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Effects of light and nutrient supply on stable isotope composition and fractionation in nitrogen-limited seagrass beds

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EFFECTS OF LIGHT AND NUTRIENT SUPPLY ON STABLE ISOTOPE COMPOSITION AND FRACTIONATION IN NITROGEN-LIMITED SEAGRASS BEDS

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Rebecca Jane Bernard

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To: Dean Kenneth Furton
College of Arts and Sciences

This thesis, written by Rebecca Jane Bernard, and entitled Effects of Light and Nutrient Supply on Stable Isotope Composition and Fractionation in Nitrogen-Limited Seagrass Beds, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Date of Defense: March 25, 2010

The thesis of Rebecca Jane Bernard is approved.

Dean Kenneth Furton
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Florida International University, 2010

DEDICATION

I dedicate this thesis to all the *Thalassia testudinum* at Grassy Key Bank in Florida Bay without which this work would not have been possible.

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ABSTRACT OF THE THESIS

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Professor James W. Fourqurean, Major Professor

This experiment investigated causes of seasonality of $\delta^{15}N$ and $\delta^{13}C$ values in *Thalassia testudinum* leaf tissue by manipulating plant demand and nutrient supply in situ for 13 months. I clearly demonstrated that seagrass elemental content, stable C and N isotopic content, morphology and the concentration of NH_4^+ in seagrass porewaters directly respond to manipulations of resources and also by the plant demand for nutrients to support growth. Isotopic values displayed marked seasonality with heavier values found in summer ($\delta^{15}N$=5.0‰ $\delta^{13}C$=-5.7‰) and lighter values in winter ($\delta^{15}N$=1.7‰ $\delta^{13}C$= -9.4‰). Calculations of Δ ($\delta^{15}N$ source DIN- $\delta^{15}N$ plant product) indicate that *T. testudinum* is able to strongly fractionate against source pool DIN. Interpretation of an enriched $\delta^{15}N$ signature as pollution-derived must first recognize the isotopic seasonality of the plant demand relative to the nutrient supply. Only when these links have been explained can the full relevance of $\delta^{15}N$ values be applied.

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1. Introduction

Coastal eutrophication and global climate change share a common pedigree; human populations are adroitly changing Earth's environmental systems. Humans are changing the planet through changes in atmospheric and hydrogeochemistry, changes in rates and balance of biogeochemical processes such as the components of the nitrogen cycle, and the diversity of life (Vitousek et al. 1997a; Vitousek et al. 1997b). Nearshore marine areas are especially affected by global climate change and anthropogenic nutrient inputs (Smith et al. 1999) with ecosystem productivity, distribution, and function markedly altered (Cloern 2001; Harley et al. 2006; Halpern et al. 2007).

Seagrasses--a group of about sixty species of marine angiosperms--are a critical component of the nearshore marine environment and can be described as the "canary" of the marine ecosystem because they are sensitive to both nutrient and light alteration that result from anthropogenic and climate perturbations. Inhabitants of shallow marine and estuary environments of all the world's continents except Antarctica (Green and Short 2003), seagrass populations are experiencing an accelerated rate of decline worldwide (Short and Wyllie-Echeverria 1996; Waycott et al. 2009) because they are sensitive to overexploitation, physical environment modification, nutrient and sediment pollution, and global climate change (Duarte 2000; Waycott et al. 2009). Seagrasses are primary producers that have a key role in ecosystem function and services and since they are fixed to the substratum seagrasses are very good indicators of their local environments. The carbon (C), nitrogen (N), and phosphorus (P) content of seagrass leaves may reflect relative nutrient and light availability (Duarte 1990; Grice et al. 1996) for a particular species in a particular place at a particular time. It is necessary to understand the

relationship between seagrasses and coastal eutrophication because of increased anthropogenic nutrient loading not only to promote the progress of science, but also to develop effective coastal management strategies and restorative measures that improve nearshore marine ecosystem health in the face of global climate change.

2. Objective of Study

Recently there has been increased use of stable isotopes of nitrogen to trace and discriminate among anthropogenic N inputs to ecosystems (Kendall et al. 2007). Studies have shown signals of nitrogen enriched with ^{15}N relative to ^{14}N in various ecosystems may be a result of anthropogenic N sources (McClelland et al. 1997; Lepoint et al. 2004; Risk et al. 2009); however, seasonal variations in nitrogen isotope fractionation may exhibit the same pattern of enrichment in summer months and confound results interpreted as pollution-derived (Anderson and Fourqurean 2003; Vizzini et al. 2003). It is paramount to understand the amount of natural variation in nitrogen isotope ratios from natural systems so the signal is not interpreted as pollution derived, when other factors such as seasonality or biological fractionation could be at work. Marine plants, including seagrasses, can indicate the stable isotopic signature of source dissolved inorganic nitrogen (DIN) —ammonium (NH_4^+) and nitrate (NO_3^-) in the water column for leaves and NH_4^+ in sediment porewater for roots—through the $\delta^{15}N$ values in their leaf tissue (Udy and Dennison 1997; Lee and Dunton 1999). Ratios of $\delta^{15}N$ in seagrasses have been shown to be more enriched (10‰) when source N was from anthropogenic sewage inputs (Costanzo et al. 2001) and lower (0‰) when source N was from biological N_2 fractionation (Lajtha and Marshall 1994). Conversely, an enriched $\delta^{15}N$ signature is also possible as a result of high light intensity promoting high photosynthetic rates in seagrass

on a seasonal basis. When rates of irradiance and productivity are high at N-limited sites, especially during the summer, plant N demand can exceed supply and lead to less isotopic discrimination against the heavier isotope (Fourqurean et al. 1997; Fourqurean et al. 2005). Thus, changes in light availability or N demand by the plant—that result from natural or anthropogenic influences—could affect nitrogen isotope fractionation as uptake rates by seagrasses change on a seasonal basis. Since biological isotope fractionation is a function of demand relative to supply, this experiment manipulated both supply (fertilization) and demand (light) of N to test whether observed ^{15}N seasonality can be explained by increased plant demand relative to supply in the summer months or conversely as function of the seasonality of ^{15}N of the source nutrients. Seagrass stable carbon isotope content has been shown to display marked seasonality also (Vizzini et al. 2003; Fourqurean et al. 2005) based upon the degree of carbon demand relative to the degree of carbon supply. At oceanic pH, carbon dioxide (CO_2) is limiting for seagrass photosynthesis (Beer 1989; Schwarz et al. 2000), but carbon becomes non-limiting if light levels are reduced to levels that limit photosynthesis (Durako and Hall 1992). Reduced light levels and subsequent reduced photosynthetic rates change the plant discrimination against ^{13}C as demand for C decreases and result in depleted carbon isotope signatures (Cooper and Deniro 1989). Increased carbon demand resulting from increased photosynthetic rates can result in reduced discrimination against ^{13}C and heavier isotope signatures. Similarly, enriched carbon isotope signatures can also be a product of decreased carbon supply (Durako and Sackett 1993) with changes in pH affecting carbon supply, and when plant demand outpaces supply there is less discrimination against ^{13}C. I hypothesize that the stable isotope composition and

fractionation of seagrass is regulated by the source nutrients and level of irradiance available to the plants and use stable isotopes from plant tissue, porewater, and water column to test this hypothesis.

3. Materials and Methods

Subjects and Setting—The in situ experiment and material collection was performed in a seagrass meadow dominated by *Thalassia testudinum* Banks ex König at Grassy Key Bank, Florida Bay, Florida, United States of America. Grassy Key Bank (N24° 49.328' W80° 54.663') (Figure 1) is located inside the boundary of the Florida Keys National Marine Sanctuary (FKNMS) which adjoins Biscayne National Park and Everglades National Park to the north and Dry Tortugas National Park to the west. Florida Bay is characterized as a shallow, saline, high light and low nutrient subtropical bay divided by shallow carbonate mud banks into discrete sub-basins located south of the Florida mainland and west of the Florida Keys (Fourqurean and Robblee 1999). The dominance of *T. testudinum* and interesting spatial patterns of nutrient limitation and availability (Fourqurean and Zieman 2002) and $\delta^{15}N$ ratios (Fourqurean et al. 2005) directed me to work in Florida Bay.

Experimental Design—I manipulated the demand for inorganic nutrients to supply the needs for seagrass growth by manipulating the intensity of light that drives photosynthesis using shade screens, and I manipulated the supply of inorganic N by fertilizing seagrass plots with nitrogen. To examine the interactions of changing supply and demand of resources on the performance of seagrass ecosystems and the fractionation of the inorganic nutrients on uptake, manipulations of both light and nutrient supply were

applied in a fully factorial design. Light and nitrogen treatments were randomized in a 3x3 factorial grid demarcated with 27 0.25m^2 polyvinyl chloride (PVC) quadrat frames secured to the benthos at 2m apart in a *T. testudinum* dominated seagrass meadow. Nitrogen levels (control, low, and high additions at a loading rate of 1.43 g N m^{-2} d^{-1} (MCSM 2001; Ferdie and Fourqurean 2004) in the form of slow release nitrogen fertilizer, Polyon^{TM}, Pursell Technologies Inc., 38-0-0 ($\delta^{15}N$=0.0 ‰), evenly sprinkled over the sediment surface (Armitage et al. 2006) and light levels (control, 25% light reduction, 75% light reduction with InterNet# xb1131 aquatic netting attached to a 1m^2 PVC frame positioned over the quadrats, see Figure 2) were experimentally manipulated at the study site. The shade net was replaced biweekly to minimize the effect of biofouling on the light penetration through the shades. Light measurements in the PAR waveband of 400 to 700 nm were made with a LI-COR 1400 data logger with a 4pi quantum sensor placed under and then outside the shade net at respective quadrats.

I hypothesized that plant morphology, plant growth rates, plant elemental, isotope, and chlorophyll content and fractionation of stable isotopes of C and N would be regulated by both the demand for resources to support growth and the supply rate of those resources to the plants. I sampled these response variables monthly for 13 months following the establishment of the experimental plots. I collected two *Thalassia testudinum* short shoots from each quadrat for stable isotope and elemental analysis and short-shoot morphology. Seasonal seagrass productivity was measured using the leaf-mark technique (Zieman 1974) during the growing season. A modified Braun-Blanquet (BB) survey (Braun-Blanquet 1972; Fourqurean et al. 2002) was performed at each

quadrat on a monthly basis to assess seagrass density. Water column samples filtered at 0.45 μm were collected in six 2 L amber high-density polyeythlyene (HDPE) narrow mouth bottles on a monthly basis for isotope and ammonium analysis. Porewater was collected by multiple 60 ml syringes modified to act as a coring apparatus (Brandsberg and Piggott 1968) from the root zone of *T. testudinum* (~40 cm) at each of the twenty-seven quadrats on a monthly basis until month seven of the project. The sediment cores were pressed for porewater using a hydrologic sediment press at the Stable Isotope Laboratory at University of Miami Rosenstiel School of Marine and Atmospheric Science (RSMAS). Following month seven, porewater was collected via sediment sippers (Fourqurean et al. 1992b) (Figure 3) into 60 ml syringes. The syringes were then filtered through GF/F filters (Whatman 1825-025) into evacuated collection bottles on the boat. Sample volumes were based on preliminary bench top ammonia colorimetric analysis (Hansen and Koroleff 1999) and the minimum amount of N needed for isotope analysis (25 μg in this case). All samples were stored on ice on the dive boat, Research Vessel "Halophila", and then deep frozen until analysis with changes in porewater ammonia concentration of the sample because of freezing deemed to be non-significant (Worm and Reusch 2000). Abiotic measurements included temperature, salinity, and turbidity. All fieldwork was conducted using Self Contained Underwater Breathing Apparatus (SCUBA).

Seagrass Analysis—At the lab, *T. testudinum* blades were gently scraped of epiphytes using a razor blade and measured for morphology. Seagrass samples, separated into the first 30 cm of the youngest leaf and all older leaf material, secured in pre-

weighed tares, were dried at a constant temperature (70° C) for 3 d in a laboratory oven. Sample dry weight was taken using a microbalance and samples were then homogenized into a fine powder using a Fritsch pulverisette. Using the homogenized samples, C and N nutrient composition were measured in duplicate in-house on a Fisons Carlo Erba Elemental Analyzer. Total phosphorous was determined in duplicate by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration in the extract (Fourqurean et al. 1992a). Elemental content was calculated on a dry weight basis; elemental ratios were calculated on a mole:mole basis.

Productivity samples were marked on randomly chosen short shoots in each treatment just above the bundle sheath with an 18 gauge hyperdermic needle and collected after 7 days. Leaf production rate per shoot was determined by dividing the dry weight of new leaf tissue produced by the number of days since marking. Areal leaf production rates were obtained by multiplying shoot leaf production rates by shoot density.

Short shoots of *T. testudinum* were harvested on the last collection day of the experiment from all treatments. Leaves were kept in a dark cooler with ice and transported to the laboratory for processing. To extract leaf chlorophyll approximately 30 mg of wet blade material was immersed in 5 ml of N, N-dimethylformamide (DMF) (Dunton and Tomasko 1994). Epiphytes were gently removed with a razor blade prior to immersion in DMF. The samples were placed in the dark at room temperature (~25° C) for 24 h and then analyzed on a Shimadzu 160 UV spectrophotometer at 664 nm and 647 nm. Absorbances were used to calculate the concentration of chl *a*, chl *b*, and total chl

using the following equations (Porra et al. 1989; Wellburn 1994) where OD is optical density:

Chl *a* ($\mu g\ ml^{-1}$)= $11.65(OD_{664})-2.69(OD_{647})$

Chl *b* ($\mu g\ ml^{-1}$)= $20.81(OD_{647})-4.53(OD_{664})$

Values were reported on a dry weight basis. A subset of leaf material was used to calculate a wet to dry conversion factor.

Isotope Analysis—Stable isotopes of carbon and nitrogen from the homogenized youngest leaf *Thalassia testudinum* samples were measured using standard elemental analyzer isotope ratio mass spectrometer (EA-IRMS) procedures (Fry et al. 1996). The EA is used to combust organic material forming N_2 and CO_2, which were measured on a Finnigan MAT Delta C IRMS coupled to a Conflo II and a Carlo Erba NC 1500 Elemental Analyzer in continuous flow mode at Florida International University's Southeast Environmental Research Center (SERC) Stable Isotope Laboratory. Nitrogen consists of two stable isotopes ^{14}N and ^{15}N (^{14}N: 99.64% ^{15}N: 0.36% (Nier 1950)). N_2 is measured for $^{15}N/^{14}N$ isotopic ratio and referred against the international standard of atmospheric nitrogen (AIR). CO_2 is used for $^{13}C/^{12}C$ isotopic ratio measurements against the international standard of Vienna Pee Dee Belemnite (V-PDB). The accepted unit of isotope ratio measurement is the delta value (δ given in per mil (‰). The δ value is defined as:

δ in ‰ $=[(R_{sample}/R_{standard})-1]*1000$

where R represents the measured isotope ratio. Using this convention, an increase in $\delta^{15}N$ value indicates the presence of a larger amount of the heavier isotope relative to the lighter isotope and such a sample is considered enriched. Isotope fractionation occurs in any thermodynamic reaction because of differences in the rates of reaction for molecular species of different mass. Standard Δ notation $^{15}N_{A-B}$ indicates the simple isotopic difference of the $\delta^{15}N$ values between two phases A and B typically in positive per mil (‰) units. These calculations were used to determine the biological fractionation between plant nutrient source (A) and product (B) at varying light and nutrient conditions.

Nitrate was isolated for isotopic measurement from monthly water column samples by the passive ammonia diffusion method (Sigman et al. 1997). Two Liters of sample containing roughly 0.5 to 1 μM NO_3^- was filtered into incubation bottles with 6g magnesium oxide (MgO) and incubated at 65°C for 5 d in a lab oven to raise the pH above 9.7 in order to remove traces of ammonia. After preincubation, samples were evaporated at 95°C to reduce the volume to ~250 ml and concentrate NO_3^- and remove NH_4^+ by volatilization. Samples were sealed after an addition of a NH_3 trap and Devarda's Alloy (75 mg per 100 ml initial sample volume) which reduced NO_3^- to NH_4^+ and absorbed NH_3 onto the trap. The NH_3 trap consists of an acidified precombusted Whatman GF/D filter sandwiched between two Teflon membranes with 10 μm pore size. Samples were incubated for 5 d at 65°C in a lab oven and 5 additional days on a shaker table. After a total incubation time of 15-18 d, the NH_3 traps were removed, placed in individual scintillation vials and dried in a desiccator with an open container of sulfuric

acid (H_2SO_4) for 2 d with the lids off, after which each filter was packed into a silver capsule and pelletized for EA-IRMS analysis.

Ammonium was isolated for isotopic measurement from monthly water column (2 L) and porewater (125 ml) samples following an adaptation of the ammonia diffusion method (Holmes et al. 1998). Filtered sample water was transferred to incubation bottles to which MgO (0.3 g per 100 ml) and the ammonia trap (as described above) were added. Samples and a standard curve of ammonium chloride (NH_4Cl) were incubated for 14 d on a shaker/incubator (Precision Shaking Water Bath Model 50) at 40°C. After incubation filter packages were removed from the incubation bottles and placed into individual scintillation vials and dried in a desiccator with an open container of H_2SO_4 for 2 d with the lids off. Once dry the filters were packed in silver capsules and pelletized for EA-IRMS analysis. Fractionation of standards (observed $\delta^{15}N$-actual $\delta^{15}N$) were calculated and added to the observed sample delta values to correct for fractionation. A fractionation of 4.03‰ was found to be associated with these methods. Calculation of Δ was made for porewater $\delta^{15}N$-NH_4^+ minus $\delta^{15}N$ *T. testudinum* tissue, water column $\delta^{15}N$-NH_4^+ minus $\delta^{15}N$ *T. testudinum* tissue, and water column $\delta^{15}N$-NO_3^- minus $\delta^{15}N$ *T. testudinum* tissue.

Ammonium Concentration—Manual colorimetric methods for determining ammonium concentration (Hansen and Koroleff 1999) in the porewater and water column samples showed a range between 2.52 and 1165 μM for the porewater (from the sediment sippers) and 0.14 to 6.66 μM for the water column over the duration of the study. Eastern Florida Bay water column ammonium levels from other studies were found to

average 3.41 μM (Boyer et al. 1999). These concentrations were used to calculate the minimum volume of water sample needed for isotope analysis.

Data Analysis—Significant differences in C:N:P ratios, elemental content, morphometric parameters, *Thalassia testudinum* tissue and source N (water column and porewater) isotope content, porewater ammonium concentration, *T. testudinum* abundance, and leaf productivity were tested using repeated measures analysis. Chlorophyll content of leaf tissue and chl *a:b* ratios were tested using full factorial analysis of variance (ANOVA). When the assumption of sphericity for repeated measures was violated, the p-values of Greenhouse-Geisser were used. Two-sample t-tests were used to determine if clean and fouled shade screens were statistically different treatments. All analyses were performed in SPSS 16.0.

4. Results

Site and Treatment—Salinity at the study site was marine and averaged 36.1 ± 0.44 with highest salinity found in Aug08 and the lowest in Dec08. Water temperature (°C) at the study site was the lowest in Feb09 (13 °C) and highest in Aug09 (33 °C). Turbidity averaged 0.3 ± 0.06 NTU for the duration of the study and reached a maximum in Dec08 and minima in April09 and May09 (Figure 4). The light reduction for the two treatments was found to be significantly different for clean screens as well as fouled screens (two-sample t-test, $p<0.01$ for clean and fouled screens).

Water Chemistry—Porewater ammonium concentration averaged 296.9 μM ± 53.4 μM for the duration of the study. Maximum porewater NH_4^+ concentrations were

observed in Jan09 and minimum NH_4^+ concentrations were observed in May09. Porewater NH_4^+ concentration was affected by both nutrient and the interaction of light and nutrients (ANOVA, Nutrient main effect $p<0.001$ and Light x nutrient main effect $p=0.017$, respectively) (Table 1). The high N addition treatments had the greatest influence on porewater NH_4^+ concentration and light reduction through time also exerted influence on porewater NH_4^+ concentration (Figure 5). Time also had an effect (time interaction $p=0.024$) on porewater NH_4^+ concentrations and data from the core syringe and sediment sipper collection methods showed similar trends that N addition to treatment plots significantly increased NH_4^+ concentration in the porewater through time relative to the controls. The high N addition treatments had the highest porewater NH_4^+ concentration (Figure 6 and Figure 7). Though light reduction treatments did not have a statistically significant effect on porewater NH_4^+ concentration, they did show a similar trend, with shade treatments increasing the NH_4^+ concentration in the porewater relative to the controls, evidence for change in supply and demand of NH_4^+ by *Thalassia testudinum* with light reduction. Plant demand for NH_4^+ was highest in the controls where light levels were ambient and NH_4^+ concentrations were the lowest. Water column NH_4^+ concentrations averaged 1.51 μM ± 0.58 μM for the duration of the study. Maximum water column NH_4^+ concentrations were observed in March09 and minima in Nov08 (Figure 8).

Porewater $\delta^{15}N\text{-}NH_4^+$ ranged from 5‰ (±0.91) in the winter to -14‰ (±2.45) in the summer and the isotope composition became more depleted during the growing season. Only N addition was found to have a significant effect ($p=0.005$) on porewater

$\delta^{15}N\text{-}NH_4^+$ values (Table 1). As N fertilization continued, $\delta^{15}N\text{-}NH_4^+$ values in the porewater became more enriched relative to the controls, likely reflecting the $\delta^{15}N$ value of the fertilizer, but during the growing season (March09-Aug09), the overall trend was a decrease in the isotopic composition of the porewater (Figure 9). Water column $\delta^{15}N\text{-}NH_4^+$ ranged from -5.2‰ in the late summer to -13‰ in the spring. Water column $\delta^{15}N\text{-}NO_3^-$ ranged from -10‰ in late summer to -21‰ in the late spring.

Isotope Content—The $\delta^{13}C$ values of *Thalassia testudinum* leaves significantly decreased in the light reduction plots (ANOVA, Light main effect $p<0.001$ Figure 10) (Table 2). The carbon isotope values in the leaf tissue also changed over time (ANOVA, Time main effect $p<0.001$), and there was a significant time by light interaction ($p=0.00$) for carbon isotope values indicating that light reduction affected the seasonality of $\delta^{13}C$ values in *T. testudinum* tissue with maximum enrichment reached in Oct08 and maximum depletion reached in March09. There was no significant effect of light or nutrient treatments on $\delta^{15}N$ of *T. testudinum* leaf tissue but there were indications that $\delta^{15}N$ values were influenced by time (ANOVA, Time main effect, $p<0.001$), time and nutrient (Nutrient by time interaction, $p=0.014$), as well as the interaction of time, light, and nutrient (Light by nutrient by time interaction, $p=0.014$) (Figure 11) with $\delta^{15}N$ values of the treatment plots showing a trend toward less enrichment through time compared to the controls. Post hoc tests revealed no significant differences between treatments, and time was the main controlling factor. However, when the data were split seasonally and analyzed for summer values only, nutrient addition was the main effect ($p=0.007$) on $\delta^{15}N$ values in *T. testudinum* tissue, and the interactions of time and time, light, and

nutrient were significant also ($p<0.001$ and $p=0.036$, respectively). Post hoc tests revealed that the controls were significantly different from the high nitrogen addition treatments ($p=0.005$). Adding N resulted in more depleted *T. testudinum* $\delta^{15}N$ new leaf tissue values relative to the controls. No significant differences between the light treatments suggest no supply-demand driven fractionation.

Calculation of biological fractionation Δ—The differences between the isotope ratios of the plant material and the source DIN (Δ) were computed from *T. testudinum* N source and tissue product for porewater $\delta^{15}N\text{-}NH_4^+$, water column $\delta^{15}N\text{-}NH_4^+$, and water column $\delta^{15}N\text{-}NO_3^-$. Nutrient addition was found to have a significant effect on the fractionation between porewater $\delta^{15}N\text{-}NH_4^+$ values and *T. testudinum* $\delta^{15}N$ tissue content (ANOVA, Nutrient main effect, $p<0.001$) (Table 3). With N addition, less fractionation occurred between porewater $\delta^{15}N\text{-}NH_4^+$ and *T. testudinum* $\delta^{15}N$ values than for those of the controls. Fractionation increased in summer months (June09-Aug09) and ranged from less than 1‰ (±1.58) to 18‰ (±1.05) (Figure 12). Neither light reduction nor nutrient addition had a significant effect on isotope fractionation between *T. testudinum* and its water column source nutrients, but there was a secondary interaction of time and nutrients ($p=0.014$ and $p=0.002$ for water column NO_3^- and NH_4^+ respectively) indicating that the nutrient addition treatments behaved differently through time. Adding N slightly decreased the fractionation between water column $\delta^{15}N\text{-}NH_4^+$ and *T. testudinum* $\delta^{15}N$ content as well as water column $\delta^{15}N\text{-}NO_3^-$ and *T. testudinum* $\delta^{15}N$ content (Figure 13 and Figure 14).

Elemental Content—The nutrient content of *Thalassia testudinum* leaves was a function of light treatment, with C:N decreasing (ANOVA, light main effect $p<0.001$), C:P decreasing ($p<0.001$), and N:P decreasing ($p<0.001$) with light reduction (Table 4). Nutrient addition also influenced elemental content. C:N declined (ANOVA, nutrient main effect $p=0.028$) and N:P increased ($p=0.033$) with N addition. Nutrient content also changed over time (ANOVA, time main effect $p<0.001$ for all ratios), and there was a significant time by light interaction for all nutrient ratios indicating that the nutrient addition affected the seasonality of nutrient content. For the duration of the study, light reduction decreased elemental ratios for C:N, C:P and N:P toward "seagrass Redfield Ratios" of 474:24:1 (Redfield 1958; Duarte 1990) compared to the controls, and faster seagrass growth rate affected plant demand for N resulting in the control plots being N limited. All elemental ratios followed similar trends with a peak ratio occurring in Feb09 (Figures 15-17).

Plant Responses—Seagrass species and composition was dominated by *Thalassia testudinum* at the experimental site for the duration of the study. Braun-Blanquet scores for *T. testudinum* density were between 2 and 5 with an average of 3 (25 to 50% cover). Maximum density occurred in May09 and June09. Minimum density occurred in Feb09 but also decreased in July09. Overall light and nutrient treatments did not have significant main effects on *T. testudinum* abundance, but there was indication that *T. testudinum* density responded to the light treatments differently through time (light x time interaction $p=0.002$) (Table 5) as the 75% light reduction treatment had lower BB scores than the other light treatments at the end of the study (Figure 18).

Morphometric characteristics of *T. testudinum* showed plasticity in their response to light and nutrient treatments. Leaf area (cm^2 SS^{-1}) and leaf mass (mg SS^{-1}) showed similar trends with a decrease in winter months that reached a minimum in Dec08 and a peak for all treatments in June09. Light treatment significantly influenced both leaf area and leaf mass (Light main effect in ANOVA $p=0.031$ and $p=0.030$, respectively) (Table 5), and light reduction affected leaf area and leaf mass through time (Light x time interaction $p=0.001$ and $p<0.001$, respectively) with the least amount of leaf area (23.52 cm^2 SS^{-1}) and leaf mass (116.33 mg SS^{-1}) in the 75% light reduction treatment by the end of the experiment (Figure 19 and Figure 20). Leaf length (mm) and width (mm) followed similar patterns with minimum values in winter and peak values in June09. Overall, light and nutrient treatments did not have a significant effect on *T. testudinum* size, but there was indication that leaf length and width responded to light treatments differently through time (Light x time interaction $p=0.004$ and $p=0.011$, respectively) with lower length (110 cm) and width (8.5 cm) in the 75% light reduction treatment at the end of the study (Figure 21 and Figure 22).

Growth rates for *T. testudinum* were significantly influenced by light levels (ANOVA Light main effect, $p<0.001$) (Table 6) and areal leaf production (g dw m^{-2} d^{-1}) responded differently to the different treatments through time (ANOVA time main effect $p<0.001$) with the least amount of production (0.27 g dw m^{-2} d^{-1}) found in the 75% light reduction treatment at the end of the study. Areal leaf production was at a minimum in Feb09 and increased during the warmer months of the growing season (Figure 23). There were no significant effects of light or nutrient treatments on specific productivity (mg g^{-1}

d^{-1}), but there was indication that specific productivity was influenced by time (Time main effect $p<0.001$) as the measured specific productivity increased through the growing season (Figure 24).

Total chlorophyll (mg g^{-1} dw), chlorophyll *a* (mg g^{-1} dw), and chlorophyll *a:b* ratios were not affected by nutrient or light treatments. Nutrient addition significantly affected chlorophyll *b* values (ANOVA Nutrient main effect $p=0.001$) (Table 7) with the highest measurements found at the highest N addition treatments (1.60 mg g^{-1} dw). Chlorophyll *a:b* ratios showed a decreasing trend with light reduction and the lowest value (2.52) coincided with the 75% light reduction treatment (Figure 25), however, this trend was not statistically significant.

5. Discussion

The experiment presented here clearly demonstrates that seagrass elemental content, stable C and N isotopic content, morphology and the concentration of NH_4^+ in seagrass porewaters directly respond to manipulations of not just supply of resources to the ecosystem, but also by the demand for nutrients and CO_2 to support plant growth. These findings are in agreement with previous studies in seagrass systems and expand the understanding of the seasonality of the stable isotope signature in *Thalassia testudinum* tissue in relation to the plant's source nutrients. Seasonality was expected to be due to either seasonal variation of $\delta^{15}N$ in source DIN or differential fractionation as plant N demand exceeded supply of DIN source with a constant $\delta^{15}N$. This experiment changed the isotopic composition of the porewater by adding slow release fertilizer with constant $\delta^{15}N$ and changed the degree of fractionation of the ammonium pool by changing the

balance between nutrient supply and plant demand with reduced light levels. Given reduced light levels or high N availability the $\delta^{15}N$ composition of *T. testudinum* leaves were expected to be depleted relative to the source DIN while an enriched signature relative to the source DIN was expected at low N availability or summer light levels. When supply of DIN, accomplished by N fertilization, exceeds plant demand nitrogen fractionation in *T. testudinum* should shift in favor of the lighter isotope. Fractionation between source nutrients and plant tissue product was expected to increase as plant demand for N exceeds supply of DIN.

Calculations of biological fractionation Δ—Fractionation of the isotopic composition of the source CO_2 and DIN changes as a function of the balance of plant nutrient supply and demand as expressed by a seasonal pattern between diminished light levels or N addition and *Thalassia testudinum* tissue product. There was a trend of more isotopic separation between source N and plant product during the summer when plant demand outstripped supply, but with N amelioration the isotopic separation between DIN source and plant product was less pronounced compared to the control plots (Figure 12) for porewater $\delta^{15}N\text{-}NH_4^+$ and water column $\delta^{15}N\text{-}NH_4^+$. In the winter isotope fractionation followed our expectations with separation between plant tissue product and DIN source more pronounced in the light reduction and N addition treatments compared to the controls for $\delta^{15}N\text{-}NH_4^+$ and water column $\delta^{15}N\text{-}NH_4^+$, indicating that by increasing supply of DIN or changing plant demand for N, the plant can be more selective against ^{15}N.

As porewater $\delta^{15}N\text{-}NH_4^+$ became more depleted in the summer months, the isotope separation between source N and plant product became larger. N addition caused less fractionation between plant product and nutrient source relative to the controls, indicating that plant demand could be amended with N supply, but still displayed large fractionation during the summer and less during the winter. Water column $\delta^{15}N\text{-}NO_3^-$ showed a fractionation pattern that may be related to the relative supply of NO_3^- available to the seagrasses in the water column. Variation in source DIN is due to seasonality of rainfall (Lapointe 1997), bottom water and interstitial water concentration (Yamamuro et al. 2003), nitrogen fertilization (Udy et al. 1999), anthropogenic inputs into marine systems (McClelland et al. 1997; McClelland and Valiela 1998a), as well as seasonal rates of denitrification and nitrification which leave the residual DIN pool enriched with ^{15}N (Mariotti et al. 1981). Composition of the DIN pool is a complex function of supply (e.g. deposition, N fixation, and reminerialization), utilization (e.g. plant uptake and denitrification), and losses both advective and diffusive which all influence the $\delta^{15}N$ composition of the DIN source (Figure 26). It is possible that the DIN isotope values show seasonal depletion in the summer due to competition for NH_4^+ by both nitrifiers and primary producers as well as competition for NO_3^- by primary producers and denitrifiers (Cornwell et al. 1999) that fractionate the DIN source pool. If active nitrification was occurring the most during the summer months and the urea fertilizer was reduced as fast as it was added, it is possible that in conjunction with the competition for the source nutrients, the residual pool would become highly deplete. The *T. testudinum* $\delta^{15}N$ content was generally more enriched in ^{15}N compared to its source DIN indicating the possibility of nutrient limitation regulating N uptake, plant source nitrogen from DON

which this study did not account for, other processes such as dissimilatory nitrate reductase acting on DIN leaving the residual pool highly deplete in ^{15}N, or inefficiencies in the sampling method for DIN. The lack of ^{15}N enrichment in the residual N source may also be the result of sedimentary denitrification caused by diffusion-limited NO_3^- flux within the reactive microsites of the sediments (Brandes and Devol 1997).

Isotope Content—Both $\delta^{13}C$ and $\delta^{15}N$ values for *Thalassia testudinum* displayed seasonal enrichment and depletion patterns with maximum enrichment occurring in summer to early fall. Interannual variation of $\delta^{13}C$ ranged from -9.4‰ (March09) to -5.7‰ (Aug08) and was found to be most influenced by light levels. Light manipulations depleted the $\delta^{13}C$ values relative to the controls and resulted in greater discrimination against the heavier isotope than non-shaded plots. By changing light levels without changing the isotopic composition or abundance of source CO_2, the plant demand for $CO_{2\,(aq)}$ was reduced and this led to greater discrimination against ^{13}C and lighter isotope value for *T. testudinum* tissue content. This observation is in agreement with other studies that report light limited seagrasses fractionate the dissolved inorganic carbon (DIC) pool by preferentially discriminating against the heavier isotope which results in isotopic depletion of the seagrass tissue and show that as light is reduced to levels that limit photosynthetic rates of *T. testudinum* carbon becomes non-limiting (Durako and Hall 1992; Hemminga and Mateo 1996; Lee and Dunton 1997). What is interesting about the carbon isotope values in relation to the reduced light levels is the application to longer lived seagrass species such as *Posidonia oceanica* that could record light history in its rhizome tissue (Ruiz and Romero 2001; Vizzini et al. 2003) as the plant meets its

$CO_{2\,(aq)}$ nutrient requirements. As light becomes limiting carbon becomes less limiting because of a decrease in diffusion-limited carbon demand at sub-saturating photosynthetic rates by affecting $CO_{2\,(aq)}$ acquisition mechanisms (Schwarz et al. 2000; Nayar et al. 2009). This then leads to an increase in enzymatic discrimination against ^{13}C at lower irradiances resulting in more negative $\delta^{13}C$ (Grice et al. 1996; Ralph et al. 2007). Interpretation of the $\delta^{13}C$ values in the rhizome tissue could reconstruct a light history for the life of the plant. Further implications of a reconstructed light history would give baseline benchmarks for water clarity restoration in euotrophic nearshore areas. Anthropogenic nutrient perturbations into aquatic systems affect seagrasses on the internal level and combined actions of nutrient uptake and assimilation processes that are photosynthetically dependent may result in a more dramatic impact on seagrass survival (Moore and Wetzel 2000; Ibarra-Obando et al. 2004).

Seasonality of *Thalassia testudinum* $\delta^{15}N$ values have been postulated to be the result of seasonal variation in the $\delta^{15}N$ of the DIN or seasonal differences in the fractionation of the DIN pool during uptake (Fourqurean et al. 1997; Anderson and Fourqurean 2003; Fourqurean et al. 2005) or the result of an increased uptake of land-derived DIN with high $\delta^{15}N$ values (Yamamuro et al. 2003). The likely cause is thought to be decreased fractionation in summer when plant growth demands outstrip N supply and draw down the pool of available N. My study corroborates the thought that plant supply and demand are driving the seasonality of $\delta^{15}N$ values in the leaf tissue. *Thalassia testudinum* in the control plots had heavier tissue $\delta^{15}N$ values in the summer to late fall and lighter $\delta^{15}N$ values in the winter and with N addition or light reduction, the plant

could be more selective against ^{15}N (Figure 11). Variations in $\delta^{15}N$ values ranged from 1.7‰ (Feb09) to 5.0‰ (Aug08) and were most influenced by N addition with N addition plots having relatively low $\delta^{15}N$ seagrass tissue values compared to controls (Figure 27). These findings indicate the importance of understanding the seasonality of *T. testudinum* standing crop production in relation to its source nutrients especially during the summer growing season when plant demand exceeds DIN supply—likely because seagrass growth rate is high enough to be nutrient limited—and the summer $\delta^{15}N$ values of the plant begin to reflect the $\delta^{15}N$ of source nutrients (0 ‰ for fertilizer). Experimental studies that do not take into account the seasonality of the plant or only sample during one season may not detect seasonal cycles of increased fractionation in summer when plant growth demands exceed nutrient supply and may erroneously conclude anthropogenic pollution based on the isotope value of the plant tissue.

Elemental Content—"Seagrass C:N:P Redfield Ratios" have been reported as 474:24:1 (Duarte 1990) and deviation away from this ratio expresses nutrient limitation. C:N:P elemental ratios for *Thalassia testudinum* tissue peaked in Feb09 and C:N ratios indicated that light reduction and N addition reduced N limitation. C:P and N:P ratios indicated P limitation, and as more N was added over the duration of the study, P limitation gave way to light limitation. Changes in nutrient availability resulted in changes in *T. testudinum* elemental ratios. As N content increased, C:N ratios decreased, indicating N amerloration. Average nutrient concentrations (as % DW) for the duration of the study were 37.04 for carbon, 2.03 for nitrogen and 0.096 for phosphorus and this agreed with P limitation and adequate supply of N to fulfill plant demand found in the

elemental ratios. Seagrass nutrient deficiency is more significant in the growing season because of the increased demand for nutrients (Pedersen and Borum 1997) as photosynthetic demand increases and because of the low availability of nutrients in the environment as well as competition with other organisms like macroalgae , phytoplankton, epiphytes, and bacteria. My study shows that with N addition or light reduction, plant N demands were met during the growing season, and the %N content in the tissue was highest in the 75% light reduction treatments and the high N treatments. Ecological theory of resource limitation and partitioning has long been debated in the literature (Grime 1977; Tilman 1985) and this study indicates the need to understand the chemical nature of plant resource sources as well as their fates to better understand resource limitation and competition among primary producers and nitrifiers and denitrifiers in a whole ecosystem context.

Plant Responses—In coastal areas nearshore seagrass beds are susceptible to anthropogenic nutrient inputs such as septic effluent, land and agricultural drainage, and municipal sewage which lead to an increase in nutrient concentrations and a decrease in available light in the water column as excessive phytoplankton growth (eutrophication) results in a sharp decline in submerged aquatic vegetation communities (Herbert 1999). In this experiment N amelioration was a proxy for anthropogenic nutrient inputs and the shade treatments were a proxy for decreased available light due to an increase in algae abundance from the increased nutrients. Morphometric parameters of the leaves generally decreased in the winter and increased with the summer growing season, reaching a maximum in June09. Light reduction and nitrogen addition treatments caused

the leaf width to increase as a response to abundant nutrients and as a measure to effectively capture light at lower irradiance levels. Specific productivity provides an indication of the plant's physiological state as evidenced by rate of growth of individual plants; areal productivity measurement is an indication of the production of new organic matter in the ecosystem and is a function of specific growth rate and plant density. As specific productivity and areal productivity increased over the growing season the $\delta^{15}N$ and $\delta^{13}C$ values of the plant tissue became more enriched indicating that as the plant growth outstripped its source nutrient pool during the summer, its $\delta^{15}N$ and $\delta^{13}C$ values reflected less discrimination against the heavier isotope (Figure 28 and Figure 29).

Seagrasses are able to adapt their photosynthetic process to low-light surroundings (Drew 1978; Baker and Mckiernan 1988) by shifting their photosynthetic-irradiance response (Dennison and Alberte 1982) and photosynthetic pigment content (Major and Dunton 2002). At the end of the experiment chl *a* and chl *b* concentrations were highest in the 75% light reduction and high N addition treatments compared to the controls. Increases in accessory pigments (chl *b*) relative to antenna pigments (chl *a*) in low light (Lamote and Dunton 2006) serve to increase the photosynthetic efficiency by harvesting a larger range of wavelengths since chl *b* absorbs wavelengths not absorbed by chl *a* (Mcpherson and Miller 1987) and this study is consistent with other studies that document a decrease in the chl *a:b* ratio in response to light reduction (Czerny and Dunton 1995; Lee and Dunton 1997). This study also documents a decrease in the chl *a:b* ratio in response to nutrient addition as well.

Water Chemistry—Seagrasses take up N by both leaves and roots in amounts depending on the relative availability in the sediment and the water column (Stapel et al. 1996; Lee and Dunton 1999). Seagrass leaves have been shown to prefer inorganic N sources and urea as a dissolved organic nitrogen source (DON) over amino acids while roots can take up amino acids at comparable rates to NH_4^+ and prefer those N sources to urea and NO_3^- (Vonk et al. 2008). Porewater NH_4^+ concentration increased with N addition but was not as pronounced in the treatments that had both N addition and light reduction. This may be due to the seagrass depleting the local source nutrient pool to maintain metabolic function in response to light reduction. Porewater NH_4^+ concentrations were influenced by light level, with lower ammonium concentration in the 25% light reduction plots, suggesting that highly productive seagrasses drew down the NH_4^+ pools in the porewater or that light reduction led to more nitrification or anammox. Higher NH_4^+ concentrations were found in the 75% light reduction treatments indicating lower utilization by *T. testudinum* could drive resource repletion (Lee and Dunton 1997). As available NH_4^+ increased in the porewater, the $\delta^{15}N$ values in *Thalassia testudinum* tissue became more isotopically depleted which indicated that by changing the amount of source N available to a seagrass, the plant could preferentially discriminate against the heavier isotope. Porewater $\delta^{15}N$-NH_4^+ became more enriched relative to the controls with both N addition and light reduction as *T. testudinum* fractionated the source pool in favor of the lighter isotope. Even with the relative enrichment compared to the controls, the porewater $\delta^{15}N$-NH_4^+ values became more deplete in the summer months. The decrease of the porewater $\delta^{15}N$-NH_4^+ values during the growing season could be explained by *Thalassia testudinum* not exhibiting any isotopic discrimination against the

heavier isotope during nutrient uptake or the plant metabolizing the heavier isotope and preferentially respiring ^{14}N. Water column $\delta^{15}N\text{-}NH_4^+$ and $\delta^{15}N\text{-}NO_3^-$ values were very deplete in ^{15}N and may be attributed to newly produced NO_3^- arising from nitrification (Sugimoto et al. 2008). To fully understand the seasonality of the nutrient content in seagrass leaves, it is important to understand the seasonality of the source N being utilized by the plant.

Experimental Restrictions—This study documented that seagrass elemental content, stable C and N isotopic content, morphology and the concentration of NH_4^+ in seagrass porewaters directly respond to manipulations of not just supply of resources to the ecosystem, but also to the demand for nutrients and CO_2 to support plant growth. However, this study only sampled one specific species in one specific area over a given amount of time, and while potentially useful in the understanding of seagrass ecology, its broad applicability may be limited. Other locations such as isolated basins with long water retention times may not display the same patterns of isotopic depletion and enrichment observed here. Caution should be taken with broad application of results from one species-specific study to other seagrass species even in the same sampling location. Other studies have observed species-specific variability in seasonal isotope content (Fourqurean et al. 2007; Campbell and Fourqurean 2009) indicating that interpretation of elemental and isotope content needs to be species specific. There is also limitation with our methods used to calculate Δ as a large fractionation (about 10‰) is reported with the ammonia diffusion method. As of yet, it is the best method we have to

work with until a laser isotope machine that can speciate N isotopes from a water sample is available to the masses.

6. Conclusion

Anthropogenic influences into a coastal ecosystem are a key source of increased nutrient loads into the marine realm and these nutrients have the potential to shift marine ecosystem function through algal blooms and decreases in water column clarity. To understand effects of global climate change and anthropogenic nutrient perturbations on shallow oligotrophic coastal systems, a better understanding of the relationship between aquatic primary producers such as *Thalassia testudinum* and their source nutrients is warranted. Stable isotopes of carbon and nitrogen are a good tool to elucidate this relationship but caution must be made on general interpretation and application. It is necessary to understand the cycles and processes controlling naturally occurring or anthropogenically derived source nutrients as well as the nutrient product in the plant tissue. The *T. testudinum* isotope values presented here are not as enriched as land-derived source N $\delta^{15}N$ values have been reported (McClelland and Valiela 1998b) but still indicate the need to understand N as a source and its fate in nearshore marine systems. This study has demonstrated that *T. testudinum* is able to strongly fractionate the source pool DIN, so it is necessary to determine the relationship between plant and source. An enriched $\delta^{15}N$ signature interpreted as pollution-derived is out of context without first understanding the $\delta^{15}N$ of the source nutrients and the seasonality of the plant demand relative to the nutrient supply. Only when these links have been explained can the full significance of $\delta^{15}N$ values be applied.

Tables

Table 1. Repeated Measures ANOVA for water chemistry constituents. Significance shown in bold. † indicates sphericity assumptions were violated and all interactions used the Greenhouse-Geisser values.

	Main Effects	df	F	p	**Interactions†**	df	F	p
pw NH_4^+ conc.	Light	2	1.266	0.307	Time	3.498	3.183	**0.024**
(μM)	Nutrient	2	18.988	**<0.001**	Time x light	6.997	0.506	0.826
	Light x nutrient	4	4.108	**0.017**	Time x nutrient	6.997	0.875	0.532
	Error	17			Time x light x nutrient	13.993	0.497	0.926
					Error	59.471		
	Main Effects	df	F	p	**Interactions†**	df	F	p
pw $\delta^{15}N$-NH_4^+	Light	2	0.170	0.845	Time	3.827	27.314	**<0.001**
	Nutrient	2	7.331	**0.005**	Time x light	7.654	0.750	0.642
	Light x nutrient	4	1.311	0.303	Time x nutrient	7.654	1.624	0.137
	Error	18			Time x light x nutrient	15.309	0.739	0.739
					Error	68.890		

Table 2. Repeated Measures ANOVA performed on *Thalassia testudinum* isotope content. Significance shown in bold. † indicates sphericity assumptions were violated and all interactions used the Greenhouse-Geisser values.

	Main Effects	df	F	p	**Interactions**	df	F	p
Tt $\delta^{13}C$	Light	2	77.928	**<0.001**	Time	12	40.946	**<0.001**
	Nutrient	2	0.165	0.849	Time x light	24	7.285	**<0.001**
	Light x nutrient	4	0.652	0.633	Time x nutrient	24	0.854	0.665
	Error	18			Time x light x nutrient	48	1.074	0.357
					Error	216		
	Main Effects	df	F	p	**Interactions**	df	F	p
Tt $\delta^{15}N$	Light	2	677.878	0.189	Time	12	18.589	**<0.001**
	Nutrient	2	1.828	0.071	Time x light	24	0.647	0.932
	Light x nutrient	4	3.075	0.930	Time x nutrient	24	1.812	**0.014**
	Error	18	0.209		Time x light x nutrient	48	1.592	**0.014**
					Error	216		
	Main Effects	df	F	p	**Interactions†**	df	F	p
Tt $\delta^{15}N$-summer	Light	2	1.984	0.166	Time	4.138	4.344	**0.003**
	Nutrient	2	6.729	**0.007**	Time x light	8.276	0.660	0.729
	Light x nutrient	4	0.303	0.872	Time x nutrient	8.276	1.010	0.437
	Error	18			Time x light x nutrient	16.552	1.867	**0.036**
					Error	74.482		

Table 3. Repeated Measures ANOVA performed for Δ. Significance shown in bold. † indicates sphericity assumptions were violated and all interactions used the Greenhouse-Geisser values.

	Main Effects	df	F	p	**Interactions†**	df	F	p
pw $\delta^{15}N\text{-}NH_4^+$ - δ15N Tt	Light	2	0.543	0.590	Time	4.271	21.926	**<0.001**
	Nutrient	2	18.674	**<0.001**	Time x light	8.543	0.586	0.797
	Light x nutrient	4	2.072	0.127	Time x nutrient	8.543	1.762	0.093
	Error	18			Time x light x nutrient	17.085	0.680	0.813
					Error	76.883		

	Main Effects	df	F	p	**Interactions**	df	F	p
WC $\delta^{15}N\text{-}NH_4^+$ - δ15N Tt	Light	2	1.341	0.287	Time	5	224.230	**<0.001**
	Nutrient	2	3.055	0.072	Time x light	10	0.298	0.980
	Light x nutrient	4	0.632	0.646	Time x nutrient	10	3.106	**0.002**
	Error	18			Time x light x nutrient	20	1.628	0.063
					Error	90		

	Main Effects	df	F	p	**Interactions**	df	F	p
WC $\delta^{15}N\text{-}NO_3^-$ - δ15N Tt	Light	2	1.826	0.190	Time	12	196.184	**<0.001**
	Nutrient	2	3.075	**0.071**	Time x light	24	0.598	0.932
	Light x nutrient	4	0.209	0.930	Time x nutrient	24	1.813	**0.014**
	Error	18			Time x light x nutrient	48	1.593	**0.014**
					Error	216		

Table 4. Repeated Measures ANOVA performed on *Thalassia testudinum* elemental content. Significance shown in bold. † indicates sphericity assumptions were violated and all interactions used the Greenhouse-Geisser values.

	Main Effects	df	F	p	**Interactions†**	df	F	p
C:N	Light	2	73.078	**<0.001**	Time	5.990	21.388	**<0.001**
	Nutrient	2	4.408	**0.028**	Time x light	11.980	3.581	**<0.001**
	Light x nutrient	4	0.575	0.684	Time x nutrient	11.980	0.784	0.666
	Error	18			Time x light x nutrient	23.960	0.974	0.540
					Error	107.821		
	Main Effects	df	F	p	**Interactions**	df	F	p
C:P	Light	2	129.510	**<0.001**	Time	12	12.039	**<0.001**
	Nutrient	2	0.381	0.689	Time x light	24	6.994	**<0.001**
	Light x nutrient	4	0.948	0.459	Time x nutrient	24	1.251	0.202
	Error	18			Time x light x nutrient	48	0.897	0.666
					Error	216		
	Main Effects	df	F	p	**Interactions†**	df	F	p
N:P	Light	2	44.533	**<0.001**	Time	4.878	8.152	**<0.001**
	Nutrient	2	4.140	**0.033**	Time x light	9.755	5.722	**<0.001**
	Light x nutrient	4	0.761	0.564	Time x nutrient	9.755	1.340	0.224
	Error	18			Time x light x nutrient	19.511	1.171	0.300
					Error	87.798		

Table 5. Repeated Measures ANOVA performed on *Thalassia testudinum* plant responses. Significance shown in bold. † indicates sphericity assumptions were violated and all interactions used the Greenhouse-Geisser values.

	Main Effects	df	F	p	**Interactions†**	df	F	p
BB abundance	Light	2	1.286	0.301	Time	4.837	16.554	**<0.001**
	Nutrient	2	0.612	0.553	Time x light	9.675	3.239	**0.002**
	Light x nutrient	4	0.241	0.991	Time x nutrient	9.675	0.156	0.985
	Error	18			Time x light x nutrient	19.350	0.324	0.927
					Error	87.074		
	Main Effects	df	F	p	**Interactions†**	df	F	p
Leaf Area (cm^2 SS^{-1})	Light	2	4.262	**0.031**	Time	5.947	25.267	**<0.001**
	Nutrient	2	0.312	0.736	Time x light	11.895	3.089	**0.001**
	Light x nutrient	4	0.914	0.477	Time x nutrient	11.895	1.213	0.284
	Error	18			Time x light x nutrient	23.789	0.843	0.674
					Error	107.052		
	Main Effects	df	F	p	**Interactions**	df	F	p
Leaf Mass (mg SS^{-1})	Light	2	4.312	**0.030**	Time	12	20.457	**<0.001**
	Nutrient	2	0.357	0.704	Time x light	24	3.200	**<0.001**
	Light x nutrient	4	0.728	0.584	Time x nutrient	24	1.341	0.140
	Error	18			Time x light x nutrient	48	0.823	0.787
					Error	216		
	Main Effects	df	F	p	**Interactions†**	df	F	p
Leaf Length (mm)	Light	2	0.405	0.673	Time	6.062	28.918	**<0.001**
	Nutrient	2	0.182	0.835	Time x light	12.123	2.658	**0.004**
	Light x nutrient	4	0.682	0.613	Time x nutrient	12.123	1.455	0.152
	Error	18			Time x light x nutrient	24.246	0.891	0.614
					Error	109.109		
	Main Effects	df	F	p	**Interactions**	df	F	p
Leaf Width (mm)	Light	2	2.840	0.085	Time	12	9.102	**<0.001**
	Nutrient	2	0.848	0.445	Time x light	24	1.869	**0.011**
	Light x nutrient	4	2.272	0.101	Time x nutrient	24	1.100	0.346
	Error	18			Time x light x nutrient	48	1.015	0.454
					Error	216		

Table 6. Repeated Measures ANOVA performed on *Thalassia testudinum* plant productivity. Significance shown in bold. † indicates sphericity assumptions were violated and all interactions used the Greenhouse-Geisser values.

	Main Effects	df	F	p	**Interactions**	df	F	p
Areal Productivity ($g\ dw\ m^{-2}\ d^{-1}$)	Light	2	20.968	**<0.001**	Time	2	34.722	**<0.001**
	Nutrient	2	0.213	0.810	Time x light	4	15.083	**<0.001**
	Light x nutrient	4	2.706	0.063	Time x nutrient	4	1.755	0.159
	Error	18			Time x light x nutrient	8	0.713	0.678
					Error	36		
	Main Effects	df	F	p	**Interactions**	df	F	p
Specific Productivity ($mg\ g^{-1}\ d^{-1}$)	Light	2	2.745	0.091	Time	2	37.685	**<0.001**
	Nutrient	2	0.032	0.969	Time x light	4	1.408	0.251
	Light x nutrient	4	0.364	0.831	Time x nutrient	4	1.732	0.164
	Error	18			Time x light x nutrient	8	0.616	0.759
					Error	36		

Table 7. Two-Way ANOVA performed on *Thalassia testudinum* leaf chlorophyll content. Significance shown in bold.

		df	F	p
chl a	Light	2	0.721	0.490
(mg/g dw)	Nutrient	2	0.585	0.560
	Light x nutrient	4	1.380	0.250
	Error	72		

		df	F	p
chl b	Light	2	0.969	0.384
(mg/g dw)	Nutrient	2	7.563	**0.001**
	Light x nutrient	4	2.241	0.073
	Error	72		

		df	F	p
total chl	Light	2	0.037	0.964
(mg/g dw)	Nutrient	2	0.242	0.785
	Light x nutrient	4	0.891	0.474
	Error	72		

		df	F	p
chl a:b	Light	2	1.488	0.233
	Nutrient	2	0.928	0.400
	Light x nutrient	4	0.494	0.740
	Error	72		

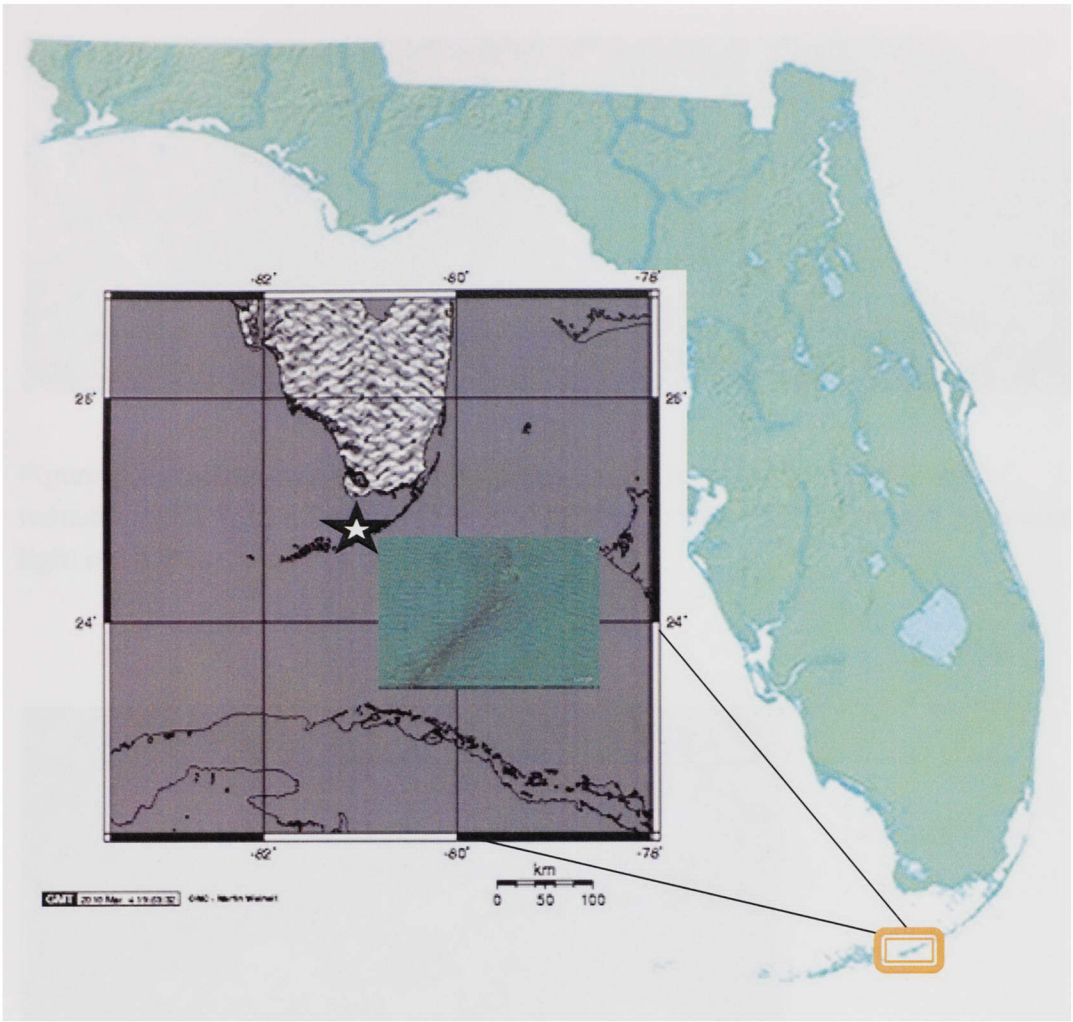

Figure 1. Grassy Key Bank

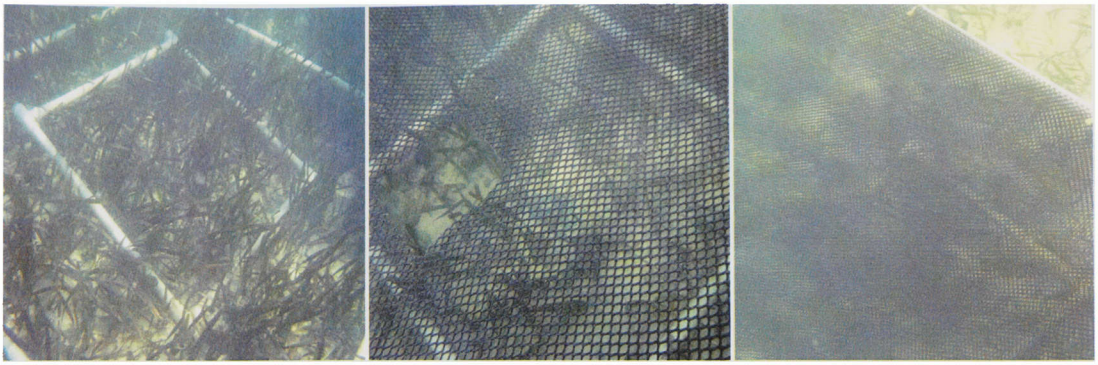

Figure 2. Experimental Treatments. From l to r: control/HN/LN; 25% light reduction/25% light reduction +LN/25% light reduction +HN; 75% light reduction/75% light reduction +LN/ 75% light reduction +HN.

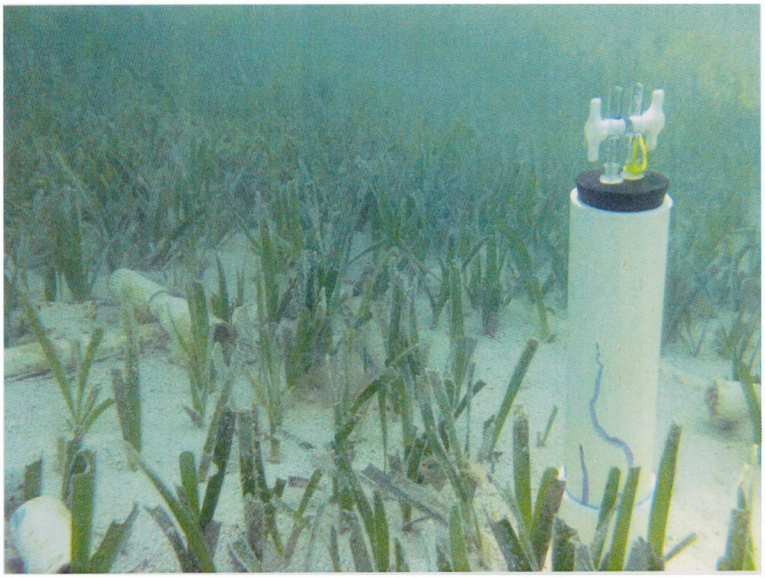

Figure 3. Sediment Sipper

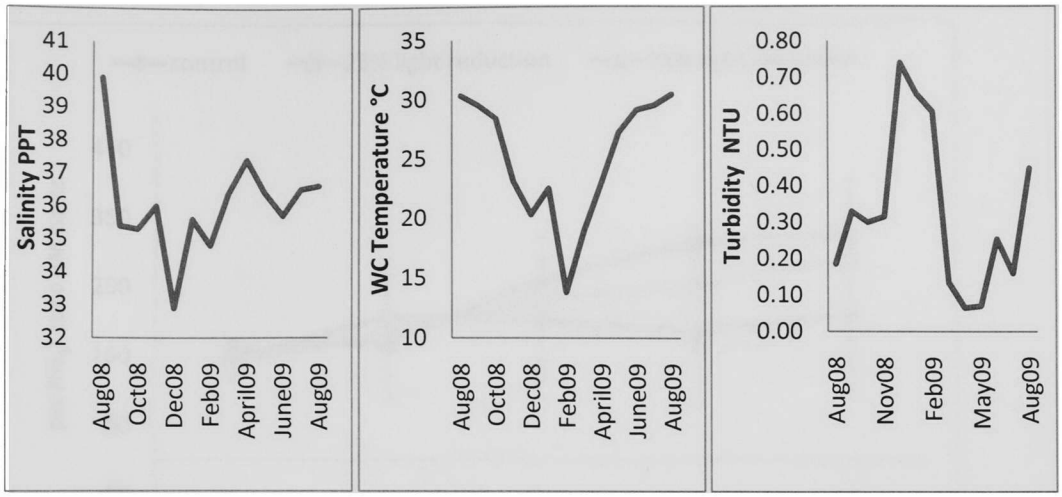

Figure 4. Abiotic Parameters

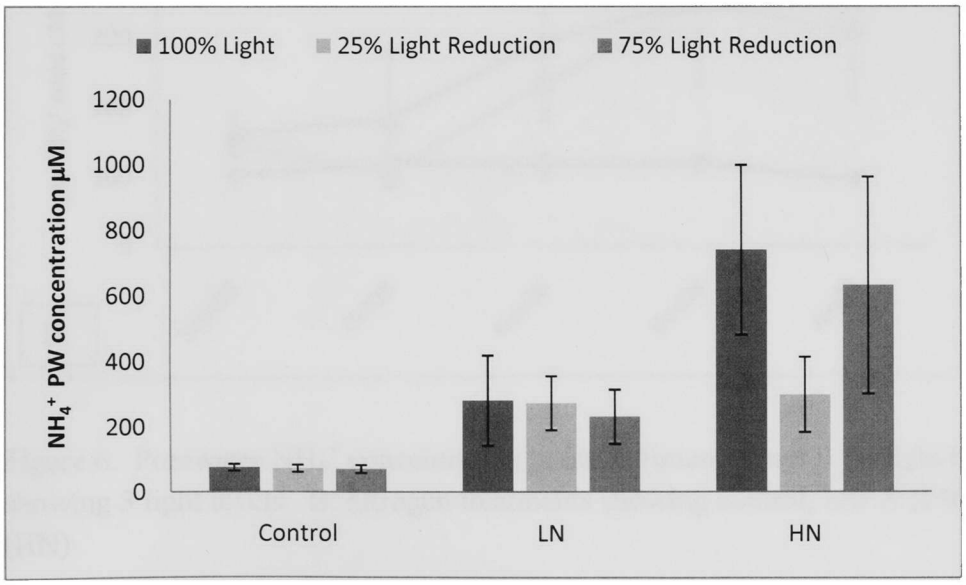

Figure 5. Porewater NH_4^+ concentration with N treatment

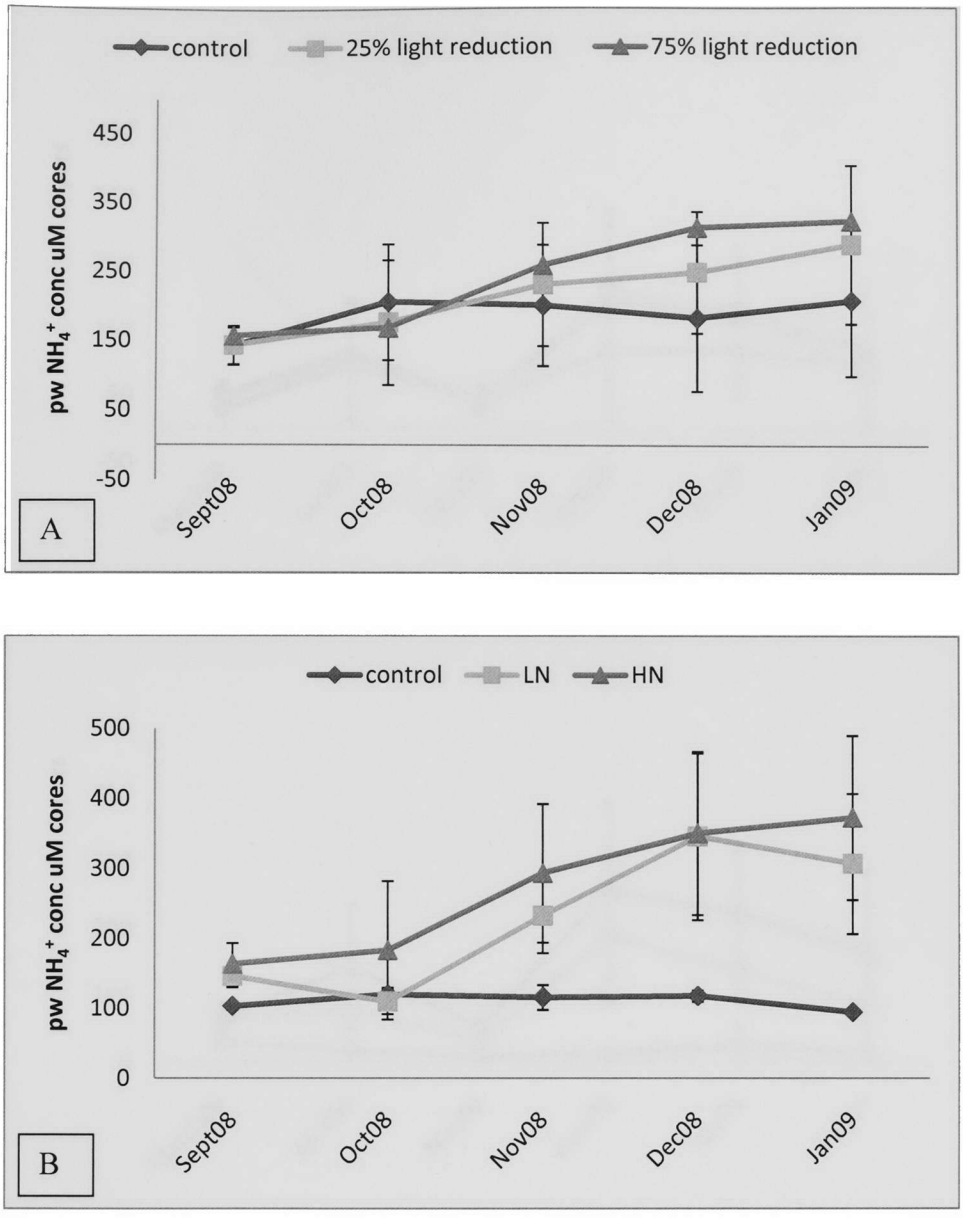

Figure 6. Porewater NH_4^+ concentration in the sediment (cores). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

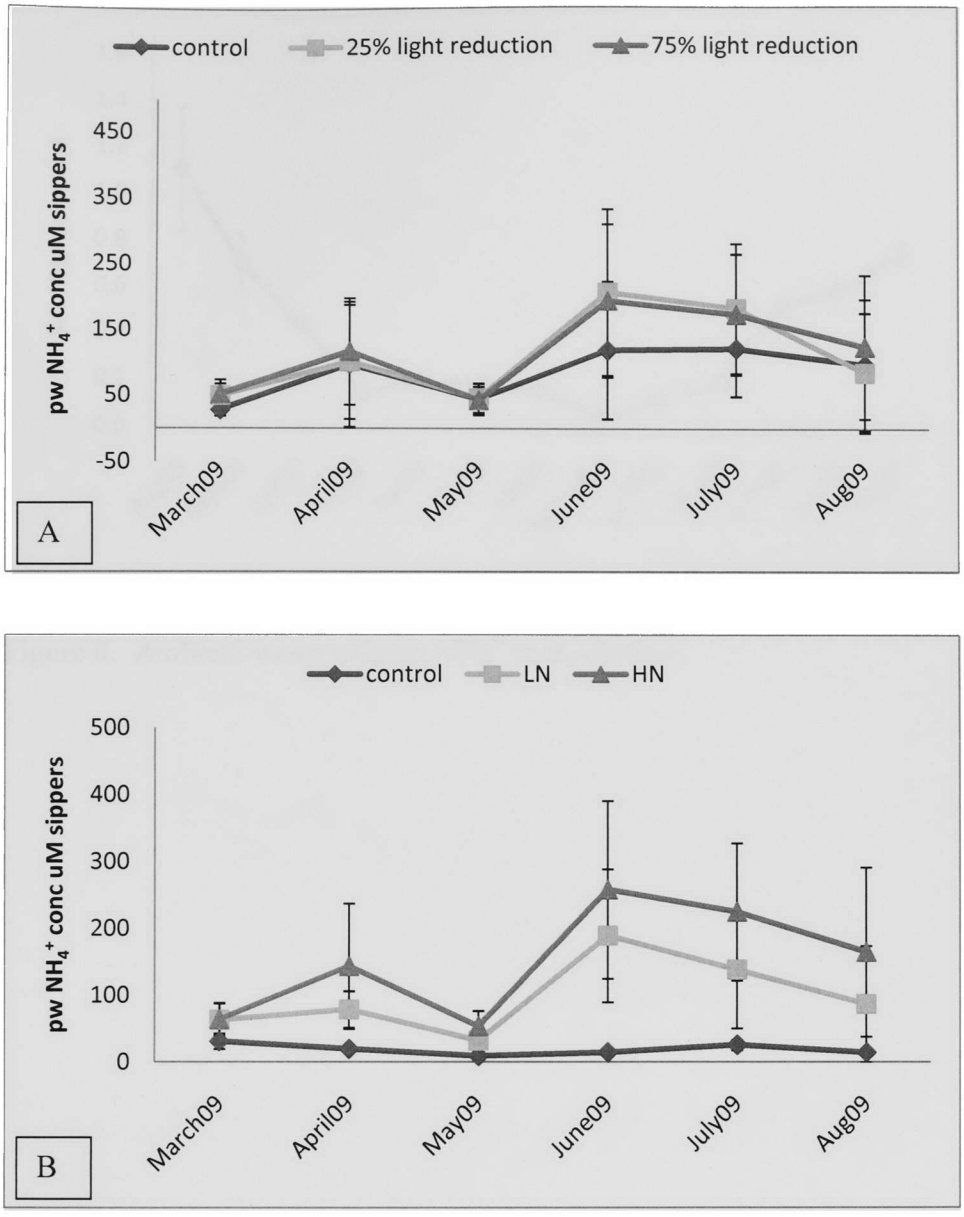

Figure 7. Porewater NH_4^+ concentration in the sediment (sippers). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

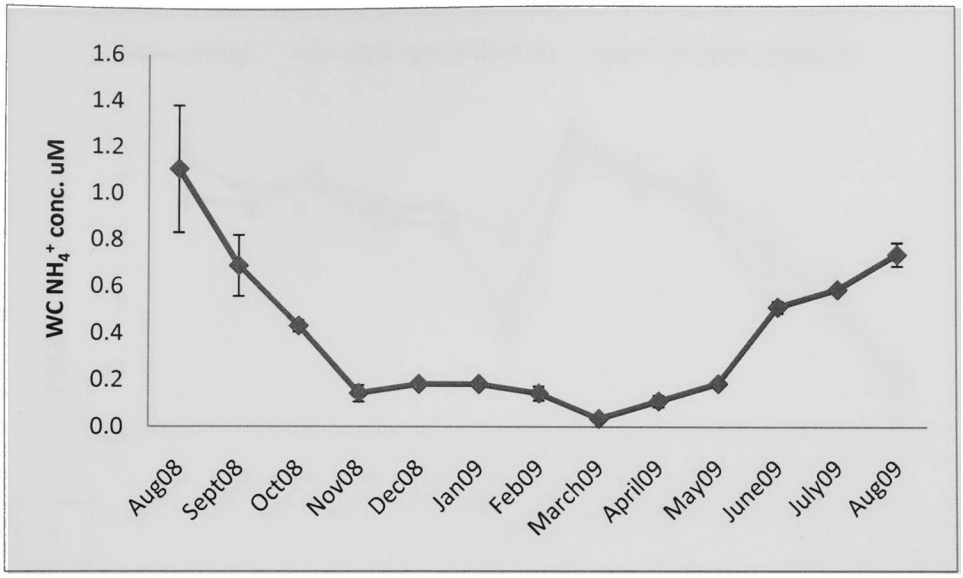

Figure 8. Ambient water column NH_4^+ concentration

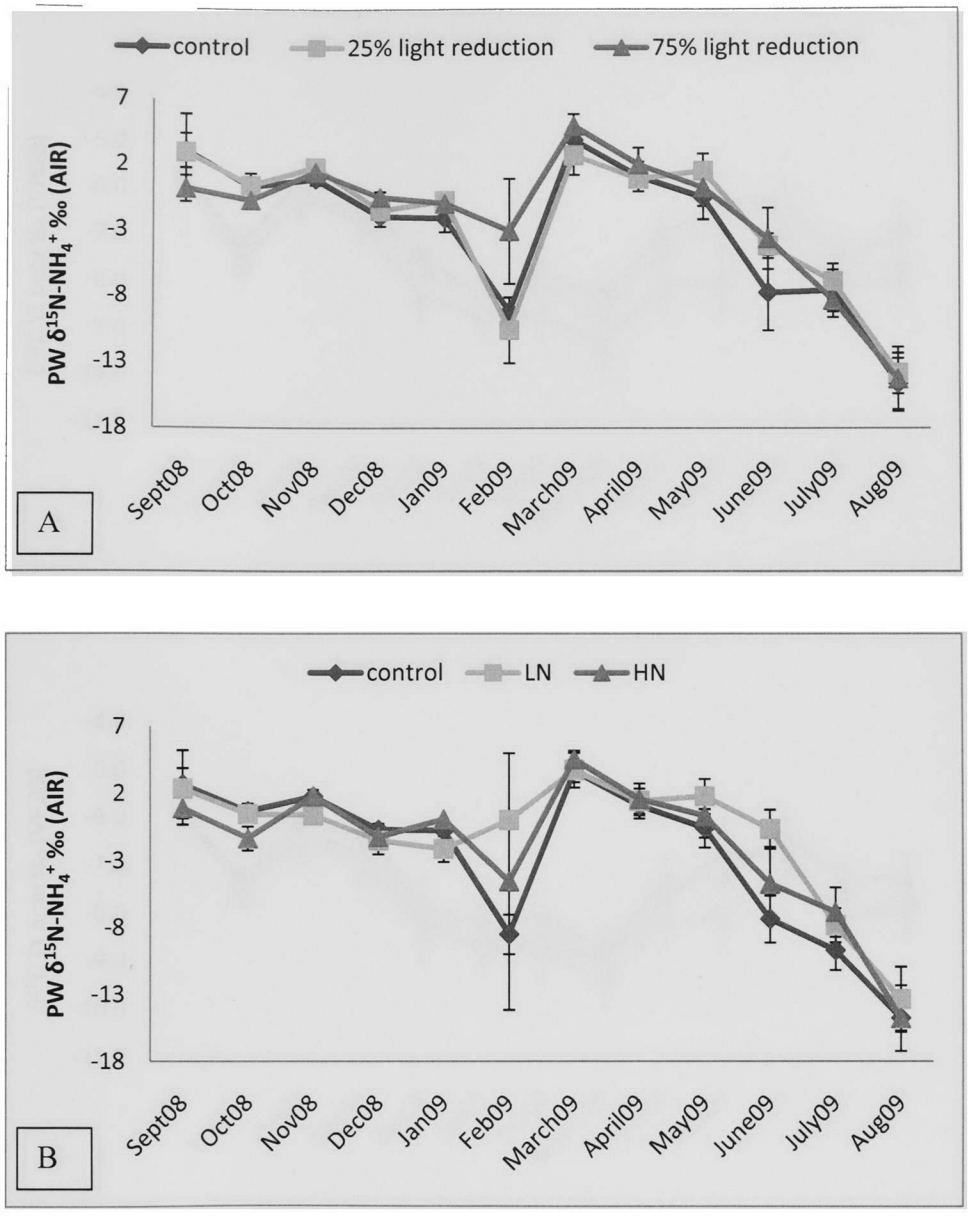

Figure 9. Porewater $\delta^{15}N$- NH_4^+ time series. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

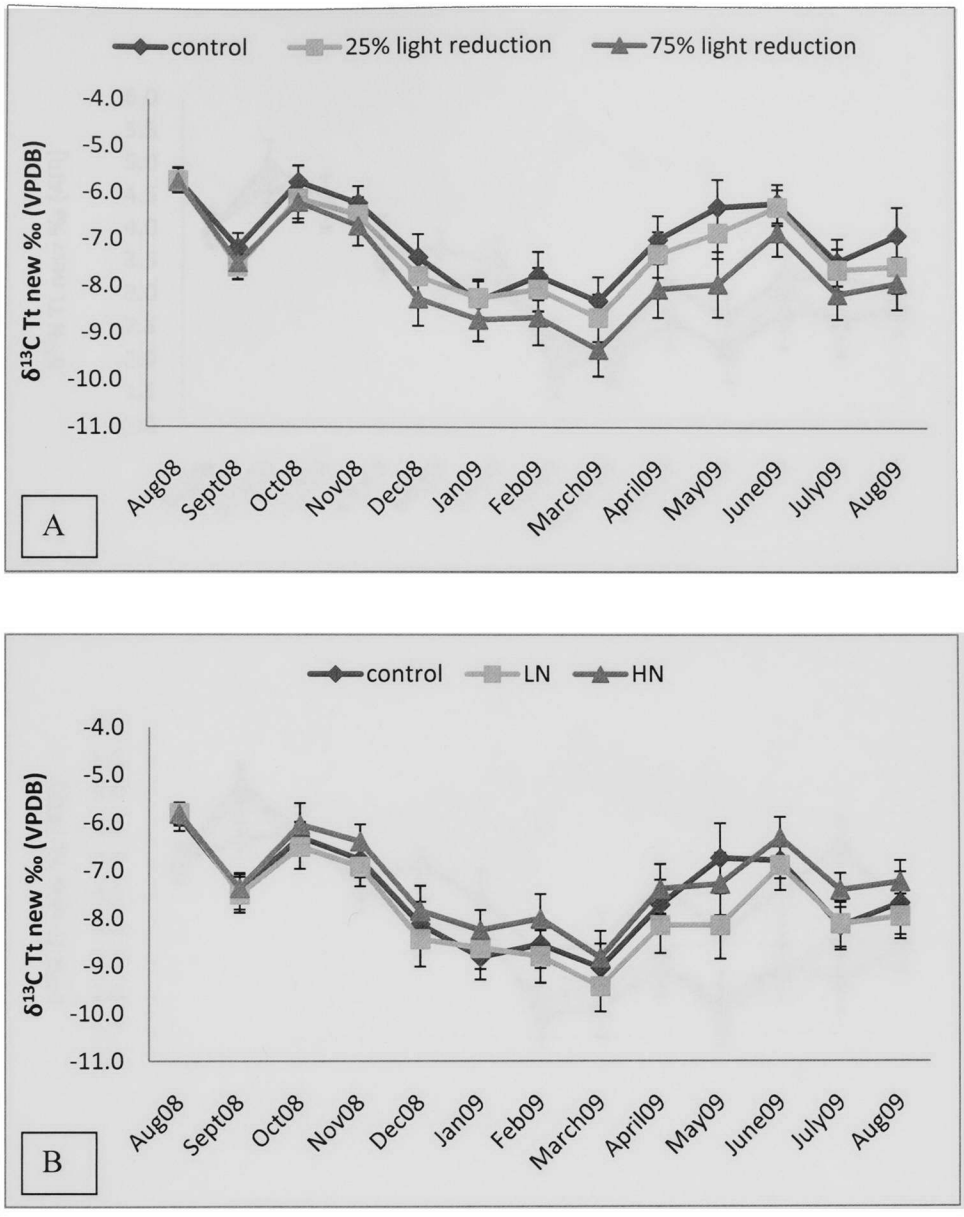

Figure 10. *Thalassia testudinum* $\delta^{13}C$ content of new leaf tissue. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

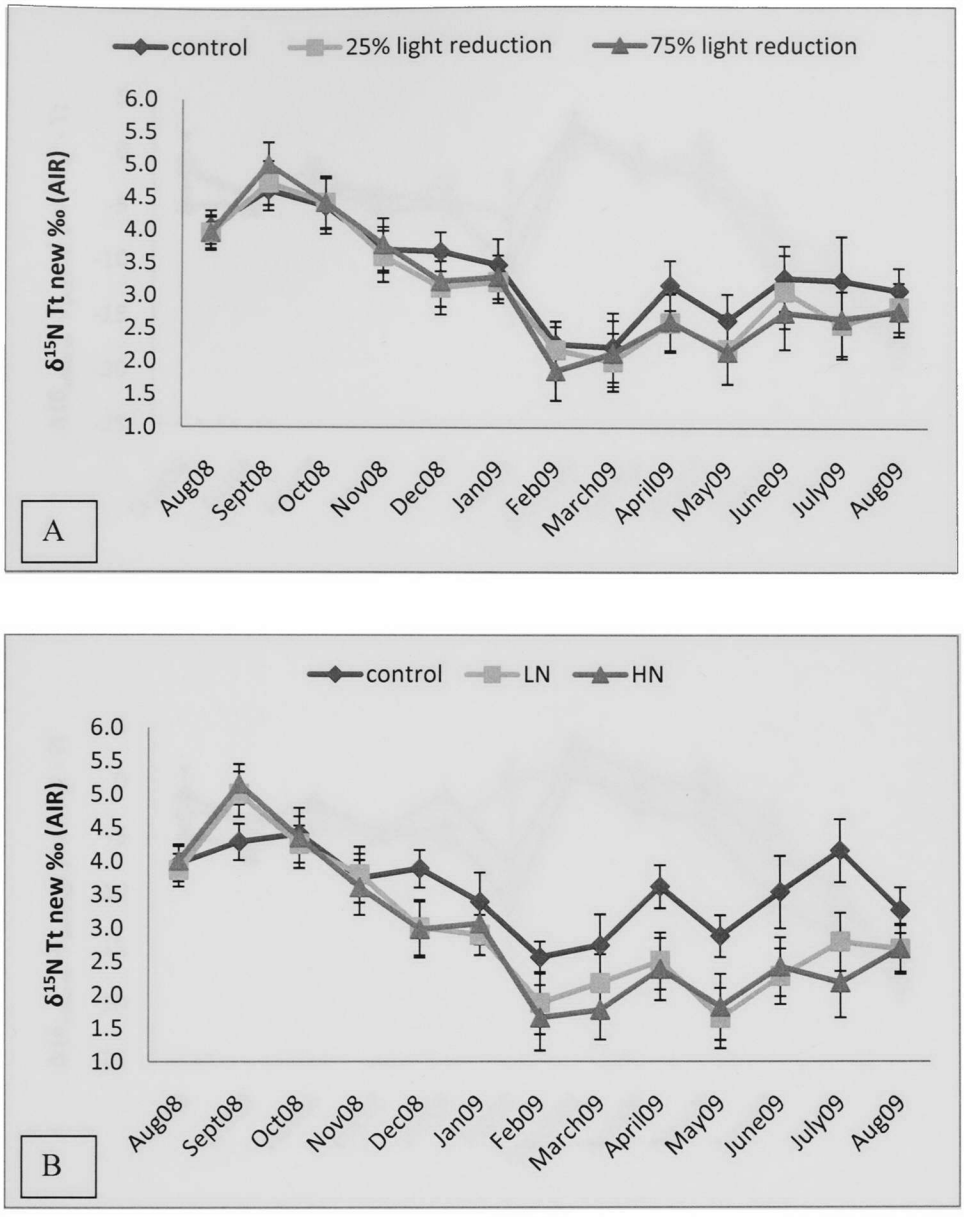

Figure 11. *Thalassia testudinum* $\delta^{15}N$ content of new leaf tissue. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

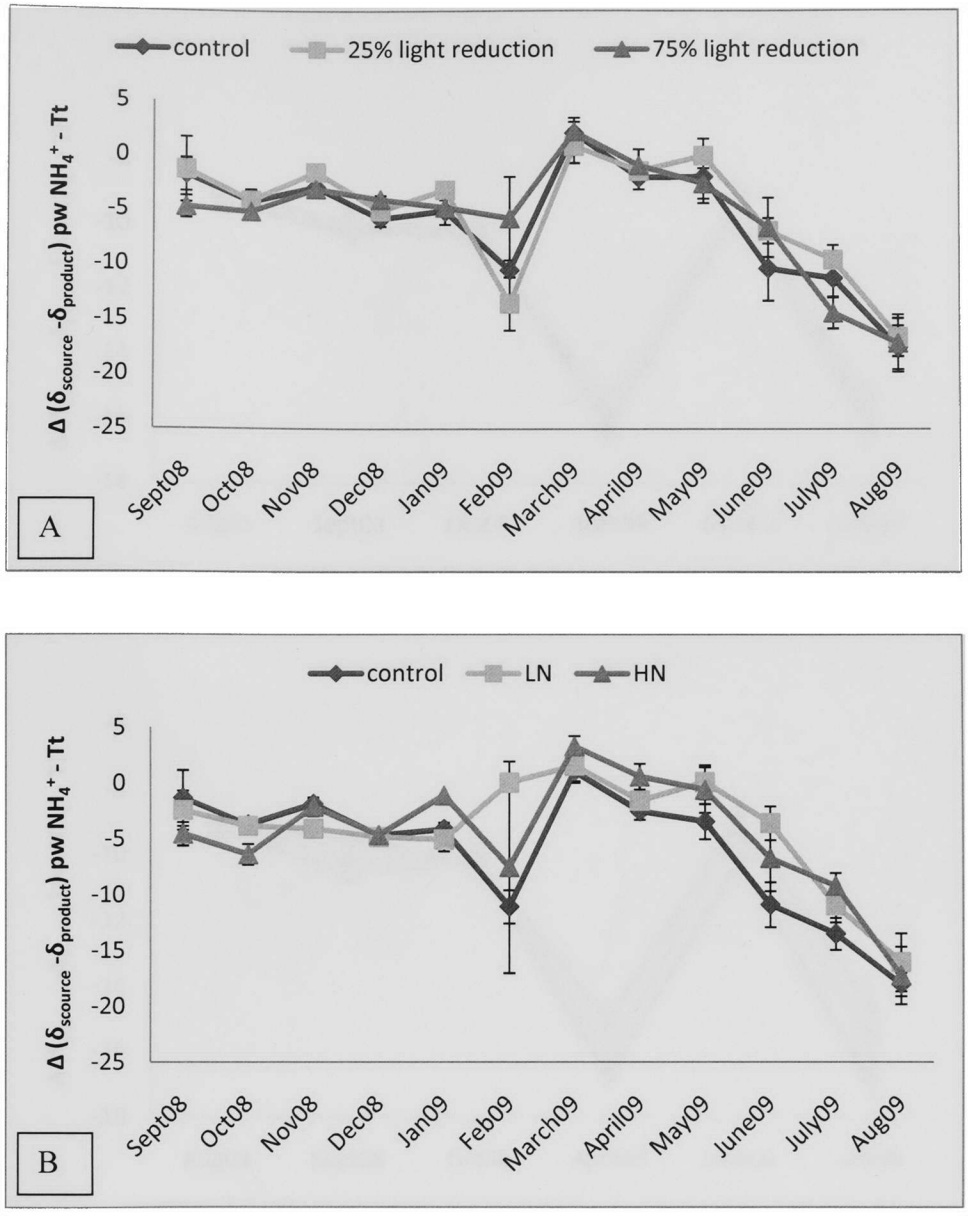

Figure 12. Δ (porewater $\delta^{15}N\text{-}NH_4^+$ - $\delta^{15}N$ *Thalassia testudinum*). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

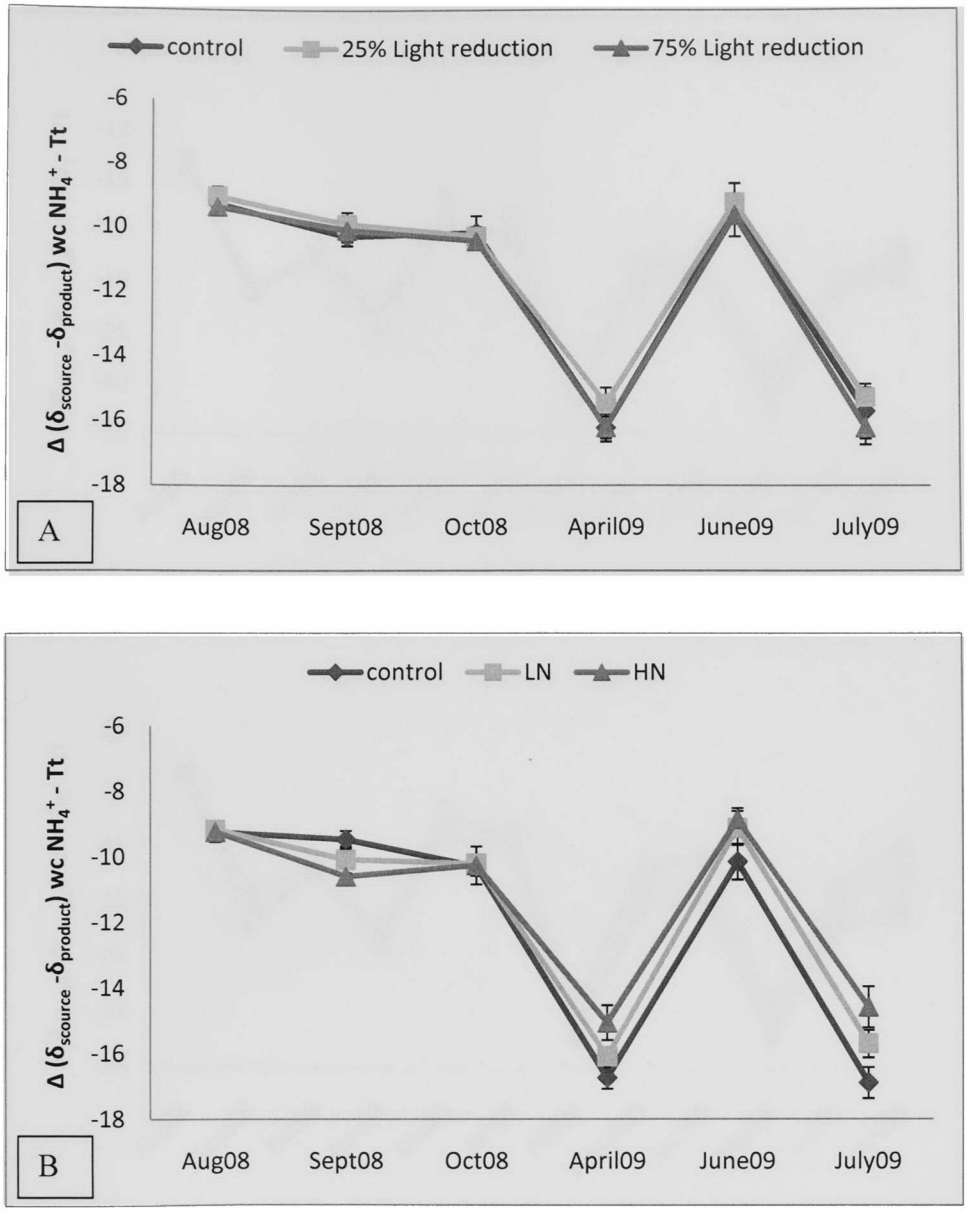

Figure 13. Δ (water column $\delta^{15}N\text{-}NH_4^+$ - $\delta^{15}N$ *Thalassia testudinum*). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

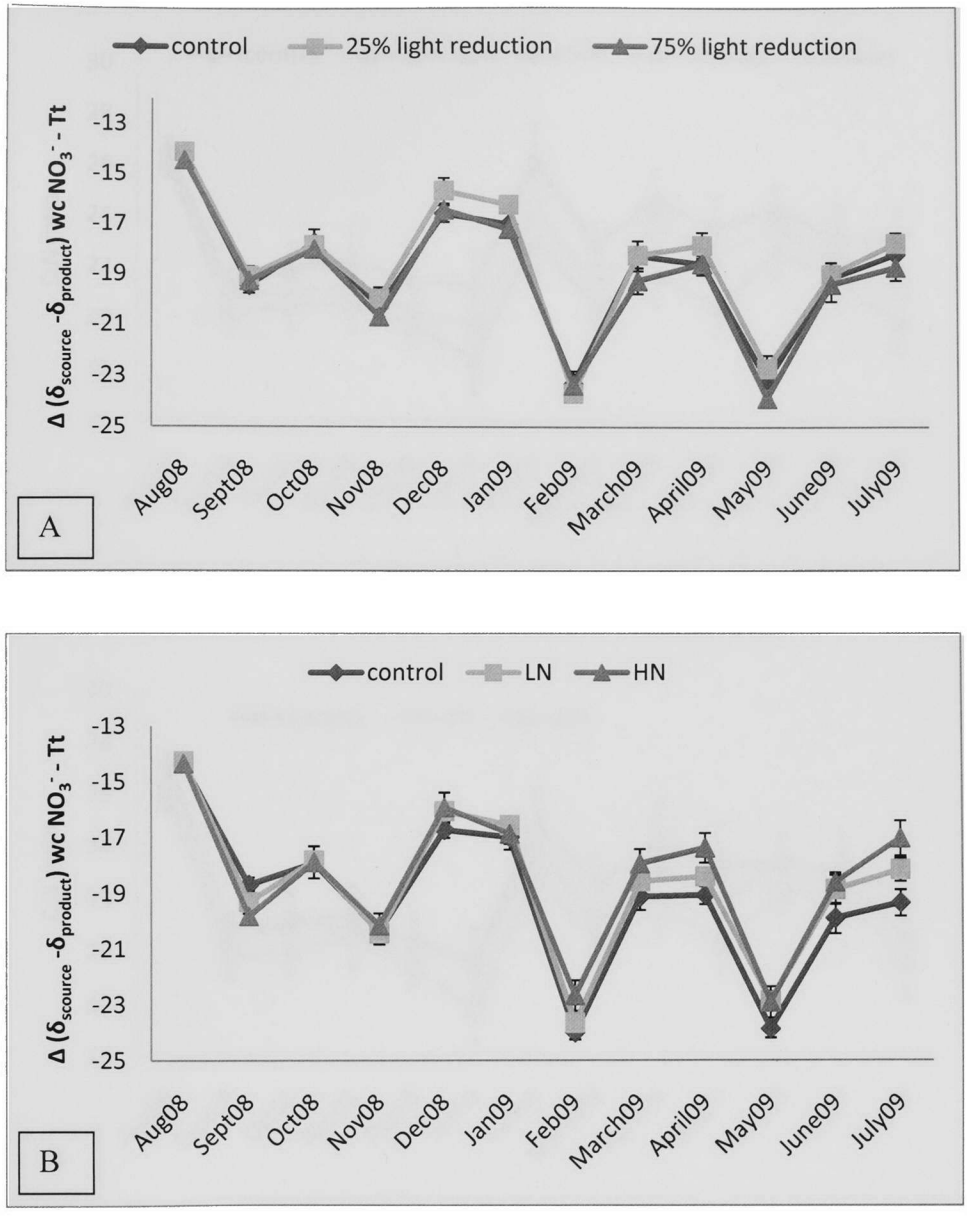

Figure 14. Δ (water column $\delta^{15}N\text{-}NO_3^-$ - $\delta^{15}N$ *Thalassia testudinum*). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

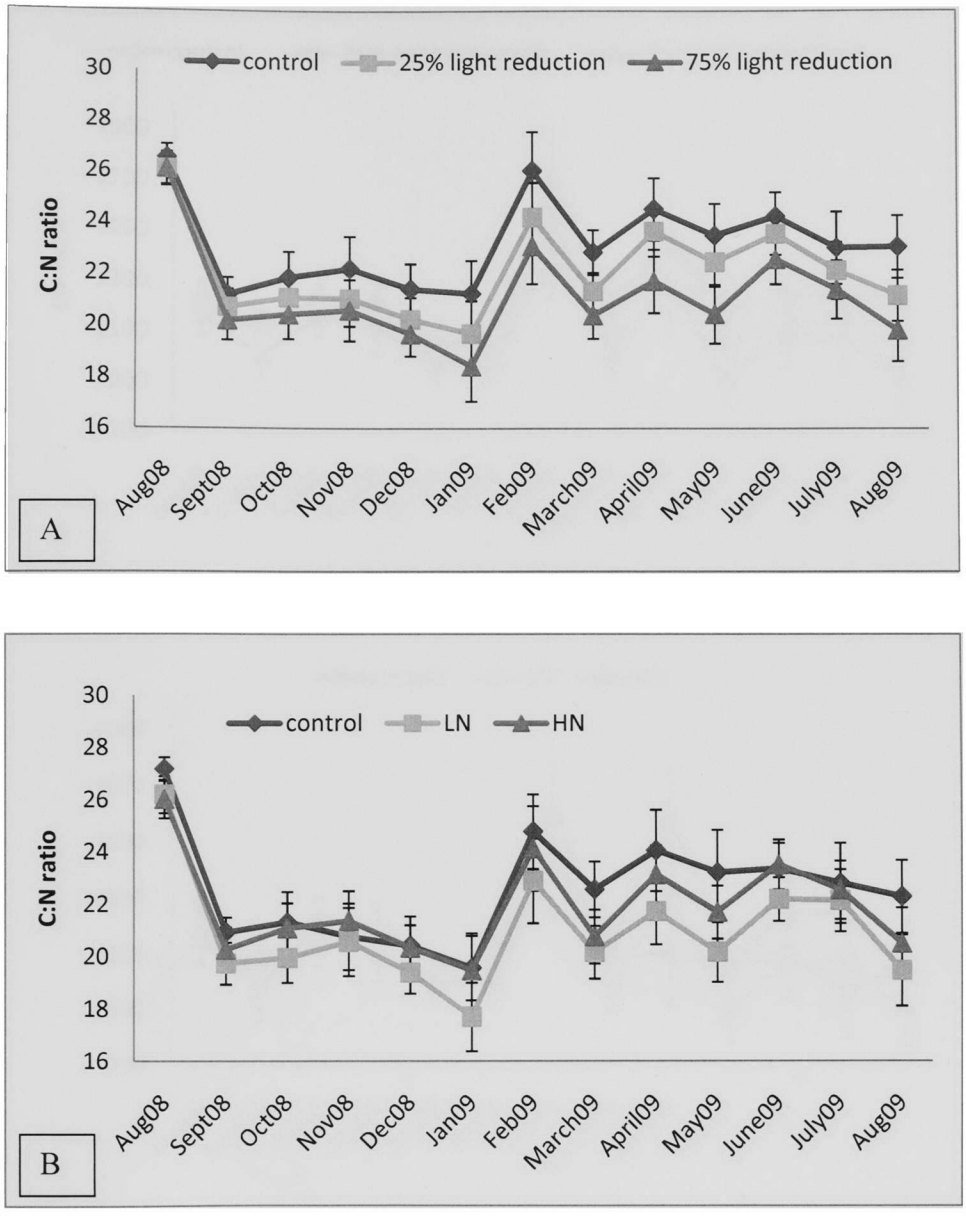

Figure 15. *Thalassia testudinum* C:N ratio. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

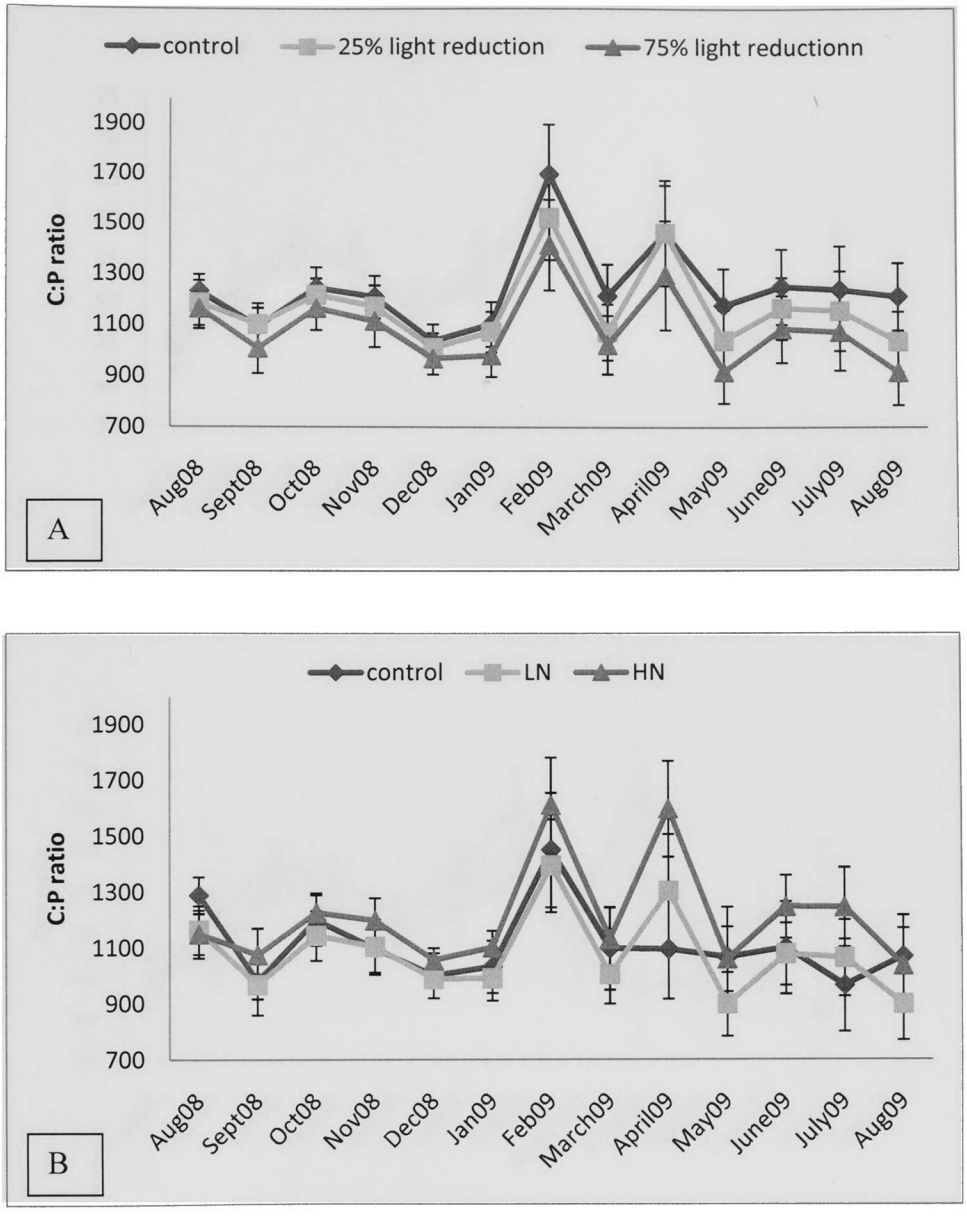

Figure 16. *Thalassia testudinum* C:P ratio. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

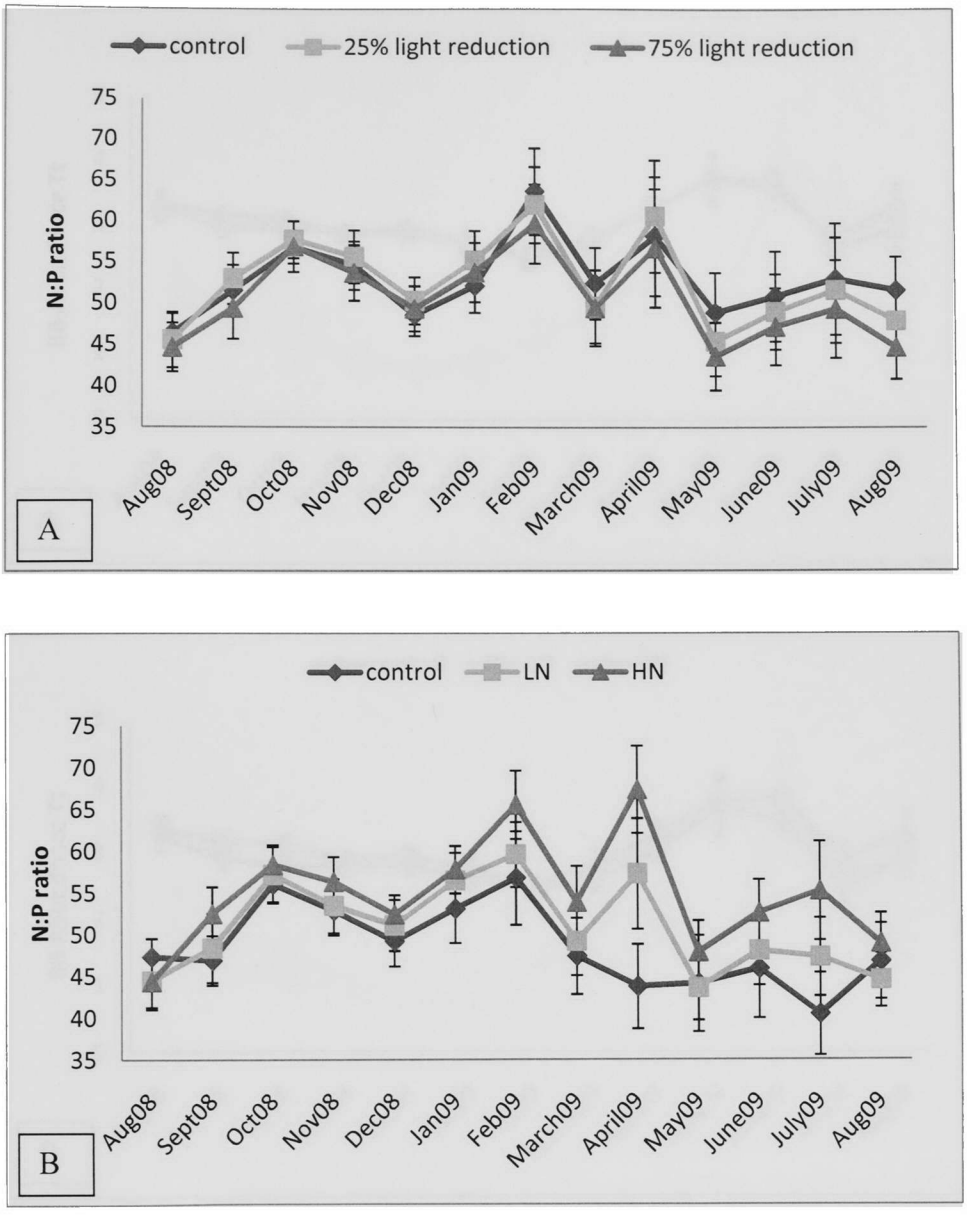

Figure 17. *Thalassia testudinum* N:P ratio. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

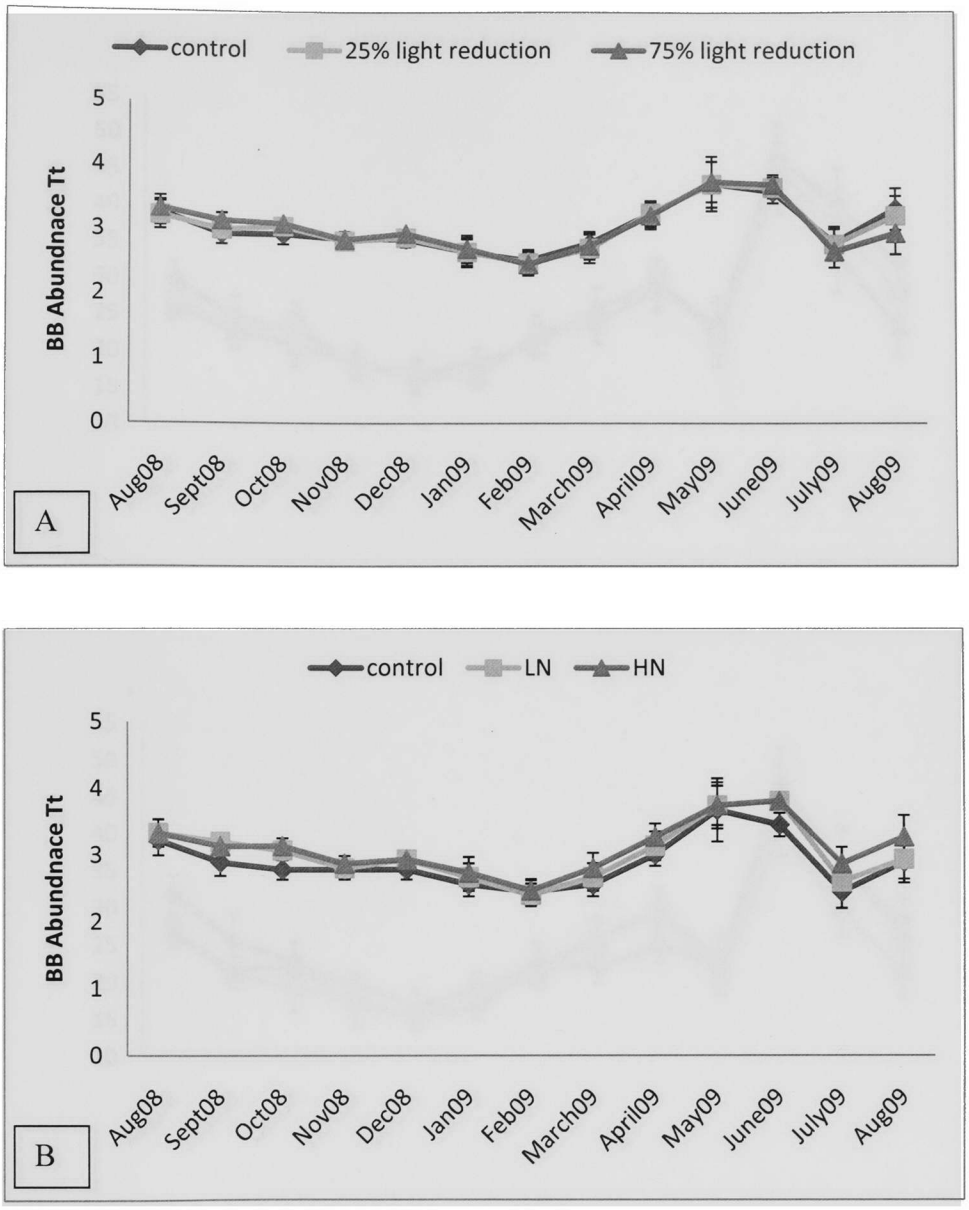

Figure 18. Braun-Blanquet abundance scores. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

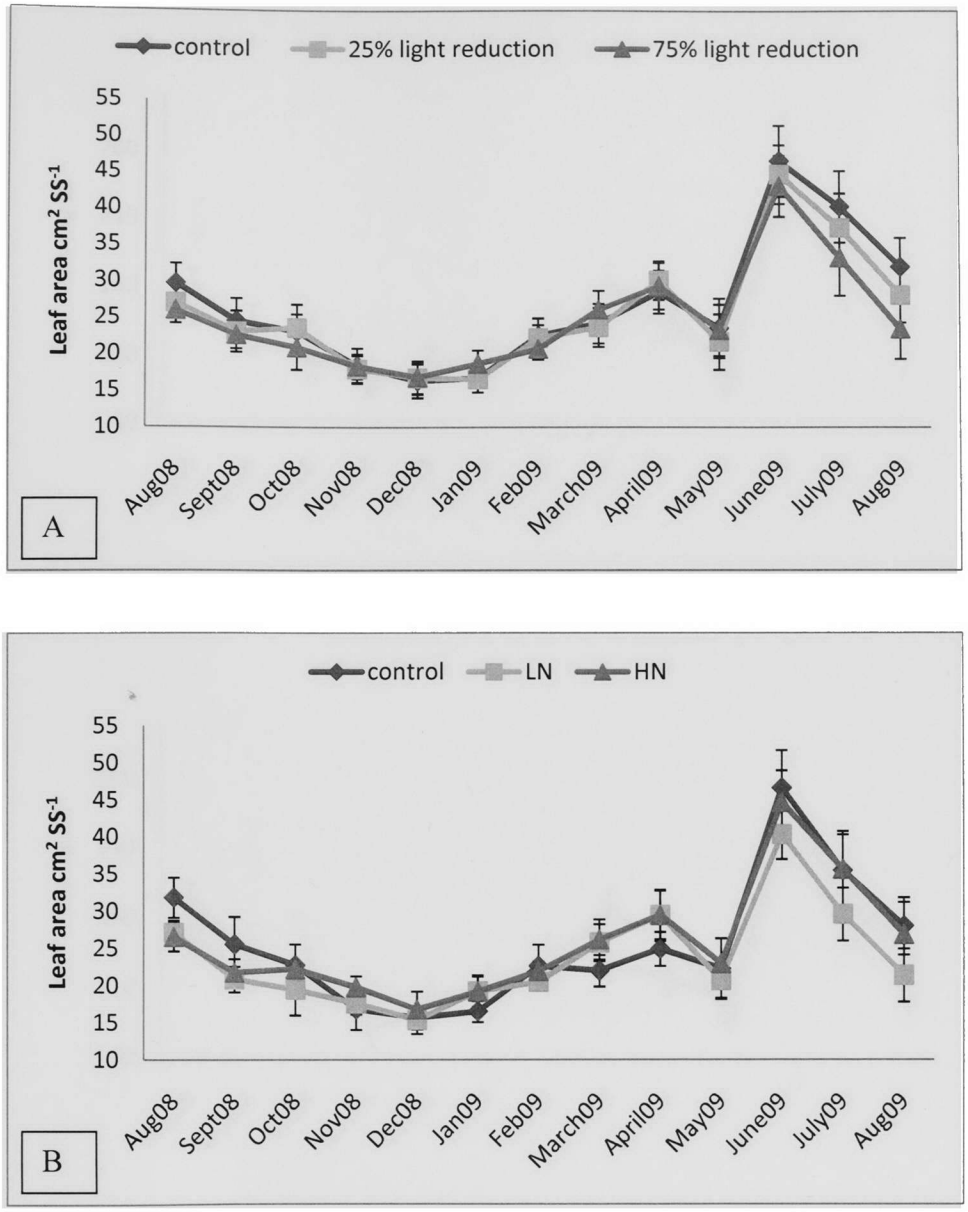

Figure 19. *Thalassia testudinum* leaf area (cm^2 SS^{-1}). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

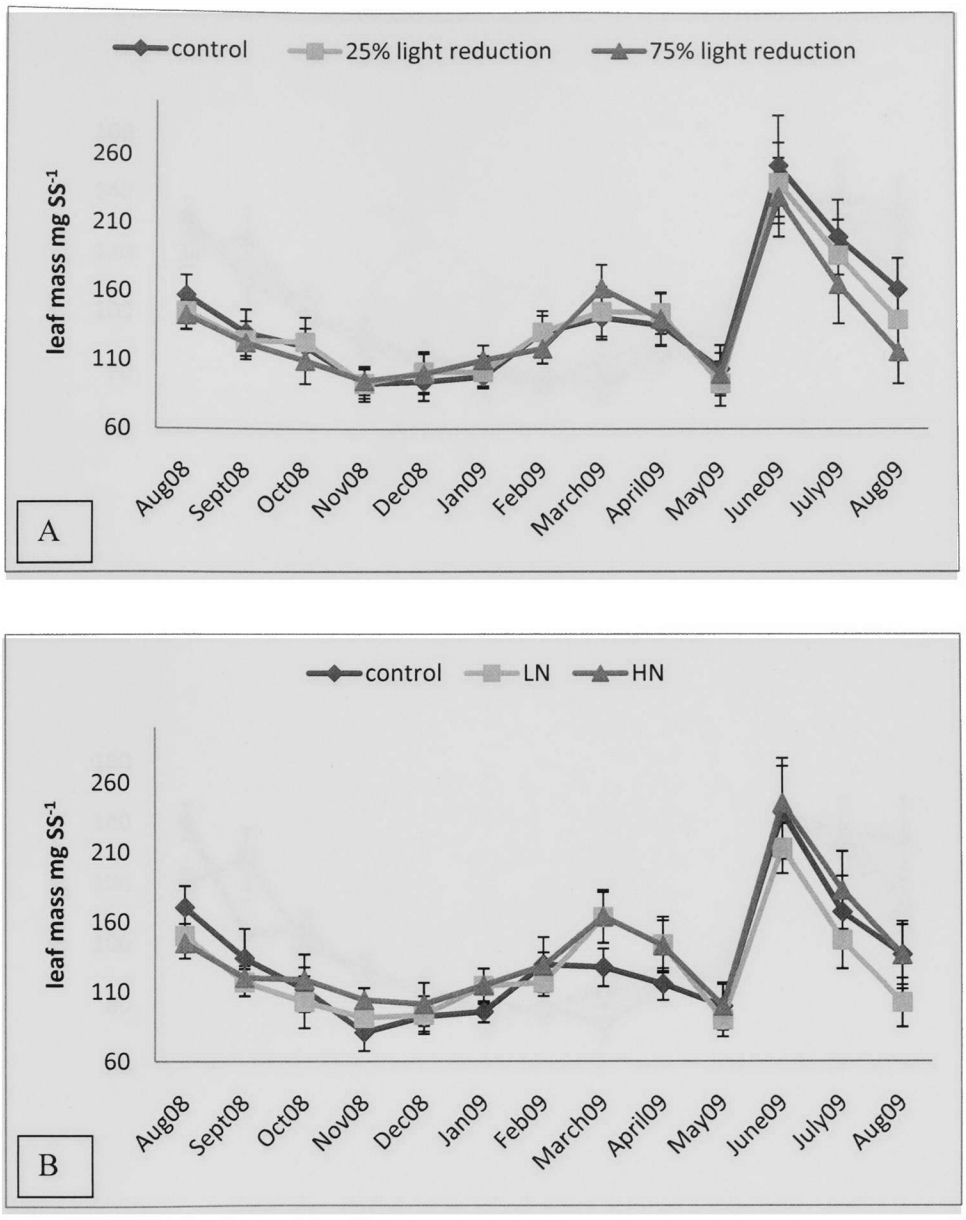

Figure 20. *Thalassia testudinum* leaf mass (mg SS^{-1}). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

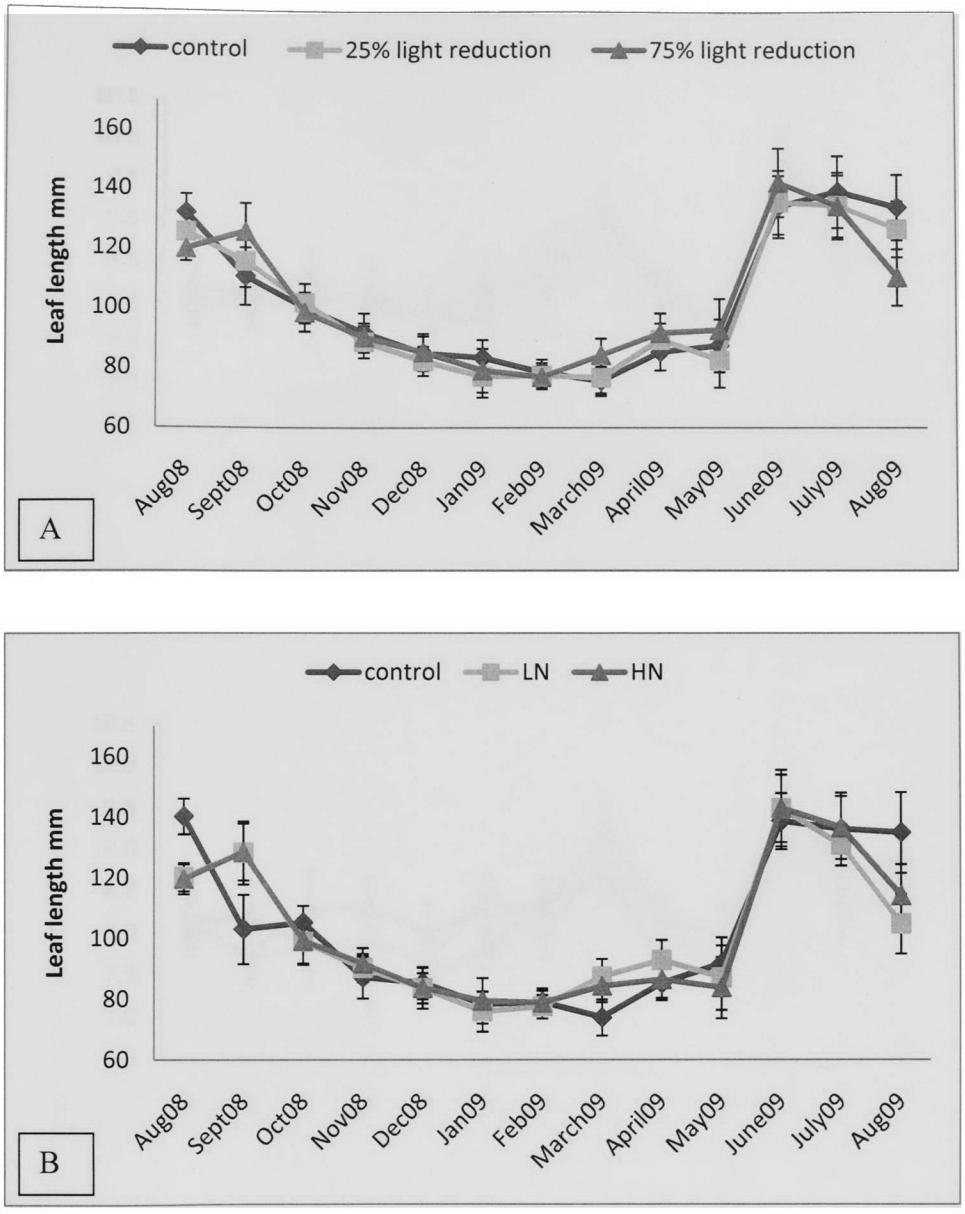

Figure 21. *Thalassia testudinum* leaf length (mm). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

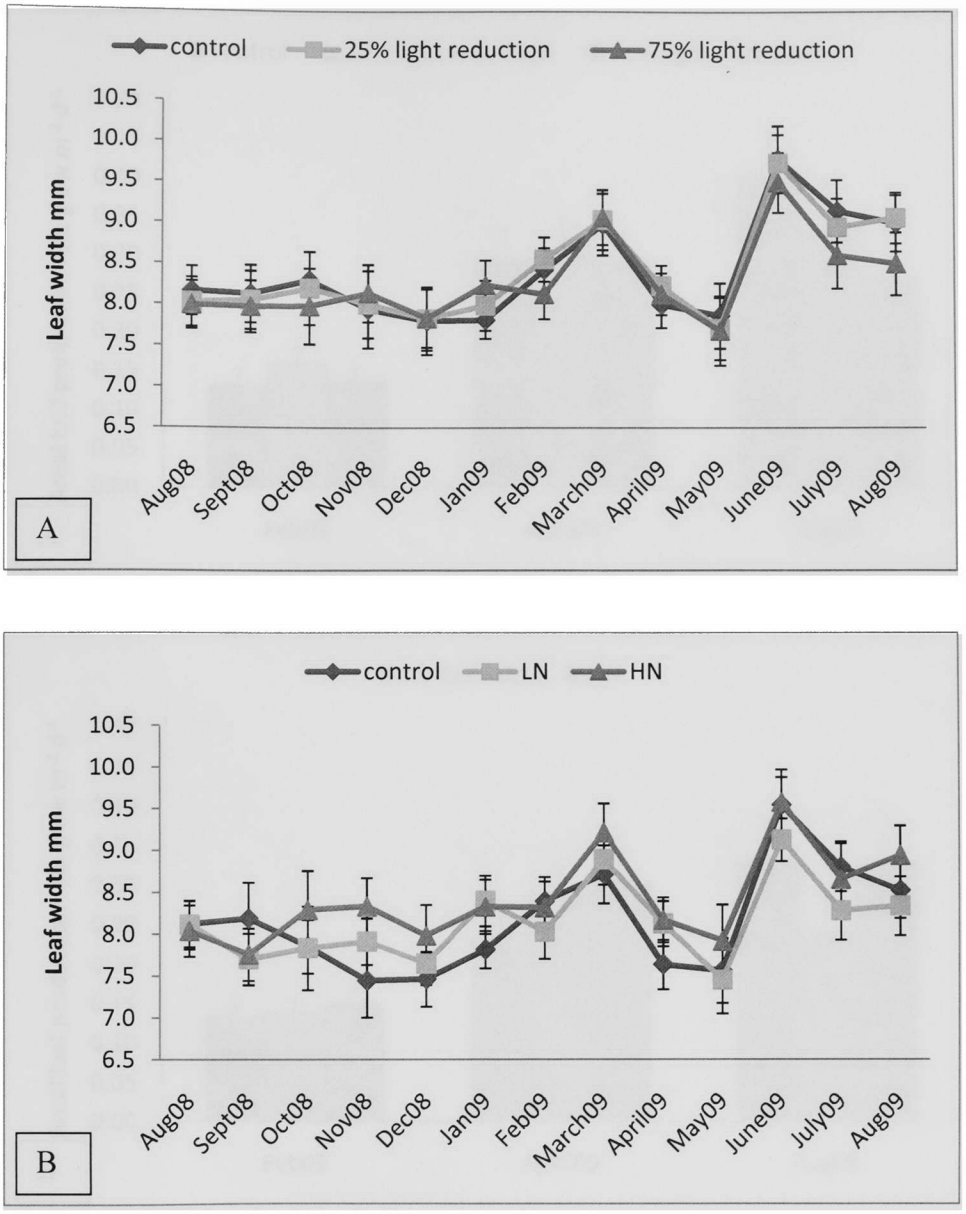

Figure 22. *Thalassia testudinum* leaf width (mm). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

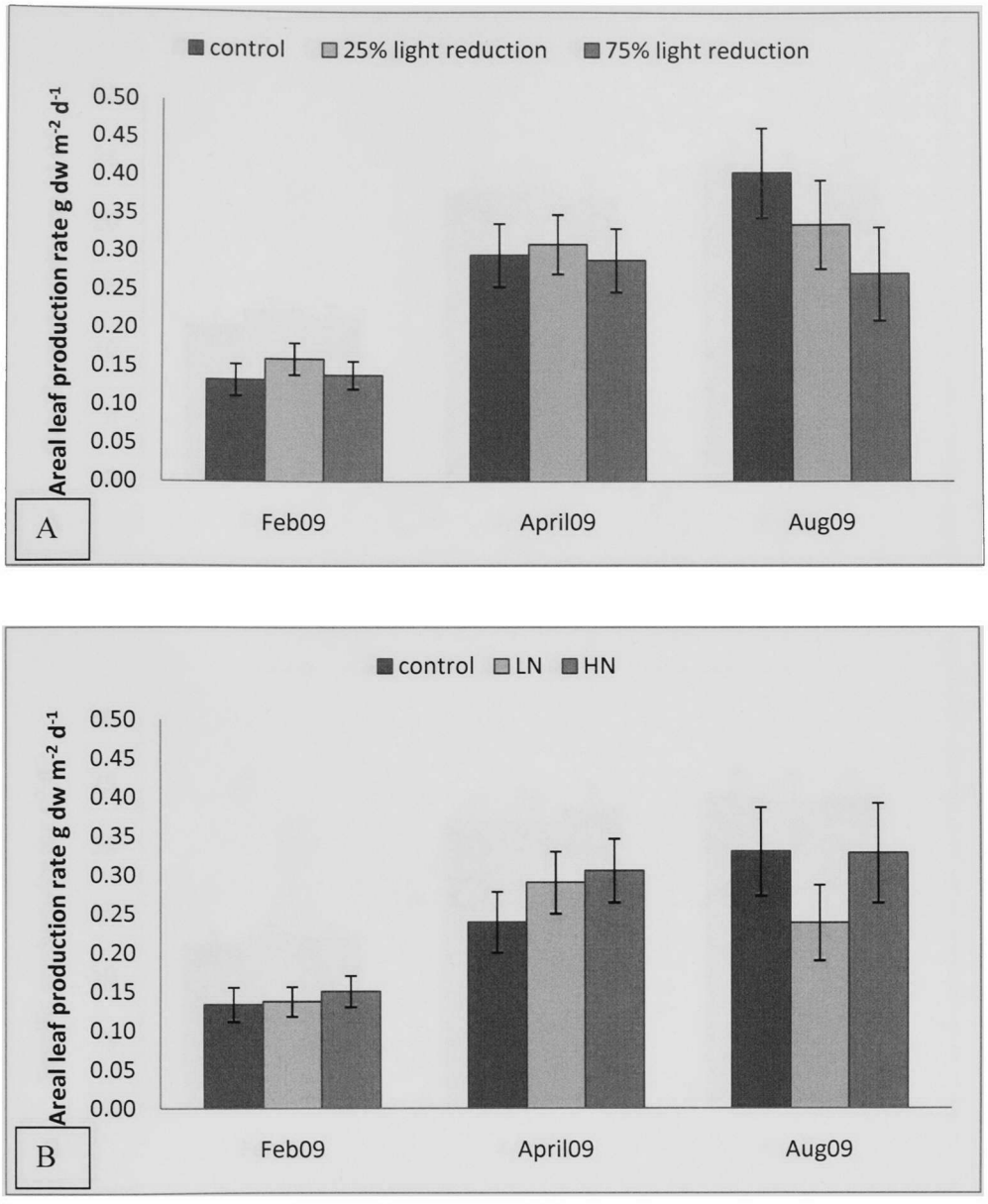

Figure 23. Areal leaf production of *Thalassia testudinum* (g dw m^{-2} d^{-1}). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

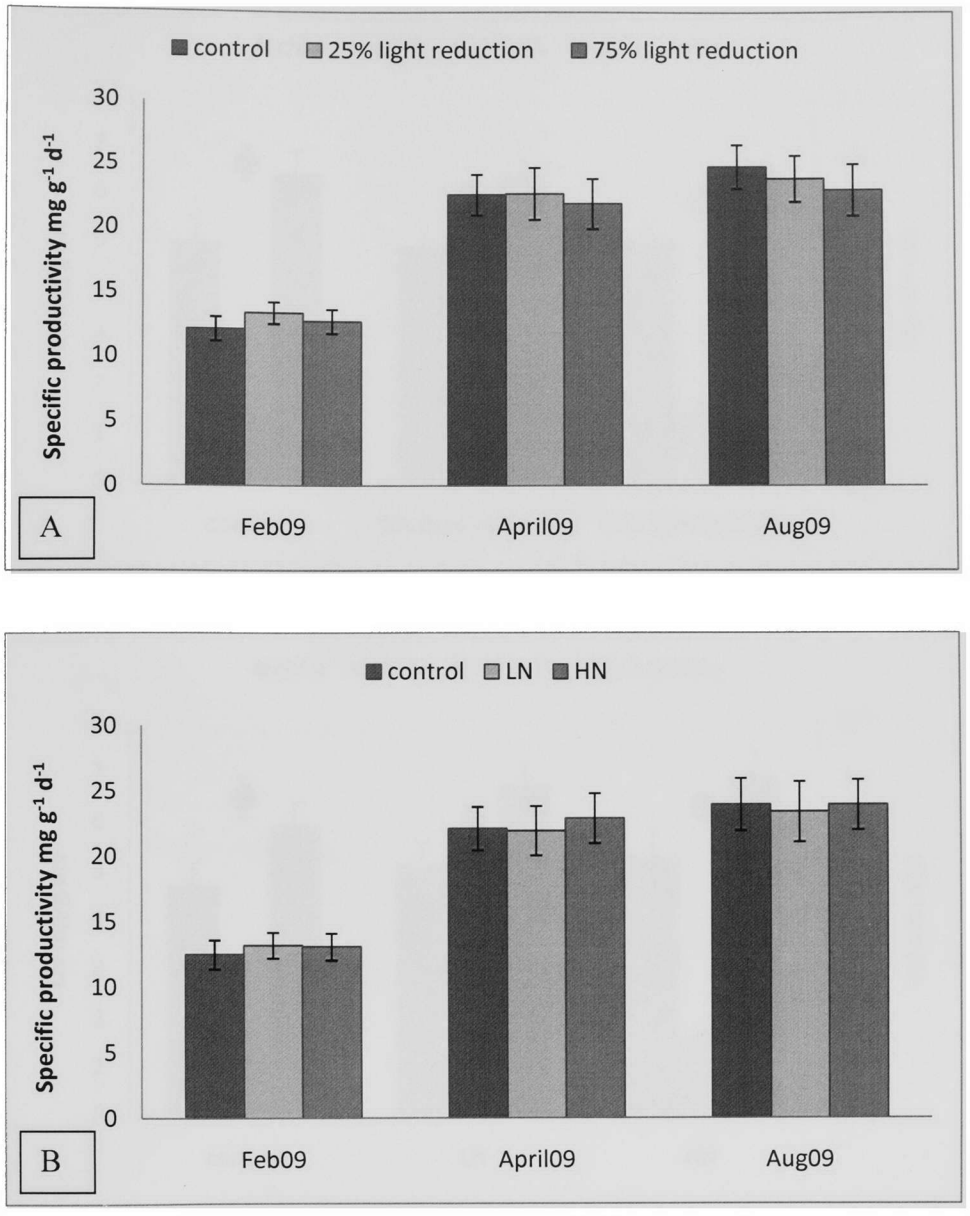

Figure 24. Specific productivity of *Thalassia testudinum* (mg g^{-1} d^{-1}). A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

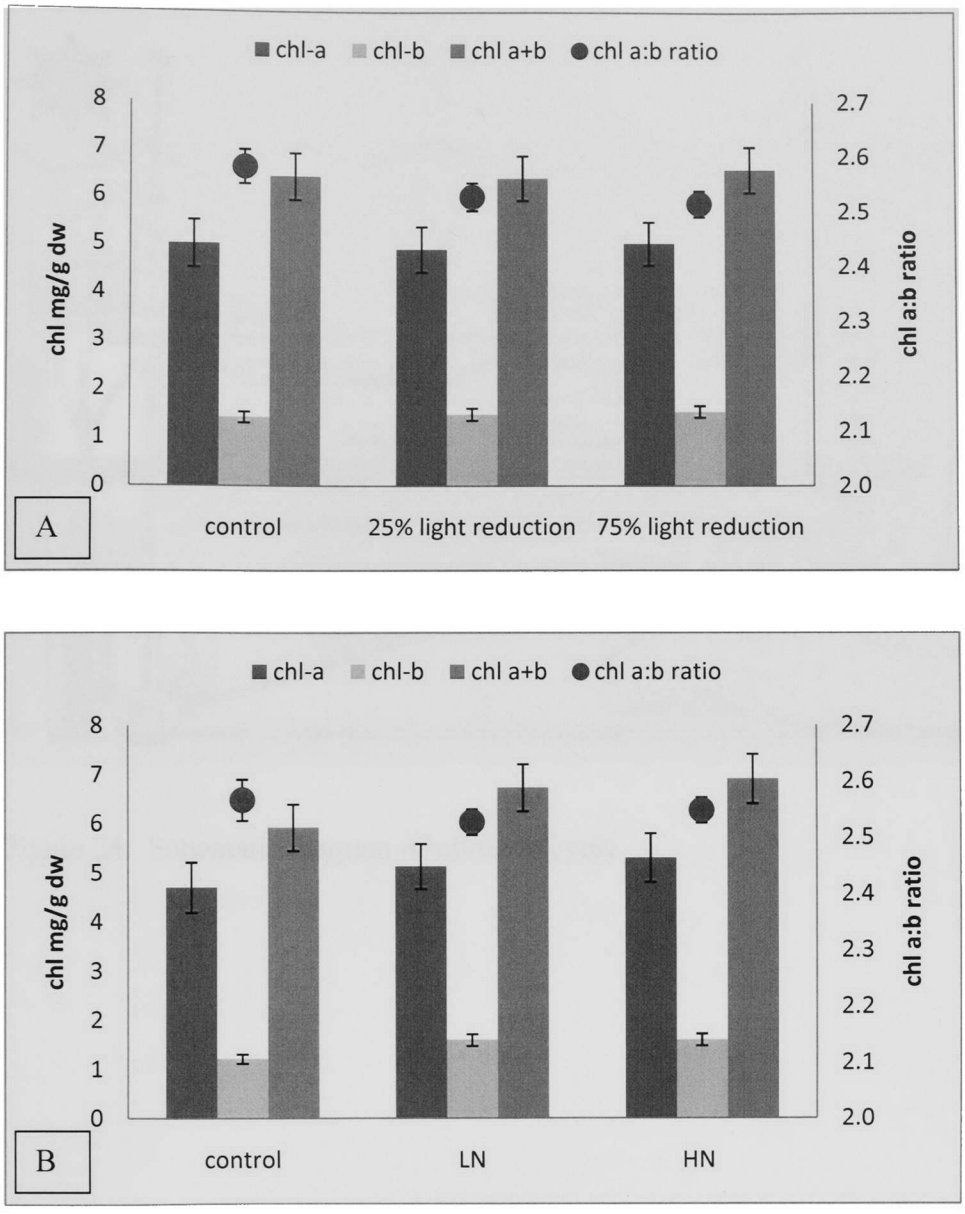

Figure 25. *Thalassia testudinum* blade chlorophyll content. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

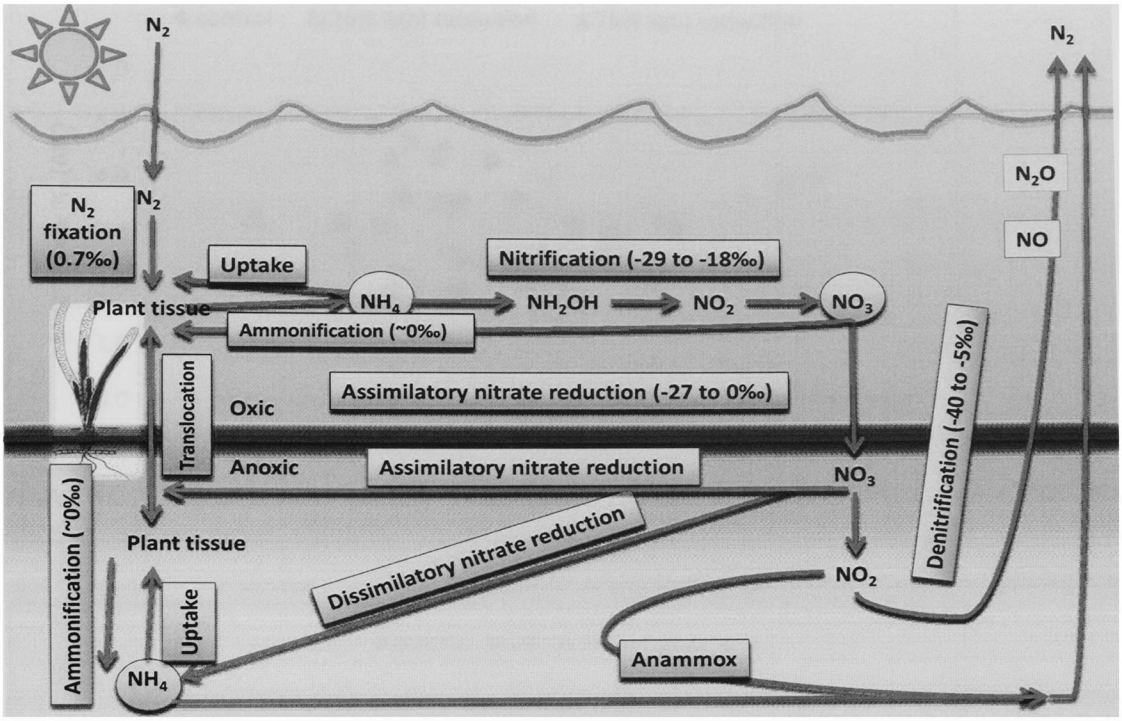

Figure 26. Schematic diagram of nitrogen cycle

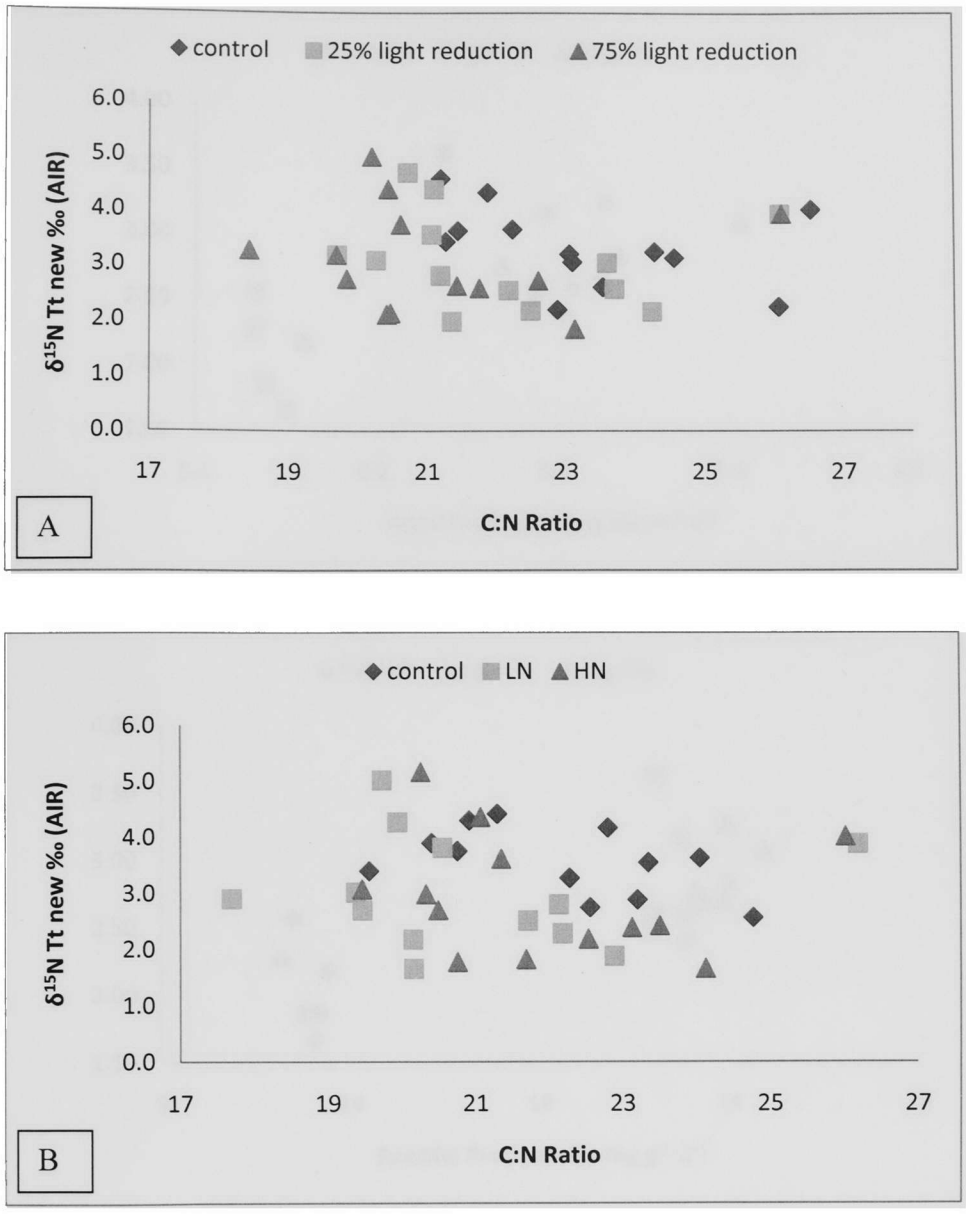

Figure 27. C:N ratio and *Thalassia testudinum* new leaf $\delta^{15}N$ values. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

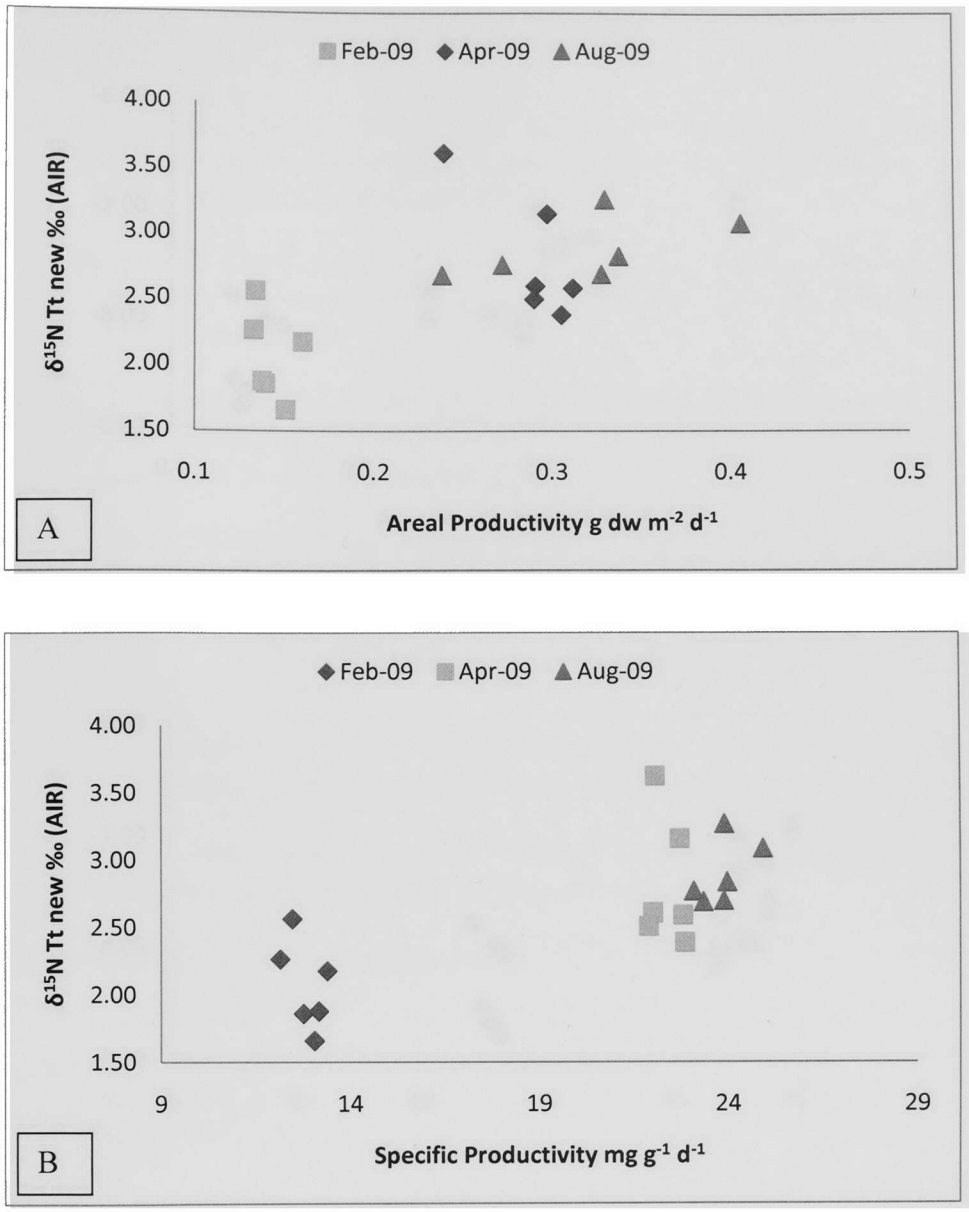

Figure 28. *Thalassia testudinum* productivity and $\delta^{15}N$ values. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

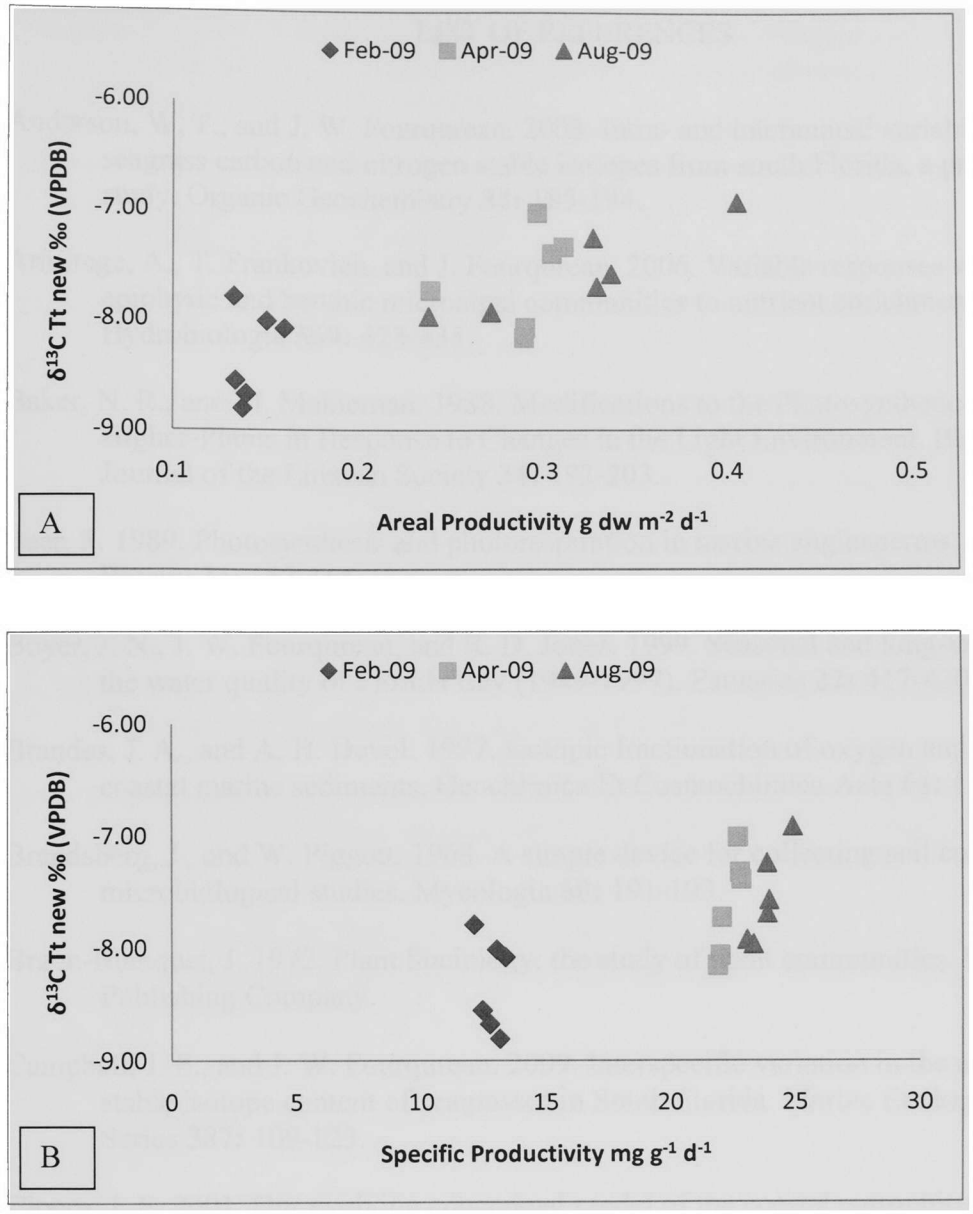

Figure 29. *Thalassia testudinum* productivity and $\delta^{13}C$ values. A: light treatments showing 3 light levels. B: nitrogen treatments showing control, low N (LN) and high N (HN)

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