<tr>
<td></td>
<td>Strength</td>
<td>30.74</td>
</tr>
<tr>
<td></td>
<td>Energy</td>
<td>20.41</td>
</tr>
<tr>
<td></td>
<td>Lipids</td>
<td>11.33</td>
</tr>
<tr>
<td>60&amp;90</td>
<td>Viscosity</td>
<td>41.85</td>
</tr>
<tr>
<td></td>
<td>Strength</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td>Energy</td>
<td>17.17</td>
</tr>
<tr>
<td></td>
<td>Lipids</td>
<td>14.85</td>
</tr>
</tbody>
</table>

Table 8. Nested PERMANOVA Results for Chilean Populations. Treatment Under Harvesting for 20 Years Against Treatment with No Harvesting (n=3)

<table>
<thead>
<tr>
<th>Nested Permanova Table results</th>
<th>Sources</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>Pseudo-F</th>
<th>p (Perm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment (Tr)</td>
<td>1</td>
<td>49.098</td>
<td>49.098</td>
<td>2.3183</td>
<td>0.105</td>
</tr>
<tr>
<td></td>
<td>Site (Tr)</td>
<td>4</td>
<td>84.714</td>
<td>21.179</td>
<td>13.245</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>Res</td>
<td>12</td>
<td>19.188</td>
<td>1.599</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17</td>
<td>153</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference at 0.05 significance level
Figure 11. Long-term alginate response following 20 years of repetitive harvesting (H) versus never harvested (UH) populations from Northern Chile. Point Loma was used as an outgroup. A = Average alginate viscosity. B = Average compressive strength of gel. (H/3 and UH/3) were lost in the analysis. C = Average alginate yield.
was found between harvesting treatments ($p=0.105$) suggesting that repetitive harvesting of *M. pyrifera* populations in northern Chile does not result in significant negative effects on chemical compounds of the final alginate product or on nutritional quality of the tissue to be used as food for abalone aquaculture. Average results of each response variable for each site and treatment (harvested and unharvested) are presented in Table 9. Notice that viscosity in Chilean populations was considerably higher than populations in San Diego (average of 1446 mPa s and 78 mPa s respectively).
Table 9. Long-Term Analysis. Average Values per Treatment/Site for Chile and the Outgroup Point Loma with their Respective Standard Deviation Values

<table>
<thead>
<tr>
<th>Sites</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Proteins (%)</th>
<th>Lipids (%)</th>
<th>Crude Fiber (%)</th>
<th>E.L.N</th>
<th>Energy (Cal/g)</th>
<th>Alginate yield (%)</th>
<th>Viscosity (m Pa s)</th>
<th>Compressive Strength (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Point Loma</strong></td>
<td>2.61 ± 0.07</td>
<td>37.97 ± 0.27</td>
<td>14.42 ± 0.32</td>
<td>0.34 ± 0.01</td>
<td>5.04 ± 0.12</td>
<td>42.56 ± 0.78</td>
<td>4540.66 ± 33.04</td>
<td>16.51 ± 0.43</td>
<td>74 ± 7.01</td>
<td>1530 ± 167.74</td>
</tr>
<tr>
<td><strong>Harvested/Site 1</strong></td>
<td>9.63 ± 0.07</td>
<td>39.51 ± 0.36</td>
<td>10.86 ± 0.13</td>
<td>0.68 ± 0.02</td>
<td>5.42 ± 0.06</td>
<td>43.53 ± 0.44</td>
<td>4145.66 ± 18.71</td>
<td>19.21 ± 0.81</td>
<td>1335 ± 121.35</td>
<td>2501 ± 636.90</td>
</tr>
<tr>
<td><strong>Harvested/Site 2</strong></td>
<td>9.04 ± 0.22</td>
<td>45.27 ± 0.34</td>
<td>10.41 ± 0.24</td>
<td>0.51 ± 0.08</td>
<td>5.51 ± 0.15</td>
<td>38.30 ± 0.50</td>
<td>3782.44 ± 37.89</td>
<td>0.76 ± 0.65</td>
<td>121.35 ± 14.36</td>
<td>3339 ± 0</td>
</tr>
<tr>
<td><strong>Harvested/Site 3</strong></td>
<td>9.57 ± 0.01</td>
<td>40.27 ± 0.24</td>
<td>10.65 ± 0.08</td>
<td>0.60 ± 0.06</td>
<td>5.62 ± 0.08</td>
<td>42.82 ± 0.30</td>
<td>4227.45 ± 20.98</td>
<td>0.71 ± 0.65</td>
<td>1673 ± 29.63</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Un-Harvested/Site 1</strong></td>
<td>10.96 ± 0.22</td>
<td>36.64 ± 0.14</td>
<td>10.62 ± 0.01</td>
<td>0.56 ± 0.05</td>
<td>5.61 ± 0.02</td>
<td>46.57 ± 0.12</td>
<td>4515.64 ± 22.10</td>
<td>0.37 ± 0.65</td>
<td>800 ± 62.45</td>
<td>2824 ± 142.49</td>
</tr>
<tr>
<td><strong>Un-Harvested/Site 2</strong></td>
<td>11.79 ± 0.13</td>
<td>37.88 ± 0.14</td>
<td>11.13 ± 0.16</td>
<td>0.57 ± 0.06</td>
<td>5.77 ± 0.01</td>
<td>44.65 ± 0.31</td>
<td>4472.40 ± 25.70</td>
<td>0.75 ± 0.65</td>
<td>1452 ± 161.13</td>
<td>3551 ± 264.72</td>
</tr>
<tr>
<td><strong>Un-Harvested/Site 3</strong></td>
<td>11.23 ± 0.16</td>
<td>41.33 ± 0.37</td>
<td>10.63 ± 0.22</td>
<td>0.46 ± 0.02</td>
<td>5.97 ± 0.02</td>
<td>41.61 ± 0.54</td>
<td>4018.27 ± 22.03</td>
<td>0.34 ± 0.65</td>
<td>1940 ± 200.75</td>
<td>ND</td>
</tr>
</tbody>
</table>

All values ± Standard Deviation
ND: No Data
DISCUSSION

Variability in seaweed chemical constituents due to oceanographic factors is well known. Of these, seasonal variation in oceanographic conditions (Kain 1982, Rodriguez-Montesinos and Hernandez-Carmona 1991), geographic distribution (Haug and Jensen 1956; Rodriguez-Montesinos and Hernandez Carmona 1991), currents, depth and temperature (Percival and McDowel 1967) are main drivers of this variability. Consequently, this likely resulted in the corresponding variability in the chemical compounds extracted from the kelp tissue through time. Specifically, average alginate yield in Point Loma ranged from 9.04% to 19.59% which is considerably low in quality, but similar to other values for this species in summer (Rodriguez-Montesinos and Hernandez-Carmona 1991). Populations from Chile did not differ much from populations of San Diego with respect to these values. In fact, values for the main chemical constituents were similar between harvested and unharvested populations, with variability between them being relatively close to natural fluctuations. This suggests that repeated harvesting did not result in significant negative effects on kelp tissue quality. In contrast, significant differences in viscosity and compressive strength were observed between harvested and unharvested populations. It appears to be caused by the location of the sites and their level of exposure to wave action. The higher alginate viscosity in Chilean populations over the average viscosity of San Diegan populations may be taken into consideration when biomass and quality of the product are being evaluated for future extraction in Chile.

Variation in alginate quality through time has been described for several species of brown algae (Rodriguez-Montesinos and Hernandez-Carmona 2008; Zubia et al. 2008) including *M. pyriforma* (Kelco 1976; Rodriguez-Montesinos and Hernandez-Carmona 1991).
These studies have shown variation within the same individual according to tissue type, and among different individuals in a population according to age structure (McKee et al. 1992) suggesting a high degree of variability in these measures. This variability can easily explain the spatial and temporal fluctuations observed in this study over short and long-term periods of harvesting.

*M. pyrifera* is a great source of carbohydrates because of sugars, soluble carbohydrates, and the higher content of alginic acid (Hong and Hien 2004). In San Diego, 42% of the total content was carbohydrates, and similar concentrations were found in Chilean populations (harvested site 41.54% and un-harvested site 44.2%). However, values were lower than the ones found by Ortiz et al. (2009), who described a 75% content of carbohydrates in samples from Chile. This lower value may be explained since sample procedure was conducted in summer when most of the individuals are older and nutrients are lowest (Rodriguez-Montesinos and Hernandez-Carmona 1991). However, there is no description of the season when the study by Ortiz collected those samples, so it cannot be compared.

The second major constituent was ash, which represents the content of minerals, cations, anions, and chloride that are assimilated by the algal tissue (Larsen 1975; Rodriguez-Montesinos and Hernandez-Carmona 1991; Ortiz et al. 2009) and even though it has high values (from 37.97% in San Diego to 41.69% in Chile), it does not represent any important nutritional value for aquaculture. Two of the most important components of the algae used for abalone food are proteins and fiber. Populations from San Diego (14.4%) shown higher content of proteins than Chilean (10.6% and 10.7%) both collected in the same season. In general concentrations are not presented in high concentrations for this species. For instance,
Kelco (1976) reported a value of 5.6% and Rodriguez-Montesinos and Hernandez-Carmona (1991), 5.13% to 12.72%. If compared with other species (Larsen 1975; Ortiz et al. 2009) numbers remained higher or equal justifying its use in the food industry. Algae are also an important source of fiber (Lahaye 1991). Properties to improve or help digestion are very important when is used as food in aquaculture. Other studies have shown values of 6% to 7% (Kelco 1976), 7.81% (Ortega and Zaragoza 1983) and 7.02% to 8.86% (Rodriguez-Montesinos and Hernandez-Carmona 1991). Our Values of 5.04% in San Diego and 5.51 to 5.78% in Chile were slightly lower but does not differ significantly with previews literature. Unfortunately we were not able to measure percentage of crude fiber for the short-term experiments.

Finally, lipids are present in very low concentrations in *M. pyrifera* tissues. For example, North (1971) reported concentrations between 0.5 to 2%, Kelco (1976) reported 1% to 1.2, and Rodriguez-Montesinos and Hernandez-Carmona (1991) reported an average of 0.87% during summer from populations of Baja California, Mexico. Our values from San Diego ranged from 0.35% at the beginning of the experiment to 0.002% at the end of summer season. These low values are consistent with the low values obtained in others constituents and again it may be because experiments were conducted over senescence individuals (Rodriguez-Montesinos and Hernandez-Carmona 2008). In Chile, Ortiz et al. (2009) reported lipids values of 0.7% in a population of *M. pyrifera* located northern Chile. This value is very similar to the averages of 0.59% in harvested sites and 0.43% in un-harvested sites described here for two different location within northern Chile which also suggest no significant variation due to harvesting activity.
The primary conclusion from this study is that repetitive harvesting of *M. pyrifera* does not result in significant changes to the main chemical constituents used for industry. Secondly, variability in these constituents through time appears more strongly regulated by oceanographic conditions, not harvesting. Consequently, repetitive harvesting of natural populations for the extraction of alginates and/or aquaculture food (as recommended in Chapter One of this thesis) is possible without negatively impacting the final products. However, it is very important to establish and regulate methods of extraction that incorporate the social, economical and ecological aspect of the species. When combined with the results from chapter one of this thesis, the author strongly suggests the extraction of only half of the stipes per boulder for *M. pyrifera* in exposed populations from Northern Chile.
ACKNOWLEDGEMENTS

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