PHYSIOLOGICAL EFFECTS OF SIMULATED SUBMARINE

GROUNDWATER DISCHARGE ON THE HAWAIIAN ENDEMIC

EDIBLE ALGA *GRACILARIA CORONOPIFOLIA*

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BOTANY

AUGUST 2009

By
Daniel William Amato

Thesis Committee:

Celia M. Smith, Chairperson
Thomas K. Duarte
Cynthia L. Hunter

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a thesis for the degree of Master of Science in Botany.

THESIS COMMITTEE
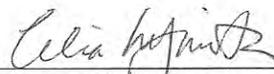
_____
Chairperson

_____

_____
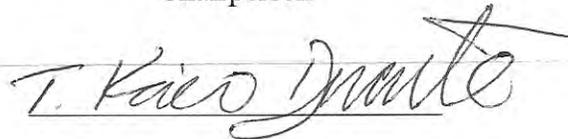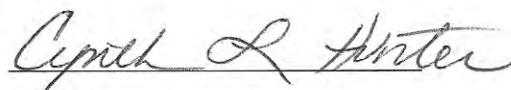
TABLE OF CONTENTS

<u>ACKNOWLEGDEMENTS</u>

ABSTRACT

Submarine groundwater discharge (SGD) is a significant source of nutrients to many coastal environments. The difficulty of experimental manipulation *in situ* has resulted in little evidence for a causal relationship between SGD and coastal biological processes. In this study, we examine the physiological response of the endemic edible Hawaiian rhodophyte, *Gracilaria coronopifolia*, to varied levels of simulated SGD. Forty-eight thalli were grown in a unidirectional flow-though mesocosm at 25ºC and 250 µmol photons $m^{-2}s^{-1}$ PAR during two replicate trials. The 27‰ SGD treatment (26.6µM-N, 1.1 µM-P, 27‰ salinity) provided optimal conditions for growth of *Gracilaria coronopifolia* and had higher mean values for all parameters measured. Regression relationships indicate simulated SGD levels predict growth rate, apical tip development, and photosynthetic parameters (ETR_{max} and E_k). Our results indicate moderate levels of SGD input may increase the growth rate, apical tip development, and photosynthetic performance of *Gracilaria coronopifolia* on otherwise oligotrophic Hawaiian reefs.

LIST OF TABLES

LIST OF FIGURES

Chapter 1:

Review of Literature

Primary Productivity and Marine Algae:

Global net primary production is defined as the amount of carbon fixed through photosynthesis, which is available to heterotrophic organisms in terrestrial and marine ecosystems. The once held paradigm that the majority of the world's oceans are essentially deserts is no longer accepted, as high photosynthetic and turn-over rates have clarified ample productivity (Sheldon and Sutcliffe 1978; Eppley 1980; Jenkins 1982; Laws et al. 1984; Laws et al. 1987). Photosynthetic marine organisms are responsible for 48 % of global net primary productivity (Field et al. 1998) and produce between 30-60 Pg of organic carbon each year (Martin et al. 1987; Charpy-Roubaud and Sournia 1990; Smith and Hollibaugh 1993; Carr et al. 2006). Ninety percent of marine net primary productivity arises from phytoplankton metabolism over 70 % of earth's surface. Benthic algae are responsible for the remaining 10 % of the world's marine primary production, sustaining shallow coastal communities that represent 1 % of Earth's surface area (Charpy and Charpy-Roubaud 1990).

In order to fix roughly half of the annual global carbon, marine photosynthetic organisms must have an adequate supply of nutrients. Nitrogen, phosphorus, and carbon are the most important macronutrients as predicted by plant physiology. Nitrogen is generally considered to be the primary limiting nutrient in marine environments although studies have shown phosphorus to be limiting for certain species or locations. Tropical

1

waters are usually considered to be oligotrophic (nutrient limited) with nutrient levels

near the limits of chemical detection. In some tropical ecosystems, there appears a sharp

contradiction between high amounts benthic primary production and extremely low levels

of dissolved nutrients in the overlying water column (Lewis 1977; Marsh 1977; D'Elia et

al. 1981; Laws et al. 1984). Net macroalgal productivity in some oligotrophic waters is

comparable to areas of high nutrient flux from coastal upwelling of deep nutrient-rich

water; standing stocks may reach 1000g dry mass m^{-2} (Valiela 1995; Stimson et al. 1996).

On some reef slopes, high macroalgal productivity is sustained despite low nutrient

concentrations (Lapointe et al. 1993; Stimson et al. 1996).

Historically, it has been assumed that the water column is the primary source of

macroalgal nutrients and thus, the majority of measurements have only considered the

water column overlying the benthos (Hanisak 1983). In order to maintain high biomass

in a coral reef, macroalgae must acquire sufficient nutrients to offset losses to herbivory,

reproduction, and detritus formation. A plausible explanation for the discrepancy of high

productivity in otherwise oligotrophic waters is that measurements of water column

nutrients are not indicative of actual concentrations available to the benthos. Larned

(1997) found that six out of nine species of macroalgae could not sustain positive growth

when cultured in seawater from the Kaneohe Bay water column. This research suggests

that there must be other sources of nutrients available to the benthos. After uptake by

phytobenthos and rapid mixing, these nutrients likely become nearly undetectable in the

water column.

Several hypotheses have been proposed to explain this contradiction of high rates of

algal production in oligotrophic waters. Many studies have shown that the growth rate of

2

marine algae is a function of water velocity, which influences the flux of nutrients to plant surfaces (Friedlander and Levy 1995; Gonen et al. 1995; Larned 1997; Ryder et al. 2004). Even at low concentrations of nutrients, sufficient water velocity can increase rates of nutrient uptake by thinning external boundary layers of an alga. Periodic pulsing of nutrients into the water column may also account for sustained algal growth (Lapointe 1985; Navarro-Angulo and Robledo 1999). Marine algae are capable of storing nutrients in excess of growth requirements for later use in the form of pigments and metabolic compounds (Ryther et al. 1981; Bird et al. 1982; Liu and Dong 2001). Nutrient efflux and remineralized organic matter from the sediment has also been proposed as a source of benthic nutrients (Hansen et al. 1987; Capone et al. 1992; Boucher et al. 1994).

The role of invertebrates as a nutrient source has been suggested for tropical phytobenthos (Hansen et al. 1987). Turf algae and seagrasses have been shown to benefit from nutrients provided by macrofaunal excretions (Williams and Carpenter 1988; Powell et al. 1989). Larned (1997) concludes that epifaunal invertebrates release about twice as much nitrogen and half as much phosphate as sediment efflux per unit area of *Dictyosphaeria cavernosa* thalli per day. Some species of tropical macroalgae capable of forming mat morphologies are able to trap benthic nutrients and under their canopy. Dissolved inorganic nitrogen (DIN) concentrations beneath mats of *Dictyosphaeria cavernosa, Gracilaria salicornia, and Kappaphycus alvarezii* have beeen measured at concentrations five-fold greater than the overlying water column (Larned, 1997).

Submarine Groundwater Discharge: A Historical Perspective:

The in-flux of submarine groundwater discharge (SGD) is now recognized as an important pathway for nutrients to reach the marine ecosystems (Johannes 1980; Hatcher 1990; Burnett et al. 2003; Paytan et al. 2006). SGD is defined as any and all flow of water on continental margins from the seabed to the coastal ocean, regardless of fluid composition or driving force (Burnett et al. 2003). This process can occur wherever there is an aquifer, with a head level above sea level, hydraulically connected to permeable marine sediments. One component of SGD is meteoric water that precipitated on land, percolated to the water table, and is forced from marine sediments by terrestrial hydraulic gradients or gravitational convection. A second major component of SGD consists of recirculated seawater driven across the ocean-sediment interface by a combination of terrestrial and marine hydraulic forces such as convection, geothermal convection, tidal pumping, and wave set up (McCoy and Corbett 2009).

While recent research has begun to focus on characterizing, differentiating (terrestrial versus recirculated), and quantifying sources of SGD to the coastal ocean (Wilson 2005; Martin et al. 2006; Martin et al. 2007; McCoy et al. 2007; Thompson et al. 2007; Cable and Martin 2008), it remains clear that SGD, and specifically the meteoric or fresh water component of SGD, has significant implications for coastal management strategies worldwide. Groundwater nutrient concentrations are typically high relative to seawater and even small groundwater fluxes may make large contributions to coastal nutrient budgets (Li et al. 1999). This is of particular importance in oligotrophic areas such as coral reefs with few other external nutrient sources (Hamner and Wolanski 1988;

Shellenbarger et al. 2006).

Knowledge of SGD and its basic driving forces are not limited to contemporary science of the last century. The first submarine springs were reported by Greek, Roman, and Persian writers (Potie and Tardieu 1977). Strabo, a Roman geographer who lived from 63 B.C. to 21 A.D., wrote of a submarine spring 2.5 miles offshore from Syria in the Mediterranean. Water was collected from this fresh spring via boat using a lead funnel and leather tube for use consumption on land (Kohout 1966). While submarine springs have been found in coastal waters of the United States, Cuba, Mexico, Chile, Jamaica, Australia, and Japan, the most reports have been from the Mediterranean basin: Libya, Israel, Lebanon, Syria, Greece, France, Spain, Italy, and Yugoslavia (Bonem 1988). The occurrence of submarine freshwater springs has long been known via Hawaiian legends. Today, these legends have been validated as sites with cool and or fresh water emanating from the sea floor (known in Hawai'i as "water holes" or lua wai) have been found on the Kona coast of the Big Island of Hawai'i and in Waikiki (which means "spouting-water" in Hawaiian) on the island of Oahu (Grossman et al. 2008).

As for modern scientific investigation, research concerning terrestrial groundwater and the marine environment from 1925 to 1980 had four important conclusions: (1) SGD was a widespread phenomenon, (2) nitrate concentrations were typically two to three orders of magnitude greater in SGD than overlying ocean water, (3) the salinity of sediment pore water was related to benthic composition and (4) discharge decreased with increasing distance from shore (Johannes 1980). In the 1980 benchmark review paper, Johannes states that the collective knowledge of SGD, though scattered and fragmentary, suggests that SGD deserves more attention than it has received from marine ecologists.

This conclusion was largely based on previous studies, the observation that SGD is a potential source of both nutrients and pollutants from the terrestrial environment, and that human activities on land could ultimately influence coastal marine ecosystems. At this time, few studies had attempted to link reduced salinity of marine sediments pore waters to the composition the benthic environment (Johannes 1980). Porewater salinity was found to be related to the composition of marine microphytes (Bruce 1925; Smith 1955; Moore 1979), of overlying algal mats (Sanders 1979), and seagrass beds and associated fauna (Kohout and Kolipinski 1967). Commercial fish and lobster yields have also been positively correlated with the rate of discharge of land-based nutrients (Sutcliffe 1972).

Heeding Johannes's call to action, oceanographers, geochemists, and marine ecologists set out to locate and quantify SGD and its interaction with costal environments. In Discovery Bay, Jamaica, a highly significant negative relationship between nitrate and salinity, with nitrate concentrations typically near 80 µM, was found for SGD (D'Elia et al. 1981). In Great South Bay, New York, nitrate and salinity in sediment cores were negatively correlated and both variables were related to the amount of rainfall (Capone and Bautista 1985). The first direct *in situ* measurements of ground water flux on a coral reef was reported from Barbados, West Indies. Using Lee style, seepage meters (Lee 1977), Lewis (1987) found a strong inverse relationship with salinity and nitrogen ($NO_2 + NO_3$). SGD was found to decrease with water depth and was influenced by tidal and seasonal factors in Barbados (Lewis 1987). In 1989, Lapointe and O'Connell reported that the productivity of *Cladophora* mats may be enhanced by nutrient rich groundwater discharge in Harrington Sound, Bermuda. Porewaters under the algal mats had reduced salinities, elevated ammonium, and high N:P ratios (Lapointe

and O'Connell 1989). In Cape Cod, MA, Giblin and Gaines observed a negative

relationship between salinity and nitrate in sandy sediments (Giblin and Gaines 1990).

More than two decades after Johannes 1980 paper, studies linking terrestrial

groundwater and the coastal environment generally concluded that SGD is likely a

significant source of nutrients (particularly nitrate) to coastal environments and that

anthropogenic activities (such as sewage disposal and agriculture) could potentially be

significant factors in eutrophication and / or increased algal biomass (Lapointe 1997;

Paerl 1997). Although comparisons of well-water nutrients to SGD and relationships of

salinity to nutrient gradients of porewaters may indicate groundwater discharge from the

sediment, researchers still needed more convincing measures to couple terrestrial

processes with the sediment-ocean interface.

In 1996, Cable and Burnett published a study that addressed this issue. They

conclude that the isotope [222]Radon is useful natural tracer of groundwater discharge to the

coastal zone because: [222]Radon is chemically conservative, easily measured, is three to

four orders of magnitude more concentrated in groundwater than seawater, and decays at

a rate comparable to many coastal processes. In 2002, Moore et al. reported that nutrients

were highly correlated with radium concentration in SGD 20km of the North Carolina

coast. Currently, a variety of radium and radon tracers are used to estimate SGD and

nutrient flux in coastal waters (Beck et al. ; Burnett et al. 2008). Using a combination of

[222]Ra and Lee-style seepage meters, SGD flux in Kahana Bay, Oahu was found to be

comparable to average annual surface runoff from Kahana River. Total dissolved

nitrogen and phosphorus was estimated to be 200% and 500% greater than fluxes via

surface runoff (Garrison et al. 2003). Hwang et al. (2005) reported radium and nutrient

concentrations in brackish groundwater to be an order of magnitude higher than stream water or the ambient water of Yeoja Bay, Korea. Although SGD is known to vary greatly on scales of space and time, many studies suggest that SGD flux may rival rivers and the atmosphere as a source of nutrients to coastal environments (Paerl 1997; Krest et al. 2000; Moore et al. 2002).

As radon tracers emerged as a method of SGD quantification, a similar method employing isotopes tracers began to gain popularity among researchers looking to link water column nutrients to anthropogenic sources. The ratio of ^{14}N:^{15}N is now widely used to indicate anthropogenic sources of nutrients in plant tissues, sediments, and the water column. Using $\delta^{15}N$ and water sampling methods, Smith et al. (2005) concluded that SGD is factor in occurrence of large-scale ephemeral blooms of the marine alga *Cladophora sericea* on coral reefs in Maui. The porewaters of sediments contained high concentrations of nutrients and low salinity relative to the overlying ambient water column. Tissue samples revealed elevated nitrogen and $\delta^{15}N$ suggesting that terrestrial based nutrients via SGD are likely to play an important role in the development of algal blooms (Smith et al. 2005). A similar study by Lapointe concluded that increased DIN and decreased salinity indicate SGD may play a role in macroalgal blooms on coral reefs in both Florida and Jamaica. Elevated $\delta^{15}N$ ratio values indicated wastewater as a contributing source of nitrogen to the blooms in Florida (Lapointe 1997). Tissue nutrient samples of the seagrass *Thalassia testudinum* from before and after heavy rains suggest submarine springs may influence nutrient processes within seagrass meadows near the Yucatan Peninsula, Mexico. Elevated $\delta^{15}N$ in the seagrass tissues suggests wastewater as a nitrogen source (Carruthers et al. 2005). Herrera-Silveira conclude that SGD has

played a major role in the shift from seagrass (*T. testudinum*) dominated cover to green filamentous algae and strongly influences primary production as well as trophic status at sites near the Yucatan Peninsula, Mexico (Herrera-Silveira 1998; Herrera-Silveira et al. 1998; Herrera-Silveira et al. 2002; Herrera-Silveira and Morales-Ojeda 2009).

Remote sensing has recently emerged as a valuable method of detection and quantification of SGD. Using low altitude thermal infrared imagery (TIR) and simultaneous water column sampling, Johnson et al. (2008) identified >50 point-sourced nutrient-rich SGD plumes on the Kona coast of the Big Island, Hawai'i. In tropical regions where groundwater is typically cooler than the coastal ocean, temperature can become an important marker for SGD. Water column profiling along a SGD plume in Honokohau Harbor and Kealakekua Bay (Hawai'i) has provided detailed information about the mixing dynamics of SGD. These surface plumes consisted of a distinct gradient of high nutrient, cool, buoyant, brackish water less than a few meters thick flowing seaward. Highly significant negative linear relationships were found between salinity and nitrate, phosphate, and silica. Temperature was also positively related with salinity (Johnson et al. 2008). Groundwater resources in this region of Hawai'i is thought to be of particular importance to reef productivity because there is little terrestrial surface water input. SGD is known to be the only significant source of freshwater to this coastal ecosystem (Kay et al. 1977; Oki and Dimeyer 1999).

Nutrient Limitation in the Marine Environment:

In the past two decades, there has been a growing concern among coastal managers as benthic communities in many tropical regions show signs of change. Shifts from coral to algal dominance have prompted many scientists to identify the factors controlling productivity on coastal reefs. SGD has been suggested to play an integral role in coastal eutrophication and reef degradation where natural groundwater nutrient concentrations are enhanced by anthropogenic sources, such as sewage or agricultural practices (D'Elia et al. 1981; Lapointe and O'Connell 1989; Valiela et al. 1990; Lapointe 1997; Herrera-Silveira 1998; González et al. 2008).

The rate of growth is generally regulated by the availability of the scarcest nutrient relative to the requirements of an alga. If the concentration of this limiting nutrient increases until there is excess relative to requirements, a different nutrient may then become relatively scarce and will thus become the growth-limiting nutrient. This relationship, known as "Liebig's law of the minimum" (Liebig 1840; Odum 1971), has become a founding theory for investigations into ecosystem ecology and plant physiology. The identity of the limiting nutrient may vary among species, locations, and seasons; thus broad generalizations may be unwarranted (Lapointe 1987; Lapointe et al. 1987; Larned 1998; Tsai et al. 2005).

Nitrogen:

Nitrogen is a crucial element in the composition of the most important structural and functional macromolecules in organisms. On average, nitrogen makes up 15% of

proteins and 13% of nucleic acids (Inokuchi et al. 2002). In marine systems, nitrogen

exists in many inorganic and organic forms. Dissolved forms of inorganic nitrogen

(DIN), such as nitrate, nitrite, and ammonium, are generally more limiting than dissolved

organic nitrogen (DON), such as urea and amino acids (Hanisak 1983). Ammonium and

nitrate, considered the most important sources of nitrogen, are more easily assimilated

into macroalgae than other forms of nitrogen (Hanisak 1983). Nitrate, the most common

form of DIN in marine systems, is present in concentrations, which are significantly

higher than either ammonium or nitrite (Kain et al. 1990). Organic forms of nitrogen

may play an important role in marine systems where inorganic forms of nitrogen become

limiting. The growth of *Gracilaria tikvahiae* was supported by organic forms of nitrogen

from different sources of organic waste (Asare 1980; Hanisak 1983). Although many

studies have measured nitrogen uptake, very few have detected nitrogen release into the

water column.   Tyler and McGlathery (2006) estimated that actively growing *Gracilaria*

vermiculophylla releases an average of 67% of gross daily nitrogen uptake back into the

water column. Naldi and Wheeler (2002) conclude that both DIN and free amino acids

were released from *Gracilaria pacifica* and *Ulva fenestrata*.

Uptake of nitrogen involves both active and passive transport into algal cells

(D'Elia and DeBoer 1978). Active transport is the most common *in situ* as the

concentrations in algal cells are typically 1000-fold higher than the water column

(Lobban and Harrison 1994). Although nitrate may be the most common form of

nitrogen available, ammonium may be preferentially assimilated and even inhibit the

uptake of nitrate in some algae (DeBoer et al. 1978; Dortch 1990). One explanation for

this occurrence is the difference in the energy requirement for assimilation. In order for

an alga to utilize nitrate, it must first convert it to ammonium. This fundamental

physiological process is a two-step reduction requiring eight electrons. In the first step,

the enzyme nitrate reductase utilizes two electrons to reduce nitrate to nitrite. NAD(P)H

serves as the electron donor in this reaction. The second step requires six electrons for

the enzyme nitrite reductase to reduce nitrite to ammonium. This step is coupled to

photosynthetic electron transport using ferredoxin, which serves as the electron donor for

nitrite reductase (Solomonson and Barber 1990). Once nitrate is reduced or ammonium

is taken up, most algae employ glutamine synthetase as the primary ammonium-

assimilating enzyme (Alwyn et al. 1998). According to Dortch (1990), a review of the

literature indicates ammonium inhibition of nitrate assimilation is neither as universal nor

as severe as is generally believed. In addition, nitrate can sometimes inhibit ammonium

uptake and small amounts of ammonium may stimulate nitrate uptake (Dortch 1990).

Phosphorous:

While nitrogen in generally considered to determine short-term production in

coastal waters, the phosphorus supply may ultimately set the long-term limit (Tyrrell

1999). Phosphorus plays an important role in producing ATP for energy metabolism,

biosynthesis of phospholipids, and the production of other cellular components.

Phosphorus in marine ecosystems occurs as dissolved inorganic P (DIP), dissolved

organic P (DOP), or particulate P (PP) (Valiela 1984). At least 90% of terrestrial

phosphorous in coastal waters is PP associated with suspended solids carried by rivers

(Follmi 1996). Because a large portion of riverine PP flux occurs during unpredictable

episodes, the amount, composition, and fate of this phosphorus source is not well known

(Jordan et al. 2008). It is likely that much of this PP is converted to more biologically available forms of dissolved inorganic P (DIP). In addition to delivering large quantities of nitrate to coastal ecosystems, SGD has been found to contain extremely high levels of phosphate. In regions of high SGD, near the Kona coast of the Big Island of Hawai'i, water column phosphate concentrations have been measured at ~ 4 µM, three orders of magnitude higher than ambient oceanic levels (Johnson et al. 2008).

Nitrogen vs. Phosphorous Limitation:

Traditionally, the ratio of nitrogen to phosphorus in tissue samples and the water column has been used to infer nutrient limitation in algae and ecosystems. Howarth (1988) suggests that three main factors which will determine whether N or P will limit algal growth are: (1) the ratio of N:P in external nutrient inputs, (2) preferential loss of N or P in the photic zone due to biogeochemical processes (denitrification, sedimentation of N or P, adsorption of P), and (3) the extent of N fixation. When marine phytoplankton are grown under conditions of maximal growth (no limiting nutrients), the molar ratio of carbon to nitrogen to phosphorus approximates the Redfield ratio of 106:16:1 (C:N:P) (Redfield 1958; Goldman et al. 1979). N:P ratios < 16:1 have been used to indicate nitrogen limitation with respect to the demands of algal metabolism (usually microalgae) (Redfield 1958). Using this criterion, water column samples in 22 of 27 estuaries, measured during peak algal growth, suggested N limitation (Boynton et al. 1982). In six of seven studies from tropical oligotrophic (less than or equal to 1.5 µM DIN and less than or equal to 0.2 µM PO_4) regions, water column $DIN:PO_4$ ratios were less than the Redfield ratio (Larned 1998). Nutrient analysis of Patuxent River estuary (Chesapeake

Bay) porewaters indicate a negative relationship between N:P ratios and salinity. Near

the freshwater end of the estuary, N:P ratios were generally > 16. At the saline end of the

gradient, N:P ratios were < 16 and as low as 1.5 (Jordan et al. 2008). Some studies on the

C:N:P ratio of macroalgal tissues have concluded that a critical N:P ratio exists between

10 to 15. Values higher than this critical ratio may indicate nitrogen limitation, while

lower values may be evidence for nitrogen storage (Hanisak 1983). However, Atkinson

and Smith (1983) conclude that the median C:N:P in benthic marine algae and seagrasses

is about 550:30:1, suggesting inferences of nutrient limitation based on the Redfield ratio

may not be applicable to these organisms. Of 96 species of phytoplankton, 46 macroalgal

species, 27 seagrasses, 11 freshwater angiosperms, and several mixed communities of

macroalgae and phytoplankton, N and P concentrations tend to be higher in

phytoplankton compared to macrophytes (Duarte 1992).

It appears that methods employing a generalized elemental ratio may not provide

accurate conclusions regarding limitation because of fundamental variability in species

optimal growth requirements, local nutrient delivery, other abiotic factors (temperature,

light, and salinity) and that manipulative experiments rarely follow. Many studies stress

that actual N:P ratios in algae are quite variable as algae will absorb more phosphorus

than predicted (McAllister et al. 1961; Vince and Valiela 1973). A more reliable

assessment of nutrient limitation in algae and ecosystems is laboratory and *in situ*

enrichment experiments (Fong et al. 1993; Larned 1998).

The results of nutrient enrichment experiments on tropical macroalgae have

concluded that both nitrogen (Lapointe 1987; Lapointe et al. 1987; Lapointe and

O'Connell 1989; Littler et al. 1991; McGlathery 1992; Larned 1998) and phosphorus

(Lapointe et al. 1987; Littler et al. 1991; Lapointe et al. 1992; Lapointe et al. 1993) limit productivity in oligotrophic waters. The addition of both N and P have greater effects on growth together than when supplied separately suggesting a secondary limitation by P; saturating amounts of N therefore cannot substitute for the P requirements of a plant (Howarth 1988). If both N and P occur at high concentrations, then trace metals may limit the growth of macrophytes (Prince 1974). Of 36 species of tropical macroalgae in seven studies, growth was enhanced by inorganic nitrogen in 22 species and by inorganic phosphorus in 17 species (Larned 1998). The freshwater macroalga *Spirogyra fluviatilis* was found to have an optimal molar C:N:P ratio of 1800: 87:1 (Townsend 2008). Townsend et al. (2008) suggest this indicates the Redfield ratio may not apply to macroalgae and tissue stoichiometry should only be used to indicate nutrient limitation when a species optimal C:N:P ratio is known.

Previous Research on Species of *Gracilaria*:

A relatively large amount of research has focused on species in the genus *Gracilaria* due to their global distribution, diversity of species (>150 species described, (Byrne et al. 2002), commercial interest in high quality agar, and potential as a bioindicator of water quality. According to Tseng (2001), *Gracilaria* is now the most important agarophyte, producing 60% of the world's agar; commercial cultivation is performed on a very large scale in several countries such as Chile, China and Taiwan. Many species of *Gracilaria* are known to display a strong response to changes in water column nutrient concentrations and are characteristically tolerant to abiotic

environmental factors such as salinity, temperature, and irradiance (Bird et al. 1979; Dawes et al. 1984; Jones et al. 1996).

In general, when concentrations of nitrogen and phosphorus increase, *Gracilaria* species increase productivity and change the chemical composition of tissues. A positive correlation was shown with uptake rate and concentration for both ammonium and phosphate in *Gracilaria tikvahiae* (Friedlander and Dawes 1985). *Gracilaria* species take up nutrients in excess of immediate growth requirements during nutrient pulses and store those nutrients for metabolic use during nutrient limited durations. Nitrogen storage in red algae occurs in the form of excess tissue N, accessory pigments, or amino acids (Ryther et al. 1981). *G. tikvahiae* was found to store excess nitrogen as amino acids and proteins (Bird et al. 1982). Stored nutrients can be metabolized for protein when internal N reserves are depleted (Jones et al. 1996). After only six hours soaking in full nutrient medium, *G. tikvahiae* was able to sustain non-nutrient-limiting growth rates for two weeks before declining (Ryther et al. 1981). Following ten days of nitrogen enrichment, *G. tenuistipitata* sustained rapid growth for more than 20 days in unenriched water (Liu and Dong 2001). Although a positive relationship between growth rate and nutrient uptake exists, chemical toxicity will develop at a certain concentration threshold specific to each element and species. The growth rate of *G. lemaneiformis* increased with increasing nutrients but dropped significantly when N/P exceeded 400/25 µM. At 600/37.5 µM, ruptured, irregularly aligned thylakoids and accumulation of starch grains gave evidence of chloroplast damage (Yu and Yang 2008).

First principles of plant physiology dictate that ammonium is preferred over nitrate; this has been supported by work with some marine algae (Nelson et al. 1980). Uptake of

nitrate and nitrite may be suppressed under conditions of elevated ammonium (D'Elia and DeBoer 1978; Ryther et al. 1981). *G. tenuistipitata* had higher uptake rates for ammonium than nitrate in outdoor tank culture with brackish seawater (Haglund and Pedersén 1993). Ammonium enrichment produced higher growth rates in *G. foliifera* than nitrate, sewage, or urea enrichment (DeBoer et al. 1978). D'Elia and DeBoer (1978) concluded that nitrate uptake became suppressed in *G. foliifera* at 5μM NH_4. NH_4 was found to be preferred over nitrate even when *G. foliifera* were preconditioned with nitrate as the only source of nitrogen (D'Elia and DeBoer 1978). However, no significant differences in growth rate were detected when *G. cornea* was cultured in nitrate, ammonium, or urea (Navarro-Angulo and Robledo 1999). In *G. conferta*, ammonium and nitrate additions were found to be equally effective, but high phosphate concentrations inhibited growth (Friedlander and Ben-Amotz 1991). However *Gracilaria tenuistipitata* was shown to have a higher growth rate, a five-fold increase in photosynthetic rate, a three-fold increase in Rubisco content, increased content of Chlorophyll *a* (Chl *a*), phycobiliprotiens, and greater total soluble proteins when grown in enriched phosphate (30 µM) compared to low phosphate treatments (3 µM) (Garcia-Sanchez et al. 1996).

It is well understood that nutrients play a major role in primary productivity in marine environments although many other abiotic and biotic factors are of importance. Salinity, irradiance, and temperature have also been shown to determine the growth rate and distribution of species.

Salinity:

Salinity is one of the most critical chemical factors affecting the growth rate, development, and distribution of seaweeds (Hoyle 1975; Koch and Lawrence 1987; Dawes et al. 1999; Israel et al. 1999). Although variation exists among the world's oceans, an average value of 34.85 ‰ is reported for the salinity of all oceanic water bodies (Kalle 1971). Physiological interactions such as the movement of ions and water molecules across cell membranes can be affected by changes in salinity (Kirst 1989; Reed 1990; Lobban and Harrison 1994; Kirst 1995). The maintenance of an organism's osmotic balance requires energy input (Kirst 1989) and productivity of an alga may be reduced as a result (Bird et al. 1979; Bird and McLachlan 1986; Edwards et al. 1988; Murthy et al. 1988; Karsten and Kirst 1989; Friedlander 1992; Kamer and Fong 2000). Gessner and Schramm (1971) concluded that decreases in the photosynthetic rate of marine algae are directly proportional to decreases in salinity levels. According to Lavery and McComb (1991), the number of species of macroalgae usually decreases with decreasing salinity.

Algae, which exploit certain coastal areas with periodic pulsation of brackish or freshwater (eg. estuaries, river mouths, SGD sites), must be able to maintain high productivity while withstanding rapid fluctuations in water column salinity. Maximal growth rates for some tropical macroalgae have been observed in water with decreased salinity from ambient oceanic levels (35 ‰). Species in the genus *Gracilaria* have long been known as euryhaline species able to adapt to and tolerate wide variations in salinity (Bird et al. 1979; Bird and McLachlan 1986; Yokoya and De-Oliveira 1992). In culture, the growth rate of *Gracilaria chorda* and *G. verrucosa* reached a maximum in 25 ‰

salinity water of 4.47 % d^{-1} at 25°C and 4.95 % d^{-1} at 30°C (Choi et al. 2006). Causey et al. (1946) report positive *G. verrucosa* growth in 15-50 ‰ salinity with optimal growth occurring between 25-35 ‰. Kishnamurthy (1954) observed *G. verrucosa* in a Madras salt marsh where salinity ranges from 2.5 to 42 ‰. Maximum growth rates for *G. vermiculophylla* were observed at 20 ‰ salinity and positive growth was measured in treatments from 5-60 ‰ salinity (Yokoya et al. 1999). Hoyle (1976) concludes that *G. coronopifolia* grow fastest at 22 ‰ salinity and 20 µM NH_4. The relative growth rate *of G. tenuistipitata* grown at 20 ‰ salinity at 20°C was significantly higher than 30 ‰ or 39 ‰ salinity treatments (Israel et al. 1999). A significant inverse correlation (R= 0.45; p< 0.05) was found between relative growth rate and salinity in *G. birdiae* when grown in an estuary over a six-month period. The results of this research indicate *G. coronopifolia* and congeners not only tolerate a wide variety of salinities but may also have increased productivity in waters with reduced salinity compared to ambient oceanic conditions.

Irradiance:

Irradiance is commonly held as the most important ecological factor in the marine environment (Hoyle 1975). Growth rates in algae generally increase with increasing irradiance until photosynthesis is saturated. As irradiance increases beyond saturating levels, a decrease in photosynthesis (photoinhibition) may occur. The growth of *Garcilaria foliifera* increased linearly with increasing irradiance to 0.43 doubling per day at high light levels *in situ* (Lapointe 1981). The quantity and composition of light is a function of water clarity, depth (Valiela 1984), season, time of day, cloud cover, and surface conditions (Hoyle 1975). When grown under blue light, *Gracilaria tenuistipitata*

had lower maximal photosynthetic rates than white light controls (Mercado et al. 2002).

Irradiance can have complex interactions with absorption of inorganic nutrients. The uptake of nitrate and nitrite has a strong interaction with irradiance when compared to ammonium (Falkowski 1983; Hanisak and Ryther 1984). Many studies have suggested that nitrate reductase expression and activity in algae and higher plants is controlled by biological clock induced by irradiance (Lillo 1984; Ramalho et al. 1995; Lopes et al. 1997; Lillo et al. 1998; Granbom et al. 2007).

Tropical regions are known to receive the highest global levels of photosynthetically active radiation (PAR) and UV irradiance (Caldwell et al. 1989; Häder 1993), which can be five-fold higher then macroalgal requirements (Beach et al. 1995). Marine primary producers possess mechanisms that efficiently capture light at low irradiance levels or minimize damage induced by excessive radiation. Some algal species subjected to low irradiance, have developed adaptations for efficient harvesting of photons such as high accessory pigment to Chlorophyll *a* ratios (Reiskind et al. 1989). Seaweeds from three taxonomic classes showed increased antenna pigment content with decreasing irradiance leading to an increase in the ratio of accessory pigment to Chl *a* (Ramus et al. 1976b; Ramus et al. 1976a; Ramus et al. 1977). In *G. foliifera*, an increased R-phycoerythrin : Chl *a* ratio was related to enhanced photosynthetic performance at sub-saturating light intensities (Rosenberg and Ramus 1982). In *G. tenuistipitata*, carotenoid pigments were found to play an important role in the protection of the photosynthetic apparatus by epoxidating reactions (Carnicas et al. 1999).

Lower respiration rates and lower light compensation points (I_c) are typical of shade-adapted primary producers when compared to sun adapted plants. Shade-tolerant

primary producers require less irradiance to saturate photosynthesis (I_k) and have higher quantum efficiencies (α) (Beach and Smith 1996). The mat-like morphology of some topical rhodophytes promotes sun-shade acclimation along thalli axes of less than 10cm. Distinct differences in the photosynthetic performance of canopy versus understudy thalli microsites has been measured in *Laurencia mcdermidiae* and *Ahnfeltiopsis concinna* (Beach and Smith 1996). *Gracilaria salicornia*, an opportunistic invasive alga in Hawaiʻi, was found to exhibit similar sun-shade acclimation characteristics within its mat-like morphology. The orange colored canopy of this alga was exposed to 2389 µmol photons $m^{-2}s^{-1}$ at a depth of 0.5m, while the red thalli within the under-story were exposed to only 0.2 to 4.9% of canopy irradiance. Significant regression relationships were found of *in vivo* absorbance for phycoerythrin and carotenoid-specific maxima with (I_k), (I_c), and P_{max} indicating variations in thalli pigmentation as a response to sun-shade acclimation within a single individual (Beach et al. 1997).

When marine algae are exposed to high levels of irradiance, a decrease in photosynthesis due to photoinhibition (a down-regulatory process at photosystem II) may occur. When *G. chilensis* was exposed to full solar radiation at noon for three hours and then incubated under shade for recovery, a 16% decrease in photosynthesis was observed and recovery was close to 88%. When algae in treatments exposed to full solar radiation without UVB, photosynthetic recovery was close to 100% indicating that UVB radiation may be damaging to macroalgae (Gomez et al. 2005). Evidence of photoinhibition and reduced productivity was observed in the canopy tissues of *Ahnfeltiopsis concinna* (Beach and Smith 1996). The increased carotenoid concentration of orange colored

canopy tissues and mat morphology is likely a mechanism to mitigate photo-induced

damage to marine algae (Beach and Smith 1996; Beach et al. 1997).

Temperature:

The effect of temperature on enzymatic processes has been extensively studied in

all aspects of biology. All organisms have an optimal range of temperatures in which

they are most efficient. In macroalgae, temperature has been found to affect the

respiration and dark photosynthetic enzyme activity (Kain et al. 1990) as well absorption

of nutrients. Higher temperatures have been shown to increase the uptake of nutrients

and the maximum photosynthetic rate of algae at a particular light level (Valiela 1984).

Higher photosynthetic and respiratory rates were measured in *Gracilaria cornea* exposed

to 25-35°C compared to 15°C (Dawes et al. 1999). Algal cells at lower temperatures

were found to have higher concentrations of pigments, enzymes, and carbon (Valiela

1984). *Gracilaria tikvahiae* was able to rapidly respond to a temperature change from

10°C to 20°C with a 20-fold increase in growth (Bird et al. 1979). Maximal growth of

Gracilaria chorda was observed between 18-24°C (Kakita and Kamishima 2006). In

outdoor laboratory culture of *Gracilaria,* nutrient enrichment was found to shift the low

temperature growth limit from 20°C to 15°C (Tsai et al. 2005). There is some evidence

that the optimal temperature for an alga varies with different stages of its life cycle. The

optimal temperature for a *G. vermiculophylla* tetrasporophyte was 15-25°C while that of

the gametophyte was 20-30°C (Yokoya et al. 1999).

Published *Gracilaria* Growth Rates:

Over the past 70 years, an extensive amount of research has focused on the growth and physiology of species in the genus *Gracilaria*. Published growth rates for *Gracilaria spp*. are as variable as the methods and experimental conditions. Daily growth rates of this genus range from less than 1% d^{-1} to more than 25% d^{-1}. An average maximal growth rate of *Gracilaria spp.,* from 21 species and 33 studies reported in the literature, is ~7 % d^{-1} (SD=0.05 % per day) (Table 1.1).

Pulse Amplitude Modulation (PAM) Fluorometry:

In the early 1990s, a novel method in the field of photophysiology emerged allowing for rapid, noninvasive, in-situ measurements of photosynthetic parameters. Pulse amplitude modulation (PAM) fluorescence is a technique that measures Chl *a* fluorescence. A technique called a rapid light curve (RLC) has become a fast and effective method for determining the photosynthetic response of algae at various levels of irradiance. This technique calculates the electron transport rate (ETR) for photosystem II via multiplying the irradiance applied by the light adapted fluorescence yield (Change in (F/ Fm') (Genty et al. 1989). RLCs can assess the present photosynthetic capacity as well as an organism's potential activity over a wide range of light intensities. This type of technique is know as a Photosynthesis vs. Irradiance (P vs. I) curve and appears similar to traditional oxygen-based curves, but should not be interpreted as exactly similar (Hawes et al. 2003). In both RLCs and traditional P vs. I irradiance curves, there can be three distinct areas of the curve: the light limited, minimum saturation and a

downturn in rate at high irradiance levels.

At low light levels, photosynthesis is limited by irradiance. The slope of the curve in this light-limiting region (α) has been shown to be proportional to the efficiency of light capture (Schreiber 2004). Saroussi and Beer (2007) conclude that α, as estimated from an RLC, may not be an accurate method of estimating maximal quantum yield and suggest that Y_0 should be used instead because PAM measures this parameter directly. The minimum saturating irradiance (E_k for PAM, I_k for O_2) is identified by finding the interception of α with the maximum rate (ETR_{max}, P_{max}). Below E_k, photochemical quenching dominates, while above E_k, fluorescence quenching dominates. At high irradiance, PAM curves may decline, suggesting apparent photoinhibition as in traditional oxygen curves. Photoinhibition was suggested from PAM and oxygen evolution measurements in *Cladophora pellucida* and *C. prolifera* (Häder et al. 1997) and *Dictyota dichotoma* (Hanelt et al. 1994). Using PAM, Hader et al. (1999) concluded that sun-adapted plants recover faster from photoinhibition than algae adapted to shaded sites. The detection of photoinhibition via RLCs has been challenged in the literature, as there may not be sufficient time for photoinhibition to occur (Ralph and Gademann 2005).

A recently developed device called Diving-PAM (Walz, Germany) has been shown to produce photosynthetic measurements *in situ* in marine angiosperms (Beer et al. 1998; Ralph et al. 1998; Beer and Bjork 2000; Beer et al. 2006), and macroalgae (Beer et al. 2000; Beer and Axelsson 2004), which closely correlate with oxygen evolution. A linear relationship was found between ETR and gross oxygen evolution in *Ulva fasciata* and *U. lactuca* with increasing irradiance up to 600 µmol photons $m^{-2}s^{-1}$ (Beer et al. 2000) and in *Ulva lactuca* at lower irradiances (Longstaff et al. 2002). Evidence of the uncoupling of

this Oxygen evolution / PAM-ETR relationship at high irradiance was found in *Ulva australis* and *Porphyra columbina* when ETRs were overestimated in comparison with gross oxygen evolution (Franklin and Badger 2001). Oxygen evolution was also found to decrease in relation to ETR in the seagrass *Halophia stipulacea* and *Zostera marina* (Beer et al. 1998). Using *Ulva fasciata* and *U. reticulata*, Carr and Bjork (2003), conclude that ETR was found to decrease at high irradiances while oxygen evolution remained constant. Gilbert et al. (2000) report that the relationship between ETR and oxygen evolution began to deviate from linearity at high irradiances in four planktonic algal species. Because the O_2:ETR ratio is relatively constant at low light levels, RLCs may well estimate photosynthetic rates at non-saturating irradiances (Carr and Bjork 2003).

In an effort to assess to what degree high irradiances would limit ETR measurements for macroalgae of different divisions, Beer and Axelsson (2004) compared PAM and oxygen evolution measurements on green, brown and red macroalgae. In agreement with previous studies, all the algae displayed a clear positive correlation between O_2 evolution and ETR. At high irradiances, all the algae show a decrease in ETR while O_2 evolution remained relatively constant. It was observed that the O_2/ETR ratio began to increase when effective quantum yield of photosystem II (Y) reached a critical value of 0.1. Thus, Beer and Axelsson recommend that ETR should only be used as a measure of photosynthetic rate when quantum yield exceeds 0.1 and note that the irradiance level associated with this value may vary with species and previous irradiance history (Beer and Axelsson 2004).

Conclusions and Study Objectives:

Three decades since Johannes first concluded that the geohydrochemical properties of SGD deserved more attention from marine ecologists, we are still left with substantial areas that remain unexplored in the current knowledge of the land-sea interface. Many studies have been successful at quantifying the flux rate and chemical composition of SGD in coastal environments. Fewer studies have found correlative evidence or even suggest that SGD in fact plays an important role determining primary productivity of coastal regions. Thus far, little evidence for a causal relationship between SGD and coastal biological processes and has emerged. This may be due in part to the inherent difficulty in the detection of SGD (Burnett et al. 2003) and the presumably even more difficult task of *in situ* manipulation and control design. Until an appropriate and robust method of *in situ* manipulation is developed, this question may best be answered within the highly controlled environment offered by growth chambers.

The collective body of knowledge summarized in this review suggests that irradiance, nutrients, salinity, and temperature are the most important factors determining the productivity and distribution of *Gracilaria* species. The relative importance of these parameters has been shown to vary among species, season, and location. Manipulative experiments are likely the most reliable method to determine optimal conditions for a species. While numerous studies have investigated the growth response and quality of agar in *Gracilaria* species, studies which measure the physiological response of this genus to combinations of abiotic factors such as salinity, light and temperature are rare (Dawes et al. 1999). Furthermore, simultaneous variation of nitrogen, phosphorous, and

salinity simulating the dynamic mixing associated with SGD and coastal waters has never been attempted.

The objective of this study was to measure the physiological effects of simulated SGD on an endemic species of macroalgae in a laboratory setting using empirical data on SGD from Hawai'i to determine treatment conditions. Based on previous research with *Gracilaria coronopifoila* and congeners, conditions of reduced salinity and increased nutrients may increase the productivity of *G. coronopifolia* in otherwise oligotrophic water. It is hypothesized that moderate levels of simulated SGD will increase the growth rate and photosynthetic performance of *G. coronopifolia* compared to ambient oceanic controls.

Tables:

Table 1.1 Maximum Growth Rates (% per day) published for species of *Gracilaria* .

Gracilaria species	Maximum Growth Rate (%d^{-1})	Reference
arcuata	3.50%	Nelson et al. 1980
balinae	1.12%	Hurtado-Ponce et al. 1997
bursapastoris	12.24%	Hoyle 1978
bursapastoris	2.72%	Hoyle 1978
bursapastoris	5.00%	Hoyle 1976
bursapastoris	8.30%	Hunt et al. 1979
chilensis	3.17%	Pickering et al. 1993
chilensis	4.60%	Santelices et al.1993
chilensis	7.00%	Troell et al. 1997
chorda	4.47%	Choi et al. 2006
cornea	5.70%	Causey et al. 1946
cornea	14.00%	Navarro- Angulo et al. 1999
coronopifolia	1.47%	Hoyle 1978
coronopifolia	6.41%	Hoyle 1978
coronopifolia	8.30%	Hunt et al. 1979
crassa	2.00%	Hoyle 1976
debilis	3.60%	Goldstein 1973b
edulis	7.55%	Nelson et al. 1980
edulis	5.10%	Gerung et al. 1999
firma	0.91%	Chirapart and Ohno 1993
fisheri	2.56%	Chirapart and Ohno 1993
foliifera	12.00%	DeBoer et al. 1978
foliifera	12.70%	Kim and Humm 1965
foliifera	12.00%	Kim and Humm 1965
sp.	10.00%	Tsai et al. 2005
gracilis	11.00%	Wakiba et al. 2001
lemaneiformis	13.90%	Yang et al. 2006
lemaneiformis	11.71%	Yang et al. 2006
lemaneiformis	11.03%	Zhou et al. 2006

Table 1.1 Maximum Growth Rates (Continued)

Gracilaria species	*Maximum Growth Rate (% d^{-1})*	*Reference*
parvispora	2.65%	Glenn et al. 1998
parvispora	4.60%	Nelson et al. 2001
parvispora	10.00%	Nagler et al. 2003
parvispora	8.90%	Ryder et al. 2004
parvispora	10.30%	Ryder et al. 2004
salicornia	0.86%	Chirapart and Ohno 1993
salicornia	4.90%	Gerung et al. 1999
salicornia	10.77%	Smith et al. 1994
tenuistipata	9.00%	Haglund and Pedersén 1993
tenuistipitata	7.00%	Hunt et al. 1979
tenuistipitata	3.30%	Wu et al. 1993
tenuistipitata var. liui	18.30%	Israel et al. 1999
tikvahiae	5.76%	Hanisak 1987
verrucosa	25.90%	Causey et al. 1946
verrucosa	4.45%	Chirapart and Ohno 1993
verrucosa	4.95%	Choi et al. 2006
verrucosa	5.33%	Jones 1959a
verrucosa	2.60%	Jones 1959b
verrucosa	8.85%	Kim et al. 1969
verrucosa	4.97%	Kim 1970
verrucosa	5.40%	Kim 1970
Mean	**7.37%**	
Standard Deviation	**± 0.05**	

Literature Cited:

Alwyn T, Rees V, Grant CM, Harmens HE, Taylor RB (1998) Measuring rates of
ammonium assimilation in marine algae: Use of the protonophore carbonyl
cyanide chlorophenylhydrazone to distinguish between uptake and assimilation.
Journal of Phycology 34: 264-272

Asare SO (1980) Animal waste as a nitrogen source for *Gracilaria tikvahiae* and
Neoagardhiella baileyi in culture. Aquaculture 21: 87-91

Beach K, Smith C, Michael T, Shin H (1995) Photosynthesis in reproductive unicells of
Ulva fasciata and *Enteromorpha flexuosa*: implications for ecological success.
Marine Ecology Progress Series 125: 229-237

Beach KS, Borgeas HB, Nishimura NJ, Smith CM (1997) *In vivo* absorbance spectra and
the ecophysiology of reef macroalgae. Coral Reefs 16: 21-28

Beach KS, Smith CM (1996) Ecophysiology of tropical rhodophytes. I. Microscale
acclimation in pigmentation. Journal of Phycology 32: 701-710

Beck AJ, Rapaglia JP, Cochran JK, Bokuniewicz HJ, Yang S (2008) Submarine
groundwater discharge to Great South Bay, NY, estimated using Ra isotopes.
Marine Chemistry 109: 279-291

Beer S, Axelsson L (2004) Limitations in the use of PAM fluorometry for measuring
photosynthetic rates of macroalgae at high irradiances. Journal of Phycology 39:
1-7

Beer S, Bjork M (2000) Measuring rates of photosynthesis of two tropical seagrasses by

pulse amplitude modulated (PAM) fluorometry. Aquatic Botany 66: 69-76

Beer S, Larsson C, Poryan O, Axelsson L (2000) Photosynthetic rates of *Ulva*

(Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry.

European Journal of Phycology 35: 69-74

Beer S, Mtolera M, Lyimo T, Bjork M (2006) The photosynthetic performance of the

tropical seagrass *Halophila ovalis* in the upper intertidal. Aquatic Botany 84: 367-

371

Beer SB, Vilenkin A, Weil M, Veste L, Eshel S (1998) Measuring photosynthetic rates in

seagrasses by pulse amplitude modulated (PAM) fluorometry. Marine Ecology

Progress Series 174: 293–300

Bird C, McLachlan J (1986) The effect of salinity on distribution of species of *Gracilaria*

Grev. (Rhodophyta, Gigartinales): An experimental assessment. Botanica Marina

29: 231-238

Bird KT, Habig C, DeBusk T (1982) Nitrogen allocation and storage patterns in

Gracilaria tikvahiae (Rhodophyta). Journal of Phycology 18: 344-348

Bird N, Chen L, McLachlan J (1979) Effects of temperature, light and salinity on growth

in culture of *Chondrus crispus, Furcellaria lumbricalis, Gracilaria tikvahiae*

(Gigartinales, Rhodophyta) and *Fucus serratus* (Fucales, Phaeophyta). Botanica

Marina 22: 521-527

Bonem R (1988) Effects of submarine karst development on reef succession Proceedings

of the 6th International Coral Reef Symposium, Australia, pp 419-423

Boucher G, Clavier J, Garrigue C (1994) Estimation of bottom ammonium affinity in the New Caledonia lagoon. Coral Reefs 13: 13-19

Boynton W, Kemp W, Keefe C (1982) A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. In: Kennedy VS (ed) <u>Estuarine Comparisons</u>. Academic Press, New York, pp 69-90

Bruce JR (1925) The metabolism of the shore-living dinoflagellates I.--salinity and carbon-assimilation, in *Amphidinium*. Journal of Experimental Biology 2: 413-426

Burnett WC, Bokuniewicz H, Huettel M, Moore WS, Taniguchi M (2003) Groundwater and pore water inputs to the coastal zone. Biogeochemistry 66: 3-33

Burnett WC, Peterson R, Moore WS, De Oliveira J (2008) Radon and radium isotopes as tracers of submarine groundwater discharge - Results from the Ubatuba, Brazil SGD assessment intercomparison. Estuarine, Coastal and Shelf Science 76: 501-511

Byrne K, Zuccarello GC, West J, Liao M-L, Kraft GT (2002) *Gracilaria* species (Gracilariaceae, Rhodophyta) from southeastern Australia, including a new species, *Gracilaria perplexa* sp. nov.: Morphology, molecular relationships and agar content. Phycological Research 50: 295-311

Cable JE, Burnett WC, Chanton JP, Weatherly GL (1996) Estimating groundwater discharge into the northeastern Gulf of Mexico using radon-222. Earth and Planetary Science Letters 144: 591-604

Cable JE, Martin JB (2008) *In situ* evaluation of nearshore marine and fresh pore water

    transport into Flamengo Bay, Brazil. Estuarine, Coastal and Shelf Science 76:

    473-483

Caldwell MM, Teramura AH, Tevini M (1989) Changing solar ultraviolet climate and the

    ecological consequences for higher plants. Trends in Evolution and Ecology 4:

    363-366

Capone D, Dunham S, Horrigan S, Duguay L (1992) Microbial nitrogen transformations

    in unconsolidated coral reef sediments. Marine Ecology Progress Series 80: 75-88

Capone DG, Bautista MF (1985) A groundwater source of nitrate in nearshore marine

    sediments. Nature 313: 214-216

Carnicas E, Jimenez C, Niell FX (1999) Effects of changes of irradiance on the pigment

    composition of *Gracilaria tenuistipitata* var. *liui* Zhang et Xia. Journal of

    Photochemistry and Photobiology B: Biology 50: 149-158

Carr H, Bjork M (2003) A methodological comparison of photosynthetic oxygen

    evolution and estimated electron transport rate in tropical *Ulva* (Chlorophyceae)

    species under different light and inorganic carbon conditions. Journal of

    Phycology 39: 1125-1131

Carr M-E, Friedrichs MAM, Schmeltz M, Noguchi Aita M, Antoine D, Arrigo KR,

    Asanuma I, Aumont O, Barber R, Behrenfeld M, Bidigare R, Buitenhuis ET,

    Campbell J, Ciotti A, Dierssen H, Dowell M, Dunne J, Esaias W, Gentili B,

    Gregg W, Groom S, Hoepffner N, Ishizaka J, Kameda T, Le Quéré C, Lohrenz S,

    Marra J, Mélin F, Moore K, Morel A, Reddy TE, Ryan J, Scardi M, Smyth T,

    Turpie K, Tilstone G, Waters K, Yamanaka Y (2006) A comparison of global

estimates of marine primary production from ocean color. Deep Sea Research Part II: Topical Studies in Oceanography 53: 741-770

Carruthers T, van Tussenbroek B, Dennison W (2005) Influence of submarine springs and wastewater on nutrient dynamics of Caribbean seagrass meadows. Estuarine, Coastal and Shelf Science 64: 191-199

Causey NB, Prytherch JP, McCaskill J, Humm HJ, Wolf FA (1946) Influence of environmental factors upon the growth of *Gracilaria confervoides*. Bulletin of Duke University Marine Station 3: 19-24

Charpy-Roubaud C, Sournia A (1990) The comparative estimation of phytoplanktonic, microphytobenthic and macrophytobenthic primary production in the oceans. Marine Microbial Food Webs 4: 31–57

Charpy L, Charpy-Roubaud C (1990) A model of the relationship between light and primary production in an atoll lagoon. Journal of the Marine Biology Association of the United Kingdom 70: 357-369

Chirapart A, Ohno, M. (1993) Growth in tank culture of species of *Gracilaria* from the southeast Asian waters. Botanica Marina 36: 9-13

Choi H, Kim Y, Kim J, Lee S, Park E, Ryu J, Nam K (2006) Effects of temperature and salinity on the growth of *Gracilaria verrucosa* and *G. chorda*, with the potential for mariculture in Korea. Journal of Applied Phycology 18: 269-277

D'Elia C, DeBoer J (1978) Nutritional studies of two red algae [*Neoagardhiella baileyi, Gracilaria foliifera*]. II. Kinetics of ammonium and nitrate uptake. Journal of Phycology 14: 266-272

D'Elia C, Webb K, Porter J (1981) Nitrate-rich groundwater inputs to Discovery Bay, Jamaica: A significant source of N to local coral reefs? Bulletin of Marine Science 31: 903-910

Dawes CJ, Chen CP, Jewett-Smith J, Marsh A, Watts SA (1984) Effect of phosphate and ammonium levels on photosynthetic and respiratory responses of the red alga *Gracilaria verrucosa*. Marine Biology 78: 325-328

Dawes CJ, Orduña-rojas J, Robledo D (1999) Response of the tropical red seaweed *Gracilaria cornea* to temperature, salinity and irradiance. Journal of Applied Phycology 10: 419-425

DeBoer JA, Guigli HJ, Israel TL, D'Elia CF (1978) Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. Journal of Phycology 14: 261-266

Dortch Q (1990) The interaction between ammonium and nitrate uptake in phytoplankton. Marine Ecology Progress Series 61: 183-201

Duarte CM (1992) Nutrient concentration of aquatic plants: Patterns Across Species. Limnology and Oceanography 37: 882-889

Edwards DM, Reed RH, Stewart WDP (1988) Osmoacclimation in *Enteromorpha intestinalis*: long-term effects of osmotic stress on organic solute accumulation. Marine Biology 98: 467-476

Eppley R (1980) Estimating phytoplankton growth rates in the central oligotrophic oceans. Primary Productivity in the Sea 19: 231–240

Falkowski P (1983) Light-shade adaptation and vertical mixing of marine phytoplankton: a comparative field study. Journal of Marine Research 41: 215-237

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the
biosphere: Integrating terrestrial and oceanic components. Science 281: 237-240

Follmi K (1996) The phosphorus cycle, phosphogenesis and marine phosphate-rich
deposits. Earth Science Reviews 40: 55-124

Fong P, Zedler JB, Donohoe RM (1993) Nitrogen vs. phosphorus limitation of algal
biomass in shallow coastal lagoons. Limnology and Oceanography 38: 906-923

Franklin LA, Badger MR (2001) A comparison of photosynthetic electron transport rates
in macroalgae measured by pulse amplitude modulated chlorophyll fluorometry
and mass spectrometry. Journal of Phycology 37: 756-767

Friedlander M (1992) *Gracilaria conferta* and its epiphytes: The effect of culture
conditions on growth. Botanica Marina 35: 423-428

Friedlander M, Ben-Amotz A (1991) The effect of outdoor culture conditions on growth
and epiphytes of *Gracilaria conferta*. Aquatic Botany 39: 315-333

Friedlander M, Dawes CJ (1985) In situ uptake kinetics of ammonium and phosphate and
chemical composition of the red seaweed *Gracilaria tikvahiae*. Journal of
Phycology 21: 448-453

Friedlander M, Levy I (1995) Cultivation of *Gracilaria* in outdoor tanks and ponds.
Journal of Applied Phycology 7: 315-324

Garcia-Sanchez MJ, Fernandez JA, Xavier NF (1996) Photosynthetic response of P-
deficient *Gracilaria tenuistipitata* under two different phosphate treatments.
Physiologia Plantarum 96: 601-606

Garrison GH, Glenn CR, McMurtry GM (2003) Measurement of submarine groundwater

    discharge in Kahana Bay, Oʻahu, Hawaiʻi. Limnology and Oceanography 48:

    920-928

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of

    photosynthetic electron transport and quenching of chlorophyll fluorescence.

    Biochemistry and Biophysics Acta 990: 87-92

Gerung GS, Ohno M, Yamamoto H (1999) Growth rates and agar properties on some

    species of *Gracilaria Grev*. (Rhodophyta, Gigartinales) from Manado, Indonesia.

    Bulletin of Marine Sciences and Fisheries Kochi University: 9-14

Gessner F, Schramm W (1971) Salinity. Marine Ecology 1: 705-820

Giblin A, Gaines A (1990) Nitrogen inputs to a marine embayment: The importance of

    groundwater. Biodegradation 10: 309-328

Glenn EP, Moore D, Brown JJ, Tanner R, Fitzsimmons K, Akutigawa M, Napolean S

    (1998) A sustainable culture system for *Gracilaria parvispora* (Rhodophyta)

    using sporelings, reef growout and floating cages in Hawaiʻi. Aquaculture 165:

    221-232

Goldman JC, McCarthy JJ, Peavey DG (1979) Growth rate influence on the chemical

    composition of phytoplankton in oceanic waters. Nature 279: 210-215

Goldstein ME (1973) The reproductive biology and mariculture of *Gracilaria*. In

    Summary record of agar and agarophytes workshop. Atlantic Regional Office

    Fisheries Research Board of Canada., pp 34-41

Gomez I, Figueroa FL, Huovinen P, Ulloa N, Morales V (2005) Photosynthesis of the red

   alga *Gracilaria chilensis* under natural solar radiation in an estuary in southern

   Chile. Aquaculture 244: 369-382

Gonen Y, Kimmel E, Friedlander M (1995) Diffusion boundary layer transport in

   Gracilaria conferta (Rhodophyta). Journal of Phycology 31: 768-773

González FUT, Herrera-Silveira JA, Aguirre-Macedo ML (2008) Water quality

   variability and eutrophic trends in karstic tropical coastal lagoons of the Yucatán

   Peninsula. Estuarine, Coastal and Shelf Science 76: 418-430

Granbom M, Lopes PF, Pedersen M, Colepicolo P (2007) Nitrate reductase in the marine

   macroalga *Kappaphycus alvarezii* (Rhodophyta): oscillation due to the protein

   level. Botanica Marina 50: 106-112

Grossman E, Logan J, Street J, Paytan A, Chavez P (2008) Ground Water and its

   Influence on Reef Evolution. In: Field ME, Cochran, S.A., Logan, J.B., and

   Storlazzi C.D., (ed) The coral reef of south Molokaʻi, Hawaiʻi; portrait of a

   sediment-threatened fringing reef. U.S. Geological Survey Scientific

   Investigations pp 111-116

Häder D-P, Lebert M, Jimenez C, Salles S, Aguilera J, Flores-Moya A, Mercado J,

   Vinegla BL, Figueroa F (1999) Pulse amplitude modulated fluorescence in the

   green macrophytes, *Codium adherens*, *Enteromorpha muscoides*, *Ulva gigantea*

   and *Ulva rigida*, from the Atlantic coast of southern Spain. Environmental and

   Experimental Botany 41: 247-255

Häder D (1993) Risks of enhanced solar ultraviolet radiation for aquatic ecosystems.

   Progress in Phycological Research 9: 1-45

Häder DP, Herrmann H, Schafer J, Santas R (1997) Photosynthetic fluorescence induction and oxygen production in two Mediterranean *Cladophora* species measured on site. Aquatic Botany 56: 253-264

Haglund K, Pedersén M (1993) Outdoor pond cultivation of the subtropical marine red alga *Gracilaria tenuistipitata* in brackish water in Sweden. Growth, nutrient uptake, co-cultivation with rainbow trout and epiphyte control. Journal of Applied Phycology 5: 271-284

Hamner W, Wolanski E (1988) Hydrodynamic forcing functions and biological processes on coral reefs: A status review Proceedings of the Sixth International Coral Reef Symposium, pp 103-113

Hanelt D, Li J, Nultsch W (1994) Tidal dependence of photoinhibition of photosynthesis in marine macrophytes of the South China Sea. Botanica Acta 107: 66-72

Hanisak MD (1983) The nitrogen relationships of marine macroalgae. In: Carpenter EJ, and D. G. Capone (ed) *Nitrogen in the Marine Environment*. Academic Press, New York, pp 699-730

Hanisak MD, Ryther JH (1984) Cultivation biology of *Gracilaria tikvahiae* in the United States. Hydrobiologia 116: 295-298

Hansen LA, Alongi DM, Moriarty DJW, Pollard PC (1987) The dynamics of benthic microbial communities at Davies Reef, central Great Barrier Reef. Coral Reefs 6: 63-70

Hatcher BG (1990) Coral reef primary productivity. A hierarchy of pattern and process. Trends in Ecology & Evolution 5: 149-155

Hawes I, Sutherland D, Hanelt D (2003) The use of pulse amplitude modulated
fluorometry to determine fine-scale temporal and spatial variation of *in situ*
photosynthetic activity within an *Isoetes*-dominated canopy. Aquatic Botany 77:
1-15

Herrera-Silveira J (1998) Nutrient-phytoplankton production relationships in a
groundwater-influenced tropical coastal lagoon. Aquatic Ecosystem Health &
Management 1: 373-385

Herrera-Silveira JA, Medina-Gomez I, Colli R (2002) Trophic status based on nutrient
concentration scales and primary producers community of tropical coastal lagoons
influenced by groundwater discharges. Hydrobiologia 475: 91-98

Herrera-Silveira JA, Morales-Ojeda SM (2009) Evaluation of the health status of a
coastal ecosystem in southeast Mexico: Assessment of water quality,
phytoplankton and submerged aquatic vegetation. Marine Pollution Bulletin 59:
72-86

Herrera-Silveira JA, Ramírez RJ, Zaldivar RA (1998) Overview and characterization of
the hydrology and primary producer communities of selected coastal lagoons of
Yucatán, México. Aquatic Ecosystem Health and Management 1: 353-372

Howarth RW (1988) Nutrient limitation of net primary production in marine ecosystems.
Annual Review of Ecology and Systematics 19: 89-110

Hoyle MD (1975) The literature pertinent to the red algal genus *Gracilaria* in Hawaiʻi.
Technical Report 3: 340

Hoyle MD (1976) Autecology of ogo (*Gracilaria bursapastoris*) and limu manauea (*G. coronopifolia*) in Hawaiʻi with special emphasis on *Gracilaria* species as indicators of sewage pollution. Ph. D. Botany, Honolulu

Hoyle MD (1978) Reproductive phenology and growth rates in two species of *Gracilaria* from Hawaiʻi. Journal of Experimental Marine Biology and Ecology 35: 273-283

Hunt JW, Windward Community College. Marine Option Program. (1979) Cooperative *Gracilaria* project : environmental factors affecting the growth of *Gracilaria bursapastoris* (ogo) and *Gracilaria coronopifolia* (limu manauea). Marine Option Program, Windward Community College, Kaneohe

Hurtado-Ponce AQ, Samonte GP, Luhan MR, Guanzon N (1992) *Gracilaria* (Rhodophyta) farming in Panay, Western Visayas, Philippines. Aquaculture 105: 233-240

Hwang D-W, Kim G, Lee Y-W, Yang H-S (2005) Estimating submarine inputs of groundwater and nutrients to a coastal bay using radium isotopes. Marine Chemistry 96: 61-71

Inokuchi R, Kuma K, Miyata T, Okada M (2002) Nitrogen-assimilating enzymes in land plants and algae: phylogenic and physiological perspectives. Physiologia Plantarum 116: 1-11

Israel A, Martinez-Goss M, Friedlander M (1999) Effect of salinity and pH on growth and agar yield of *Gracilaria tenuistipitata* var. *liui* in laboratory and outdoor cultivation. Journal of Applied Phycology 11: 543-549

Jenkins W (1982) Oxygen utilization rates in North Atlantic subtropical gyre and primary production in oligotrophic systems. Nature 300: 246-248

Johannes RE (1980) The ecological significance of the submarine discharge of

    groundwater. Marine Ecology Progress Series 3: 365-373

Johnson AG, Glenn CR, Burnett WC, Peterson RN, Lucey PG (2008) Aerial infrared

    imaging reveals large nutrient-rich groundwater inputs to the ocean. Geophysical

    Research Letters 35

Jones AB, Dennison WC, Stewart GR (1996) Macroalgal responses to nitrogen source

    and availability: amino acid metabolic profiling as a bioindicator using *Gracilaria*

    edulis (Rhodophyta). Journal of Phycology 32: 757-766

Jones WE (1959a) Experiments on some effects of certain environmental factors on

    Gracilaria verrucosa (Hudson) Papenfuss. Journal of the Marine Biological

    Association of the United Kingdom 38: 153-167

Jones WE (1959b) The growth and fruiting of *Graciliaria verrucosa* (Hudson) Papenfuss.

    Journal of the Marine Biological Association of the United Kingdom 38: 47-56

Jordan T, Cornwell J, Boynton W, Anderson J (2008) Changes in phosphorus

    biogeochemistry along an estuarine salinity gradient: The iron conveyer belt.

    Limnology and Oceanography 53: 172-184 is this 1 pg or missing additional pgs?

Kain J, Norton T, Cole K, Sheath R (1990) Biology of the red algae. Cambridge

    University Press Cambridge

Kakita H, Kamishima H (2006) Effects of environmental factors and metal ions on

    growth of the red alga *Gracilaria chorda* (Holmes) (Gracilariales, Rhodophyta).

    Journal of Applied Phycology 18: 469-474

Kalle K (1971) Salinity: general introduction. In: Kinne O (ed) Marine Ecology. Wiley-

    Interscience, New York, pp 683-688

Kamer K, Fong P (2000) A fluctuating salinity regime mitigates the negative effects of

    reduced salinity on the estuarine macroalga, *Enteromorpha intestinalis*. Journal of

    Experimental Marine Biology and Ecology 254: 53-69

Karsten U, Kirst G (1989) The effect of salinity on growth, photosynthesis and

    respiration in the estuarine red alga *Bostrychia radicans* Mont. Helgoland Marine

    Research 43: 61-66

Kay E, Lau L, Stroup E, Dollar S, Fellows D, Young R (1977) Hydrologic and ecologic

    inventories of the coastal waters of West Hawaiʻi. University of Hawaiʻi,

    Honolulu

Kim CS, Humm, HJ (1965) The red alga, *Gracilaria foliifera* with special reference to

    cell wall polysaccharides. Bulletin of Marine Science 15: 1036-1050

Kim DH (1970) Economically important seaweeds in Chile - I *Gracilaria*. Botanica

    Marina 13: 140-162

Kim DH, Mendoza H, Wilkomirsky W, Ohme U, Overdick L, Espinoza R (1969)

    Resultados preliminares sobre crecimiento de *Gracilaria lemanaeformis* y

    contribucion a la industrializacion de esta alga y otras agarofitas In Hoyle 1975,

    pg. 220

Kirst GO (1989) Salinity tolerance of eukaryotic marine algae. Annual Review of Plant

    Physiology and Plant Molecular Biology 41: 21-53

Kirst GO (1995) Osmotic adjustment in phytoplankton and macroalgae. In: Kiene P,

    Visscher PT, Keller D, Kirst GO (eds) <u>Biological and Environmental Chemistry</u>

    <u>of [DMSP] and Related Sulfonium Compounds.</u> Plenum Press, New York, pp

    121–129

Koch E, Lawrence J (1987) Photosynthetic and respiratory responses to salinity changes in the red alga *Gracilaria verrucosa*. Botanica Marina 30: 327-329

Kohout F (1966) Submarine springs: A neglected phenomenon of coastal hydrology. Hydrology 26: 391-413

Kohout F, Kolipinski M (1967) Biological zonation related to groundwater discharge along the shore of Biscayne Bay, Miami, Florida. In: Lauff G (ed) <u>Estuaries. Advanced Science</u>, Washington, DC, Jekyll Island, GA, pp 488-499

Krest J, Moore W, Gardner L, Morris J (2000) Marsh nutrient export supplied by groundwater discharge: Evidence from radium measurements. Global Biogeochemical Cycles 14: 167-176

Krishnamurthy V (1954) Ecology and seasonal succession of the algal flora of a salt marsh at Madras. Madras University 24: 161-178

Lapointe BE (1981) The effects of light and nitrogen on growth, pigment content, and biochemical composition of *Gracilaria foliifera* v. *angustissima* (Gigartinales, Rhodophyta). Journal of Phycology 7: 90-95

Lapointe BE (1985) Strategies for pulsed nutrient supply to *Gracilaria* cultures in the Florida Keys: Interactions between concentration and frequency of nutrient pulses. Journal of Experimental Marine Biology and Ecology 93: 211-222

Lapointe BE (1987) Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. Marine Biology 93: 561-568

Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on

    coral reefs in Jamaica and southeast Florida. Limnology and Oceanography 42:

    1119-1131

Lapointe BE, Littler MM, Littler DS (1987) A comparison of nutrient-limited

    productivity in macroalgae from a Caribbean barrier reef and from a mangrove

    ecosystem. Aquatic Botany 28: 243-255

Lapointe BE, Littler MM, Littler DS (1992) Nutrient availability to marine macroalgae in

    siliciclastic versus carbonate-rich coastal waters. Estuaries 15: 75-82

Lapointe BE, Littler MM, Littler DS (1993) Modification of benthic community structure

    by natural eutrophication: the Belize barrier reef Proceedings of the Seventh

    International Coral Reef Symposium, pp 323-334

Lapointe BE, O'Connell J (1989) Nutrient-enhanced growth of *Cladophora prolifera* in

    Harrington Sound, Bermuda - Eutrophication of a confined, phosphorus-limited

    marine ecosystem. Estuarine Coastal and Shelf Science 28: 347-360

Larned ST (1997) Nutrient-limited growth and sources of nutrients for coral reef

    macroalgae. Ph.D. Botany, Honolulu

Larned ST (1998) Nitrogen- versus phosphorus-limited growth and sources of nutrients

    for coral reef macroalgae. Marine Biology 132: 409-421

Lavery P, McComb A (1991) Macroalgal-sediment nutrient interactions and their

    importance to macroalgal nutrition in a eutrophic estuary. Estuarine, Coastal and

    Shelf Science 32: 281-295

Laws E, DiTullio G, Redalje D (1987) High phytoplankton growth and production rates

    in the North Pacific subtropical gyre. Limnology and Oceanography: 905-918

Laws EA, Redalje DG, Haas LW, Bienfang PK, Eppley RW, Harrison WG, Karl DM, Marra J (1984) High phytoplankton growth and production rates in oligotrophic Hawaiian coastal waters. Limnology and Oceanography 29: 1161-1169

Lee D (1977) A device for measuring seepage flux in lakes and estuaries. Limnology and Oceanography 22: 140-147

Lewis J (1977) Processes of organic production on coral reefs. Biological Reviews 52: 305-347

Lewis J (1987) Measurements of groundwater seepage flux onto a coral reef: spatial and temporal variations. Limnology and Oceanography: 1165-1169

Li L, Barry DA, Stagnitti F, Parlange JY (1999) Submarine groundwater discharge and associated chemical input to a coastal sea. Water Resources Research 35: 3253–3259

Liebig J (1840) Die organische chemie in ihrer anwendung auf agricultur und physiologie. Vieweg, Braunschweig

Lillo C (1984) Circadian rhythmicity of nitrate reductase activity in barley leaves. Physiologia Plantarum 61: 219-223

Lillo C, Provan F, Appenroth KJ (1998) Photoreceptors involved in the regulation of nitrate reductase in *Spirodela polyrhiza*. Journal of Photochemistry and Photobiology B: Biology 44: 205-210

Littler MM, Littler DS, Titlyanov EA (1991) Comparisons of N-limited and P-limited productivity between high granitic islands versus low carbonate atolls in the Seychelles archipelago - a test of the relative-dominance paradigm. Coral Reefs 10: 199-209

Liu J, Dong S (2001) Comparative studies on utilizing nitrogen capacity between two macroalgae *Gracilaria tenuistipitata* var. *liui* (Rhodophyta) and *Ulva pertusa* (Chlorophyta): II. Feedback control of intracellular nitrogen pools on nitrogen uptake. Journal of Environmental Sciences (China) 13: 323-327

Lobban C, Harrison P (1994) <u>Seaweed ecology and physiology</u>. Cambridge University Press

Longstaff B, Kildea T, Runcie J, Cheshire A, Dennison W, Hurd C, Kana T, Raven J, Larkum A (2002) An *in situ* study of photosynthetic oxygen exchange and electron transport rate in the marine macroalga *Ulva lactuca* (Chlorophyta). Photosynthesis Research 74: 281-293

Lopes PF, Oliveira MC, Colepicolo P (1997) Diurnal fluctuation of nitrate reductase activity in the marine red alga *Gracilaria tenuistipitata* (Rhodophyta). Journal of Phycology 33: 225-231

Marsh JA (1977) Terrestrial inputs of nitrogen and phosphorus on fringing reefs of Guam. In: Taylor DL (ed) <u>Proceedings of the Third International Coral Reef Symposium,</u> pp 331-336

Martin J, Cable J, Jaeger J, Hartl K, Smith C (2006) Thermal and chemical evidence for rapid water exchange across the sediment-water interface by bioirrigation in the Indian River Lagoon, Florida. Limnology and Oceanography 51: 1332-1341

Martin J, Cable J, Smith C, Roy M, Cherrier J (2007) Magnitudes of submarine groundwater discharge from marine and terrestrial sources: Indian River Lagoon, Florida. Water Resources Research 43: W05440

Martin J, Knauer G, Karl D, Broenkow W (1987) VERTEX: carbon cycling in the

northeast Pacific. Deep-sea research. Part A. Oceanographic research papers 34:

267-285

McAllister CD, Parsons TR, Stephens K, Strickland JDH (1961) Measurements of

primary production in coastal sea water using a large-volume plastic sphere.

Limnology and Oceanography 6: 237-258

McCoy CA, Corbett DR (2009) Review of submarine groundwater discharge (SGD) in

coastal zones of the Southeast and Gulf Coast regions of the United States with

management implications. Journal of Environmental Management 90: 644-651

McCoy CA, Corbett DR, Cable JE, Spruill RK (2007) Hydrogeological characterization

of southeast coastal plain aquifers and groundwater discharge to Onslow Bay,

North Carolina (USA). Journal of Hydrology 339: 159-171

McGlathery KJ (1992) Physiological controls on the distribution of the macroalga

Spyridea hypnoides - patterns along a eutrophication gradient in Bermuda. Marine

Ecology Progress Series 87: 173-182

Mercado JM, Sanchez P, Carmona R, Niell FX (2002) Limited acclimation of

photosynthesis to blue light in the seaweed *Gracilaria tenuistipitata*. Physiologia

Plantarum 114: 491-498

Moore C (1979) The zonation of psammolittoral harpacticoid copepods around the Isle of

Man. Journal of the Marine Biological Association of the United Kingdom 59:

711-724

Moore W, Krest J, Taylor G, Roggenstein E, Joye S, Lee R (2002) Thermal evidence of

    water exchange through a coastal aquifer: Implications for nutrient fluxes.

    Geophysical Research Letters 29: 49-41

Murthy MSM, Sharma CLNS Rao YN (1988) Salinity induced changes in peroxidase

    activity in the great seaweed *Ulva lactuca*. Botanica Marina 31: 307–310

Nagler PL, Glenn EP, Nelson SG, Napolean S (2003) Effects of fertilization treatment

    and stocking density on the growth and production of the economic seaweed

    Gracilaria parvispora (Rhodophyta) in cage culture at Molokai, Hawai'i.

    Aquaculture 219: 379-391

Naldi M, Wheeler P (2002) ^{15}N measurements of ammonium and nitrate uptake by *Ulva*

    fenestrata (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta): comparison of

    net nutrient disappearance, release of ammonium and nitrate, and [15]N

    accumulation in algal tissue. Journal of Phycology 38: 135-144

Navarro-Angulo L, Robledo D (1999) Effects of nitrogen source, N:P ratio and N-pulse

    concentration and frequency on the growth of *Gracilaria cornea* (Gracilariales,

    Rhodophyta) in culture. Hydrobiologia 398: 315-320

Nelson SG, Glenn EP, Conn J, Moore D, Walsh T, Akutagawa M (2001) Cultivation of

    Gracilaria parvispora (Rhodophyta) in shrimp-farm effluent ditches and floating

    cages in Hawai'i: a two-phase polyculture system. Aquaculture 193: 239-248

Nelson SG, Tsutsui RN, Best BR (1980) Evaluation of seaweed mariculture potential of

    Guam I. Ammonium uptake by and growth of 2 species of *Gracilaria*

    (Rhodophyta), 0379-5403, University of Guam

Odum E (1971) <u>Fundamentals of ecology</u>. WB Saunders, Philadelphia

Oki N, Dimeyer P (1999) Assessment of land surface models by runoff in major river basins of the globe using Total Runoff Integrating Pathways (TRIP). Journal of the Meteorological Society of Japan 77: 235-255

Paerl HW (1997) Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnology and Oceanography 42: 1154-1165

Paytan A, Shellenbarger G, Street J, Gonneea M, Davis K, Young M, Moore W (2006) Submarine groundwater discharge: An important source of new inorganic nitrogen to coral reef ecosystems. Limnology and Oceanography 51: 343-348

Pickering TD, Gordon ME, Tong LJ (1993) Effect of nutrient pulse concentration and frequency on growth of *Gracilaria chilensis* plants and levels of epiphytic algae. Journal of Applied Phycology 5: 525-533

Potie L, Tardieu B (1977) Development of submarine springs. In: Karst Hydrogeology. University of Alabama at Huntsville Press, Huntsville, Alabama

Powell G, Kenworthy W, Fourqurean J (1989) Experimental evidence for nutrient limitation of seagrass growth in a tropical estuary with restricted circulation. Bulletin of Marine Science 44

Prince J (1974) Nutrient assimilation and growth of some seaweeds in mixtures of seawater and secondary sewage treatment effluents. Aquaculture 4: 69-79

Ralph PJ, Gademann R (2005) Rapid light curves: A powerful tool to assess photosynthetic activity. Aquatic Botany 82: 222-237

Ralph PJ, Gademann R, Dennison WC (1998) *In situ* seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. Marine Biology 132: 367-373

Ramalho CB, Hastings JW, Colepicolo P (1995) Circadian oscillation of nitrate reductase activity in *Gonyaulax polyedra* is due to changes in cellular protein levels. Plant Physiology 107: 225-231

Ramus J, Beale S, Mauzerall D (1976a) Changes in photosynthetic pigment concentration in seaweeds as a function of water depth. Marine Biology 37: 223-229

Ramus J, Beale S, Mauzerall D (1976b) Correlation of changes in pigment content with photosynthetic capacity of seaweeds as a function of water depth. Marine Biology 37: 231-238

Ramus J, Lemons F, Zimmerman C (1977) Adaptation of light-harvesting pigments to downwelling light and the consequent photosynthetic performance of the eulittoral rockweeds *Ascophyllurn nodosum* and *Fucus vesiculosus*. Marine Biology 42: 293-303

Redfield A (1958) The biological control of chemical factors in the environment. American Scientist 46: 205–221

Reed R (1990) Solute accumulation and osmotic adjustment. In: Cole KM, Sheath RG (eds) <u>Biology of the Red Algae.</u> Cambridge University Press, Cambridge, pp 147–170

Reiskind JB, Beer S, Bowes G (1989) Photosynthesis, photorespiration and ecophysiological interactions in marine macroalgae. Aquatic Botany 34: 131-152

Rosenberg G, Ramus J (1982) Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva sp.* (Chlorophyceae): photosynthesis and antenna composition. Marine Ecology Progress Series 8: 233-241

Ryder E, Nelson SG, McKeon C, Glenn EP, Fitzsimmons K, Napolean S (2004) Effect of water motion on the cultivation of the economic seaweed *Gracilaria parvispora* (Rhodophyta) on Molokai, Hawai'i. Aquaculture 238: 207-219

Ryther JH, Corwin N, DeBusk TA, Williams LD (1981) Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). Aquaculture 26: 107-115

Sanders J (1979) The importance of salinity in determining the morphology and composition of algal mats. Botanica Marina 22: 159-162

Santelices B, Westermeier R, Bobadilla M (1993) Effect of water motion on the cultivation of the economic seaweed *Gracilaria parvispora* (Rhodophyta) on Molokai, Hawai'i. Journal of Applied Phycology 5: 517-524

Saroussi S, Beer S (2007) Alpha and quantum yield of aquatic plants derived from PAM fluorometry: Uses and misuses. Aquatic Botany 86: 89-92

Schreiber U (2004) Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: An overview. In: Papageorgiou G, Govindjee (eds) Chlorophyll a fluorescence: A Signature of photosynthesis; advances in photosynthesis and respiration series. Kluwer Academic Dordrecht, pp 279

Sheldon R, Sutcliffe W (1978) Generation times of 3 h for Sargasso Sea microplankton determined by ATP analysis. Limnology and Oceanography: 1051-1055

Shellenbarger G, Monismith S, Genin A, Paytan A (2006) The importance of submarine

    groundwater discharge to the nearshore nutrient supply in the Gulf of Aqaba

    (Israel). Limnology Oceanography 51: 1876-1886

Smith JE, Hunter CL, Conklin EJ, Most R, Sauvage T, Squair C, Smith CM (2004)

    Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on Oʻahu,

    Hawaiʻi. Pacific Science 58: 325-343

Smith JE, Runcie JW, Smith CM (2005) Characterization of a large-scale ephemeral

    bloom of the green alga *Cladophora sericea* on the coral reefs of West Maui,

    Hawaiʻi. Marine Ecology Progress Series 302: 77-91

Smith R (1955) Salinity variation in interstitial water of sand at Kames Bay, Millport,

    with reference to the distribution of *Nereis diversicolor*. Journal of the Marine

    Biological Association of the United Kingdom 34: 33-46

Smith S, Hollibaugh J (1993) Coastal metabolism and the oceanic organic carbon cycle.

    Reviews of Geophysics 31: 75–89

Solomonson LP, Barber MJ (1990) Assimilatory nitrate reductase: Functional properties

    and regulation. Annual Review of Plant Physiology and Plant Molecular Biology

    41: 225-253

Stimson J, Larned S, McDermid K (1996) Seasonal growth of the coral reef macroalga

    Dictyosphaeria cavernosa (Forskål) Børgesen and the effects of nutrient

    availability, temperature and herbivory on growth rate. Journal of Experimental

    Marine Biology and Ecology 196: 53-77

Sutcliffe WH (1972) Some relations of land drainage, nutrients, particulate material, and
fish catch in two eastern Canadian bays. Journal of the Fisheries Research Board
of Canada 29: 357-362

Thompson C, Smith L, Maji R (2007) Hydrogeological modeling of submarine
groundwater discharge on the continental shelf of Louisiana. Journal of
Geophysical Research 112

Townsend SA, J. H. Schult M. M. Douglas S. Skinner (2008) Does the Redfield ratio
infer nutrient limitation in the macroalga *Spirogyra fluviatilis*? Freshwater
Biology 53: 509-520

Troell M, Halling C, Nilsson A, Buschmann AH, Kautsky N, Kautsky L (1997)
Integrated marine cultivation of *Gracilaria chilensis* (Gracilariales, Rhodophyta)
and salmon cages for reduced environmental impact and increased economic
output. Aquaculture 156: 45-61

Tsai C-C, Chang J-S, Sheu F, Shyu Y-T, Yu AY-C, Wong S-L, Dai C-F, Lee T-M (2005)
Seasonal growth dynamics of *Laurencia papillosa* and *Gracilaria coronopifolia*
from a highly eutrophic reef in southern Taiwan: temperature limitation and
nutrient availability. Journal of Experimental Marine Biology and Ecology 315:
49-69

Tseng C (2001) Algal biotechnology industries and research activities in China. Journal
of Applied Phycology 13: 375-380

Tyler A, McGlathery K (2006) Uptake and release of nitrogen by the macroalgae
Gracilaria vermiculophylla (Rhodophyta). Journal of Phycology 42: 515-525

Tyrrell T (1999) The relative influences of nitrogen and phosphorus on oceanic primary
    production. Nature 400: 525-531

Valiela I (1984) Marine ecological processes. Springer-Verlag, New York, pp 346

Valiela I (1995) Marine ecological processes. Second edition. Springer-Verlag, New
    York, pp 700

Valiela I, Costa J, Foreman K, Teal JM, Howes B, Aubrey D (1990) Transport of
    groundwater-borne nutrients from watersheds and their effects on coastal waters.
    Biogeochemistry 10: 177-197

Vince S, Valiela I (1973) The effects of ammonium and phosphate enrichments on
    chlorophyll a, pigment ratio and species composition of phytoplankton of
    Vineyard Sound. Marine Biology 19: 69-73

Wakibia JG, Anderson RJ, Keats DW (2001) Growth rates and agar properties of three
    gracilarioids in suspended open-water cultivation in St. Helena Bay, South Africa.
    Journal of Applied Phycology 13: 195-207

Williams S, Carpenter R (1988) Nitrogen-limited primary productivity of coral reef algal
    turfs: potential contribution of ammonium excreted by *Diadema antillarum*.
    Marine Ecology Progress Series 47: 145-152

Wilson A (2005) Fresh and saline groundwater discharge to the ocean: a regional
    perspective. Water Resources Research 41: W02016-W02016.02011

Wu CY, Li RZ, Lin GH, Wen ZC, Dong LF, Zhang JP, Huang XH, Wei SQ, Lan GB
    (1993) Some aspects of the growth of *Gracilaria tenuisitipitata* in pond culture.
    Hydrobiologia 261: 339-343

Yang Y-F, Fei X-G, Song J-M, Hu H-Y, Wang G-C, Chung IK (2006) Growth of
Gracilaria lemaneiformis under different cultivation conditions and its effects on
nutrient removal in Chinese coastal waters. Aquaculture 254: 248-255

Yokoya N, De-Oliveira E (1992) Effects of salinity on the growth rate, morphology and
water content of some Brazilian red algae of economic importance. Ciencias
Marinas 18: 49

Yokoya NS, Kakita H, Obika H, Kitamura T (1999) Effects of environmental factors and
plant growth regulators on growth of the red alga *Gracilaria vermiculophylla*
from Shikoku Island, Japan. Hydrobiologia 398/399: 339-347

Yu J, Yang Y-F (2008) Physiological and biochemical response of seaweed *Gracilaria
lemaneiformis* to concentration changes of N and P. Journal of Experimental
Marine Biology and Ecology 367: 142-148

Zhou Y, Yang H, Hu H, Liu Y, Mao Y, Zhou H, Xu X, Zhang F (2006) Bioremediation
potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into
fed fish culture in coastal waters of north China. Aquaculture 252: 264-276

Chapter 2:

Physiological Effects of Simulated Submarine Groundwater Discharge on the Hawaiian Endemic Edible Alga *Gracilaria coronopifolia*

Abstract:

Submarine groundwater discharge (SGD) is a significant source of nutrients to many coastal environments. The difficulty of experimental manipulation *in situ* has resulted in little evidence for a causal relationship between SGD and coastal biological processes. In this study, we examined the physiological response of the endemic edible Hawaiian rhodophyte, *Gracilaria coronopifolia*, to varied levels of simulated SGD in a laboratory setting. Forty-eight thalli were grown in a unidirectional flow-though mesocosm at 25ºC and 250 µM $m^{-2}s^{-1}$ PAR during two replicate trials. To simulate increasing levels of SGD, four treatments were established, ranging from high salinity / low nutrient to low salinity / high nutrient water. Treatment levels were determined from empirical relationships among salinity, nitrate, and phosphate from known sites of SGD near Kona, Hawai'i. After 16 days, the growth rate, apical tip development (Tip Score and Tip Index), and photosynthetic parameters (ETR_{max}, α, E_k) via Pulse Amplitude Modulated (PAM) fluorometry were determined. The 27 ‰ SGD treatment (26.60 µM-N, 1.10 µM-P, 27 ‰ salinity) provided optimal conditions for growth of *Gracilaria coronopifolia* and had higher mean values for all parameters measured. The mean growth rate in the 27 ‰ SGD treatment (3% day^{-1}) was 3-fold greater than controls (0.20 µM-N, 0.05 µM-P, 35 ‰ salinity). ETR_{max}, E_k, Tip Index and growth rate were significantly greater in the 27

‰ SGD treatment compared to controls. Regression relationships indicate simulated

SGD levels have some ability to predict growth rate, Tip Score, ETR_{max}, and E_k. The

parameters Tip Score and Tip Index are informative approaches to quantify development

of new apices for complex morphologies.

The results of this study indicate moderate levels of SGD input to the coastal

environment may increase the growth rate, apical tip development, and photosynthetic

performance of *G. coronopifolia* on otherwise oligotrophic Hawaiian reefs. This work

has important implications for coastal ecosystems and management of terrestrial

groundwater resources.

Introduction:

Terrestrial groundwater can discharge directly into the marine environment wherever a

coastal aquifer is connected to the sea (Burnett et al. 2003). Until recently, this process

known as submarine groundwater discharge (SGD), has been largely ignored by coastal

managers and scientists because of the difficulty in assessment and the perception that

this process rarely occurred. With renewed interest and advances in detection techniques,

many studies have recently concluded that SGD is a significant source of nutrients to

numerous coastal environments (Johannes 1980; D'Elia et al. 1981; Giblin and Gaines

1990; Valiela et al. 1990; Simmons 1992; Moore 1996; Lapointe 1997; Paerl 1997;

Corbett et al. 1999; Burnett et al. 2001; Burnett et al. 2003; Moore et al. 2009). While

the quality and quantity of groundwater input to marine environments has been

documented in the literature, little is known about the effects SGD on biological

processes in coastal ecosystems.

The influx of SGD to the benthic environment increases nutrient concentrations while decreasing temperature and salinity of local waters compared to ambient oceanic conditions (Burnett et al. 2003; Johnson et al. 2008). The pore waters of coastal sediments have repeatedly been found to contain nitrate and phosphate concentrations several orders of magnitude greater than that of the overlying water column (Valiela et al. 1990). In some locations, nutrient input via SGD rivals local rivers and streams. On the island of Oahu, total nutrient loading in Kahana Bay via SGD, was equal or greater than that carried by surface runoff (Garrison et al. 2003). Total SGD flux (total nutrient addition / bottom area) for Yeosu Bay, Korea is 26 mM DIN $m^{-2} d^{-1}$ and 0.11 DIP mM $m^{-2} d^{-1}$, an order of magnitude greater than measurements from both stream water and ocean sediments (Hwang et al. 2005). In arid areas, SGD may be the only source of fresh water to coastal environments; on the west coast of the Island of Hawai'i, nearly all the fresh water entering the ocean was in the form of SGD (Kay et al. 1977) with similar observations in the Yucatan Peninsula, Mexico (Hanshaw and Back 1980). .

The biological effects of SGD in tropical oliogotrophic (nutrient deficient) environments such as these may be amplified as nutrient availability largely controls primary productivity. Although phosphorus limitation has be shown in some species (Lapointe 1987; Lapointe et al. 1987; Littler et al. 1991; Lapointe et al. 1992), additions of nitrogen will generally increase macroalgal biomass and abundance (McGlathery 1992; Valiela et al. 1992; Fong et al. 1993; Peckol et al. 1994; Fong et al. 1996; Hernandez et al. 1997; Valiela et al. 1997; Larned 1998; Kamer and Fong 2001). Numerous studies have concluded that the type and severity of nutrient limitation may be habitat or species specific (Lapointe et al. 1987; Larned 1998).

Salinity is one of the most critical chemical factors affecting the growth rate, development, and distribution of marine plants (Hoyle 1975; Koch and Lawrence 1987; Dawes et al. 1999; Israel et al. 1999). Maximal growth rates for some tropical macroalgae have been observed in water with decreased salinity from ambient oceanic levels (35 ‰). A maximum growth rate of 13 % d^{-1} for *Grateloupia filicina* of was observed at 20 ‰ salinity (Wong and Chang 2000). In culture, the growth rate of *Gracilaria chorda* and *G. verrucosa* reached a maximum in 25 ‰ salinity of 4.47 % d^{-1} at 25°C and 4.95 % d^{-1} at 30 °C (Choi et al. 2006). Causey et al. (1946) report optimal growth of *G. verrucosa* occurring between 25-35 ‰ salinity. Maximum growth rates for *G. vermiculophylla* were observed at 20 ‰ salinity (Yokoya et al. 1999). Hoyle (1976) concludes that *G. coronopifolia* and *G. bursapastoris* grow fastest at 22 ‰ salinity. The relative growth rate of *G. tenuistipitata* grown at 20 ‰ salinity was significantly higher than in 30 or 39 ‰ salinity treatments (Israel et al. 1999).

Increases in nutrients and decreases in salinity associated with SGD influx may synergistically increase the productivity of some tropical marine species. Herrera-Silveira suggest that SGD has played a major role in the shift from seagrass (*Thalassia testudinum*) dominated cover to green filamentous algae and strongly influences primary production as well as trophic status at sites near the Yucatan Peninsula, Mexico (Herrera-Silveira 1998; Herrera-Silveira et al. 1998; Herrera-Silveira et al. 2002; Herrera-Silveira and Morales-Ojeda 2009). Elevated $\delta\ ^{15}N$ levels in *Cladophora sericea* suggest terrestrial based nutrients via SGD are likely to play an important role in the development of algal blooms on Maui reefs (Smith et al. 2005). A similar study by Lapointe (1997)

concluded that increased δ ^{15}N, DIN and decreased salinity indicate SGD may play a role in macroalgal blooms on coral reefs in both Florida and Jamaica.

Many studies have been successful at quantifying the flux rate and chemical composition of SGD in coastal environments. Fewer studies show correlative evidence or even suggest that SGD plays an important role determining primary productivity of coastal regions. Thus far, little evidence for a causal relationship between SGD and coastal biological processes and has emerged. This may be attributable to inherent difficulties in the detection of SGD (Burnett et al. 2003; Leote et al. 2008) and the presumably even more difficult task of implementing *in-situ* manipulations and controls. The objective of this study was to measure the physiological effects of simulated SGD on an endemic Hawaiian species of macroalgae in a controlled laboratory setting using empirical SGD data from Hawaiʻi to determine treatment conditions. Moderate levels of simulated SGD are expected to increase the growth rate, branching morphology (apical tip development) and photosynthetic performance of *Gracilaria coronopifolia* when compared to ambient controls.

Methodology:

Algae Collections and Pretreatment:

Two replicate experiments were conducted at the University of Hawaiʻi at Manoa. *Gracilaria coronopifolia* was collected from the south shore of Oahu on October 19th 2007 and October 11th 2008 at Ala Moana Beach. Thalli were then transported to the University of Hawaiʻi at Manoa in a cooler with seawater. One thallus was cut from each collected sample and placed in a three-liter beaker containing a mixture of distilled water,

Instant Ocean® sea salt at 35 ‰ salinity, and nutrients at ambient oceanic levels (0.02 µM

NO_3 , 0.05 µM P_i). Thalli were then placed into a growth chamber for at least ten days

to heal and acclimate to growth chamber conditions.

Experimental Growth Chamber Setup:

A growth chamber (Environmental Growth Chambers, model GC-15) was

modified to accommodate a unidirectional flow-through saltwater system. Four 200-liter

HDPE drums held treatment water and were located next to the EGC. A digital

Ismatec™ variable speed peristaltic pump (ISM 444) with 24 independent channels

provided consistent flow of treatment water from the drums into 24 replicate 800 ml

beakers. The flow rate was set to allow 1.6 liters of treatment water to enter each beaker

d^{-1}. Treatment water entered the bottom of each beaker via pharmaceutical grade tubing

(2.06 mm diameter) and slowly spilled from the top into a common water bath. The water

bath, consisting of two clear plastic storage bins, buffered extremes in temperature for 12

sample beakers within each bin. Ten centimeters from the bottom of each bin, a hole

allowed for gravity feed of wastewater to a drain outside of the EGC. Each beaker was

equipped with aeration from a clear tube and air stone. Irradiance was set at 250 µM

photon $m^{-2} s^{-1}$ PAR as determined by a spherical Li-Cor® quantum sensor (model LI-

193SA, Li-Cor, NE, USA), on a 12h:12h light:dark cycle. For a single trial, this

mesocosm design supported four treatments of six samples for a total of 24 beakers.

Selection of Experimental Thalli:

Twenty-four thalli were selected for a single experimental trial. The criterion for thalli selection was $50 \leq$ (Sample wet mass / apical tip number) ≥ 100. This measure of mass to tip ratio, known hereafter as "Tip Score", was devised to control for variability among samples . Pilot studies confirmed that Tip Score was positively correlated with growth rate (data not shown). Samples were cleaned of epiphytes and inspected with a dissecting microscope to ensure that no reproductive tissues were present.

Chemical Composition of Treatments:

Four different treatments were chosen to simulate varied levels of SGD. Using empirical relationships (Johnson et al. 2008), the concentration of nitrate, phosphate, and salinity was determined. From measurement of SGD plumes on the Kona coast of the Big Island, Hawai'i, Johnson et al. (2008) calculated highly significant regression equations of both nitrate and phosphate with salinity, which were used to determine the nutrient concentration of each treatment at four levels of salinity. The nutrient equations in micromoles are $[NO_3^-] = (-3.3)*Salinity + 115.7$ ($R^2 = 0.93$, n = 99) and $[PO_4^{3-}] = (-0.14)*Salinity + 4.9$ ($R^2 = 0.98$, n = 72). Treatment water was a mixture of distilled water, Instant Ocean® sea salt to a desired salinity, and reagent grade nutrients (NaNO, NaPO$_4$). The four treatments were:

A) 35 ‰ salinity + (0.20 μM nitrate, 0.05 μM phosphate)

B) 27 ‰ salinity + (7.51 μM nitrate, 0.15 μM phosphate)

C) 19 ‰ salinity + (23.36 μM nitrate, 1.65 μM phosphate)

D) 11 ‰ salinity + (82.68 μM nitrate, 3.75 μM phosphate)

When the phosphate equation was solved using 35 ‰ salinity, $[PO_4^{3-}] = 0.00$. To avoid P limitation, 0.05 µM P was chosen for the 35 ‰ treatment. This was determined to be an ecologically relevant concentration for ambient ocean water. The N:P ratios of Johnson et al.'s (2008) data set revealed an inverse correlation of N:P with salinity (Figure 2.1). An N:P ratio of four was chosen for the 35 ‰ treatment as this value was smaller than the mean (5.2) and larger than the median (3.0) of the Johnson et al. data for salinities 34.1-35 ‰. The N:P ratio of the remaining three treatments were ~ 23.6.

Diving PAM Modifications:

In trial two, a Diving-PAM (Underwater PAM, Walz Co., Germany) was modified by constricting the exposed surface area of the fiber optic cable. A rubber cap with a 1 mm x 2 mm slit cut in the center was placed over the cable's end. As a result of this slight modification, ETR measurements were less variable when compared to trial one. PAM measurements are area dependent and thus calculated parameters cannot be pooled between trials. The PAM results of trial two will be reported in this study.

Data Collection Protocol:

On day zero, each sample was blotted dry with a Kimwipe® and the initial wet mass was determined using a Sartorius balance (model A200S). Apical tip number was recorded, and the sample was photographed with a Canon digital camera (Rebel XT). A rapid light curve (RLC) was conducted using Pulse Amplitude Modulated (PAM) Fluorometry (Underwater PAM, Walz Co., Germany). All samples were then randomly assigned to a treatment.

Every four days, for 16 days, samples were removed from the growth chamber and placed in a sampling tray using a blind sampling method. The tray was then placed on shaker table under 50 μmol photon meter^{-2} s^{-1} PAR. On days 0, 4, 8, 12, and 16, the wet mass of all samples was measured and the number of live apical tips for each plant was recorded. RLCs were performed on days 0, 8, and 16. On the final day of experimentation, all sample thalli were photographed.

Calculations and Statistical Analysis:

Two indices were developed to quantify the growth of algal apical tips. "Tip Score" is a measure of a sample's apical tip number in reference to its mass. "Tip Index" is a measure of the production of new apical tips normalized to initial tip number. These parameters were calculated as follows:

Tip Score= (# of live apical tips / wet mass)

Tip Index= ((final # of live apical tips - initial # of live apical tips) / initial # of live apical tips) x 100

Raw PAM data was imported using WinControl software (Walz GmbH, Effeltrich, Germany). Yield values below 0.1 were not used in analysis as ETR may be underestimated below this critical value (Beer and Axelsson 2004). PAM-RLC parameters (ETR$_{max}$, α) were calculated by nonlinear regression curves using SigmaPlot (version 11, Systat Inc.) software. Empirical data were fitted to a double exponential decay function as suggested by Ralph and Gademann (2005) in the absence of photoinhibition. A measure of the minimum saturating irradiance (E$_k$) was calculated by dividing ETR$_{max}$ by α.

Growth rates were calculated as percent growth per day (((final wet mass - initial wet mass) / initial wet mass) / 16 days) x 100. Fisher's ANOVA, Tukey's post-hoc comparisons and quadratic regressions were conducted using SPSS software (SPSS Inc.) if the distribution of parameter data was normal and homoscedastic. Welch's ANOVA and Games-Howell post-hoc comparisons were performed using SPSS software (SPSS Inc.) if the distribution of parameter data was normal and heteroscedastic. Non-parametric one-way Kruskal-Wallis ANOVA on ranks, Tukey's post-hoc comparisons on ranks, and Spearman rank order correlations were performed on ranked data using SigmaPlot software (Systat Inc.) if the assumption of normality was violated, but distributions were of similar shape. A value of $\alpha = 0.05$ was retained as the probability of a type I error for all statistical tests.

Results:

Trial 1 and 2 Pooled Data

Growth Rate:

A maximal mean growth rate of 3.01 % d^{-1} was observed in the 27 ‰ treatment, which is ~ 3 fold greater than the 35 ‰ (1.11 % d^{-1}) or 11 ‰ (1.35 % d^{-1}) treatments (Table 2.1). A quadratic trend was observed between SGD treatment (independent variable) and growth rate and a significant regression was calculated (R^2=0.39, P=0.000; Figure 2.2). Welch's ANOVA analysis detected significant differences among treatments (F=30.827, P=0.000; Table 2.2). Games-Howell post-hoc comparisons confirmed the mean growth rate for both 19 ‰ (2.23 % d^{-1}) and 27 ‰ treatments was significantly greater than the 35 ‰ treatment (P=0.000, P=0.000; Table 2.3). The mean growth rate of

27 ‰ treatment was also determined to be significantly greater than the 19 ‰ treatment (P=0.008, Table 2.3). At low salinity and high nutrients, many samples became visually bleached after only four days and gradually lost mass. However, some thalli recovered and grew at rates approaching other treatments. Although changes in sample mass would indicate thalli in the 11 ‰ treatment were growing nearly as rapidly as other treatments, 45 % of samples in the 11 ‰ treatment experienced negative growth by day eight. By day 16, more than half of samples were experiencing negative growth and sections of the thalli were visibly bleached.

Apical Tip Development:

Both measures of apical tip development produced a curve very similar in shape to the quadratic function of percent daily growth rate vs. SGD treatment. Major increases in apical tip development were evident after day eight in treatments 19 ‰ and 27 ‰. New apical tips commonly grew from existing tips while others appeared randomly along the thalli axes. New apical tip development was uncommon in treatments 35 ‰ and 11 ‰; the 11 ‰ treatment began to show signs of thalli bleaching after four days. By day eight, 83 % of samples in the 11 ‰ treatment had evidence of bleaching and a decrease in the total number of live apical tips from initial values.

Tip Score

Prior to experimentation, Tip Scores of samples ranged from 50 to 100; no significant difference was detected among treatments (F=0.626, P=0.602). A maximal mean Tip Score of 117.56 was observed in the 27 ‰ treatment (Table 2.4) and one-way Kruskal-Wallis ANOVA analysis detected significant differences among treatments (H=31.494, P<0.001; Table 2.5). Tukey's post-hoc comparisons on ranks found significant differences between the 11 ‰ treatment and both 27 ‰ and 19 ‰ treatments (Table 2.6). By day 16, 100 % of samples in the 11 ‰ treatment experienced a decrease in Tip Score from initial values. The shape of the Tip Score vs. SGD treatment curve displays a similar quadratic trend to the growth rate curve (Figure 2.3) and a significant regression was calculated (R^2=0.62, P=0.000; Figure 2.3). Tip Score increases from a minimum at 11 ‰ to a maximum value at 27 ‰ and then declines in the 35 ‰ treatment. A small but significant quadratic regression (R^2=0.32, P=0.000; Figure 2.4) was found between Tip Score (independent variable) and percent growth per day (dependant variable).

Tip Index

A maximal mean Tip Index of 132.70 was observed in the 27 ‰ treatment (Table 2.7) and one-way Kruskal-Wallis ANOVA analysis detected significant differences among treatments (H=32.720, P<0.001; Table 2.8). Tukey's post-hoc comparisons on ranks found significant differences between the 27 ‰ treatment and both 35 and 11 ‰ treatments (Table 2.9). Significant differences were also detected between the 19 and 11 ‰ treatments. By day 16, 91 % of samples in the 11 ‰ treatment experienced apical tip

necrosis and thus had a negative value for Tip Index. Figure 2.5 displays a quadratic

trend as Tip Index increases from a negative value at 11 ‰ to a maximum value at 27 ‰

and then declines in the 35 ‰ treatment. A Spearman Rank Order correlation indicated a

significant linear relationship (R= 0.59, P=0.000) between Tip Index and percent growth

per day (Figure 2.6).

Trial Two Data

Growth Rate:

A maximal mean daily growth rate of 2.82 % d^{-1} was observed in the 27 ‰

treatment during trial two (Table 2.10). Fisher's ANOVA detected significant

differences among treatments (F=6.610, P=0.003; Table 2.11). Tukey's poc-hoc

comparisons concluded that both 27 and 19 ‰ (2.45 % d^{-1}) treatments had a greater mean

growth rate than the control 35 ‰ (1.10 % d^{-1}) treatment (P=0.003, P=0.023; Table 2.12).

A quadratic trend is evident for the growth rate vs. SGD treatment curve and a significant

regression was calculated (R^2=0.46, P=0.000; Figure 2.7). By day eight, almost all thalli

the 11 ‰ treatment showed signs of bleaching; two of the six samples also decreased in

mass. At the end of the experiment, almost all samples in the 11 ‰ treatment were

experiencing negative growth.

PAM Measurements and Calculated Parameters:

Figure 2.8 shows a representative rapid light curve (RLC) with non-linear

regression lines fitted to mean ETR at a given irradiance for each treatment. The RLC of

the 27 ‰ treatment displays higher mean ETR values at nearly every irradiance level

than all other treatments. The RLC for the 19 ‰ treatment lies below that of both 27 and

35 ‰ treatments.

The parameters ETR_{max}, α, and E_k were calculated for each sample thallus by

non-linear regression (all $R^2 > 0.99$). A quadratic trend similar was evident for ETR_{max}

vs. SGD treatment and a significant regression was calculated ($R^2=0.49$, P=0.000; Figure

2.9). A maximal mean ETR_{max} of 44.72 µmol e^- m^{-2} s^{-1} was calculated for the 27 ‰

treatment (Table 2.13). Fisher's ANOVA analysis of ETR_{max} confirms that significant

differences exist among treatments (F= 25.73, P=0.000; Table 2.14). Tukey's post-hoc

comparisons reveal that the 27 ‰ treatment had a significantly higher mean ETR_{max} than

all other treatments. Samples in the 35 ‰ treatment had a significantly higher mean

ETR_{max} than both 19 and 11 ‰ treatments (p=0.025, P=0.004; Table 2.15).

The slope of the light limited part of the RLC (α) did not have as clear a

relationship with SGD treatments as seen with other parameters (Figure 2.10). A

maximal mean α of 0.274 (µmol e^- m^{-2} s^{-1}) (µmol photon m^{-2} s^{-1}) $^{-1}$ was observed in the 27

‰ treatment and was slightly higher than the 35 ‰ control treatment (mean= 0.238,

Table 2.16). A minimum α (mean=0.213) was observed calculated for the 19 ‰

treatment (Table 2.16). Fisher's ANOVA analysis was not able to detect significant

differences among treatments (F=2.601, P=0.081; Table 2.17)

Figure 2.11 shows the quadratic trend was retained for the E_k vs. SGD treatment

and a significant regression was calculated ($R^2=0.49$, P=0.00). A maximal mean E_k

value of 165.95 µmol photon $meter^{-2}$ s^{-1} PAR was calculated for the 27 ‰ treatment

(Table 2.18) and Fisher's ANOVA analysis indicates significant differences among

treatments (F=25.48, P=0.000; Table 2.19). Tukey's post-hoc comparisons reveal the

mean E_k of 27 ‰ treatment is significantly greater than all other treatments (Table 2.20). The mean E_k value of the 35 ‰ treatment (124.08 µmol photon $m^{-2} s^{-1}$ PAR) is significantly greater than both 19 and 11‰ treatments (P=0.009, P=0.000; Table 2.20). While the curve of the parameters ETR_{max}, α, and E_k appear similar to that of growth rate vs. SGD treatment, no statistical relationship was detected between growth rate and these parameters.

Discussion:

Almost three decades after Johannes's benchmark review (1980) suggested that SGD deserves more attention from marine ecologists, little evidence of a relationship between SGD and coastal biological processes has been published. Numerous studies have been successful at measuring the quality and quantity of SGD in coastal waters. Fewer studies have reported correlative evidence or even suggested terrestrial groundwater may play a role in marine productivity. The diffuse, large-scale nature of SGD presents a major challenge to scientists seeking to establish a causal relationship via *in situ* manipulative experiments. This study was undertaken to measure the physiological effects of simulated SGD on a coastal marine alga in a controlled laboratory setting. Our results support the hypothesis that moderate levels of simulated SGD increase the growth rate, apical tip development, and photosynthetic performance of *Gracilaria coronopifolia* compared to ambient controls.

The results of this study display a quadratic trend for the mean of all variables when plotted against simulated SGD treatment (increasing salinity, decreasing nutrients). Significant regression relationships indicate simulated SGD levels can predict growth

rate, Tip Score, ETR_{max}, and E_k. This trend and statistical analysis identify the simulated 27 ‰ SGD treatment (27 ‰ salinity, 7.51 µM nitrate, 0.15 µM phosphate) as an optimal combination of salinity and nutrients for *Gracilaria coronopifolia* within this study. When compared to controls, this treatment had greater mean values in every parameter measured (significance was detected in four of six parameters). A maximal mean growth rate for *G. coronopifolia* of ~3 % d^{-1} was observed in the 27 ‰ SGD treatment. This 3-fold increase in growth rate compared to controls was concurrent with increased apical tip development. Maximal mean values in both Tip Score and Tip Index were observed in the 27 ‰ treatment. Maximal mean values for ETR_{max}, α, and E_k were also observed in the 27 ‰ treatment. This suggests that these samples had a greater maximum rate of electron transport, were able to use low irradiance more efficiently and required higher irradiance to saturate electron flow than samples in other treatments; this combination of performance traits suggests a physiological state that is more than just a sun/ shade acclimation but rather an elevated nutritional status over plants in other treatments. The mean ETR_{max} and α of samples in the 27 ‰ treatment were generally higher than values reported for 12 marine rhodophytes from southern Chile (Gomez et al. 2004).

The effect of changes in salinity on the productivity of osmotically adjusted marine algae commonly involves a transient increase in respiration and a stimulation or inhibition of photosynthesis (Kirst 1990). Reduced salinity has been shown to decrease photosynthesis in several marine algae (Otaga and Matsui 1965; Koch and Lawrence 1987; Simon et al. 1999; Wong and Chang 2000). Gessner and Schramm (1971) report that decreases in photosynthetic rate is proportional to decreases in salinity. Munda and Kremer (1977) conclude that reduced salinity decreases photosynthesis but increases

72

respiration in *Fucus serratus* and *F. vesiculosus*. Lapointe (1984) finds a similar result

for *Gracilaria tikvahiae*. However, a significant decrease in respiration is found in

Gracilaria cornea in 20 ‰ salinity compared to 30 ‰ salinity. Maximal growth rate and

photosynthetic capacity (P_{max}) of *Grateloupia filicina* is observed at 20 ‰ salinity but

declines as salinity decreases (Wong and Chang 2000). Photosynthesis may decline in

hypo-osmotic conditions due to loss of Cl^- ions (Gessner and Schramm 1971), the ex-

osmosis of ionic photosynthetic co-factors (Lapointe et al. 1984), or reductions in

biocarbonate (HCO_3^-) concentrations (Imai et al. 1997). Elevated levels of nitrate

ameliorate the negative effects of reduced salinity in some macroalgae. When cultured in

water with high nitrate and 15 ‰ salinity, *Enteromorpha intestinalis* has the greatest

biomass, lowest wet:dry biomass ratios, and greater tissue N and P than when grown in

low levels of nitrate (Kamer and Fong 2001).

In this study, a significant reduction in photosynthesis as well as an increase in

growth rate and apical tip development was measured in the 19 ‰ treatment compared to

controls. The 19 ‰ treatment contained two-fold higher nitrate and phosphate

concentrations than the 27 ‰ treatment and two orders of magnitude greater nutrients

than controls. Because nitrate and phosphate are not likely to inhibit growth at these

concentrations, the salinity of the 19 ‰ treatment may be limiting productivity. This

hypothesis is supported by the results of photosynthetic parameters ETR_{max} and E_k.

The low salinity and high nutrient concentrations of the 11 ‰ treatment is at or

near the viability threshold for *G. coronopifolia*. Although some samples in this

treatment were slowly dying, others were able to resist the initial shock and continue to

gain mass at rates similar to other treatments. Elevated levels of nutrients in SGD may

mitigate the negative effects of reduced salinity in *G. coronopifolia* and other species of marine algae. Some species of algae may be able to persist and thrive in high nutrient, hypo-osmotic conditions if SGD rate exhibits periodic cycling (eg. tidal pumping) or variability. A fluctuating salinity regime mitigates negative effects of reduced salinity on *Enteromorpha intestinalis* (Kamer and Fong 2000). In Hawai'i, the invasive marine alga *Gracilaria salicornia* remains viable and able to photosynthesize after a one-week immersion in fresh water (0 ‰) (Smith et al. 2004). High rates of SGD may help promote the persistence and productivity of alien algae at sites where low salinity limits the distribution of less tolerant native species.

Salinity alone influences apical tip formation in macroalgae. The mean number of algal branches increases with decreasing salinity in *Grateloupia filicina* from of 35 to 15 ‰ (Zablackis 1987) and in an unknown species of *Gracilaria* from 34 to 18 ‰ salinity (Zablackis 1986). Increased branching is also observed in *Fucus vesiculosus* with decreasing salinity (Jordan and Vadas 1972). In this study, increases in Tip Score and Tip Index were observed in the 19 and 27 ‰ treatments compared to controls. Increased apical tip formation is clearly a morphological response of this species to hypo-salinity / nutrient combinations. Tip Score and Tip Index are simple but informative measures with a clear relationship to growth rate over varied levels of simulated SGD.

Similar to other species in the genus *Gracilaria, G. coronopifolia* was found to be a euryhaline species capable of growth over a wide range of nutrients and salinity. The maximal growth rate of ~3 % d^{-1} observed in this study was within the range of reported growth rates for this species. A survey of *Gracilaria* research from 21 species and 33 studies show a mean growth rate of ~7 % d^{-1} (SD=0.05; Table 1.1). Somewhat

surprisingly, no correlation between growth rate and the photosynthetic parameters ETR_{max}, α, and E_k was detected. This is likely due to the relatively high rate of growth and poor photosynthetic performance of thalli in the 19 ‰ treatment. This result is contrary to other studies, which indicate PAM derived photosynthetic rate can be used as a proxy for growth. Smith et al. (2004) report a significant positive relationship between growth and ETR_{max} in *Gracilaria salicornia* ($R^2 = 0.465$, $p < 0.001$) when grown in treatments of various temperatures and salinities and algicide. The results of this study indicate the relative level of simulated SGD flux can be used to predict growth rate, branching, and photosynthetic parameters in *G. coronopifolia.*

In summary, the volume and quality of SGD to the benthic environment is a function of complex interactions including local groundwater chemistry, hydraulic gradients, tidal cycles, wave regime, wind force, thermal gradients and sediment type (Burnett et al. 2003). The ability of an organism to tolerate fluctuating salinity regimes and store nutrients while maintaining optimal respiratory and photosynthetic performance is likely a key factor in the distribution and productivity of an alga. The interaction of abundant nutrients coupled with changes of osmotic pressure in algal thalli may change the morphology and increase the productivity of some species under optimal conditions. The results of this study suggest that moderate amounts of simulated SGD (27 ‰ salinity, 7.51 µM nitrate, 0.15 µM phosphate) may increase the growth rate, apical tip development and photosynthetic performance of *Gracilaria coronopifolia* in an otherwise oligotrophic environment. It is likely that *G. coronopifolia* and other species of marine algae in coastal environments have adapted to the SGD driven hyposaline-high nutrient conditions as simulated in the 27 ‰ salinity treatment. The importance of this natural

source of nutrients may be greater in arid regions, such as the Kona Coast, Hawaiʻi, where SGD is the only significant source of freshwater and nutrients to coastal environments.

The results of this study have important implications for coastal ecosystem and terrestrial groundwater management strategies. Anthropogenic additions of nutrients to groundwater are likely increase primary productivity in areas of SGD. Recent studies have suggested anthropogenic nutrients via SGD may play a role in the occurrence of invasive algae blooms and phase shifts (Herrera-Silveira 1998; Herrera-Silveira et al. 1998; Herrera-Silveira et al. 2002; Smith et al. 2005; Herrera-Silveira and Morales-Ojeda 2009). Historically, groundwater management strategies have not considered marine biological processes as a constraint when determining the rate of extraction from a coastal aquifer (Pongkijvorasin et al. 2008). As the demand for fresh water increases with the human population, particularly in coastal areas, terrestrial groundwater should be considered an important resource for terrestrial and marine organisms alike.

Tables:

Pooled Growth Rate (% per day)

Table 2.1. Descriptive statistics for pooled percent growth rate per day per treatment.

Treatment (‰)	n	Mean	Standard Deviation	Median	Min	Max
11	12	1.35	1.484	1.83	-1.95	3.69
19	12	2.23	0.456	2.28	1.55	3.18
27	12	3.01	0.595	3.03	2.01	3.88
35	12	1.11	0.397	1.07	0.31	1.78

Table 2.2. Welch's ANOVA results for pooled percent growth rate per day (a) asymptotically distributed.

	F-Statistic(a)	df1	df2	P-value
Growth Rate	30.827	3	23.468	**0.000**

Table 2.3. Games-Howell test of pooled daily growth rate (*) indicates the mean difference is significant at the $\alpha = 0.05$ level.

(I) (‰)	(J) (‰)	Mean Difference (I-J)	Std. Error	95% Confidence Interval		P-value
		Lower Bound	Upper Bound	Upper Bound	Lower Bound	Lower Bound
11	19	-0.89	0.45	-2.200	0.429	0.245
	27	-1.67(*)	0.46	-3.004	-0.332	**0.013**
	35	0.24	0.44	-1.068	1.547	0.948
19	11	0.89	0.45	-0.429	2.200	0.245
	27	-0.78(*)	0.22	-1.386	-0.178	**0.008**
	35	1.13(*)	0.17	0.640	1.611	**0.000**
27	11	1.67(*)	0.46	0.332	3.004	**0.013**
	19	0.78(*)	0.22	0.178	1.386	**0.008**
	35	1.90(*)	0.21	1.327	2.487	**0.000**
35	11	-0.24	0.44	-1.547	1.068	0.948
	19	-1.13(*)	0.17	-1.611	-0.640	**0.000**
	27	-1.90(*)	0.21	-2.487	-1.327	**0.000**

Pooled Tip Score

Table 2.4. Descriptive statistics for pooled tip score per treatment.

Treatment (‰)	n	Mean	Standard Deviation	Median	Minimum	Maximum
11	12	31.04	21.404	28.93	0.00	79.95
19	12	110.33	32.708	101.04	81.12	189.91
27	12	117.56	35.669	99.57	85.11	178.61
35	12	79.14	15.348	74.27	58.05	99.94

Table 2.5. Pooled data tip score Kruskal-Wallis one-way ANOVA on ranks test statistics.

	H-statistic	df	P-value
Tip Score	31.494	3	**<0.001**

Table 2.6. Tukey's pairwise multiple comparison of ranked pooled data for tip score. (*) Indicates the significance at the $\alpha=0.05$ level. A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison.

Treatment comparison	Difference of Ranks	Q-statistic	P<0.05
27ppt vs 11ppt(*)	333	6.866	Yes
27ppt vs 35ppt	157	3.237	No
27ppt vs 19ppt	6	0.124	Do Not Test
19ppt vs 11ppt(*)	327	6.743	Yes
19ppt vs 35ppt	151	3.114	Do Not Test
35ppt vs 11ppt	176	3.629	No

Pooled Tip Index

Table 2.7. Descriptive statistics for pooled tip index per treatment.

Treatment (‰)	n	Mean	Standard Deviation	Median	Minimum	Maximum
11	12	-50.41	32.373	-53.85	-100.00	8.33
19	12	83.78	53.834	69.44	21.43	166.67
27	12	132.70	105.781	109.17	34.04	380.00
35	12	30.24	41.025	20.00	-20.00	115.38

Table 2.8. Pooled data tip index Kruskal-Wallis one-way ANOVA on ranks test statistics.

	H-statistic	df	P-value
Tip Index	32.720	3	**<0.001**

Table 2.9. Tukey's pairwise multiple comparison of ranked pooled data for Tip Index. (*) Indicates the significance at the $\alpha=0.05$ level. A result of "Do Not Test" occurs for a comparison when no significant difference is found between the two rank sums that enclose that comparison.

Treatment comparison	Difference of Ranks	Q-statistic	P<0.05
27ppt vs 11ppt(*)	357.0	7.361	Yes
27ppt vs 35ppt(*)	183.5	3.784	Yes
27ppt vs 19ppt	47.5	0.979	No
19ppt vs 11ppt(*)	309.5	6.382	Yes
19ppt vs 35ppt	136.0	2.804	No
35ppt vs 11ppt	173.5	3.578	No

Table 2.10. Trial two percent daily growth rate descriptive statistics per treatment.

Treatment (‰)	n	Mean	Standard Deviation	Median	Minimum	Maximum
11	6	1.67	1.188	1.91	0.57	3.69
19	6	2.45	0.433	2.50	1.89	3.18
27	6	2.82	0.667	2.73	2.01	3.85
35	6	1.10	0.369	1.05	0.71	1.78

Table 2.11. Trial two percent daily growth rate one-way Fisher's ANOVA statistics.

	Sum of Squares	df	Mean Square	F-statistic	P-value
Between Groups	55763.17	3	18587.72	24.492	**0.000**
Within Groups	33393.31	44	758.94		
Total	89156.48	47			

Table 2.12. Tukey's pairwise comparison of trial two daily percent growth rate. (*) indicates significance at $\alpha = 0.05$.

(I) (‰)	(J) (‰)	Mean Difference (I-J)	Std. Error	95% Confidence Interval		P-value
		Lower Bound	Upper Bound	Upper Bound	Lower Bound	Lower Bound
11	19	-79.29(*)	11.25	-109.317	-49.259	0.286
	27	-86.52(*)	11.25	-116.551	-56.493	0.060
	35	-48.10(*)	11.25	-78.126	-18.068	0.551
19	11	79.29(*)	11.25	49.259	109.317	0.286
	27	-7.23	11.25	-37.263	22.795	0.821
	35	31.19(*)	11.25	1.162	61.220	**0.023**
27	11	86.52(*)	11.25	56.493	116.551	0.060
	19	7.23	11.25	-22.795	37.263	0.821
	35	38.43(*)	11.25	8.396	68.454	**0.003**
35	11	48.10(*)	11.25	18.068	78.126	0.551
	19	-31.19(*)	11.25	-61.220	-1.162	**0.023**
	27	-38.43(*)	11.25	-68.454	-8.396	**0.003**

Trial Two ETR$_{max}$

Table 2.13. Trial two ETR$_{max}$ rate descriptive statistics per treatment. The units of ETR$_{max}$ are $\mu mol\ e^-\ m^{-2}\ s^{-1}$.

Treatment (‰)	n	Mean	Standard Deviation	Median	Minimum	Maximum
11	6	13.84	4.141	13.52	8.75	20.54
19	6	17.07	9.388	15.81	5.93	31.16
27	6	44.72	8.125	41.99	35.68	57.31
35	6	29.31	3.432	30.00	24.69	33.32

Table 2.14. Trial two ETR$_{max}$ Fisher's one-way ANOVA statistics.

	Sum of Squares	df	Mean Square	F-statistic	P-value
Between Groups	3532.97	3	1177.66	25.73	**0.000**
Within Groups	915.29	20	45.77		
Total	4448.26	23			

Table 2.15. Tukey's pairwise comparison of trial two ETR$_{max}$. (*) indicates significance at alpha = 0.05.

(I) (‰)	(J) (‰)	Mean Difference (I-J)	Std. Error	95% Confidence Interval		P-value
		Lower Bound	Upper Bound	Upper Bound	Lower Bound	Lower Bound
11	19	-3.23	3.91	-14.162	7.702	0.841
	27	-30.88(*)	3.91	-41.812	-19.948	**0.000**
	35	-15.48(*)	3.91	-26.411	-4.547	**0.004**
19	11	3.23	3.91	-7.702	14.162	0.841
	27	27.65(*)	3.91	-38.582	-16.718	**0.000**
	35	12.25(*)	3.91	-23.181	-1.317	**0.025**
27	11	-30.88(*)	3.91	19.948	41.812	**0.000**
	19	27.65(*)	3.91	16.718	38.582	**0.000**
	35	15.40(*)	3.91	4.468	26.332	**0.004**
35	11	-15.48(*)	3.91	4.547	26.411	**0.004**
	19	12.25(*)	3.91	1.317	23.181	**0.025**
	27	-15.48(*)	3.91	-26.332	-4.468	**0.004**

Trial Two α

Table 2.16. Trial two α descriptive statistics per treatment. The units of alpha are $(\mu mol\ e^-\ m^{-2}\ s^{-1})(\mu mol\ photon\ m^{-2}\ s^{-1})^{-1}$.

Treatment (‰)	n	Mean	Standard Deviation	Median	Minimum	Maximum
11	6	0.233	0.025	0.23	0.210	0.260
19	6	0.213	0.069	0.22	0.090	0.290
27	6	0.274	0.014	0.27	0.260	0.300
35	6	0.238	0.019	0.24	0.210	0.260

Table 2.17. Trial two α one-way Fisher's ANOVA statistics.

	Sum of Squares	df	Mean Square	F-statistic	P-value
Between Groups	0.012	3	0.004	2.601	0.081
Within Groups	0.030	20	0.000		
Total	0.042	23			

Trial Two E_k

Table 2.18. Descriptive statistics for trial two E_k. The units of E_k are $(\mu mol\ photon\ m^{-2}s^{-1})$.

Treatment (‰)	n	Mean	Standard Deviation	Median	Minimum	Maximum
11	6	59.51	18.206	47.88	42.50	91.17
19	6	77.05	23.329	68.08	43.97	105.75
27	6	162.95	27.905	155.83	133.52	200.80
35	6	124.08	20.211	126.13	93.83	143.36

Table 2.19. Trial two E_k one-way Fisher's ANOVA statistics.

	Sum of Squares	df	Mean Square	F-statistic	P-value
Between Groups	39415.88	3	13138.63	25.48	**0.000**
Within Groups	10314.30	20	515.72		
Total	49730.18	23			

Table 2.20. Tukey's pairwise comparison of trial two E_k. (*) indicates significance at alpha = 0.05.

| (I) (‰)	(J) (‰)	Mean Difference (I-J)	Std. Error	95% Confidence Interval		P-value
		Lower Bound	Upper Bound	Upper Bound	Lower Bound	Lower Bound
11	19	-17.54	13.11	-54.235	19.161	0.551
	27	-103.44(*)	13.11	-140.135	-66.740	**0.000**
	35	-64.57(*)	13.11	-101.263	-27.868	**0.000**
19	11	17.54	13.11	-19.161	54.235	0.551
	27	-85.90(*)	13.11	-122.598	-49.203	**0.000**
	35	-47.03(*)	13.11	-83.726	-10.331	**0.009**
27	11	103.44(*)	13.11	66.740	140.135	**0.000**
	19	85.90(*)	13.11	49.203	122.598	**0.000**
	35	38.87(*)	13.11	2.174	75.569	**0.035**
35	11	64.57(*)	13.11	27.868	101.263	**0.000**
	19	47.03(*)	13.11	10.331	83.726	**0.009**
	27	-38.87(*)	13.11	-75.569	-2.174	**0.035**

Figures:
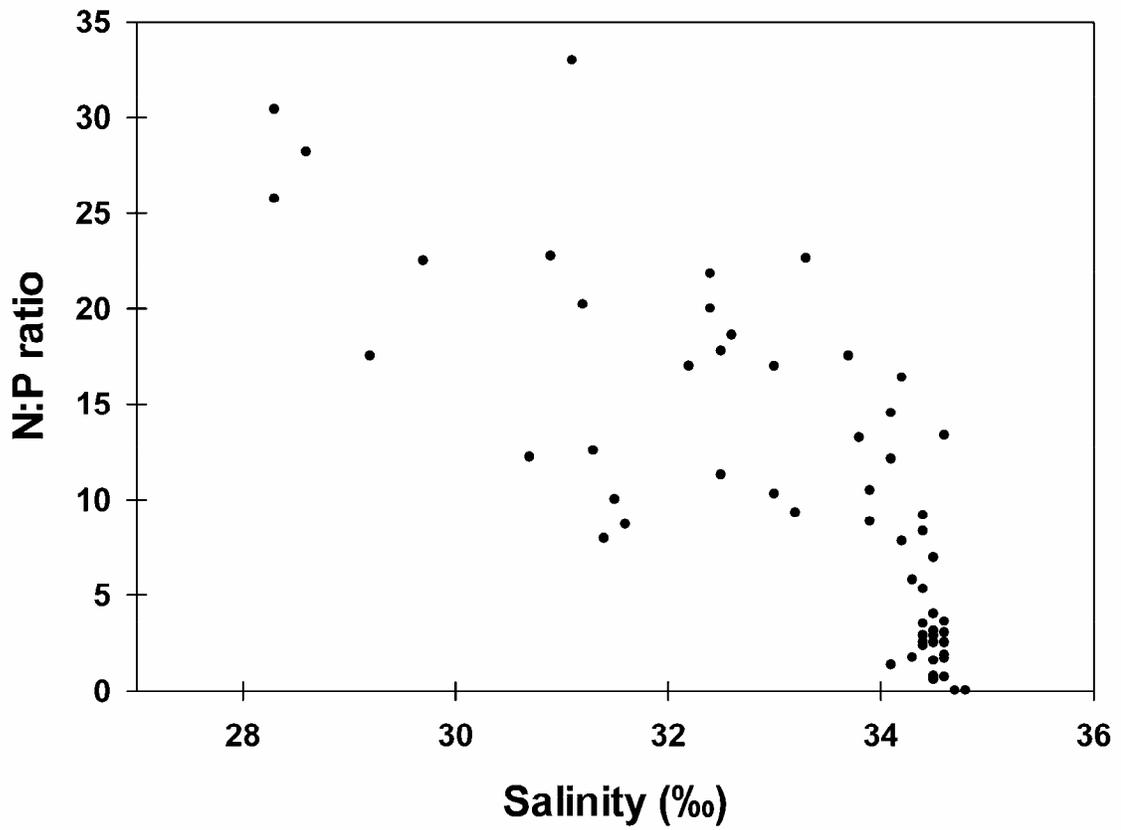
Figure 2.1. Scatter plot of N:P ratio vs. salinity from Kona, Hawaiʻi. N=62 Raw data from Johnson et al. (2008)
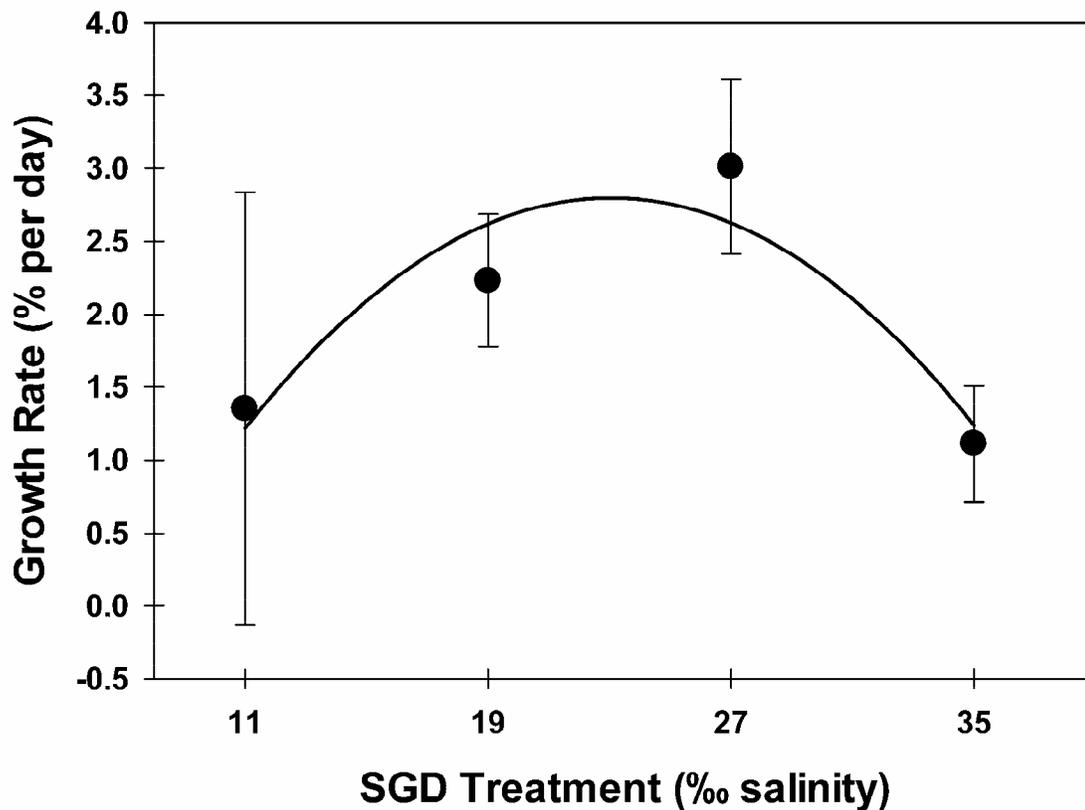
Figure 2.2. Pooled mean growth rate (% per day) vs. SGD treatment of *Gracilaria coronopifolia*. Error bars indicate ± 1 standard deviation of the mean. The solid line represents the best-fit line from quadratic regression (f= -2.9923 + 0.5027(x) -0.0109(x)^2 ; R^2=0.39, P=0.000).
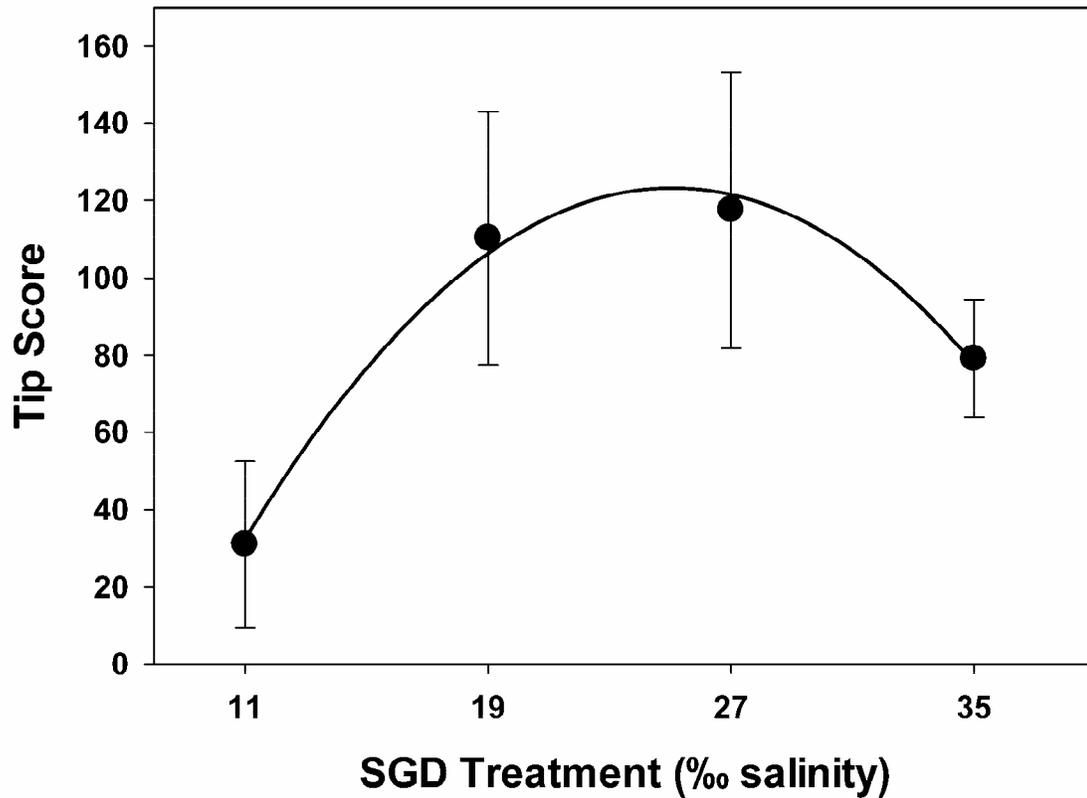
Figure 2.3. Pooled mean tip score vs. SGD treatment of *Gracilaria coronopifolia*. Error bars indicate ± 1 standard deviation of the mean. The solid line represents the best-fit line from quadratic regression (f= -165.5022 + 23.0457(x) - 0.4598(x)^2; R^2= 0.62, P=0.000).
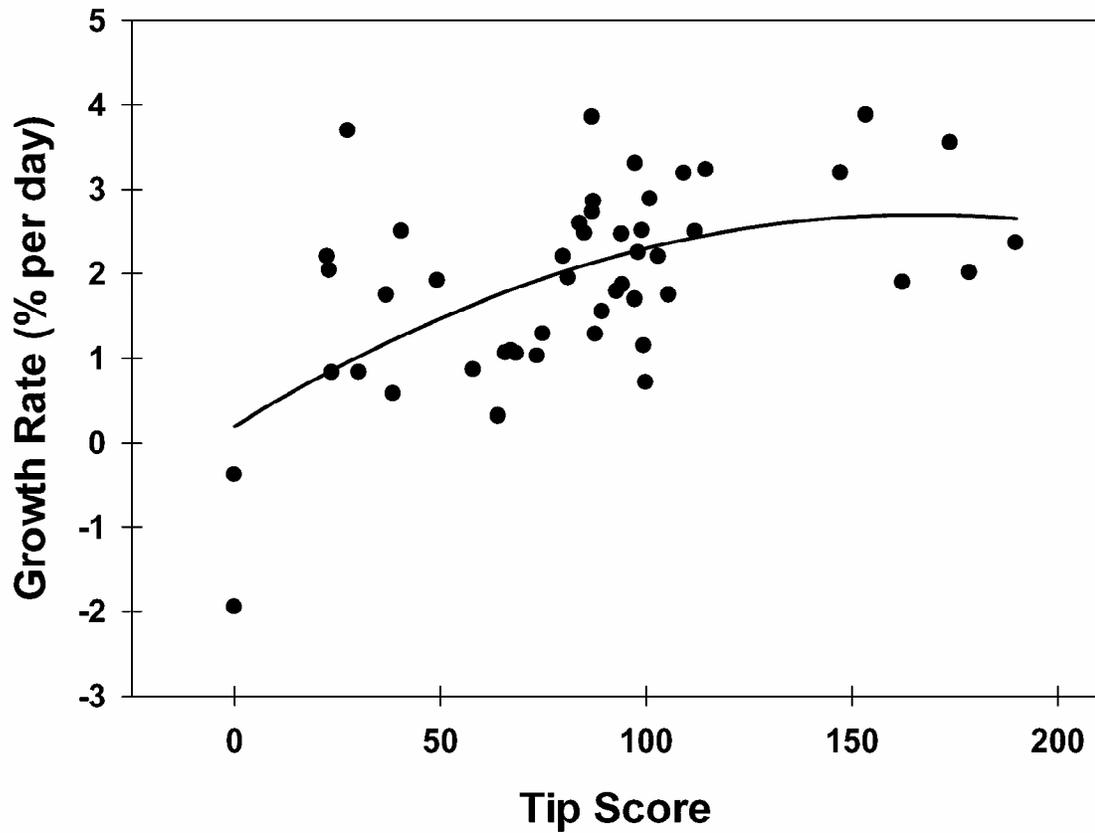
Figure 2.4. Pooled scatter plot of tip score vs. growth rate (% per day) for *Gracilaria coronopifolia* in all treatments. The solid line represents the best-fit line from quadratic regression (f= 0.1962 + 0.0300(x) -8.9985E-5(x)^2 ; R^2=0.32, P=0.000).
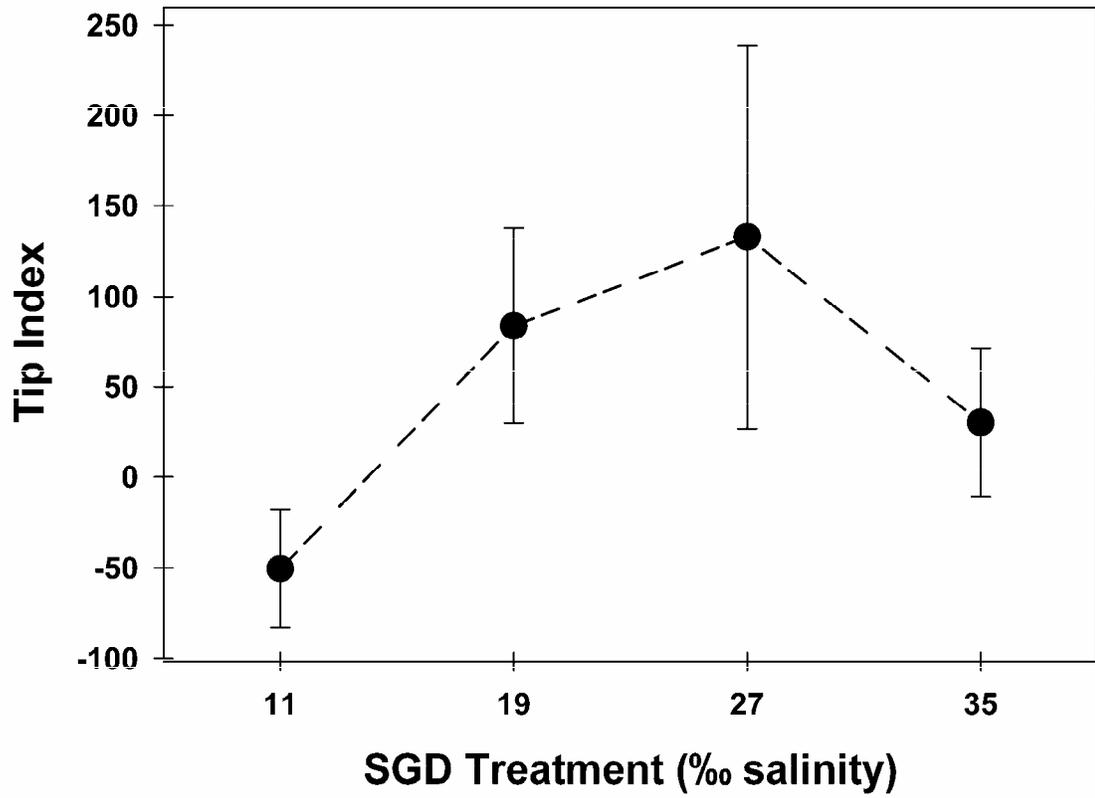
**Figure 2.5.** Pooled mean tip index vs. SGD treatment of *Gracilaria coronopifolia*. Error bars indicate ± 1 standard deviation of the mean. The broken line indicates the general trend.
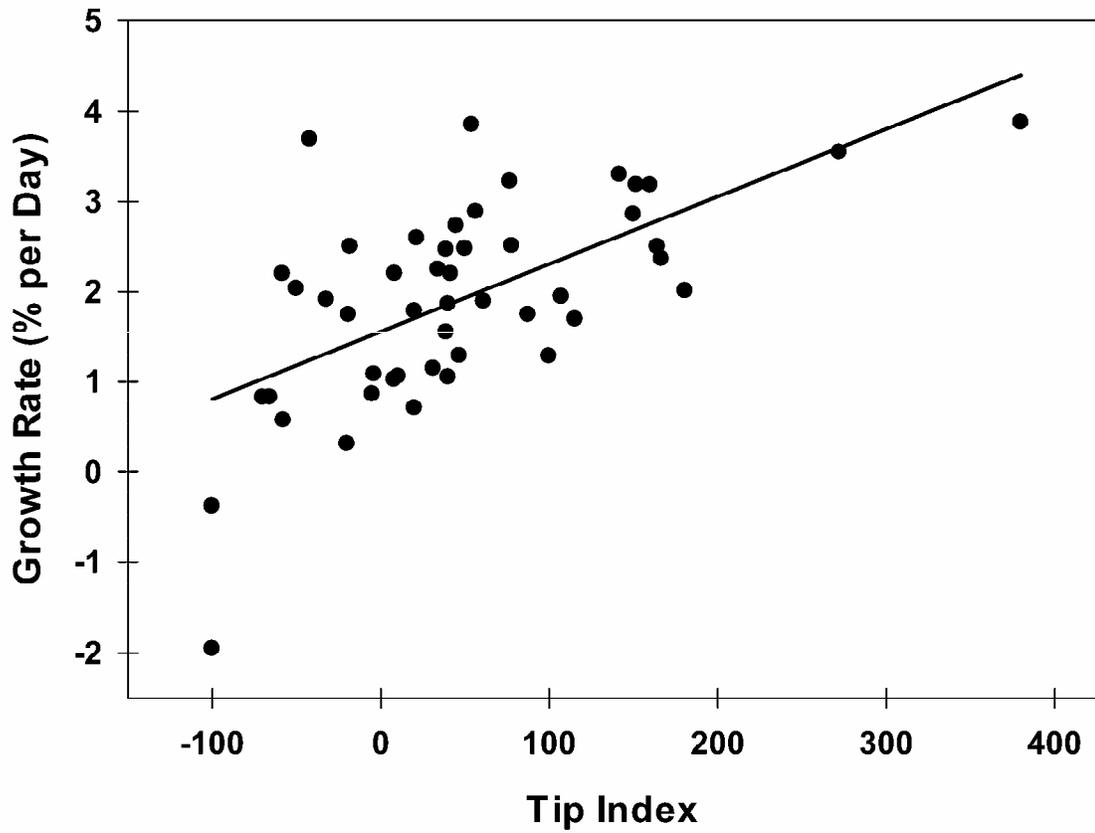
Figure 2.6. Pooled scatter plot of tip index vs. growth rate (% per day) of *Gracilaria coronopifolia* in all treatments. The solid line represents the best-fit linear line.
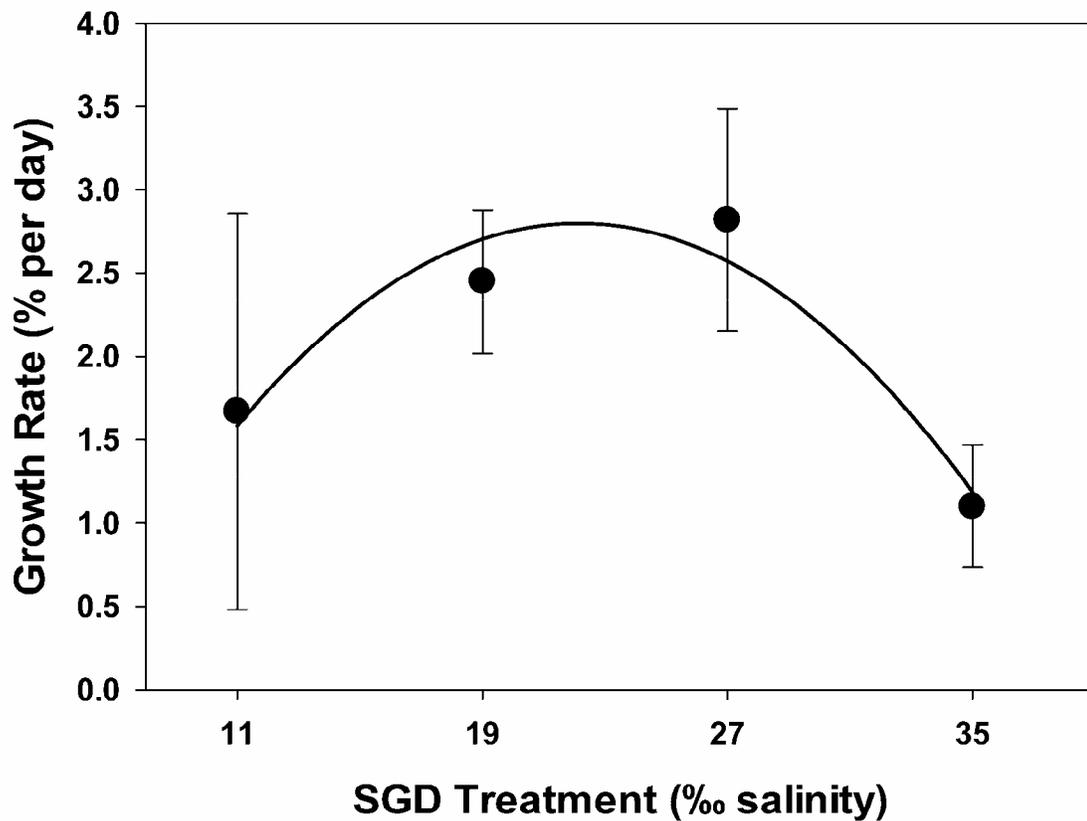
Figure 2.7. Mean growth rate (% per day) vs. SGD treatment of *Gracilaria coronopifolia* in trial two. Error bars indicate ± 1 standard deviation of the mean. Solid line represents the best-fit line from quadratic regression (f= -1.9979 + 0.4335(x) -0.0098(x)^2 ; R^2=0.46, P=0.000).
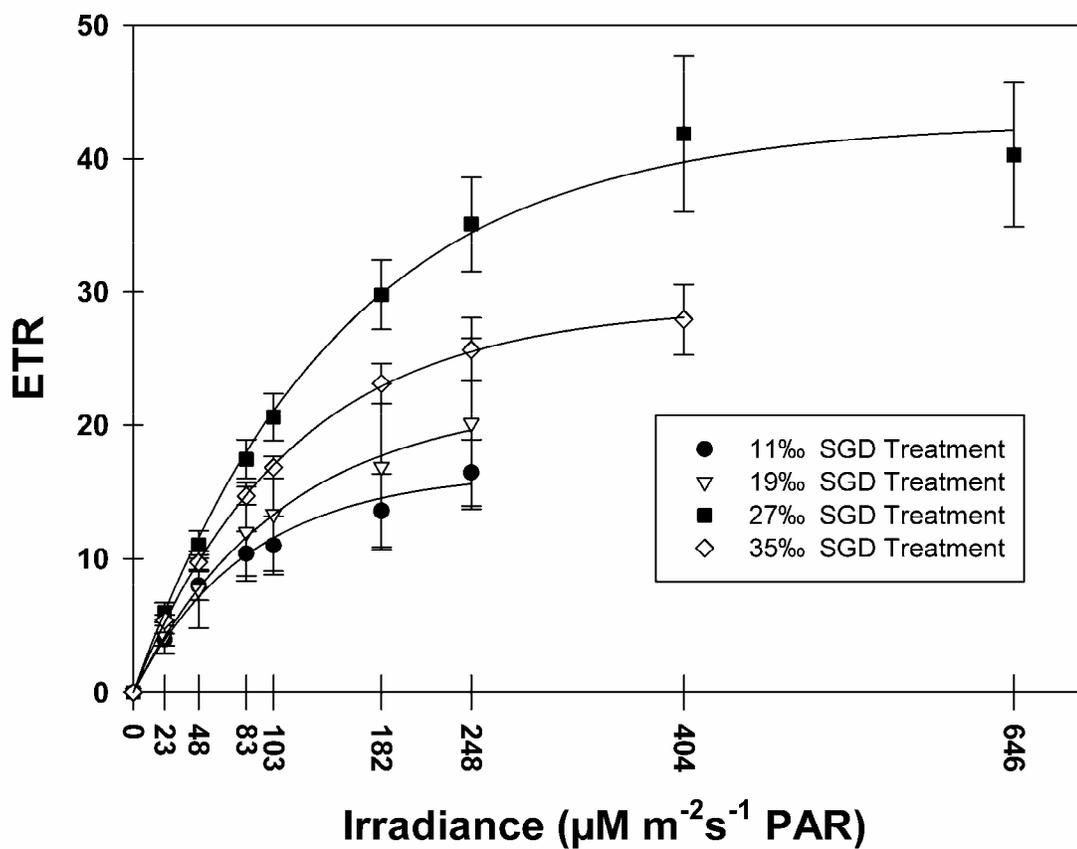
Figure 2.8. Rapid light curve (RLC) for replicates of *Gracilaria coronopifolia* in trial two. The mean ETR value of each treatment at given irradiance is shown. Error bars indicate ± 1 standard deviation of the mean. A non-linear regression line is fit to each treatment RLC ($R^2 > 0.99$ for all samples).
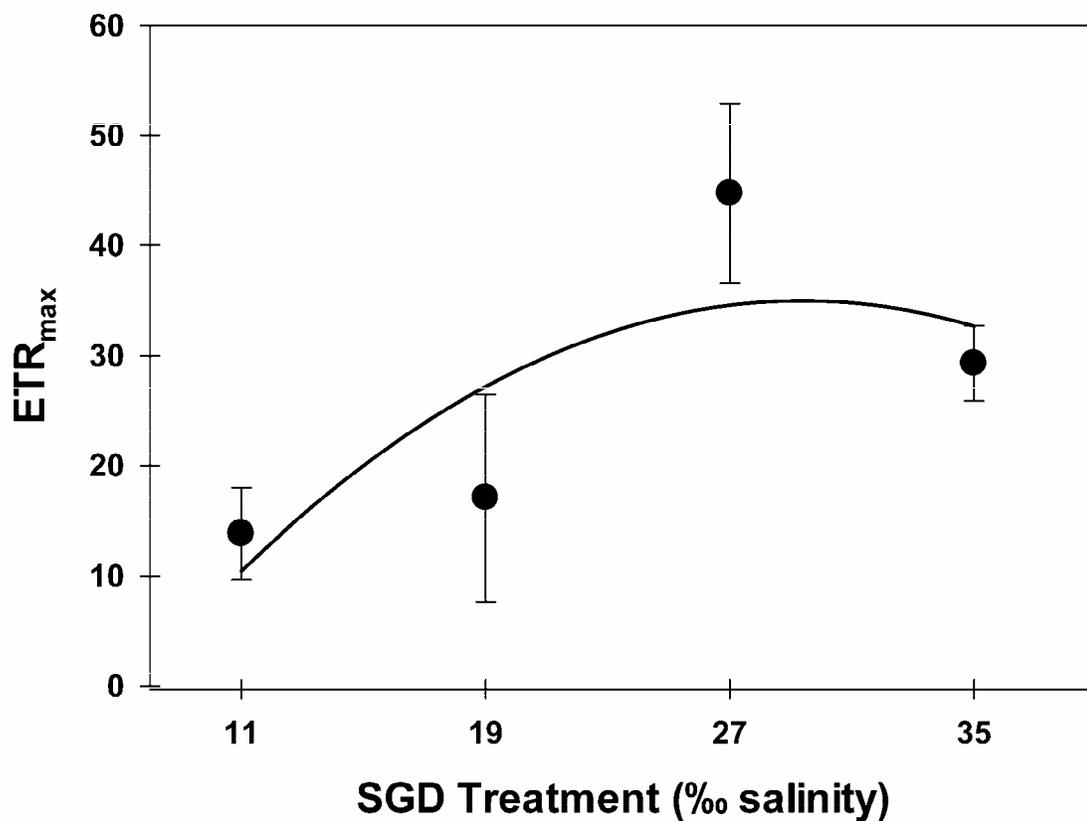
Figure 2.9. Mean ETR_{max} vs. SGD treatment of *Gracilaria coronopifolia* for trial two. The units of ETR_{max} are μmol e$^-$ m^{-2} s^{-1}. Error bars indicate $\pm$ 1 standard deviation of the mean. Solid line represents the best-fit line from quadratic regression (f= -27.7433 + 4.2737(x) -0.0728(x)^2 ; R^2=0.49, P=0.000).
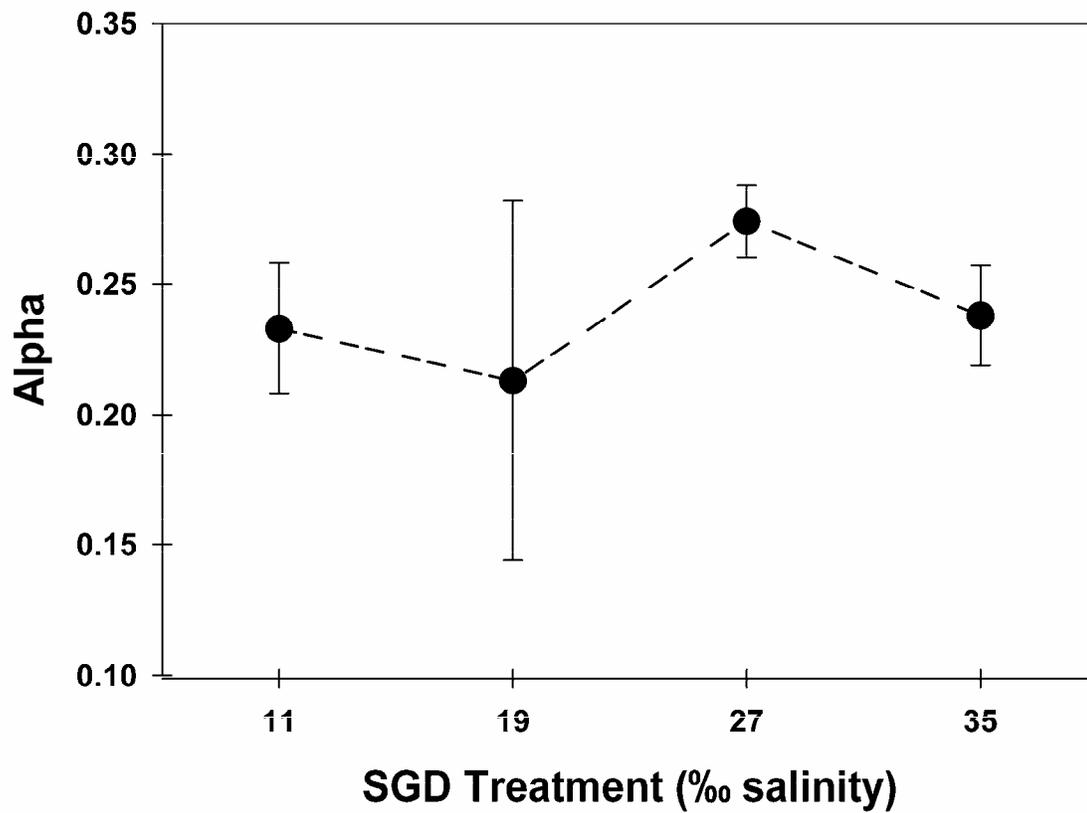
Figure 2.10. Mean alpha (α) vs. SGD treatment of *Gracilaria coronopifolia* for trial two. The units of alpha are (μmol e$^-$ m^{-2} s^{-1}) (μmol photon m^{-2}s^{-1})$^{-1}$. Error bars indicate $\pm$ 1 standard deviation of the mean.
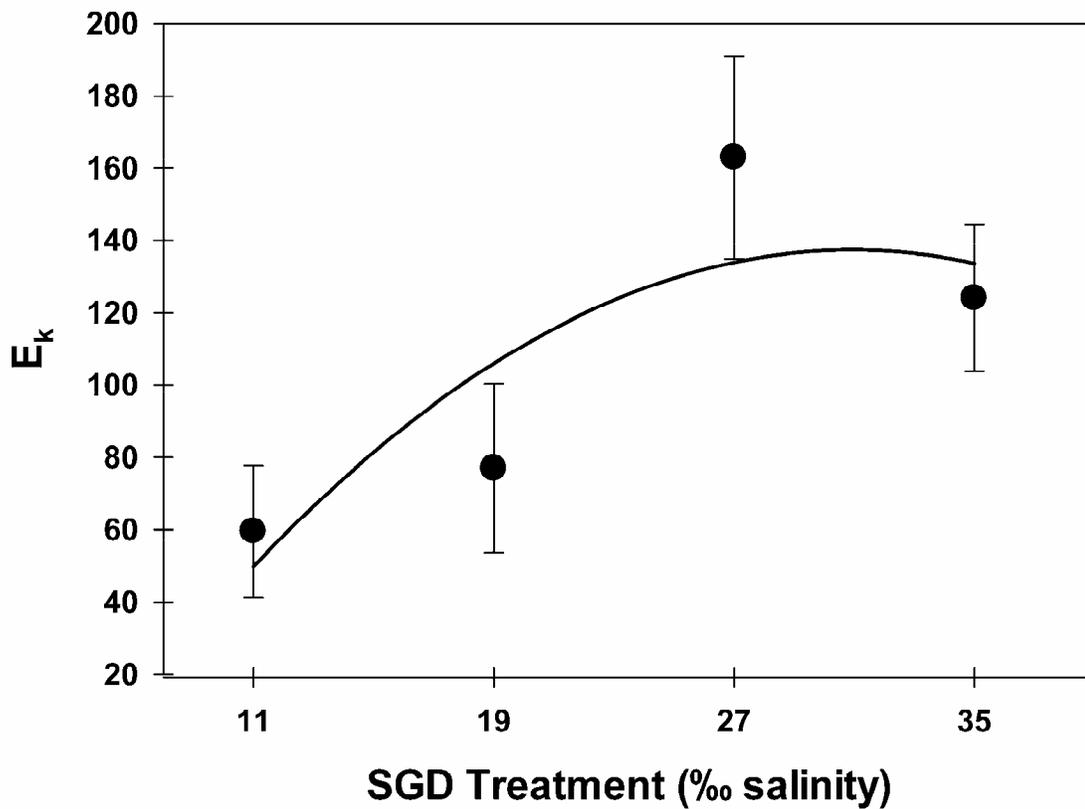
Figure 2.11. Mean E_k vs. SGD treatment of *Gracilaria coronopifolia* for trial two. The units of E_k are μmol photon $m^{-2} s^{-1}$ PAR. Error bars indicate ± 1 standard deviation of the mean. Solid line represents the best-fit line from quadratic regression (f= -73.4211 + 13.6310(x) - 0.2203(x)^2 ; R^2=0.57, P=0.000).

Literature Cited:

Burnett WC, Taniguchi M, Oberdorfer J (2001) Measurement and significance of the direct discharge of groundwater into the coastal zone. Journal of Sea Research 46: 109-116

Burnett WC, Bokuniewicz H, Huettel M, Moore WS, Taniguchi M (2003) Groundwater and pore water inputs to the coastal zone. Biogeochemistry 66: 3-33

Causey NB, Prytherch JP, McCaskill J, Humm HJ, Wolf FA (1946) Influence of environmental factors upon the growth of *Gracilaria confervoides*. Bulletin of Duke University Marine Station 3: 19-24

Chirapart A, Ohno, M (1993) Growth in tank culture of species of *Gracilaria* from the southeast Asian waters. Botanica Marina 36: 9-13

Choi H, Kim Y, Kim J, Lee S, Park E, Ryu J, Nam K (2006) Effects of temperature and salinity on the growth of *Gracilaria verrucosa* and *G. chorda*, with the potential for mariculture in Korea. Journal of Applied Phycology 18: 269-277

Corbett DR, Chanton J, Burnett W, Dillon K, Rutkowski C, Fourqurean JW (1999) Patterns of groundwater discharge into Florida Bay. Limnology and Oceanography 44: 1045-1055

D'Elia C, Webb K, Porter J (1981) Nitrate-rich groundwater inputs to Discovery Bay, Jamaica: A significant source of N to local coral reefs? Bulletin of Marine Science 31: 903-910

Dawes CJ, Orduña-rojas J, Robledo D (1999) Response of the tropical red seaweed *Gracilaria cornea* to temperature, salinity and irradiance. Journal of Applied Phycology 10: 419-425

DeBoer JA, Guigli HJ, Israel TL, D'Elia CF (1978) Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. Journal of Phycology 14: 261-266

Fong P, Zedler JB, Donohoe RM (1993) Nitrogen vs. phosphorus limitation of algal biomass in shallow coastal lagoons. Limnology and Oceanography 38: 906-923

Fong P, Boyer KE, Desmond JS, Zedler JB (1996) Salinity stress, nitrogen competition, and facilitation what controls seasonal succession of two opportunistic green macroalgae? Journal of Experimental Marine Biology and Ecology 206: 203-221

Garrison GH, Glenn CR, McMurtry GM (2003) Measurement of submarine groundwater discharge in Kahana Bay, O'ahu, Hawai'i. Limnology and Oceanography 48: 920-928

Gerung GS, Ohno M, Yamamoto H (1999) Growth rates and agar properties on some species of *Gracilaria Grev*. (Rhodophyta, Gigartinales) from Manado, Indonesia. Bulletin of Marine Sciences and Fisheries Kochi University: 9-14

Gessner F, Schramm W (1971) Salinity. Marine Ecology 1: 705-820

Giblin A, Gaines A (1990) Nitrogen inputs to a marine embayment: The importance of groundwater. Biodegradation 10: 309-328

Glenn EP, Moore D, Brown JJ, Tanner R, Fitzsimmons K, Akutigawa M, Napolean S (1998) A sustainable culture system for *Gracilaria parvispora* (Rhodophyta)

using sporelings, reef growout and floating cages in Hawaiʻi. Aquaculture 165: 221-232

Goldstein ME (1973) The reproductive biology and mariculture of *Gracilaria*. In Summary record of agar and agarophytes workshop. Atlantic Regional Office Fisheries Research Board of Canada., pp 34-41

Gomez I, Figueroa FL, Ulloa N, Morales V, Lovengreen C, Huovinen P, Hess S (2004) Patterns of photosynthesis in 18 species of intertidal macroalgae from southern Chile. Marine Ecology Progress Series 270: 103–116

Haglund K, Pedersén M (1993) Outdoor pond cultivation of the subtropical marine red alga *Gracilaria tenuistipitata* in brackish water in Sweden. Growth, nutrient uptake, co-cultivation with rainbow trout and epiphyte control. Journal of Applied Phycology 5: 271-284

Hanisak MD, Samuel MA (1987) Growth-rates in culture of several species of *Sargassum* from Florida, USA. Hydrobiologia 151: 399-404

Hernandez I, Peralta G, PerezLlorens JL, Vergara JJ, Niell FX (1997) Biomass and dynamics of growth of *Ulva* species in Palmones river estuary. Journal of Phycology 33: 764-772

Herrera-Silveira J (1998a) Nutrient-phytoplankton production relationships in a groundwater-influenced tropical coastal lagoon. Aquatic Ecosystem Health & Management 1: 373-385

Herrera-Silveira J (1998b) Nutrient-phytoplankton production relationships in a groundwater-influenced tropical coastal lagoon Aquatic Ecosystem Health & Management 1

Herrera-Silveira JA, Ramírez RJ, Zaldivar RA (1998) Overview and characterization of the hydrology and primary producer communities of selected coastal lagoons of Yucatán, México. Aquatic Ecosystem Health and Management 1: 353-372

Herrera-Silveira JA, Medina-Gomez I, Colli R (2002) Trophic status based on nutrient concentration scales and primary producers community of tropical coastal lagoons influenced by groundwater discharges. Hydrobiologia 475: 91-98

Herrera-Silveira JA, Morales-Ojeda SM (2009) Evaluation of the health status of a coastal ecosystem in southeast Mexico: Assessment of water quality, phytoplankton and submerged aquatic vegetation. Marine Pollution Bulletin 59: 72-86

Hoyle MD (1975) The literature pertinent to the red algal genus *Gracilaria* in Hawai'i. Marine Agronomy Program, University of Hawai'i, Honolulu

Hoyle MD (1976) Autecology of ogo (*Gracilaria bursapastoris*) and limu manauea (*G. coronopifolia*) in Hawai'i with special emphasis on *Gracilaria* species as indicators of sewage pollution. Ph. D. Botany, Honolulu

Hoyle MD (1978) Reproductive phenology and growth rates in two species of *Gracilaria* from Hawai'i. Journal of Experimental Marine Biology and Ecology 35: 273-283

Hunt JW, (1979) Cooperative *Gracilaria* project : environmental factors affecting the growth of *Gracilaria bursapastoris* (ogo) and *Gracilaria coronopifolia* (limu manauea). Marine Option Program, Windward Community College, Kaneohe HI

Hwang D-W, Kim G, Lee Y-W, Yang H-S (2005) Estimating submarine inputs of groundwater and nutrients to a coastal bay using radium isotopes. Marine Chemistry 96: 61-71

Imai M, Katayama N, Yamaguchi Y (1997) Effects of salinity on growth, photosynthesis and respiration in a freshwater alga *Rhizoclonium riparium* (Chlorophyceae, Cladophorales). Phycological Research 45: 233-237

Israel A, Martinez-Goss M, Friedlander M (1999) Effect of salinity and pH on growth and agar yield of *Gracilaria tenuistipitata* var. *liui* in laboratory and outdoor cultivation. Journal of Applied Phycology 11: 543-549

Johannes RE (1980) The ecological significance of the submarine discharge of groundwater. Marine Ecology Progress Series 3: 365-373

Johnson AG, Glenn CR, Burnett WC, Peterson RN, Lucey PG (2008) Aerial infrared imaging reveals large nutrient-rich groundwater inputs to the ocean. Geophysical Research Letters 35

Jones WE (1959a) Experiments on some effects of certain environmental factors on *Gracilaria verrucosa* (Hudson) Papenfuss. Journal of the Marine Biological Association of the United Kingdom 38: 153-167

Jones WE (1959b) The growth and fruiting of *Graciliaria verrucosa* (Hudson) Papenfuss. Journal of the Marine Biological Association of the United Kingdom 38: 47-56

Jordan AJ, Vadas RL (1972) Influence of environmental parameters on intraspecific variation in *Fucus vesiculosus*. Marine Biology 14: 248-&

Kamer K, Fong P (2000) A fluctuating salinity regime mitigates the negative effects of reduced salinity on the estuarine macroalga, *Enteromorpha intestinalis*. Journal of Experimental Marine Biology and Ecology 254: 53-69

Kamer K, Fong P (2001) Nitrogen enrichment ameliorates the negative effects of reduced salinity on the green macroalga *Enteromorpha intestinalis*. Marine Ecology-Progress Series 218: 87-93

Kay E, Lau L, Stroup E, Dollar S, Fellows D, Young R (1977) Hydrologic and ecologic inventories of the coastal waters of West Hawaiʻi. Technical Report-Water Resources Research Center, University of Hawaiʻi (USA)

Kim CS, Humm, HJ (1965) The red alga, *Gracilaria foliifera* with special reference to cell wall polysaccharides. Bulletin of Marine Science 15: 1036-1050

Kim DH, Mendoza H, Wilkomirsky W, Ohme U, Overdick L and Espinoza R (1969) Resultados preliminares sobre crecimiento de *Gracilaria lemanaeformis* y contribucion a la industrializacion de esta alga y otras agarofitas In Hoyle 1975, pg. 220

Kim DH (1970) Economically important seaweeds in Chili-I *Gracilaria*. Botanica Marina 13: 140-162

Kirst GO (1990) Salinity tolerance of eukaryotic marine algae. Annual Review of Plant Physiology and Plant Molecular Biology 41: 21-53

Koch E, Lawrence J (1987) Photosynthetic and respiratory responses to salinity changes in the red alga *Gracilaria verrucosa*. Botanica Marina 30: 327-329

Lapointe BE (1987) Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. Marine Biology 93: 561-568

Lapointe BE (1997) Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnology and Oceanography 42: 1119-1131

Lapointe BE, Littler MM, Littler DS (1987) A comparison of nutrient-limited productivity in macroalgae from a Caribbean barrier reef and from a mangrove ecosystem. Aquatic Botany 28: 243-255

Lapointe BE, Littler MM, Littler DS (1992) Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters. Estuaries 15: 75-82

Lapointe BE, Rice DL, Lawrence JM (1984) Responses of photosynthesis, respiration, growth and cellular constituents to hypo-osmotic shock in the red alga *Gracilaria tikvahiae*. Comparative Biochemistry and Physiology Part A: Physiology 77: 127-132

Larned ST (1998) Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae. Marine Biology 132: 409-421

Leote C, Ibánhez J, Rocha C (2008) Submarine groundwater discharge as a nitrogen source to the Ria Formosa studied with seepage meters. Biogeochemistry 88: 185-194

Littler MM, Littler DS, Titlyanov EA (1991) Comparisons of N-limited and P-limited productivity between high granitic islands versus low carbonate atolls in the Seychelles archipelago - a test of the relative-dominance paradigm. Coral Reefs 10: 199-209

McGlathery KJ (1992) Physiological controls on the distribution of the macroalga

   Spyridea hypnoides - patterns along a eutrophication gradient in Bermuda. Marine

   Ecology Progress Series 87: 173-182

Moore WS (1996) Large groundwater inputs to coastal waters revealed by ^{226}Ra

   enrichments. Nature 380: 612-614

Moore WS, John HS, Karl KT, Steve AT (2009) Submarine Groundwater Discharge. In:

   editors? <u>Encyclopedia of Ocean Sciences</u>. Academic Press, Oxford, pp 4530-4537

Nagler PL, Glenn EP, Nelson SG, Napolean S (2003) Effects of fertilization treatment

   and stocking density on the growth and production of the economic seaweed

   Gracilaria parvispora (Rhodophyta) in cage culture at Molokai, Hawai'i.

   Aquaculture 219: 379-391

Navarro-Angulo L, Robledo D (1999) Effects of nitrogen source, N:P ratio and N-pulse

   concentration and frequency on the growth of *Gracilaria cornea* (Gracilariales,

   Rhodophyta) in culture. Hydrobiologia 398: 315-320

Nelson SG, Tsutsui RN, Best BR (1980) Evaluation of seaweed mariculture potential of

   Guam I. Ammonium uptake by and growth of 2 species of *Gracilaria*

   (Rhodophyta), 0379-5403, University of Guam

Otaga F, Matsui T, (1965) Photosynthesis in several marine plants in relation to carbon

   dioxide supply, light and inhibitors. Japanese Journal of Botany 19: 83-97

Paerl HW (1997) Coastal eutrophication and harmful algal blooms: Importance of

   atmospheric deposition and groundwater as "new" nitrogen and other nutrient

   sources. Limnology and Oceanography 42: 1154-1165

Peckol P, DeMeo-Anderson B, Rivers J, Valiela I, Maldonado M, Yates J (1994) Growth, nutrient uptake capacities and tissue constituents of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae* related to site-specific nitrogen loading rates. Marine Biology 121: 175-185

Pickering TD, Gordon ME, Tong LJ (1993) Effect of nutrient pulse concentration and frequency on growth of *Gracilaria chilensis* plants and levels of epiphytic algae. Journal of Applied Phycology 5: 525-533

Pongkijvorasin S, Roumasset J, Duarte T, Burnett K (2008) Renewable Resource Management with Stock Externalities: Coastal Aquifers and Submarine Groundwater Discharge. University of Hawai'i at Manoa, Department of Economics Honolulu, pp 30

Ryder E, Nelson SG, McKeon C, Glenn EP, Fitzsimmons K, Napolean S (2004) Effect of water motion on the cultivation of the economic seaweed *Gracilaria parvispora* (Rhodophyta) on Molokai, Hawai'i. Aquaculture 238: 207-219

Santelices B, Varela D (1993) Intra-clonal variation in the red seaweed G*racilaria chilensis*. Marine Biology 116: 543-552

Simmons GM (1992) Importance of submarine groundwater discharge (SGWD) and seawater cycling to material flux across sediment water interfaces in marine environments. Marine Ecology Progress Series 84: 173-184

Simon C, Gall EA, Levavasseur G, Deslandes E (1999) Effects of short-term variations of salinity and temperature on the photosynthetic response of the red alga *Grateloupia doryphora* from Brittany (France). Botanica Marina 42: 437-440

Smith JE, Hunter CL, Conklin EJ, Most R, Sauvage T, Squair C, Smith CM (2004) Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on O'ahu, Hawai'i. Pacific Science 58: 325-343

Smith JE, Runcie JW, Smith CM (2005) Characterization of a large-scale ephemeral bloom of the green alga *Cladophora sericea* on the coral reefs of West Maui, Hawai'i. Marine Ecology Progress Series 302: 77-91

Troell M, Halling C, Nilsson A, Buschmann AH, Kautsky N, Kautsky L (1997) Integrated marine cultivation of *Gracilaria chilensis* (Gracilariales, Rhodophyta) and salmon cages for reduced environmental impact and increased economic output. Aquaculture 156: 45-61

Tsai C-C, Chang J-S, Sheu F, Shyu Y-T, Yu AY-C, Wong S-L, Dai C-F, Lee T-M (2005) Seasonal growth dynamics of *Laurencia papillosa* and *Gracilaria coronopifolia* from a highly eutrophic reef in southern Taiwan: temperature limitation and nutrient availability. Journal of Experimental Marine Biology and Ecology 315: 49-69

Valiela I, Costa J, Foreman K, Teal JM, Howes B, Aubrey D (1990) Transport of groundwater-borne nutrients from watersheds and their effects on coastal waters. Biogeochemistry 10: 177-197

Valiela I, Foreman K, LaMontagne M, Hersh D, Costa J, Peckol P, DeMeo-Andreson B, D'Avanzo C, Babione M, Sham C-H, Brawley J, Lajtha K (1992) Couplings of watersheds and coastal waters: Sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries and Coasts 15: 443-457

Valiela I, Collins G, Kremer J, Lajtha K, Geist M, Seely B, Brawley J, Sham CH (1997a) Nitrogen loading from coastal watersheds to receiving estuaries: New method and application. Ecological Applications 7: 358-380

Valiela I, McClelland J, Hauxwell J, Behr PJ, Hersh D, Foreman K (1997b) Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnology and Oceanography 42: 1105-1118

Wakibia JG, Anderson RJ, Keats DW (2001) Growth rates and agar properties of three gracilarioids in suspended open-water cultivation in St. Helena Bay, South Africa. Journal of Applied Phycology 13: 195-207

Wong S-L, Chang J (2000) Salinity and light effects on growth, photosynthesis, and respiration of *Grateloupia filicina* (Rhodophyta). Aquaculture 182: 387-395

Wu CY, Li RZ, Lin GH, Wen ZC, Dong LF, Zhang JP, Huang XH, Wei SQ, Lan GB (1993) Some aspects of the growth of *Gracilaria tenuisitipitata* in pond culture. Hydrobiologia 261: 339-343

Yang Y-F, Fei X-G, Song J-M, Hu H-Y, Wang G-C, Chung IK (2006) Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. Aquaculture 254: 248-255

Yokoya NS, Kakita H, Obika H, Kitamura T (1999) Effects of environmental factors and plant growth regulators on growth of the red alga *Gracilaria vermiculophylla* from Shikoku Island, Japan. Hydrobiologia 398/399: 339-347

Zablackis E (1986) Responses to salinity in the red algae *Gracilaria* and *Grateloupia*. Botany Department. Thesis (M. S.). University of Hawaiʻi

Zablackis E (1987) The effect of salinity on growth rate and branch morphology in tank cultivated *Grateloupia filicina* (Rhodophyta) in Hawaiʻi. Aquatic Botany 27: 187-193

Zhou Y, Yang H, Hu H, Liu Y, Mao Y, Zhou H, Xu X, Zhang F (2006) Bioremediation potential of the macroalga *Gracilaria lemaneiformis* (Rhodophyta) integrated into fed fish culture in coastal waters of north China. Aquaculture 252: 264-276