TESTING LINKS AMONG EUTROPHICATION, BLOOM ALGAE, AND GREEN TURTLE FIBROPAPILLOMATOSIS

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Abstract

Fibropapillomatosis (FP) is a tumor forming disease which poses a major threat to green turtles (*Chelonia mydas*). Increasing evidence suggests that FP results from the proliferative growth of the herpes virus. Hawaiian green turtles have shifted their diet from native to alien invasive algae, especially in regions with high anthropogenic impacts result in blooms of these invasive species. Some invasive algae appear to store excess nitrogen as arginine, which is an essential component of envelope for this virus. Growth experiments of algae with nutrient enrichment and tissue analyses for field collected algae indicated that excess nutrient input can increase algal growth rates, and algal tissue arginine levels are higher in eutrophic regions than from tissues collected in less impacted regions. Thus, anthropogenic land-based sources of pollution such as elevated nutrient inputs appear to negatively impact marine hervibores by stimulating a nutrient storage metabolism of bloom species of marine plants.

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Chapter 1. Literature Review

Introduction

Anthropogenic nutrient enrichment and loss of herbivores are considered the driving force of the phase shift in coral reef ecosystems, with fast growing algae overtaking corals and dominating the benthic habitats (Lapointe, 1997, 1999; Littler & Littler, 1984; Smith et al., 2001, 2002, Stimson et al., 2001). The consequences of this phase shift from coral to algal dominance include possible loss of palatable foods for herbivorous animals, loss of suitable habitats, and decreased biodiversity (Hughes, 1994; McCook, 1999; Smith et al., 2002). In Hawai‘i, phase shifts often involve blooms of native and non-native invasive algae (Smith et al. 2002), especially in regions with excess nutrient inputs which accelerates growth of these algae (Dailer et al., 2010). Invasive algae have significant negative economic and ecological impacts on native marine ecosystems (Vermeij et al., 2009; Williams and Smith, 2007).

The green turtle, *Chelonia mydas*, is one of the largest herbivores in marine ecosystems and, as a species, is distributed throughout tropical and subtropical seas. This is the most common sea turtle species found in Hawai‘i (Balazs, 1980), even though it is listed as endangered by the International Union for the Conservation of Nature as well as threatened in 1978 under the U.S. Endangered Species Act and throughout its Pacific Range (Balazs and Chaloupka, 2004).

Juvenile green turtles are pelagic and thought to be omnivores. They recruit to benthic foraging grounds and become herbivorous when they reach about 35 cm straight carapace length (about 6 years of age) in Hawai‘i and Australia (Balazs and Chaloupka,

2004; Bjorndal, 1997). Pacific populations mainly feed on algae, while Caribbean populations feed almost solely on the sea grass *Thalassia testudinum*, also known as turtle grass (Gulko and Eckert, 2004).

Adults spend almost their entire lifespans in the foraging grounds (Bjorndal, 1999), thus these foraging grounds are critical for their conservation. The main Hawaiian Islands (MHI) contain numerous feeding grounds for the green turtles. Since 1950, alien invasive algae have caused serious problems to the coastal ecosystems in Hawai‘i, especially in regions with excess anthropogenic nutrient input (Smith et al, 2002; Dailer et al., 2010). Hawaiian green turtles living in such regions have shifted their diets from native to alien invasive algae (Russell and Balazs, 2009). More studies are needed to evaluate how the diet shifts to alien algae affect the status of Hawaiian green turtles, as a relationship between eutrophication, alien algae in diet and the onset of fibropapillomatosis has been proposed (Van Houtan et al., 2010).

Diet of green turtles

Diet in Hawai‘i

There are about 400 species of red algae (Abbott, 1999; Huisman et al., 2007), about 170 species of green and brown algae (Abbott and Huisman, 2004) and three species of seagrass in Hawai‘i (Russell et al., 2003; Huisman et al 2007). Over the 24 years of their study, Russell and Balazs (2000) found that more than 275 species of algae, seagrass and blue-green algae are associated (either eaten or attached) with green turtles. An early study of nearly 2,000 green turtles throughout the Hawaiian Archipelago during 1972 and 1980 revealed that turtles grazed on 56 algal species, one seagrass and nine invertebrates (Balazs, 1980). Despite the large number eaten, only nine species of algae

make up the majority of their diet; *Pterocladia* (*Pterocladiella*) *capillacea* and *Amansia glomerata* in the MHI *Codium edule, Codium arabicum, Codium phasmaticum* and *Ulva fasciata* (*U. lactuca*) both in MHI and the North Western Hawaiian Islands (NWHI), and *Caulerpa racemosa, Spyridia filamentosa* and *Turbinaria ornata* in NWHI. *Ulva reticulata* and *Ahnfeltia (Ahnfeltiopsis) concinna* are also eaten when turtles were in waters around O‘ahu. One native seagrass, *Halophila hawaiiana*, often contributes significant amount to the diet of the Hawaiian green turtles (Balazs et al., 1987; Arthur and Balazs, 2008). A second native seagrass, *Halophila decipiens*, was first reported in 2001 and was considered to be an alien species until reexamination of the green turtle stomach samples collected between 1978 and 2002 revealed that Hawaiian green turtles foraged on *H. decipiens* as early as 1998 (Russell et al., 2003). That study also found that *H. hawaiiana* and *H. decipiens* do not co-occur in the turtle stomach samples or samples collected in the field.

The diet of green turtles appears to strongly reflect what macroalgae are common to abundant in their habitat. For instance, green turtles grazing in Waikīkī (Miya and Balazs, 1993) had eaten *U. fasciata* (*U. lactuca*)*, P. capillacea, Hypnea musciformis, S. filamentosa, Gelidium pusillum* and *Sargassum* sp., algal species that were common in Waikīkī (Doty, 1969). Further, stomach samples collected from three dead turtles found in the area consisted of *U. reticulata, U. fasciata, P. capillacea*, and *H. musciformis*. Habitat surveys indicated that *U. reticulata and U. fasciata* were highly abundant and that floating plants of *Codium* spp., *Sargassum* spp. and *H. musciformis* were also collected from the study site. In sum, this study supports the view that the green turtle grazes on common to abundant algae in localized regions.

In summer of 1989, large numbers of green turtle feces washed ashore in Kualoa Beach Park, O‘ahu (Balazs et al., 1993). Those fecal pellets mostly consisted of partially digested *C. edule* and *A. glomerata*, adding two more taxa to the list of readily consumed algae.

By 2008, the diet of Hawaiian green turtles was dominated by red algae, which were found in 99.5 % of the stomach samples (Arthur and Balazs 2008). When classified by morphological characteristics, complex branching macroalgae are the major diet items at most of the study sites except two Hawai‘i Island sites (both on the Kona side), where turf and filamentous algae dominate the diet. In 78.9 % of the stomach samples, only one food accounted for more than 50 % of the relative volume. Those included *Halophila* sp., *Acanthophora* sp., *Centroceras* sp., *Gelidiella* sp., *Gracilaria* sp., *Hypnea* sp., *Pterocladiella* sp., *Amansia glomerata, Cladophora* sp., *Codium* sp. and *Dictyosphaeria* sp.

Diet items in other regions

Stomach samples have been analyzed for other green turtles populations in the world, and their diets tend to incorporate different food items from those of Hawaiian green turtles. Thirty-eight species of Rhodophyta, 21 species of Chlorophyta, and 10 species of phaeophytes were identified from the stomach samples of 518 green turtles in Heron Island, Australia (Forbes, 1993). Green turtles in Moreton Bay, Australia, feed on seagrasses *Zostera caprocorni* and *Halodule uninervis* as well as *Halophila ovalis* and algae *Gracilaria* sp. and *Hypnea* sp. (Brand-Gardner et al., 1999). Turtles in Moreton Bay also feed on the fruits of Grey Mangrove (*Avicennia marina*) (Read and Limpus, 2002).

As mentioned earlier, the diet of Caribbean green turtles is mostly constituted of *Thalassia testudinum*, which accounts for 87 % of the diet by dry weight (Bjorndal, 1995). A seagrass, *Syringodium filiforme* is also often found in the stomach samples of Caribbean turtles (Mortimer, 1981, 1995).

Many studies found that Rhodophyta are the most common diet items for the green turtles feeding on algae. Among the 40 stomach samples of juvenile green turtles in Indian River County, Florida, red algae are found in 56.4 % of the samples and constitute 89.9 % of the sampled volume (Gilbert, 2005).

Diets of green turtles in other sites than Hawai‘i are summarized in Table 1.1, and reveal similarities in their food items across different habitats.

Food selectivity

The diet of maturing sea turtles is determined by the local food availability. After juveniles return from pelagic portion of their life, they settle into living and grazing in a much smaller range. For example, the most common diet items of juvenile green turtles in Indian River County, Florida, are *Hypnea* spp., which are also the most abundant species in their habitat (Gilbert, 2005). But other studies also found that green turtles show food selectivity. Turtles from the southern part of the Nicaraguan feeding grounds preferably graze on red algae *Hypnea musciformis* and *Gracilaria* spp., which are not abundant in the habitat (Mortimer, 1981). In addition, *Halimeda* spp. consists 20.6 % of the biomass in the habitat but accounts for only 0.2 % of the stomach samples. Stomach samples of the turtles from Oman contain relatively large volume of *Sargassum illicifolium* (Ross, 1985). However, two other *Sargassum* spp., *S. vulgare* and *S.*

grandifolium commonly occur in the habitat but are not found in the stomach sample. Hawaiian green turtles also show selective feeding. At Palaʻau, on the Island of Molokaʻi, *Asparagopsis taxiformis* and *Galaxaura rugosa,* the two common marine plants, are never found in turtle diets, even in trace amounts (Balazs et al. 1987). A summary of food selectivity studies is summarized in Table 1.2.

Only a few studies investigate the effects of nutritional content on green turtles' food selectivity. A classic example is the case of the Caribbean green turtles feeding on *Thalassia testudinum*; grazing selectively recrops young blades with high nutrients (Bjorndal, 1980). Selective feeding on younger *Thalassia* blades is also reported (Mortimer, 1981, 1995). Turtles seem to avoid older parts of the blades laden with epiphytes; old or dead *Thalassia* blades account for only 7.8 % of stomach samples compared to *Thalassia* biomass in the vegetation of which 56 % is older blades. Green turtles in Moreton Bay, Australia, selectively consume *Gracilaria* sp., which has the highest nitrogen and lowest fiber contents (Table 1.2) (Brand-Gardner et al., 1999). On the other hand, *Z. capricorni*, the least selected item, has the lowest nitrogen and highest fiber contents. The gross energy, however, is highest in *Z. capricorni* and lowest in *Gracilaria sp.* Gilbert (2005) reports that *Hypnea* spp*.,* which is the most common diet item among the green turtles in Florida, has the highest levels of protein and gross energy (GE), and the lowest acid detergent fiber (ADF, a measure of indigestible carbohydrate). On the other hand, brown algae and *Bryothamnion seaforhi*, both of which are avoided by turtles, can be low in protein, GE and high in ADF. These results suggest that green turtles may select foods based on the food's nutritional content.

Effects of diet on growth and reproduction

The quality of diet directly affects the growth rate and reproductive output of organisms. Throughout the Hawaiian Archipelago, the growth rates of the green turtles significantly differ among the feeding grounds (Balazs, 1979; Balazs, 1995; Balazs and Chaloupka, 2004). At Kau, on the Island of Hawai‘i, the mean growth rate of four recaptured turtles is 0.44 cm m^{-1}. Their major food is *Pterocladiella capillacea*. Turtles at Bellows Beach, O‘ahu, mainly ate *Codium* spp. and *Ulva fasciata* (*U. lactuca*), and maintain a mean growth rate of 0.2 cm m^{-1} (n= 2). At French Frigate Shoals in NWHI, the mean growth rate of 19 turtles is 0.08 cm m^{-1}, and their main foods are *Caulerpa racemosa*, *Codium* spp. and *Turbinaria ornata*. Turtles at Lisianski Island grow at a mean rate of 0.13 cm m^{-1} and their major foods are *C. racemosa* and *T. ornata*. At Kure and Midway Atolls, the mean growth rate of nine turtles was 0.1 cm m^{-1}. Turtles at these atolls eat invertebrates (*Velela*, *Ianthina* and *Physalia*) as well as *Codium edule* and *Spyridia filamentosa*. Table 1.3 summarizes the growth rates and the major diets at different sites in Hawai‘i and illustrates that the differences in growth rates result from differences in the available food sources in those habitats (Balazs, 1995). One of their major food items in MHI, *P. capillacea* is rare in NWHI, while the principal food items in NWHI, *C. racemosa*, *T. ornata*, and *S. filamentosa*, are commonly found in MHI but not eaten by turtles living in the MHI. This pattern would suggest that turtles in NWHI eat these three species because more preferable foods such as *P. capillacea* are absent from their habitats. Abbott (1989) reports that those preferable algae were less available in NWHI. The site specific growth rates of green turtles found in these studies are a clear example of how the community of plants in a feeding ground may affect the status of this

animal.

Captive green turtles generally grow faster because they are fed animal food such as fish or invertebrates with high nutrients (Bjorndal, 1985). They can reach sexual maturity as early as nine years of age (Bjorndal, 1995), while the Hawaiian green turtles need 35-40 years in MHI and more than 50 years in NWHI (Midway Atoll) (Balazs and Chaloupka, 2004), although some of these may be substantial underestimates (K. Van Houtan, pers. comm.). Captive turtles also have the capacity to breed annually with more nests than the wild turtles, while wild turtles nest with a three or four-year intervals (Bjorndal, 1985). Bjorndal (1985, 1995) attributed the slow growth rate and low reproductive output of the wild turtles to the low quality of their natural food. Despite having a cellulolytic gut microflora that can produce volatile fatty acid for their energy source to compensate their low quality diet (Bjorndal, 1980; Bjorndal et al., 1991) wild turtles appear to be nutrient limited and cannot achieve their maximum potential reproductive output.

Alien algae in Hawai‘i

Problems of alien algae

Since 1950, more than 19 species of algae have been introduced to the Hawaiian waters, either intentionally or by accident (Russell and Balazs, 1994a; Smith et al., 2002). Not all of the alien species became invasive, but some have been very successful and have spread rapidly throughout the islands, outcompeting native algae and smothering coral reefs. Their long-term effects include loss of biodiversity and alteration of the community structure in coral reef ecosystems, and reduced intrinsic value of the coastal

area (Smith et al., 2002). The five most successful invasive alien algae are *Acanthophora spicifera*, *Avrainvillea amadelpha*, *Gracilaria salicornia*, *Hypnea musciformis* and the *Kappaphycus*/*Eucheuma* complex of at least two species in two closely related genera. The traits that allow these taxa to be invasive have been examined in several ways for several species (Smith et al., 2002; Vermeij et al., 2009a, 2009b). However, simple growth rates in controlled conditions remain unavailable.

Alien algae in Hawaiian green turtle diets

Of the 19 alien algal species introduced to Hawaii, Hawaiian green turtles have incorporated seven into their diets (Russell and Balazs, 1994a). These seven species are all red algae: *Acanthophora spicifera*, *Hypnea muciformis, Gracilaria salicornia*, *Gracilaria tikvahiae*, *Eucheuma denticulatum*, *Kappaphycus alvarezii* and *Kappaphycus striatum*.

Balazs et al. (1987) reports that *Acanthophora spicifera* was an important food item for Hawaiian green turtles along with the native algal food items identified earlier. In 10 of the 12 stomach contents he obtained in Kawela Bay, O‘ahu, more than 99 % were consisted of *A. spicifera*. A more recent study (Arthur 2008) reports that *A. spicifera* was present in 49.2 % of the stomach samples.

Three years after its introduction to Hawai‘i in 1974, *H. muciformis* was part of the diet of a green turtle caught in Kāne‘ohe Bay, representing 80 % of the wet mass (Russell and Balazs, 1994b). The spread of *H. muciformis* from Kāne‘ohe Bay to other places in the Hawaiian Islands could be tracked using diet samples from turtles (Russell and Balazs, 1994a). As with *A. spicifera, H. muciformis* make up 99-100 % of the stomach

samples of some turtles. *H. muciformis* could be found in 11 %, and *A. spicifera* in 20 % of the 754 samples. On average, these algae constitute 27.2 % and 34.3 % of the wet weight of the samples, respectively.

For the other five species, the number of samples in which these aliens were present was still low (one to seven turtles).

Ecology and impact of the three alien algae most common in the green turtle diet

Green turtles in Kāne'ohe Bay appear to have shifted their diet to include more alien species over the past decades (Russell and Balazs, 2009). The three most common foods are *Acanthophora spicifera, Hypnea muciformis* and *Gracilaria salicornia*, all of which are alien species to O'ahu. *A. spicifera* was unintentionally introduced to Hawai'i in the 1950's perhaps as barge fouling from Guam (Doty, 1961). This red alga is the most widespread and successful of the alien algae in Hawai'i (Smith et al., 2002). It has a strong ability to adapt to different conditions and invade wide variety of habitats, and has replaced many native algae such as *Laurencia nidifica*, *Hypnea cervicornis*, and *Chondoria* spp. (Huisman et al., 2007). The brittle branches of *A. spicifera* fragment easily, and the whole plant can be regenerated from a small fragment (M. Kawachi, unpub. data; Kilar and McLachlan, 1986).

H. musciformis was intentionally introduced from Florida to Kāne'ohe Bay in 1974 for carrageenan production (Russell and Balazs, 1994b). On the Island of Maui, thousands of tons of this red alga often wash up on the beaches and emit a foul smell as they decompose (Van Beukering and Cesar, 2004). Hotel and condominium owners in the Kihei area pay $50,000 each year to clean up the alga, and potentially lose $20 million

because of the reduced property values and occupancy rates (Van Beukering and Cesar, 2004). As reported for *A. spicifera, H. musciformis* also spreads quickly by fragmentation and has a strong ability to regenerate from small fragments.

G. salicornia was first found in Hilo Bay, the Island of Hawai‘i, in 1971 (Abbott 1999). This red alga was intentionally introduced to the Island of O‘ahu (Kāne‘ohe Bay and Waikīkī) for research on agar production in the late 1970’s (Russell and Balazs, 1994a; Smith et al., 2002). It forms large, dense mats and often overgrows corals and native algae such as the closely related *Gracilaria coronopifolia*. *G. salicornia* has wide ranging phenotypic acclimation to irradiance (Beach et al., 1997). Moreover, like *A. spicifera* and *H. muciformis*, it has a high growth rate, spreads widely by fragmentation, and easily regrows from tiny fragments. In Waikīkī, large amounts of drift *G. salicornia* are often found to accumulate on beaches after large swells (Smith et al., 2004; Huisman et al., 2007), which mar the scenery and negatively affect the tourist industry.

In light of the overfishing of reef fish and urchins combined with the regulations protecting green turtles, the green turtles emerge as one of the most likely herbivores that could control the abundance of invasive algae. Bringing the population sizes of green turtles back to healthy levels might help control and manage these invasive marine weeds.

Further, it seems unlikely that green turtles would contribute to the spread of the alien algae, as none of the epiphytic algae growing on their carapace or skin are alien species (Russell and Balazs, 1994b). Further, Russell and Balazs (1994b) observe no algal growth from the culturing of fecal pellets. These factors - lack of invasive weed fouling and complete digestion of food - should minimize the possibility that turtles

spread alien algae. However, green turtles are sloppy feeders, nipping numerous small fragments of algae with their beaks (Russell and Balazs, 2000). The three alien algae favored by green turtles have similar competitive strategies such as rapid growth rate and exceptional abilities to regenerate from small fragments (Smith et al., 2002, 2004). By scattering small fragments while eating, green turtles contribute to spread and increase the biomass of these alien algae at local scales. It remains unknown how much they eat versus how many fragments are formed as well as the persistence of grazing-generated fragments in the field.

Effects of alien algae on Hawaiian green turtles

Russell and Balazs (1994a) argue that alien algae may help sustain Hawaiian green turtles' population, because alien algae are supplying abundant foods and would be as nutritious as native species. They also recently stated that the nutritional contents of the three most common alien algae in the green turtle diet are similar to that of the native species and supplying the turtles with an abundant source of energy and protein (Russell and Balazs, 2009). These arguments, however, overemphasized positive aspects of the alien algae to green turtles. A study of nutritional contents of Hawaiian algae (Table 1.4) indicates that *A. spicifera* has the lowest protein value (2.6 ± 0.1 % dry weight) among the 16 macroalgal species studied (McDermid et al., 2007). The protein content of *G. salicornia* is also not very high (3.9 ± 0.4 % dry weight). Among the alien species, only *H. muciformis* has a protein value of over 10 % (11.1 ± 0.4 % and 11.6 ± 0.4 % dry weight from two sites), but it's protein content is still lower than the values of the native species such as *P. capillacea* (13.4 ± 0.3 % dry weight) and *A. glomerata* (12.3 ± 0.4 %

dry weight). Therefore, shifting diets from the native algae to these alien species could have negative effects on green turtles' fitness, even though the alien species are more abundant in their feeding habitats.

Another possible adverse effect of alien algae on Hawaiian green turtles is the loss of suitable habitat for activities including resting (Gulko and Eckert, 2004). Green turtles spend most of their time foraging and resting on the bottom with fine-grained sand or powdery silt (Balazs et al., 1987). Their resting sites are usually close to their foraging sites. Invasive alien species such as *G. salicornia*, *Kappaphycus* spp., and *E. denticulatum* form dense mats and cover these resting sites and disrupt this behavior, changing the quality and availability of these sites and potentially the health of the green turtles.

Fibropapillomatosis disease

Overview

Fibropapillomatosis (FP) is a tumor forming disease mainly found in the green turtles, but has also reported in other sea turtle species such as loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*) turtles (Herbst, 1994). This disease forms cutaneous and visceral lesions up to 30 cm in diameter (Brill et al., 1995; Herbst and Klein, 1995) and, although the lesions are benign themselves, they can impede the animal's movement, feeding activity or organ functions (Gulko and Eckert, 2003; Arthur et al., 2008). FP is the most common identified cause of green turtle strandings in Hawai‘i during 1982 and 2003 (Chaloupka et al., 2008). No effective treatment has been identified for turtles developing this disease (George, 1997), although FP afflicted turtles

can have intensive therapeutic methods applied, including surgical removal of tumors.

History

FP in green turtles was first observed in Florida in 1938 (George, 1997). The disease was first reported in Hawaiian green turtles in 1958, but the incidence increased dramatically in the 1980s (Balazs, 1991). By 1998, more than 50 % of the green turtles in Kāneʻohe Bay, Oʻahu and nearly 35 % of the Island of Molokaʻi were affected by this disease (Gulko and Eckert, 2003). FP prevalence is still high (54 % of 23 turtles captured) in Kāneʻohe Bay in 2003 (Arthur et al., 2008). Van Houtan et al. (2010) analyze the combined spatial and temporal variability of disease rates in Hawaiʻi and report that when all islands are grouped, the disease rate is highest in the mid 1990's and decline gradually thereafter. However, the time series of disease rates varies significantly among different regions, suggesting FP is caused locally.

Etiology of FP

Earlier studies of FP etiology focused on the parasitic trematodes and naturally occurring tumor promoting toxins (Dailey et al., 1992; Dailey and Morris, 1995; Aguirre et al., 1998; Landsberg et al., 1999; Arthur et al., 2008), but none of them are proved to be the direct cause of the disease. Increasing evidence suggests that a herpes virus is the primary cause of FP (Jacobson et al., 1991; Herbst, 1994; Herbst et al. 1995; Herbst et al., 1998; Lackovish et al., 1999; Quackenbush et al., 2001). However, herpes virus DNA is also found in the green turtles without FP (Quackenbush et al., 2001), suggesting that multiple factor may be involved in the tumor development (Arthur et al, 2007) or that the

virus has a latent period of residence in the host before rapid proliferation.

FP, Eutrophication and invasive algae

High FP incidence is often found in areas with urbanization of coastal zones both in Hawai‘i and elsewhere (Herbst, 1994; dos Santos et al., 2010; Van Houtan et al., 2010). FP is not observed in pelagic juvenile green turtles and is only found in turtles that have already recruited to the nearshore feeding grounds (Ehrhart, 1991; Aguirre et al., 1998; Van Houtan et al., 2010). Moreover, adults at their nesting grounds show lower disease rates (George, 1997). These findings indicate that the condition of their feeding ground has some causative effects on the development of this disease.

Coastal eutrophication is a primary cause of excessive growth of fleshy macroalgae (Dailer et al., 2012). In Hawai‘i, extreme eutrophication caused by anthropogenic sources of pollution (especially nitrogen) results in the rapid growth and large blooms of specific invasive algae (Dailer et al., 2010, 2012). Hawaiian green turtles' diet shifts from native to invasive algae in the last four decades (Russell and Balazs, 1994, 2009) would be the consequence of changes in the benthic algal community associated with the emergence of these invasive species. It is proposed that these invasive plants store excess nitrogen from the environment as arginine, which is a tetra-amine amino acid (Van Houtan et al., 2010). Arginine is critical for the growth of the herpes virus, as it is an essential component of the chaperone proteins that allow the virus to attach to new cell lines (Van Houtan et al., 2010). Results of an earlier, limited sampling suggest that invasive algae favored by Hawaiian green turtles have higher arginine content on some reef regions but lower in other regions (Table 1.4; McDermid et

al., 2007). A full interpretation of that study is limited as the sampling was not systematically conducted or with sample replication.

Blooming invasive algae in eutrophic region may provide green turtles with abundant food sources, but grazing on such invasive algae could expose turtles to elevated arginine and stimulate FP formation.

Objectives of this study

To assess the relationship among coastal eutrophication, blooming invasive algae, and green turtle FP, it is crucial to investigate the responses of these algae to excess nutrients. The objectives this study are 1) to study the physiological responses, namely the comparative growth rates and the photosynthetic parameters, of native and invasive algae favored by Hawaiian green turtles to experimentally increased nutrient inputs, and 2) to examine arginine accumulation and the source of tissue nitrogen for algae in eutrophic and less-impacted regions.

Appendix

Table 1.1. Marine plants commonly consumed by green turtles outside Hawai‘i.

Country	Location	Consumed plants	References
Australia	Heron Island	*Gelidiella* *Polysiphonia* *Laurencia* *Caulerpa* *Codium* *Enteromorpha* *Turbinaria*	Forbes (1993)
	Moreton Bay	*Gracilaria* sp. *Hypnea* sp. *Zostera capricorni* *Halophila ovalis* *Halodule uninervis*	Brand-Gardner et al. (1999)
		Halophila ovalis *Gracilaria cylindrica* *Hypnea spinella* *Grey mangrove fruits*	Read and Limpus (2002)
	Green Island, Great Barrier Reef	*Thalassia hemprichii* *Cymodocea* sp. *Halodule* sp. *Gracilaria* spp. *Gelidiella* sp. *Acanthophora* sp.	Fuentes et al. (2006)
Belize	Robinson Point	*Thalassia testudinum*	Searle (2003)
India	Gulf of Mannar Biosphere Reserve	*Gelidiella acerosa* *Thalassia hemprichii* *Halophila ovalis* *Halimeda macroloba* *Dictyota dichotoma*	Kannan and Rajagopalan (2004)
Nicaragua		*Thalassia testudinum* *Syringodium filiforme* *Hypnea musciformis* *Gracilaria* spp.	Mortimer (1981, 1995)
Oman	Masirha Channel	*Halophyla ovalis* *Halodule uninervis*	Ross (1985)

	Ra's Al Hadd	*Sargassum illicifolium* *Chaetomorpha aerea* *Hypnea* sp. *Gelidium* sp. *Nizamudinnia zanardinii* *Cladophoropsis javanica* *Halophila ovalis* *Halodule uninervis*	Ferreira et al. (2006)
Uruguay		*Ulva lactuca* *Chondracanthus teedei* *Polysiphonia* sp. *Pterocladiella capillacea*	Calvo et al. (2003)
U.S.A.	Indian River County, FL	*Hypnea* spp. *Chondria* spp. *Gelidium* spp. *Polysiphonia* spp.	Gilbert (2005)

Table 1.2. Food selectivity of green turtles in literature. Dw = dry weight.

Location	Diet items	spp. abundant in habitat	spp. preference (selectivity index)	Method of selectivity calculation	Reference
Nicaragua	*Thalassia testudinum* (78.9 % dw contribution) *Syringodium filiforme* (9.2 %) Total *Gracilaria* spp. (1.1 %)	*Thalasia testudinum* (41.6 % dw biomass) *Halimeda* spp. (20.6 %) *Syringodium filiforme* (18.2 %)			Mortimer (1981)
Moreton Bay, Australia	*Gracilaria* sp. (41.2 % mean volume) *Zostera capricorni* (19.1 %) *Halophila ovalis* (19.0 %) *Hypnea* sp. (5.8 %) *Halodule uninervis* (7.6 %)	*Zostera capricorni* (65 % cover) *Halophila ovalis* (15%) *Halodule uninervis* (12 %) *Polysiphonia* sp. (7%) *Hydroclathrus clathratus* (1 %)	*Gracilaria* sp. (0.61) *Hypnea* sp. (-0.26) *Halophila ovalis* (-0.77) *Halodule uninervis* (-0.88) *Zostera capricorni* (-0.94)	Vanderploeg and Scavia electivity index (1979) -1.0 (least preferred) to 1.0 (most preferred)	Brand-Gardner et al. (1999)
Green Island, Australia	*Thalassia hemprichii* (37.78 % volume) *Cymodocea* sp. (31.5 %) *Halodule* sp. (8.05 %) *Gracilaria* spp. (6.19 %) *Syringodium* sp. (3.02 %)	*Cymodocea* sp. (29.7 %) *Halodule* sp. (11.1 %) *Halimeda* spp. (10.2 %) *Galaxaura* sp. (7.25 %) *Thalassia* sp. (6.4 %)			Fuentes et al. (2006)
Indian River County, FL	*Hypnea* spp. (52.6 % volume) *Chondria* spp. (8.4 %) *Gelidium* spp. (7.6 %) *Polysiphonia* spp. (4.0 %) *Laurencia poiteau* (3.2 %)	*Hypnea* spp. (35.5 % volume) *Bryothamnion seaforthi* (22.6 %) *Padina* spp. (8.4 %) *Dictyota* spp. (7.0 %) *Botryocladia* spp. (4.6 %)	*Chondria* spp. (0.91) *Gelidium* spp. (0.88) *Laurencia poiteau* (0.84) *Caulerpa prolifera* (0.69) *Gracilaria mammilaris* (0.55)	Ivlev's electivity index (1961) -1 (total avoidance) to 1 (exclusive feeding)	Gilbert (2005)
Estero Banderitas Bahía Magdalena, Mexico	Winter *Gracilaria textorii* (51.3 % volume) *Codium ampliveiculatum* (27.8 %) *Gracilaria pacifica* (17.6 %) *Ulva lactuca* (2.2 %) *Chondria nidifica* (1.0 %) Spring *Codium ampliveiculatum* (78.6 %) *Gracilaria textorii* (13.6 %) *Laurencia pacifica* (4.7 %) *Gracilaria pacifica* (1.5 %)	*Amphiloa beauvoisii* (27.5 % volume) *Gracilaria vermicullophylla* (15.7 %) *Asparagopsis taxformis* (14.6 %) *Gracilaria textorii* *Caulerpa sertularioides* *Caulerpa sertularioides* (28.2 %) *Amphiloa beauvoisii* (26.5 %) *Gracilaria vermicullophylla* (25.7 %) *Codium ampliveiculatum*	*Codium ampliveiculatum* (-4.7) *Gracilaria textorii* (-3.1) *Ulva lactuca* (-2.0) *Chondria nidifica* (-0.9) *Codium ampliveiculatum* (-2.9) *Gracilaria textorii* (-2.4) *Laurencia pacifica* (-1.1) *Gracilaria pacifica* (1.5 %)	Johnson T-bar value (1980) high selectivity with smaller value	López-Mendilaharsu et al. (2008)

Table 1.3. Growth rate and major diet items at different feeding grounds in Hawai‘i (Balazs, 1995).

Location	Growth rate (cm/month)	Major diet items
Ka‘u, Hawai‘i	0.44	*Pterocladiella capillacea*
Bellows, O‘ahu	0.2	*Codium* spp. *Ulva fasciata* (*U. lactuca*)
French Frigate Shoals NWHI	0.08	*Caulerpa racemosa* *Codium* spp. *Turbinaria ornata*
Lisianski Island NWHI	0.13	*Caulerpa racemosa* *Turbinaria ornata*
Kure and Midway Atolls NWHI	0.1	*Codium edule* *Spyridia filamentosa* invertebrates

Table 1.4. Nutritional contents of major diet items for Hawaiian green turtles reported in McDermid et al. (2007). Values are expressed as % dry weight except energy.

Species	Site	Total protein	carbohydrate	Crude lipid	energy (kJ/g)	Arginine
Chlorophyta						
Codium hawaiiense	Midway Atoll, NWHI	4.0 ± 0.3	27.4 ± 0.4	2.6 ± 0.3	4.18 ± 0.11	0.14
Codium reediae	Kanahā, Maui	7.0 ± 0.3	8.2 ± 1.3	6.1 ± 0.2	3.10 ± 0.05	0.28
Ulva fasciata	Papaʻiloa, Oʻahu	12.3 ± 0.5	20.6 ± 0.7	3.6 ± 0.1	11.55 ± 0.04	0.74
Ulva fasciata	Māʻalaea Bay, Maui	8.8 ± 0.4	17.1 ± 1.3	5.1 ± 0.2	9.95 ± 0.30	1.26
Rhodophyta						
Acanthophora spicifera	Kaneohe, Oʻahu	2.6 ± 0.1	31.5 ± 1.1	2.4 ± 0.2	7.85 ± 0.17	0.17
Amansia glomerata	Leleiwi, Hawaiʻi	12.3 ± 0.4	20.3 ± 1.1	3.7 ± 0.5	8.12 ± 0.32	0.41
Gracilaria salicornia	Kaneohe, Oʻahu	3.9 ± 0.4	24.6 ± 0.6	1.5 ± 0.1	6.05 ± 0.22	0.18
Hypnea musciformis	Kanahā, Maui	11.1 ± 0.2	16.1 ± 0.2	3.9 ± 0.1	7.01 ± 0.16	2.23
Hypnea musciformis	Laniākea, Oʻahu	11.6 ± 0.7	19.9 ± 1.0	1.9 ± 0.2	7.71 ± 0.08	1.87
Laurencia nidifica	Lualualei, Oʻahu	3.2 ± 0.2	16.0 ± 1.1	3.4 ± 0.1	10.07 ± 0.16	0.42
Pterocladiella capillacea	Punaluʻu, Hawaiʻi	13.4 ± 0.3	33.2 ± 0.3	2.3 ± 0.5	14.70 ± 0.49	0.46

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Chapter 2. Physiological responses of four red algae favored by Hawaiian green turtles to simulated eutrophication

Introduction

Anthropogenic nutrient inputs to coastal waters are reported to increase primary productivity and increasingly, to drive large macroalgal blooms (Lapointe et al., 2005; Smith et al., 2005; Dailer et al., 2010). On the island of Maui, Hawai‘i, macroalgal blooms are observed only in areas with high nutrient inputs caused mainly by nearby injection wells (Dailer et al., 2010, 2012a). Those regions with chronic invasive algal blooms also coincide with regions that have high disease rates of green turtle fibropapillomatosis (FP) (Van Houtan et al., 2010). Because Hawaiian green turtles have shifted their diet to include more alien invasive algae, especially in regions where these invasive species form large blooms (Van Houtan et al., 2010), we expect a link between rapid bloom growth by invasive algae and FP development. Our first step is to examine the growth rates of suspected bloom species under a eutrophic condition.

Algal responses to nutrient additions have been studied in Hawai‘i and other tropical waters, but relatively low to moderate nutrient concentrations are used in most of these studies (Larned, 1998; Larned and Stimson, 1996; Fong et al., 2003, Smith et al., 2005). Impacted regions of Maui coastal waters are likely to have dissolved inorganic nitrogen levels well above 100 μM (M. L. Dailer, pers. comm.). Few studies have investigated the algal responses to such a high level of nutrient enrichment comparable to eutrophic conditions observed in Maui. It is very important to understand the mechanism of macroalgal blooms in eutrophic regions and how it affects the health of herbivores

including green turtles.

This chapter describes growth experiments on four red algal species commonly eaten by Hawaiian green turtles. The physiological responses of these marine plants to the nutrient enrichment that simulates eutrophic conditions are reported. Preliminary results for arginine accumulation and the source of tissue nitrogen in algae from a eutrophic region on Maui are also reported.

Materials and methods

Sample collection and preparation

Four red algal species known to be eaten by *C. mydas* were collected from Kaʻalaʻwai, Oʻahu, Hawaiʻi: two natives, *Amansia glomerata* C. Agardh and *Pterocladiella capillacea* (S.G. Gmelin) Sant. and Homm. and two invasives *Acanthophora spicifera* (Vahl) Børgensen and *Hypnea musciformis* (Wulfen in Jacquin) Lamouroux. These algae frequently appear in Hawaiian green turtles' diet (Russell and Balazs, 2000, 2009). Samples were brought to Ānuenue Fisheries Research Center (AFRC), Honolulu, Hawaiʻi, within 1 h of collection, cleaned of sand and epiphytes, and rinsed three times with filtered seawater. Samples were kept in 5 L glass aquaria with running filtered seawater up to 18 h after cleaning. Algae were then spun in a salad spinner and cut into experimental units, ca 3 g wet weight and exact weights recorded. An artificial anchoring device was attached to each sample. This device was made of clear Nalgene tubing (15 cm long and 11 mm outside and 6 mm inside diameters) and had one or two slits into which that alga was placed; the tubing closed back on itself trapping the marine plants. Rust resistant nuts were attached to the both ends of the hose

to weight the assembly on the bottom of aquaria during the experiment. *P. capillacea* was tied together near the holdfast for each 3 g experimental unit, using unwaxed dental floss to avoid the alga slipping off the anchoring devices. For *A. glomerata*, each sample was cut to contain the stipe that was held in the slits in the anchoring device. All algae were dipped in an ultrasonic jewelry cleaner (Brason 1200, Branson Ultrasonic Corporation) containing filtered seawater for five seconds before they were placed in the aquaria to reduce the load of epiphytic diatoms. This process was repeated at each weight and photosynthesis measurement (day 4 and day 8).

Experimental set up

The algal culturing set up was constructed based on Larned (1998) and modified to suit this experiment (Fig 2.1). Each algal sample with an anchoring device was placed in a 5 L aquarium that was held in a fiberglass water bath. Seawater was pumped from several meters off the bottom of Honolulu Harbor in front of AFRC, filtered through a sand filter (Hayward Pool Products Inc., Model No. S310T) followed by pressurized flow through three polypropylene filter cartridges (25.4 cm x 6.35 cm, two at 5μm and the final at 1μm pore size). Filtered seawater was first delivered to the nutrient mixing chambers set at 1 m above ground and then delivered to each 5 L aquarium by gravity flow. Each aquarium was supplied with flowing seawater (360 - 380 ml min^{-1}) and independent aeration. Each water bath housed up to 16 aquaria (a total of 48 aquaria).

Nutrient enrichment experiment

A total of five experimental runs were performed from June to October 2011.

Each experiment ran for eight days. Four different nutrient treatments were used in this experiment: enriched nitrogen (100 μM), enriched phosphorus (5.6 μM), nitrogen and phosphorus enriched together (100 μM + 5.6 μM), and unenriched ambient seawater (control). Each treatment was delivered to up to four aquaria housed in the same water bath. Algal samples were randomly placed in aquaria. One water bath housed one replicate for one species for each nutrient treatment. Throughout the five experimental runs, each plant species had 12 replicates for each treatment.

Algae were preconditioned for three days in the aquaria with flowing ambient seawater before nutrient enrichment. The purpose of this preconditioning process was to reduce the initial variability of growth rate caused by nutrients stored in the algae following Dailer et al. (2010). On day 4, after three days of preconditioning, algae received nutrient enrichment for four days. Nutrient stock solutions were delivered to the mixing chambers using a peristaltic pump (Masterflex, Cole-Parmer) with the flow rate of 2.1 to 2.2 ml m^{-1} and mixed with the filtered seawater for the nutrient enrichment.

Nutrient stock solutions were prepared in 23 L plastic carboys with distilled water and reagent grade sodium nitrate (nitrogen enrichment) and sodium phosphate (phosphorus enrichment) one to two days before the start of nutrient enrichment and stored in the refrigerator until the start of the enrichment. These stock solutions were calculated to provide nutrient treatment samples with 100 μM nitrate and 5.6 μM phosphate. The mixing chamber for the control treatment received only distilled water without any nutrient. Water samples were taken 30 min after the start of nutrient enrichment and sent to the Agricultural Diagnostic Service Center, University of Hawai‘i at Mānoa, to determine the actual nutrient concentrations in the enriched and unenriched

seawater.

Photosynthetically active radiation (PAR) received inside the aquaria was recorded using a LI-1400 datalogger and a LI-193 spherical quantum sensor (LI-COR Environmental) and corrected for underwater measurements. Temperature of flowing seawater in the aquaria was recorded using HOBO Onset Pendant Temperature/Light data loggers.

Growth and photosynthesis measurements

Changes in weight and photosynthetic parameters were measured before enrichment (day 4) and four days after enrichment (day 8). Before the measurement of photosynthetic parameters, each sample was placed in the ultrasonic cleaner containing filtered seawater for five seconds to remove the epiphytic diatoms. A Junior PAM (Pulse Amplitude Modulation Fluorometer) Photosynthesis Yield Analyzer and WinControl-3 Software (Heinz Walz GmbH, Germany) were used to measure photosynthetic parameters. After the PAM measurements, samples were spun in a salad spinner and blotted dry for weighing. All weight measurements were accomplished with Ohaus Scout Pro Balance model SPE123, with 0.001 g capability.

Data analysis

Specific growth rate of each sample was calculated based on the relative changes in wet weight per day after the start of nutrient enrichment (day 4), normalized for initial weight and numbers of days in growth period.

Specific growth rate = (weight at day 8 – weight at day 4) / weight at day 4 / 4 (days of nutrient enrichment)

Two photosynthetic parameters, ETR_{max} and E_k, were chosen for analyses. ETR_{max} is the maximum electron transport rate, which predicts the maximum photosynthetic rate. E_k is the minimum saturating irradiance. Both of these factors are useful indicator of the photosynthetic ability of the plants (Ralph and Gademann, 2005).

All statistical analyses were performed using SigmaPlot Version 11.0. Data were analyzed with Two-way analysis of variance (ANOVA) to determine if there are significant differences among nutrient treatments and among experimental runs. Experimental runs were treated as blocks in the analysis. Tukey's multiple pairwise comparisons were used to determine the differences between the four treatments for each species.

Results

Temperature, PAR and water chemistry

Temperatures of sea water in the aquaria containing algal samples ranged from 25.1 to 34.0 °C throughout the five experimental runs. Daily maximum PAR during the day time (0900 to 1700) ranged from 1617.7 to 2683.4 μmol photons m^{-2} s^{-1}. PAR often exceeded 2000 μmol photons m^{-2} s^{-1} full sun levels during the day, associated with upwelled light energy in experimental water baths. Daily fluctuations of temperature and PAM were consistent throughout the five experimental runs.

Nutrient concentrations of the ambient harbor water were 0 - 0.3 μM for nitrate

and 1.7 - 2.0 μM for phosphate. During the fourth run, the background seawater nutrient levels were elevated to 26.45 μM nitrate and 3.2 μM phosphate, possibly because nutrients levels in the harbor water were elevated. The data from the fourth run were not included in the data analyses (i.e., n =9), because the background seawater nutrient level was high enough to promote a higher growth rate for the control samples.

Growth rate

Significant differences in the specific growth rates among nutrient treatments were found in all four species (Table 2.1, Fig. 2.2-2.4). Differences among experimental runs were also significant for *Acanthophora spicifera*, *Amansia glomerata*, and *Pterocladiella capillacea*. Post hoc pairwise comparisons of *A. glomerata* indicated that the specific growth rate of combined treatment samples was significantly greater than phosphate enriched and control samples, and that of nitrate enriched samples was significantly greater than phosphate enriched samples. For *Hypnea musciformis*, the specific growth rate of nitrate enriched samples was significantly greater than those of the control, phosphate and combined treatments. The specific growth rate of nitrate enriched *P. capillacea* was significantly greater than that of phosphate enriched samples. Nutrient enrichment, especially nitrate, did not enhance the specific growth rate of *A. spicifera* (Table 2.1, Fig 2.2). For this species, control samples had the highest specific growth rate, which was significantly higher than that of the nitrate and combined enrichment samples. Phosphate enriched samples also had a significantly higher specific growth rate than the combined enrichment samples.

Photosynthetic parameters

Results of the photosynthetic parameters are shown in Table 2.2 and Fig 2.6-2.13. The WinControl-3 Software could not calculate ETR_{max} and E_k for several samples, possibly because of unexpected measurement error associated with Junior PAM handling. Thus, sample sizes were reduced to eight for the control and nitrogen treatment samples of *A. spicifera* and the nitrogen and combined treatment samples of *A. glomerata*. For ETR_{max}, significant differences among nutrient treatments were found only in *H. musciformis* (Fig 2.8). No differences among nutrient treatments were found in E_k for all species. However, overall trends of both photosynthetic parameters were similar to SGR. For *A. spicifera*, ETR_{max} and E_k were higher in control and phosphate enriched samples (Fig 2.6, 2.10). ETR_{max} and E_k of *A. glomerata* and *H. musciformis* were higher in nitrogen enriched and combined samples (Fig 2.7-8, 2.11-12). Only E_k of nitrogen enriched *P. capillacea* did not follow the same pattern as SGR and was lower than phosphate-enriched samples (Fig 2.13). There were significant differences among experimental runs both in ETR_{max} and E_k for *A. spicifera*.

Preliminary evaluation of the arginine accumulation with eutrophication

To investigate if algae accumulate arginine in a eutrophic area, algal tissue analyses of amino acid content were conducted for three invasive species, *A. spicifera*, *H. musciformis* and *U. lactuca*, collected from Kihei, Maui. Kihei is the site of high anthropogenic nutrient input, chronic invasive algal blooms, and also high FP disease rate (Dailer et al., 2010; Van Houtan et al., 2010). Algal $\delta^{15}N$ (stable isotope of N) values were also analyzed to determine the source of tissue N. The use of $\delta^{15}N$ is well

established for the determination of the source of algal tissue N, with sewage derived N has a high $\delta^{15}N$ value (Dailer et al., 2010). Preliminary results indicate that tissue arginine and $\delta^{15}N$ levels of all three species are significantly higher in Kihei than Olowalu, a less-impacted area (Table 2.3, Fig 2.14 - 2.15). These results indicate that algae in eutrophic regions incorporate wastewater derived nitrogen and accumulate arginine in their tissue.

Discussion

Eutrophication of coastal waters by anthropogenic nutrient inputs causes blooms of algae with rapid growth rates, and drives phase shifts from healthy coral reef to a macro algae dominant state. Understanding the consequences of algal blooms under eutrophic conditions is critical for the management of coastal waters and organisms living in the affected regions, especially herbivores that forage on those blooming algae as well as those that eat other algal species displaced by the blooming algae.

Nitrogen enrichment significantly increased the growth rate of *Hypnea musciformis*, *Amansia glomerata*, and *Pterocladiella capillacea*. The growth of all four species was not limited by phosphate, as the phosphate enrichment did not have significant effects on their growth rates.

Mean specific growth rates of four species with nitrate enrichment ranged from 0.024 g g^{-1} d^{-1} (*A. glomerata*) to 0.052 g g^{-1} d^{-1} (*A. spicifera*). Larned (1998) reports algal growth experiments with a supplement of only 6 μM ammonium for nitrogen enrichment - much lower nutrient concentration than this experiment. He reports mean specific growth rates of nine algal species with ammonium treatment ranging from 0.003 g g^{-1} d^{-1} (*Dictyosphaeria versluysii*) to 0.08 g g^{-1} d^{-1} (*Ulva fasciata* (*lactuca*)). While Larned (1998) and this present study use different algal species, these results are clearly similar.

This indicates that very high nutrient concentrations such as the one used in the present experiment do not necessarily result in a very high algal growth rate. Low to moderate concentrations would be enough to saturate algae with nutrients and to induce higher growth. Alternatively, the very high levels of ambient irradiances may have slowed growth rates.

Vermeij et al. (2009) found no differences in the growth rates among *H. musciformis* grown in three different nutrient concentrations (no nutrient addition, + 10.0 μM ammonium + 1.0 μM phosphate, and + 30.0 μM ammonium + 3.0 μM phosphate) for plants that were not stripped during preconditioning. Instead, increased nutrient concentration increased survival of the fragments. Similar results occurred for fragments of *A. spicifera*. No significant differences were found for the % growth in weights between the nutrient added and the control (no nutrient added) fragments (M. S. Kawachi, unpub. data). These findings suggest that algae would not use all available nutrients for their immediate growth, but they may store the excess nutrients for survival and maintenance once the nutrients exceed their saturating levels.

Note that the mean specific growth rates of *P. capillacea* for all treatments and those of *A. glomerata* for the control, phosphate and combined treatments were higher than those of *H. musciformis* (Fig 2.2 – 2.4). *H. musciformis* forms large blooms in coastal regions with excess nutrient inputs on Maui (Dailer et al.. 2010). This invasive alga has not been observed to reproduce sexually in Hawai‘i; fragmentation is the predominant mode for reproduction (Smith et al., 2002). Fragments generally grow faster than sexually reproduced spores (Vermeij et al., 2009). *H. musciformis* readily fragments; this observation plus nutrient acquisition may explain bloom formation of this species.

A. spicifera appears to have a nearly unique strategy for propagation. The specific growth rate of the control treatment of this species was the highest among all samples studies in this experiment. *A. spicifera* is the most abundant invasive alga in Hawai‘i, yet it does not form a large blooms like *H. musciformis* (Smith et al. 2002). The large invasive algal blooms often occur in the region with high nutrient inputs, but in this experiment, the nutrient enrichment suppressed the growth rate of *A. spicifera*. *A. spicifera* fragments without nutrient addition were found to grow significantly more in length than nutrient added fragments (M. S. Kawachi, unpub. data). Detailed studies, especially focusing on its sexual reproductive strategies, are needed to determine why *A. spicifera* slows its growth under nutrient enriched conditions, and how this alga uses and stores available nutrients.

The measurements of the photosynthetic parameters have an implication that they could be used to predict the growth rates of algae. Significant differences were not found in ETR_{max} and E_k for most of the samples, and this could be because of the variability in the PAM measurements. Junior PAM is very sensitive to variables associated with measurements such as where its fiber optic is attached on the plants (M.S. Kawachi, pers. obs.). We could expect that by keeping these variables constant, the precision of the PAM measurements could be increased.

This study demonstrated that excess nutrient inputs, especially nitrogen, can significantly increase the growth of macroalgae favored by Hawaiian green turtles. Nitrogen is particularly important for *H. musciformis*, a bloom-forming invasive species. Considering the slow growth rate of samples without nitrogen input, this species may not persist in regions with low anthropogenic impacts. Success of invasive algae in their

newly invaded region is often linked to nutrient enrichment (Schaffelke et al., 2006). Two bloom forming algae in Maui, *H. musciformis* and *Ulva lactuca*, are more responsive to wastewater effluent addition than a non-blooming species, *Dictyota acutiloba* (Dailer et al., 2012b). Rapid responses of invasive species to available resources would be an advantageous trait for their success over native species.

Anthropogenic nutrient inputs may provide abundant foods for green turtles by stimulating the growth of select, weedy algae, but this appears to put turtles at risk of FP development, via arginine accumulation. Results of this study indicate that land-based management is very important for the conservation of marine herbivores and their ecosystem services. Future studies should examine the arginine accumulation in algal tissues under experimentally nutrient enriched conditions.

Table 2.1. Results of Two-way ANOVA for the specific growth rate.

	Run		Treatment		
Species	*F*	*P*	*F*	*P*	Pairwise comparison
Acanthophora spicifera	46.443	<0.001	14.787	<0.001	C vs N (*P*=0.001), C vs NP (*P*<0.001), P vs NP (*P*=0.001)
Amansia glomerata	12.748	<0.001	6.602	0.002	N vs P (*P*=0.016), P vs NP (*P*=0.005), C vs NP (*P*=0.041)
Hypnea musciformis	2.531	0.101	11.249	<0.001	C vs N (*P*<0.001), N vs P (*P*<0.001), N vs NP (*P*=0.003)
Pterocladiella capillacea	17.069	0.030	3.526	0.030	N vs P (*P*=0.037)

Table 2.2. Results of Two-way ANOVA for ETR_{max} and E_k.

	Run		Treatment		
Species	*F*	*P*	*F*	*P*	Pairwise comparison
ETR_{max}					
Acanthophora spicifera	3.740	0.040	2.592	0.078	
Amansia glomerata	0.127	0.881	1.538	0.233	
Hypnea musciformis	0.178	0.838	3.709	0.025	C vs NP (*P*=0.034)
Pterocladiella capillacea	0.207	0.815	2.429	0.090	
E_k					
Acanthophora spicifera	3.587	0.045	2.253	0.110	
Amansia glomerata	0.164	0.850	0.415	0.744	
Hypnea musciformis	1.641	0.215	1.276	0.305	
Pterocladiella capillacea	0.124	0.884	2.094	0.128	

Table 2.3. Results of t-test for algal tissue analyses. N=3.

	Arginine	$\delta^{15}N$
Species	*P*	*P*
Acanthophora spicifera	0.005	<0.001
Hypnea musciformis	<0.001	<0.001
Ulva lactuca	<0.001	<0.001

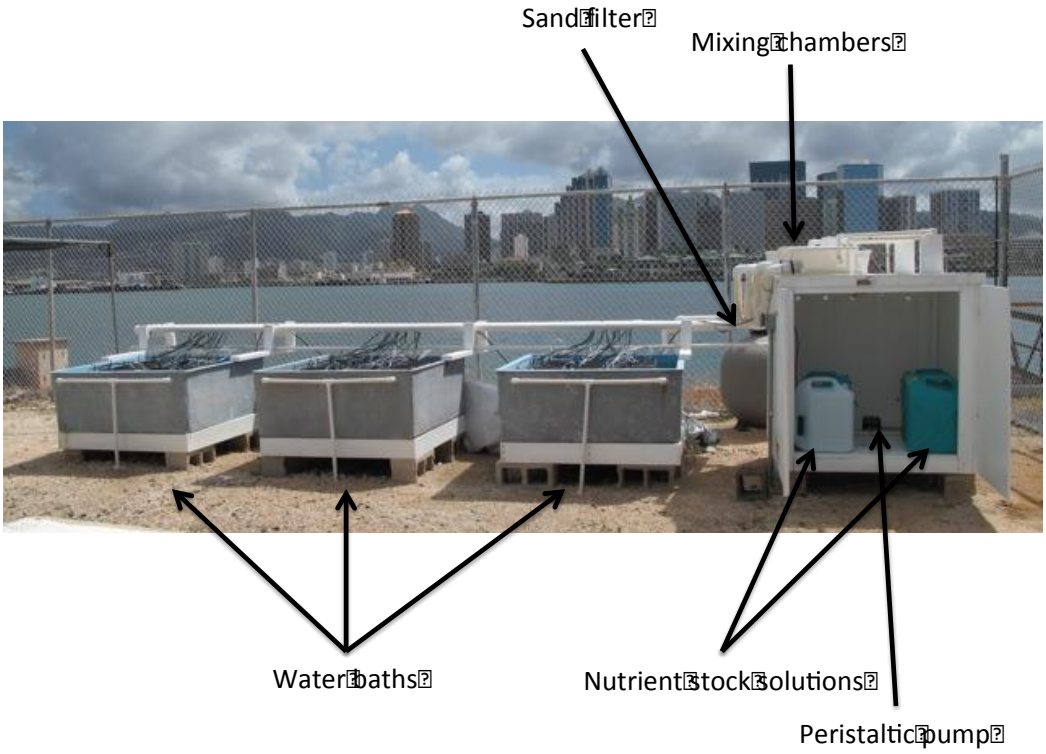

Fig 2.1. Flowing sea water system at Ānuenue Fisheries Research Center. Three polypropylene filter cartridges are located behind the housing of the nutrient stock solution/peristaltic pump.

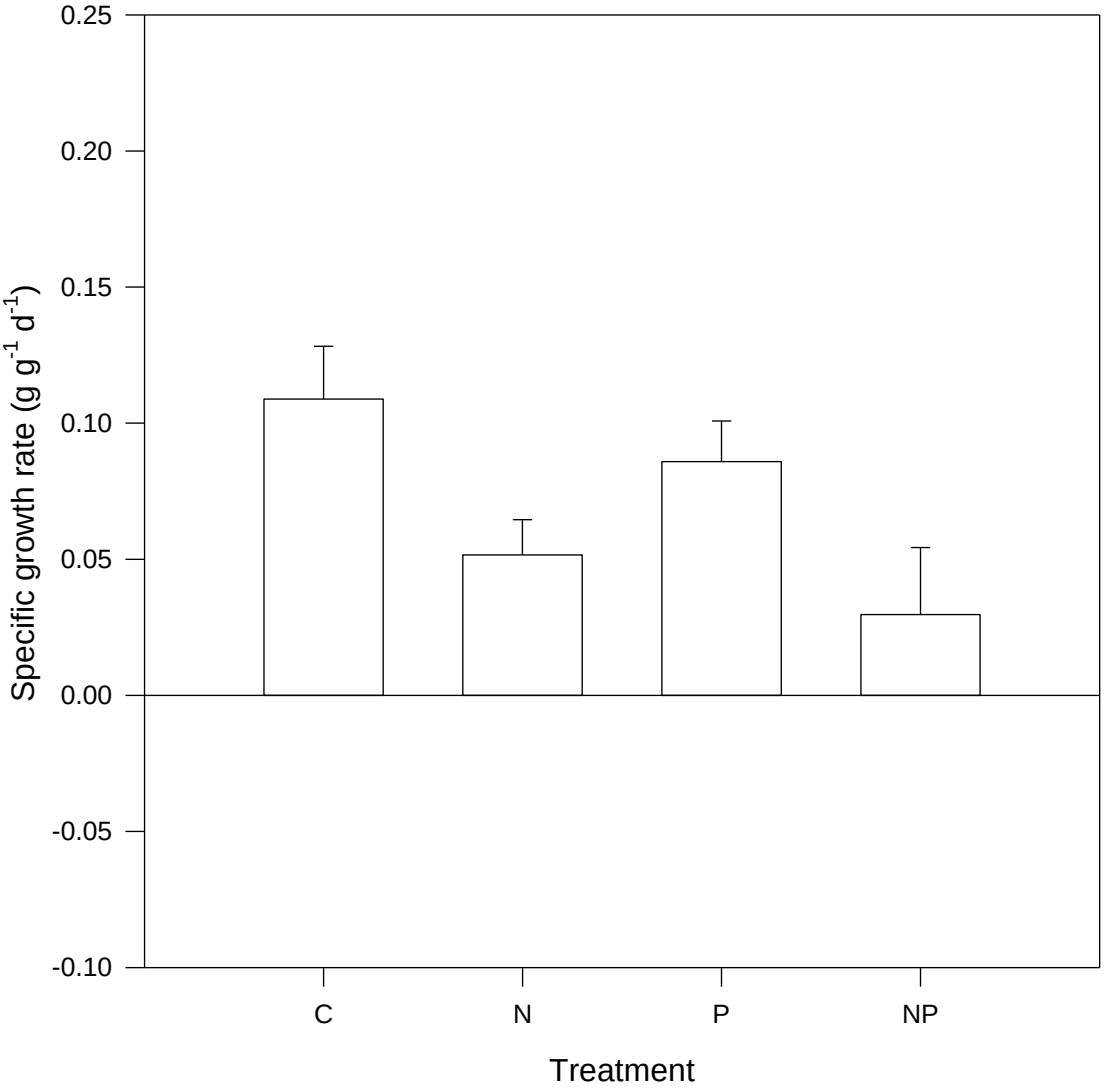

Fig 2.2. Specific growth rates of *Acanthophora spicifera* with four nutrient treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

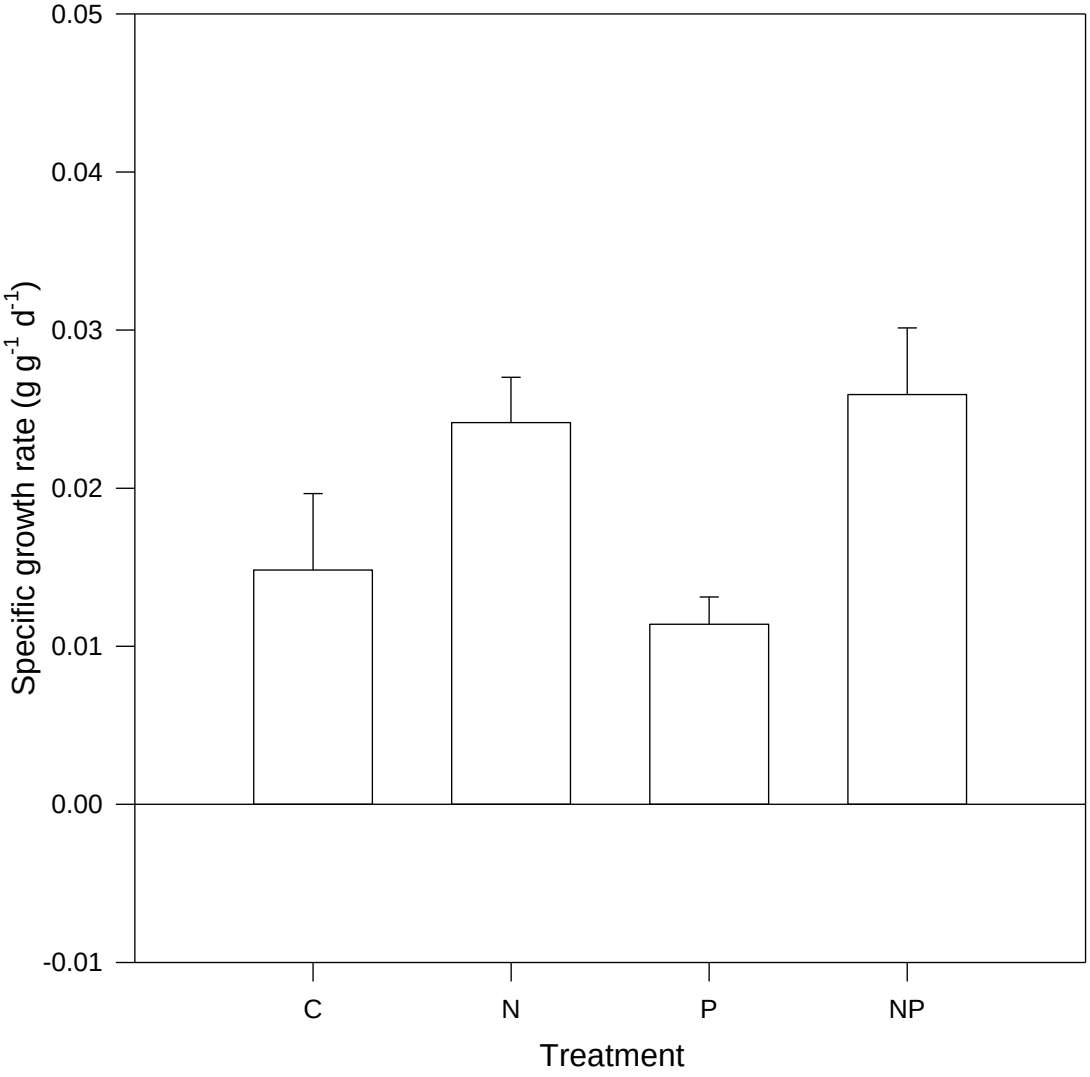

Fig 2.3. Specific growth rates of *Amansia glomerata* with four nutrient treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

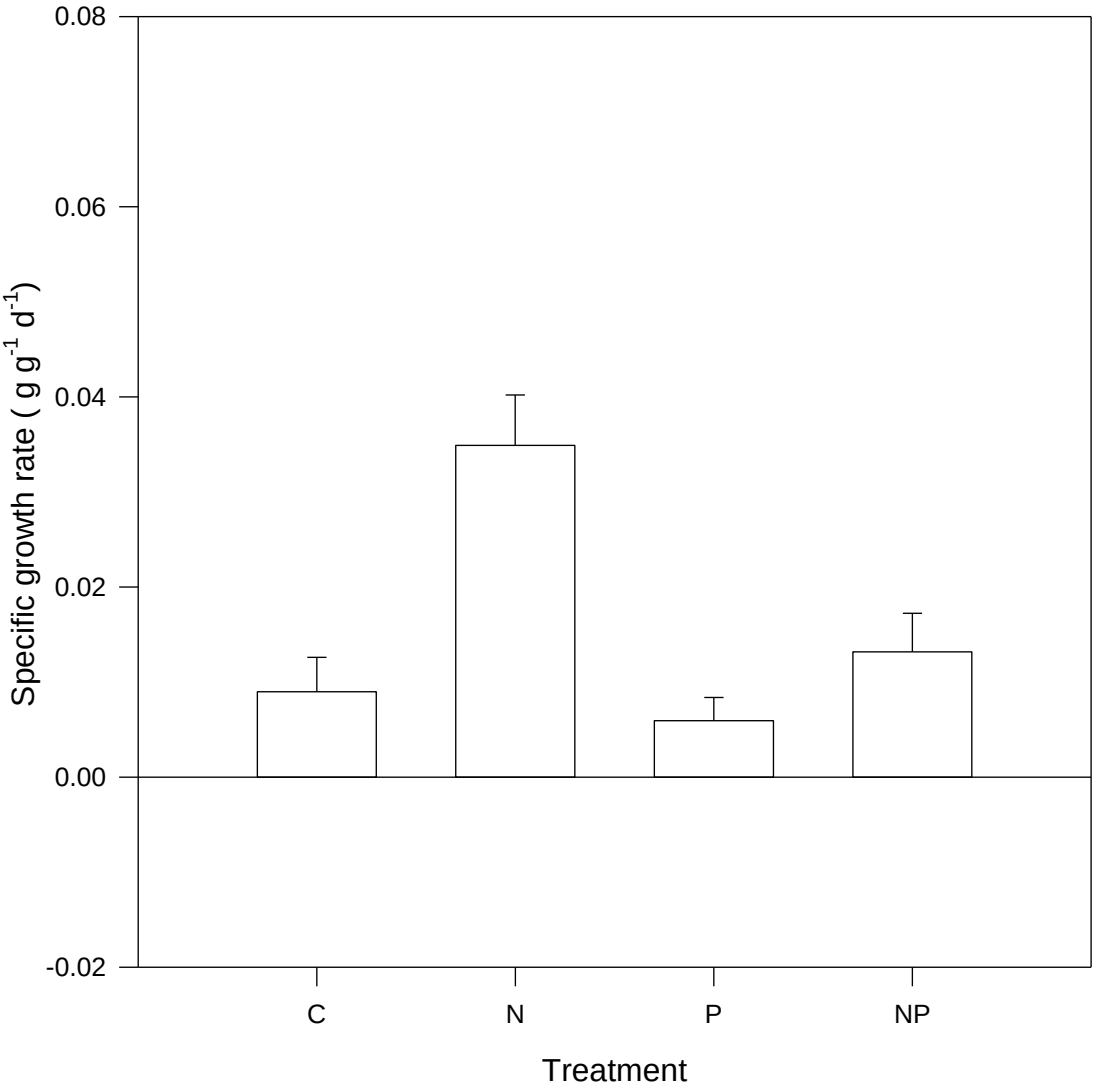

Fig 2.4. Specific growth rates of *Hypnea musciformis* with four nutrient treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

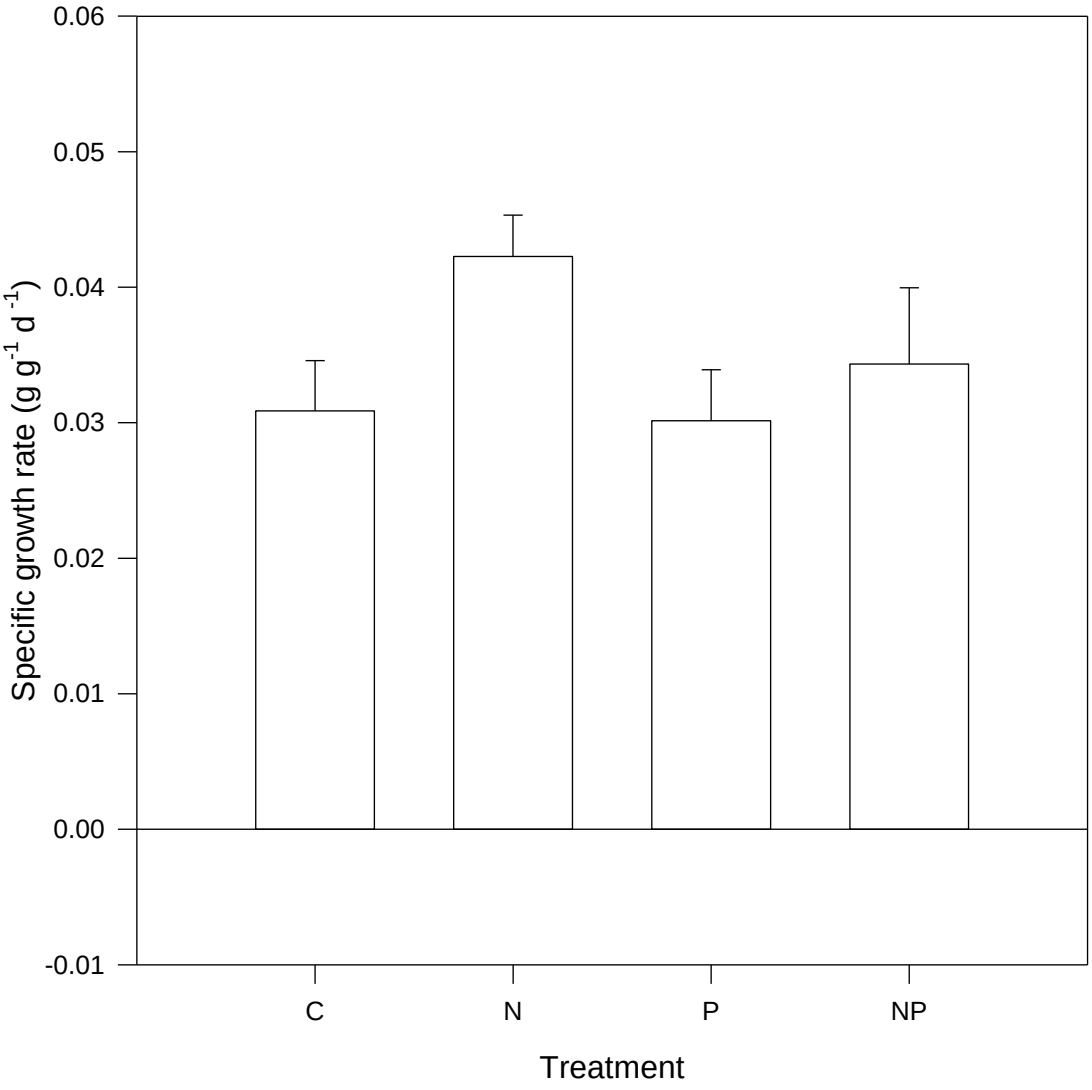

Fig 2.5. Specific growth rates of *Pterocladiella capillacea* with four nutrient treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

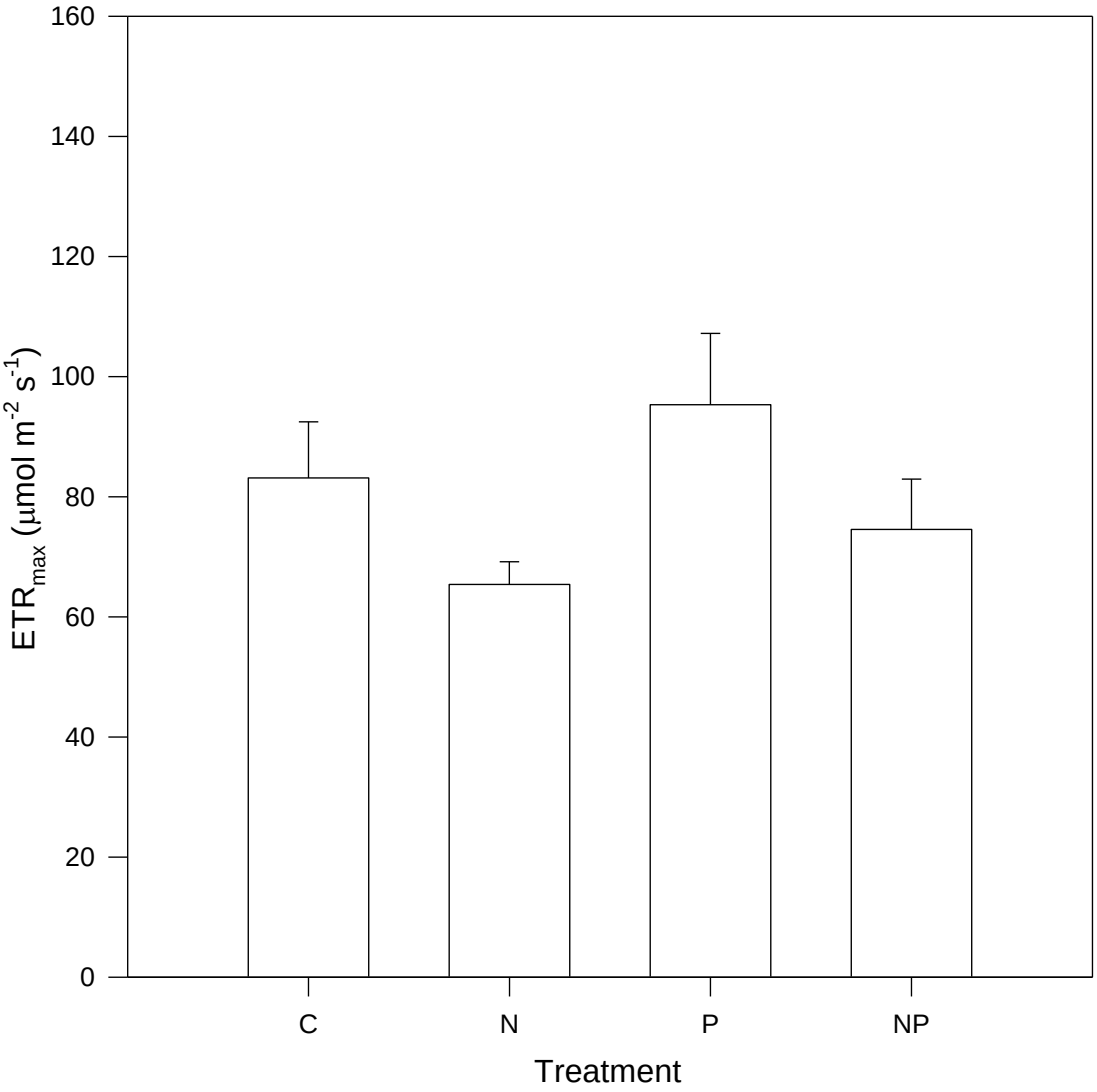

Fig 2.6. Maximum electron transport rate (ETR_{max}) of *Acanthophora spicifera* with four treatments (n=9 except C and N for which n = 8). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

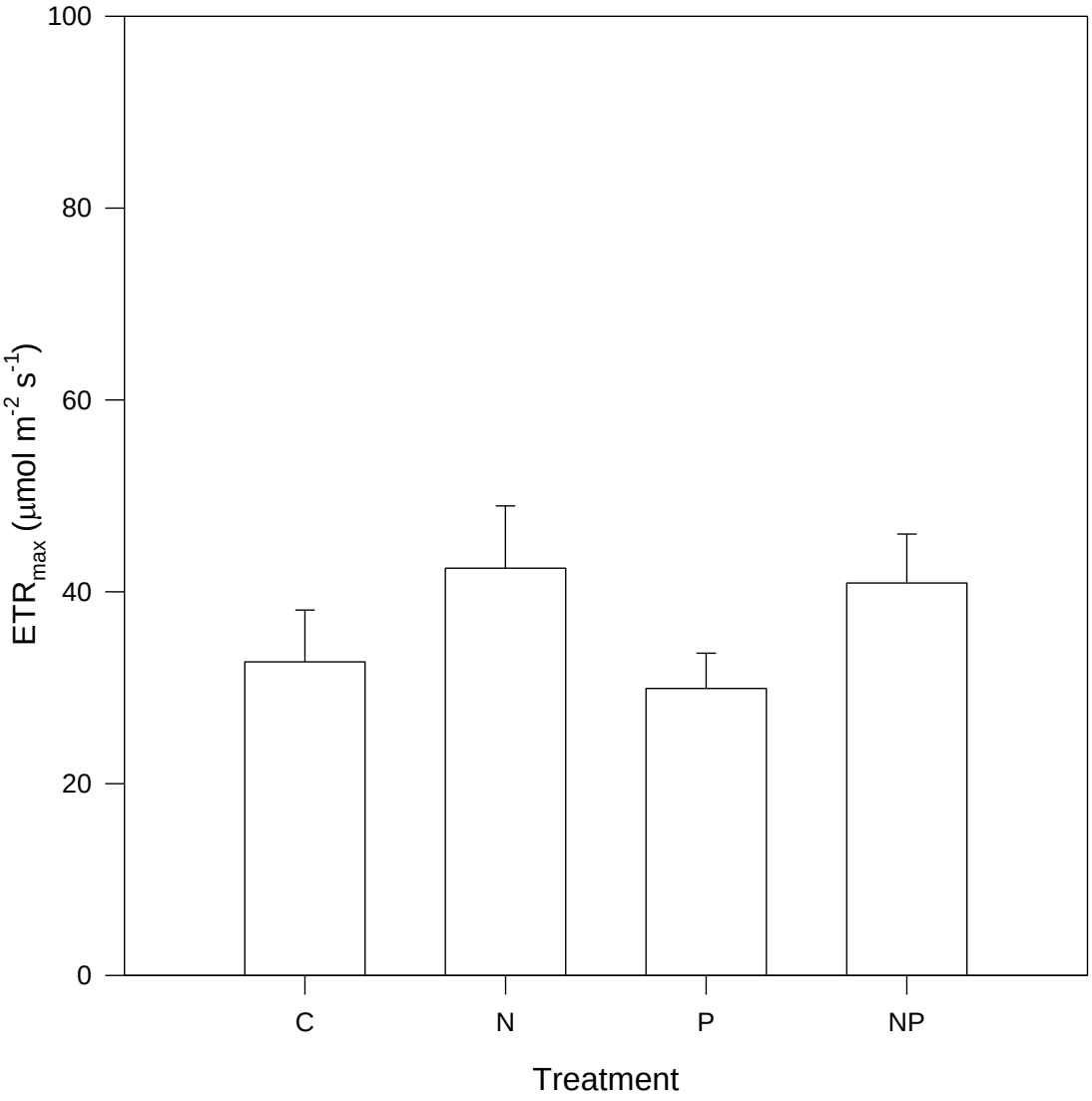

Fig 2.7. Maximum electron transport rate (ETR_{max}) of *Amansia glomerata* with four treatments (n=9 except N and NP for which n = 8). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

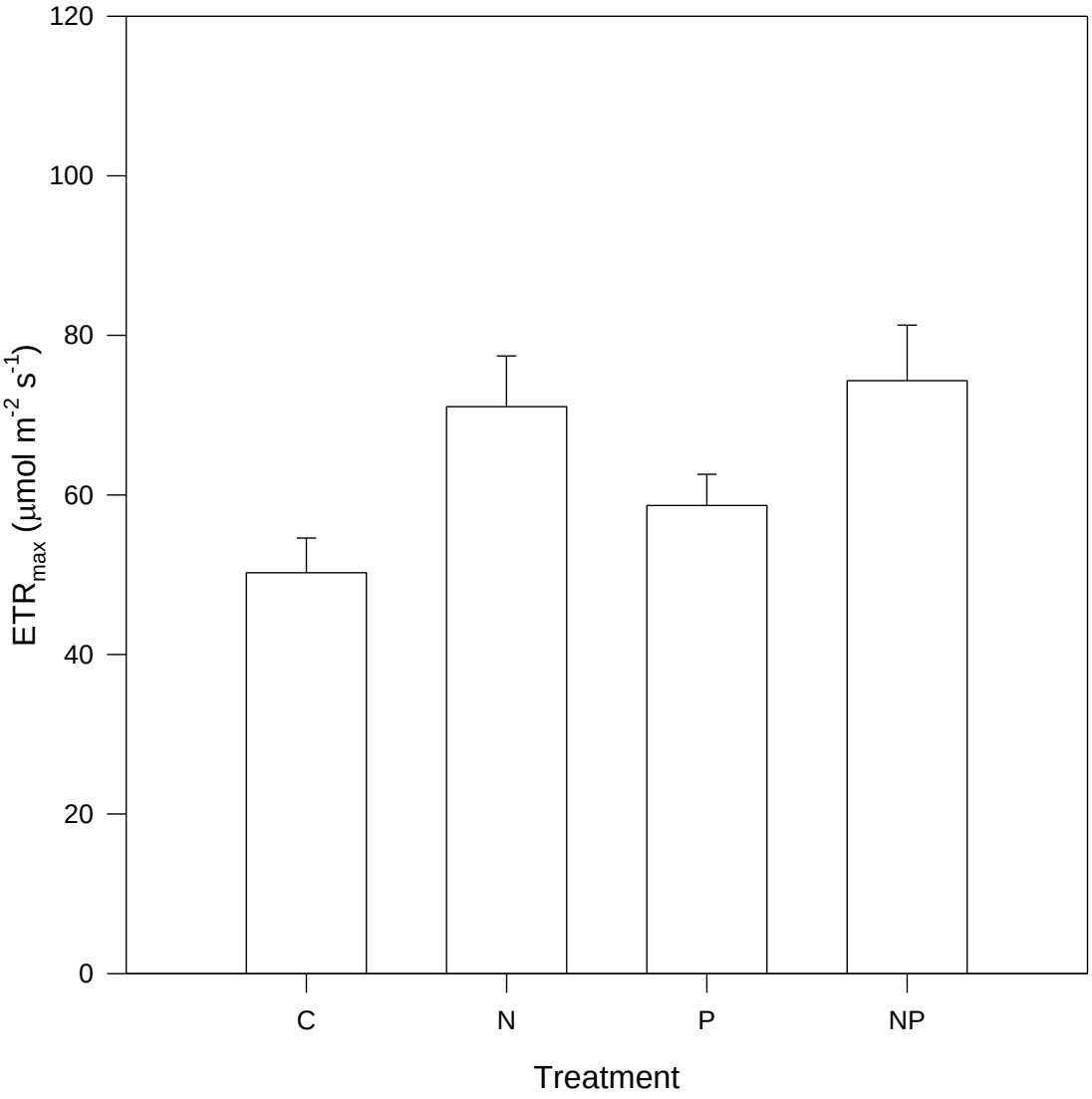

Fig 2.8. Maximum electron transport rate (ETR_{max}) of *Hypnea musciformis* with four treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

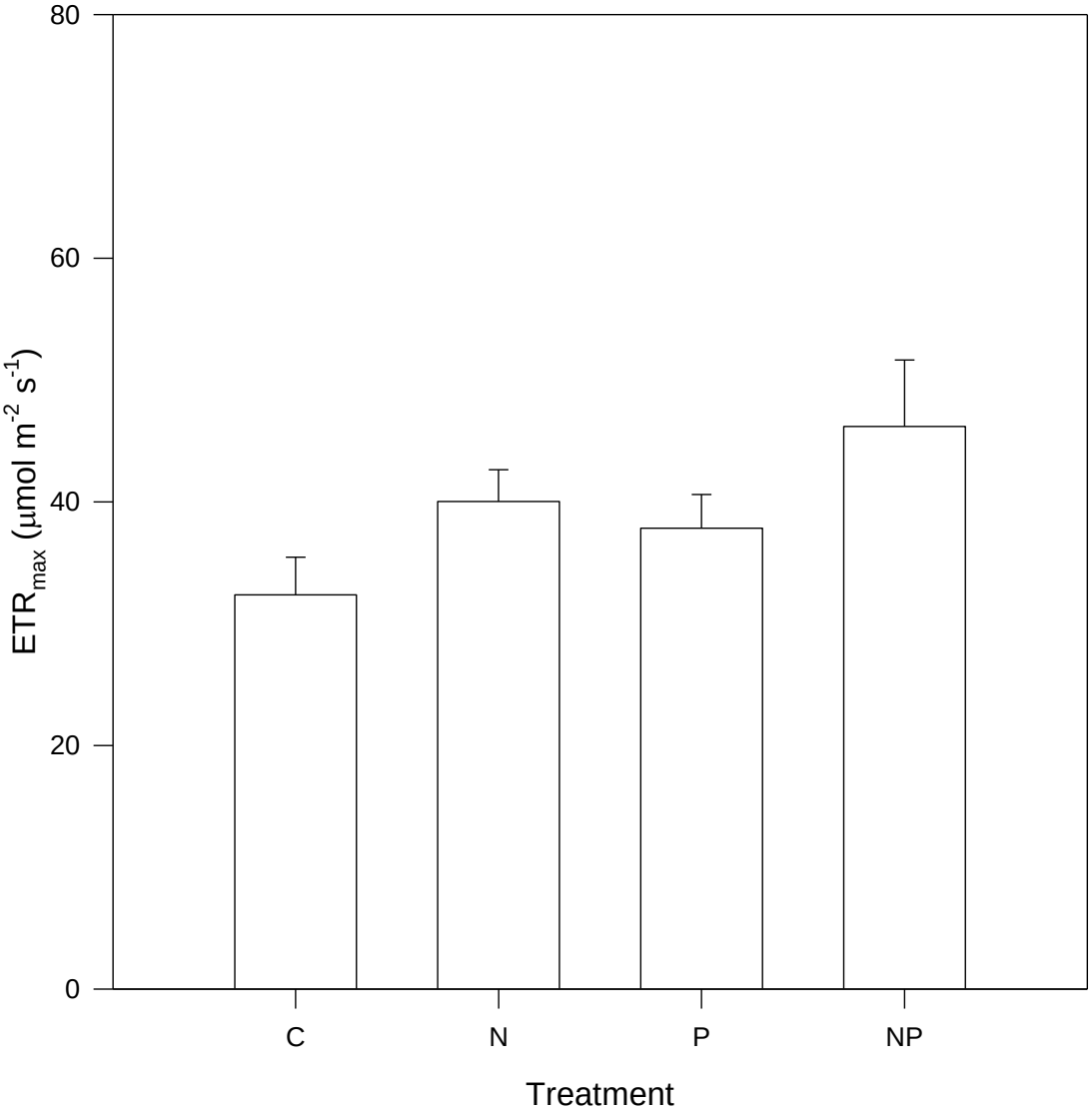

Fig 2.9. Maximum electron transport rate (ETR_{max}) of *Pterocladiella capillacea* with four treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

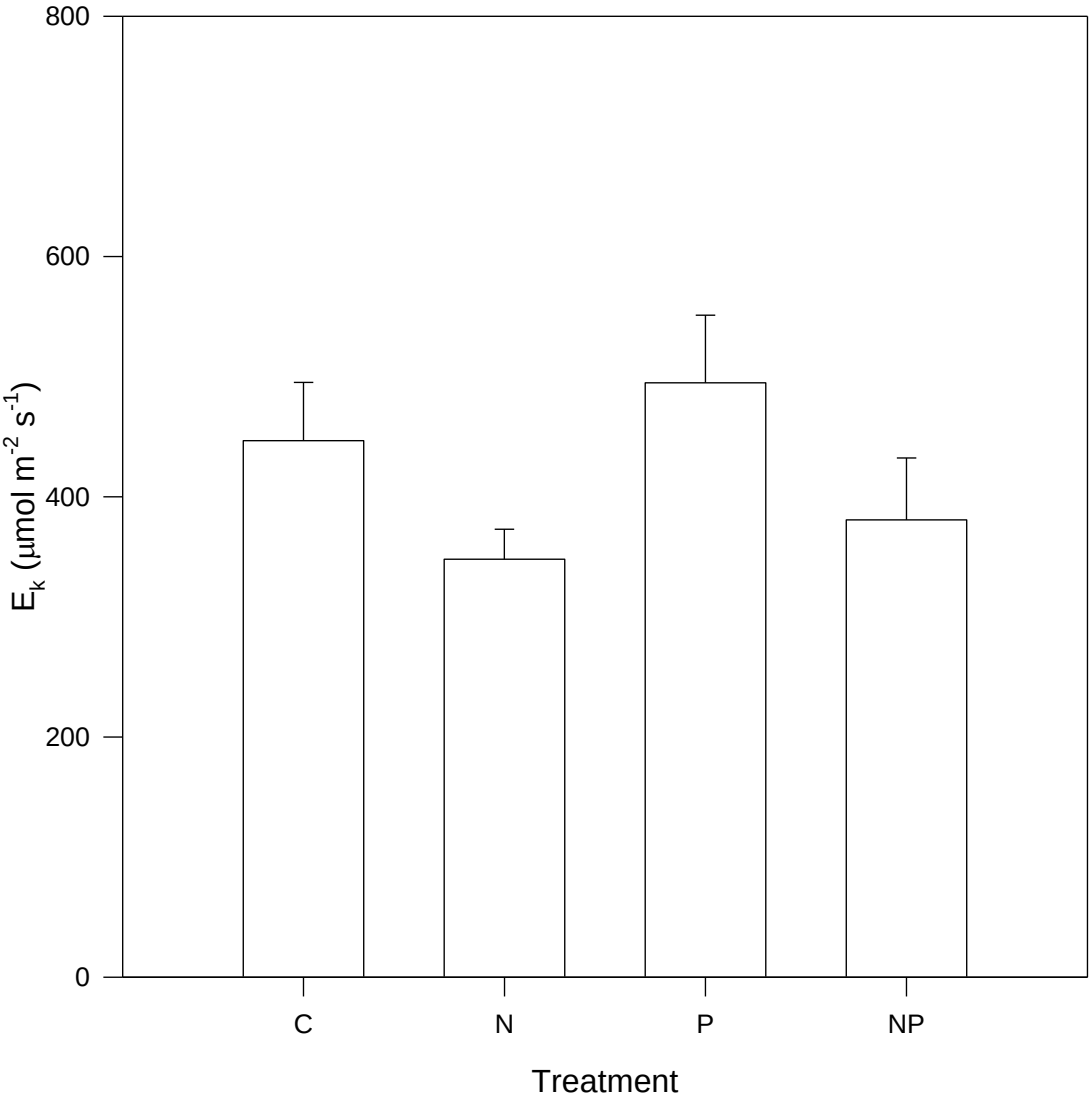

Fig 2.10. Minimum saturating irradiance (E_k) of *Acanthophora spicifera* with four treatments (n=9 except C and N for which n = 8). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

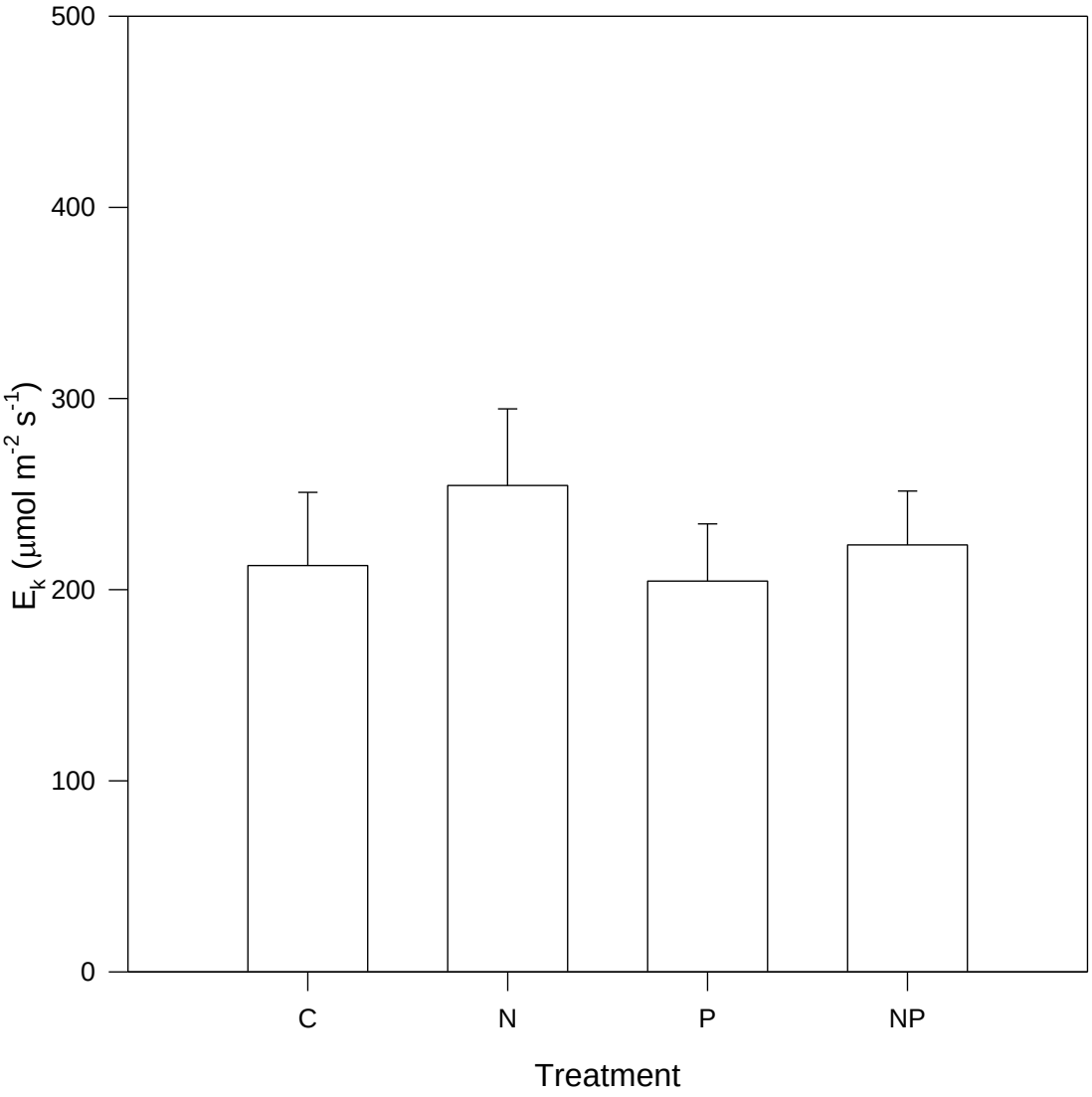

Fig 2.11. Minimum saturating irradiance (E_k) of *Amansia glomerata* with four treatments (n=9 except N and NP for which n = 8). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

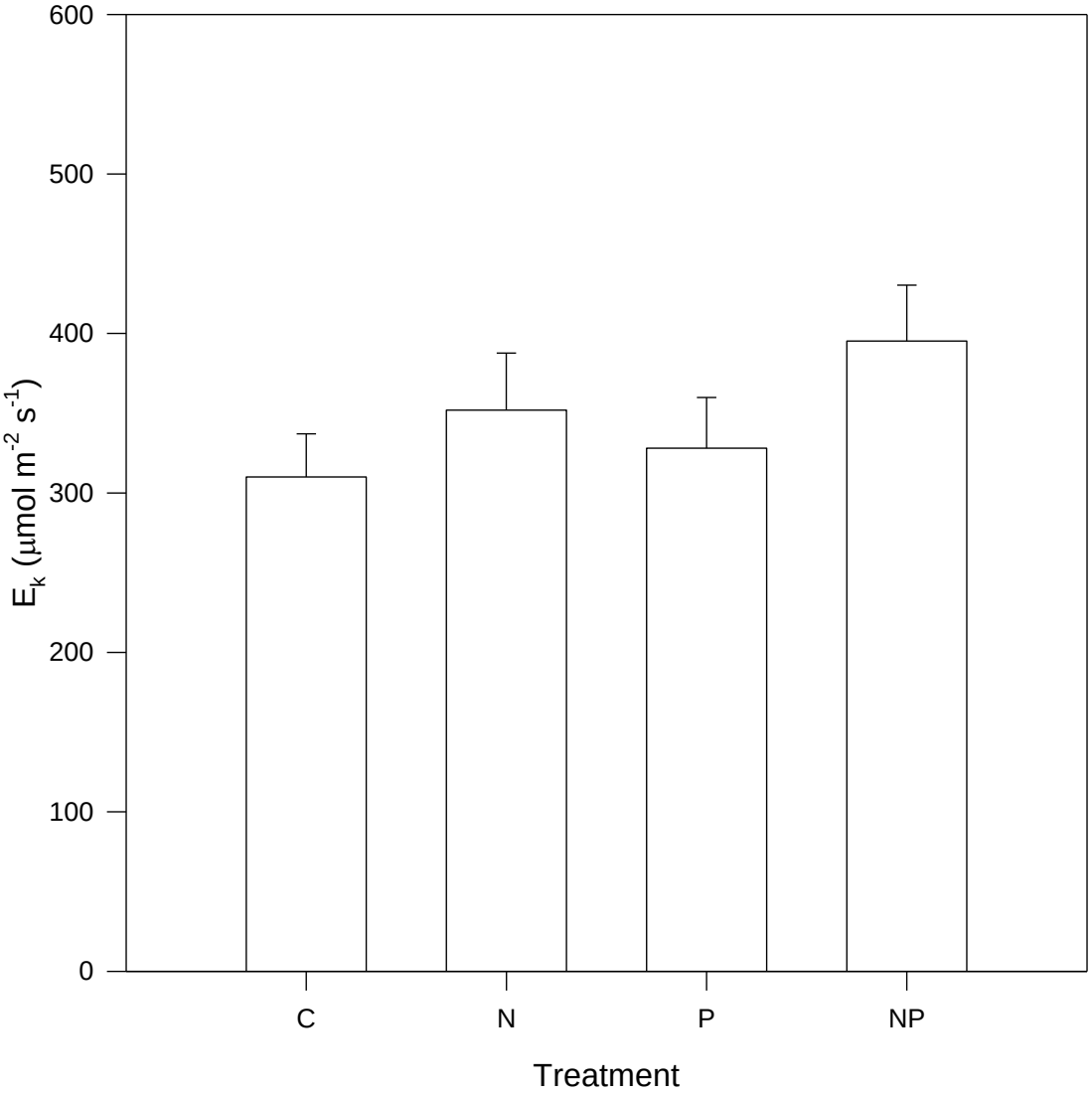

Fig 2.12. Minimum saturating irradiance (E_k) of *Hypnea musciformis* with four treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

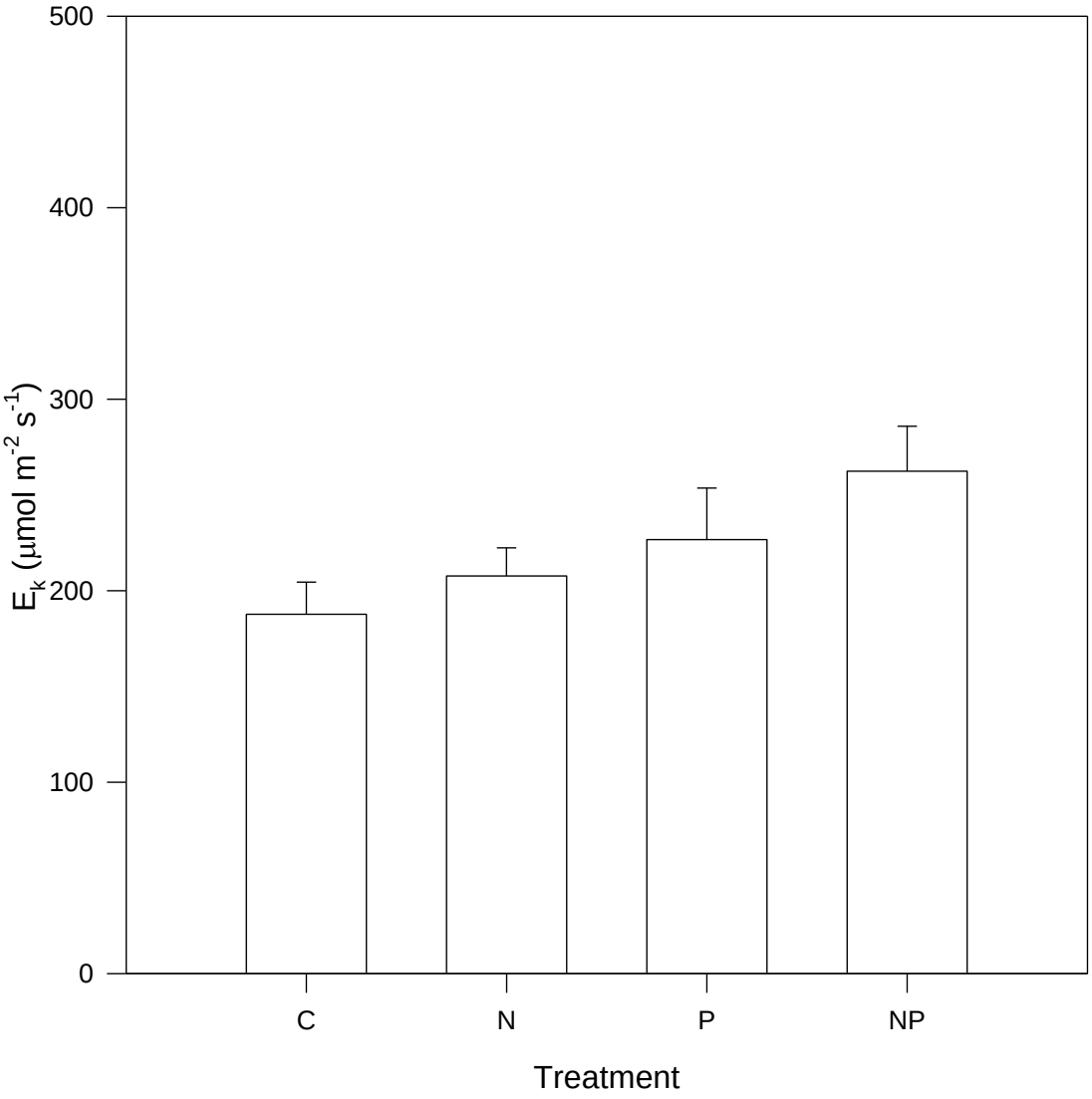

Fig 2.13. Minimum saturating irradiance (E_k) of *Pterocladiella capillacea* with four treatments (n=9). C: control, N: nitrate, P: phosphate, NP: nitrate and phosphate combined. Bars are SE.

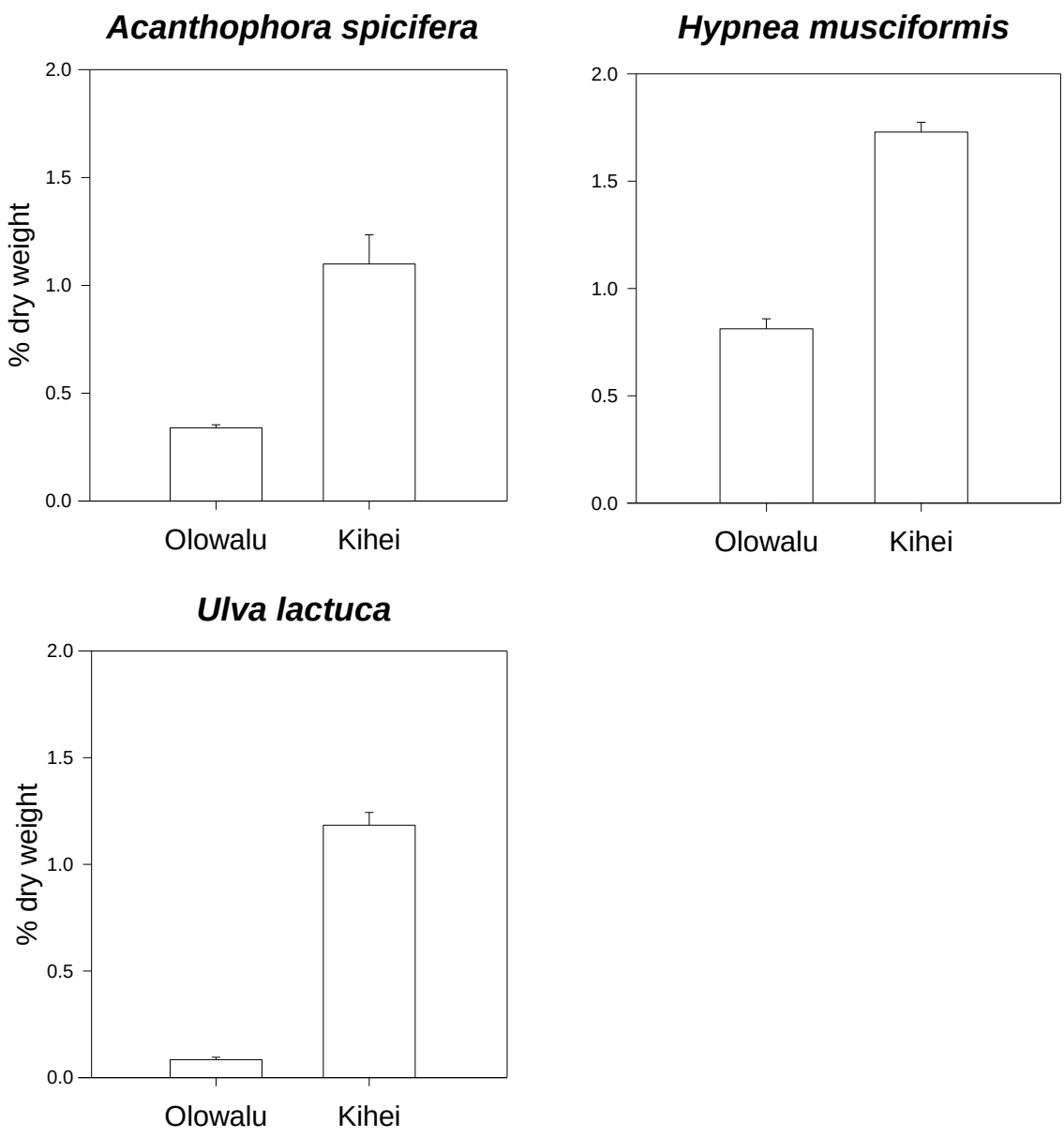

Fig 2.14. Arginine contents of three invasive algae collected from a eutrophic (Kihei) and a less-impacted (Olowalu) sites in Maui. Bars are SE.

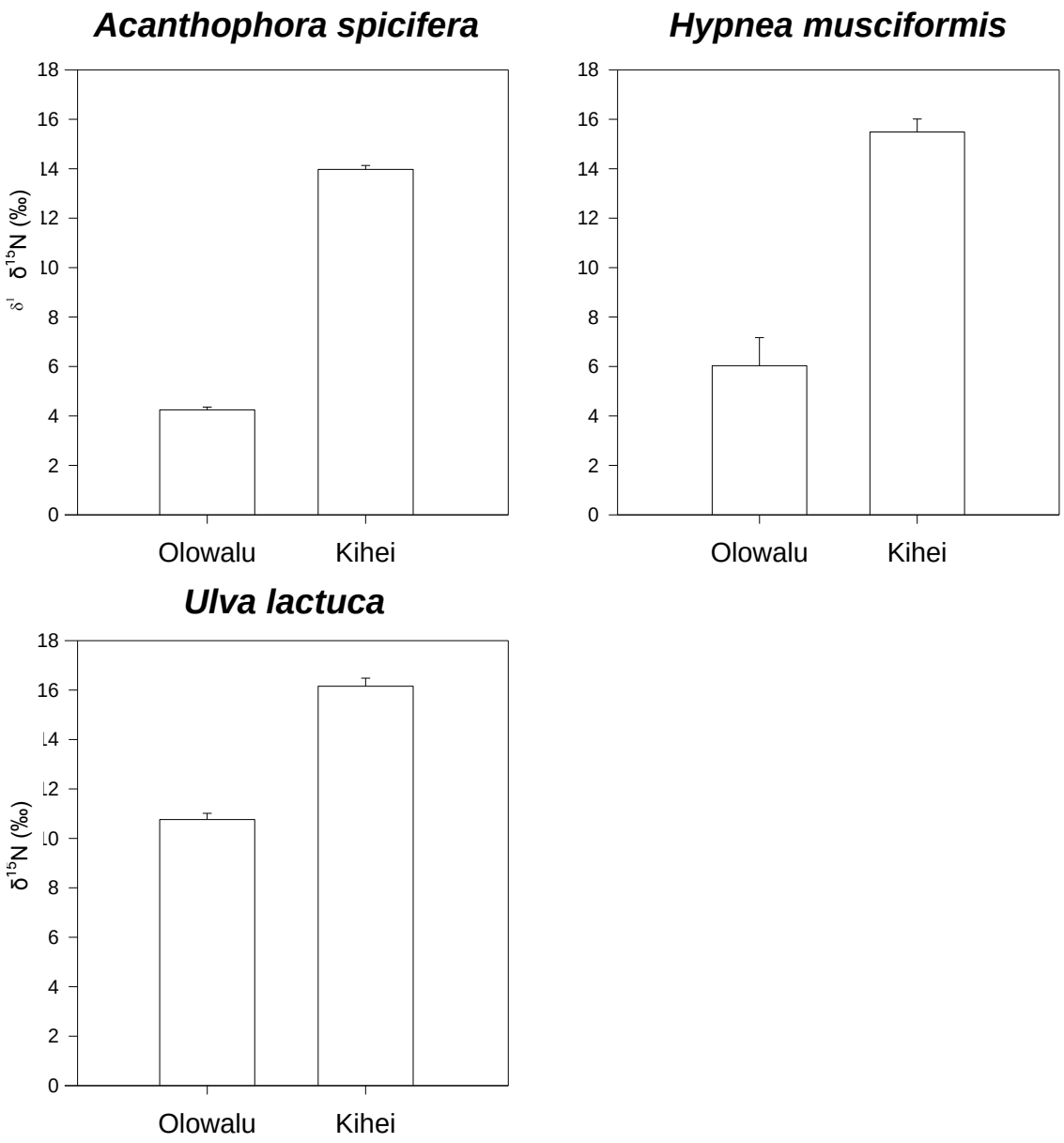

Fig 2.15. $\delta^{15}N$ values of three invasive algae collected from a eutrophic (Kihei) and a less-impacted (Olowalu) sites in Maui. Bars are SE.

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