GATHERING, CONSUMPTION AND ANTIOXIDANT POTENTIAL OF CULTURALLY SIGNIFICANT SEAWEEDS ON OʻAHU ISLAND, HAWAIʻI

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BOTANY

AUGUST 2012

by
Georgia M. Hart

Thesis Committee

Tamara Ticktin, Chairperson
Heather McMillen
Celia Smith

Keywords: *limu*, macroalgae, traditional knowledge, Native Hawaiian, antioxidant, eutrophication

To the beauty and diversity of our shared human heritage

ACKNOWLEDGEMENTS

I would first like to express my gratitude for the support and guidance of my thesis committee, the Department of Botany, fellow graduate students and members of the Ticktin Laboratory. Tom Ranker and Alison Sherwood for their leadership within the department during my degree program. My chairperson, Dr. Tamara Ticktin, for providing me holistic support and for having a positive and enthusiastic attitude that kept me moving forward. Also to Dr. Ticktin for creating a welcoming and rigorous atmosphere for interdisciplinary research. Dr. Heather McMillen for teaching me ethnographic approaches to research and for consistently having high standards for my work, including the detailed feedback on this manuscript. Dr. Celia Smith for instruction in algal ecology, for sharing her own expertise as well as the knowledge passed to her through Dr. Isabel Aiona Abbott, and for consistently upholding the importance of my work. Ticktin lab members Anita Varghese, Isabel Schmidt, Lisa Mandle, Tamara Wong, Katie Kamelamela, Daniela Dutra, Shimona Quazi, Dr. Ivone Manzali and Clay Trauernicht for sharing knowledge and resources as well as providing feedback on my work at each stage in its development. A special thanks to Katie Kamelamela for helping to connect me to community members and for teaching me about Native Hawaiian gathering through her own work. Dr. Ivone Manzali for general feedback on the antioxidant component of my work and for helping me to explore the ethnopharmacological relevance of the macroalgal species investigated in this study.

A heartfelt thank you to the public high school science teachers, Dana Hoppe, Suzie Wallace, Matt Dillon, Lynette Low, Jeff McKeown, Channing Llaneza, Mandy Llamedo, Tim Harrison and JJ Feurer who welcomed me into their classrooms and made this thesis work possible. To the high school students who allowed their experience and knowledge to form the basis of much of the work incorporated into this study and especially those students who undertook an interview with a community member. Thank you also to those community members who took the time to share their knowledge with student interviewers.

Thank you to the cultural practitioners, Luwella Leonardi, Eric Nourrie, Pililua Keopuhiwa, Leimomi, Constance Castillo, and the many adults interviewed by students involved in this study, who openly and generously shared their knowledge and who allowed me to record our conversations together. Thank you to my teachers and friends Wally Ito and Uncle Henry Chang-Wo for patiently and generously spending time with me and explaining key concepts to me and for their dedication to *limu* and Native Hawaiian cultural practice. Orion Ostanbro for spending time at the ocean to share his *limu* knowledge.

Thank you to the National Science Foundation Graduate Research Fellowship Program for funding my tuition and living expenses and allowing me the flexibility to pursue this research topic. The Beatrice Krauss Fellowship for supporting Ethnobotany and providing me with funding for research supplies and travel. The

University of Hawaiʻi at Mānoa Graduate Student Organization for supporting my travel to meetings and cultural gatherings.

Thank you to Dr. Anthony Wright for informal lessons on chemistry and for use of his lab and materials at the College of Pharmacy in Hilo. Dr. Dovi Kelman for teaching me laboratory methods, helping with analysis and providing me a place live for part of my stay in Hilo. Nicole Tabandera, for key support with lab procedures, for assisting in data collection, and for spending time with me during my stay in Hilo. Dr. Karla McDermid for working with me to identify my voucher specimens and for advising me on wet voucher collection. Dr. Alison Sherwood's lab (and particularly Kimberly Conklin) for molecular confirmation of specific specimens. Thank you to Dr. Wen Sun of Marine Agrifuture, LLC. (Olakai Hawaiʻi) for providing cultivated *Gracilaria* spp. samples used in this study.

Thank you to Haley Hendricks for lab and field assistance on the eutrophication project. Brian Chrysler for transcribing interviews with *limu* gatherers. Jennifer Bufford for lab assistance and for helping me to problem solve on multiple occasions. Fred Reppun for connecting me with Dana Hoppe. Kasha Ho for allowing me to participate in her afterschool garden club in Waiʻanae and for brainstorming project ideas with me. Kenneth Hamel and Amy Carlisle for allowing me to tag along for snorkeling excursions and for helping me formulate and troubleshoot my research plans. Marianela Zanolla Balbuena for carefully explaining to me the precise ways in which she measured the photosynthetic rate of *Asparagopsis taxiformis*.

William Aila for providing suggestions for my socio-cultural study, for helping me to understand the cultural context of my work and for encouraging the work shared herein. Dr. Al Keali‘i Chock for providing me with resources to begin my study and for teaching me about Hawaiian Ethnobotany. Dr. Kasey Barton for advice on chemical approaches and alerting me to limitations of colorimetric assays. Dr. Inderjit for methodological support with polyphenolic determination. Dr. John Paul Bingham for providing Folin Reagent for the eutrophication study. Stephanie Saephan for assistance in creating maps. Dr. Jonathan Price for feedback on my study and advice through the graduate school process. To all my family and friends for their love and support.

I would like to thank the late Dr. Isabel Aiona Abbott. While I did not have the opportunity to meet her, this study would not have been possible without her passion, dedication and enormous contributions to the taxonomy and ethnobotany of the Hawaiian macroalgae.

ABSTRACT

Gathering wild plants provides multiple benefits to indigenous cultures along physical, spiritual and psychological dimensions. Wild-gathered seaweeds (*limu*) are a prominent component of Native Hawaiian diet and culture, but have been under-studied for their nutritional benefits and cultural use. In order to investigate the contemporary levels of wild seaweed gathering and consumption, the factors influencing the prevalence of gathering, and to explore potential disease-preventative benefits wild seaweeds provide, this study uses a combination of ethnographic, pharmacological and ecological approaches to address the following questions: (1) How common is wild seaweed gathering and consumption among youth on Oʻahu today?, (2) Which demographic and familial characteristics predict gathering?, (3) What is the perception of change among gatherers in the abundance of wild seaweeds over time?, (4) How does the antioxidant capacity of wild seaweeds differ from that of cultivated seaweeds consumed on Oʻahu?, and (5) How may eutrophication influence the antioxidant values of wild seaweeds? Levels of gathering and consumption were assessed with surveys of public high school juniors and seniors as well as through semi-structured interviews with adult *limu* gatherers. Antioxidant activity was assessed with laboratory assays. One-fifth of surveyed students had gathered wild seaweeds and one-third had consumed them, with a larger proportion of gathering and consumption among Native Hawaiian students. Familial gathering was the strongest predictor of student gathering, with Hawaiian ethnicity being a stronger predictor among male students compared to female students. As opposed to pre-Contact Hawaiʻi, more male than female

Hawaiian students reported gathering wild seaweeds. There was a consistent perception of decline in abundance of wild seaweeds among *limu* gatherers with harvest by non-traditional means and pollution as the most commonly cited reasons for this decline. Wild seaweeds provided higher levels of antioxidants than cultivated seaweeds, and eutrophication was correlated with a decline in antioxidant power. Taken together, these results demonstrate that traditional gathering practice has persisted and adapted through time despite urbanization, commercialization and environmental degradation and also that wild seaweeds likely provide a greater level of a particular disease-preventative property than their cultivated counterparts, with nitrogen loading potentially decreasing this benefit. This suggests that conservation of nearshore environments to promote native seaweeds would also support Native Hawaiian cultural practice and health and that promoting traditional gathering protocol would support more sustainable harvest.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DPPH – 2, 2-diphenyl-1-picrylhydrazyl stable radical

FRAP - ferric reducing antioxidant power

TEK - traditional ecological knowledge

WEP – wild edible plant

PREFACE

My motivation for this work stems from a desire to elevate the place of traditional knowledge held by local or indigenous people within the western scientific discourse. It is a means of helping to make visible that which has often been invisible. While we cannot undo the wrongs of the past, they can be acknowledged as we slowly make amends.

This work focuses on indigenous health and cultural practice, with regard to wild gathered edible seaweeds (*limu*). I undertook this project while a resident of Oʻahu Island. Though I do not have a cultural or ancestral tie to the island or to *limu*, I have a great amount of love and appreciation for both.

Working with students in public high schools was a deliberate component of the design of this study. I intended to make use of my prior experience as a public school teacher in order to expand the contribution I could make to the broader community as a research scientist. Teaching students about science and *limu* and including students in the research process was my attempt to give back while at the same time receiving the help I needed from students and their communities.

This work is a small contribution that only touches upon the richness of knowledge and practice relating to *limu* alive on Oʻahu and the complexity of the chemistry, flavor and health benefits of this wild-gathered traditional food. I am grateful for the lessons I have learned and the knowledge I have gained during this process and hope to continue to enrich my understanding and appreciation for the biocultural diversity around us.

CHAPTER 1

PREVALENCE AND ANTIOXIDANT BENEFIT OF SEAWEED GATHERING AND CONSUMPTION AMONG PUBLIC HIGH SCHOOL STUDENTS ON OʻAHU ISLAND

INTRODUCTION

Traditional diets are place-based combinations of plant, animal and mineral foods, acquired from the local environment and consumed by a specific cultural group (Kuhnlein and Receveur 1996). Among indigenous people, gathering wild foods and preparing traditional cuisine is an expression of cultural heritage. Gathering provides health benefits and often accompanies an understanding of the location, seasonality, growth and reproduction of gathered species, as well as the knowledge of the proper means of processing and preparing specific dishes. Traditional knowledge of this kind has been recognized for its intrinsic value (UNESCO 2003) and for its potential to help address world health and environmental crises (Alexander *et al.* 2011, Peloquin and Berkes 2009, Cunningham *et al.* 2008, Gari 2004, Gadgil *et al.* 1993, Redford and Padoch 1992, Schultes 1989).

As political, economic, environmental and cultural changes have caused a shift in the availability, quality and valuation of traditional foods, many indigenous peoples have begun to consume more processed and refined foods, fewer plant foods and diets higher in fat—characteristics of a more ‘westernized’ diet (Throw *et al.* 2011, Williams *et al.* 2001, Brand-Miller and Holt 1998, Whiting and MacKenzie 1998, Kuhnlein and Receveur 1996). Many of the indigenous groups who have undergone this dietary transition have experienced over-representative levels of nutrition-related "diseases of affluence" –obesity, diabetes and heart disease (Alberti *et al.* 2007, Zimmet *et al.* 2003, Rowley *et al.* 2000, Thorburn *et al.* 1987, Hughes 1998, Odom 1998). These inter-related diseases share common risk factors including poor diet, physical inactivity and genetic components (Alberti *et al.* 2007, Rowley *et al.* 2000). For indigenous people, the loss of land (with concomitant loss of access to traditional foods and gathering rights) is also inextricably tied to a diminished health status (Trinidad 2012, Cunningham 2010, Zimmet *et al.* 2003, Casken 1999, Kuhnlein 1992). Therefore, an interdisciplinary approach that considers nutrition and lifestyle choices in combination with the cultural, political and environmental context will be better positioned to address these health problems and disparities than disciplinary studies.

Nutritional and lifestyle intervention programs aimed at reversing the progression of chronic illness have shown great success in reducing symptoms in obese and diabetic participants (Zimmet 2003, Tuomilehto *et al.* 2001). Intervention studies in which indigenous participants with chronic illness revert back to traditional foods (Shintani 1991, 1994) or back to a combination of traditional foods and traditional lifestyles (O'Dea 1984) have also attained rapid and impressive reductions in the symptoms of diabetes, obesity and heart disease (Rowley 2000, 2001). Given the demonstrated benefit of traditional diets in preventing disease, investigating the specific characteristics of traditional foods that distinguish them from market (‘western’) foods may clarify the sources of these health benefits and can contribute dietary suggestions for treatment of epidemic diseases such as obesity and diabetes.

The market foods that are available and affordable to indigenous communities tend to emphasize refined carbohydrate, saturated fat and sugar and contain low levels of fiber, factors known to contribute to diminished health status (Popkin and Gordon-Larsen 2004). For example, among the Inuit, increased consumption of market food and decreased consumption of traditional foods (e.g., marine mammals, caribou, berries) is correlated with increased intake of saturated fat (Egeland *et al.* 2009); a well-established risk factor in development of cardiovascular disease (Popkin and Du 2003). Wild fruits consumed by Australian Aboriginals have been shown to provide more fiber than commercially available fruits (Brand-Miller and Holt 1998), suggesting that traditional foods may provide greater protection from cancers such as colon cancer (Patarra *et al.* 2011).

Differences in the nature of the carbohydrates found in traditional plant foods and market foods are understood to have important health consequences. Many traditional plant foods have lower glycemic indices than ‘western’ foods (Willet *et al.* 2002, Brand *et al.* 1990, Thorburn *et al.* 1987), meaning the carbohydrates are released more slowly into the blood, avoiding spikes in blood sugar and subsequent insulin response which have been linked to the onset and progression of diabetes (Cordain *et al.* 2005). For example, one study found that the majority of traditional Pacific Islander and Australian Aboriginal starchy foods were digested more slowly than starchy market foods (potato, white bread, wholemeal bread, spaghetti, white rice, and corn) (Thorburn *et al.* 1987). Among the Pima of the Southwestern United States, increased consumption of traditional foods (e.g., tepary beans, corns, mesquite pods, acorns) was associated with greater intake of complex carbohydrates, fiber and vegetable protein, as well as with decreased risk of developing diabetes (Williams *et al.* 2001).

Traditional plant foods, and particularly wild edible plants (WEP), have also been recognized for their unique contribution to health, including the contribution of non-nutrient phytochemicals. Wild edible plants—plants not influenced by human behavior—are integral to the diet of traditional communities and provide important health benefits that are only beginning to be explored (Jeambey *et al.* 2009, Grivetti and Ogle 2007, Orech *et al.* 2007, Johns *et al.* 1999, Etkin 1996). They are often a key source of micronutrients, fiber and protein, particularly for women and children, and can provide food security, particularly in the agricultural off-season (Termote *et al.* 2011, Gari 2004, Freiberger *et al.* 1998, Eder 1978). For example, among the Wet'suwet'en of northwest British Columbia, medicinal teas, green vegetables and rice root bulbs provide vitamin C in the late winter and spring when it is not readily available from other traditional sources (Gottesfeld 1995). WEP are also increasingly recognized for their contribution of non-nutrient phytochemicals, or secondary metabolites, with pharmacological action (Chapman *et al.* 1997, Etkin 1996, Johns 1996, Vera-Guzman *et al.* 2011) including antioxidant (McCune and Johns 2007, Simopoulos 2001, Trichopoulou *et al.* 2000), anti-cholesterolemic

(Johns *et al.* 1999), antidiabetic (Ooi *et al.* 2011, Baldea *et al.* 2010), and antimalarial (Etkin and Ross 1997) activities. Wild plants, because they have not been altered through domestication or other human influence, may be expected to have more potent chemical content and stronger medicinal value compared to cultivated plants (Etkin 1996, Johns 1996).

Seaweeds are a wild gathered food and medicine used by traditional people in many parts of the world (Hong 2011, Dillehay *et al.* 2008, Ostaff 2007, Turner 2003, Xia and Abbott 1987, Abbott 1978). Seaweeds are also known to be rich sources of bioactive secondary compounds with anti-cancer, anti-diabetic, anti-inflammatory, and anti-bacterial activities (Lee *et al.* 2011, Maschek and Baker 2008, Smit 2004). Increasingly, seaweeds have also been recognized as rich sources of antioxidants (Souza *et al.* 2011). Despite the potential to draw links between the disease-preventative qualities of seaweeds recognized by nutritional and chemical scientists and the cultural uses of seaweeds as components of traditional diet and medicine, the field of marine ethnopharmacology remains largely unexplored (McClatchey 2009).

One possibility, with supportive evidence, is that secondary compounds in marine macroalgae ("seaweeds") have protective effects against obesity and diabetes. Greater consumption of macroalgae was correlated with decreased risk of type-II diabetes in Korean men (Lee *et al.* 2010), and this anti-diabetic effect was attributed to the non-digestible carbohydrate in algae, as well as to the secondary compounds which inhibit starch and sugar digestion and act as antioxidants. The polyphenolic compounds in marine macroalgae have anti-diabetic activity by inhibiting alpha-glucosidase and alpha-amylase which break down sugar and starch (Nwosu *et al.* 2011, Zhang *et al.* 2007, Zhang *et al.* 2006), while many compounds such as phlorotannins and bromophenol have demonstrated antioxidant activity, meaning they reduce damaging free radicals (Lee *et al*. 2011, Zhang *et al.* 2006, Yangthong *et al*. 2009, Kim *et al.* 2008, 2010). Antioxidants have been associated with the benefits of consuming fruits and vegetables and are thought to play a role in preventing the onset and progression of disease (Balasumdram *et al.* 2006). While oxidative stress has clearly been linked with the onset and progression of obesity (Lee *et al.* 2011), diabetes (Sheik-Ali *et al.* 2011), cancer and heart disease (Salvatore *et al.* 2005), the probable role of dietary antioxidants in disease prevention remains to be fully substantiated (Benzie and Wachtel-Galor 2012, Sheik-Ali *et al.* 2011, Belch *et al.* 2008, Manach 2004).

Prior to European contact (typically marked by the arrival of Captain James Cook in 1778), the Hawaiian diet consisted primarily of complex carbohydrates such as taro, sweet potato, yam, breadfruit and wild gathered greens such as seaweeds (~78% of calories from carbohydrate, Fujita *et al.* 2004, Hughes *et al.* 1998). Seaweeds are one of the traditional wild greens in Hawaiʻi that were used for food, medicine and ceremony and that continue to be gathered for these purposes today (Abbott 1996, 1992, 1978). Native Hawaiian seaweeds or macroalgae (also called *limu*[1]) had a particularly prominent role in pre-Contact Hawaiian diet as compared with other Pacific Island societies with comparable numbers of available species (Abbott 1992). The importance of *limu* in the Hawaiian diet is thought to have arisen because of the *kapu* system of social taboos. Under this system, women were forbidden from eating many types of food (e.g., pork, coconuts, most bananas, many fish), and so they went to the ocean and explored other sources of sustenance (Abbott 1978, 1996). While *limu* may have composed only a small portion of the total calories prior to western contact, it is likely that *limu* were consumed with most meals (Reed 1907) as an important condiment, adding flavor as well as necessary micronutrients, protein (McDermid and Stuerke 2003), and fiber (McDermid *et al.* 2005). Despite its low caloric contribution, *limu* could have significant impacts on human health through its secondary compounds and overall antioxidant potential, and, pre-Contact, this impact would have been most pronounced for Hawaiian women, who prepared and consumed their meals separately from men (Kamakau 1992).

Receiving health benefits from wild gathered foods necessitates being able to access them, having the knowledge to gather and process them and having the desire and motivation to do so. Despite cultural, economic and health benefits of utilizing wild plants (Nabhan *et al.* 2010, Gari *et al*. 2004), the rapid loss of traditional ecological

---

[1] The term "*limu*" in Hawaiian may refer to a diverse set of mostly aquatic and semi-aquatic photosynthetic organisms including marine and freshwater algae, mosses, liverworts and lichens. For most Hawaiians (from ancient to contemporary times) the word means "edible seaweed" and this is how it is used throughout the text. (Abbott 1996)

knowledge (TEK) surrounding wild plants is well documented (Rijal 2008, Ohmagari and Berkes 1997). Important factors in this loss include participation in the formal education system (which reduces time for plant gathering as well as valuation of the practice) (McCarter and Gavin 2011, Cruz Garcia 2006, Ladio and Lozado 2004, Turner 2003, Ohmagari and Berkes 1997, Kuhnlein and Moody 1989), participation in the wage economy (Kuhnlein and Moody 1989, Eder 1988), commercialization of natural resources (Turner 2003), environmental degradation (Toledo and Barrera-Bassols 1984) including pollution (Turner 2003), lack of access to gathering areas, including legislation that restricts gathering (Kuhnlein and Moody 1989), lack of local autonomy concerning resource use (Johannes 2002, Byer *et al.* 2001), changes in taste appreciation, the influence of introduced culture (Rijal 2008) including introduced educational and community services (Benz *et al.* 2000), the introduction of "easy" western foods (Schonfeld-Leber 1979), and social stigma (Cruz Garcia 2006). In Hawai'i, for example, the import of western foods (Abbott 1978), western culture, the loss of land and the lack of access and tenure over land and sea (Gruelle 1946, Johannes 2002) contribute to the loss of TEK surrounding resource use. It is important to recognize, however, that traditional ecological knowledge and traditional resource management systems also adapt, and have continually been adapting, to changing social, political, economic and ecological conditions (Poepoe *et al.* 2007, Ticktin *et al.* 2006).

Among Native Hawaiians[2], as with many indigenous peoples, traditional knowledge is passed to younger generations orally through storytelling and chants, as well as through practice, imitation and demonstration, or "learning by doing." It is through apprenticeship and repetition that new skills are acquired (Poepoe *et al.* 2007, McGregor *et al.* 2003). This transmission occurs primarily through *'ohana* (family or kin groups—consanguine, affinal or fictive) with an emphasis on the knowledge of elders, or *kupuna* (McCubbin and Marsella 2009) and therefore intergenerational ties, which transfer Hawaiian ways of knowing, are critical to Hawaiian well-being and cultural continuity (McGregor *et al.* 2003). In investigating the current state and the health benefits of seaweed consumption and gathering in Hawai'i, it is critical to consider intergenerational transmission of the knowledge and skills necessary to gather and consume wild seaweeds, as well as changes in the availability of the natural resource and changes in the level of valuation of this cultural practice.

To examine factors regulating cultural continuity of seaweed gathering and to assess the potential role of edible Hawaiian seaweeds as antioxidants in the Hawaiian diet, this chapter of the study uses a combination of ethnographic and pharmacological approaches to address the following questions: (1) How common is wild seaweed gathering and consumption among youth on O'ahu today?, (2) Which demographic and familial characteristics predict gathering?, (3) What is the perception of change

---

[2] Throughout the text, the terms "Native Hawaiian" and "Hawaiian", when used in reference to people, refer to the original Polynesian settlers of the Hawaiian Islands and their descendents.

among gatherers in the abundance of wild seaweeds over time?, and (4) How does the antioxidant capacity of wild seaweeds differ from that of cultivated seaweeds consumed on Oʻahu? I hypothesized that gathering and consumption of wild seaweeds would be rare among non-Hawaiians (<10%) and relatively common among Hawaiians (~50%) with greater prevalence among female youth, and youth with family members who have gathered. I also hypothesized that adult gatherers would report a decline in wild seaweed abundance, given that most people I spoke to informally on this topic had this perception. Based on other studies comparing wild plants to their cultivated counterparts, I hypothesized that the antioxidant value would be higher among wild seaweeds compared to those that are cultivated.

METHODS

Antioxidant Determination

Collections

In order to determine the antioxidant power of wild and cultivated seaweeds eaten in Hawaiʻi, macroalgal specimens were collected and acquired with the aim to maximize the number of edible species important in pre-Contact or contemporary diet. This sampling strategy is important given that food analyses that base their sample selection on population intake are rare (Floegel *et al.* 2011). On June 21st and 22nd 2011, seven wild edible species, representing several of` the most important wild edible species available today, were collected by snorkeling at six sites on Oʻahu Island by G.H. (Figure 1). Seven co-occurring species, not known to be edible or very seldom used for food, were also collected for comparison with edibles. Four additional cultivated species (as well as one cultivated species that was also collected wild) were acquired from a seaweed cultivation farm or marketplace on June 28th 2011 (Table 1). At the time of collection or acquisition, both dry and wet vouchers were prepared for each specimen for identification. Morphological species identification was completed with the help of Dr. Karla McDermid (UH Hilo) using wet and dry vouchers for all wild-gathered specimens. Cultivated species of *Gracilaria* had been previously identified by Dr. Alison Sherwood's laboratory (UH Mānoa), or were confirmed during this study, using molecular techniques. Dry vouchers for all samples were deposited at the Joseph Rock Herbarium at the University of Hawaiʻi at Mānoa.

For each wild-collected species at each location, an aggregate sample of six to ten plants were collected and transported to the lab in seawater in a cooled container to protect the plants from thermal stress. The upper portion of plants (the portion that would typically be consumed) was used for analysis. Cultivated samples were purchased or acquired in ½ lb bags of wet material. Within five hours of collection, all samples were thoroughly cleaned in seawater to remove any sand and epiphytes and then washed again in freshwater. Samples were pat dry and their wet weights were recorded with an analytic balance. The wet weight of cultivated *Gracilaria parvispora*, *G. salicornia* and *G. tikvahiae* were not recorded and therefore wet weight was obtained by conversion of dry weight measurements using average percent-water values for these species obtained from McDermid and Stuerke (2004) (n=2, 5, and 3 respectively for each species). Wet weight for *Pyropia yezoensis* (i.e., nori, purchased dry) was calculated by soaking the species in water for 2 minutes, patting it dry and then weighing it on an analytic balance (n=5). After wet weight was recorded, all samples were stored for 12-24 hours in chilled coolers, and then partially freeze-dried at the Chemistry Department at UH Mānoa. Two days later they were transported to the School of Pharmacy at Hilo where they were dried completely (1-5 days) in a LABCONCO FreeZone 12 freeze drier. Dry weight was recorded with an analytic balance.

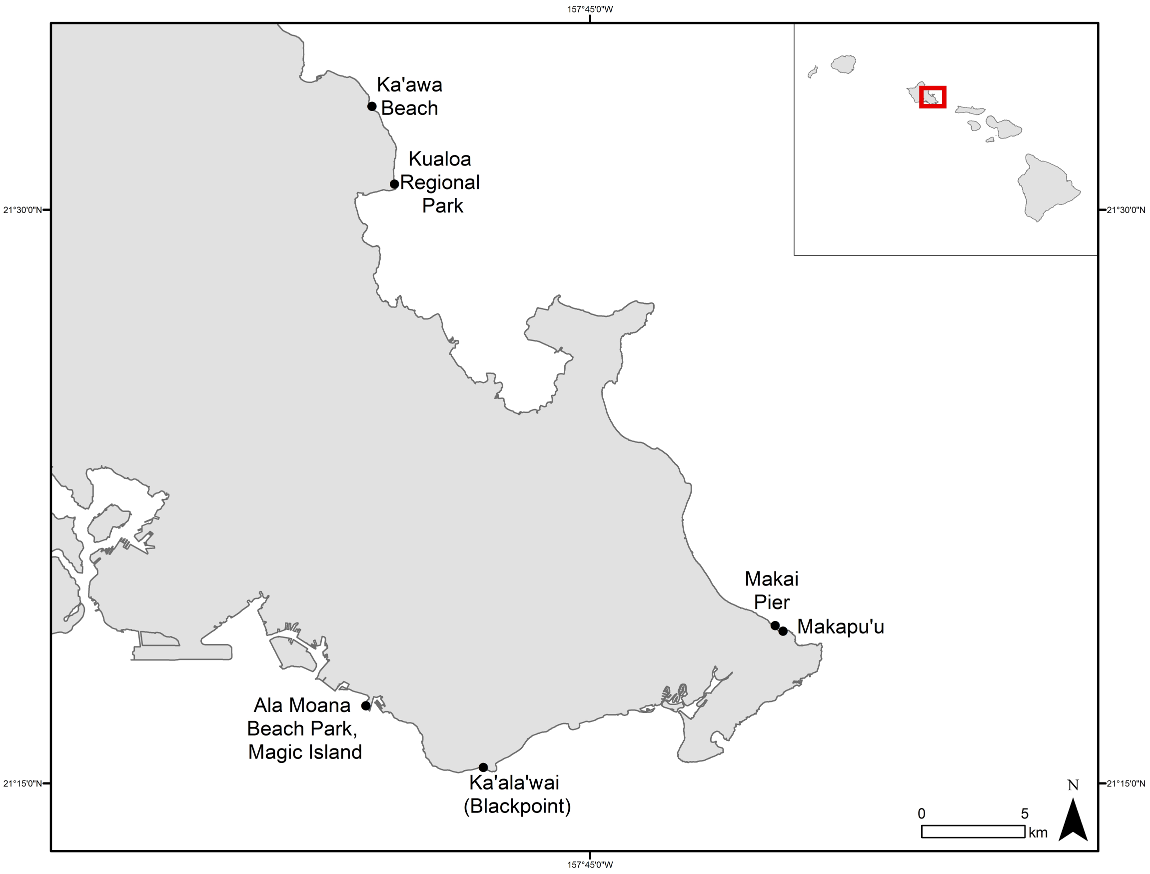


Figure 1. Map of eastern O‘ahu Island showing collection sites for wild specimens.

Table 1. Macroalgal sample information for antioxidant determination

Species	Common names*	Use for food in Hawai‘i	Native	Site or source on O‘ahu	Date of collection or acquisition
Rhodophyta					
Asparagopsis taxiformis (Delile) Trevisan de Saint-Léon	*limu kohu,* *limu līpehu,* *limu līpehe,* *limu koko* *limu līpa‘akai*	common	Yes	Ka‘ala‘wai (Black Point)	June 21st, 2011
Asparagopsis taxiformis (Delile) Trevisan de Saint-Léon	*limu kohu,* *limu līpehu,* *limu līpehe,* *limu koko* *limu līpa‘akai*	common	Yes	Makapu‘u beach	June 21st, 2011
Chondrophycus cartilaginous (Yamada) Garbary & J.T.Harper	not documented	not documented	Yes	Kualoa Regional Park	June 22nd, 2011
Hydropuntia spp. Montagne	not documented	not documented	No	Ala Moana Beach Park	June 21st, 2011
Gracilaria parvispora I.A.Abbott	ogo	common	Yes	Kahuku Olakai Hawaii, Marine Agrifuture, LLC.	June 28th, 2011
Gracilaria parvispora I.A.Abbott	ogo	common	Yes	Tamashiro’s Market, Honolulu	June 22nd, 2011
Gracilaria salicornia (C.Agardh) E.Y.Dawson	Gorilla ogo, ogo, robusta	common	No	Ala Moana Beach Park	June 21st, 2011
Gracilaria salicornia (C.Agardh) E.Y.Dawson	Gorilla ogo, ogo, robusta	common	No	Kahuku Olakai Hawaii, Marine Agrifuture, LLC.	June 28th, 2011
Gracilaria tikvahiae Mclachlan	ogo	common	No	Kahuku Olakai Hawaii, Marine Agrifuture, LLC.	June 28th, 2011
Hypnea spinella (C.Agardh) Kützing¥	limu huna	Seldom**	Yes	Ka‘awa Beach	June 22nd, 2011
Laurencia majuscula (Harvey) A.H.S.Lucas	not documented	not documented	Yes	Ka‘ala‘wai (Black Point)	June 21st, 2011
Martensia fragilis Harvey	*limu hā‘ula*	not documented	Yes	Makapu‘u	June 21st, 2011
Pyropia yezoensis (Ueda) M.S.Hwang & H.G.Choi¥¥	nori	common	No	Tamashiro’s Market (imported-Japan)	June 28th, 2011

Table 1. (Continued) Macroalgal sample information for antioxidant study

Phaeophyceae					
Dictyopteris plagiogramma (Montagne) Vickers	*limu līpoa*	common	Yes	Makai Pier	June 21st, 2011
Dictyopteris plagiogramma (Montagne) Vickers	*limu līpoa*	common	Yes	Tamashiro's Market (gathered on Maui)	June 28th, 2011
Dictyota acutiloba J.Agardh	*limu alani*	Seldom	Yes	Makai Pier (epiphytic)	June 21st, 2011
Dictyota acutiloba J.Agardh	*limu alani*	Seldom	Yes	Kualoa Regional Park	June 22nd, 2011
Sargassum aquifolium (Turner) C.Agardh[¥¥¥]	*limu kala*	somewhat common	Yes	Kualoa Regional Park	June 22nd, 2011
Sargassum polyphyllum J.Agardh	*limu kala*	Seldom	Yes	Kualoa Regional Park	June 22nd, 2011
Turbinaria ornata (Turner) J.Agardh	*limu kāhili*	not documented	Yes	Kualoa Regional Park	June 22nd, 2011
Chlorophyta					
Codium edule P.C.Silva	*limu wāwae'iole,* *limu a'ala'ula,* *limu a'ala,* *limu ala'ula*	common	Yes	Ka'ala'wai (Black Point)	June 21st, 2011
Ulva flexuosa[†] Wulfen	*limu 'ele'ele*[†]	somewhat common[†]	Yes	Ka'ala'wai (Black Point)	June 21st, 2011
Ulva lactuca Linnaeus[¥¥¥¥]	*limu pālahalaha,* *limu pāpahapaha,* *limu pahapaha,* *limu pakaiea*	Seldom	Yes	Ka'ala'wai (Black Point)	June 21st, 2011

*following Abbott (1996) and Aiona (2003); [¥]formerly *H. cervicornis*; [¥¥] formerly *Porphyra yezoensis*; [¥¥¥]formerly *S. echinocarpum*; [¥¥¥¥] formerly *U. fasciata*; **name and consumption documented for native species in genus *Hypnea* only (Aiona 2003); [†]*Limu 'ele'ele* has been linked specifically the species *Enteromorpha prolifera* (Abbott and Huisman 2004) and generally to the genus *Enteromorpha* (Abbott 1978, 1996, Reed 1907). The genus *Enteromorpha* now falls within *Ulva* (Hayden 2003). *Ulva* is undergoing taxonomic revision in Hawai'i using genetic markers. None of the morphological species designations match the molecular designations (O'Kelly *et al.* 2010). *Limu 'ele'ele* was at one time one of the most abundant and widely used of all *limu* (Pukui 1960) and common at *lū'au* (Hawaiian feasts) (Abbott 1978), but is now more difficult to locate on O'ahu (interviews with *limu* gatherers, this paper). Species designations follow Guiry and Guiry (2012).

Extractions

Dried algal material was extracted for two and then for twenty-four hours in methanol. The solution was filtered though cotton, then the solvent was evaporated off at low pressure using a rotary evaporator. Extract weight was recorded with an analytic balance. Extracts were then dissolved in dimethyl sulfoxide (DMSO) at 10 mg/mL and extract solutions were held at 4°C until use.

FRAP Assay

The ferric reducing antioxidant power (FRAP) assay measures the ability of the sample mixture to reduce ferric- tripyridyltriazine (FeIII-TPTZ) complex to the ferrous (II) form, at low pH, which results in an intense blue color with an absorption maximum at 593 nm (Benzie and Strain 1996). The FRAP assay was modified from the Benzie and Strain protocol (1996, 1999), also following Griffin and Bhagooli (2004). FRAP reagent (10 mL of 300mM acetate buffer (pH 3.6), 1 mL of 10mM TPTZ and 1 mL 20 mM $FeCl_3{\cdot}6H_2O$) was prepared and heated to 37°C for 30 minutes. The 300 mM acetate buffer was prepared by mixing 3.1 g of sodium acetate trihydrate ($NaOAc{\cdot}3H_2O$) with 16 mL glacial acetic acid and made to 1 L with ddH_2O. The TPTZ solution was prepared by mixing equal volumes of 10 mM TPTZ with 40 mM HCl.

FRAP reagent (150 μL) was added to each well in a 96-well plate. A blank reading was taken at 595 nm. Triplicate samples of 20 μL of each standard or sample were added to the wells. After 8 minutes, a second reading was taken at 595 nm. The difference in the absorbance between the two time periods, minus absorbance from of the DMSO (insignificant), was compared with a standard curve using known concentrations of Fe(II) ($FeSO_4 \cdot 7H_2O$, 25-1000 μmol, $r^2 > 0.99$) and then recorded as the FRAP value in μmol Fe(II) per unit wet weight (see Appendix C for values per unit extract and by dry weight).

DPPH assay

The DPPH assay measures the reducing potential of a sample solution towards the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) stable radical. The DPPH test solution turns from purple to yellow when reduced. Therefore the percent DPPH radical scavenging can be determined by measuring the reduction in the absorbance of the sample solution. This protocol used in this study was modified from Brand-Williams *et al.* (1995). The stock solution was prepared by dissolving 24mg of DPPH (2, 2-diphenyl-1-picrylhydrazyl) with 100ml MeOH and was stored at -20°C in brown bottle covered in foil. The working solution was prepared by making a 1:3 DPPH stock sol: HPLC grade MeOH to obtain an absorbance of 1.1±0.02 units at 490 nm. For each plate, 15 μL sample or standard was added to each well in triplicate. A reading was taken at 490 nm for each plate at 2 and 24 hours and the %DPPH quenching was calculated for each sample as the difference in the initial and final absorbances, divided by the initial absorbance. Antioxidant power was expressed as %DPPH scavenging at 24 hrs at (150 μg/μl) per mg wet weight (see Appendix C for values per μg extract and per mg dry weight).

Data Analysis

When comparing the antioxidant potential of wild species not eaten, wild edibles and cultivated edibles, the antioxidant value of two separate collections for *A. taxiformis*, *D. acutiloba* and *G. parvispora* were averaged before use in analysis. The sample of *Dictyopteris plagiogramma* from Tamashiro's Market was not used for these analyses since its age and processing varied from other wild samples and these variables are known to impact macroalgal antioxidants (Jimenez-Escrig *et al.* 2001). The antioxidant values for wild and cultivated species were square-root transformed to obtain normality (Shapiro-Wilk normality test) and then compared with Welch's t-test using R (R Core Development Team 2008). For the comparison of wild species not eaten with wild and cultivated edibles, see Appendix C.

Socio-cultural study

Classroom collaborations and in-class survey

In order to examine the frequency and determinants of seaweed consumption and gathering, the level of taste appreciation as well as to assess intergenerational changes in the frequency of gathering, high school juniors and seniors from six public high schools were asked to complete an in-class survey focusing on their seaweed consumption and gathering practices.

Approximately 350 Marine Science and Science elective high school junior and senior students at six public high schools on O'ahu Island (Figure 2) were involved in this research collaboration. G.H. visited classrooms as a science expert, provided lesson materials, references, and delivered interactive lectures and hands-on activities related to the biological and cultural importance of marine macroalgae in the Hawaiian Islands as well as careers in science. These collaborations were established in order to ensure community involvement and community benefit during the research process itself and not only through the research findings. High schools were selected for this study in an attempt to represent the island's regional diversity and population density. Students were selected based on age (juniors or seniors only) and based on the course of study (marine science in all cases except for Waiʻanae High School, where students were engaged in a science elective course involving seaweed cultivation). Students who participated in the study were asked to complete an in-class survey focusing on their seaweed gathering and consumption patterns, as well as knowledge of seaweed names, and the occurrence of seaweed gathering among the student's parents and grandparents. The survey included 16 questions (Appendix A) and took students approximately 10-15 minutes to complete. It was completed in-class, while G.H. and the teacher were present. The survey was intended to address the research questions of this study, as well as to indicate gaps in knowledge among high school students that could potentially be addressed through the public school system.

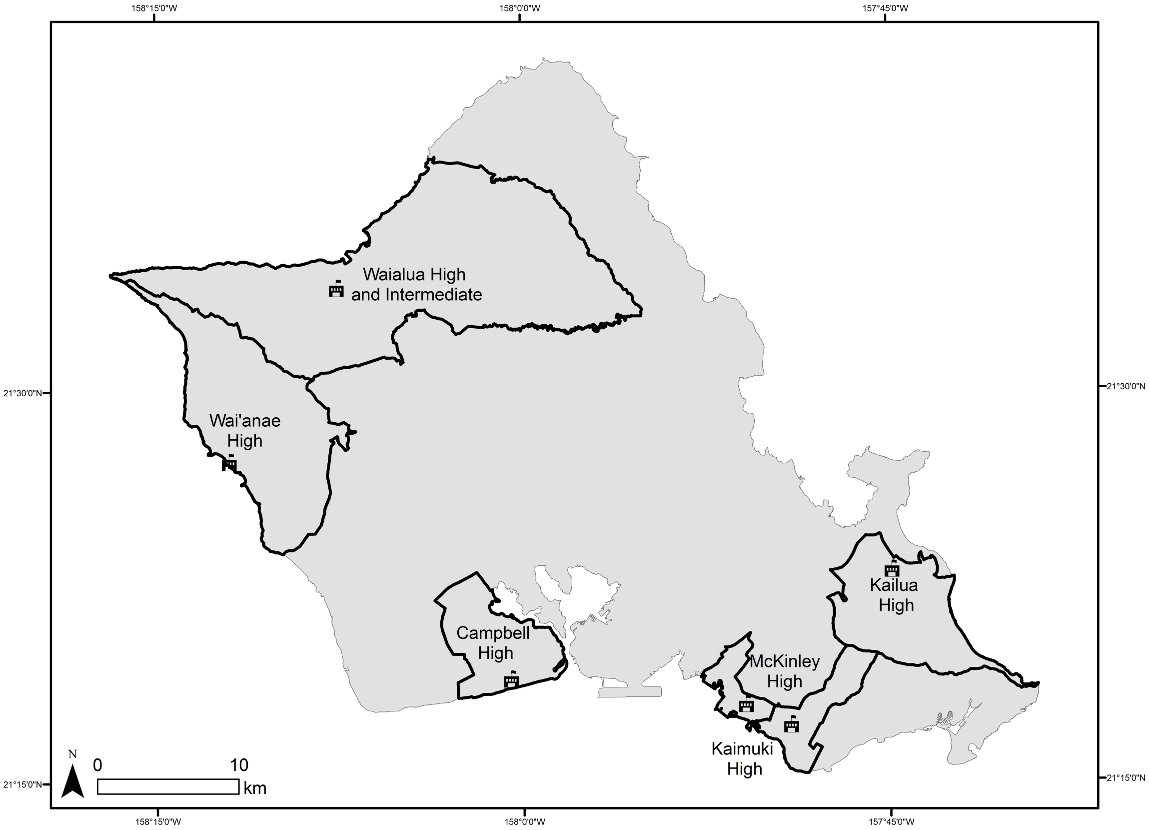


Figure 2. Location of public high schools and school districts where collaborations were undertaken and surveys were administered.

Student interview homework assignment

In order to provide a broader understanding of wild seaweed gathering and consumption on Oʻahu Island, to investigate changes in the quality and quantity of wild seaweeds over time, and to increase student level of understanding of the importance of seaweed gathering and consumption in their community or family, students at four high schools (Campell, Kailua, McKinley and Waiʻanae) were asked to complete an interview homework assignment. Fifty-five students interviewed members of their communities, and 30 of these interviewees were used for further analyses because that set of interviewees were familiar with wild seaweeds (through gathering, familial gathering or consumption; hereafter referred to as "*limu*-knowledgeable adults") and were was least 20 years older than the student (participants ranged from 36 to 68 years old). The interview was approximately 10-20 minutes and it focused on changes observed to the nearshore environment, gathering decisions and seaweed name knowledge. Students were assigned a list of questions for the interview and were also asked to come up with at least one question on their own (Appendix A). Interviewees were also asked to indicate if they would be interested to participate in a follow-up interview with the investigator.

Adult seaweed gatherer semi-structured interviews

In order to provide a more in-depth context of contemporary seaweed gathering and changes in seaweed abundance, adult seaweed gatherers were identified through the homework assignment described above or through online video commentaries (YouTube), the annual Hana Limu Festival on Maui, or by recommendation of one interviewee by another ("snowballing"). Seven semi-structured interviews (4 audio-recorded and transcribed) were completed by G.H. The interviews were 1-2 hours in length. Visual prompts included fresh *limu* specimens and photographs as well as the use of a map to identify places on Oahu where *limu* populations are or were at one time located (Appendix A).

Prior informed consent

Participants in this study (students, parents/guardians, and adult interviewees) were provided a written description of the nature of the project, the extent of participation being asked of them, their privacy and confidentiality, the voluntary nature of their participation, and the ways in which the survey information would be used and distributed. Participants were asked to provide their signature to indicate consent/assent to participate (or have their child participate) in the project (Appendix A). This project was approved by the University of Hawai‘i, Committee on Human Subjects (CHS#19082), and by the Department of Education for the State of Hawai‘i.

Data analysis

Data analysis and resultant findings are based on the sample of students for whom full consent (parent/guardian as well as student assent) were obtained. Although 350 students were involved in the classroom collaborations, full consent was obtained for 180 students (out of 350 potential students), with a range of 15 to 36 at each school. A low return rate for parent/guardian consent forms, not lack of consent, limited the potential sample size for this study. In addition to descriptive statistics, student survey data were analyzed using a generalized linear mixed model (lme4 package, Bates *et al.* 2009) in order to identify the factors that best predict gathering and consumption of wild seaweeds among high school students. The student's high school was incorporated as a random factor with Hawaiian ethnicity, gender and parental gathering as fixed factors. The best model was selected using

Akaike Information Criteria (AIC). In the case of survey responses for parental and grandparental gathering, students who indicated they did not know if their parents or grandparents had gathered seaweed were excluded from the analysis since it could not be determined if their family members had gathered seaweed. All analyses were performed in R (R Development Core Team 2008).

RESULTS

Antioxidant determination

FRAP assay

The FRAP values ranged from 0.04 to 2.7 (mean of 0.5) μmol $FeSO_4$ equivalents per gram wet weight. The species with the highest values were among those not documented as edibles (*Martensia fragilis*, *Dictyota acutiloba* (from two sites), and *Laurencia majuscula*), followed by edible *limu līpoa* (*Dictyopteris plagiogramma*) (Figure 3). Among the edible species, *limu līpoa* (*Dictyopteris plagiogramma*) and *limu ʻeleʻele* (*Ulva flexuosa*) had the highest values (Figure 4). As a group, wild edible species had significantly higher FRAP values then cultivated edibles (Fig. 5). No significant differences were detected between wild species not eaten and wild edibles (Appendix B).

DPPH assay

The DPPH values ranged between 0.32 and 4.12 (mean of 1.46) %DPPH radical scavenging per mg of wet weight. The highest %DPPH scavenging values occurred for *Martensia fragilis*, *Dictyota acutiloba* (not documented as edibles), followed by the edible *limu kohu* (*Asparagopsis taxiformis*), gorilla ogo (*Gracilaria salicornia*), *Chondrophycus cartilaginous*, and then edible *limu līpoa* (*Dictyopteris plagiogramma*)(Fig. 3). Among the edible species, *limu kohu* (*Asparagopsis taxiformis*) and gorilla ogo (*Gracilaria salicornia*) had the highest %DPPH scavenging (Figure 4). There was no significant difference detected between wild edibles and cultivated edibles (Fig. 6), nor between wild species not eaten and wild edibles (Appendix B).

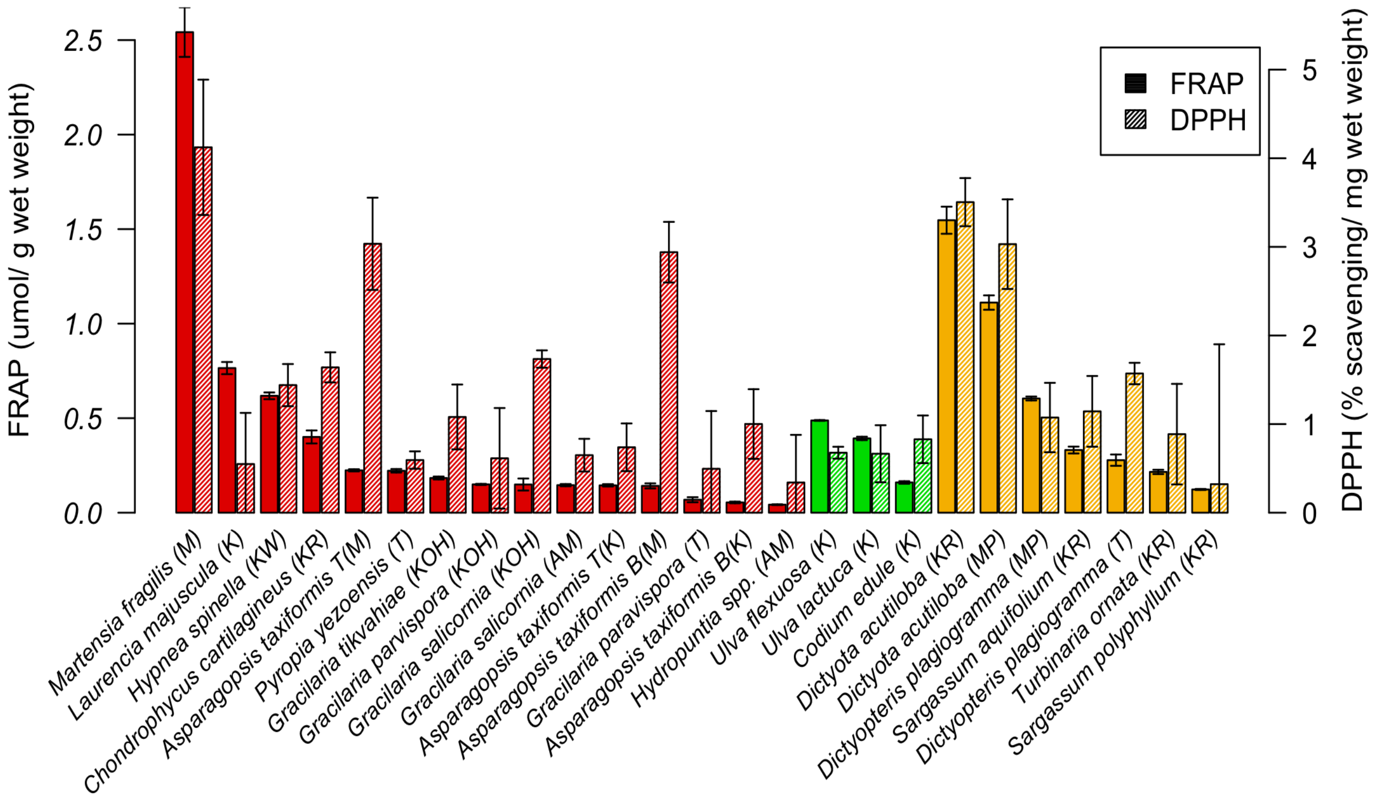


Figure 3. FRAP value (μmol $FeSO_4$ equivalents) per gram wet weight and %DPPH radical scavenging per milligram wet weight for all samples. Error bars indicate one standard deviation. Bar colors indicate macroalgal phyla or class (red= Rhodophyta, green= Chlorophyta, and brown = Phaeophyceae). The letters following each species name in parentheses represent collection site or place of acquisition: M= Makapuʻu, KR = Kualoa Regional Park, KOH= Kahuku Olakai Hawaii Farms, T= Tamashiro's Market, K= Kaʻalaʻwai, MP= Makai Pier, and AM= Ala Moana Beach Park. The letter designations after *Asparagopsis taxiformis* indicate the portion of the plant: T= upper thallus, B= stolons.

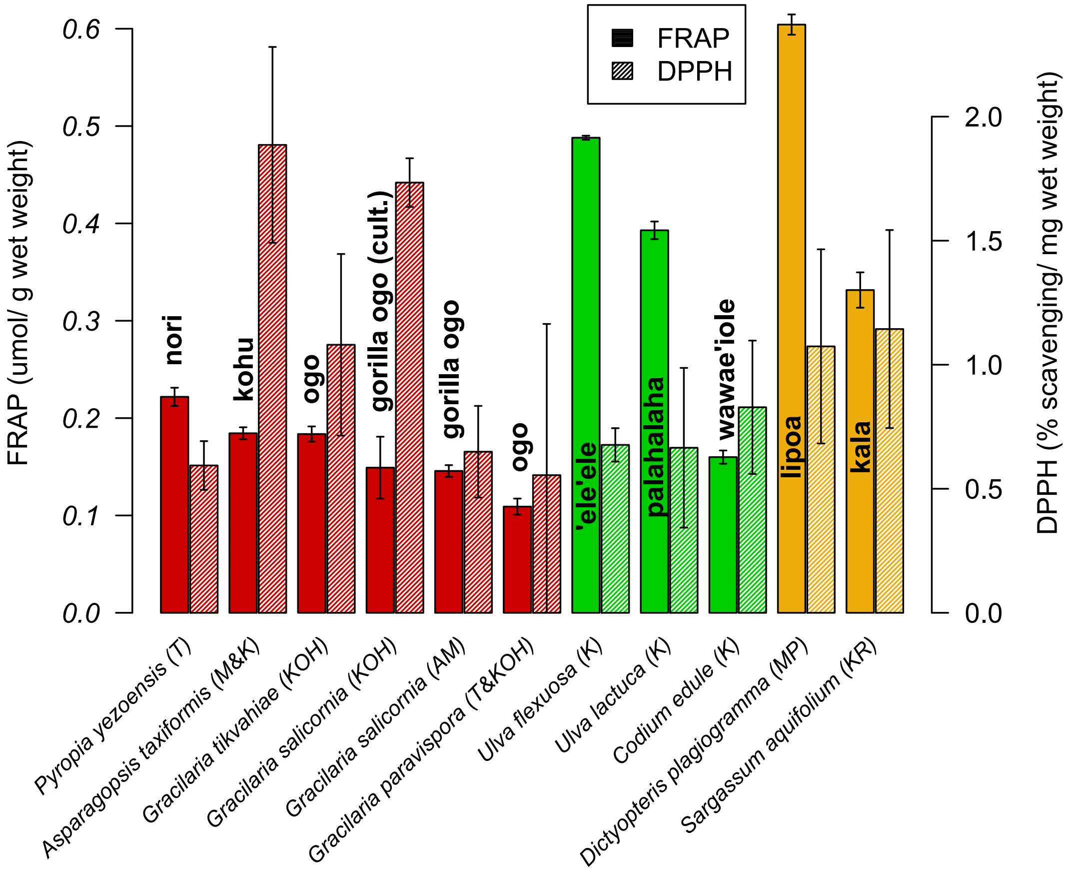


Figure 4. FRAP value (μmol $FeSO_4$ equivalents) per gram of wet weight and %DPPH radical scavenging per mg wet weight for all edible species. Error bars indicate one standard deviation. Abbreviated common names are shown in bold next to FRAP bars. Cultivated species are those labeled "nori", "ogo" or "gorilla ogo (cult.)."

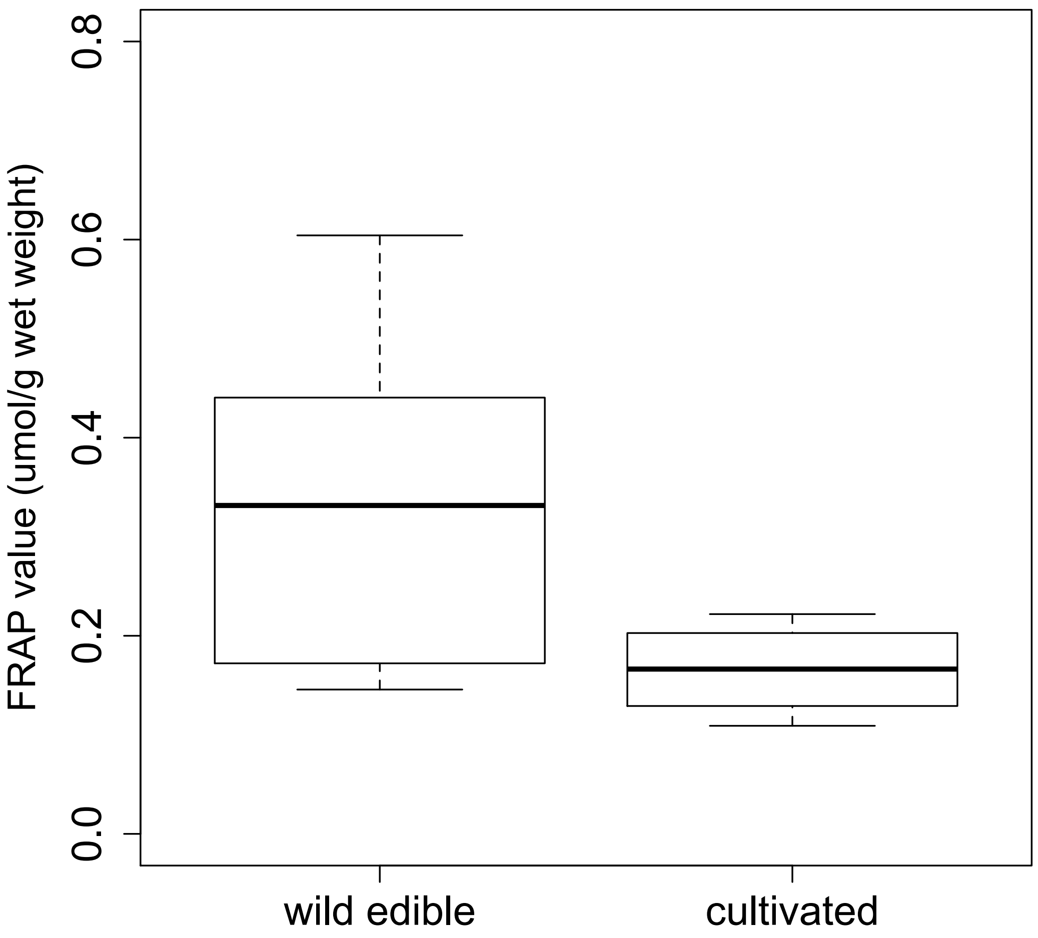


Figure 5. Comparison of wild edible (n=7) and cultivated edible (n=4) species by FRAP value (µmol $FeSO_4$ per g wet weight). (Welch's t-test: t = 2.29, df = 8.39, p= 0.049)**.**

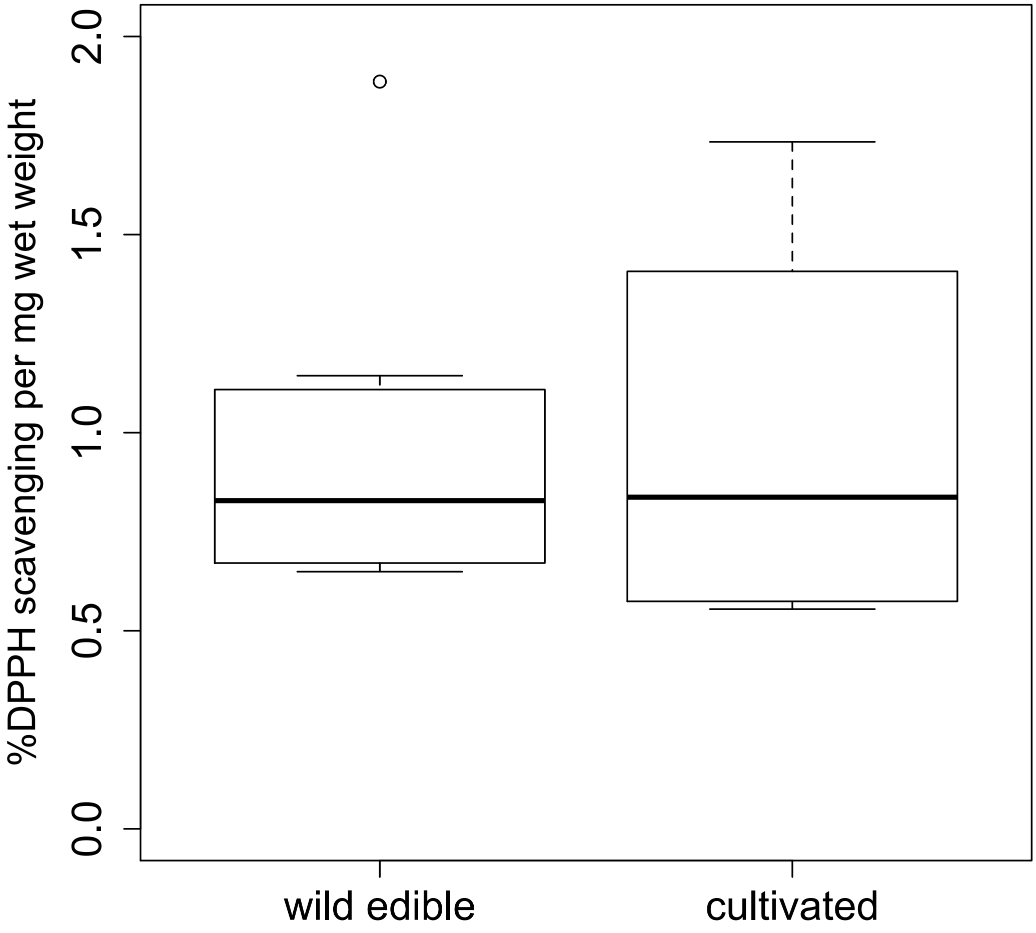


Figure 6. Comparison of %DPPH radical scavenging per mg fresh algal weight for wild edibles (n=7) and cultivated edibles (n=4). (Welch's t-test: t = 0.0053, df = 5.06, p= 0.96).

Socio-cultural study

Student surveys

Characterization of surveyed population

Surveys from a total of 180 students with full consent from parent/guardians and the student, were included in this study. The survey population was 55% female (F=99, M=80, NA=1) and included juniors and seniors from six public high schools (Campbell 35, Kailua 15, Kaimuki 36, McKinley 35, Waianae 28, Waialua 31). The survey provided four blank lines for students to identify their ethnicities and those students who did not wish to identify their ethnicities were asked to write "NA" (Appendix A). Within the survey sample, 37% of students reported Hawaiian and 36% reported Filipino as one of their ethnicities. Forty-three percent of the surveyed students who were Filipino were also Hawaiian, while 41% of the Hawaiian students were also Filipino. First ethnicity mentions included: Hawaiian 49, other Pacific Islander 19, Filipino 35, other Asian 30, Caucasian (including Portuguese) 22, African American/Black 4, Hispanic 6, Native American 2, and NA 13. For the remainder of the report, "Hawaiian students" or "Filipino students" refer to any student who indicated Hawaiian or Filipino ethnicity, respectively, in any part of their ethnicity response.

Consumption of seaweeds

Among all students surveyed, nearly every student reported they consume cultivated seaweed, while 29% indicated that they had consumed wild-gathered seaweed, including 40% Hawaiian students and 25% of non-Hawaiian students (Figure 7). In addition, 36% of Filipino students reported having consumed wild seaweeds. Students consumed an average of 3 (range from 0 and 21) meals or snacks per week that contained seaweed, with significantly more frequent consumption of seaweed among Hawaiian students (Figures 8 and 9). The most commonly consumed foods containing seaweed were sushi and musubi (spam sushi), followed by poke (raw fish salad)(Figure 10).

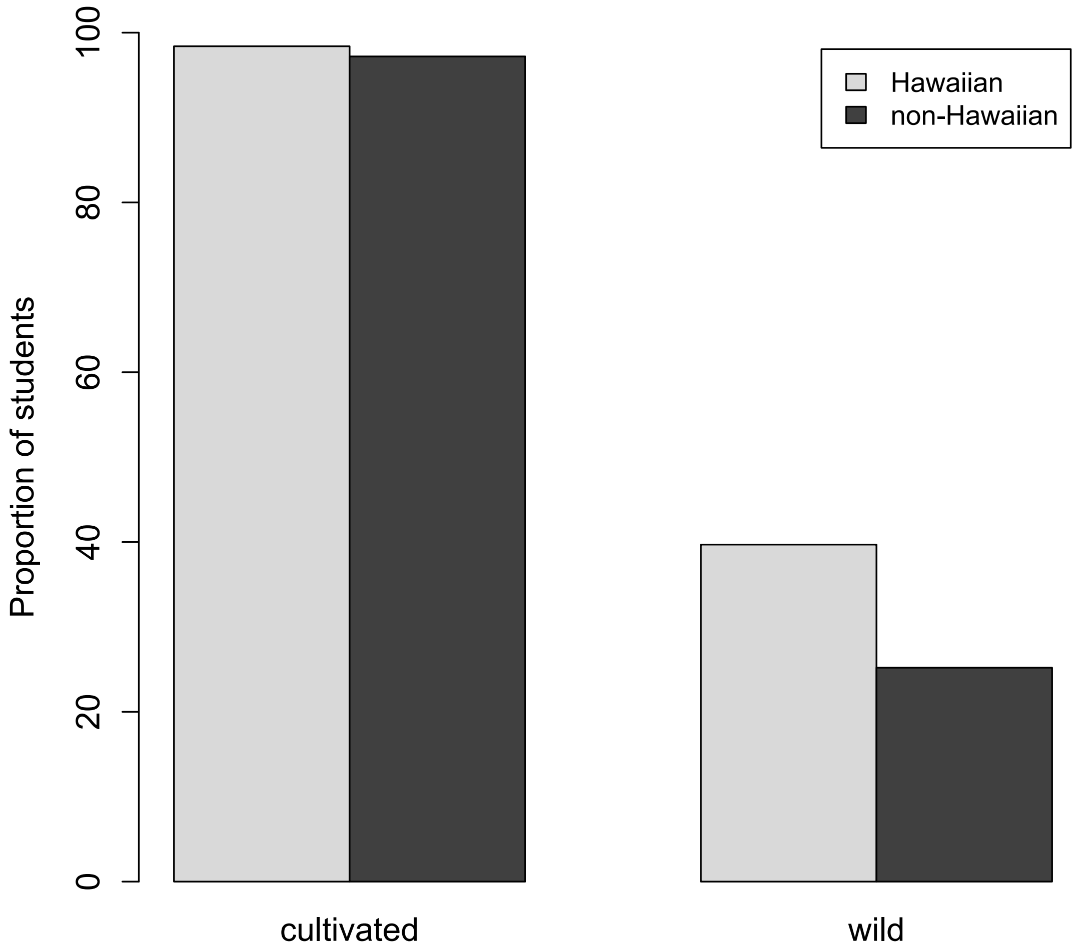


Figure 7. Proportion of Hawaiian and non-Hawaiian students who have consumed cultivated and wild seaweeds (n=166, 63 Hawaiian and 103 non-Hawaiian).

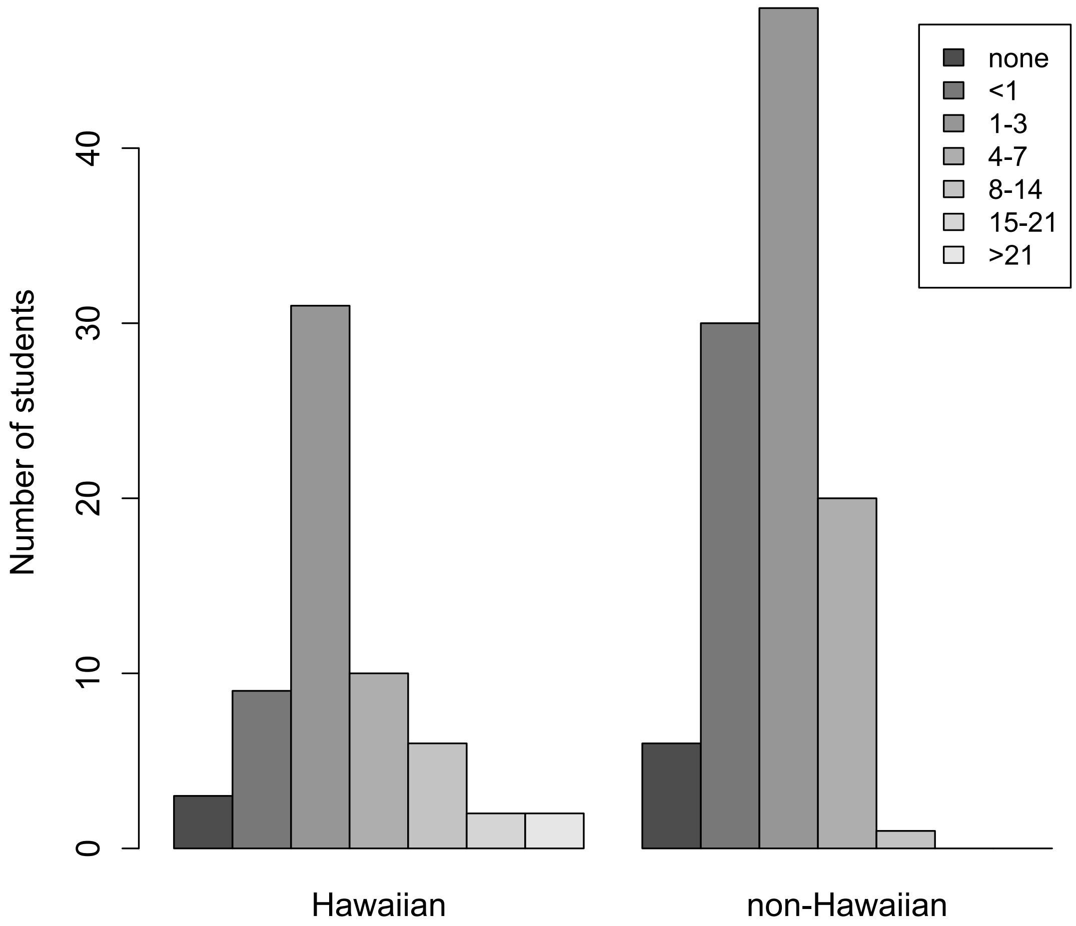


Figure 8. Number of meals or snacks per week that contain seaweed consumed by surveyed students who did or did not report Hawaiian ethnicity (n=169, 63 Hawaiian, 106 non-Hawaiian).

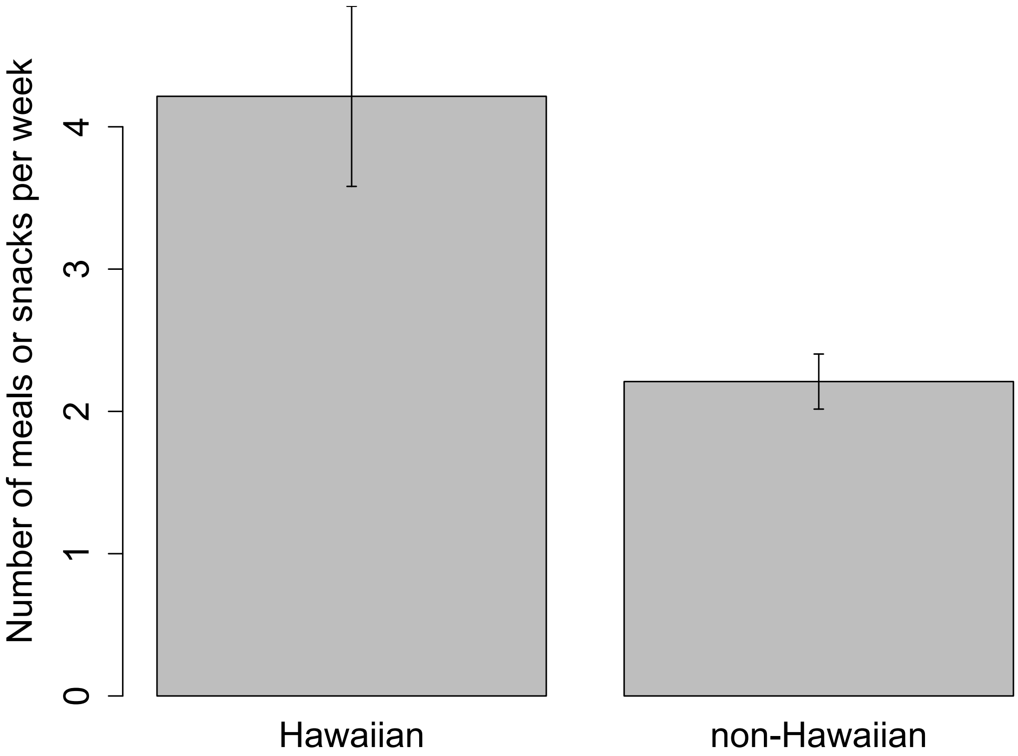


Figure 9. Frequency of consumption of seaweed-containing foods among Hawaiian and non-Hawaiian students. Error bars indicate one standard error (n=169, 63 Hawaiian, 106 non-Hawaiian). Welch's t-test (t=3.02, df=73.9, p=0.003).

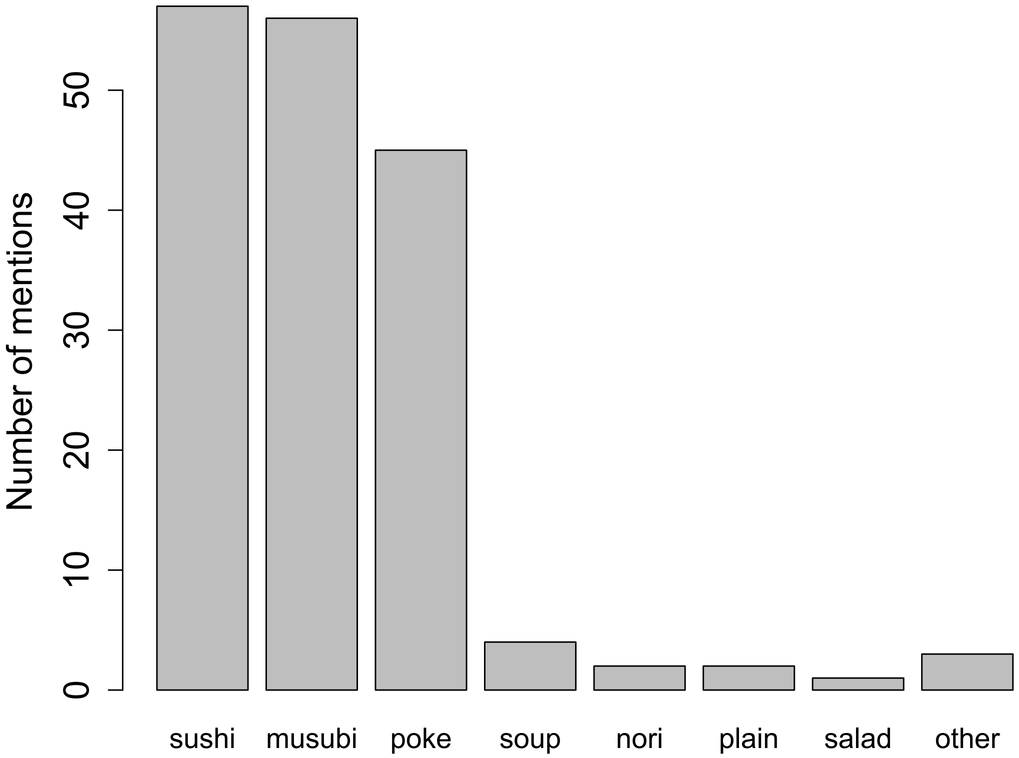


Figure 10. Number of student mentions for most commonly consumed food that contains seaweed (n=180).

Taste appreciation

The seaweed most often reported as the favorite was *Pyropia* spp. (nori), followed by *Gracilaria* spp. (ogo, etc.). Of the wild seaweeds, *limu kohu* (*Asparagopsis taxiformis*), *limu wāwaeʻiole* (*Codium edule*) and *limu līpoa* (*Dictyopteris* spp.) were reported by students as favorite seaweeds. A larger proportion of Hawaiian students than non-Hawaiian students listed *Gracilaria* spp./ogo or any native Hawaiian seaweeds as a favorite seaweed and a smaller proportion of Hawaiian students left the question blank or reported an answer that could not be linked to a specific taxon. (Figure 11)

When asked to describe the taste of seaweed or *limu*, one-third of students mentioned how good/delicious/tasty/*ono* it was, while only 2% indicated it tasted bad or nasty, and 4% indicated it was tasteless, plain or bland (Figure 12). The most common descriptor was salty, followed by good/delicious/tasty/*ono* and then crunchy. Twenty percent of students described the texture, including 13% who mentioned seaweed was crunchy. Forty-five percent of students mentioned that seaweed is salty, 11% "oceany" or fishy, 5% sweet, 1% tangy or sour, and <1% bitter. Several students mentioned that seaweeds have unique or distinct flavors and that they complement other foods well. Hawaiian students were equally likely as non-Hawaiian students to mention that seaweed was good or bad tasting, or oceany tasting or to describe the texture, but less likely to describe seaweed as sweet, and more likely to describe the seaweed as tangy.

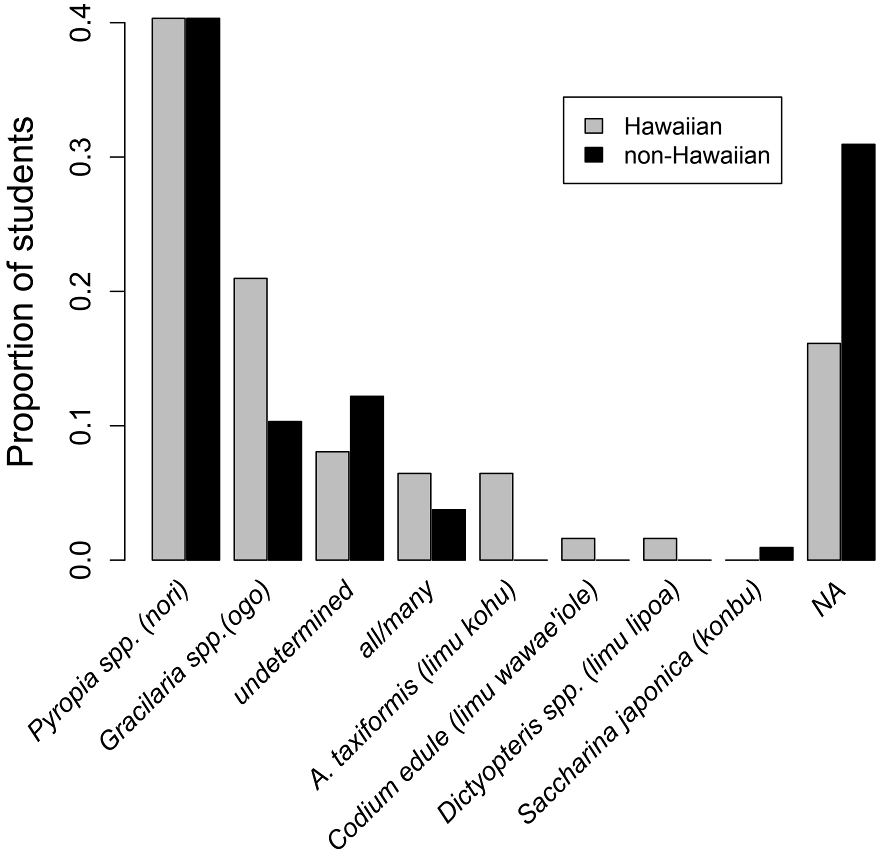


Figure 11. Proportion of Hawaiian and non-Hawaiian student mentions for favorite seaweed. Undetermined species are those whose description could not be matched to a species, such as "the green one", or "I don't know its name". NA refers to students who left the question blank, or who indicated they had no favorite seaweed (n=169, 63 Hawaiian and 106 non-Hawaiian).

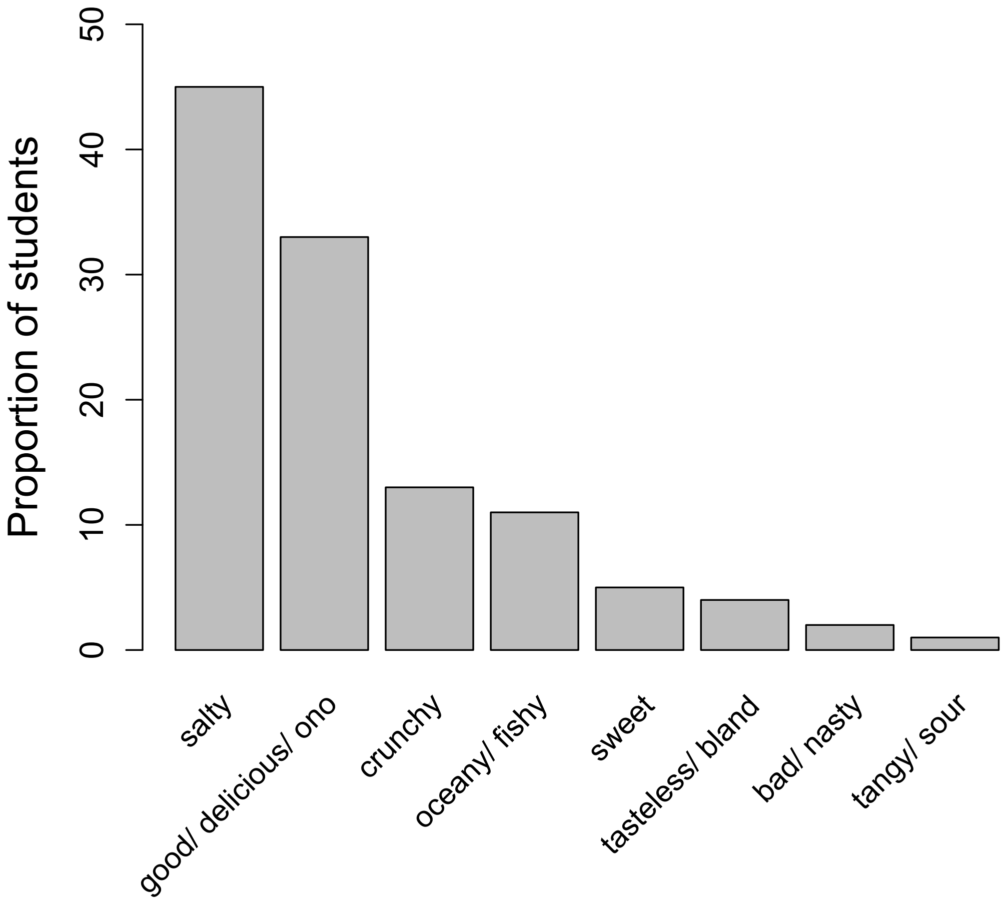


Figure 12. Proportion of students who described seaweed in each flavor or texture category (open-ended response) (n= 166).

Gathering of wild seaweeds

Approximately one in five students overall had gathered wild seaweed including one-third of Hawaiian students and one-tenth of non-Hawaiian students (Figure 13). Just under one-third of Filipino students indicated they have gathered wild seaweeds. Four out of ten Hawaiian students indicated one of their parents had gathered wild seaweed, while 6 out of 10 indicated that one of their grandparents had gathered wild seaweed. These proportions were lower for non-Hawaiians (Figure 14). Familial (parent or grandparental) gathering was reported by 37.4% of all students and 62.8% of Hawaiian students. Students who indicated that they did not know if their family members had gathered wild seaweed were excluded from these analyses.

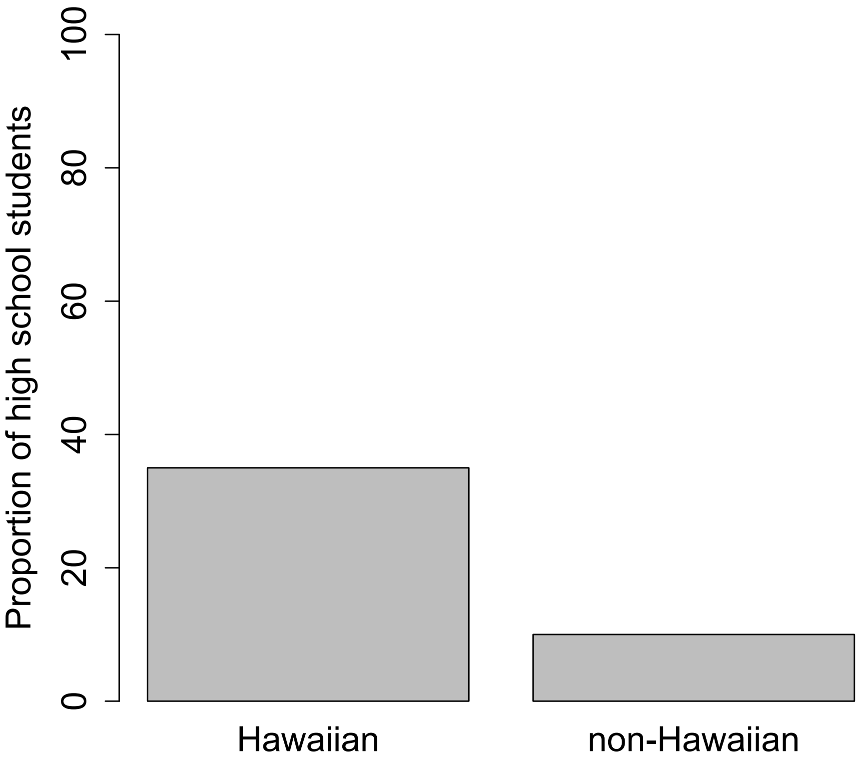


Figure 13. Proportion of students who have gathered wild seaweed (n=169, 63 Hawaiian and 106 non-Hawaiian).

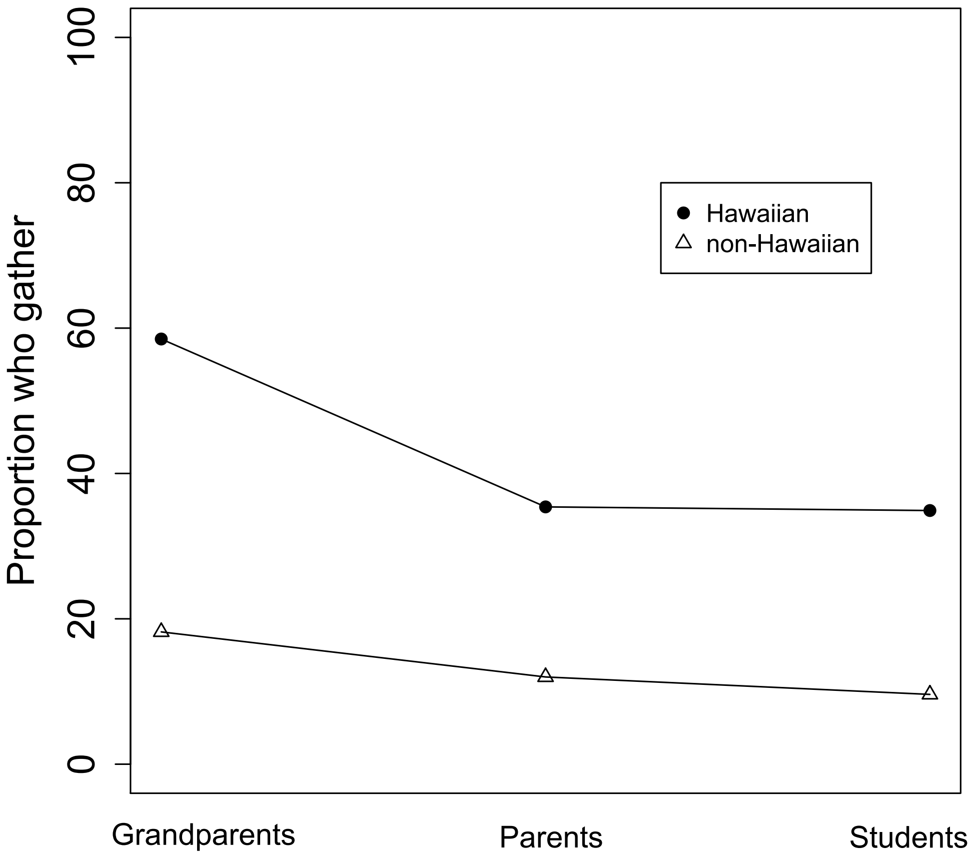


Figure 14. Proportion of grandparents, parents and students who have gathered seaweed as reported by student surveys and excluding students who did not know if their parents or grandparents had gathered seaweed. Samples sizes for grandparental, parental and student gathering are 41, 48 and 63 for Hawaiians and 67, 84 and 106 for non-Hawaiians, respectively.

Predictors of wild seaweed gathering

According to the generalized linear mixed model, having a parent who the student knew had gathered seaweed was a significant predictor of student gathering ($p<0.001$, Table 2). Gender and Hawaiian ethnicity were not a significant predictors of gathering, however the best model included their interaction, which indicated that Hawaiian ethnicity was a stronger predictor of gathering among male compared to female students (Figure 15).

Table 2. Generalized linear mixed model to predict wild seaweed gathering among high school students

GLMM	*Estimate*	*Std. Error*	*z value*	*Pr(>|z|)*
(Intercept)	-2.1567	0.4700	-4.588	4.47e-06 ***
genderM	-17.8913	2722.3648	-0.007	0.995
parents_gatherY	2.4185	0.5718	4.230	2.34e-05 ***
hawaiianY	0.4199	0.6405	0.656	0.512
genderM:hawaiianY	18.5339	2722.3649	0.007	0.995

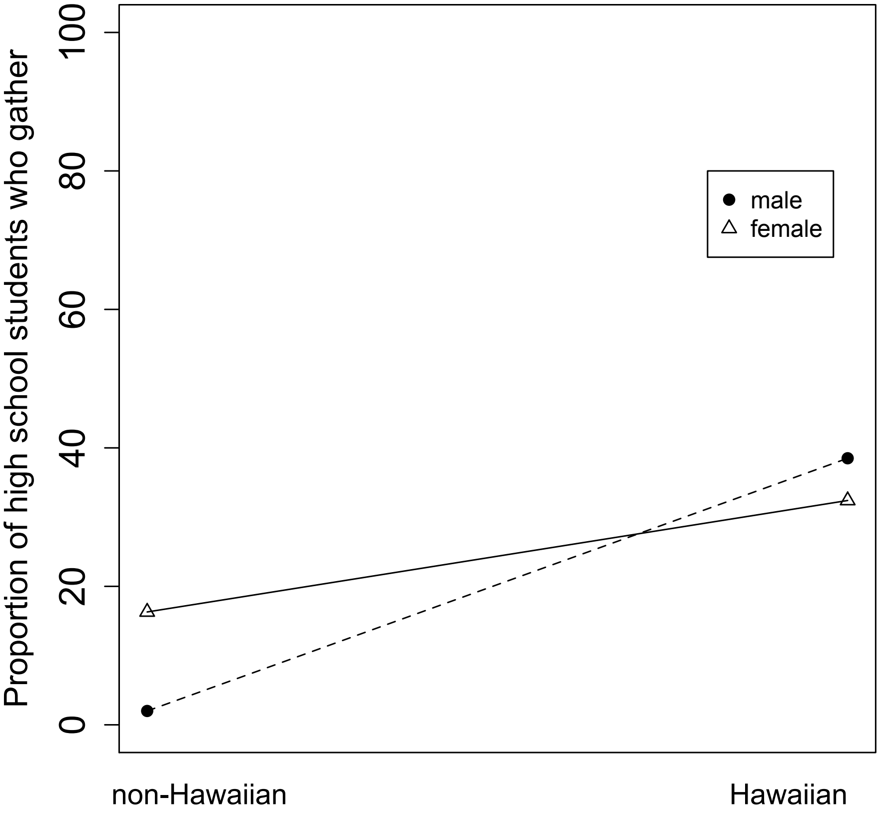


Figure 15. Proportion of gathering among Hawaiian and non-Hawaiian males and females surveyed at public high schools. Sample sizes included 26 male and 37 female Hawaiians, as well as 50 male and 56 female non-Hawaiians.

Adult interviewees

Characterization of interviewed population

Out of over 50 interviews that were completed by high school students, 30 were with adults who were knowledgeable about *limu* gathering ("*limu* –knowledgeable adults", see Methods). G.H. also completed 6 in-depth interviews with *limu* gatherers (2 who were also interviewed by a student). The age of interviewees from both sources ranged from 30 to 77 (average age of 45) and approximately 74% of those interviewed were female (F=25, M=8, NA=1). Sixty-five percent (n=22) of interviewees reported Hawaiian ethnicity, 18% indicated they were Filipino (n=6). Approximately half of the adults in the interview population lived on the Waiʻanae coast.

Change in wild seaweed abundance

Of the 34 adults interviewed by students and G.H., all those who were aware of changes in the abundance of wild seaweeds reported a decline (Figure 16). Some interviewees noted a decline in specific species of cultural importance (e.g., *limu huluhuluwaena*, *limu manauea*, *limu līpoa*) and also noted a change in the quality (e.g., smaller plants or plants not being as clean, diminished taste quality). More interviewees cited environmental reasons than harvest-related reasons for the decline (Figure 17). Over-picking (harvesting more than needed or harvesting to sell) was the most often cited harvest-related reason for the decline in abundance of wild seaweeds (Figure 18). Pollution was the most often cited environment-related reason for the decline in abundance of wild seaweeds (Figure 19).

Adult interviewees shared knowledge of traditional gathering protocol when discussing the reason for the decline in *limu* abundance. They shared that when gathering *limu*, it should be removed by clipping or cutting the upper portions of plants so that they may regrow, and that harvesters should take only the amount needed for personal or family use. Picking *limu* to sell, or picking more than needed are referred to as “over-picking.” Lack of regulation and homelessness were also mentioned in the context of over-picking to help explain to the decline in the abundance of wild seaweed on Oʻahu. As one interviewee explained, a patch of reef covered with *limu kohu* (*Asparagopsis taxiformis*) could represent $100-200, a significant sum or money for someone in economic need or suffering from chemical dependency.

Within the category of development, specific attention was given to the diversion of freshwater and to the pollution and channelization of surface water before it entered the ocean. It was explained that many marine macroalgae depend upon nutrients available from freshwater for their growth and maintenance (also mentioned in Poepoe *et al.* 2007). The diversion of freshwater for development, channelization of waterways and the increase in pollutants and sediment in runoff were perceived to be decreasing the ability of many *limu* to survive in the nearshore environment. The following macroalgal species were identified as indicators of fresh water, or as being more dependent upon freshwater: *limu ʻeleʻele* (*Enteromorpha* spp.), *limu pālahalaha* (*Ulva lactuca*), and *limu huluhuluwaena* (*Grateloupia filicina*). Also mentioned was the loss of *limu līpoa* (*Dictyopteris plagiogramma*) and the particularly strong and distinctive fragrance (i.e., dictyoterpenes, Moore 1977) it once imparted on many of the beaches on Oʻahu. Some interviewees explained that *limu* are seasonal and respond to factors such as rainfall and the seasonal movement of the sand. *Limu kala* was identified as one of the most culturally important *limu* by one interviewee and described as having approximately 50 types. It was described to have been more abundance in the past, when it would "tickle" you when you swam at particular beaches.

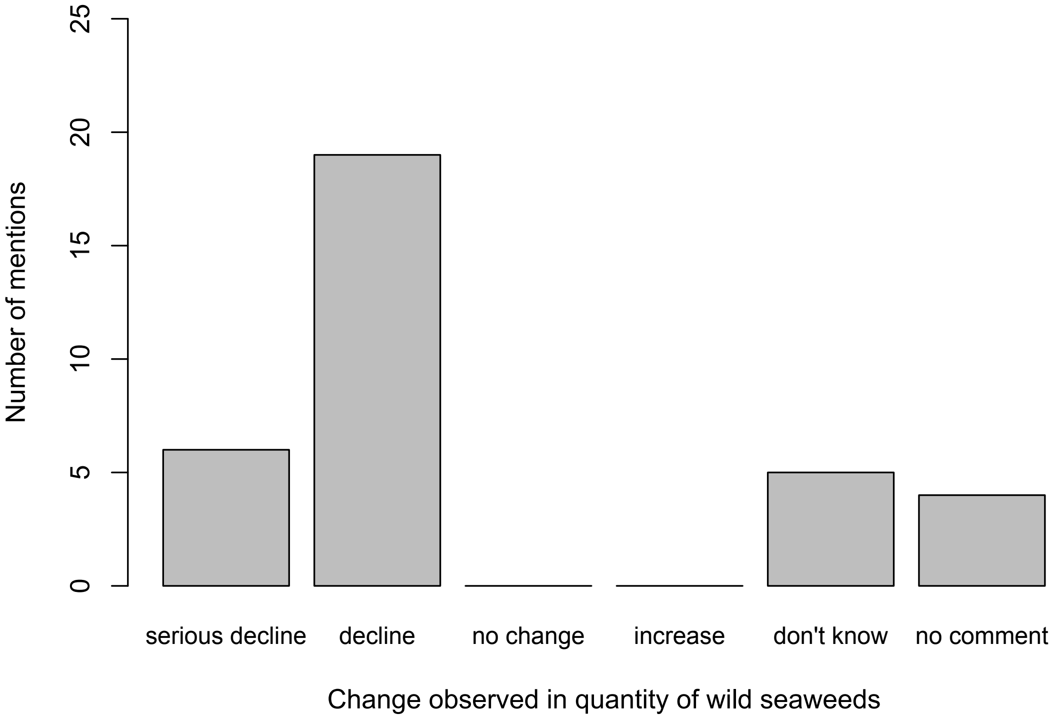


Figure 16. Responses of adult *limu*-knowledgeable interviewees to the question of how the abundance of wild seaweeds on Oʻahu had changed over time. No interviewee mentioned an increase or a lack of change in abundance (n=34).

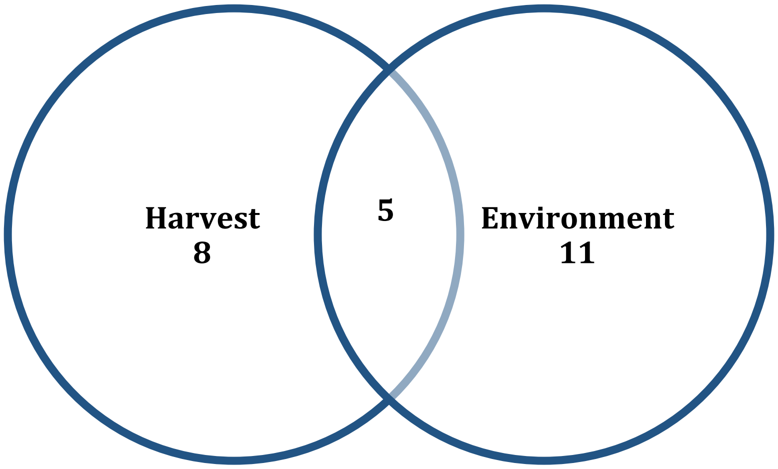


Figure 17. Number of adult *limu*-knowledgeable interviewees who mentioned harvest-related, environment-related, or both reasons for an observed decline in wild seaweeds on O‘ahu.

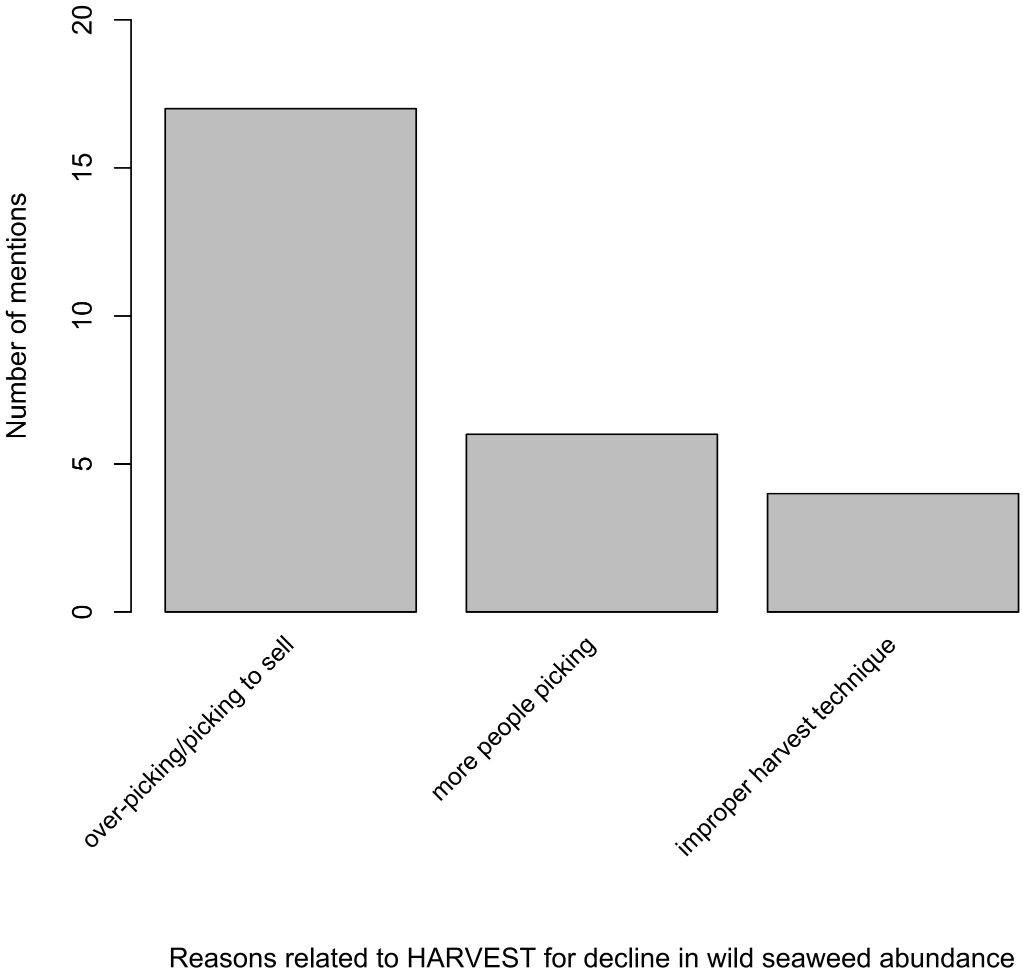


Figure 18. Mentions by adult *limu*-knowledgeable interviewees of harvest-related reasons for decline in the abundance of wild seaweeds on O‘ahu (n=34).

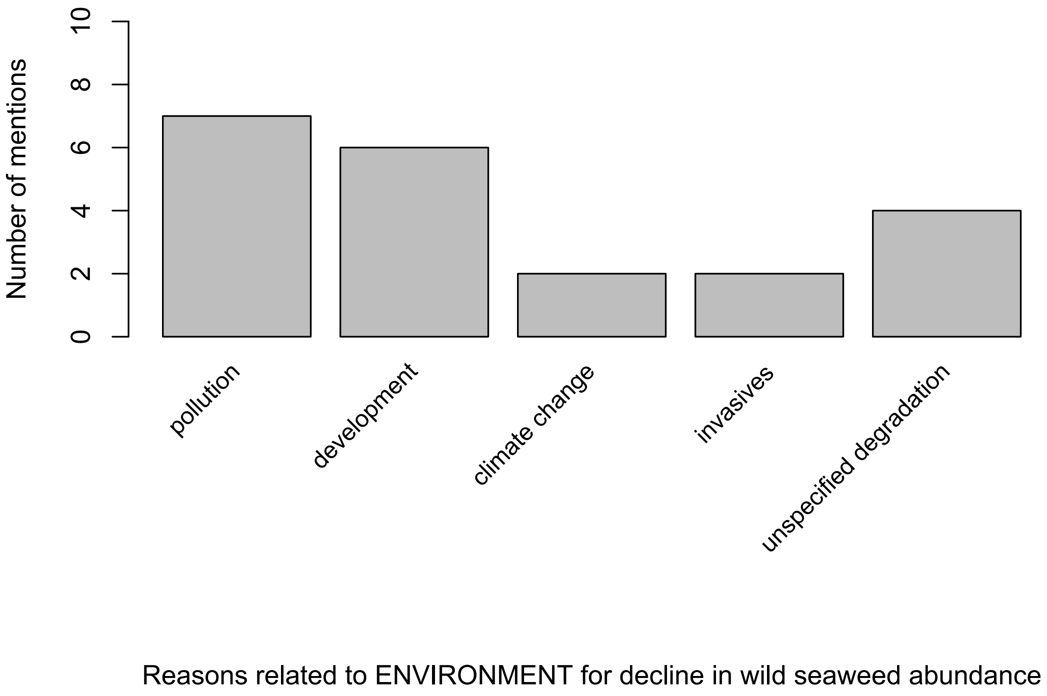


Figure 19. Mentions by adult *limu*-knowledgeable interviewees of environment-related reasons for decline in the abundance of wild seaweeds on O‘ahu (n=34).

DISCUSSION

Hawaiian wild seaweeds continue to be gathered and consumed in the urban, multi-ethnic context of O‘ahu Island and these wild seaweeds appear to contribute higher levels of antioxidant activity than cultivated seaweeds consumed in Hawai‘i today. While nearly every student consumes cultivated seaweed, nearly one-third also consume wild seaweeds and most students demonstrated taste appreciation for edible seaweeds in general. While the gender roles associated with wild seaweed gathering seem to have shifted over time, the practice of wild seaweed gathering and consumption, with concurrent health benefits, continues today. Its future depends upon familial practice within Native Hawaiian families as well as the environmental integrity of nearshore ecosystems.

Hawaiian seaweeds as sources of antioxidants to diet

The higher antioxidant power found in this study for wild compared to cultivated seaweeds by the FRAP assay (though not the DPPH assay) indicates that people consuming wild seaweeds are more likely to receive disease-preventative benefits than those who consume only cultivated seaweeds. Nearly every student indicated they consumed cultivated seaweeds, and nori (*Pyropia* spp.) was the most commonly listed favorite species, followed by ogo (*Gracilaria* spp.) which today comes from predominantly cultivated sources. These favorites coincide with the foods most commonly eaten: sushi and musubi (both contain nori) and poke (contains ogo, or, more rarely, a wild native seaweed). The average student reported consuming 3 meals or snacks a week containing seaweed, with a range from 0 to more than 21, indicating that antioxidant contribution will vary greatly by individual. Further, even though the most commonly consumed seaweeds are not native to Hawaiʻi, Hawaiian students consumed them more frequently than non-Hawaiian students, perhaps indicating a cultural preference for this food category carried over from earlier eras of Hawaiian history.

The difference in antioxidant power between wild and cultivated species could be explained by phylogenic differences, or by different growth conditions. All cultivated seaweeds in this study belong to the phylum Rhodophyta, and four out of five belong to the genus *Gracilaria*. Therefore, the results could reflect a lower antioxidant potential in red algae or *Gracilaria* spp. in general, rather than a specific effect of cultivation. Another possibility is that wild seaweeds produce a larger amount of secondary compounds with antioxidant activity than cultivated species because they experience higher levels of environmental stress in the open ocean compared to cultivated seaweeds, which are grown in tanks with flowing seawater. For example, macroalgal antioxidants such as polyphenols may be produced in response to herbivory, desiccation and UV radiation (Pavia and Brock 2000, Van Alsytne 1988, Cronin and Hay 1996, Peckol *et al.* 1996) and these stressors may be more present for wild compared to cultivated seaweeds. While several studies have determined that wild edible plants have higher (Bunea *et al.* 2011, Halvorsen *et al.* 2002) or similar (van der Walt *et al.* 2009, Spina *et al.* 2008) antioxidant potential compared to their cultivated counterparts, to our knowledge, no other study compares antioxidant power in wild and cultivated seaweeds.

The different results obtained in this study by the FRAP and DPPH assays highlight the importance of employing multiple assays in order to characterize the antioxidant power of a set of species (Szabo *et al.* 2007). Antioxidant activity in macroalgae is attributed to a diverse set of compounds including carotenoids, vitamin E, alpha-tocopherol and chlorophylls (lipidic fraction), as well as polyphenols, vitamin C and phycobiliproteins within the aqueous fraction (Rodriguez-Bernaldo de Quiros *et al.* 2008). The methanolic extracts used in this study would be expected to extract mostly polar or aqueous-fraction compounds

(Floegel *et al.* 2011). Given the diversity of antioxidant compounds present in macroalgae, the multiple *in vivo* antioxidant mechanisms they follow (Pelligrini *et al.* 2003), as well as the mechanistic differences among the two antioxidant assays (Prior *et al.* 2005), it is not surprising that the FRAP and DPPH assay produced somewhat different results. Red algae (Rhodophyta) in this study tended to have higher DPPH values relative to FRAP values, while green (Chlorophyta) and brown (Phaeophyceae) algae tended to have higher FRAP than DPPH values. While multiple explanations are possible, the relatively higher DPPH values among Rhodophyta could be attributed to the action of phycobiliproteins (only found in red algae), or to the size of antioxidant compounds, since the DPPH is reduced more easily by smaller molecules (Prior *et al.* 2005).

The antioxidant power of the edible species of seaweed tested in this study may be relatively low compared with other dietary sources of antioxidants, but direct comparisons are complicated by experimental differences across studies. When the seaweeds in this study were compared directly with high antioxidant foods such as blueberry, strawberry, broccoli and apple using the FRAP (Halvorsen *et al.* 2002) and DPPH (Floegel *et al.* 2011) values obtained from other studies, the values were much lower for the seaweeds in this study. It is difficult, however, to determine if these differences are due to real differences in the foods tested, or due to methodological differences, such as the different extraction procedures (Wang *et al.* 2010). A study by Lako *et al.* (2007) testing a variety of Fijian traditional foods, including two seaweeds, found seaweeds to have moderately low levels of antioxidants compared to other common plant components of Fijian diet. This would seem to support the conclusion that edible seaweeds are not among the largest sources of antioxidants to diet. This conclusion would run counter to the often-cited statement that macroalgae are rich sources of antioxidants (Souza *et al.* 2011, Rodriguez-Bernaldo de Quiros *et al.* 2010).

On the other hand, in Hawaiian traditional diet, macroalgae (*limu*) were eaten with more consistency than a particular vegetable might be consumed today, meaning the antioxidant or other disease-preventative benefit may be underestimated without considering the frequency of consumption. *Limu* are often used like a condiment or spice, imparting strong flavors and fragrances to the dish, and, in pre-Contact Hawaiʻi, were eaten regularly, with most meals (Reed 1907, Abbott 1992). Spices often have strong flavors, are used in small quantities and known for their pharmacological action (Etkin 1996). Therefore, the disease-preventative effects of *limu* may also need to be considered from the standpoint of its medicinal value as a spice. For example, cinnamon and tumeric are two common spices that are antimicrobial (Raffatullah *et al.* 1990, cited in Etkin 1996). Further, wild foods such as *limu* may be particularly high in unpalatable or potentially toxic substances, “secondary” metabolites, compared with cultivated or semi-cultivated species (Johns 1996, Etkins 1996), which may increase their pharmacological potential. In fact, more species of *limu* are documented for their use in Hawaiian traditional medicine than are documented as edibles (Napoleon 2004). While there are some complications matching Hawaiian and scientific names, nearly every species tested in the antioxidant assays in this study are also documented for use in Hawaiian traditional medicine (Reed 1907, Handy *et al.* 1934, Abbott 1996, Napoleon 2004). While important work has been done (e.g., Moore 1977), much remains to be investigated with regard to the chemistry and pharmacological action of the diverse edible Hawaiian macroalgae.

Comparisons with species tested by Kelman *et al.* (2012) from Hawaiʻi Island, using identical procedures resulted in a similar range of antioxidant values for all species tested (by extract weight), but the ranking of particular species was different. Also, in comparing the present study to Vijayavel and Martinez (2010), who tested two of the same edible Hawaiian species as this study by DPPH, the rank order of %DPPH scavenging by species is different. This difference in ranking between studies, even with identical procedures and/or species, could indicate variation caused by any number of biological or environmental factors. It likely indicates that sample sizes are not adequate to characterize intraspecies variation. Larger sample sizes (multiple individually extracted and processed samples per species) and consistent procedures will be necessary in order to disentangle the causes responsible for this variation across sites, species and studies.

In addition to the antioxidant intake associated with wild seaweed gathering and consumption, it is important to consider these practices as indicators of a set of behaviors with wider-reaching consequences for human health. Gathering is a practice done together, with family and community members. It is a means of strengthening intergenerational ties and promoting social cohesion and psychological well-being. Gathering also provides physical exercise and connection with the natural environment. Further, when *limu* are consumed, they are often combined with other traditional foods such as raw fish or seafood, stews or traditional starches, which also impart important health benefits to the consumer. Eating a larger amount of traditional foods may also indicate less consumption of market foods.

Continuance of seaweed gathering practice

Having a parent who gathers wild seaweed was the strongest predictor that a student would have gathered seaweed themselves. While the Native Hawaiian concept of family (*ʻohana*) extends well beyond parents and grandparents, the study supports the accepted understanding that family practice is critical to intergenerational transmission of Native Hawaiian cultural practice (McCubbin and Marsella 2009, Poepoe *et al.* 2007, McGregor *et al.* 2003). While future studies should expand questionnaires to address the impact of all family and extended kin members, this study suggests that parents may be particularly important in transmitting traditional knowledge concerning seaweed gathering. This is not surprising given that vertical transmission (parent to child) has been shown to be important in traditional societies (Ohmagari and Berkes 1997, Hewlett and Sforza 1986) and is considered the most important means of transmitting traditional ecological knowledge across all cultures (Ruddle 1993). However, given that this study did not ask students how they first learned to gather wild seaweeds, it cannot be determined if parents were the teachers or if having a parent who gathered seaweed increased the chances of interacting with and learning from other seaweed gatherers. If vertical transmission is in fact the main means of transmitting the practice of seaweed gathering, this would be expected to lead to high heterogeneity in the specifics of the practice across families, as well as high levels of cultural conservation (Cavalli-Sforza and Feldman 1981). While vertical transmission is expected to be important, Native Hawaiian practice is likely also transmitted within *'ohana* that extend beyond parent-child relationship, as well as through culturally-based curricula and other media. The implications of these various means of transmission for the evolution of Native Hawaiian cultural practice could be explored with future research.

Hawaiian students reported wild seaweed gathering for approximately 60% of their grandparents compared to 40% of their parents. While this may represent a decline in cultural practice, given that each student could potentially be aware of the gathering practices of four grandparents, but of only two parents, this data should not be immediately interpreted as a generational decline. Even if gathering was not transmitted from the grandparents to parents of a particular child, the practice may still be transmitted within other members of the family or kin group. Gathering, for example, may have been taken up by aunts and uncles other than the parent, or by a different sibling other than the student themselves. Transmission of cultural knowledge is understood to depend upon values, personality traits and attitudes (Hewlett and Cavalli-Sforza 1986), and to be gender specific (Ruddle 1993), so that not all children would be expected to be interested in gathering, or be of the appropriate gender for learning a given skill. As one Hawaiian seaweed gatherer shared during an in-depth interview, certain family members may be selected to fulfill particular roles (e.g., marine or terrestrial gathering) based on inclination and ability.

Hawaiian high school students reported a similar level of wild seaweed gathering among their parents as they reported for themselves. These results suggest that cultural practice is not declining and may even be increasing when we consider that siblings of students may gather, even if the student themselves does not. In fact, closer analyses of the survey data from this study revealed an increase in gathering from parents to students for particular regions of Oʻahu (data not shown due to constraints of research approval). Impacts of the Hawaiian cultural renaissance, “eat local” movements, and statewide efforts to maintain and revitalize Native Hawaiian culture over the last several decades may be responsible for this trend. Further, if Native Hawaiian traditional practice is learned through less traditional means, such as through school, media, or cultural experts (oblique and one-to-many transmission), new or re-introduced cultural traits can spread rapidly, though the knowledge and practice will tend to be more homogenous (Cavalli-Sforza and Feldman 1981).

The level of cultural practice reported in this study was likely influenced by the choice to survey students in public schools and in Marine Science classrooms.

Students enrolled in Marine Science (an elective science course), may tend to have greater interaction with the ocean and greater knowledge of marine resource use within their families, possibly leading to an overestimate of levels of cultural practice. On the other hand, the choice to survey students from public schools excluded the students who are enrolled in one of many cultural charter schools, or private schools such as Kamehameha Schools, which may be favored by families with a strong interest to preserve and practice Hawaiian cultural traditions. This would suggest the results may underestimate the level of cultural practice among Oʻahu youth.

While wild seaweed gathering is considered traditionally to be the work of women and children in Hawaiʻi (Setchell 1905, Abbott 1978), as many male as female students in my survey population indicated they had gathered wild seaweeds. There was a tendency for more Hawaiians than non-Hawaiians to gather, this tendency was particularly pronounced for males. The higher proportion of male than female Hawaiian students who gather may be explained by the co-occurrence of seaweed gathering with fishing, which is most often the work of men and boys (Abbott 1978). Several Hawaiian males who indicated they had gathered wild seaweeds explained that this was done when they went fishing, in some cases it was specified that fishing was with their father. Further, given that wild seaweeds are more difficult to locate today than in the past, beach gathering by women may be less common and gathering while fishing may be proportionally more important in the contemporary context of Oʻahu.

It is interesting to note that when students were asked to identify an adult in their community who was knowledgeable on *limu* for the interview assignment, three-fourths of those identified were female. This tendency to identify females as *limu* experts (ages 36-77) contrasts with the slightly higher proportion of male than female students indicating they gather today. It could suggest that a gender shift in gathering practice has occurred in recent years, or that despite a shift in gender roles that may have occurred sometime before, the perception of women as *limu* experts persists.

Wild seaweed abundance

The continuation of wild seaweed gathering and associated traditional knowledge also depends on the availability and quality of the seaweeds themselves. Given the sharp decline in this natural resource observed among all adult seaweed gatherers interviewed, transmission of this practice could be expected to be increasingly difficult. In fact, several interviewees indicated they no longer gathered due to lack of availability of the seaweeds themselves. Pollution was a major reason cited for the decline in seaweeds and also as a reason to discontinue gathering based not only on the safety of the food source, but also because of the way in which it altered its taste. Development, and particularly its impact on hydrology and water quality, was also a top environment reason given for the decline in wild seaweed abundance.

The introduction and spread of several invasive seaweed species has mirrored the decline in many native edible seaweeds. Notably, *Gracilaria salicornia*, has overtaken many areas on Oʻahu where native seaweeds used to be found. This invasive can tolerate the stressful and sometimes extreme conditions of degraded marine ecosystems, and is less favored by herbivores (Smith *et al.* 2004). While *G. salicornia* is used locally in foods such as poke, and while it was listed as a favorite seaweed by one student in this study, it was also mentioned by gatherers as a cause of the decline in native edible species due to outcompetition.

Over-picking (harvesting more than need or harvesting to sell) was the most commonly reported reason for the decline in wild seaweeds. This was considered by some as greedy and often coincided with improper harvest technique, such as ripping up the seaweeds entirely, rather than cutting or pinching only the upper thallus, allowing the seaweed the potential for regrowth. The sheer number of people picking and consuming wild seaweed was also cited as a factor in the decline. While more research is necessary to determine the extent and cause of decline, several native wild edible seaweeds, which were once more abundant, are now considered by some gatherers to be rare. Interviews from this study suggest *limu huluhuluwaena* (*Grateloupia filicina*), *limu ʻeleʻele* (*Ulva* spp., formerly *Enteromorpha prolifera*), *limu līpoa* (*Dictyopteris* spp.), are among those that have declined greatly in abundance in particular regions. Also expressed by some adult interviewees was a deep sadness with the loss of wild seaweeds from the marine environment over the course of their lives.

Taste appreciation

Despite these challenges, many interviewees and students showed a high level of appreciation for the taste of seaweeds, including wild seaweeds, with very few students providing negative descriptors for the taste of seaweeds. Hawaiian and non-Hawaiian students showed an equal appreciation for imported and cultivated nori (*Pyropia* spp.), while Hawaiian students were more likely to identify locally cultivated (sometimes gathered) *Gracilaria* spp. (ogo) and native wild seaweeds as their favorite. These results demonstrate cultural diversity in food preference, but again the near ubiquity of the appreciation for and consumption of cultivated seaweeds.

Seaweed gathering among Filipinos

In addition to gathering by Hawaiian students, nearly as many Filipino students indicated they had gathered wild seaweeds. Seaweed gathering and consumption is a traditional practice among several ethnic groups in the Philippines (Tito and Liao 2000). Abbott observed that Filipinos in Hawaiʻi were as likely as Native Hawaiians to gather seaweeds and were perhaps more curious and more likely to be encountered gathering seaweeds at the ocean, and that they probably gathered a larger quantity of seaweed than Hawaiians (1978). Filipino traditional gathering and its continuance and evolution among the Filipino community in Hawaiʻi are topics worthy of further study from both a socio-cultural and ecological standpoint.

In conclusion, wild edible Hawaiian seaweeds showed antioxidant activity at levels equal or higher than cultivated edibles in Hawaiʻi, depending on the assay employed. Based on research linking antioxidants to disease, the antioxidants in wild and cultivated Hawaiian seaweeds are likely to be disease-preventative, particularly in combating the diseases of affluence which are over-represented among indigenous peoples including Native Hawaiians. Engaging in the gathering and consumption of wild seaweed is an indicator of consumption of other traditional foods given that native seaweeds (*limu*) take on the role as condiment or spice in traditional dishes. Gathering also provides other health benefits including opportunities for physical exercise and community or family interaction. While antioxidant assays provide one means to categorize bioactivity, the edible wild seaweeds in this study have diverse and distinctive flavors that should be more thoroughly and individually investigated for the health implications of their chemistry beyond antioxidant activity. In order for the health benefits of consuming wild seaweeds to be realized, cultural practice must continue. While Native Hawaiian cultural practices have continually adapted to changing ecological, economic and political settings, the continuation of wild seaweed gathering and consumptions depends on intergenerational transmission within families or extended kin groups, as well as the availability of wild seaweeds in areas free of pollutants. Conservation efforts that incorporate the goal to maintain and increase populations of wild edible seaweeds would help to encourage cultural practice and are likely to be supported by local people and particularly Native Hawaiians, given their appreciation for this culturally significant resource.

CHAPTER 2

INFLUENCE OF NEARSHORE NITROGEN LOADING ON THE PHOTOSYNTHETIC PERFORMANCE AND SECONDARY METABOLISM OF THE EDIBLE WILD-HARVESTED MACROALGA, *ASPARAGOPSIS TAXIFORMIS* (BONNEMAISONIALES, RHODOPHYTA) (DELILE) TREVISAN

INTRODUCTION

Increased nutrient loading is a major human-mediated impact on coastal ecosystems (McGowan 2004). The concentration of nitrogen in nearshore environments is believed to have doubled due to human activities, primarily urbanization and agriculture (Howarth *et al.* 2002, Seitzinger *et al.* 2002). This increase in nutrients and resultant ecosystem effects, such as reduced water quality and increased algal growth, are referred to as eutrophication (Andersen *et al.* 2006). The degree of nitrogen loading, or eutrophication, has been quantified for the Hawaiian Islands using a nitrogen-footprint index ("N footprint") that takes into account several factors that generate, deliver and retain nitrogen in the nearshore environment (Van Houtan *et al*. 2010). Coastal macroalgae are typically nitrogen limited in their growth (Larned 1998) and may show strong, species-specific growth responses to pulses of nitrogen from land sources (Pederson and Borum 1996). For a given alga, nitrogen addition may be expected to increase growth and may alter other aspects of algal metabolism as well.

In addition to the primary metabolites that are necessary for growth, maintenance and reproduction, algae also produce an array of secondary metabolites or "natural products" which may function as attractants, deterrents or UV-absorbers (Smit 2004). The carbon nutrient balance hypothesis (CNBH) posits that when nitrogen is not limiting for a plant, most available carbon will be partitioned off for primary growth, leaving little carbon for production of secondary compounds (Stamp 2003). When nitrogen is a limiting factor for growth, more available carbon is used to produce secondary compounds. This suggests that nitrogen enrichment of shorelines could decrease production of secondary compounds in nitrogen-limited algae.

Polyphenolics are ubiquitous secondary metabolites in terrestrial plants and algae, are the most potent class of antioxidants in the human diet, and are often the active components in herbal medicine (Catoni *et al.* 2008, Manach *et al.* 2004). There are several thousand polyphenolic compounds including several hundred that are known in the human diet (Bravo 1998). They are distinguished from other secondary metabolites based on their biosynthetic origin and are composed of at least two carbon-containing benzene rings, each with at least one hydroxyl (OH) group (Crozier *et al.* 2008). When consumed, polyphenolic compounds inhibit starch and sugar digesting enzymes, which has the effect of lowering blood sugar responses to meals (Zhang *et al.* 2007). Polyphenols are also antioxidants; they are understood to be donators of hydrogen atoms, which reduce free radicals, limiting oxidative damage in the body, thereby potentially preventing disease (Aruoma

1998, Nwosu *et al.* 2011, Lee *et al.* 2010, but see Benzie and Wachtel-Galor 2012 and Sheik-Ali *et al.* 2011).

Marine macroalgae, or *limu*, are culturally significant plants in Hawaiʻi and are an important component of traditional Hawaiian diet (Abbott 1996). Prior to European contact (typically marked by the arrival of Captain James Cook in 1778), the Hawaiian diet consisted primarily of complex carbohydrates such as taro, sweet potato, yam, breadfruit and wild greens such as *limu* (~78% of calories from carbohydrate), supplemented by animal foods (Fujita *et al.* 2004, Hughes *et al.* 1998). *Limu* were also used for medicine and ceremony and continue to be gathered for these purposes today (Abbott 1996). While *limu* may have composed only a small portion of the total calories, plant foods such as *limu*, which are used as spices or condiments, are known to a have significant impact on human health, by way of the actions of their secondary compounds (Etkin 1996). For example, the Maasai pastoralists of east Africa add leaves and bark from wild plants as additives into milk and soups. Even though these make only minor contributions to energy intake, the anticholesterolic effect of secondary compounds (saponins and phenols) present in these additives may help explain why their diet, which was at one time based nearly exclusively on the consumption of meat and milk (with as much 66% of calories from fat), has not led to incidence of cardiovascular disease (Johns *et al.* 1999). Algae are known for their diverse array of natural products (Smit 2004) and the secondary metabolites, such as polyphenols, present in edible Hawaiian macroalgae may have played an under-appreciated role in Hawaiian health. To our knowledge, only two studies have investigated antioxidant power in a limited number of edible macroalgae collected in Hawaiʻi (Vijayavel and Martinez 2010, Kelman *et al.* 2012) and no study has determined their levels of polyphenols

The synthesis of the majority of polyphenolic compounds requires the precursor phenylaline, which is produced through the shikimate biosynthetic pathway (Wright *et al.* 2010). After phenylaline is produced, it may be converted to secondary phenolic compounds such as hydroxycinnamic acids, flavonoids, condensed tannins and lignins through the phenylpropanoid pathway, or it may be used directly in the synthesis of proteins for primary metabolism (Haukioja *et al.* 1998). While the occurrence of the secondary phenolic compounds and the pathways producing them are better-characterized in terrestrial plants than in macroalgae, the shikimate pathway which produces phenylaline has been experimentally confirmed in red (Rhodophyta) and brown (Phaeophyceae) algae (Pelletreau and Targett 2008), and the phenylpropanoid pathway that leads to the secondary phenolic compounds has been demonstrated in red algae (Bourarb *et al.* 2004). Further, macroalgae have been shown to contain both hydroxycinnamic acids and flavonoids (Stewart and Stewart 2010) and recent work may have identified lignins in some red algae (Martone *et al.* 2009). The red alga that is the focus of this study contains both hydroxycinnamic acids and flavonoids as major components of its phenolic profile (Abd El Mageid *et al.* 2009).

The competition for phenylaline between primary and secondary metabolism is the basis of the Protein Competition Model (PCM) of phenolic allocation (Wright *et al.* 2010). According to this model, when N is plentiful, phenylaline would be expected to be used in the production of primary metabolites such as proteins. This would reduce production of phenolic compounds. All else being equal, the concentration of polyphenolic compounds in macroalgae would therefore be expected to be lower in coastal areas experiencing high levels of nitrogen loading. While this model has so far only been tested for terrestrial plants, shared biosynthetic and secondary products suggest this trade-off could also be present in red algae.

Growth in algae requires light harvesting pigments including chlorophylls and several accessory pigments. In cyanobacteria and red algae such as *Asparagopsis taxiformis* (Bonnemaisoniaceae (Delile) Trevisan), there is a special class of accessory pigments called phycobiliproteins which are arranged into phycobilisome complexes. Phycobiliproteins are nitrogen-rich compounds that may make up as much as 50% of the soluble protein in the cell (Grossman *et al.* 1993). The energy harvested through the help of phycobilisomes in red algae will be used for primary metabolism including growth, maintenance and reproduction. Under macronutrient limitation (i.e., lack of nitrogen, sulfur, carbon or iron), the phycobilisomes in cyanobacteria will degrade (Grossman *et al.* 1993). It is therefore reasonable to expect that in a nitrogen-limited alga, increasing nitrogen would increase production of photosynthetic machinery, so long as other nutrients are not also limiting. Pulse Amplitude Modulated fluorometry (PAM) is a tool to measure photosynthetic performance by measuring electron transport activity (e.g., Padilla-Gamiño and Carpenter 2007). Measuring the rate of the electron transport in photosystem II provides an approximation of metabolic rate. Increasing nitrogen would be expected to increase the photosynthetic performance, as measured by PAM.

Polyphenolic compounds in macroalgae are known to vary with exposure to herbivory (Pavia and Brock 2000, Van Alsytne 1988, Cronin and Hay 1996), UV radiation (Pavia *et al.* 1997) nitrogen availability (Yates and Peckol 1993, Arnold *et al.* 1995), age, plant part, and desiccation stress and season (Peckol *et al.* 1996). The impacts of these factors on polyphenolic level may be relatively rapid; algal polyphenolics in several brown algae responded to environmental changes within 2-7 days, as indicated by metabolic turnover (Arnold and Targett 2000). Algal polyphenolics have highly variable concentrations, can be induced over a short period of time and vary along environmental or temporal gradients (Targett and Arnold 1998). Previous studies in temperate brown algae have demonstrated, under both lab and field conditions, that the production of the major polyphenol phlorotannin is inversely related to available nitrogen (Yates and Peckol 1993, Arnold *et al.* 1995). This suggests that a trade-off could also be present in the tropical red alga of interest in this experiment, *A. taxiformis*.

This study evaluates the Protein Competition Model of phenolic allocation by measuring photosynthetic performance and secondary metabolism of the native red alga *Asparagopsis taxiformis*, from coastal sites with high, medium and low nitrogen footprint values on Oʻahu Island, Hawaiʻi. In order to understand how eutrophication (particularly, an increased level of nitrogen) influences the photosynthetic rate and polyphenolic concentration of *A. taxiformis*, this study includes: (1) measuring the photosynthetic maximum (Pmax) of live field-collected plants from each of three sites that vary in their nitrogen footprint using Junior PAM, and (2) extracting and testing these same algal samples for their polyphenolic content using the colorimetric Folin-Ciocalteau test. I hypothesized that the photosynthetic maximum of the plants would increase with increasing nitrogen because this nitrogen can be utilized in the production of the light harvesting chlorophylls and phycobiliproteins. I also hypothesized that the polyphenolic content of the alga would decrease as the nitrogen content increases because under high nitrogen conditions more phenylaline will be used in primary growth and less will be available for production of polyphenolic compounds. These hypotheses follow from the Protein Competition Model of phenolic allocation.

METHODS

Study species

Asparagopsis taxiformis is known in Hawaiian as *limu kohu* (the supreme seaweed) and is the favorite edible seaweed of many Hawaiians and is one of the most commonly consumed wild algae in Hawaiʻi in contemporary times (Abbott 1996, 1999). Its essential oil contains a large number of halogenated compounds, primarily bromoform, which is only slightly soluble in water (Burreson *et al.* 1976). *Limu kohu* is typically soaked overnight in water before it is prepared for consumption in order to alter the taste; for example to reduce the "iodine taste" in the seaweed (Abbott 1996). Polyphenols in the water extract of *Asparagopsis taxiformis* include: cinnamic acid, caffic acid, dihydroxybenzoic, vanillic acid, protocatechuic, catechin, quercetin, chlorogenic acid, and hydroxytyrosol (Abd El Mageid *et al.* 2009).

Collections

Seven to ten plants were collected from each of three populations of *Asparagopsis taxiformis* along a nitrogen gradient on Oʻahu Island. Mokuleia Public Beach Access on the northshore has a medium-low N footprint (hereafter referred to as "Mokuleia"), Kaʻalaʻwai, or Diamond Head, has a medium-high N footprint, ("Diamond Head"), and Kaiaka Beach Park has the highest possible N footprint ("Kaiaka")(Figure 20). One-third to one-half of each *A. taxiformis* clump at 0.5 to 1 m depth were collected with care, by hand, to maximize the amount of holdfast removed and minimize stress to the plant. This material was transferred to the lab in plastic bags with ample seawater in a cooler with a small amount of ice to avoid thermal stress. Photosynthetic measurements were initiated immediately after returning to lab. Mokuleia and Kaiaka collections and measurements were made on November 26th, 2011. Diamond Head collections and measurements were made on November 27th, 2011. Weather was similar (partly cloudy) on both days.

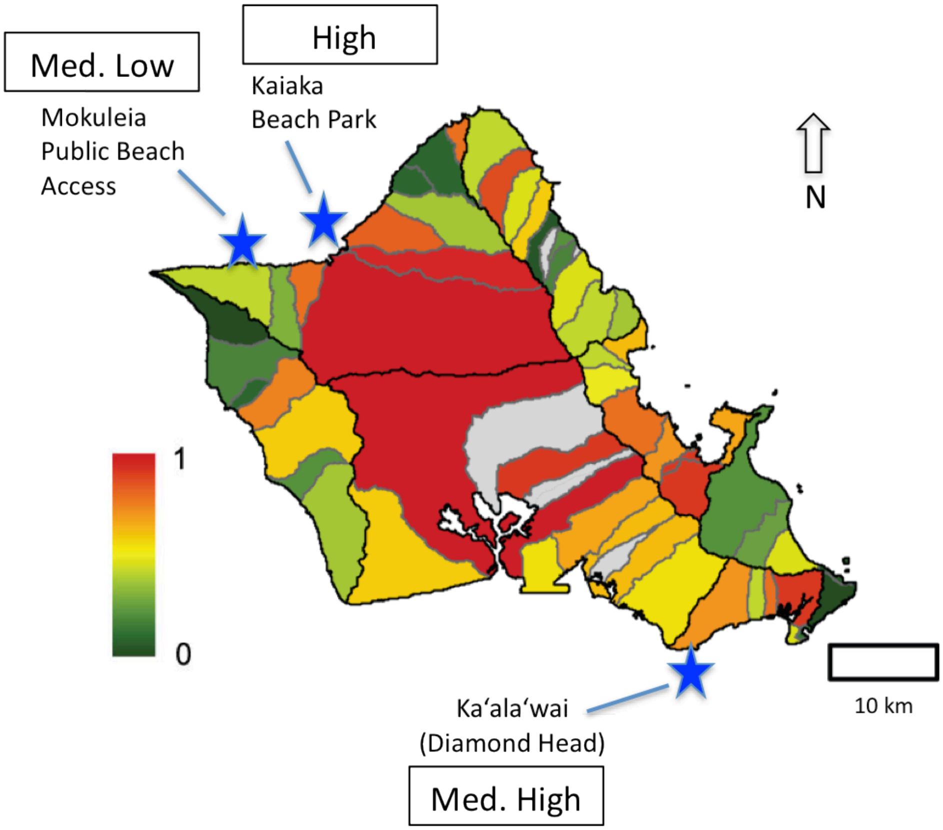


Figure 20. Nitrogen-footprint map for O'ahu Island with collection sites. Red (1) indicates the highest level of nitrogen and dark green the lowest (0). Blue stars indicate the three collection sites for this study and the boxes indicate the N footrpint level for the watershed where the sites are found. Modified from Figure 4 in Van Houtan *et al.* 2010.

Photosynthetic performance

Photosynthetic performance was measured using pulse amplitude modulated fluorometry (Junior PAM). Measurements were taken under laboratory light conditions while the plants were submerged in seawater. A glass microscope slide cover was inserted between the fiber optic cable and the alga to standardize the measurement distance. Measurements were taken on one axis per individual plant, just below the branching portion of the thallus. Light response curves were taken with appropriate yield (>0.9) and appropriate initial Ft (~300) and were used to estimate the ETRmax, which is the activity along the electron transport chain that is observed at the light saturation level for that individual. The settings for the Junior PAM were as follows: Gain =2, measuring light intensity= 5, light curve intensity= 2. In addition to the photosynthetic measurements, the diameter of the main axis at the point of measurement, the width of the longest lateral branch and the length of the axis being measured were recorded as covariates. Mean ETRmax was compared among the three sites using ANOVA. Homogeniety of variances was tested using the Bartlett and Fligner tests, and normality was tested with the Shapiro-Wilk test (Crawley 2007). All analyses were performed in R (R Core Development Team 2008).

Extractions

After photosynthetic measurements, plants were carefully washed in seawater to remove any epiphytes, sand or invertebrates. Plants were pat dry and set to air dry on aluminum foil in a dark, ventilated room for 72 hrs. Algal water extracts were then prepared by placing 0.42 (+/- 0.01) grams dry weight of the upper, branched portion of one algal thallus per plant in 50 mL of deionized water. Algae were extracted for 24 hours at room temperature in the dark and then held for 10 days at -20°C before assaying. Extracts were then brought to room temperature, filtered and used in the Folin-Ciocalteu assay as described below.

Polyphenolic determination

The total content of phenolic and polyphenolic compounds of the water extract was determined by a modification of the Folin–Ciocalteu method (Swain and Hillis 1959, Inderjit 1996). Five milliliters of filtrate was combined with 0.5 mL of Folin–Ciocalteu reagent and 1 mL of saturated $NaCO_3$ solution in a glass test tube. Deionized water was added to bring the mixture to 10 mL. This was vortexed briefly and allowed to stand for 30 minutes. The absorbance was then measured on a Diode Array Spectrophotometer at 725 nm. A standard curve was prepared with known concentrations of tannic acid. The content of phenolic and polyphenolic compounds was calculated in mg tannic acid equivalents per gram of dry weight of algae (mg TAE/g dry weight). The standard curve (Absorbance = 5.7997*Concentration) had an r^2 of 0.995 (Figure 21).

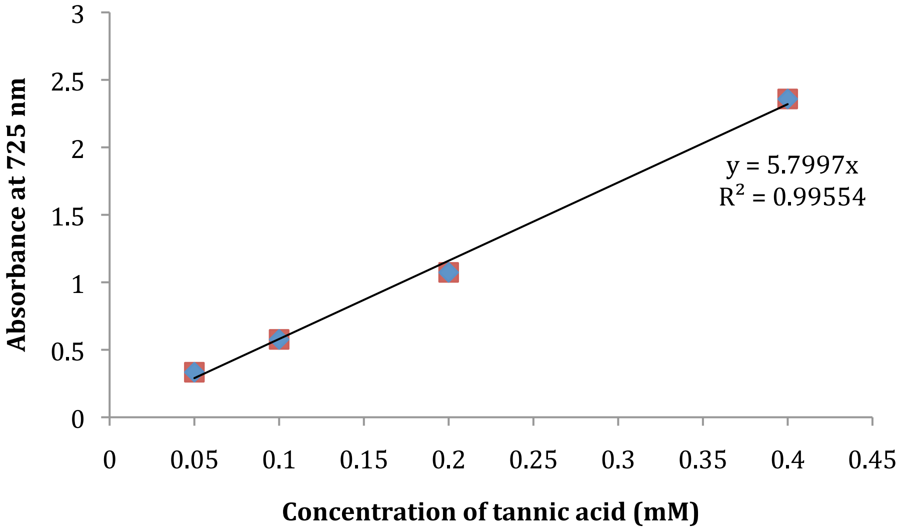


Figure 21. Calibration curve showing the absorbance at 725 nm for four known concentrations of tannic acid. Error bars indicate one standard deviation (not visible). Concentration (in mM tannic acid equivalents) is equal to the absorbance, divided by 5.7997. Measurements were taken in triplicate.

Data analysis

Milligrams TAE per gram dry algae were compared across the three sites using ANOVA. Homogeniety of variances was tested using the Bartlett and Fligner tests and normality was tested with the Shapiro-Wilk test (Crawley 2007). All analyses were performed in R (R Core Development Team 2008).

RESULTS

Photosynthetic performance

Maximum electron transport rate (ETR max) did not vary significantly between sites (ANOVA: df=2, F= 1.14, p= 0.338) (Figure 22). ETR max ranged from 7.5 to 22.3. No clear trend was observed for the influence of nitrogen footprint on photosynthetic performance.

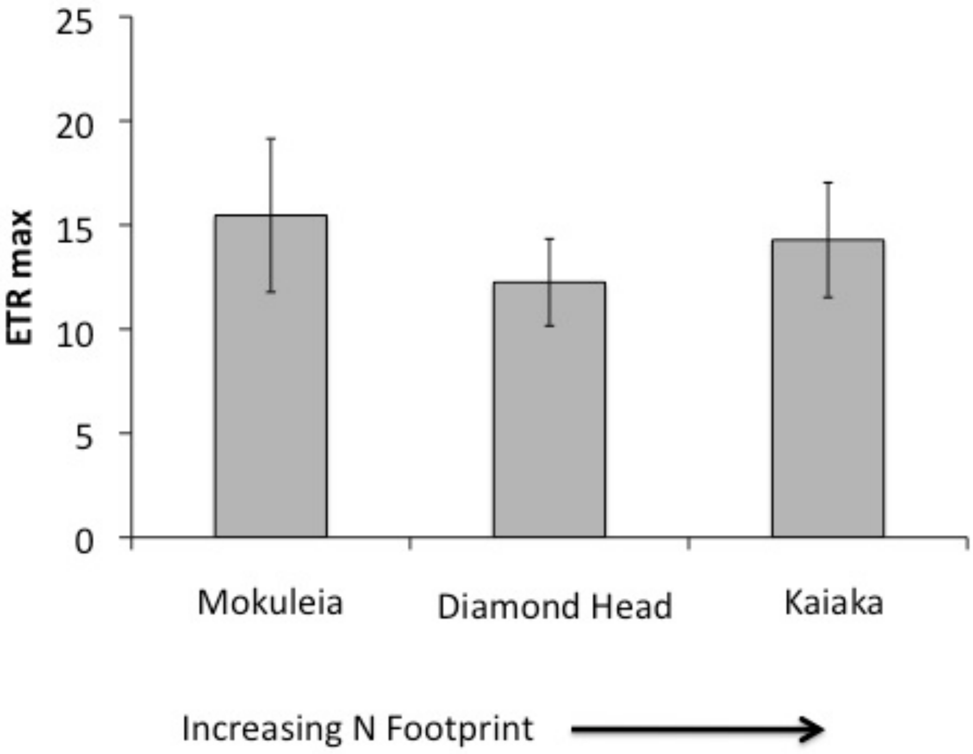


Figure 22. Mean ETR max by site. Error bars indicate 2 standards errors (n= 6, 3 and 2). (ANOVA: df=2, F= 1.14, p= 0.338).

Polyphenolic content

The total content of polyphenolic compounds did not vary significantly by site when compared across the three sites (ANOVA: df=2, F= 3.02, p= 0.104) (Figure 23). Total polyphenolic content, under the extraction procedures described, was estimated to vary from 8.8 to 29.2 mg TAE per g dry weight of algae. There was a trend for polyphenolic content to decrease with increasing N footprint, though this trend is not statistically significant.

Given the small sample size from Mokuleia and Diamond Head (due to experimental error that caused some samples to be discarded), these two sites were combined for a second analysis. Grouped in this manner, the medium nitrogen sites (Mokuleia and Diamond Head) had significantly higher polyphenolic concentration that the high nitrogen site (Kaiaka) (Welch's t-test: t= 3.99, df= 14.9, p= 0.001)(Figure 24).

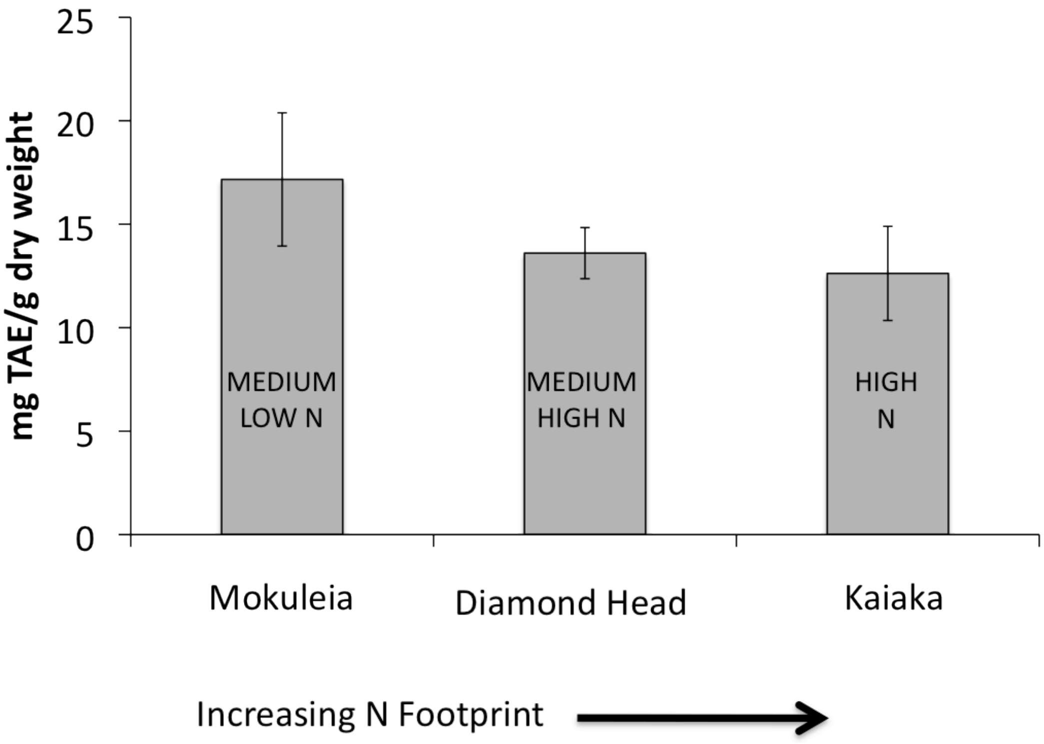


Figure 23. The mean level of polyphenols, in mg TAE per g of dry weight of algae across three sites. Errors bars indicate 2 standard errors (n= 3, 2 and 5). (ANOVA: Df=2, F=3.02, p= 0.104).

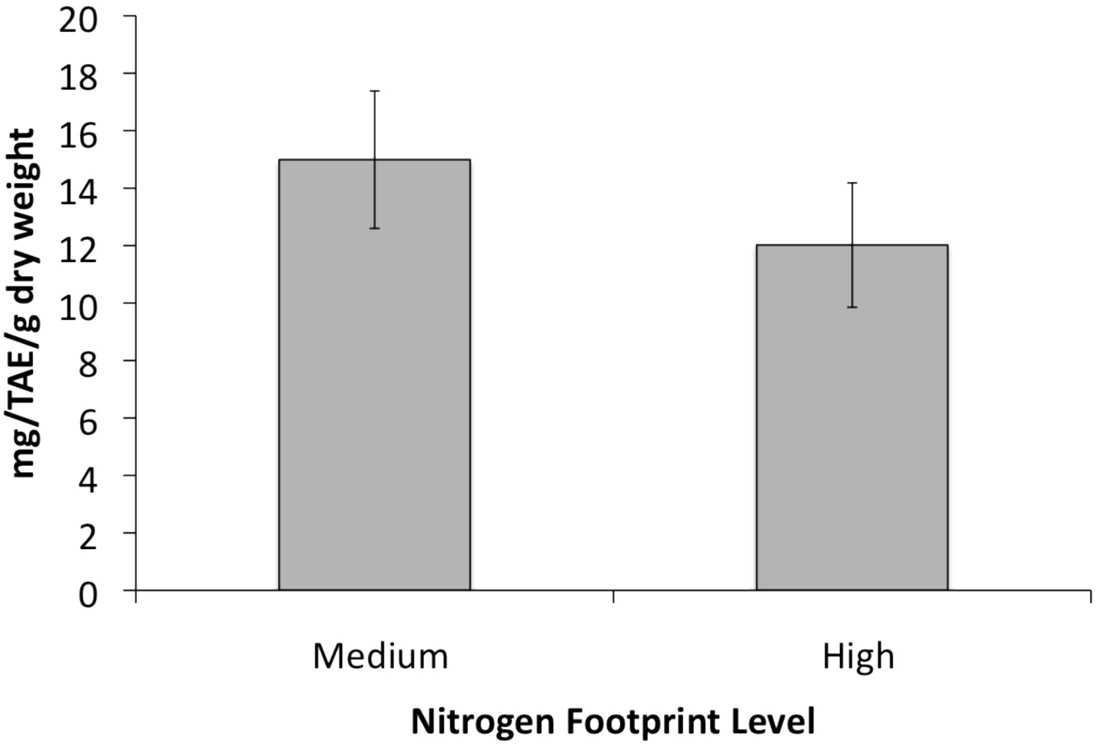


Figure 24. The mean level of polyphenols, in mg TAE per g of dry weight of algae between two nitrogen-footprint categories. Errors bars indicate 2 standard errors (n= 6 and 5). (Welch's t-test: t= 3.99, df= 14.9, p= 0.001).

DISCUSSION

No difference was detected between *limu kohu* (*Asparagopsis taxiformis*) plants at low, medium and high nitrogen sites in photosynthetic performance, while for polyphenolic concentration, plants at medium nitrogen sites had significantly higher levels of polyphenolics than those at the high nitrogen site. A large sample size, different experimental methods, and/or greater consideration for covariates may be necessary to disentangle the factors regulating photosynthetic performance. With regard to polyphenolic concentration, these results provide some preliminary evidence to support the hypothesis that increased nitrogen loading (eutrophication) of coastal waters decreases polyphenolic concentration (a source of antioxidants) in *A. taxiformis*.

Photosynthetic performance

Unmeasured biotic and abiotic variation could have contributed to the large intrasite variation observed in photosynthetic performance (Figure 22). Factors such as the age, life stage and size could contribute to inter-plant variation in photosynthetic rate (Dunton and Jodwalis 1988, Altamirano *et al.* 2003). Microsite abiotic variation in available light, temperature (Padillo-Gamiño and Carpenter 2007), salinity (Martins *et al.* 1999), wave action and other nutrients such as phosphorus (Larned 1998) could also help to explain the observed variation.

The temporal scale of measurement may also help explain the lack of accordance between observed and expected results. Measurements were taken at a single point in time, while N footprint is an index that provides a longer-range, average characterization of a given site (see Van Houtan *et al.* 2010). Polyphenolic phlorotannins in brown algae are known to have a turnover rate as fast as 2-7 days (Arnold and Targett 2000). Therefore, the polyphenolics measured in the plants in this study may have been formed in response to nitrogen conditions at a smaller temporal scale than that which was used to classify the nitrogen loading of watersheds.

Another possibility is that *A. taxiformis* did not show a response to nitrogen loading because it is less nutrient limited in its growth, or because it differs from other macroalgae in nitrogen requirements and nitrogen storage. Macroalgae are known to show highly species-specific growth responses to pulses of nitrogen from land sources (Pederson and Borum 1996). While Larned demonstrated nitrogen limitation in the majority of Hawaiian macroalgae tested, other macroalgae are known to be phosphorous limited (2008). Due to the difficulties of maintaining *A. taxiformis* in laboratory settings, to my knowledge, its nutrient growth limitations have not been tested in a controlled setting.

Intersite, as well as some intrasite variation in photosynthetic performance or secondary metabolism could also be explained by evolutionary history. *Asparagopsis taxiformis* is known to have at least 4 lineages, 3 of which occur in Hawai'i (Sherwood 2008). The lineage of the samples utilized in this study is not known, though past sampling suggests Kaiaka and Mokuleia may have the same lineage, while Diamond Head plants would belong to a different lineage. Differences between lineages could have contributed to variation measured in this study.

Asparagopsis taxiformis plants had ETR max rates similar to other studies (Padillo-Gamiño and Carpenter 2007). Given that photosynthetic rate varied significantly along different portions of the thallus (data not shown), in the future, measurements could be taken in various different locations on the thallus and the mean or maximum value could be used to approximate photosynthetic performance.

Another option would be to determine relative ETR max, as in Padillo-Gamiño and Carpenter, by measuring the branching portions and multiplying the yield by the PAR (2007). These more robust measurements would allow for a clearer characterization of intra-plant, inter-plant and between-site variation.

Polyphenolic content

This study demonstrated that the water extract of *Asparagopsis taxiformis* has detectable levels of polyphenols as measured by the Folin-Ciocalteu assay method. While the concentration in this study was lower than that of other studies (El-Baroty *et al.* 2007), this is likely due to differences in extraction procedure (e.g., samples were not ground before extraction in this study). Despite the small and uneven sample sizes (n= 6, 3, and 2 per site), there was a trend for polyphenolic content to decrease as the N footprint increased across the three sites, and a significant difference was shown between medium and high nitrogen sites. Sampling over a larger number of watersheds, while measuring important covariates, would help to elucidate the role of nitrogen and other factors in determining the polyphenolic concentration of individual plants. If these preliminary results relating to polyphenolic concentration in *limu kohu* (*Asparagopsis taxiformis*) could be confirmed with larger-scale or more controlled studies, that would further support the hypothesis that human-caused degradation to coastal ecosystems is altering the chemistry and decreasing the nutritional benefit in a culturally significant edible marine resource.

The Folin-Ciocalteu assay has been used extensively to measure total phenolic content of plant material (Inderjit 1996). Given that this test may be measuring the reducing capacity of other compounds apart from polyphenols, the assay is most useful for relative comparisons rather than quantitative determinations (Prior *et al.* 2005). In addition, each polyphenol will have a different reducing capacity that will in most cases not correspond with the reducing potential of the standard used in the calibration curve (Appel 2001). Further, if plants at one site have a larger proportion of a particular polyphenol with greater reducing capacity, this site will be identified as one with plants with greater polyphenolic content, though they may in fact have a lower content of high-activity polyphenols (Appel 2001). Given these complications, a more accurate means of measuring polyphenols would be the use of high-profile liquid chromatography (HPLC). In this case, a particular class of polyphenols, with particular relevance to health, could be quantified more directly and reliably.

Establishing a link between the identity and quantity of secondary metabolites in edible algae and their taste would be another useful step forward. This would be particularly interesting for secondary compounds that have health benefit for the consumer. While many polyphenols have known health benefits as well as bitter or astringent taste, the taste profile of *Asparagopsis taxiformis* is likely to be primarily formed by compounds such as halogenated hydrocarbons. Future research could explore the link between taste, chemistry and biology of the native wild-harvested macroalgae of Hawai‘i.

CHAPTER 3

CONCLUSIONS AND RECOMMENDATIONS

This study used a combination of pharmacological, ethnographic and ecological methods to: (1) evaluate current levels and determinants of seaweed consumption and of wild seaweed (*limu*) gathering across generations on Oʻahu, (2) document perceptions of change in *limu* abundance Oʻahu Island, (3) identify differences in antioxidant potential between wild and cultivated seaweeds, and (4) explore how eutrophication of coastal waters may influence antioxidant potential of edible *limu*. While the results and some conclusions are given in the preceding two chapters, below I give more specific conclusions and recommendations for resource management and educational practice from my perspective as a research scientist.

RESOURCE MANAGEMENT

Conclusions for resource management

- Populations of native seaweeds (*limu*) are perceived by resource users to have undergone a severe decline in recent years.
- Cultural practitioners, and particularly *limu* gatherers, have ecological knowledge, including knowledge of changes in abundance of native macroalgae, that may not be otherwise known, recorded, or available.
- Over-picking (harvest by non-traditional means or for non-traditional purposes) and pollution were the top-cited reasons for decline in *limu* abundance on Oʻahu.

- Pollution (in the form of increased nitrogen loading) may also decrease the antioxidant health benefit of seaweeds to human consumers.
- Wild seaweed gathering is relatively common among Oʻahu youth, particularly among Hawaiian and Filipino families, and may be increasing in some regions of the island.

Recommendations for resource management

- Continue and increase efforts to restore *limu* populations, particularly projects that involve *limu* gatherers and other community members.
- From a harvest perspective, sustainable use can be encouraged by recognizing and supporting harvest for cultural and family use, promoting education on proper harvest technique, and regulating harvest for commercial purposes.
- From an environmental perspective, the impact of the alteration of water quality and flow on nearshore ecosystems due to development could be assessed by taking biological and/or cultural surveys of macroalgal abundance and population locations (or perceived abundance and locations) several months before and after development projects.
- The cultural value and health benefits of marine resources can be used synergistically with biological arguments to support species conservation and ecosystem management goals.

EDUCATIONAL PRACTICE

Conclusions for educational practice

- Cultural practice of *limu* gathering continues and may be increasing in some regions of the island.
- Name knowledge held by students is low and does not always accompany cultural practice, indicating a loss of traditional knowledge.

- Community members and particularly cultural practitioners have knowledge of value to science, and knowledge not held by younger generations.
- Incorporating traditional knowledge into the science classroom can support intergenerational transmission, can broaden student understanding of multiple ways of knowing, can engage community members in the learning process and can elevate indigenous knowledge systems, supporting greater social equity.

Recommendations for educational practice

Incorporate traditional knowledge into the science classroom through:

- Revision of standards to require understanding of multiple ways of knowing, emphasizing Hawai‘i’s multiethnic population and indigenous heritage
- Encouraging incorporation of traditional knowledge through:
  - Student-conducted interviews with community members
  - Guest lectures from cultural practitioners
  - Greater student contact with the natural environment

APPENDIX A

SURVEY, INTERVIEW AND CONSENT FORMS

In-class student survey

Limu in-class survey

Directions: Please answer all of the following questions. If you do not wish to answer one or more of the questions, please write "N/A" next to the question.

Age ___________ City and Country of birth ______________________

Gender __________ Ethnicities ___________________ ___________________
___________________ ___________________

1. Which foods do you eat that contain limu or seaweed? *Check all boxes that apply.*

- ☐ Sushi
- ☐ Musubi
- ☐ Salads with limu or seaweed
- ☐ Poke
- ☐ Soups with limu or seaweed
- ☐ Opihi (limpet) with limu
- ☐ Limu or seaweed plain/alone
- ☐ Others- please list ______________
- ☐ I DO NOT eat any foods that contain seaweed or limu because __________________________
____

2. Which food that contains limu or seaweed would you say that you eat the most? *Write the name of the food on the line below, or check the box.*

____

☐ I do not eat any foods with limu or seaweed in them

3. How many meals or snacks do you eat in a week that include some kind of limu or seaweed? *Check one box.*

- ☐ none
- ☐ less than 1 per week
- ☐ 1 to 3 per week
- ☐ 4 to 7 per week
- ☐ 8 to 14 per week
- ☐ 15 to 21 per week
- ☐ more than 21 per week

4. Which edible limu or seaweed is your favorite?

5. Please list the names of all the kinds of limu or seaweed that you know.

6. Have you ever used limu or seaweed to heal an injury or illness? If so, which have you used?

7. How many years have you lived in XXX *(region of survey)*?

Turn over ↓

8. How many years have you lived in the Hawaiian Islands?

(survey page break)

9. Do you ever gather limu or seaweed from the ocean or beach? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ________________
☐ No

10. Do (or did) your <u>parents</u> gather limu or seaweed? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ________________
☐ No
☐ I don't think so
☐ I don't know

11. Do (or did) your <u>grandparents</u> gather limu or seaweed? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ________________
☐ No
☐ I don't think so
☐ I don't know

12. In which part of the island (or in what other place) did your **parents** grow up?

____

13. In which part of the island (or in what other place) did your **grandparents** grow up?

____

14. Do you ever eat limu or seaweed that has been gathered or picked up from the ocean or beach? *Check one box and answer on the lines next to the box you checked.*

☐ Yes If "Yes", how often? ____________________________________
If "Yes", which kinds of limu or seaweed? ____________________
☐ No If "No", why not? _______________________________________

15. Do you ever buy limu, ogo or seaweed at the store or market? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ____________________________________
If "Yes", which kinds of limu or seaweed? ____________________
☐ N

16. How would you describe the taste of limu or seaweed?

Mahalo for completing this survey!

Student interview homework assignment

*Limu interview homework assignment*

Directions: For this assignment you will need to interview someone who lives in your community and who is **at least 20 years older** than you. It can be a family member, but it does not have to be.

Please ask the person you interview all of the following questions and record their answers on this paper. If he or she does not wish to answer one or more of the questions, please write "N/A" next to the question.

Age ___________ Place of birth ______________________

Gender __________ Ethnicities ___________________ ___________________

___________________ ___________________

1. Which foods do you eat that contain limu or seaweed? *Check as many boxes as apply.*

- ☐ Sushi
- ☐ Musubi
- ☐ Salads with limu or seaweed
- ☐ Poke
- ☐ Soups with limu or seaweed
- ☐ Opihi (limpet) with limu
- ☐ Other- please list ______________
- ☐ Other- please list ______________
- ☐ I DO NOT eat any foods that contain seaweed or limu because __________________________ ____________________________________

2. Which food that contains limu or seaweed would you say that you eat the most? *Write the name of the food on the line below, or check the box.*

____

☐ I do not eat any foods with limu or seaweed in them

3. How many meals or snacks do you eat in a week that include some kind of limu or seaweed? *Check one box.*

- ☐ None
- ☐ 1 to 3 per week
- ☐ 4-7 per week
- ☐ 8-14 per week
- ☐ 15-21 per week
- ☐ more than 21 per week

4. Which edible limu or seaweed is your favorite?

5. Please list the names of all the kinds of limu or seaweed that you know.

6. Have you ever used limu or seaweed for medicine? If so, which have you used?

7. Do you ever gather limu or seaweed from the ocean or beach? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ______________
☐ No

8. Do (or did) your <u>parents</u> gather limu or seaweed? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ______________
☐ No
☐ I don't know

9. Do (or did) your <u>grandparents</u> gather limu or seaweed? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ______________
☐ No
☐ I don't know

10. Do you ever eat limu or seaweed that has been gathered or picked up from the ocean or beach? *Check one box and answer on the lines next to the box you checked.*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ______________
☐ No If "No", why not? ________________________________

11. Do you ever buy limu, ogo or seaweed at the store or market? *Check one box and answer on the lines if you check "Yes".*

☐ Yes If "Yes", how often? ______________________________
If "Yes", which kinds of limu or seaweed? ______________
☐ No

12. How long have you lived in XXX *(region of survey)?* Where did you grow up?

13. What changes have you noticed over time to the availability or quality of the limu or seaweed on Oahu (or elsewhere that you are familiar with)?

14. What seems to be the reason for the changes you mentioned in question 13?

15. If you are going to gather limu or seaweed, how do you decide **when** you will gather?

16. If you are going to gather limu or seaweed, how do you decide **where** to gather?

17. Is there any other information you would like to share about limu or seaweed?

18. (*Ask the person at least one additional question that you are curious about. Record the question and answer below*.)
Question: ____
____

Answer:

19. Can we contact you again for this limu study? If so, please list a contact phone number or email.

Mahalo for completing this interview!

Please direct questions or comments to Georgia Hart at gmhart@hawaii.edu, or by calling XXX-XXX-XXXX.

Semi-structured interviews with adults

Semi-structured interview questions

Part I – initial questions
The structure of the interview is flexible and may not include all questions listed, nor follow the particular order shown here.

"What is the difference for you between the words 'limu" and 'seaweed'?"
"Tell me how you first learn about gathering seaweeds."
"Who do you gather with?"
"Did you gather as a child, as a young adult? Have you changed how often you gather over the course of your lifetime?"
"How often did your mother or grandmother use to gather?"
"When did you last gather seaweed?"
"How do you know if it is the right time to gather?"
"How do you decide where to gather?"

→Show map with sectors of the island
"I won't ask you exactly where you gather because I realize you may not want to share that information, but I would like to know approximately what part of the island you go to gather. Would you be willing to identify the sections of the island on this map where you gather?" (Interviewee may then circle regions of the map and identify when he or she had gathered in that region and what they had gathered)

"What are the names of seaweeds that you gather?"
"Are there any kinds of seaweed that you would like to gather, but cannot? Why? Is this different than in the past? When did these changes begin to occur?"
"Would you like to see the practice of seaweed gathering continue among the younger generations? Why or why not?"

Part II – Samples and pictures
→ the interviewee was shown pictures of edible macroalgae one at a time (and actual samples when possible) and asked to share information on each species:

"Do you recognize this type of seaweed?"
"Do you gather this type of seaweed?"
"How do you use the seaweed?"
"Has this type become more common or more rare over time?"
"What are the reasons for the change in abundance you mentioned?"
"Have you noticed any changes in the taste of this type of seaweed over time or from different areas?"

"From the time you arrive at the site to collect, walk me through all the steps you follow in gathering the seaweed." (this question would only be repeated for a second gathered species if the method was different)

“Which part of the plant do you gather?”
“How is it removed/collected?”
“How is it processed and eaten?”

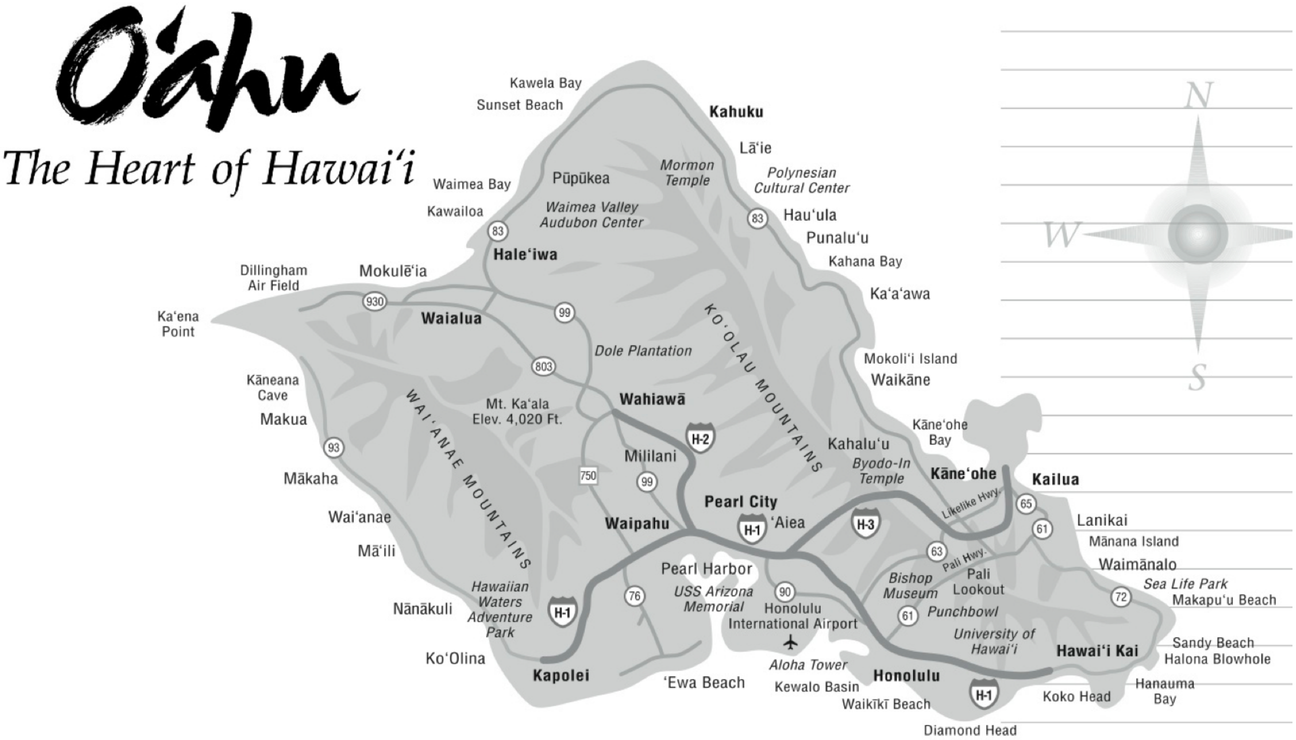
Part III – health benefits of seaweeds
“Why do people eat seaweeds?”
“How do they affect the body?”
“Is there any way to predict the taste of a seaweed before you gather it?”

Semi-structured interview species table for note taking

Species	**recognize?**	**gather?**	**change in abundance**	**reason**	**taste variation**	**part gathered**	**method**	**preparation/ processing**	**foods**
kohu									
līpoa									
wāewae'iole									
lipe'epe'e									
mane'one'o									
ʽeleʽele									
pālahalaha									
kala									
paheʽe									
huluhuluwaena									
lepe-o-hina									
manauea									
parvispora									
salicornia									
ʽakiʽaki									

Semi-structured interview map for recording limu *locations*

The map below was used for adults to identify *limu* populations and gathering locations during semi-structured interviews.



“Oahu, Hawaii Tourist Map” accessed from mappery.com

Assent and consent forms

Minor assent

University of Hawai'i

Minor assent form to Participate in Research Project:

Contemporary seaweed gathering and consumption in the Hawaiian Islands

Aloha, my name is Georgia Hart. I am a researcher from the University of Hawaiʻi at Manoa in the Department of Botany. I am originally from Corvallis, Oregon and I have been on Oʻahu since August of 2010. As part of my Masters degree I am researching the practice of seaweed gathering and consumption in the Hawaiian Islands. My research questions are: (1) How often is seaweed eaten and gathered by high school students and adults (35 yrs+) in the Hawaiian Islands? and (2) What are the main reasons for any generational changes in seaweed consumption and gathering?

I have recently visited and observed your high school classroom. Your science teacher has agreed to allow me to distribute a survey to your class and has agreed to give a homework assignment to your class as part of my research. The purpose of this form is to ask your permission for the information from your survey and your interview assignment to be included, anonymously, in my research results.

Project Description - Activities and Time Commitment: The class survey asks you about your patterns of seaweed consumption and seaweed gathering. It will be completed in class and will take approximately 10 minutes. For the homework assignment, I am asking you to interview someone in the community who is at least 20 years older. It may be a family member or not. The interviewee does not have to gather or eat seaweed in order to be interviewed. I have a form that you should use that has specific questions to be completed, but you are encouraged to also design one of your own questions. The interview also focuses on the gathering and consumption of edible seaweeds. One example of the type of question I will ask is, “How many meals or snacks do you eat per week that contain seaweed?” The interview should take approximately 10-20 minutes to complete.

Benefits and Risks: The investigator believes there is little or no risk for you in participating in this research project. Participating in this research may be of no direct benefit to you. However, you may appreciate the process of learning more about your community. It is believed, also, that the results from this project will help identify changes in the dietary patterns in your community and that the study will provide suggestions for improvement of diet and general health. If you feel uncomfortable or stressed by any of the questions, you may skip the question, or take a break, or withdraw from the project altogether.

Confidentiality and Privacy: During this research project, I will keep all data from the surveys and interviews in a secure location. Only my University of Hawaii advisor and I will have access to the data, although legally authorized agencies, including the University of Hawai'i Committee on Human Studies, have the right to review research records. Your name will not be used in my data or written report. If you would like a copy of my final report, please contact me at the number listed near the end of this consent form.

Voluntary Participation: Participation in this research project is voluntary. You can choose freely to participate or not to participate. In addition, at any point during this project, you can withdraw your permission, and can stop participating without any penalty or loss of benefits.

Questions: If you have any questions regarding this research project, please contact the researcher, Georgia Hart, at XXX-XXX-XXXX, 808-956-3931, 808-956-8369 or gmhart@hawaii.edu. If you would like to be interviewed by the researcher, or would like to be otherwise involved, or would like to suggest someone to be interviewed, please contact the researcher at the email or phone number above.

If you have any questions regarding the rights of your child as a research participant, please contact the University of Hawai'i Committee on Human Studies (CHS) by phone at (808) 956-5007, or by email at uhirb@hawaii.edu

Please keep the prior portion of this consent form for your records.
If you consent for your child to participate in this project, please sign the following signature portion of this consent form and return it to your science teacher.

Tear or cut here

Signature for Assent:

I agree to participate in the research project entitled, *Contemporary seaweed gathering and consumption in the Hawaiian Islands.* I understand that I can change my mind about participating in this project, at any time, by notifying the researcher.

Your Name (Print): ____

Your Signature: ____

Date: ______________________________

Parent/guardian consent

University of Hawai'i

Parental/Guardian's Consent for Child to Participate in Research Project:
Contemporary seaweed gathering and consumption in the Hawaiian Islands

Aloha, my name is Georgia Hart. I am a researcher from the University of Hawaiʻi at Manoa in the Department of Botany. I am originally from Corvallis, Oregon and I have been on Oʻahu since August of 2010. As part of my Masters degree I am researching the practice of seaweed gathering and consumption in the Hawaiian Islands. My research questions are: (1) How often is seaweed eaten and gathered by high school students and adults (35 yrs+) in the Hawaiian Islands? and (2) What are the main reasons for any generational changes in seaweed consumption and gathering?

I have recently visited and observed your child's high school classroom. Your child's science teacher has agreed to allow me to distribute a survey to his/her class and has agreed to give a homework assignment to his or her students as part of my research. The purpose of this form is to ask your permission for your child's survey and interview information to be used for my thesis research. I also will ask your child if s/he agrees to allow his or her information to be included, anonymously, in my research results.

Project Description - Activities and Time Commitment: The class survey asks your child about his or her own patterns of seaweed consumption and seaweed gathering. It will be completed in class and will take approximately 10 minutes. For the homework assignment, I am asking your child to interview someone in the community who is at least 20 years older. It may be a family member or not. The interviewee does not have to gather or eat seaweed in order to be interviewed. I have a form that students should use that has specific questions to be completed, but students are encouraged to also design one of their own questions. The interview also focuses on the gathering and consumption of edible seaweeds. One example of the type of question I will ask is, "How many meals or snacks do you eat per week that contain seaweed/limu/ogo?" If you would like to see a copy of all of the questions that I will ask, please contact me via the phone number or email address listed near the end of this consent form.

Benefits and Risks: The investigator believes there is little or no risk for the student in participating in this research project. Participating in this research may be of no direct benefit to the student. However, the student may appreciate the process of learning more about their community. It is believed, also, that the results from this project will help identify changes in the dietary patterns in your community and that the study will provide suggestions for improvement of diet and general health. If your child feels uncomfortable or stressed by any of the questions, he or she may skip the question, or take a break, or withdraw from the project altogether.

Confidentiality and Privacy: During this research project, I will keep all data from the surveys and interviews in a secure location. Only my University of Hawaii advisor and I will have access to the data, although legally authorized agencies, including the University of Hawai'i Committee on Human Studies, have the right to review research records. Your child's name will not be used in my data or written report. If you would like a copy of my final report, please contact me at the number listed near the end of this consent form.

Voluntary Participation: Participation in this research project is voluntary. Your child (and you) can choose freely to participate or not to participate. In addition, at any point during this project, you can withdraw your permission, and your child can stop participating without any penalty or loss of benefits.

Questions: If you have any questions regarding this research project, please contact the researcher, Georgia Hart, at XXX-XXX-XXXX, 808-956-3931, 808-956-8369 or gmhart@hawaii.edu. If you would like to be interviewed by the researcher, or would like to be otherwise involved, or would like to suggest someone to be interviewed, please contact the researcher at the email or phone number above.

If you have any questions regarding the rights of your child as a research participant, please contact the University of Hawai'i Committee on Human Studies (CHS) by phone at (808) 956-5007, or by email at uhirb@hawaii.edu

Please keep the prior portion of this consent form for your records.
If you consent for your child to participate in this project, please sign the following signature portion of this consent form and return it to your child's science teacher.

Tear or cut here

Signature(s) for Consent:

I give permission for my child to participate in the research project entitled "*Contemporary seaweed gathering and consumption in the Hawaiian Islands.*" I understand that, in order to participate in this project, my child must also agree to participate. I understand that my child and/or I can change our minds about participation, at any time, by notifying the researcher of our decision to end participation in this project.

Name of Child (Print): ____________________________________
Name of Parent/Guardian (Print): ______________________________
Parent/Guardian's Signature: ________________________________
Date: ____________________

Adult interviewee consent

University of Hawai'i

Consent to Participate in Interview for Research Project:

Contemporary seaweed gathering and consumption in the Hawaiian Islands

Aloha, my name is Georgia Hart. I am a researcher from the University of Hawaiʻi at Manoa in the Department of Botany. I am originally from Corvallis, Oregon and I have been on Oʻahu since August of 2010. As part of my Masters degree I am researching the practice of seaweed gathering and consumption in the Hawaiian Islands. My research questions are: (1) How often is seaweed eaten and gathered by high school students and adults (35 yrs+) in the Hawaiian Islands? and (2) What are the main reasons for any generational changes in seaweed consumption and gathering?

Students in a science class at your local high school have been assigned a homework assignment that I will use to help answer the questions above. The assignment asks the student to interview someone in his or her community. You have been selected by one of those students to be interviewed. The purpose of this form is to ask your permission for the information obtained in that interview to be used for my thesis research.

Project Description - Activities and Time Commitment: For the homework assignment, I am asking the high school student to interview someone in his or her community who is at least 20 years older. It may be a family member or not. The interviewee does not have to gather or eat seaweed in order to be interviewed. I have a form that students should use that has specific questions to be completed, but students are encouraged to also design one of their own questions. The interview also focuses on the gathering and consumption of edible seaweeds. One example of the type of question I will ask is, "How many meals or snacks do you eat per week that contain seaweed?" The interview should take approximately 10-20 minutes to complete.

Benefits and Risks: The investigator believes there is little or no risk for you as the interviewee in participating in this research project. Participating in this research may be of no direct benefit to you. However, you may appreciate the process of communicating with and transmitting information to a younger generation. It is believed, also, that the results from this project will help identify changes in the dietary patterns in your community and that the study will provide suggestions for improvement of diet and general health. If you feel uncomfortable or stressed by any of the questions, you may skip the question, or take a break, or withdraw from the project altogether.

Confidentiality and Privacy: During this research project, I will keep all data from the interviews in a secure location. Only my University of Hawaii advisor and I will have access to the data, although legally authorized agencies, including the

University of Hawai'i Committee on Human Studies, have the right to review research records. Your name will not be used in my data or written report. If you would like a copy of my final report, please contact me at the number listed near the end of this consent form.

Voluntary Participation: Participation in this research project is voluntary. You can choose freely to participate or not to participate. In addition, at any point during this project, you can withdraw your permission without any penalty.

Questions: If you have any questions regarding this research project, please contact the researcher, Georgia Hart, at XXX-XXX-XXXX, 808-956-3931, 808-956-8369 or gmhart@hawaii.edu. If you would like to be interviewed by the researcher, or would like to be otherwise involved, or would like to suggest someone to be interviewed, please contact the researcher at the email or phone number above.

If you have any questions regarding the rights of your child as a research participant, please contact the University of Hawai'i Committee on Human Studies (CHS) by phone at (808) 956-5007, or by email at uhirb@hawaii.edu

Please keep the prior portion of this consent form for your records.
If you consent to participate in this project, please sign the following signature portion of this consent form and return it to your interviewer's science teacher.

Tear or cut here

Signature(s) for Consent:

I agree to participate in the research project entitled, *Contemporary seaweed gathering and consumption in the Hawaiian Islands.* I understand that I can change my mind about participating in this project, at any time, by notifying the researcher.

Your Name (Print): ________________________________

Your Signature: ________________________________

Date: ______________________

APPENDIX B

ADDITIONAL FINDINGS FROM SOCIO-CULTURAL STUDY

Wild species consumed and/or gathered

Student surveys
The wild species most commonly reported to be gathered were *Asparagopsis taxiformis*, *Gracilaria* spp., *Codium* spp., tubular *Ulva* spp. (formerly *Enteromorpha*) and *Dictyopteris* spp. Also reported were *Sargassum* spp.

The wild species most commonly reported to be consumed were *Asparagopsis taxiformis*, *Gracilaria* spp., *Codium* spp., tubular *Ulva* spp. (formerly *Enteromorpha*) and *Dictyopteris* spp.

Adult interviews
The wild species most commonly reported to be gathered were *Codium* spp., *Gracilaria* spp., *Asparagopsis taxiformis* and *Dictyopteris* spp. Also reported for the grandparents of interviewees were *Sargassum* spp. and *Chondrophycus* spp.

The wild species most commonly reported to be consumed were *Asparagopsis taxiformis*, *Gracilaria* spp., *Codium* spp., and *Dictyopteris* spp.

Medicinal use of seaweeds

Student surveys
No student indicated they had used *limu* to cure an injury or illness.

Adult interviews
Several interviewees indicated that an older relative had used *limu* for medicine, but they did not know or did not mention the kind of *limu* (one oblique mention of *limu huluhuluwaena*) or the specific medical uses. Several interviewees indicated that foods which contain cultivated and imported nori were consumed when sick (e.g., miso soup, saimin).

Knowledge of Hawaiian seaweed names

Student surveys
When asked to list the names of all the kinds of seaweed or *limu* they knew, the majority of students could not provide the name of any seaweeds. The names most commonly listed were nori or ogo (Japanese terms), followed by *limu kohu*. One in ten students gave a Hawaiian common name of a seaweed. One in five Hawaiian students gave a Hawaiian common name with an average of one Hawaiian common name per Hawaiian student.

Adult interviewees

Those interviewees who gathered or consumed *limu* named as many as seven Hawaiian seaweeds by Hawaiian name, though may have known more (e.g., he or she ended the list with the word “etc.”). Interviewees who had gathered *limu* listed and average of 2.25 Hawaiian names for seaweeds. The most commonly listed seaweed names, in order from the most to the fewest mentions were: ogo, *limu kohu*, *limu ʻele ʻele*, *limu wāwaeʻiole*, nori, *lipeʻepe*, *limu līpoa*, furikake, *limu kala* and *limu huluhuluwaena*.

APPENDIX C

ANTIOXIDANT ASSAY FIGURES

Extract yield

Macroalgal samples yielded an average of 67 (ranging from 18 to 148) mg of crude extract per gram dry algae (Figure A1), and an average of 7.6 (ranging from 3.1-16.5) mg of crude extract per gram wet algae (Figure A2).

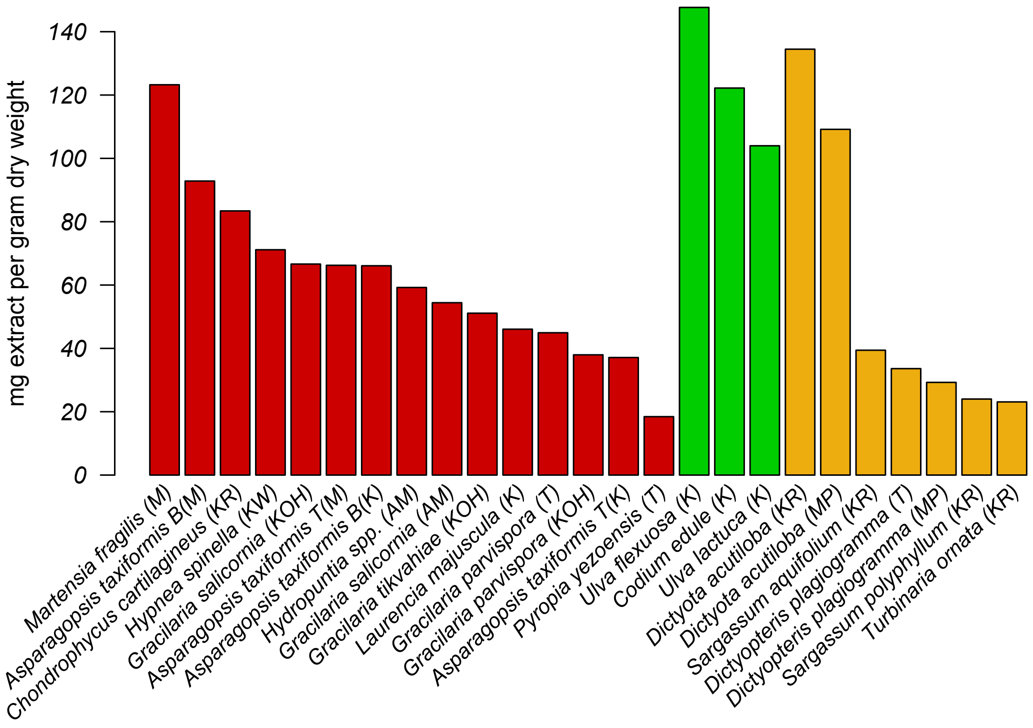


Figure 25. Extract yield in milligrams per gram dry weight of algae for each sample included in FRAP and DPPH antioxidant assays. Bar colors indicate macroalgal phyla or class (red= Rhodophyta, green= Chlorophyta, and brown = Phaeophyceae). The letters following each species name in parentheses represent collection site or place of acquisition: M= Makapu'u, KR = Kualoa Regional Park, KOH= Kahuku Olakai Hawaii Farms, T= Tamashiro's Market, K= Ka'ala'wai, MP= Makai Pier, and AM= Ala Moana Beach Park. The letter designations after *Asparagopsis taxiformis* indicate the portion of the plant: T= upper thallus, B= stolons.

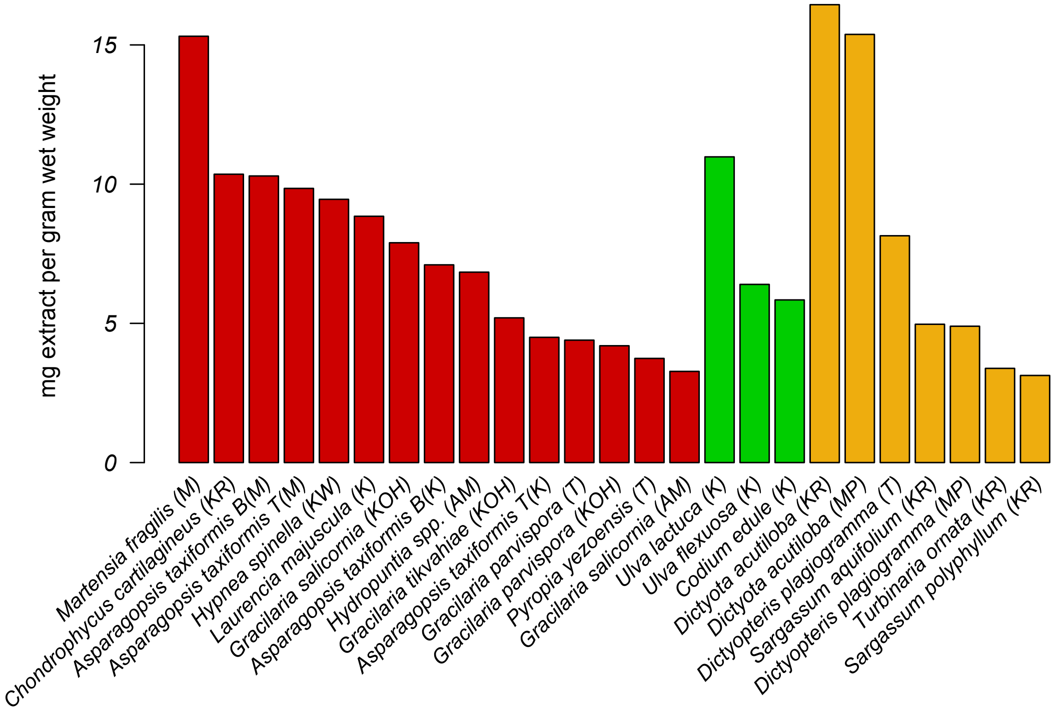


Figure 26. Extract yield in milligram per gram of fresh algae for each sample included in FRAP and DPPH antioxidant assays. Bar colors indicate class or phyla and letters following scientific names indicate source (see Figure 25).

Antioxidant values by extract weight

The FRAP value ranged from 0.41 to 11.1 (mean of 3.4) μM $FeSO_4$ equivalents per μg of extract (Figure A3), and from 6.2 to 166.0 (mean of 51.3) μmol $FeSO_4$ equivalents per gram of extract (Figure A4). Percent DPPH radical scavenging ranged from 4.9 to 30.8 (mean of 17.4) per 100μg of extract (Figure A4). *Martensia fragilis*, *Dictyopteris plagiogramma* and *Dictyota acutiloba* had the highest FRAP values. *Asparagopsis taxiformis* (upper thallus portion from Makapu'u), *Asparagopsis taxiformis* (stolons from Makapu'u), and *Martensia fragilis* had the highest %DPPH scavenging, closely followed by *Turbinaria ornata*.

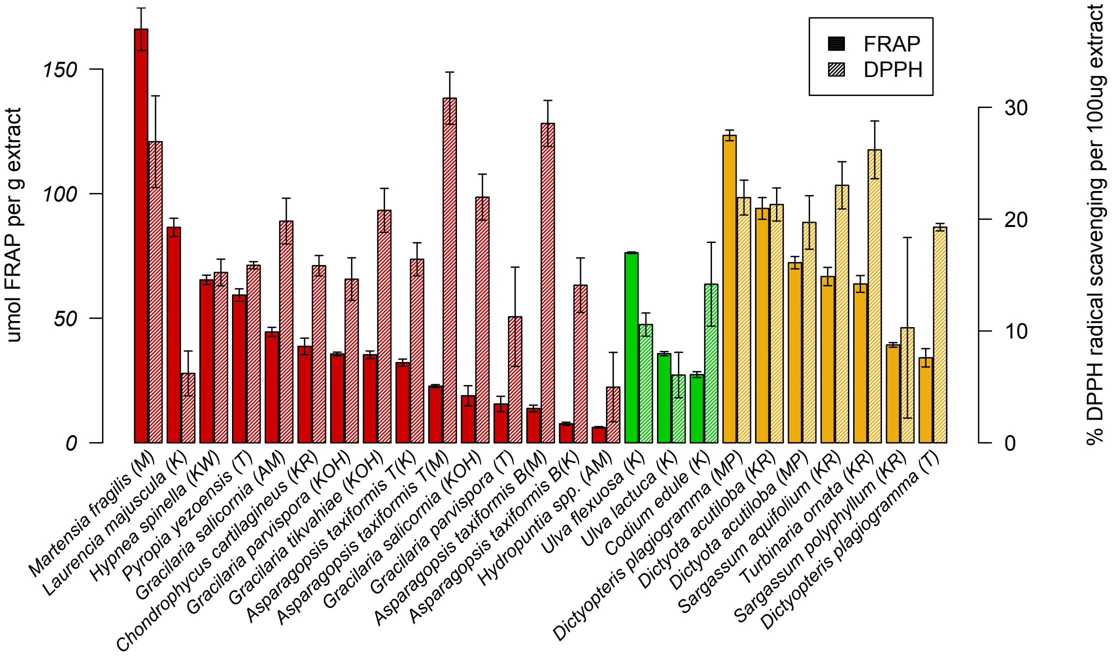


Figure 27. FRAP value in μmol $FeSO_4$ per gram of extract and as percent DPPH radical scavenging per 100 μg extract (+/- 1 StDev). Bar colors indicate class or phyla and letters following scientific names indicate source (see Figure 25).

Antioxidant values by dry weight

FRAP

The FRAP value ranged from 0.04 to 2.0 (mean of 0.2) µmol $FeSO_4$ equivalents per g of dry algae. *Martensia fragilis*, *Dictyota acutiloba* (from Kualoa Regional Park), and *Ulva flexuosa* had the highest FRAP values (Figure A5).

DPPH

Values ranged between 2.5 and 33.2 (mean of 11.8) % DPPH radical scavenging per milligram of dry algae. *Martensia fragilis*, *Dictyota acutiloba* (from Kualoa Regional Park), and *Asparagopsis taxiformis* (stolons from Makapuʻu) had the highest %DPPH scavenging. (Figure A5).

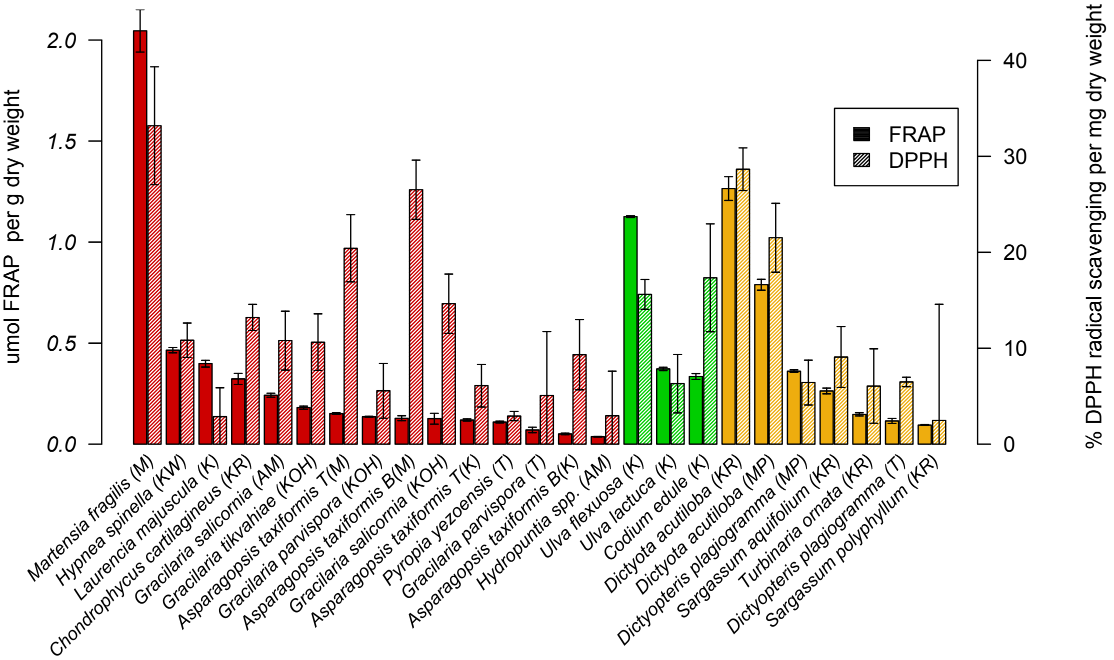


Figure 28. FRAP value in µmol $FeSO_4$ equivalents per gram of dry algal mass and as percent DPPH radical scavenging per mg dry algal mass (+/- 1 StDev). Bar colors indicate class or phyla and letters following scientific names indicate source (see Figure 25).

Antioxidant values among wild not eaten, wild edible and cultivated samples

Wild species that are not documented as food in Hawaii, or which are documented to be only infrequently eaten (n=7), were compared to wild edible species more commonly consumed (n=7) and to cultivated edibles (n=4). No significant differences were found across these three categories in FRAP values or %DPPH scavenging (Figures 29-32).

Figure 29. FRAP value in μmol per gram fresh weight of wild not eaten (n=7), wild edible (n=7) and cultivated edible (n=4) species. (ANOVA: df = 2, F= 2.73, p= 0.097).

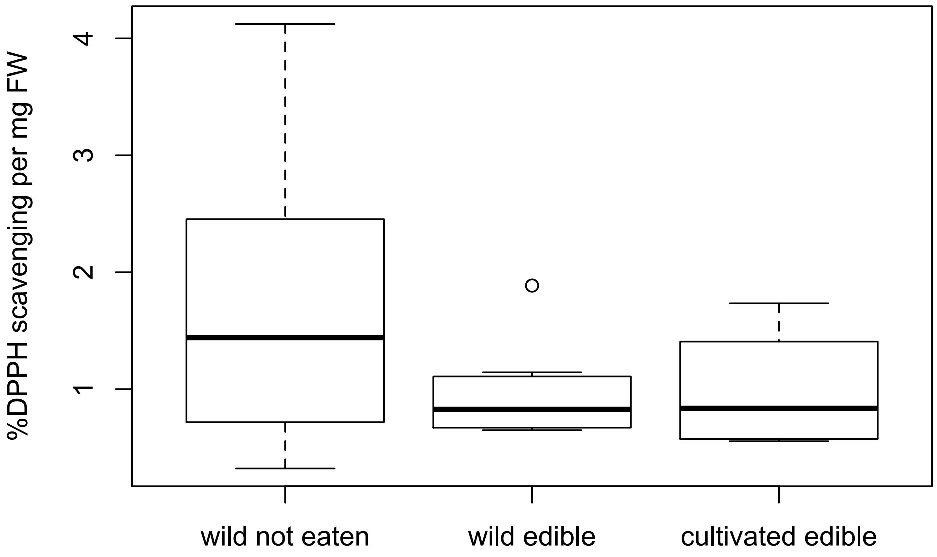


Figure 30. %DPPH scavenging by wild not eaten (n=7), wild edible (n=7) and cultivated edible (n=4) species of marine macroalgae per mg fresh weight. (ANOVA: df=2, F=1.28, p= 0.31).

Figure 31. FRAP value in μmol of wild not eaten (n=7), wild edible (n=7) and cultivated edible (n=4) species of marine macroalgae per gram dry weight. (ANOVA: df = 2, F= 1.61, p= 0.23).

Figure 32. %DPPH scavenging by wild not eaten (n=7), wild edible (n=7) and cultivated edible (n=4) species of marine macroalgae per gram dry weight. (ANOVA: df=2, F= 0.47, p= 0.63).

Antioxidant values compared to other foods

Comparisons across studies, even for the same assay, are complicated by procedural differences. Particularly important are the different extraction procedures which influence the amount and the type of compounds which are extracted from the sample material. The extraction procedures in the papers used for comparison below differed from those of my own study, and should therefore be interpreted with significant caution.

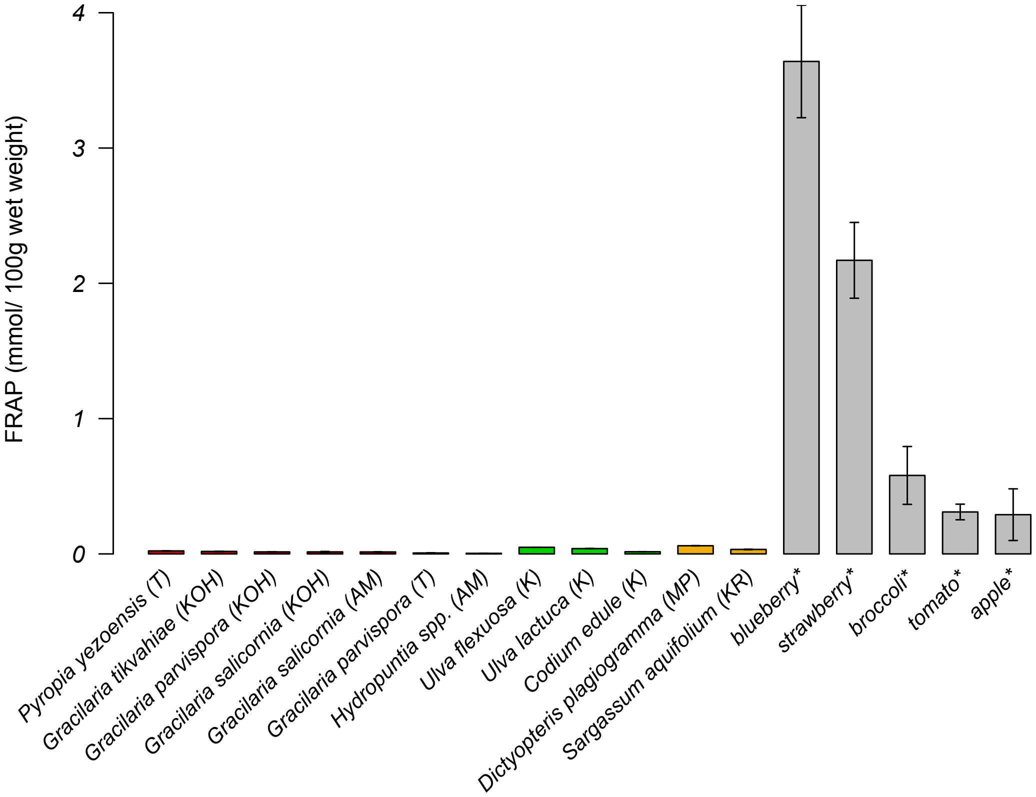


Figure 33. FRAP value in mmol $FeSO_4$ per 100 grams wet weight of edible macroalgae (+/- 1 StDev) compared to other foods (+/-SE). Bar colors indicate class or phyla and letters following scientific names indicate source (see Figure 25). *from Halvorsen *et al.* 2002

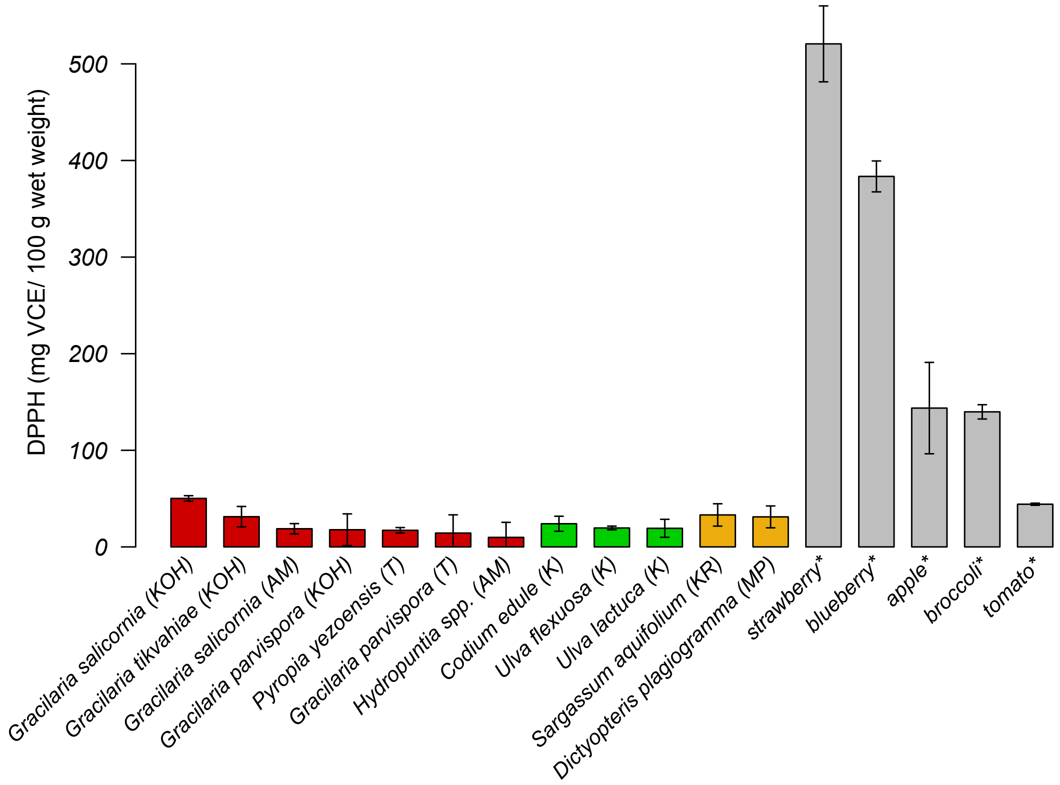


Figure 34. %DPPH radical scavenging in milligrams vitamin C equivalents (VCE) per 100 grams wet weight of edible macroalgae (+/- 1 StDev) or other food (+/-SE) Bar colors indicate class or phyla and letters following scientific names indicate source (see Figure 25). * from Floegel *et al.* 2011

Antioxidant values by collection site

Site comparisons

Makapu'u had the highest mean FRAP and DPPH values by dry weight, followed by Makai Pier (Figures A12 & A13). Number of samples per site ranged from 1 to 5 (see text in Figures A12 & A13).

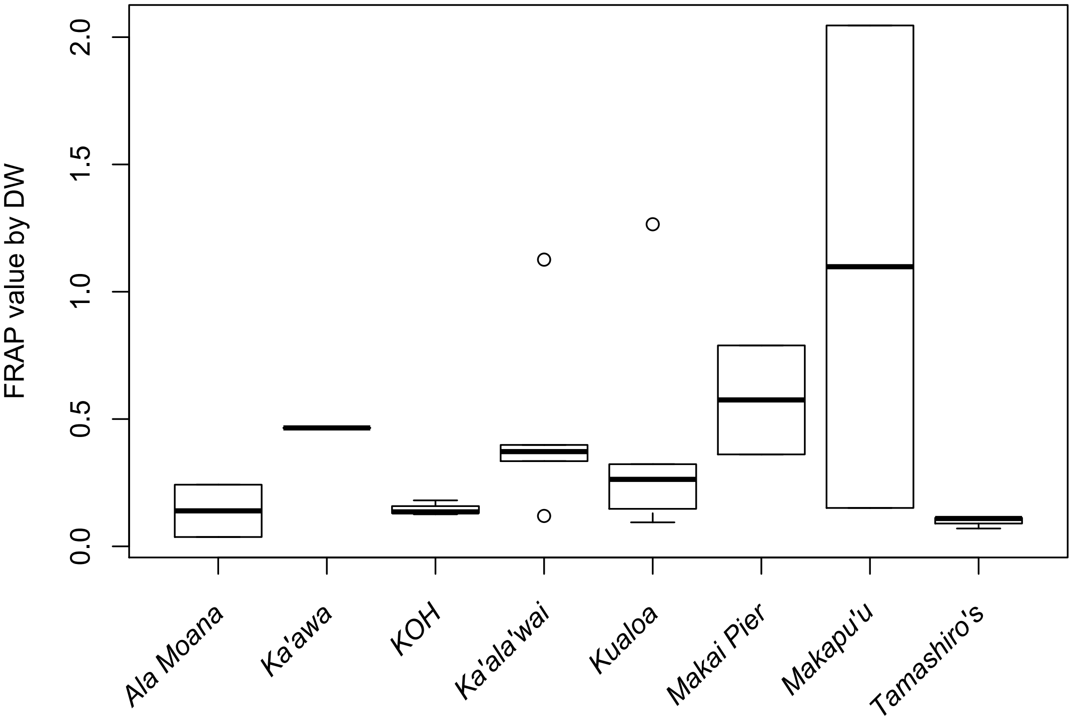


Figure 35. Mean site FRAP values by site in μmol per gram dry weight (n= 2, 1, 3, 5, 5, 2, 2, 3).

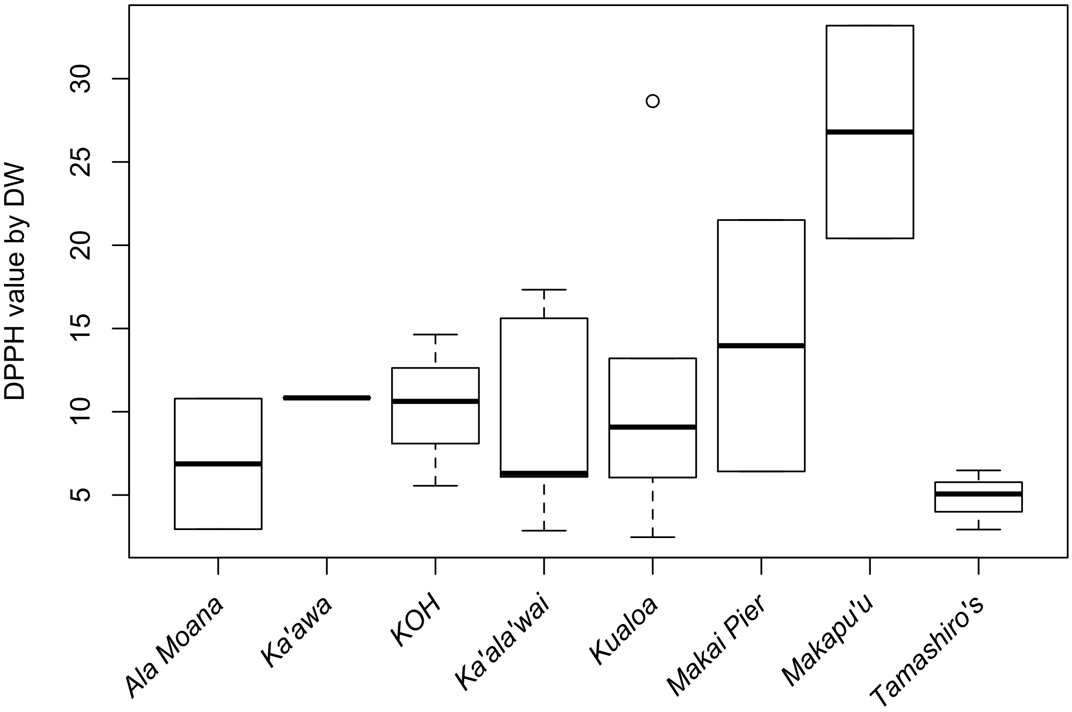


Figure 36. %DPPH scavenging by site by dry weight by site (n=2, 1, 3, 5, 5, 2, 2, 3).

APPENDIX D

ADDITIONAL FINDINGS FROM ANTIOXIDANT STUDY

Within species comparisons of antioxidant values

Four samples of *Asparagopsis taxiformis* were assayed in this study. Plants were collected from two sites and these samples were split between top (upper thallus) and bottom (holdfast) portions of the plant. Upper portions had higher FRAP values than lower portions of the plant (mean values: 67.5 and 44.6 by dry weight; 9.2 and 4.9 by wet weight). For the DPPH assay, however, upper portions had lower DPPH values compared to lower portions of the plant (mean values: dry weight 13.3 and 17.9, wet weight 1.88 and 1.97). The plants collected from Makapuʻu had higher FRAP values (upper 75.39, lower 63.99, mean 69.69 by dry weight and upper 11.21, lower 7.10, mean 9.16 by wet weight) compared to those at Kaʻalaʻwai (upper 59.69, lower 25.25, mean 42.47 by dry weight and upper 7.23, lower 2.71, mean 4.97 by wet weight). The plants collected from Makapuʻu also had approximately 3X greater DPPH values (upper 20.41, lower 26.52, mean 23.46 by dry weight and upper 3.03, lower 2.94, mean 2.99 by wet weight) compared to those at Kaʻalaʻwai (upper 6.09, lower 9.31, mean 7.70 by dry weight and upper 0.74, lower 1.00, mean 0.87 by wet weight).

Two samples of *Dictyopteris plagiogramma* were included in the assays in this study. One sample was wild-collected at Makai Pier, Oʻahu Island (Figure 1), while the other sample was purchased from Tamashiro's Market and was wild-collected on Maui (Guy Tamashiro, personal communication). The sample at Tamashiro's Market had been washed and salted for preservation and was sold in the refrigeration section. The fresh sample had approximately 3X greater FRAP values than the purchased samples (180.6 compared to 57.3 by dry weight and 30.2 compared to 13.9 by wet weight). The purchased sample had slightly higher DPPH values compared to the fresh sample (6.47 compared to 6.42 by dry weight and 1.57 compared to 1.07 by wet weight).

Two samples of *Gracilaria salicornia* were assayed by FRAP and DPPH. One set of plants was wild-collected at Ala Moana Beach Park, Oʻahu Island (Figure 1). The other sample was cultivated at Kahuku Olakai Hawaiʻi Marine Agrifuture, LLC on Oʻahu. The wild sample had FRAP values twice that of the cultivated sample (121.1 compared to 62.9 by dry weight). The cultivated sample had a higher DPPH value than the wild sample (14.6 compared to 10.8 % by dry weight). Wet weight was not available.

Two samples of *Dictyota acutiloba* were included in this study. Both were wild collected. One set of plants was collected at Makai Pier, where it was growing epiphytically on *Dictyopteris* spp., while the other set was collected at Kualoa

Regional Park (Figure 1). The samples collected from Kualoa Regional Park had consistently higher FRAP and DPPH values (~25-30% higher). Kualoa Regional Park plants had FRAP values of 632.55 by dry weight and 77.35 by wet weight, while plants at Makai Pier had values of 394.56 by dry weight and 55.58 by wet weight. DPPH values were 28.7% by dry weight and 3.5% by wet weight for Kualoa Regional Park, and 21.5% by dry weight and 3.03% by wet weight for Makai Pier.

Within genus comparisons of antioxidant values

Two species within the genus *Sargassum* were included in the FRAP and DPPH assays. Both species were collected at Kualoa Regional Park. *Sargassum aquifolium* had FRAP and DPPH values 3-4X greater than *Sargassum polyphyllum*. FRAP values were 131.6 and 47.1 by dry weight and 16.6 and 6.1 by wet weight. DPPH values were 9.1 and 2.5 % by dry weight and 1.1 and 0.3 % by wet weight.

Two species within the genus *Ulva* were included in this study. Both species were collected wild from Kaʻalaʻwai (Figure 1). *Ulva flexuosa* had higher FRAP and DPPH values than *Ulva lactuca*. The difference was more pronounced for dry weight comparisons (FRAP 563.1 to 186.1, DPPH 15.6 to 6.3) than for wet weight comparisons (FRAP 24.4 to 19.6, DPPH 0.68 to 0.67).

Three species within the genus *Gracilaria* were included in the assays. Wild *G. salicornia* had the highest FRAP value by dry weight (121.1). The cultivated samples had lower values: *G. salicornia* 62.9, *G. parvispora* 67.7 and *G. tikvahiae* 90.3. Cultivated *G. salicornia* had the highest DPPH value by dry weight (14.6%) followed by wild *G. salicornia* (10.8%), *G. tikvahiae* (10.6%), and lastly *G. parvispora* (5.6%).

LITERATURE CITED

Abbott IA. 1978. The uses of seaweed as food in Hawaii. *Economic Botany* 32: 409–412

Abbott, IA. *Lāʻau Hawaiʻi: Traditional Hawaiian Uses of Plants*. Honolulu: The Bishop Museum Press, 1992.

Abbott IA. *Limu. An Ethnobotanical Study of Some Hawaiian Seaweeds*. Rev. ed. Kauai: National Tropical Botanical Garden, 1996.

Abbott IA. *Marine Red Algae of the Hawaiian Islands*. Honolulu: Bishop Museum Press, 1999.

Abbott, IA and JM Huisman. *Marine Green and Brown Algae of the Hawaiian Islands*. Bishop Museum Bulletin in Botany 4. Honolulu: Bishop Museum Press, 2004. p53

Abd El Mageid, MM; Salama, NA; Saleh, MAM and HM Abo Taleb. 2009. Antioxidant and antimicrobial characteristics of red and brown algae extracts. *4th Conference on Recent Technologies in Agriculture* 818-28

Aiona, Kamaui. "Ike Kuuna Limu: Learning About Hawaiʻi's Limu." MS Thesis U of Hawaiʻi at Mānoa, Honolulu, 2003.

Alberti, KGMM; Zimmet, P and J Shaw. 2007. International Diabetes Federation: a consensus on Type 2 diabetes prevention. *Diabetic Medicine* 24: 451–63

Alexander, C; Bynum, N; Johnson, E; King, U; Mustonen, T; Neofotis, P; Oettlé, N; Rosenzweig, C; Sakakibara, C; Shadrin, V; Vicarelli, M; Waterhouse, J and B Weeks. 2011. Linking indigenous and scientific knowledge of climate change. *BioScience* 61(6): 477-84

Altamirano, M; Murakami, A and H Kawai. 2003. Photosynthetic Performance and Pigment Content of Different Developmental Stages of *Ecklonia cava* (Laminariales, Phaeophyceae). *Botanica Marina* 46: 9-16

Andersen, JH; L Schluter and G Aertebjerg. 2006. Coastal eutrophication: recent developments in definitions and implications for monitoring strategies. *Journal of Plankton Research* 28(7): 621-8

Appel, HM; Govenor, HL: D'ascenzo, M; Siska, E and JC Schultz. 2001. Limitations of folin assays of foliar phenolics in ecological studies. *Journal of Chemical Ecology* 27(4): 761-78

Arnold, TM; CE Tanner and WI Hatch. 1995. Phenotypic variation in polyphenolic content of the tropical brown alga *Lobophora variegata* as a function of nitrogen availability. *Marine Ecological Progress Series* 123: 177–83

Arnold, TM and NM Targett. 2000. Evidence for metabolic turnover of polyphenolics in tropical brown algae. *Journal of Chemical Ecology* 26(6): 1393-410

Aruoma, OI. 1998. Free Radicals, Oxidative Stress, and Antioxidants in Human Health and Disease *Journal of the American Oil Chemists' Society* 75(2): 199-212

Balasundram, N; Sundram K and S Samman. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry* 99: 191–203

Baldea, LAN; Martineaua, LC; Benhaddou-Andaloussia, A; Arnason, JT; Lévyc, E and PS Haddad. 2010 Inhibition of intestinal glucose absorption by anti-diabetic medicinal plants derived from the James Bay Cree traditional pharmacopeia *Journal of Ethnopharmacology* 132: 473-82

Bates, D; Maechler M and B Bolker. 2009. lme4: Linear mixed effect models using S4 classes. R package version 0.999375-32. http://CRAN.R-project.org/package=lme4.

Belch, J; MacCuish, A; Campbell, I; Cobbe, S; Taylor, R; Prescott, R; Lee, R; Bancroft, J; MacEwan, S; Shepherd, J; Macfarlane, P; Morris, A; Jung, R; Kelly, C; Connacher, A; Peden, N; Jamieson, A; Matthews, D; Leese, G; McKnight, J; O'Brien, I; Semple, C; Petrie, J; Gordon, D; Pringle, S and R MacWalter. 2008. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. *British Medical Journal* 337:a1840

Benz B, Cevallos J, Santana F. Rosales J and S Graf. 2000. Losing knowledge about plant use in the Sierra de Manantlan Biosphere Reserve, Mexico. *Economic Botany* 54: 183–191

Benzie, IFF and JJ Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Analytical Biochemistry* 239: 70–76

Benzie, IFF and JJ Strain. 1999. Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* 299: 15-27

Benzie, IFF and S Wachtel-Galor. 2012. Increasing the antioxidant content of food: a personal view on whether this is possible or desirable. *International Journal of Food Sciences and Nutrition* 63(S1): 62-70

Bouarab, K; Adas, F; Gaquerel, E; Kloareg, B; Salaün, J-P; and P Potin. 2004. The innate immunity of a marine red alga involves oxylipins from both the eicosanoid and octadecanoid pathways. *Plant Physiology* 135: 1838-48

Bravo, L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* 56(11): 317-33

Brand, JC; Snow, BJ; Nabhan, GP and AS Truswell. 1990. Plasma glucose and insulin responses to traditional Pima Indian meals. *American Journal of Clinical Nutrition* 51: 416-20

Brand-Miller, JC and SHA Holt. 1998. Australian Aboriginal plant foods: a consideration of their nutritional composition and health implications. *Nutrition Research Reviews* 11: 5-23

Brand-Williams, W; Cuvelier, ME and C Berset. 1995. Use of a Free Radical Method to Evaluate Antioxidant Activity. *LWT-Food Science and Technology* 28(1): 25-30

Bunea, A; Rugina, DO; Pintea, AM; Sconta, Z; Bunea, CI and C Socaciu. 2011. Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania. *Notulae Botanicae Horti Agrobotanici* 39(2): 70-76

Burreson, BJ; Moore, RE and PP Roller. 1976. Volatile halogen compounds in the alga *Asparagopsis taxiformis* (Rhodophyta). *Journal of Agricultural and Food Chemistry* 24(4): 856-61

Byers BA; Cunliffe RN and AT Hudak. 2001. Linking the conservation of culture and nature: a case study of sacred forests in Zimbabwe. *Human Ecology* 29: 187–218

Casken, J. “Pacific Island health and disease: An Overview.” Chapter 22 in Huff, RM and MV Kline (Eds.). *Pacific Islander Populations*. Thousand Oaks, CA: Sage Publications Inc., 1999. pp 397-417.

Catoni, C; Peters, A and HM Schaefer. 2008 Life history trade-offs are influenced by the diversity, availability and interactions of dietary antioxidants. *Animal Behavior* 76: 1107-19

Cavalli-Sforza LL and MW Feldman. *Cultural Transmission and Evolution: A Quantitative Approach*. Princeton, NJ: Princeton University Press; 1981.

Chapman, L; Johns, T and RLA Mahunnah. 1997. Saponin-like in vitro characteristics of extracts from selected non-nutrient wild plant food additives used by Maasai in meat and milk-based soups. *Ecology of Food and Nutrition* 36: 1-22

Connan, S; Delisle, F; Deslandes, E and EA Gall. 2006. Intra-thallus phlorotannin content and antioxidant activity in Phaeophyceae of temperate waters. *Botanica Marina* 49: 39–46

Cordain, L; Boyd Eaton, S; Sebastian, A; Mann, N; Lindeberd, S; Watkins, BA, O'Keefe, JH and J Brand-Miller. 2005. Origins and evolution of the Western diet: health implications for the 21st century. *The American Journal of Clinical Nutrition* 81: 341–54

Crawley, MJ. *The R Book*. West Sussex, England: Wiley, 2007.

Cronin, G and ME Hay. 1996. Effects of light and nutrient availability on the growth, secondary chemistry, and resistance to herbivory of two brown seaweeds. *Oikos* 7: 93–106

Crozier, MN; IB Jaganath and MN Clifford. "Phenols, Polyphenols and Tannins: An Overview" in *Plant Secondary Metabolites Occurrence, Structure and Role in the Human Diet*. Crozier, A *et al.* (Eds.). Oxford, UK: Blackwell Publishing, 2006.

Cruz Garcia, GS. 2006. The mother-child nexus: knowledge and valuation of wild food plants in Wayanad, Western Ghats, India. *Journal of Ethnobiology and Ethnomedicine* 2(1): 39

Cunningham, AB; Shanley, P and S Laird. "Health, habitats and medicinal plant use." In Pierce Colfer, CJ (Ed.) *Human Health and Forests: A global overview of issues, practice and policy*. London: Earthscan, 2008. pp. 35-62.

Cunningham, CK. "Hawaiʻi's food policy council: A tool for food system planning." An area of concentration paper. Master of Urban and Regional Planning: University of Hawaiʻi at Mānoa, Honolulu, 2010.

Demmig-Adams, B and W Adams. 2002. Antioxidants in photosynthesis and human nutrition. *Science* 298: 2149-53

Dillehay, TD; Ramírez, C; Pino, M; Collins, MB; Rossen, J and JD Pino-Navarro. 2008. Monte Verde: seaweed, food, medicine, and the peopling of South America. *Science* 5877: 784-6.

Dunton, KH and CM Jodwalis. 1988. Photosynthetic performance of *Laminaria solidungula* measured *in situ* in the Alaskan High Arctic. *Marine Biology* 98(2): 277-85

El-Baroty, GS; Moussa, MY; Shallan, MA; Ali, MA; Sabh, AZ and E Shalaby. 2007. Contribution to the aroma, biological activities, minerals, protein, pigments and lipid contents of the red alga: *Asparagopsis taxiformis* (Delile) Trevisan. *Journal of Applied Sciences Research* 3(12): 1825-34

Eder, JF. 1978. The caloric returns to food collecting: disruption and change among the Batak of the Philippine tropical forest. *Human Ecology* 6(1): 55-69

Eder, JF. 1988. Batak foraging camps today: a window to the history of a hunting-gathering economy. *Human Ecology* 16(1): 35-55

Egeland, GM; Charbonneau-Roberts, G; Kuluguqtuq, J; Kilabuk, J; Okalik, L; Soueida, R and HV Kuhnlein. "Back to the future: using traditional food and knowledge to promote a healthy future among Inuit." Chapter 1 in Kuhlein, HV *et al.* (Eds.) *Indigenous Peoples and Their Food Systems: the many dimensions of culture, diversity and environment for nutrition and health*. Rome: Food and Agriculture Association of the United Nations, 2009. pp 9-22

Etkin, N. 1996. Medicinal cuisines: Diet and ethnopharmacology. *International Journal of Pharmacology* 34(5): 313-326

Etkin NL and PJ Ross. 1997. "Malaria, medicine and meals: a biobehavioral perspective." In Romanucci-Ross, DE *et al.* (Eds.) *The Anthropology of Medicine*, 3rd ed. New York: Praeger Publishers, 1997. pp 169-209

Floegel A; Kim, D; Chung, S; Koo, SI and OK Chun. 2011. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis* 24: 1043–8

Folin, O and V Ciocalteu. 1927. On tyrosine and tryptophane determinations in proteins. *Journal of Biological Chemistry* 73: 627–50

Freiberger, CE; Vanderjagt, DJ; Pastuszyn, A; Glew, RD; Mounkaila, G; Millson, M and RH Glew. 1998. Nutrient content of the edible leaves of seven wild plants from Niger. *Plant Foods for Human Nutrition* 53: 57–69

Friedman, M. 2006. Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. *Agricultural and Food Chemistry* 54: 8655–81

Fujita, R; KL Braun, and CK Hughes. 2004. The traditional Hawaiian diet: a review of the literature. *Pacific Health Dialog* 11(2): 250-259
Gadgil, M; Berkes, F and C Folke. 1993. Indigenous knowledge for biodiversity conservation. *Ambio* 22: 151-6

Gari, J. Plant diversity, sustainable rural livelihoods and the HIV/AIDS crisis. Bangkok: UNDP and Rome: FAO, 2004.

Gottesfeld, LMJ. 1995. The role of plant foods in traditional Wet'suwet'en nutrition *Ecology of Food and Nutrition* 34(2): 149-69

Griffin, SP and R Bhagooli. 2004. Measuring antioxidant potential in corals using the FRAP assay. *Journal of Experimental Marine Biology and Ecology* 302(2): 201-11

Grivetti, LE and BM Ogle. 2000. Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. *Nutrition Research Reviews* 13: 31-46

Grossman, AR; Schaefer, MR; Chiang, GG, and J Collier. 1993. The phycobilisome, a light-harvesting complex responsive to environmental conditions. *Microbiological Reviews* 57(3): 725-49

Gruelle, KB. 1946. Effect of war on food habits in Hawaiʻi. *Journal of Home Economics* 38(2): 91-4

Guiry, MD and GM Guiry. 2012. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 05 April 2012.

Halvorsen, BL; Holte, K; Myhrstad, MCW; Barikmo, I; Hvattum, E; Remberg, SF; Wold, A; Haffner, K; Baugerød, H; Andersen, LF; Moskaug, JO; Jacobs, Jr., DR; and R Blomhoff. 2002. A Systematic Screening of Total Antioxidants in Dietary Plants. *The Journal of Nutrition* 132: 461–71

Handy, ESC; Pukui, MK and K.Livermore. *Outline of Hawaiian Physical Therapeutics*. Honolulu: B.P. Bishop Museum, Bulletin 126, 1934.

Haukioja, E; Ossipov, V; Koricheva, J; Honkanen, T; Larsson, S. and KS Lempa. 1998 Biosynthetic origin of carbon-based secondary compounds: cause of variable responses of woody plants to fertilization? *Chemoecology* 8: 133–9

Hayden HS; Blomster, J; Maggs, CA; Silva, PC; Stanhope, MJ and JR Waaland. 2003. Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *European Journal of Phycology* 38**:** 277–94

Hewlett BS, and LL Cavalli-Sforza. 1986. Cultural transmission among Aka Pygmies. *American Anthropologist* 88: 922-34

Hong, S. 2011. Biocultural diversity and traditional ecological knowledge in island regions of Southwestern Korea. *Journal of Ecological Field Biology* 34(2): 137-47

Howarth, RW; Sharpley, A and D Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. *Estuaries* 25(4b): 656-76

Hughes, CK. 1998. Traditional Hawaiian diet programs: a culturally competent chronic disease intervention. *Pacific Health Dialogue* 5(2): 328-31

Inderjit**.** 1996. Plant phenolics in allelopathy. *Botanical Review* 62: 186-202

Jeambey, Z; Johns, T; Talhouk, S and M Batal. 2009. Perceived health and medicinal properties of six species of wild edible plants in north-east Lebanon. *Public Health Nutrition* 12(10): 1902–11

Jimenez-Escrig, A; Jimenez-Jimenez, I; Pulido, R and F Saura-Calixto. 2001 Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food and Agriculture* 81: 530-534

Johannes, RE. 2002. The renaissance of community-based marine resource management in Oceania. *Annual Review of Ecology and Systematics* 33: 317–40

Johns, T. 1996. Phytochemicals as evolutionary mediators of human nutritional physiology. *International Journal of Pharmacognosy* 34(5): 327-34

Johns, T; Mahunnah, RLA; Sanaya, P; Chapman, L and T Ticktin. 1999. Saponins and phenolic content in plant dietary additives of a traditional subsistence community, the Btemi of Ngorongoro District, Tanzania. *Journal of Ethnopharmacology* 66: 1-10

Kamakau, Samuel M. *Ruling Chiefs of Hawai'i*. Rev. ed. Honolulu: Kamehameha Schools Press, 1992. p222

Kelman, D; Posner, EK; McDermid, KJ; Tabandera, NK; Wright PR and AD Wright. 2012. Antioxidant Activity of Hawaiian Marine Algae. *Marine Drugs* 10(2): 403-416

Kim, KY; Nam, KA; Kurihara, H and SM Kim. 2008**.** Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. *Phytochemistry* 69(16): 2820-25

Kim, KY; Nguyen, TH; Kurihara, H and SM Kim. 2010. α-glucosidase inhibitory activity of bromophenol purified from the red alga *Polyopes lancifolia*. *Journal of Food Science* 75(5): H145-50

Kuda, T; Tsunekawa, M; Goto, H and Y Araki. 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *Journal of Food Composition and Analysis* 18: 625–33

Kuhnlein HV. 1992. Change in the use of traditional foods by the Nuxalk native people of British Columbia. *Ecology of Food and Nutrition* 27(34): 259-82

Kuhnlein HV and SA Moody. 1989. Evaluation of the Nuxalk food and nutrition program: traditional food use by a native Indian group in Canada. *Journal of Nutrition Education* 21(3): 127-32

Kuhnlein, HV and O Receveur. 1996. Dietary change and traditional food systems of indigenous peoples. *Annual Reviews of Nutrition* 16:417-42

Larned, ST. 1998. Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. *Marine Biology* 132(3): 409-21

Ladio, AH and M Lozada. 2004. Patterns of use and knowledge of wild edible plants in distinct ecological environments: a case study of a Mapuche community from northwestern Patagonia. *Biodiversity and Conservation* 13: 1153–73

Lako, J; Trenerry, VC; Wahlqvist, M; Wattanapenpaiboon, N; Sotheeswaran, S and R Premier. 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry* 101: 1727–41

Lee, HJ; Kim, HC; Vitek, L and CM Nam. 2010. Algae consumption and risk of Type 2 diabetes: Korean National Health and Nutrition Examination Survey in 2005. *Journal of Nutritional Science and Vitaminology* 56(1): 13-18

Lee, O; Yoon, K; Kim, K; You, S. 2011. Seaweed extracts as a potential tool for the attenuation of oxidative damage in obesity-related pathologies. *Journal of Phycology* 47: 548-56

Lee, CM; Barrow, CL; Kim, S; Miyashtia, K and F Shahidi. 2011. Global trends in marine nutraceuticals. *Food Technology* 65(12): 22-31

Manach, C; A Scalbert, A; Morand, C; Rémésy, C and L Jimenez. 2004. Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition* 79: 727–47

Martins, R; Miguel Oliveira, J; Flindt, MR and JC Marques. 1999. The effect of salinity on the growth rate of the macroalgae *Enteromorpha intestinalis* (Chlorophyta) in the Mondego estuary (west Portugal). *Acta Oecologica* 20(4): 259-65

Martone, PT; Estevez, JM; Lu, F; Ruel, K; Denny, MW; Somerville, C and J Ralph. 2009. Discovery of lignin in seaweed reveals convergent evolution in cell-wall architecture. *Current Biology* 19(2): 169-75

Maschek, JA and BJ Baker. "The Chemistry of Algal Secondary Metabolism" Chap. 1 in Amsler, CD (Ed.) *Algal Chemical Ecology*. Berlin and Heidelberg: Springer-Verlag, 2008. p. 1-24.

Marsella, AJ; Johnson, JL; Watson, P and J Gryczynski (Eds.). *Ethnocultural perspectives on disaster and trauma: Foundations, issues and applications.* New York, NY: Springer, 2008.

McCarter, J and MC Gavin. 2011. Perceptions of the value of traditional ecological knowledge to formal school curricula: opportunities and challenges from Malekula Island, Vanuatu. *Journal of Ethnobiology and Ethnomedicine* 7:38

McClatchey, SC; Mahady, GB; Bennett, BC; Shiels, L and V Savod. 2009. Ethnobotany as a pharmacological research tool and recent developments in CNS-active natural products from ethnobotanical sources. *Pharmacology and Therapeutics* 123(2): 239–254

McCubbin, LD; Ishikawa, M and HI McCubbin. 2008. "Kanaka Maoli: Native Hawaiians and Their Testimony of Trauma and Resilience." Chapter 9 in Marsella, AJ *et al.* (Eds). *Ethnocultural perspectives on disaster and trauma: Foundations, issues and applications.* New York, NY: Springer, 2008. pp 271-298.

McCubbin, LD and A Marsella. 2009. Native Hawaiians and psychology: the cultural and historical context of indigenous ways of knowing. *Cultural Diversity and Ethnic Minority Psychology* 15(4): 374–87

McCune, LM and T Johns. 2007. Antioxidant activity relates to plant part, life form and growing condition in some diabetes remedies. *Journal of Ethnopharmacology* 112: 461-6

McDermid, KJ and B Stuercke. 2003. Nutritional composition of edible Hawaiian seaweeds. *Journal of Applied Phycology* 15: 513–24

McDermid, K and B. Stuerke. "A comparison of the nutritional content of Hawaiian *Gracilaria* species." In Abbott, IA and KJ McDermid (Eds.). *Taxonomy of Economic Seaweeds with Reference to the Pacific and other Locations*. Volume IX. Honolulu: University of Hawaiʻi Sea Grant College Program, 2004. pp. 211-26.

McDermid, K. J., B. Stuercke, and OJ Haleakala. 2005. Total dietary fiber content in Hawaiian marine algae. *Botanica Marina* 48(5-6): 437-40

McGowan, MP. "Submarine groundwater discharge: Freshwater and nutrient input into Hawaii's coastal zone." MS Thesis U of Hawaiʻi at Mānoa, Honolulu, 2004.

McGregor, DP; Morelli, PT; Matsuoka, JK; Rodenhurst, R; Kong, N and MS Spencer. 2003. An ecological model of Native Hawaiian well-being. *Pacific Health Dialog Viewpoints & Perspectives* 10(2): 106-28

Moore, RE. 1977. Volatile compounds from marine algae. *Accounts of Chemical Research* 10(2): 40-7

Nabhan, GP; Walker, D and AM More. 2010. Biocultural and ecograstronomic restoration: The renewing America's food traditions alliance. *Ecological Restoration* 28(3): 266-279

Napoleon, K. “He kālailaina i ka limu ma ka lāʻau lapaʻāu: he nīnauele me hulu kupuna Henry Allen Auwae” (An analysis of *limu* used in Hawaiian medicine: An interview with esteemed elder Henry Allen Auwae). MS Thesis U of Hawaiʻi at Mānoa, Honolulu, 2004.

Naud, J; Oliver, A; Belanger, A and L Lapointe. 2010. Medicinal understory herbaceous species cultivated under different light and soil conditions in maple forests in southern Québec, Canada. *Agroforestry Systems* 79(3): 303-26

Ness, AR and JW Powles. 1997. Fruit and vegetables, and cardiovascular disease: a review. *International Journal of Epidemiology* 26(1): 1-13

Nguyen, ML. 2003. Comparison of food plant knowledge between urban Vietnamese living in Vietnam and in Hawaiʻi. *Economic Botany* 57(4): 472-80

Nwosu, F; Morris, J; Lund, VA; Stewart, D; Ross, HA and GJ McDougall. 2011. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae*. Food Chemistry* 126: 1006–12

O’Dea K. 1984. Marked improvement in carbohydrate and lipid metabolism in diabetic Australian Aborigines after temporary reversion to traditional life-style. *Diabetes* 33: 596-603

O’Kelly, CJ; Kurihara, A; Shipley, TC and AR Sherwood. 2010. Molecular assessment of *Ulva* spp. (Ulvophyceae, Chlorophyta) in the Hawaiian Islands*. Journal of Phycology* 46: 728–35

Odom, SK. 1998. Hele Mai ʻAi: Developing a culturally competent nutrition education program. *Pacific Health Dialogue* 5(2): 383-5

Ohmagari K and F Berkes. 1997. Transmission of indigenous knowledge and bush skills among the Western James Bay Cree women of Subarctic Canada. *Human Ecology* 25: 197-223

Ooi, KL; Muhammad, TST; Tan, ML and SF Sulaimana. 2011. Cytotoxic, apoptotic and anti- alpha-glucosidase activities of 3,4-di-O-caffeoyl quinic acid, an antioxidant isolated from the polyphenolic-rich extract of *Elephantopus mollis* Kunth. *Journal of Ethnopharmacology* 135: 685–95

Orech, FO; Aagaard-Hansen, J and H Friis. 2007. Ethnoecology of traditional leafy vegetables of the Luo people of Bondo district, western Kenya. *International Journal of Food Sciences and Nutrition* 58(7): 522-30

Ostaff, M. 2006. Limu: Edible seaweed in Tonga, an ethnobotanical study. *Journal of Ethnobiology* 26(2): 208-27

Padilla-Gamiño, JL and RC Carpenter. 2007. Seasonal acclimatization of *Asparagopsis taxiformis* (Rhodophyta) from different biogeographic regions. *Limnology and Oceanography* 52(2): 833–42

Patarra, RF; Paiva, L; Neto, AI; Lima, E and J Baptista. 2011. Nutritional value of selected macroalgae. *Journal of Applied Phycology* 23(2): 205-8

Pavia, H and E. Brock. 2000. Extrinsic factors influencing phlorotannin production in the brown alga *Ascophyllum nodosum*. *Marine Ecology Progress Series* 193: 285-94

Pavia, H; Cervin, G; Lindgren, A and P Aberg. 1997. Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum. Marine Ecology Progress Series* 157: 139–46

Peckol, P; JM Krane and JL Yates. 1996. Interactive effects of inducible defense and resource availability on the phlorotannins in the North Atlantic brown alga *Fucus vesiculosus. Marine Ecology Progress Series* 138: 209–17

Pellegrini, N; Serafini, M; Colombi, B; Del Rio, D; Salvatore, S; Bianchi, M and F Brighenti. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. *Journal of Nutrition* 133: 2812–19

Pelletreau, KN and NM Targett. "New perspectives for addressing patterns in secondary metabolites". Chapter 6 in Amsler, CD (Ed.) *Algal Chemical Ecology*. Berlin: Springer-Verlag, 2008. pp 121-146.

Peloquin, C and F Berkes. 2009. Local knowledge, subsistence harvests, and social–ecological complexity in James Bay. *Human Ecology* 37: 533–45

Poepoe, KK; Bartram, BK and AM Friedlander. "The use of traditional Hawaiian knowledge in the contemporary management of marine resources." Chap. 6 in Haggan *et al.* (Eds.) *Fishers' Knowledge in Fisheries Science and Management*. Coastal Management Sourcebooks. Paris: UNESCO Publishing, 2007. pp: 119-43.

Popkin, BM and S Du. 2003. Dynamics of the Nutrition Transition towards the Animal Foods Sector in China and its implications: A worried perspective. *The Journal of Nutrition* 133: 3898S-3906S

Popkin, BM and P Gordon-Larsen. 2004. The nutrition transition: Worldwide obesity dynamics and their determinants. *International Journal of Obesity* 28: S1-S9

Prior, R; Wu, X and K Schaich. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* 53: 4290-4302

Pukui, Mary Kawena. Interview with Winifred Sanborn, Alice Aki, I. Ashdown. Honolulu: Bernice Pauahi Bishop Museum Audiotape Archives, 1960. A Haw 84.6.1.

R Core Development Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Raffatullah, S; Tariq, M; Al-Yahya MA. Mossa, JA and AM Ageel. 1990. Evaluation of tumeric (*Curcuma longa*) for gastric and duodenal antiulcer activity in rats. *Journal of Ethnopharmacology* 29: 25-34

Redford, K and C Padoch. *Conservation of Neotropical Forests: Working from Traditional Resource Use.* New York: Columbia University Press, 1992.

Reed, Minnie. "The economic seaweeds of Hawaii and their food value." Annual Report of the Hawaii Agricultural Experiment Station, 1907. pp 61-88.

Richards, TA; Dacks, JB; Campbell, SA; Blanchard, JL; Foster, PG; McLeod R and CW Roberts. 2006. Evolutionary origins of the eukaryotic shikimate pathway: gene fusions, horizontal gene transfer, and endosymbiotic replacements. *Eukaryotic Cell* 5: 1517–31

Rijal, Arun. 2008. A quantitative assessment of indigenous plant uses among two Chepang communities in the central mid-hills of Nepal. *Ethnobotany Research and Applications* 6:395-404

Rodriguez-Bernaldo de Quiros, A; Frecha-Ferreiro, S; Vidal-Perez, AM and J Lopez-Hernández. 2010. Antioxidant compounds in edible brown seaweeds. *European Food Research and Technology* 231: 495-98

Rowley, KG; Daniel, M; Skinner, K; Skinner, M; White, GA and K O'Dea. 2000. Effectiveness of a community-directed 'healthy lifestyle' program in a remote Australian Aboriginal community. *Australian and New Zealand Journal of Public Health* 24(2): 136-44

Rowley, KG; Su, Q; Cincotta, M; Skinner, M; Skinner, K; Pindan, B; White, GA and K O'Dea. 2001. Improvements in circulating cholesterol, antioxidants, and homocysteine after dietary intervention in an Australian Aboriginal community. *American Journal of Clinical Nutrition* 74: 442–8

Ruddle, K. "The transmission of traditional ecological knowledge." Chapter 3 in Inglis, JT (Ed.) *Traditional Ecological Knowledge: Concepts and Cases*. Ottawa: Canadian Museum of Nature and International Development Research Centre, 1993. pp 17-31.

Salvatore, S; Pellegrini, N; Brenna, OV; Del Rio, D; Frasca, G; Brighenti, Fand R Tumino. 2005**.** Antioxidant characterization of come Sicilian edible wild greens. *Journal of Agriculture and Food Chemistry* 53: 9465–71

Schonfeld-Leber, B. 1979. Marine algae as human food in Hawaii, with notes on other Polynesian Islands. *Ecology of Food and Nutrition* 8(1): 47-59

Schultes RE. "Reasons for ethnobotanical conservation." In Johannes RE, (Ed.) *Traditional Ecological Knowledge: A Collection of Essays*. Gland, Switzerland and Cambridge, UK: International Conservation Union, 1989. pp 31-7.

Scourse, A and C Wilkins. 2009. Impacts of modernization on traditional food resource management and food security on Eauripik atoll, Federated States of Micronesia. *Food Security* 1:169–76

Seitzinger, SP; Kroeze; Bouman, AF; Caraco, N; Dentener, F and RV Styles. 2002. Global patterns of dissolved inorganic and particulate nitrogen inputs to coastal systems: recent conditions and future projections. *Estuaries* 25: 620-55

Setchell, WA. "Limu." In Setchell, WA (Ed.) *Botany*. Berkeley: University of California Publications, 1905. 2(3): 91-113.

Shahidi, F. 2009. Nutraceuticals and functional foods: whole versus processed foods. *Trends in Food Science and Technology* 20(9): 376-87

Sheikh-Ali, M; Chehade, JM and AD Mooradian. 2011. The antioxidant paradox in Diabetes Mellitus. *American Journal of Therapeutics* 18: 266–78

Sherwood, A. 2008. Phylogeography of *Asparagopsis taxiformis* (Bonnemaisoniales, Rhodophyta) in the Hawaiian Islands: two mtDNA markers support three separate introductions. *Phycologia* 47(1): 79-88

Shintani, T; Hughes, C; Beckham, S and HK 0'Connor. 1991. Obesity and cardiovascular risk intervention through the ad libitum feeding of traditional Hawaiian diet. *American Journal of Clinical Nutrition* 53: l647S-1650S

Shintani, T; Beckwith, S; O'Connor, HK; Hughes, C and A Sato. 1994. The Wai'anae diet program: culturally-sensitive, community-based obesity and clinical intervention program for the Native Hawaiian population. *Hawaii Medical Journal* 53: 138-47

Simopoulos, AP. 2001.The Mediterranean diets: What is so special about the diet of Greece? The scientific evidence. *Journal of Nutrition* 131: 3065S-73S

Smit, AJ. 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review**.** *Journal of Applied Phycology* 16: 245–262

Smith, JE; Hunter, CL; Conklin, EJ; Most, R; Sauvage, T, Squair, C and CM Smith. 2004. Ecology of the Invasive Red Alga *Gracilaria salicornia* (Rhodophyta) on Oʻahu, Hawaiʻi. *Pacific Science* 58(2): 325-43

Souza, BWS; Cerqueira, MA; Marins, JT; Quintas, MAC; Ferreira, ACS; Teixeira, JA and AA Vicente. 2011. Antioxidant potential of two red seaweeds from the Brazilian coasts. *Journal of Agriculture and Food Chemistry* 59: 5589-94

Spina, M; Cuccioloni, M; Sparapani, L; Acciarri, S; Eleuteri, AM; Fioretti, E and M Angeletti. 2008. Comparative evaluation of flavonoid content in assessing quality of wild and cultivated vegetables for human consumption*. Journal of the Science of Food and Agriculture* 88: 294–304

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses*. The Quarterly Review of Biology* 78(1): 23-55

Stewart, AJ and RF Stewart. "Phenols". Chapter 37 in Jorgensen, SE (Ed.) *Ecotoxicology*. Amsterdam: Academic Press, 2010. pp. 285-92.

Swain, T and WE Hillis. 1959. The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture* 10(1): 63-8

Szabo, MR; Idit, A; Chambre, D and AX Lupea. 2007. Improved DPPH determination for antioxidant activity spectrophotometric assay. *Chemical Papers* 61(3): 214-6

Termote, C; Van Damme, P and BD Djailo. 2011. Eating from the wild: Turumbu, Mbole and Bali traditional knowledge on non-cultivated edible plants, District Tshopo, DR Congo. *Genetic Resources and Crop Evolution* 58: 585–618

Thorburn, AW; Brand, JC and AS Truswell. 1987. Slowly digested and absorbed carbohydrate in traditional bushfoods: a protective factor against diabetes? *American Journal of Clinical Nutrition* 45: 98-106

Thow, AM; Heywood, P; Schultz, J; Quested, C; Jan, S and S Coalguiri. 2011. Trade and the nutrition transition: Strengthening policy for health in the Pacific*. Ecology of Food and Nutrition* 50(1): 18-42

Ticktin, T; Whitehead, AN and H Fraiola. 2006. Traditional gathering of native hula plants in alien-invaded Hawaiian forests: adaptive practices, impacts on alien invasive species and conservation implications. *Environmental Conservation* 33(3): 185-94

Tito, OD and LM Liao. 2000. Ethnobotany of *Solieria robusta* (Gigartinales, Rhodophyta) in Zamboanga, Philippines. *Science Diliman* 12(2): 75-7

Toledo VM and N Barrera-Bassols. *Ecologia y Desarrollo Rural en Patzcuaro: Un modelo para el Analisis Interdisciplinario de Comunidades Campesinas.* Mexico City, Mexico: Instituto de Biologıa, Universidad Nacional Autónoma de Mexico, 1984.

Trichopoulou, A; Costacou, T; Bamia, C, and D Trichopoulos. 2003. Adherence to a Mediterranean diet and survival in a Greek population. *New England Journal of Medicine* 348(26): 2599-608

Trinidad, AMO. 2012. Critical indigenous pedagogy of place: a framework to indigenize a youth food justice movement. *Journal of Indigenous Social Development* 1(1): 1-17

Tuomilehto, J; Lindström, J; Eriksson, JG; Valle, TT; Hämäläinen, H; Ilanne-Parikka, P; Keinänen-Kiukaanniemi, S; Laakso, M; Louheranta, A; Rastas, M; Salminen, V and M Uusitupa. 2001. Prevention of Type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *New England Journal of Medicine* 344(18): 1343-50

Turner, NJ. 2003. The ethnobotany of edible seaweed (*Porphyra abbottiae* and related species; Rhodophyta: Bangiales) and its use by First Nations on the Pacific coast of Canada. *Canadian Journal of Botany* 81(2): 283-93

UNESCO, 2003. Convention for the safeguarding of the intangible cultural heritage. Paris, 17 Oct. 2003, pp.1–15. Available: http://www.unesco.org/culture/ich/en/convention/

Van Alstyne, KL. 1988. Grazing increases polyphenolic defenses in the intertidal brown alga *Fucus distichus. Ecology* 69: 655–63

van der Walt, AM; Loots, DT; Ibrahim, MIM and CC Bezuidenhout. 2009. Minerals, trace elements and antioxidant phytochemicals in wild African dark-green leafy vegetables (morogo). *South African Journal of Science* 105: 444-8

Van Houtan KS, Hargrove SK, Balazs GH. 2010. Land use, macroalgae, and a tumor-forming disease in marine turtles. *PLoS ONE* 5(9): e12900

Vera-Guzmán, AM; Chávez-Servia, JL; Carrillo-Rodríguez, JC and MG López. 2011. Phytochemical evaluation of wild and cultivated pepper (*Capsicum annuum* L. and *C. pubescens* Ruiz & Pav.) from Oaxaca, Mexico. *Chilean Journal Of Agricultural Research* 71(4): 578-85

Vijayavel, K and JA Martinez. 2010. In vitro antioxidant and antimicrobial activities of two Hawaiian marine limu. *Journal of Medicinal Food* 13(6): 1494–9

Wang, T; Jonsdottir, R; Kristinsson, HG; Hreggvidsson, GO; Jonsson, JO; Thorkelsson, G and F Olafdottir. 2010. Enzyme-enhanced extraction of antioxidant ingredients from red algae *Palmaria palmate*. *Food Science and Technology* 43: 1387-93

Whiting, SJ and ML MacKenzie. 1998. Assessing the changing diet of indigenous peoples. *Nutrition Reviews* 56(8): 248-50

Willett, W; Manson, J and S Liu. 2002. Glycemic index, glycemic load, and risk of type 2 diabetes. *American Journal of Clinical Nutrition* 76: 274S–80S

Williams, DE; Saremi, A; Knowler, WC; Kriska, AM; Smith, CJ; Bennett, PH; Hanson, RL; Nelson, RG and J Roumain. 2001. The effect of Indian or Anglo dietary preference on the incidence of diabetes in Pima Indians. *Diabetes Care* 24(1): 811-816

Wright, DM; GJ Jordan; WG Lee; RP Duncan; DM Forsyth and DA Coomes. 2010. Do leaves of plants on phosphorus-impoverished soils contain high concentrations of phenolic defence compounds? *Functional Ecology* 24: 52–61

Xia, B and IA Abbott. 1987. Edible seaweeds of China and their place in the Chinese diet. *Economic Botany* 41(3): 341-53

Yangthong, M; Hutadilok-Towatana, N and W Phromkunthon. 2009. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. *Plant Foods for Human Nutrition* 64: 218–23

Yates, JL and P Peckol. 1993. Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus. Ecology* 74: 1757–66

Zhang, Q; Zhang, J; Shen, J; Silva, A; Dennis, DA and CJ Barrow. 2006. A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology* 18: 445–50

Zhang, J.; Tiller, C; Shen, JK; Wang, C; Girouard, GS; Dennis, D; Barrow, CJ; Miao, MS and HS Ewart. 2007. Antidiabetic properties of polysaccharide- and polyphenolic-enriched fractions from the brown seaweed *Ascophyllum nodosum*. *Canadian Journal of Physiology And Pharmacology* 85(11): 1116-23

Zimmet, P; Shaw, J and KGMM Alberti. 2003. Preventing Type 2 diabetes and the dysmetabolic syndrome in the real world: a realistic view. *Diabetic Medicine* 20: 693–702