An investigation of *Thalassia testudinum* below-ground structure and productivity.

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Abstract

A large percentage of *Thalassia testudinum* biomass is located below ground, yet many papers published on seagrass ecology focus on above-ground structure and productivity. This is likely due to the fact that viable estimates of below-ground biomass production are logistically difficult to obtain.

In 1975 Patriquin published a paper proposing a method to estimate below-ground productivity in *Thalassia testudinum*. This method requires complete extraction of the plant and it may also be error prone due to variability in developmental growth.

This study investigated alternative methods for estimating below-ground productivity in *Thalassia testudinum*. The study had two major goals: 1) Determine how changes in above-ground leaf growth correspond to changes in below-ground structure; 2) Measure and attempt to exploit structural consistencies in order to improve the estimation of below-ground productivity.

During the summers of 2000 and 2001 *Thalassia testudinum* plants growing in Florida Bay were tagged with bird bands and leaf length repeatedly measured. At the end of the field season the plants were extracted. The extracted, tagged plants were taken to a lab in Charlottesville, Virginia where a comprehensive series of structural measurements were recorded. The record leaf dynamics was coupled with the structural measurements and the data analyzed.

The amount of leaf area added per day per short shoot had a moderate correspondence (R^2=0.42) with below-ground rhizome volume. Short shoot scar age had a high correspondence (R^2=0.67) with the length of the rhizome runner. These results

were an indication that there was a correspondence between above-ground growth and below-ground structure.

Roots attached and growing from the short shoot were found to have three distinct patterns of growth. Roots classified as "fast growers" had an estimated elongation rate of 2.62 cm for every short shoot leaf scar ($R^2=0.63$). These roots were estimated to be elongating at a rate of 0.15 cm per day. This rate translates to 0.0003 grams dry weight biomass per day.

The unexplained variance in the relationship between above-ground dynamics and below-ground structure may lie in resource and energy translocation within the plant. The structural patterns of the below-ground plant components displayed natural variability, but a pattern of consistency was documented. These consistencies could be utilized to allow a refinement of methods used to estimate below-ground productivity in *Thalassia testudinum*.

Future research needs to expand to a multivariate experimental approach. Future studies also need to explore other geographical locations to determine how the patterns observed in this study differ from patterns within other populations based on spatial, temporal, and characteristic differences in the surrounding ecosystems.

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Introduction

Recent literature reviews noted an increasing trend in the number of published scientific papers related to seagrass ecology (Duarte, 1999). Of these, a majority of the studies focused on above-ground biomass (Duarte and Chiscano, 1999) This imbalance is due to the fact that direct observation of below-ground production in seagrasses is logistically difficult (Van Tussenbroek, 1998). Tagging of rhizomes is possible in some species (Brouns, 1985), but the rhizome depth, sediment, and the growth matrix make this difficult or impossible in many seagrass species. This is especially true for *Thalassia testudinum*, where below-ground production estimates have to be calculated using indirect methods (Patriquin, 1973).

Effective methods for directly assessing above-ground productivity in *Thalassia testudinum* have been available for more than 25 years (Zieman, 1974). In contrast, methods for estimation of below-ground productivity rely on formation rates of various plant parts as temporal markers (Gallegos, et al., 1993; Patriquin, 1973). The differences in formation rates are used to create a Δ-time that corresponds to a below-ground biomass. This method assumes no variation in formation rate. However, variance in formation rates related to seasonal and physical parameters have been observed in *Thalassia testudinum* (Van Tussenbroek, 1995) which means this method could be subject to extensive error.

The lack of a robust method for estimating below-ground production represents a rather important disconnect in the field of seagrass ecology. As the pool of data becomes larger, the ability to develop effective models, whether whole plant or whole system, becomes increasingly feasible. Yet because viable estimates of below-ground

productivity in *Thalassia testudinum* are logistically difficult to obtain, the development of these models is hindered by lack of data on below-ground processes. These models are increasingly important from the stand point of applied science, and to the seagrass research community's efforts to coalesce the data of the past 100 years into meaningful whole plant models (Duarte, 1999).

Seagrass research, especially the work done on *Thalassia testudinum* over the past 30 years, has established the capacity of this species to provide essential food and habitat in tropical coastal marine systems (den Hartog, 1970; Tomlinson and Vargo, 1966; Zieman, 1982). Studies suggest that *Thalassia testudinum* is the foundation for a detritus based food chain (Zieman, Macko, and Mills, 1984). It is also important to the fish ecology of these ecosystems (Thayer and Chester, 1989) serving as a nursery ground for many fish species, also providing a location for both foraging and refuge from predation (Zieman, 1982). These same factors appear to be applicable to other commercially important marine species such as Pink Shrimp. *Thalassia testudinum* also plays an important role in stabilizing sediment (Fonseca and Fisher, 1986; Patriquin, 1975). In a related issue, the presence of this species of seagrass affects the rate of deposition of suspended particles in the water column. This, in turn would affect the clarity of the water column. So a variety of factors related to the productive capacity of the entire proximal ecosystem are directly affected by *Thalassia testudinum*. These data also would suggest that the impact of the loss of this species could be felt on both a large and small spatial scale.

The loss of *Thalassia testudinum* in its indigenous habitats, and especially Florida Bay, would completely change the structure and function of the surrounding ecosystem.

In the case of a catastrophic loss, no other species could replicate its ecological function (Zieman, Florida Bay Science Conference, 2001). With major restoration projects in Everglades National Park poised to change water flow patterns, ecosystem managers and the U.S. Army Corp. of Engineers publicly called for development of ecologic models (Florida Bay Science Conference, 2001). The effective development of any model related to seagrass would be affected by the lack of data on below-ground processes. This lack of data leaves the modeler little choice but to represent many components of their model as essentially unknown processes. In cases where the model requires some flow of data or some representation of the below-ground process, the author is obliged to make a reasonable, or even an unreasonable guess.

Further, the general understanding of the biological processes of *Thalassia testudinum* are hampered by lack of understanding of below-ground mechanisms. Study of the below-ground growth matrix, resource foraging, species competition, translocation of nutrients, just to name a few, will all be aided by a better understanding of below-ground production.

Measurement of below-ground productivity has been a disquieting problem in terrestrial, wetland, and aquatic botanical studies. A widely used method is the extraction of soil cores and separation and quantification of live and dead material (Neill, 1992). These methods lead to a probable underestimation of production because seasonal issues, and loss due to grazing and other factors. Other estimation methods include root ingrowth into mesh bags. This method is problematic because of disturbance involved with the method. Further, it likely does not address the issue of production verses loss well. A study, using this ingrowth method was used to measure below-ground

production of *Spartina alterniflora* in a salt marsh (Blum, 1993). The study yielded variable below-ground production rates as high as 5.5 g dry wt m^{-2} day^{-1} and as low as 0.27 g dry wt m^{-2} day^{-1}. Spatial and temporal variability clearly can affect below-ground estimates. In contrast to the salt marsh measurements, Patriquin (1973), using plant formation rates, observed a low spatial variation in the below-ground estimates of *Thalassia testudinum*. He observed a production value of 4.4 g dry wt m^{-2} day^{-1} for total underground production at both Bermuda and Barbados. However, when production of rhizome tissue was considered separately, he measured .76 g dry wt m^{-2} day^{-1} in Barbados and 1.5 g dry wt m^{-2} day^{-1} in Bermuda. Previously mentioned factors in Patriquin's methods have noted the possible error in these estimates, but the dynamic nature of the below-ground system is clearly seen in these studies.

Therefore, the need exists to improve or develop alternate methods that will allow functional estimation of below-ground productivity of *Thalassia testudinum*. The most useful tool for estimating these values would be direct assessment, however difficulty of direct below-ground measurement means that alternate non-direct methods need to be developed This study is a first step in testing the theory that allocation of mass is related to plant productivity and structure. If consistencies in plant architecture can be identified and related to above-ground structure and productivity, the error in estimation of below-ground productivity may be reduced. Review of the primary literature offered a supportive framework for such an assumption.

Background

A necessary starting place is an understanding of primary productivity. This is the rate at which energy is stored through the process of photosynthesis (excepting chemotrophs) (Allaby, 1992). This stored energy is in the form of organic matter that can be used as nourishment to meet the basic metabolic and growth demands of the primary producer. In *Thalassia testudinum* all primary production takes place in the leaves, and more specifically the part of the leaf in the field of photosynthetically active radiation (PAR). This allows the important assumption that allocation of biomass has to be related to above-ground productivity in *Thalassia testudinum.*

Understanding how primary productivity relates to the allocation of biomass in *Thalassia testudinum* is complicated by a number of issues. Chief among them are clonal integration and the resource storage capacity of this plant. Clonal integration is the process by which the organism integrates, and presumably disseminates resources within a biological scheme that maximizes survival potential (Tomasko and Dawes, 1989). *Thalassia testudinum* grows in the marine sediment along a horizontal rhizome. The rhizome also acts as a storage device as well as transport medium of resources throughout the plant. As the rhizome grows it produces short shoots at quasi-regular intervals (den Hartog, 1970; Tomlinson and Vargo, 1966). At some point in its development, if a clonal plant is going to propagate, all or most of its ramets have to reach a point where the net primary productivity is greater than zero. The metabolic demand of the plant increases as the percentage of non photosynthetic tissue rises. This consideration is especially significant in *Thalassia testudinum* where the below-ground biomass may equal or even

be greater than that above-ground. There is data to suggest that ramets with a net energy yield greater than zero may support or supplement the energy needs of ramets with low or negative net energy yields. The notable consideration here is that consistently high levels of photosynthetic activity in a leaf might not correspond to a predictable addition of biomass. The shunting of resources complicates any postulates about the allocation of mass. This suggests the need for a hypersensitive assessment of growth pattern.

Another complication is the storage of resources in the below-ground portion of the plant. The rhizome of *Thalassia testudinum* has a significant resource storage capacity. Studies have observed a seasonal change in the distribution of soluble carbohydrates in the rhizome (Dawes and Lawrence, 1980). These researchers observed a pattern of increased soluble carbohydrates in the summer, where high productivity allowed the storage of starch. They observed a corresponding decline in carbohydrates in the winter which they suggest occurred as the plant used its reserve energy supply to keep up maintenance and growth in a period of reduced primary productive capacity. These data also suggest a complication in the allocation of mass issue. The immediacy of productive response to disturbance or change in resources has the potential of being very slow in *Thalassia testudinum*. Studies have shown that the productivity potential of seagrasses is limited by resource availability and the capacity for internal recycling can affect primary production (Hemminga, et al., 1999).

Storage and recycling of resources in *Thalassia testudinum* are points of concern. Productivity measures are expressed as rates, for example grams of dried weight biomass added per day. In below-ground measures where the plant is extracted, only a single

point for assessment exists. As earlier stated, some studies have used age differences in

subsequent short shoots for estimation of rhizome elongation and biomass added

(Patriquin, 1973). However Dawes and Lawrence (1980) observed a dynamic in rhizome

constituents. The temporal dynamics of rhizome biomass has to be considered in any

below-ground estimation of productivity.

Structural consistency in *Thalassia testudinum* morphology is the next

consideration. Differing species of seagrass, though distinct in their morphology, appear

to have consistent patterns of growth which allometric scaling equations can describe

(with certain inherent error) (Duarte, 1991). These scale differences in growth rates and

productivity appear to be related to plant size. Other studies have also found similar

results (Vermaat, et al., 1995), and it has been theorized that plant size is important to

ecological succession. Further, it has been hypothesized that a greater relative allocation

of reserves is appropriated to below-ground structure as a population becomes more

productive (Duarte and Chiscano, 1999). Density dependence appears to affect spacing

of new short shoots as well as foliar development (Tussenbroek, et al., 2000). So

structural consistencies have to be viewed in light of how ecological pressures affect

maximum structural potential (Bell and Tomlinson, 1980). *Thalassia testudinum*

meadows of Florida Bay are interesting in this regard as below-ground production is

thought to be equal to or greater than above-ground production. Generally though, when

considering all seagrass species above-ground production = 2.81 below-ground

production.$^{5+/-.07}$ (R^2=.45; F=39; p<.0001) (Duarte and Chiscano, 1999). These data,

when considered in light of scale differences, offer evidence support the assumption that

the allocation of mass is not random.

Mindful of the expressed considerations, I propose an initial exploration of the feasibility of indirect below-ground productivity estimates. The data presented in this background section would suggest that a concurrent investigation into the patterns of structure and productivity could be coupled to clarify the relationship between those factors. If meaningful, that relationship could be used as a robust proxy for direct below-ground measurement of *Thalassia testudinum*.

Objectives and hypotheses of study

The objective of this study was to conduct a series of detailed assessments of plant structure and productivity, and synthesize those measures to improve old methods to estimate the production of below-ground biomass of Thalassia testudinum. A secondary objective is to test the feasibility of using a new approach of using leaf dynamics to estimate below-ground productivity.

The specific objectives of this study are to:

- quantify relevant patterns in structure, rates of growth, demographics, as well as other life table measures (leaf plastochron interval, length of leaf life, etc) for Thalassia testudinum;

- determine the covariance between measured variables;

- explore allometric relationships that describe structural relationships and consistencies;

- use the structural relationships in combination dynamic and static measures to estimate below-ground production in Thalassia testudinum.

This project was expected to contribute to several ongoing research and modeling projects supervised by Joseph C. Zieman and Thomas Smith. It is also expected to contribute to the understanding of whole plant production and respiration budgets. This study was also expected to provide information needed in the development of species competition models.

Hypothesis:

- Allocation of biomass is related to above-ground morphological structure and productivity in Thalassia testudinum.

- There are observable consistencies in productivity and morphological structure.

- Those consistencies can be assessed, analyzed and converted to a function that reduces the error associated with the estimation of below-ground production in Thalassia testudinum.

- Viable estimates of below-ground productivity for Thalassia testudinum can be made indirectly by measurement of above-ground structure and productivity.

Methods

Site characterization

This study was conducted at a mono-specific seagrass meadow in Rabbit Key Basin. The site was located within Florida Bay, a shallow, lagoon type estuary. A major portion of Florida Bay lies within the jurisdictional boundary of Everglades National Park. Location within a national park means the ecosystems are monitored and protected by law enforcement agencies more extensively than would be expected at sites outside the park boundaries. This was one rationale for selecting Florida Bay as the study site.

Research in this area required permits which were obtained by The University of Virginia. The specific study sites were located in a 20 by 20 meter area about half a mile

west of Rabbit Key. The research site was also located near an ongoing seagrass monitoring station. Geographic location near a known and well studied site was a secondary rationale for site selection.

Florida Bay has uneven and erratic topography due to a complex series of carbonate mud banks. While the banks do not completely restrict water flow, they are boundaries for nearly discrete aquatic systems called basins or lakes. Rabbit Key Basin is located near the center of Florida Bay. A sizeable percentage of the benthic area in this basin is populated by *Thalassia testudinum*. Rabbit Key Basin also has a monitoring station owned by the South Florida Water Management District. These factors were further rationale for selecting Rabbit Key Basin as the specific study site.

Determining the population demographics for *Thalassia testudinum* at Rabbit Key Basin was necessary to establish sampling requirements. At the beginning of each field season the short shoot density was assessed. The method used was a haphazard selection of positions within the study area followed by short shoot density count. To ensure that the density estimates were not dependent on the size of the sample area, three quadrat sizes were used to isolate the sample area. The smallest was a 10 by 10 cm quadrat, the next size was 10 by 20 cm, and the largest was a 20 by 20 cm quadrat. These estimates were used to determine the density and pattern of dispersion of the short shoot population within the overall study area. That characterization was used to determine the size of the individual sample area and the number of sampling sites.

The specific study sites were selected haphazardly. Quadrats were tossed over the side of a boat and study sites established exactly where the quadrats landed. In year-one five quadrats were established, each 10 by 10 cm. At the end of the first field season

sampling proficiency had improved. Therefore, the quadrat size was increased to 10 by 20 cm and the number of sites was increased to six in year-two. Unfortunately, two quadrats were destroyed during year-two, reducing the actual total to four sites. This means total study area of 500 square centimeters was monitored during year-one, and total area of 800 square centimeters was measured during year-two. The total combined study area was 1300 square centimeters. The quadrats were painted a bright orange to make them easy to spot. The site of each quadrat was marked by two flags, one at the upper right corner, and a second safety flag 0.5 meters due north of the quadrat location. Another series of flags marked a course from the University of Virginia monitoring site to the study site. The flags were used to locate the quadrats upon the return visits to the study sites. Local particle deposition, dead leaves and drifting algae often covered the quadrats, making them difficult to find without the flags. The redundant flagging system was established because site disturbance by weather and fishermen was a common occurrence.

Monitoring of leaf dynamics

Once the specific sites were selected the quadrats were secured in the sediment. All short shoots within the boundary of the established quadrat were tagged with a plastic bird band. The band encircled the upper part of the short shoot and generally sat on the sediment surface. The bands were not in a location where they could hinder leaf growth. The bird bands were numbered, and a sequential series were applied to each quadrat. This

was done to reduce data loss. Because the band numbers ran in a sequence, lost band or lost short shoots could be easy be identified.

After the short shoots were banded, each leaf on all tagged short shoot were stabbed with a hypodermic needle to create a punch mark. Because the leaves would be monitored over an extended period of time, a system of marking was employed to ensured the non redundant and accurate measurement of each leaf. All marks were made at the top of the leaf.

All underwater data was recorded on waterproof paper. For the initial set up, the quadrat number was recorded along with the exact number of short shoots within the quadrat. The numbering sequence for the bird bands was also recorded. The final step of the initial set up was to record baseline lengths and widths for all leaves.

Pre-prepared data sheets were used in the ongoing assessment of leaf lengths. The data sheets were prepared with the date and a pre-printed list all bird band numbers expected at each individual quadrat. If a short shoot was present without a band it was important to determine if it was a new short shoot or if it was a short shoot that had lost its band. If a band was missing it was important to determine if the shoot had died, been damaged or whether the band had simply fallen off.

For each assessment, all leaves on all short shoots within each quadrat were measured. If a new leaf was present it was measured and marked. Data sheets with pre-prepared information on leaf counts for each short shoot helped determine whether old leaves had detached due to damage or senescence.

Upon return to the field station the data was entered into an Excel spreadsheet. Each leaf data series had a date, quadrad number, short shoot number, leaf number, and values for length and width.

Plant extraction and measurement

At the end of the field season the plants were extracted. A one meter squared area was extracted for each quadrat. The quadrat was always located at the center of the extraction sod. The extraction started with the sawing of the rhizosphere along the perimeter of the sod. Once the sawing was complete the sod was extracted by hand. This required forcing hands down into the sediment and then underneath the cut area in order to pull the sod free of the sediment. The sods were hoisted into a boat and immersed in a tub of water for transport.

The sods were transferred to the National Park Service research center. Upon return to the research center the sods were immediately washed down and individual plants extracted from the growth matrix. The greatest care was taken to extract the tagged short shoots. Because the growth matrix was so intertwined it was difficult to extract rhizome runners. All other plants were broken or sacrificed in order to extract the tagged plants. A large sample of untagged plants were also extracted to be studied as a statistical control. During the extraction, the exact sediment depth of the tagged short shoots was recorded. The process of disentangling a single sod took twelve hours. In all nine sods were extracted and disentangled.

The individual samples were stored in zip-lock plastic bags and then frozen for transport. Special care was taken for rhizome runners that had tagged short shoots attached. The bagged and frozen samples were transported up to Charlottesville, Virginia.

The samples were unfrozen individually and a comprehensive series of measurements were recorded. Once measured, the individual plant part, such as a rhizome segment, was put into labeled tares and dried overnight in an oven set at $60^{\circ}C$. The following day the dry weights of the tissues were recorded. The weights were ultimately coupled with the structural measurements. The list of structural measurements is as follows:

Leaf (each leaf on the short shoot)

Green leaf length

Lower leaf length

Leaf width

Short shoot

Length

Diameter

Number of leaf scars

Number of roots and root scars

Location of root scars

Number of leaves attached

Total leaf area

Presence of attached rhizome runner

Volume was computed using length and diameter

Rhizome

Length of segment

Average diameter computed from multiple measurements along the segment.

Number of scale leaf scars

Number of roots and root scars

Location of root scars

Volume was computed using length and multiple diameter measurements

Roots

Length

In some cases diameter

Attachment point on rhizome

Scar attachment point on short shoot

The data were initially recorded on paper, and then transferred to Excel spread sheets. The join and match process that linked a sample to its weight was performed in SPSS (Statistical Package for the Social Sciences). Analysis of the data used both Excel and SPSS.

Data analysis

Initial analyses were exploratory. For in-depth analysis a variety of statistical tests were employed. During the course of the study a paired t-test, an independent t-test, and a ONEWAY ANOVA were used to test for differences in means. These statistical tests were run using SPSS and SAS (SAS Institute Inc.). Linear and non linear regressions were used to generate model summaries. These were also run in SPSS and SAS. SPSS has a function called curve estimation that was used to determine which linear or non-linear function best fits a series of points. Multiple regressions, principal components analysis, discriminant analysis, and other advanced statistics available through SPSS were also used. Not all results were considered worthy of report. The data was analyzed and presented graphically using the Excel software package. Program errors associated with Excel's trend line and regression equation required that the values presented in the figures were verified using SPSS.

Sediment profiles

During year-two of the study five cores were extracted to establish a sediment depth profile for Rabbit Key Basin. The coring tools were constructed from PVC plumbing pipe. The edges of the pipe were sharpened using a dremal tool. Handles were attached at the top of the device and a rubber end piece acted to cork the upper end of the tool. The coring took place within the general study area. The cores were extracted and carefully capped for transport. During the boat ride to the National Park Service dock the

cores were situation so as to reduce vertical compression. Once back at the research center the cores were immediately frozen still encased in the PVC pipe. After the cores had been deep frozen, they were transported in thermal jackets up to Charlottesville, Virginia. The cores were deep frozen to the extent that minimal thawing occurred during transport. The cores were placed in freezers at the University of Virginia and stored there until analysis.

To systematically analyze the sediment profile, each core was segmented along its length at five centimeter intervals. The cores were still encased inside the PVC and so the casing was marked and a band saw was used to cut through the pipe and the core. Since the core was still in a deep freeze, the cuts were clean and accurate. The core number and sample depth were recorded on the side of the PVC pipe, and the disk-like samples returned to the freezer. The sediment disks were retrieved and thawed individually as needed for the sorting of tissue types. Each individual thaw and sort of the sediment disks took one to three hours. In all 76 sediment disks were sorted by tissue types.

The individual sediment depth samples (disks) were thawed and the tissue types sorted. There were six major classifications of tissue type: green leaf, lower leaf, short shoot; rhizome, root, and general unidentifiable biomass. The tissue also sorted based on a criterion of live verses dead tissue, meaning, in all, there were 12 tissue types. Once the tissues from an individual sediment depth were sorted, the tissues were dried and then weighed. Weights were recorded in an SPSS spread sheet. Analysis was done using SPSS.

Organic content, elemental analysis, and other analyses

During the extractions a great deal of extra plant tissue was made available. The plant tissue was positively identified as being from the *Thalassia testudinum* plant. This extra tissue allowed for analyses pertaining to organic content and determination of the elemental make up of the plant tissue. The organic content was determined using a muffle furnace where plant tissues could be heated to temperatures of $600^{o}C$. Ash weights were used to determine percentage of organic material.

The elemental percentages were determined using a Carlo Erba gas chromatograph machine. Multiple replication provided a stable and consistent indication of the percentages of Carbon and Nitrogen. The Carlo Erba was re-tasked to test for Sulfur, but logistical difficulties limited the number of runs and limited the number of samples used to generate the overall average. Analyses centered around comparisons of elemental level for the different tissue types. A second group of analyses explored elemental changes due to the ageing of tissue.

Phosphorus was also analyzed but, again, logistics limited the number of replications. Even though the number of samples was low, the phosphorus levels of the control standard (citrus leaves) provided evidence that the values generated by the analysis were reliable.

The pattern of root attachment and growth direction was carefully studied. The first analysis was determination of the direction of root growth. In this sub-study the angle of the root, or the placement of the root scar was carefully documented. The second sub-study was concerned with the clustering of roots on the short shoots. The placement of

the root was associated with a leaf scar number. The data were entered into an SPSS

spreadsheet and analyzed. The final analysis was a measure of root length as a function of

attachment point. In this sub-study complete roots were required. A complete root is

defined as a root with a visible tip that is still attached to the plant. Once identified as

being complete the root attachment point was noted and then the root length recorded.

Results and Discussion

The four major below-ground components of *Thalassia testudinum* are the roots, rhizome, short shoots, and the lower portion of the leaves. Quantification of below-ground biomass consists of the summation of these four major plant parts. Seagrass productivity is expressed as a rate of biomass added per unit of time. Presently, there are viable, accurate methods to determine above-ground production. The above-ground estimates are reliable because the production of biomass and the period of growth are directly measurable quantities.

The biomass of below-ground portions of *Thalassia testudinum* is logistically difficult to measure. The minimum requirement for a direct assessment of below-ground productivity would be an accurate demarcation of the existing tissue. This method would require that, after a period of time, the old below-ground tissue would be easily distinguishable from the new tissue. This marking process is essential because it establishes a time zero point (t_0) at a specific location on the plant. Without an accurate t_0, the time interval for growth has to be estimated.

The alternate requirement for accurate productivity estimates would be direct observation of plant growth. The below-ground portion of the plant is buried, so measurement necessitates destructive extraction of the plant. Therefore, any assessment will be a static measure of the plant structure. This effectively prevents dynamic observation of growth.

Researchers investigating terrestrial plants have developed methods, such as rhzitrons, to directly observe below-ground plant structure and growth (Cite).

Researchers investigating wetlant plants have employed in-growth methods where below-ground structures penetrate an artificial below-ground growth chamber (Blum). For seagrass habitats, the sediment type and the growth matrix affects the feasibility of either of these approaches. Therefore, this study attempted to scientifically bypass these limitations. This results and discussion section will report on the structural characteristics of the below-ground components *of Thalassia testdudinum*. Advancing the understanding of below-ground biomass allocation is an early step in improving below-ground productivity estimates. This project will also report on the link between static below-ground measures and above-ground dynamics. From that linkage, relationships will be used to construct a model that will generate estimates for below-ground dynamics that are not directly observable.

The results and discussion of the data are separated into six sections. The first section reviews the individual variables and explores the reliability of the data set. Following that, the individual below-ground components are addressed separately. Section two explores the data on rhizomes. Section three, roots. Section four short shoots. Section five covers the lower portion of the leaf. The final section will summarize and synthesize the interaction of the system. Section six also discusses directions for future research.

Section One

Data Overview

Results

Population Description

Data was gathered during the summers of 2000 and 2001 from Rabbit Key Basin in Florida Bay. The study sites were positioned near an established seagrass monitoring location. The year-two study sites were located approximately 30 meters away from the year-one sites. The structural characteristics of *Thalassia testudinum* vary throughout Florida Bay (Zieman, 1982), so the estimates presented in this section apply exclusively to the population at Rabbit Key Basin.

Short shoot density for Rabbit Key Basin was estimated at the beginning of each study year (Table 1). In 2000 there were 46 density counts, and in 2001 there were 40 counts. In order to determine the appropriate sample size, three different quadrat sizes were used in these assessments. The dimensions of the quadrats were either 10 by 10 cm, 10 by 20 cm, or 20 by 20 cm. These quadrat sizes were standard dimensions for previous monitoring projects. The density counts suggested an evenly distributed population of short shoots at the study sites (Table 2, Table 3). There were no statistical differences in the density estimates between the first and the second year of the study (Table 2). There were no statistical differences in the density estimates based on quadrat size (Table 3). The density counts yielded an average estimate of 1318 short shoots/meter^2 (n=86). In the first year of the study six 10 by 10 cm quads were used. In

Table 1. Group statistics for short shoots per meter^2 classified by year of study.

Year of study	N	Mean	Standard Deviation	Standard error of the mean
Short shoots per meter^2 2000	46	1315.22	177.83	26.22
Short shoots per meter^2 2001	40	1321.88	182.42	28.84

Table 2. Independent t-test of short shoot density means grouped by year of study.

t-test for equality of means					
	t	df	Significance (2-tailed)	Mean difference	Standard error of the difference
Short shoot per meter2 – Equal variances not assumed	-0.171	84	0.865	-6.65	38.91

Table 3. ONEWAY ANOVA testing for differences short shoots per meter^2 based on quadrat size.

Short shoot density assessed by quadrat size	Sum of squares	df	Mean square	F	Sig.
Between groups	690.02	2	345.01	.01	.99
Within groups	2721091	83	32784.22		
Total	2721781	85			

year-two of the study the quad size was increased to 10 by 20 cm. Two of the second year quadrats were lost leaving four quadrats functional until the plants were extracted.

Plant Structure

Leaves

Photosynthesis occurs in the leaves, however, the entire leaf is not photosynthetically active. For analytical purposes, an abscission layer located 4-7 cm above the short shoot is used to divide the leaf into functional units. According to Tomlinson (1966) this abscission layer is the point of maximum leaf width. Once the leaf reaches this point, cellular structure and function changes. The abscission layer is the location where elongation of the leaf takes place. While not absolute, this selection criterion is a good approximation for separating the leaf into photosynthetic and non-photosynthetic areas. The green photosynthetic area will be called the upper leaf. The area below the abscission layer will be called the lower leaf.

The average leaf length was 24.9 cm (n=1073). The upper leaf had an average length of 18.3 cm (Table 5), and an average width of 0.67 cm. The average area for the upper leaf was 12.9 cm^2. There were statistically significant differences in average leaf width based on year of the study (Table 6). The maximum length of the upper leaf was measured by selecting the longest complete leaf attached to the short shoot (n=437). The upper leaf grows to an average maximum length of 23.2 cm (Table 7).

Table 5. Summary of the upper leaf (photosynthetically active) length, width, and area.

Year of study		Upper leaf length (cm)	Leaf width (cm)	Upper leaf area (cm^2)
2000	Mean	18.70	0.69	13.41
	n	587	586	586
	Std. Dev.	10.09	0.15	8.55
2001	Mean	17.81	0.64	12.26
	n	492	492	492
	Std. Dev	10.58	0.18	9.29
Total	Mean	18.29	0.67	12.88
	n	1079	1078	1078
	Std. Dev	10.32	0.16	8.91

Table 6. Independent t-test of leaf width grouped by year.

	t	df	Sig. (2-tailed)	Mean dif.	Std. Error dif.	95% Conf. interval (lower)	95% Conf. interval (upper)
Leaf width, equal variances assumed	4.18	1076	.00	0.04	0.01	0.02	0.06
Leaf width, equal variances not assumed	4.13	975	.00	0.04	0.01	0.02	0.06
Levene's test for equality of variances							
	F	Sig.					
Equal variances assumed	18.29	.00					

Table 7. Data for the upper leaf length of the longest leaf still attached to the short shoot.

	n	Minimum	Maximum	Mean	Std. Dev.
Upper leaf length (cm)	437	1.10	41.90	23.16	9.22

The mean length for non-photosynthetic portion of the leaf was 6.3 cm (Table 8). The lower leaf width was identical to the upper leaf. The average area for the lower leaf was 4.2 cm^2.

Short Shoots

Data from short shoots can be divided into characteristics pertaining to the attached leaves, and secondly, the stem-like shoot,. The short shoot is the clonal unit that connects to the rhizome at the base and the leaves at its crest (Figure 1). The short shoot has been described as a vertically growing rhizome, though there are functional differences between the short shoot and the rhizome (Tomlinson, 1972).

Table 8. Summary data of lower leaf (non photosynthetic) length and area.

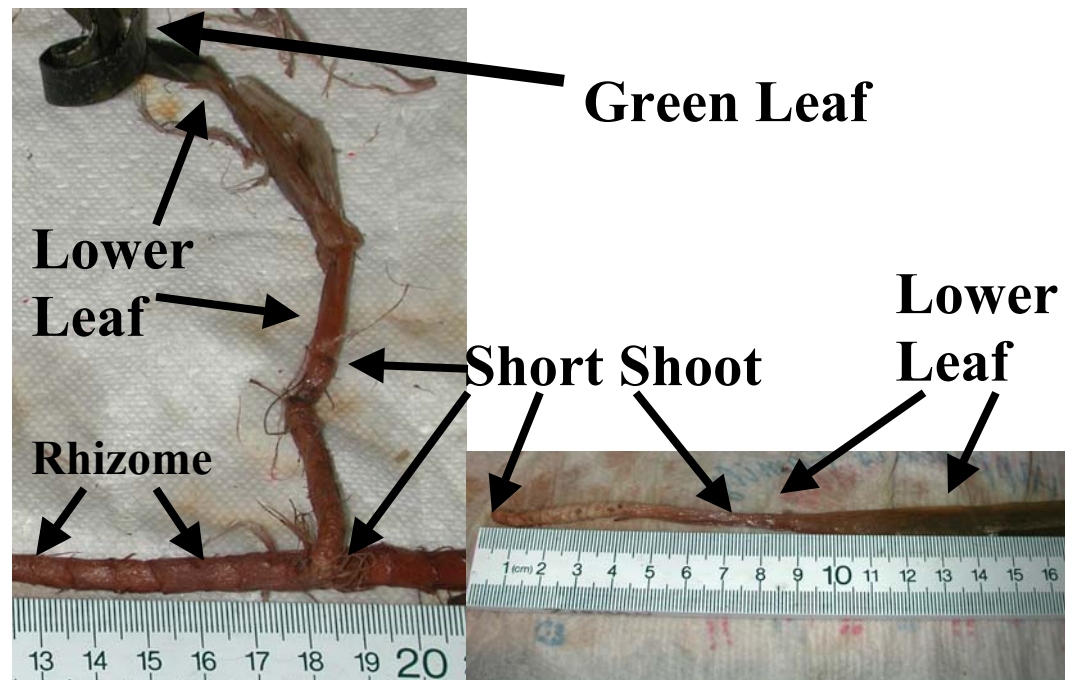| Year of study		Lower leaf length (cm)	Lower leaf area (cm^2)
2000	Mean	6.40	4.25
	n	356	356
	Std. Dev.	2.26	2.11
2001	Mean	6.26	4.13
	n	559	560
	Std. Dev	2.35	2.30
Total	Mean	6.32	4.17
	n	915	916
	Std. Dev	2.32	2.23

Figure 1. Photographs of two segments of *Thalassia testudinum*. Individual components are noted.

The average short shoot had 3.01 leaves attached (Table 9). The number of attached leaves per short shoot varied over the two years of the study, being higher in year-two (Table 10). The average leaf plastochrone interval for the study population was 24.5 days (Table 11). On average, leaves stayed attached to the short shoot for 66.05 days (Table 12).

The season long measurement of leaf elongation provided evidence that, for the study population, leaf growth exhibited an asymptotic pattern. There were two distinct periods in leaf development: 1) from leaf initiation to maximum leaf length; 2) period of slower to no growth. Figure 2 illustrates these growth periods for a single short shoot (number 1566, Quad 2, Year 1), including the period of maximum leaf growth. The mean maximum elongation rate for single leaves was 0.71 cm/day (Table 13). Elongation data is coupled with leaf width data to compute the leaf area added. The average maximum leaf area added was 0.51 cm^2/day (Table 13). The average short shoot had 38.94 cm^2 of attached green leaf area (Table 14). Leaf area added per day is a summation of area added for all attached leaves. Figure 2 shows that this summation of leaf area added is primarily influenced by the growth of the youngest leaf, however older leaves do continue to elongate and affect this measure. Leaf area was added an average of 0.71 cm^2 per short shoot, per day (Table 15). This figure also shows a trend of progressively lower maximum leaf lengths. This tendency was observed in approximately one-quarter of the study population.

Table 9. Number of leaves attached to short shoots, broken down by year.

Number of leaves attached to short shoot broken down by year	Mean	n	Std. deviation
2000	2.72	199	0.69
2001	3.37	155	0.77
Total	3.01	354	0.79

Table 10. Independent t-test of number of leaves attached to short shoot grouped by year of study.

	t	df	Sig. (2-tailed)	Mean dif.	Std. Error dif.	95% Confidence interval (lower)	95% Confidence interval (upper)
Number of leaves attached to short shoot, equal variances assumed	-8.40	352	.00	-0.65	0.08	-0.80	-0.50
Number of leaves attached to short shoot, equal variances not assumed	-8.29	313	.00	-0.65	0.08	-0.81	-0.50
Levene's test for equality of variances							
	F	Sig.					
Equal variances assumed	2.53	.11					

Table 11. Summary of leaf plastochrone interval data.

Leaf plastochrone interval broken down by year	Mean	n	Std. deviation
2000	24.43	121	5.07
2001	24.63	137	5.38
Total	24.53	258	5.23

Table 12. Summary of leaf life span data.

Age of leaf at senescence broken down by year	Mean	n	Std. deviation
2000	65.69	36	9.30
2001	66.56	25	9.19
Total	66.05	61	9.20

Table 13. Summary of maximum leaf elongation and leaf area added per day.

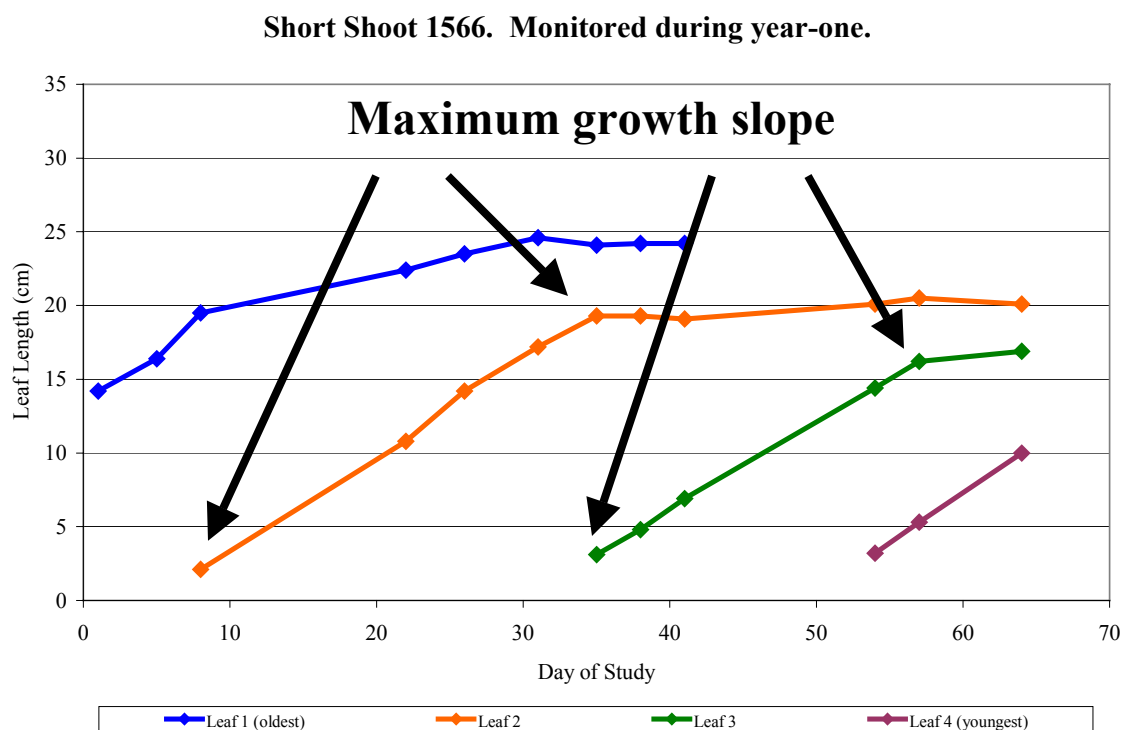| Year of study		Average maximum leaf elongation (cm/day)	Average maximum leaf area added (cm^2/day)
2000	Mean	0.70	0.52
	n	95	95
	Std. Dev.	0.20	0.20
2001	Mean	0.72	0.51
	n	1555	155
	Std. Dev	0.23	0.27
Total	Mean	0.71	0.51
	n	250	250
	Std. Dev	0.22	0.24

Figure 2. Chart showing data from an individual, tagged short shoot. The maximum

growth slopes are indicated by arrows.

Table 14. Summary of average attached leaf area for individual short shoots.

Year of study	Total leaf area (cm^2)	Green leaf area (cm^2)	Lower leaf area (cm^2)
2000 mean	52.73	40.25	12.76
n	145	153	145
Std. Dev.	29.69	22.04	8.12
2001 mean	51.29	37.75	13.66
n	170	170	169
Std. Dev	38.05	29.53	9.17
Total mean	51.95	38.94	13.25
n	315	323	314
Std. Dev	34.41	26.24	8.69

Table 15. Summary of average leaf area added per day for individual short shoots.

Leaf area added per day (cm^2/day) broken down by year	Mean	n	Std. deviation
2000	0.69	48	0.30
2001	0.72	82	0.45
Total	0.71	130	0.40

The short shoot is the stem-like structure shown in Figure 1. The average short shoot length was 2.56 cm (Table 16), and the average diameter was 0.41 cm. The mean volume was 0.39 cm^3 (Table 16). The population age distribution was estimated by counting the leaf scars on the short shoots. Each new leaf creates a scar allowing an aging technique analogous to counting tree ring. Unlike the yearly production of a single ring, the leaf scar rate is variable and influenced by several factors. First, individual short shoots have distinctive leaf plastochrone intervals (Table 11). Second, those individual rates are known to be influenced by seasonal factors. Lower temperatures and light levels slow leaf production in the winter months. Therefore scars are a general, but not exact indication of age. The average number of scars for short shoots in the study was 25.79 (Table 17). Figure 3 is a graphic representation of the population distribution. There were no statistical differences for any of the short shoot measures when the data were grouped by year of study.

Rhizome

The rhizome segment length is the distance between successive short shoots. The average rhizome segment length was 6.01 cm (Table 18). To capture the within segment variation, diameter measurements were taken at each scale leaf scar along length of the rhizome segment. The scale leaf scars can be seen in Figure 1 as the dark vertical bands on the rhizome. These individual measurements were used to generate a mean diameter for the segment. In turn, a mean of the means was computed. The average diameter was 0.46 cm (Table 18). A second, overall, average was computed using all 5277 diameter measurements. This estimate also yielded a value of 0.46 cm. The mean volume for the rhizome segments in the study population was 1.10 cm^3 (Table 18). The average number

Table 16. Short shoot length, diameter (cm), and volume (cm^3) grouped by year of study.

Year of study		Short shoot length (cm)	Short shoot diameter (cm)	Short shoot volume (cm^3)
2000	Mean	2.37	0.43	0.41
	n	277	274	274
	Std. Dev.	1.97	0.08	0.46
2001	Mean	2.85	0.37	0.35
	n	177	177	177
	Std. Dev	2.46	0.10	0.33
Total	Mean	2.56	0.41	0.39
	n	454	451	451
	Std. Dev	2.18	0.09	0.41

Table 17. Number of short shoot scars grouped by year. This data is an indicator of the age of the short shoot.

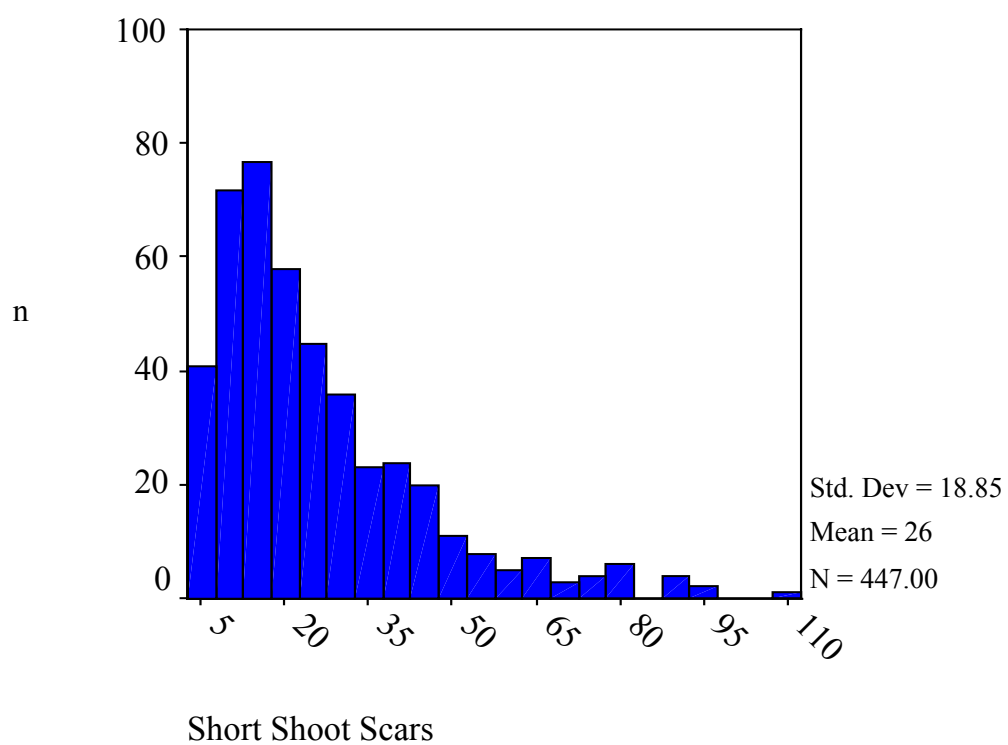| Short shoot scars broken down by year	Mean	n	Std. deviation
2000	25.28	273	18.42
2001	26.59	174	19.54
Total	25.79	447	18.85

Figure 3. Histogram of short shoot scars. This figure is a proxy for age distribution of short shoots.

Table 18. Summary of rhizome segment length and diameter (cm). Also included are data on the rhizome segment volume (cm^3).

Year of study	Rhizome length (cm)	Average rhizome segment diameter (cm)	Average of all rhizome diameter measurements in study (cm)	Rhizome segment volume (cm^3)
2000 mean	5.87	0.47	4.68	1.09
n	256	256	3148	256
Std. Dev.	2.18	0.10	1.05	0.66
2001 mean	6.18	0.45	4.49	1.10
n	181	181	2129	181
Std. Dev	1.88	0.11	1.13	0.63
Total mean	6.00	0.46	4.60	1.10
n	437	437	5277	4.37
Std. Dev	2.07	0.10	1.09	0.65

of scale leaf scars was 12.19 scars (Table 19).   The average number of root scars was

2.20 scars (Table 19). The number of rhizome roots and root scars was not measured in

year-one of the study.

With the exception of the overall average rhizome diameter, there were no

statistical differences in these measures based on the year of the study. In the case of

diameter data, the number of observations was so high (n=5277) that small group

differences exceed the threshold of statistical significance.

Roots

Roots attach to the *Thalassia testudinum* plant at two locations, on the short shoot

and on the rhizome. The investigation of roots was added prior to the second year of the

study so multi-year comparisons are not available. The destructive extraction process

tended to select for shorter complete root segments. This means that population estimates

of root length are likely to be biased. Because of that bias, population estimates on root

length were bypassed. Root length is presented and discussed in section three.

Population estimates of root segment diameter did not depend on obtaining

complete samples. This estimate used both complete and partial segments for analysis.

The average diameter for the study population was just slightly above one millimeter, and

appears normally distributed (Figure 4).

Belowground Dry Weight Biomass Estimates

The quantification of biomass not only describes the population, it also provides

the foundation for useful predictive equations. For example, Figure 5 shows the

relationship of leaf area (cm^2) to dry weight biomass (g). Table 20 contains coefficients

Table 19. Scale leaf scars on rhizome segments, and root scars per rhizome segment.

Year of study		Rhizome scale leaf scars	Rhizome root scars
2000	Mean	12.35	
	n	256	No data
	Std. Dev.	3.93	
2001	Mean	11.76	2.19
	n	181	181
	Std. Dev	3.02	1.33
Total	Mean	12.10	2.19
	n	437	181
	Std. Dev	3.59	1.33

Std. Dev = .26
Mean = 1.01
N = 286.00

Root Diamter (mm)
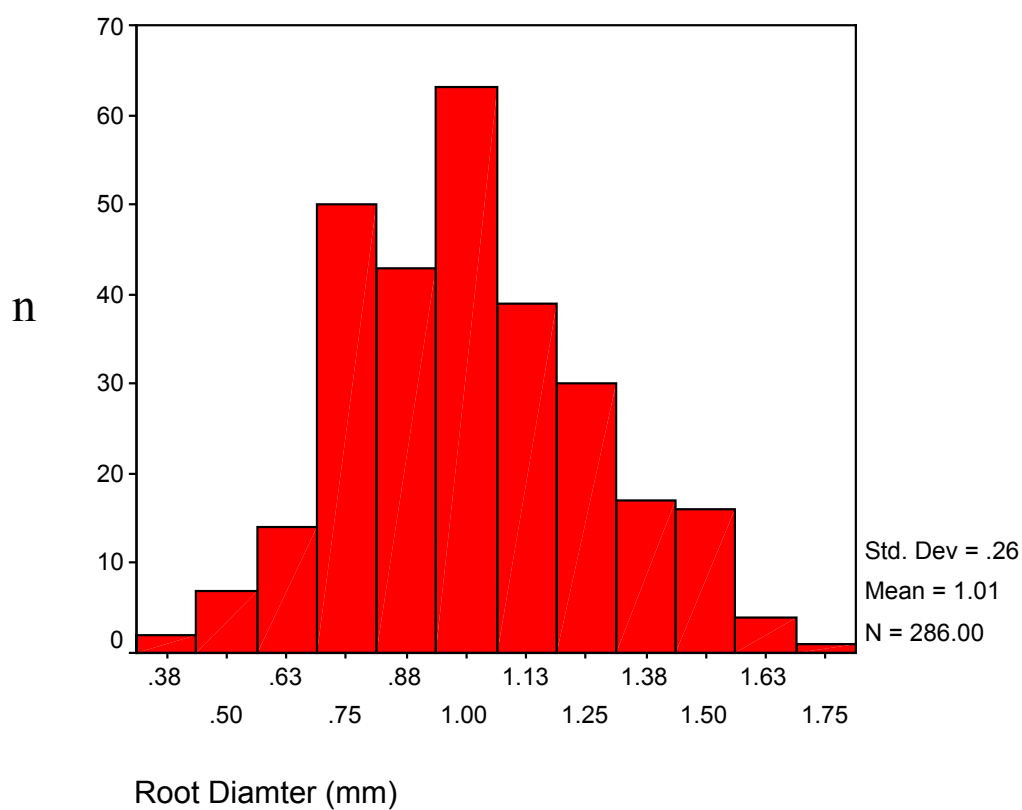
Figure 4. Distribution of root segment diameter (mm) for the study population.

Dry weight biomass plotted against leaf area
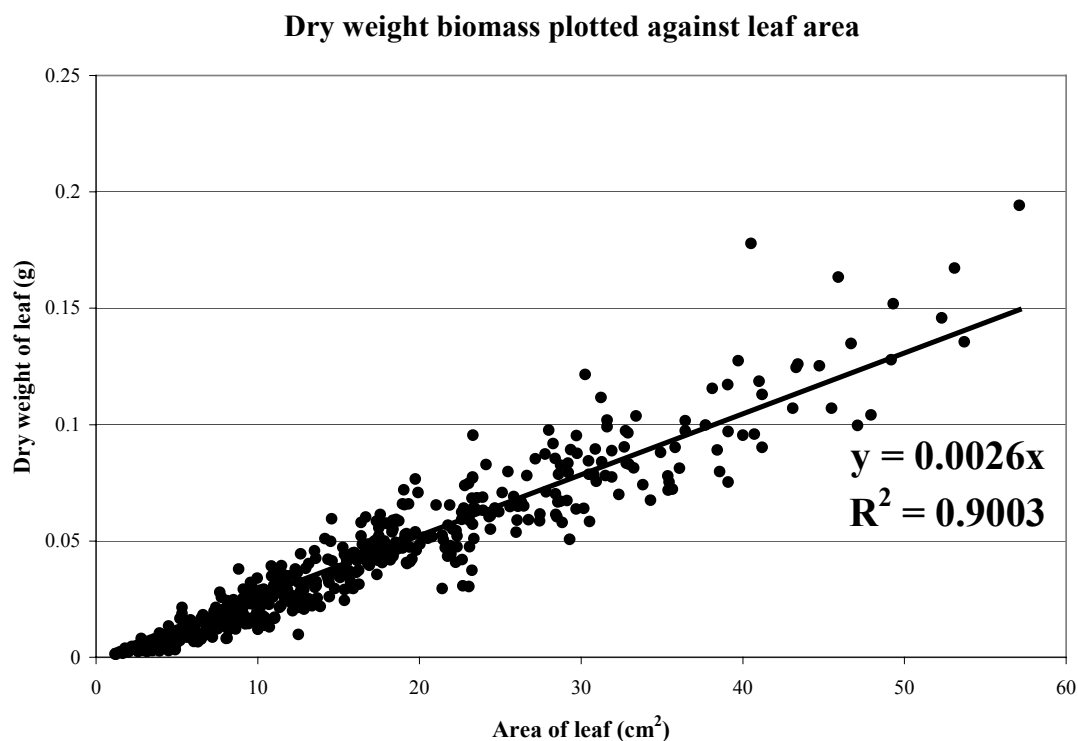
Figure 5. Chart of total leaf area (cm^2) plotted against dry weight biomass (g).

Table 20. Regression model leaf area (cm2) verses leaf weight (g), including coefficients used to predict dry weight biomass of leaf tissue.

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	-1.64E-04	.001		-.218	.827
	Leaf Area (cm^2)	3.381E-03	.000	.952	69.124	.000

a. Dependent Variable: Leaf Weight (g)

used in Equation 1.1 to predict leaf biomass. This equation is a site specific predictive

equation.

Dry Weight Leaf Biomass = 0.00+(.002814*leaf area)   Equation 1.1

In cases where it is appropriate, the regression line will be forced through the

origin.   In the case of the data in Table 20, zero leaf area forces the assumption of zero

biomass, and the constant in this model does not significantly differ from zero.   The

development of these predictive equations is described in the Appendix A. A summary

of the equations and a population description of dry weight biomass are included in Table

21.

Table 22 compares three different estimates of standing biomass at Rabbit Key

Basin. Comparing the predictive equations to estimates derived from direct measurement

provides an indication of the robustness of a model. The first estimate in the table is

derived from the predictive equations. The second estimate uses the mean weights for

individual plant parts. For example, the average green leaf is 0.042 g. That value is

multiplied by 3.01, which is the average number of leaves per short shoot. That product is

multiplied again by 1318, which is the number of short shoots per meter^2. This method

was called the mean of individual weights. The third method of estimating biomass uses

the sum weight for the individual plant parts. That sum is then multiplied by a population

density factor. For example, 418 individual short shoots were weighed and the total dry

weight biomass was 27.34 g. Those 418 samples represent 31.71% of the number of

short shoots expected in a meter^2 area of Rabbit Key Basin. Therefore, the sum weight

Table 21. Summary of dry weight biomass estimates for Rabbit Key Basin.

Tissue	Dry Weight Biomass Prediction Equation	Variance Explained	Grams of Dry Weight Biomass Per Meter^2
Total Leaf	(0.002814*Leaf Area)	91%	233.29 g
Green Leaf	(0.002814*Leaf Area)	91%	173.98 g
Lower Leaf	(0.002814*Leaf Area)	91%	59.31 g
Rhizome	.1489*Rhizome volume$^{0.9352}$	78%	207.97 g
Short Shoot	.1336*Short shoot volume$^{0.7968}$	79%	80.36 g

Table 22. Comparison of three different dry weight biomass estimates.

Tissue	Type of Dry Weight Biomass Estimate	Grams of Dry Weight Biomass Per Meter^2
Total Leaf	Predictive Equation	233.29 g
	Mean of Individual Weights	213.23 g
	Sum of Individual Weights	212.85 g
Green Leaf	Predictive Equation	173.98 g
	Mean of Individual Weights	166.62 g
	Sum of Individual Weights	163.48 g
Lower Leaf	Predictive Equation	59.31 g
	Mean of Individual Weights	49.36 g
	Sum of Individual Weights	49.37 g
Rhizome	Predictive Equation	207.97 g
	Mean of Individual Weights	210.60 g
	Sum of Individual Weights	212.43 g
Short Shoot	Predictive Equation	80.36 g
	Mean of Individual Weights	86.10 g
	Sum of Individual Weights	86.21 g

was multiplied by 3.15 to reflect grams per meter^2. This method is called the sum of individual weights. The dry weight biomass estimates for all three methods are presented in Table 22. The predictive equations tended to overestimate values for leaf tissue and underestimate for rhizome and short shoot.

Sediment Cores

Five sediment cores were extracted from the study area in Rabbit Key Basin. Each core was extracted to a depth of 0.8 meters then transported to the National Park Service lab. The samples were then frozen for transport to Charlottesville. The core tube consisted of PVC pipe, and the length of the core was measured and marked off in 5 cm intervals. It was expected that the integrity of the belowground matrix would be better maintained if the core was cut (by a band-saw) while frozen. Each individual 5 cm segment remained frozen until the time of analysis. At that point, the core segment was thawed and the individual plant parts were classified and separated. The separation criterion was based on tissue type and whether the sample was living or dead. The bulk samples were then dried and weighed. The data from these cores are presented in Table 23. The most striking result is the sharp zonation of rhizome growth. A comparison of depth profile for same tissue types reveals a phase shift in the live and dead biomass distribution. In the case of root tissue, the maximum live biomass occurred at a depth of 16-20 cm, and the maximum dead biomass occurred at 21-25 cm.

Table 23. Sediment profile constructed from cores extracted from the Rabbit Key

Basin study site. Weight is expressed in grams.

Tissue type	Depth	n	Mean weight for cores (g)	Standard deviation	Estimate of biomass (g) per meter^2
Green leaf	Above-ground	5	4.06	0.54	221.11
	0-5 cm	2	0.17	0.10	9.26
	6-10 cm	5	0.21	0.03	11.44
Lower leaf	0-5 cm	5	0.81	0.05	44.11
	6-10 cm	3	0.18	0.09	9.80
	11-15 cm	1	0.33	-	17.97
Dead leaf	0-5 cm	5	1.98	0.38	107.83
	6-10 cm	5	0.11	0.08	5.99
Live Rhizome	0-5 cm	5	1.79	0.25	97.48
	6-10 cm	5	2.78	0.55	151.40
	11-15 cm	5	4.45	1.43	242.35
	16-20 cm	3	0.39	0.16	21.24
Dead Rhizome	0-5 cm	2	0.27	0.07	14.70
	6-10 cm	5	1.03	0.19	56.09
	11-15 cm	5	3.35	0.50	182.44
	16-20 cm	4	2.39	0.68	130.16
	21-25 cm	4	0.54	0.49	29.41
	26-30 cm	3	0.22	0.13	11.98
	31-35 cm	2	0.01	0.00	0.54
	41-45 cm	3	0.08	0.06	4.36

Table 23 continued

Tissue type	Depth	n	Mean weight for cores (g)	Standard deviation	Estimate of biomass (g) per meter^2
Live root	0-5 cm	5	0.25	0.17	13.62
	6-10 cm	5	0.68	0.29	37.03
	11-15 cm	5	1.70	0.33	92.58
	16-20 cm	5	1.12	0.13	61.00
	21-25 cm	5	0.91	0.12	49.56
	26-30 cm	5	0.61	0.12	33.22
	31-35 cm	5	0.38	0.08	20.69
	36-40 cm	5	0.13	0.02	7.08
	41-45 cm	3	0.17	0.06	9.26
	46-50 cm	5	0.05	0.01	2.72
	51-55 cm	3	0.06	0.04	3.27
	56-60 cm	2	0.08	0.07	4.36
	61-65 cm	1	0.04	-	2.18
Dead root	0-5 cm	2	0.10	0.02	5.45
	6-10 cm	5	0.56	0.17	30.50
	11-15 cm	5	0.78	0.12	42.48
	16-20 cm	5	1.23	0.21	66.99
	21-25 cm	5	1.83	0.81	99.66
	26-30 cm	5	1.00	0.36	54.46
	31-35 cm	4	0.47	0.08	25.60
	36-40 cm	2	0.57	0.01	31.04
	41-45 cm	4	0.30	0.09	16.34
	46-50 cm	3	0.24	0.08	13.07
	51-55 cm	3	0.22	0.09	11.98
	56-60 cm	3	0.16	0.05	8.71
	61-65 cm	1	0.04	-	2.18
	65-70 cm	1	0.16	-	8.71

Table 23 continued

Tissue type	Depth	n	Mean weight for cores (g)	Standard deviation	Estimate of biomass (g) per meter^2
Live short shoot	0-5 cm	5	0.47	0.38	25.60
	6-10 cm	5	2.28	0.17	124.17
	11-15 cm	5	0.87	0.51	47.38
	16-20 cm	1	0.15	-	8.17
Dead short shoot	0-5 cm	2	0.99	0.08	53.92
	6-10 cm	5	1.13	0.38	61.54
	11-15 cm	5	0.51	0.20	27.77
	16-20 cm	4	0.12	0.03	6.54
Sheath	0-5 cm	5	3.12	0.25	169.92
	6-10 cm	5	2.21	0.14	120.36
	11-15 cm	1	0.16	-	8.71
General biomass	0-5 cm	5	1.36	0.41	74.07
	6-10 cm	5	2.66	0.60	144.86
	11-15 cm	5	1.61	0.34	87.68
	16-20 cm	5	2.04	0.37	111.10
	21-25 cm	5	1.91	0.92	104.02
	26-30 cm	5	1.79	0.29	97.48
	31-35 cm	4	2.05	1.22	111.64
	36-40 cm	4	1.19	0.60	64.81
	41-45 cm	4	1.89	0.95	102.93
	46-50 cm	2	0.86	0.45	46.84
	51-55 cm	3	0.75	0.94	40.85
	56-60 cm	3	0.06	0.02	3.27
	61-65 cm	1	0.19	-	10.35

Section one discussion

The short shoot density estimates suggests that Rabbit Key Basin has an evenly distributed population of *Thalassia testudinum*. *T. testudinum* is not the only seagrass present in this basin, but is the predominant species. This characterization of an evenly distributed population is important because logistics limited the number of short shoots that could be tagged and monitored.

Sources of bias

Under-sampling of a population can be a critical source of error in a research study. Two factors reduce this concern. First, since the population is evenly distributed, statistical theory would hold that the error associated with the estimates is also evenly distributed. Second, the specific study sites were haphazardly selected meaning error was not introduced due to a systematic selection of the study population. These factors reduce the probability that the data are biased by community structure or sampling technique.

General Population Description

The population demographic parameters show that the seagrass community remained stable across the two years of the study. Differences that did occur could be due to natural fluctuation within the system. Even the fluctuation that was noted appears to occur within the stable framework of an integrated physiological system. For example, when comparing the second to the first year estimates, the average length and width of the leaf declined (Table 5). In a similar comparison, the average number of attached leaves per short shoot increased. The short shoots kept the amount (area) of attached leaf

tissue consistent over the two years of the study. Some factor, perhaps density, may be forcing a physiological change. The short shoots may be lowering single leaf area, but compensating by increasing the number of attached leaves. Likewise, when looking at leaf elongation rates, the thinner leaves of the second year population grew at a greater rate (Table 13). Therefore, the average maximum leaf area added per day was approximately the same for the two years. In another example, Table 1 shows that short shoot density increased which might be another factor forcing changes in plant structure.

The data on short shoot length and age suggested a population in a growth mode. If the average number of scars was significantly higher, this would indicate an aging population. This would mean that younger short shoots were not being added to the population commensurate with older demographic patterns. If the average number of scars stayed the same between the two years it would suggest a population at zero growth. A lower scar average for year-two would suggest one of two things: a population in decline, or the occurrence of an extremely large new cohort. The distribution was positively skewed which suggests a young population.

Change in the average rhizome diameter across the two years of the study met the threshold of statistical significance. Since the number of observations is so large, statistical theory holds that this small change represents an actual trend in below-ground structure. These data may also point to an increase in density. If short shoot population is increasing, there could be a corresponding increase in the rhizome tissue below-ground. If, as the core data suggest the rhizosphere is restricted to a fixed below-ground depth, then there is likely to be competition for space within the livable sediment volume. This

furthers the hypothesis that the observed changes reflect an integrated physiological response to resource availability.

The average length of the rhizome segment increased in year-two of the study. There were no statistical differences detected between the two years, but the higher year-two average raises a question. This observation is not consistent with the corresponding increase in short shoot density. In a stable system with an unrestricted resource base an increase in short shoot density should correspond to a decrease in rhizome segment length. The competition for living space in the growth matrix can be hypothesized to be the factor that explains the longer rhizome segment. The rhizome segments might need to grow longer to find space in the matrix. They may also need to occupy deeper or shallower sediment space. The characteristics of shallow or deep sediment might cause a physiological change in the rhizome segment structure.

One notable structural change was observed when the younger leaves were measurably shorter than older leaves. For example, Figure 2 shows data from a single short shoot tagged and monitored during year-one of the study. A trend towards a progressively shorter maximum leaf length can clearly be seen. This is likely due to disturbance associated with the study methodology. The repeated handling of the leaves effectively cleaned them of epiphytic growth. Frankovich (personal communication) has demonstrated that epiphytes affect the light attenuation coefficient. Cleaning of the leaves may have increased their photosynthetic capacity. This issue is important in considering clonal integration. This decreasing trend in leaf area could suggest that there is an energy threshold that the leaf must meet. Once the threshold is met, energy is translocated out to

support other areas of the plant. The issue of clonal integration will be covered in detail in the following sections.

Biomass estimates

The data in Table 21 contained a comparison of three different biomass estimates. The first was derived from a predictive equation and the second two were based on direct measures. The fact that the predictive equations over or under estimated the population parameters isn't surprising. These predicative equations can't exploit the shape of the distribution. Also, the natural variation displayed by this species does introduce some error in these relationships. But the tightly patterned distribution around the regression line gives an indication that it is viable to use area or volume to predict biomass. The predictive equations are important because they allow a backwards computation of existing data where weights were not measured independently. Alternatively, studies where bulk weights were measured can reverse these equations to compute volume or area from weights.

In general, the within year and between year comparison of the data points provide evidence that the sampling technique provided high-quality population estimates. The data collection and measurement have captured consistent patterns in the population of *Thalassia testudinum* living in Rabbit Key Basin.

Section Two

Rhizome

Results

The rhizome is the axial component of the *Thalassia testudinum* plant. It is, in effect, the foundation from which all other tissue types grow. This section begins by exploring rhizome structure, and more specifically the within subject variation. This required the measurement of multiple segments on a single runner.   The rhizome runner is a continuous individual, however, in this study the runner will be treated as a series of sequential segments. For analytical purposes the short shoot acts as the dividing line between segments. Figure 6 shows four sequential rhizome segments. This figure also includes one partial segment extending from the newest short shoot out to the apical meristem.   The tags used to identify individual short shoots are also shown in this photograph. Segments one through three appear to be approximately the same length, while segment four is much shorter. Segments extending from the newest short shoot out to the rhizome tip are considered a special classification, the apical meristem segment.

The within subject comparison contrasted the length, diameter and volume of sequential segments. A paired t-test determined that there are no statistical differences in the length of sequential segments (Table 24). This table also includes a comparison of non-sequential rhizome lengths for segments on the same runner. For example, this is a comparison of segments one and three (or two and four) as they are labeled in Figure 6. There were also no statistical differences in the means of these two groups. This is an
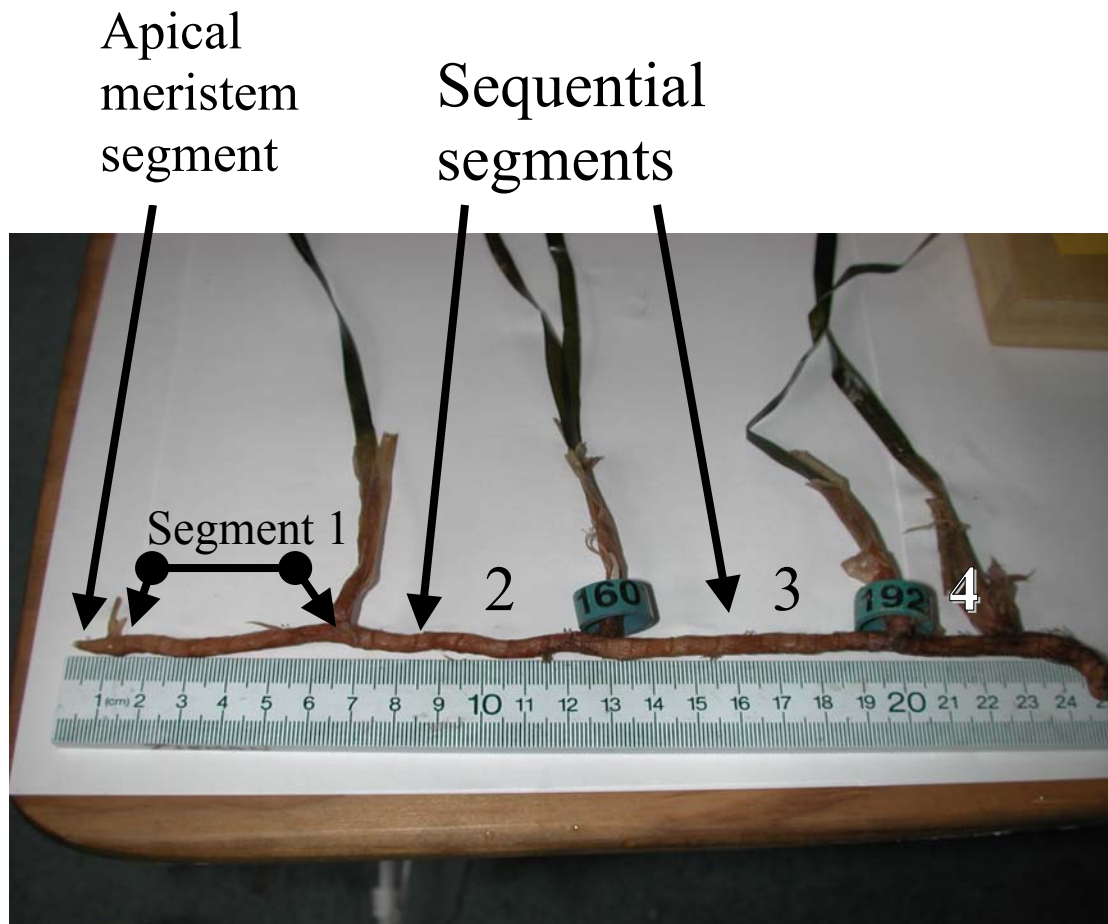
Figure 6. Photograph of rhizome components.

Table 24. Paired t-test comparison of sequential rhizome segment lengths (cm). Also included (Pair 3) are comparisons of lengths for rhizome sections one and three in a multi segment sequence.

	Mean	n	Std. deviation	Std. error mean	t	df	Sig. (2-tailed)
Pair one – First segment length (cm)	5.88	183	1.80	0.13	-0.63	182	.53
Second sequential length (cm)	5.97	183	1.97	0.15			
Pair two – Second sequential segment length (cm)	5.82	101	1.93	0.19	-0.62	100	.54
Third sequential segment length (cm)	5.95	101	1.86	0.19			
Pair one – First segment length (cm)	5.78	101	1.88	0.19	-0.92	100	.36
Third sequential length (cm)	5.95	101	1.86	0.19			

indication that in terms of population means, the plant is producing sequential

segments approximately the same length.

The linear relationship between consecutive segments on individual plants shows

that length can be variable (Figure 7). This figure gives a better indication of the within

subject variation of this population. The length of the first segment only explains 25% of

the variance in the length of the following segment. However, a subset of the population

displayed a systematic alteration of length between sequential segments on the same

mini-runner. These cases displayed an A-B-A pattern, which means the rhizome segment

lengths were short-long-short or long-short-long (Figure 8). Almost all of the 27 cases

presented in this figure display a pattern where the length of the first and the third

segments are approximately equal.

Sequential rhizome segments displayed a greater structural consistency in the

measurement of diameter.   To reiterate, this is the mean diameter of the entire rhizome

segment and not a single point measurement. As noted in section one, the average

segment diameter was 0.46 cm. Since multiple measurements were made along the

length of the segment, a within subject standard deviation was computed. The mean of

all standard deviations was 0.05 cm. This is an indication that the rhizome diameter is

somewhat variable along the length of a segment. Some of this variation was due to a

thickening of the rhizome in the area of the short shoot. Generally though, rhizome

diameter remained consistent along the length of the segment. The consistency of

rhizome diameter also was observed in the comparison of sequential segments. Figure 9

illustrates this relationship. In summary, the diameter of an existing segment will be a

good predictor of the average diameter of the following segment.
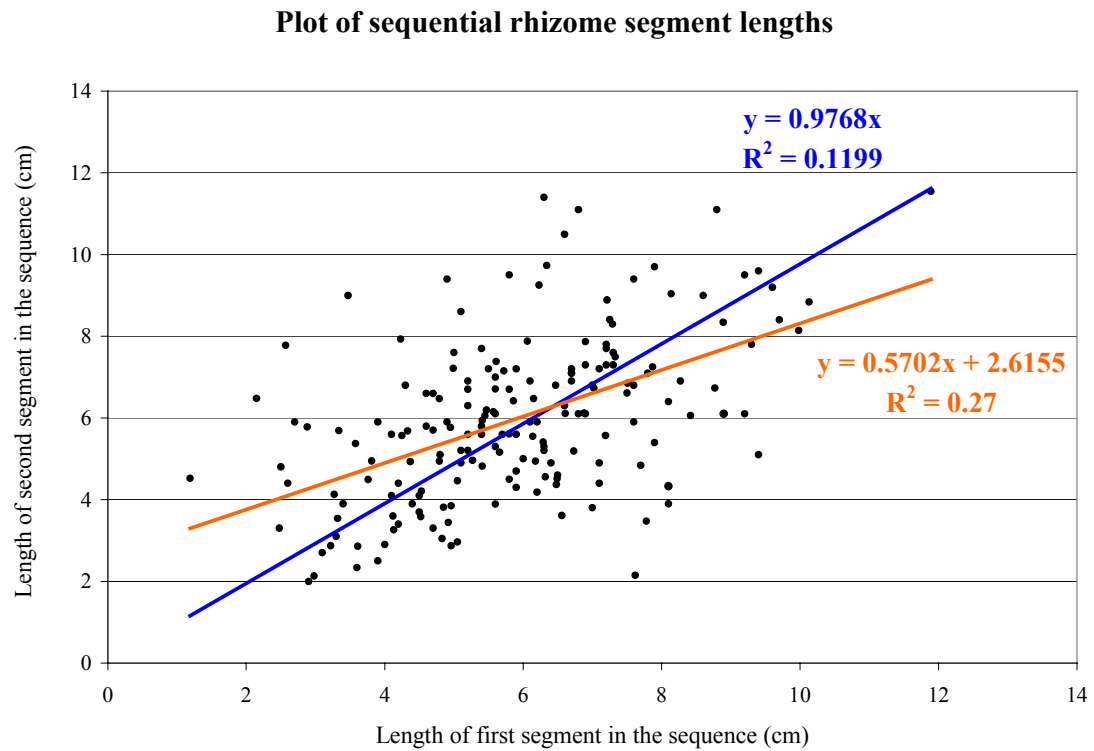
Figure 7. Chart of rhizome length (cm) for first segment in a sequence plotted against the length of the next segment in the sequence. Blue regression line is forced through the origin.
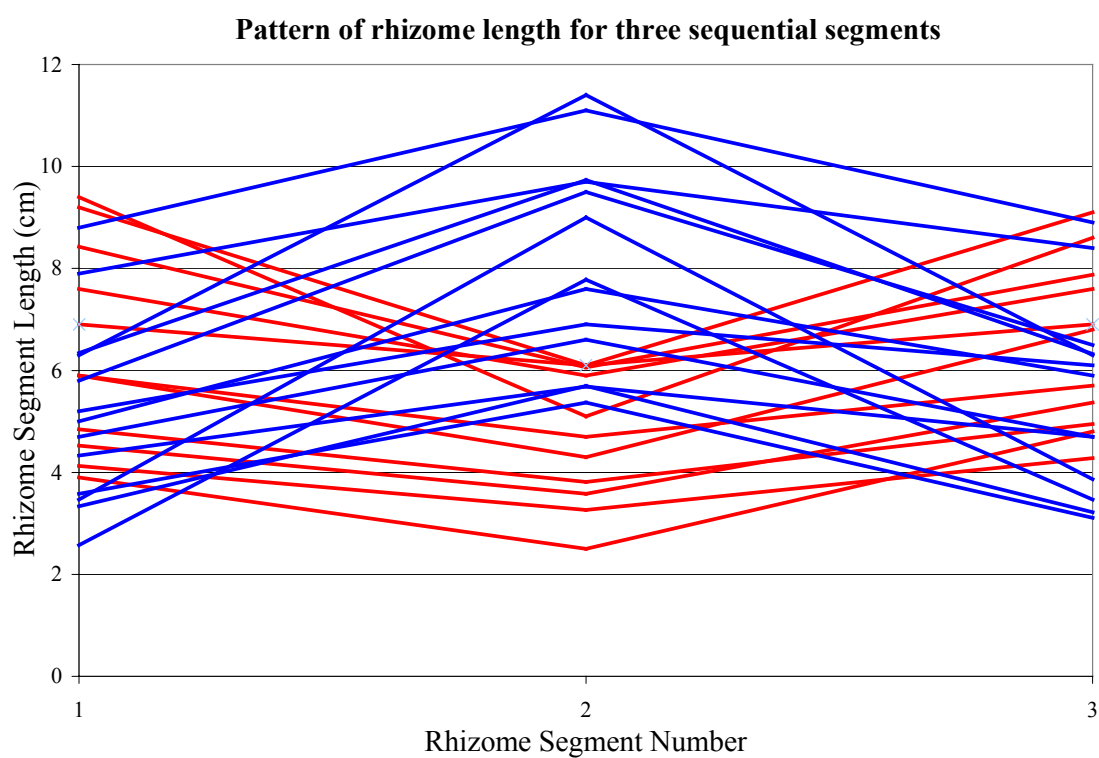
56

Figure 8. Plot of individual rhizome runners comparing the lengths of sequential segments 1-2-3 in cm. Blue lines represent cases of short-long-short segment lengths. Red represents long-short-long.
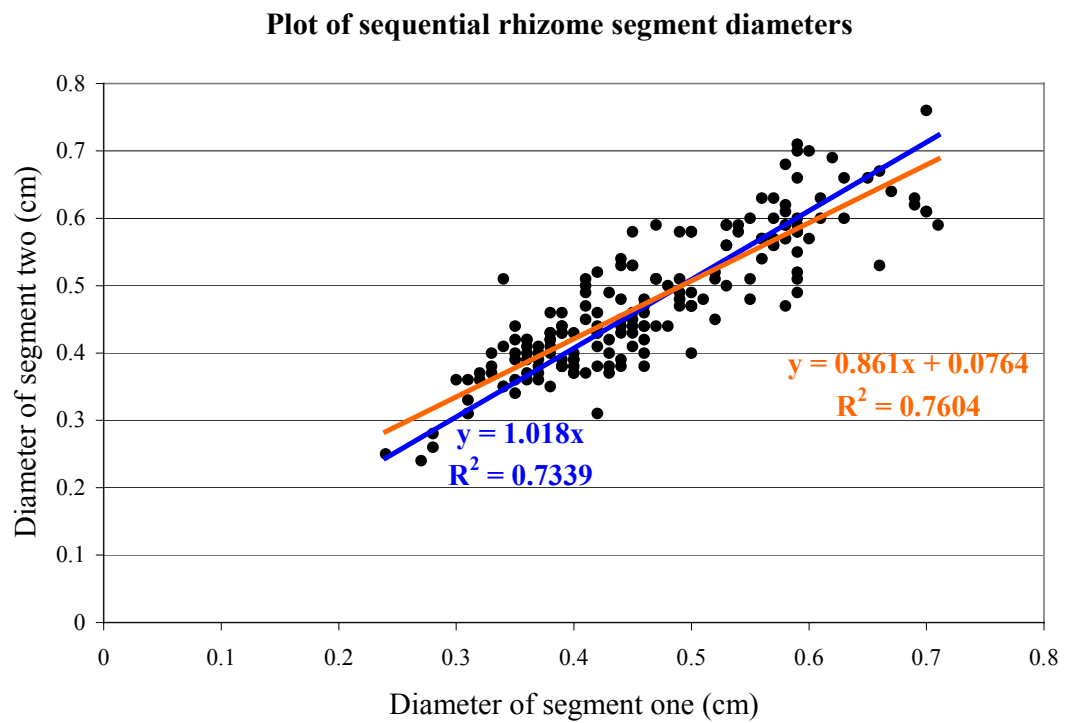
Plot of sequential rhizome segment diameters

Figure 9. Chart of average rhizome segment diameter (cm). The first segment in a sequence is plotted against diameter of adjacent rhizome segment. Regression lines are included, blue line is forced through the origin.

The volume of a rhizome segment is a product of the length and diameter of a segment (Summation from 1 to n (pi*R^2*Length of segment n), where n= the number of scale leaf scars on the rhizome). The two variables used in the volume computation had very different R^2 values. The R^2 for length was 0.25, and diameter was 0.76. The low R^2 of sequential lengths could have affected the relationship between sequential segment volumes. Figure 10 shows that the linear relationship of sequential volumes was only mildly affected by length (R^2=0.70). This could be an indication that consistent rhizome volume is important to plant survival. The plant may be adapting diameter to compensate for changes in segment length. In summary sequential rhizome segments have similar structural properties primarily due to consistency in diameter.

Determining the relationship of above ground structure and dynamics to rhizome structure was an important element of this study. Table 25 summarized rhizome length in relation to above-ground measures, Table 26 summarizes rhizome diameter, and Table 27 rhizome volume. In general the relationships are not strong. For instance, total leaf area connected to a short shoot (cm^2) explains only 16% of the variance in rhizome length and 10% of rhizome diameter. It is notable how leaf area added per day (cm^2/day) relates to rhizome volume (cm^3) (Table 27). Table 27 presents the relationship between these two variables as both a linear and a power function. The rhizome radius is squared in the computation of volume so it is most appropriate to use the power function in explaining the relationship. However, the linear relationship is the more powerful of the two methods. In summary, the leaf area added per day explains 47% of the variance in the volume of a rhizome segment. A graphic display of this relationship is included in Appendix B.
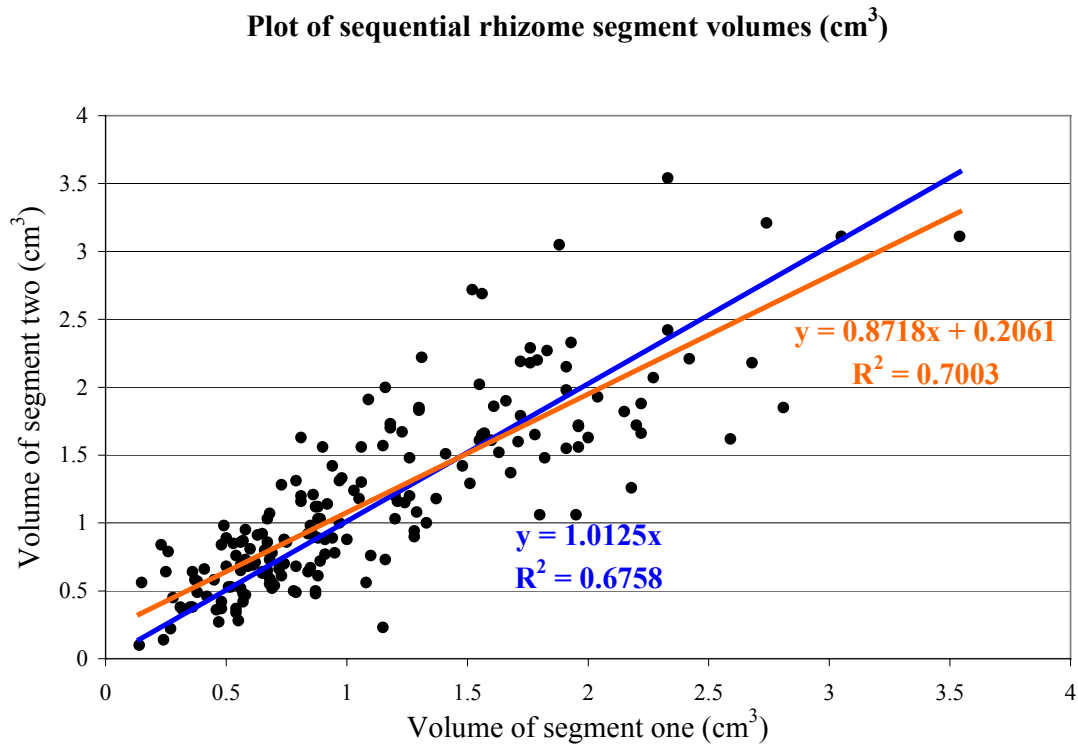
Plot of sequential rhizome segment volumes (cm^3)

Figure 10. Chart of rhizome volume (cm^3) for first segment in a sequence plotted against the volume of the next segment in the sequence. Blue regression line is forced through the origin.

Table 25. Linear relationships of rhizome length (cm) to total leaf area, green leaf area, lower leaf area (cm^2), and leaf area added per day (cm^2/day).

Independent variable: Rhizome length (cm)				
Dependent Measure	R^2	Degrees of freedom	F	Significance
Total leaf area (cm^2)	0.17	109	22.00	.00
Green leaf area (cm^2)	0.17	109	21.73	.00
Lower leaf area (cm^2)	0.16	109	20.04	.00
Leaf area added per day (cm^2 day^{-1})	0.13	109	16.60	.00

Table 26. Linear relationships of rhizome diameter (cm) to total leaf area, green leaf area, lower leaf area (cm^2), and leaf area added per day (cm^2/day). Coefficients are also included.

Independent variable: Rhizome diameter (cm)				
Dependent Measure	R^2	Degrees of freedom	F	Significance
Total leaf area (cm^2)	0.10	109	12.67	.00
Green leaf area (cm^2)	0.11	109	13.11	.00
Lower leaf area (cm^2)	0.09	109	10.08	.00
Leaf area added per day (cm^2 day^{-1})	0.33	109	52.40	.00

Table 27. Curve estimation (Linear and Power) of dependent rhizome volume (cm^3) against independents total leaf area, green leaf area, lower leaf area (cm^2), and leaf area added per day (cm^2/day).

Independent variable: Rhizome volume (cm^3)				
Dependent measure: Linear	R^2	Degrees of freedom	F	Significance
Total leaf area (cm^2)	0.25	109	36.00	.00
Green leaf area (cm^2)	0.26	111	38.21	.00
Lower leaf area (cm^2)	0.21	109	28.29	.00
Leaf area added per day (cm^2 day^{-1})	0.47	123	107.85	.00
Dependent measure: Power function	R^2	Degrees of freedom	F	Significance
Total leaf area (cm^2)	0.27	109	40.61	.00
Green leaf area (cm^2)	0.28	111	42.86	.00
Lower leaf area (cm^2)	0.23	109	32.71	.00
Leaf area added per day (cm^2 day^{-1})	0.45	123	99.41	.00

Rhizome volume is a product of the length and diameter, therefore it reliably describes the structure of a segment. Productivity requires quantification of weight   The data from this study suggest that the best predictor of rhizome segment weight is segment volume (Figure 11).   With 28% of the variance in this relationship unexplained, it appears there is a fluctuating weight to volume ratio. In this data set leaf area added per day had a lower capacity (R^2=0.33) to predict rhizome segment weight (Figure 12).

Length of the rhizome segment was not strongly related to of dry weight biomass. This is due to the variability of the diameter and the density of the rhizome segment. When the measurement scale is increased to multiple segments (a mini-runner), length becomes a better predictor of weight. Because they have leaf scars, short shoots attached to the mini-runner are periodic indicators of the age of the rhizome segemts. There was a strong linear relationship (R^2=0.69) between the age of a short shoot and distance to the apical meristem (Figure 13). A power function (R^2=0.79) accounted for more of the variance than the linear function. The extraction methodology may have introduced a ceiling effect. However, if these data are free from bias, the power function would suggest that the leaf plastochrone interval becomes longer over time. The premise in this interpretation is that the rhizome segment is not continually elongating. Once a new short shoot is established, the position of the short shoot defines the length of the segment. This study found no evidence that segments continue to elongate as they age. In summary, a short shoot younger than 30 scars offers a good estimate of runner length.

Rhizome Segment Weight Plotted Against Volume
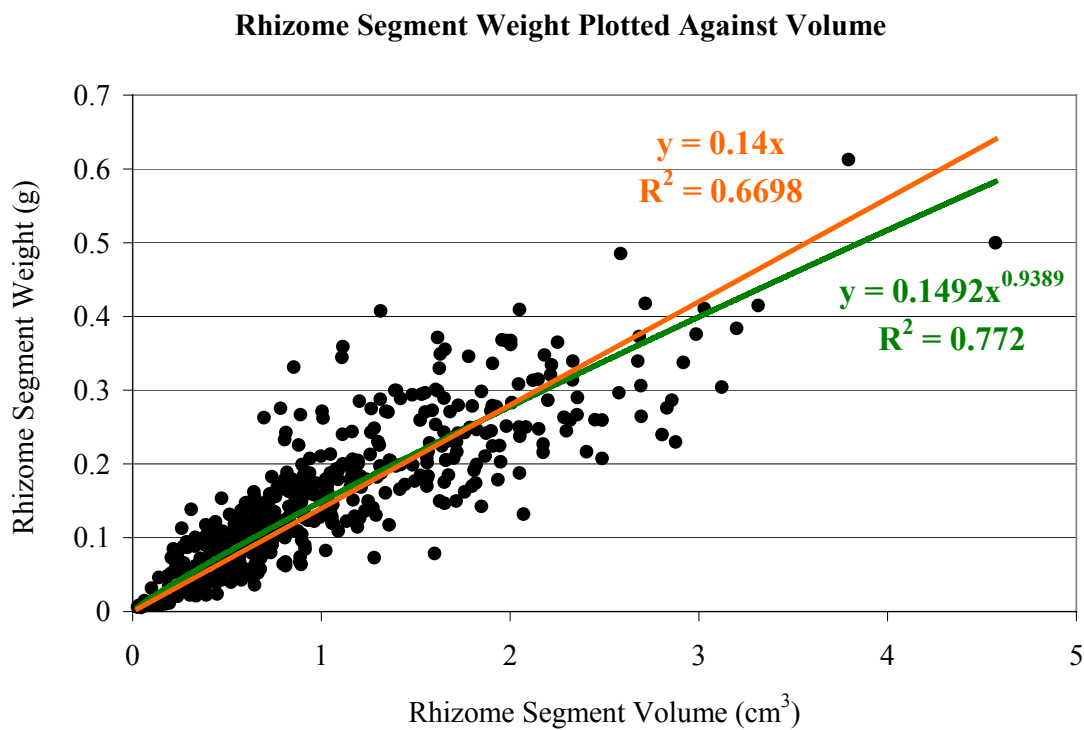
Figure 11. Chart of rhizome segment weight (g) plotted against rhizome segment volume
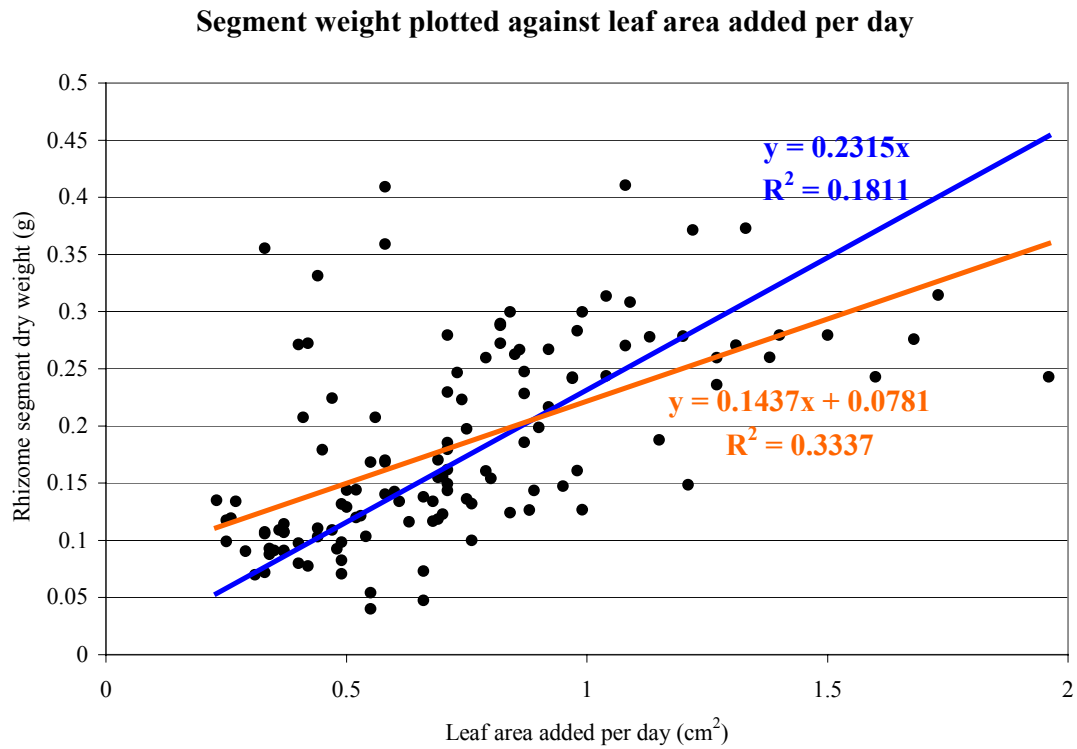(cm3). Trend lines for linear (orange) and power function (green) are included.

Figure 12. Chart of rhizome weight (g) plotted against leaf area added per day (cm^2/day). Trend lines for linear regression included. Blue regression line is forced through the origin.
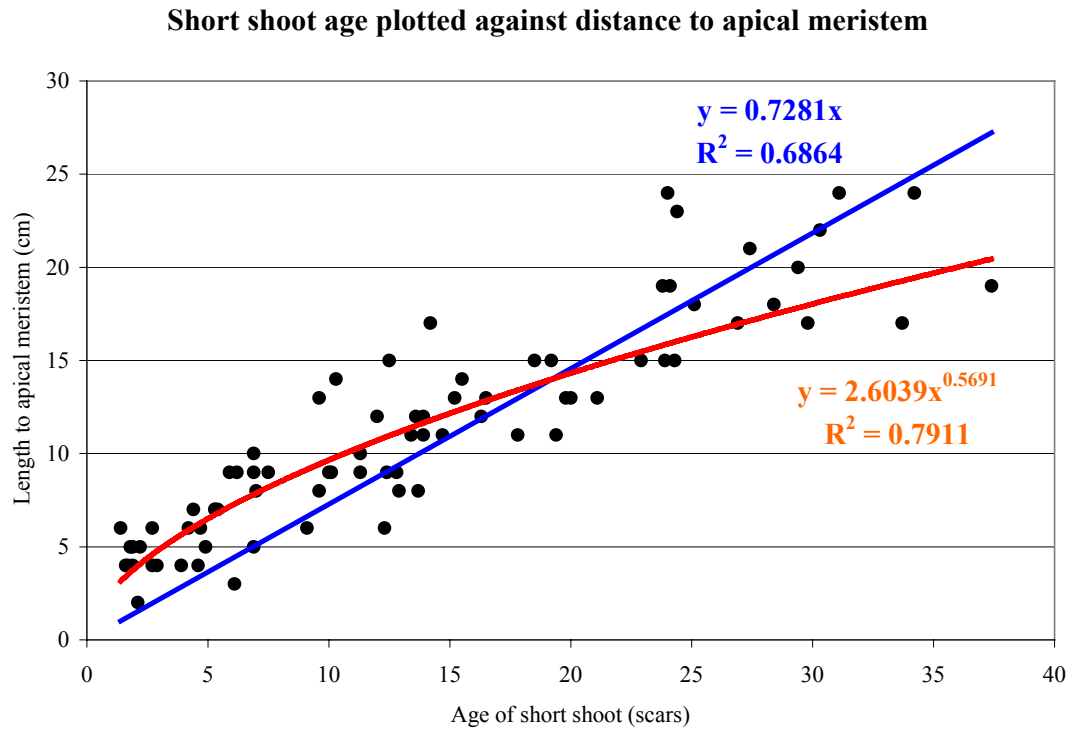
Short shoot age plotted against distance to apical meristem

Figure 13. Chart of age of short shoot (scars) plotted against length to apical meristem (cm). A linear function (blue line) and a power function (orange line) are included in the chart.

Figure 14 shows all the cases where four or more short shoots were present on an individual mini-runner. The regression lines in this figure show the individual growth slopes. The slopes vary from 0.78 cm/scar, to 1.69 cm/scar. The plants do vary in their individual elongation rates, but with the R^2 values near or above 0.90, these cases are all displaying near steady state growth.

Figure 11 showed that volume is the best predictor of weight. Converting a rhizome elongation rate to volume added rate requires quantification of segment diameter. One solution is to use mean diameter in the estimate, but significant error would be expected. Another option is to use above ground measures to estimate rhizome diameter. Leaf area added per day describes 31% of the variance in the associated rhizome diameter (Table 28). In a multiple regression leaf area added per day and the diameter of the short shoot describe 41% of the variance. The coefficients for this model are included in Table 29. The equation to estimate rhizome diameter (Equation 2.1) is calibrated specifically for Rabbit Key Basin.

Rhizome Diameter (cm) = 0.18+.12*LAA+0.49*SS Diameter (cm)   Equation 2.1

LAA=leaf area added per day   *SS*=short shoot

Finally, estimation of rhizome productivity requires a time interval. This interval is the period of time between the initiation of successive short shoots. Leaf area added per day accounts for 40% of the variance in this interval (Figure 15). The adjusted R^2 for this relationship is 0.39.

Relationship between short shoot age and distance to apical meristem.
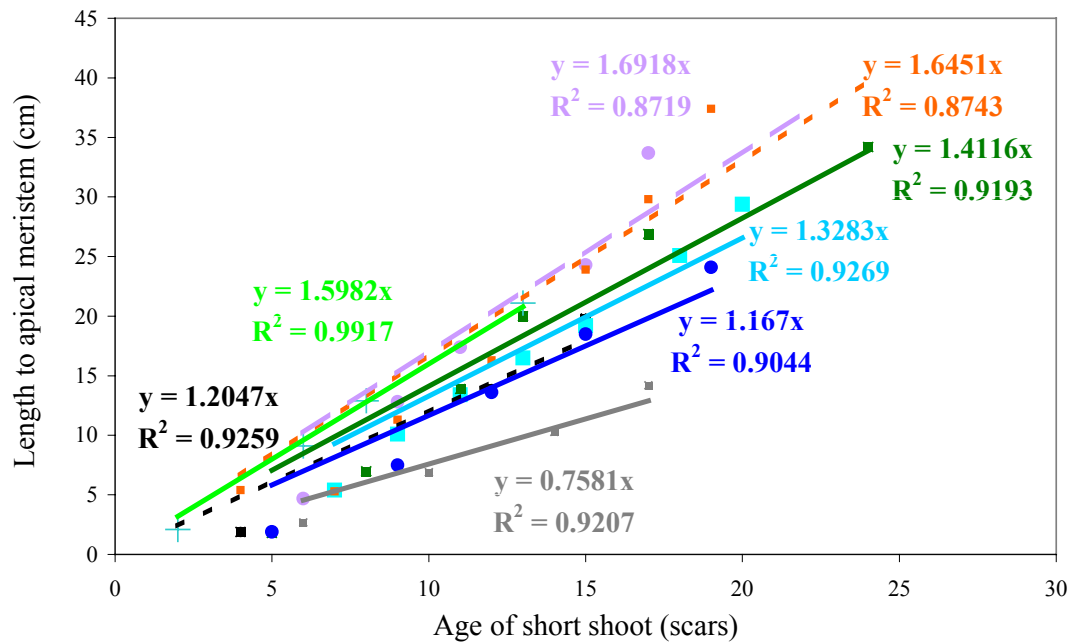Indiviudal growth slope indicated for eight individual mini-runners.

Figure 14. Chart of short shoot age (scars) plotted against length to apical meristem. The regression lines are individual growth slopes for individual plants.

Table 28. Model summary for multiple regression where rhizome diameter was the dependent measure, and leaf area added per day (cm^2/day) and short shoot diameter (cm) are the independent measures.

Multiple regression model	R	R^2	Adjusted R^2	Std. Error of the estimate
Dependent: Rhizome Diameter Independent: Leaf area added per day	0.57	0.32	0.32	0.08
Dependent: Rhizome Diameter Independents: Leaf area added per day and short shoot diameter	0.65	0.41	0.41	0.08

Table 29. Coefficients to be used in estimates of rhizome segment diameter (cm). * is the unstandardized coefficient. ** is the standardized coefficient.

	*		**		
Multiple regression model Dependent: Rhizome diameter	B	Std.Error	Beta	t	Sig.
Constant	0.34	0.02		19.61	0.00
Leaf area added per day (cm^2/day)	0.17	0.02	0.57	7.64	0.00
Constant	0.18	0.04		4.49	0.00
Leaf area added per day (cm^2/day)	0.12	0.02	0.40	5.02	0.00
Short shoot diameter (cm)	0.49	0.11	0.35	4.38	0.00

Number of days between short shoots plotted against leaf area added per day

$y = -63.345x + 148.8$
$R^2 = 0.404$
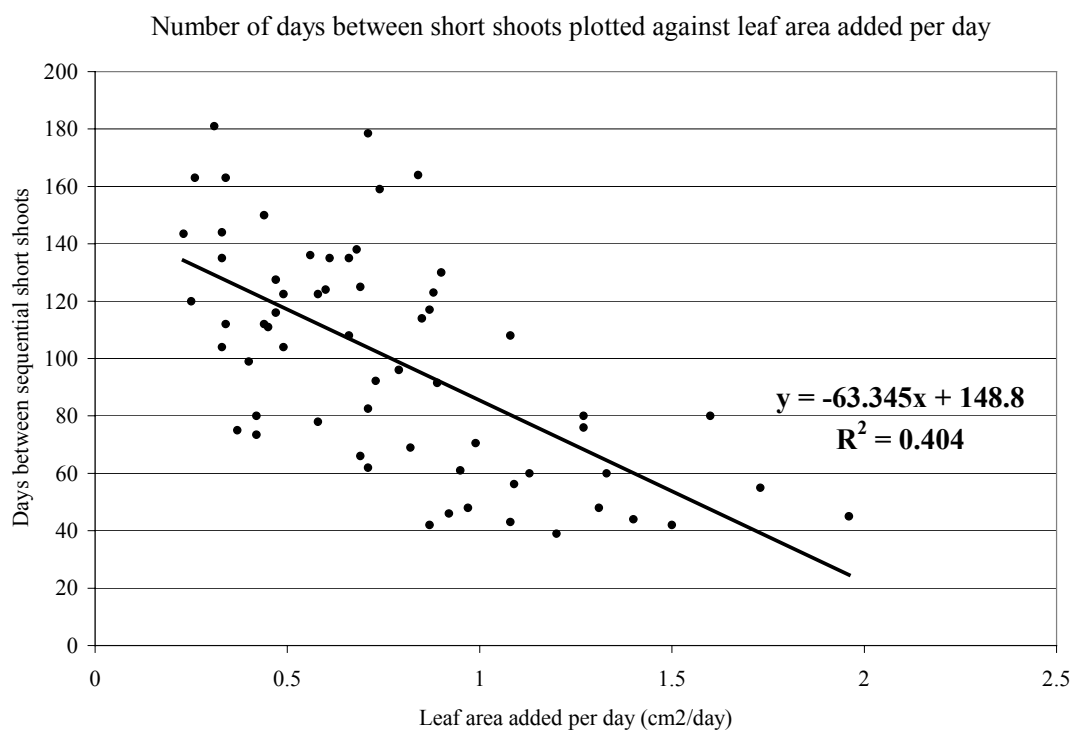
Figure 15. Chart of leaf area added per day (cm2/day) plotted against the estimated number of days between short shoots.

One of the questions addressed in this study was how the sediment depth of the plant affects the structure. Sediment depth is measure of the z vector in an x, y, z coordinate system. Depth is an indication of distance below the sediment surface, therefore, values will be indicated using the label –z. The analyses showed weak relationships between structure and depth of the plant (Table 30). The strongest relationship (R^2=0.14) was depth verses rhizome segment length (Figure 16).

There was unexpected variability in the correspondence between rhizome segment weight and volume (Figure 17), suggesting a fluctuating mass density for individual segment. Analyses determined that the apical meristem segments had different weight to volume ratio than older segments. Figure 17 shows two trend lines, one for apical meristem segments and another for all other cases. The difference in the two functions suggests that apical meristem segments have a lower dry weight per unit volume than older segments.

To better understand the changing weight to volume ratio, segments were analyzed for percentages of carbon, nitrogen, sulfur and phosphorus. The data show that carbon was the most abundant of these elements (Table 31). Nitrogen was present in much smaller percentages. The percentage of sulfur increased with the age of the tissue. Percentages of carbon remained the most consistent of the elements. Nitrogen percentage declined with age and was primarily responsible for the change in C:N. Nitrogen amounted to 2% of the total elemental percentage of the apical meristem segments. The percentages consistently drop until approximately the fifth segments where it comprises about 1% of the tissue sample. Phosphorus declined along the length of the rhizome runner.

Table 30. Adjusted R^2 values for depth vs. length (cm), diameter (cm), and volume (cm^3).

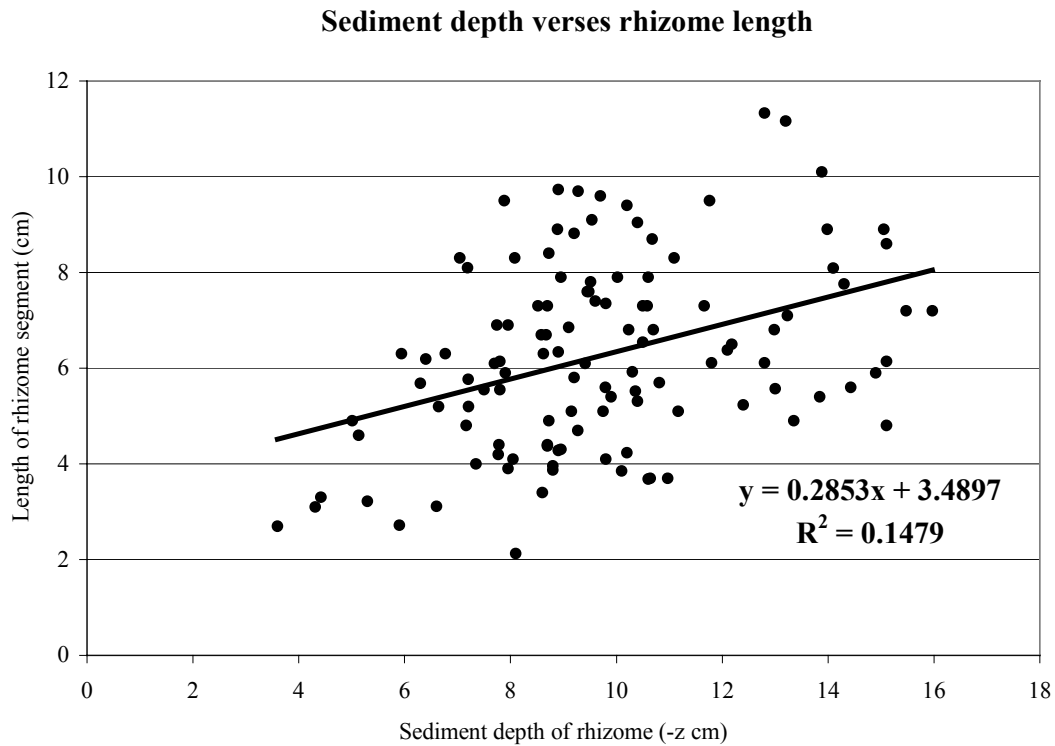| Measure	R^2
Depth (cm) vs. Length (cm)	0.14
Depth (cm) vs. Diameter (cm)	0.00
Depth (cm) vs. Volume (cm^3)	0.05

Sediment depth verses rhizome length

Figure 16. Chart of average rhizome segment length (cm) plotted against rhizome depth
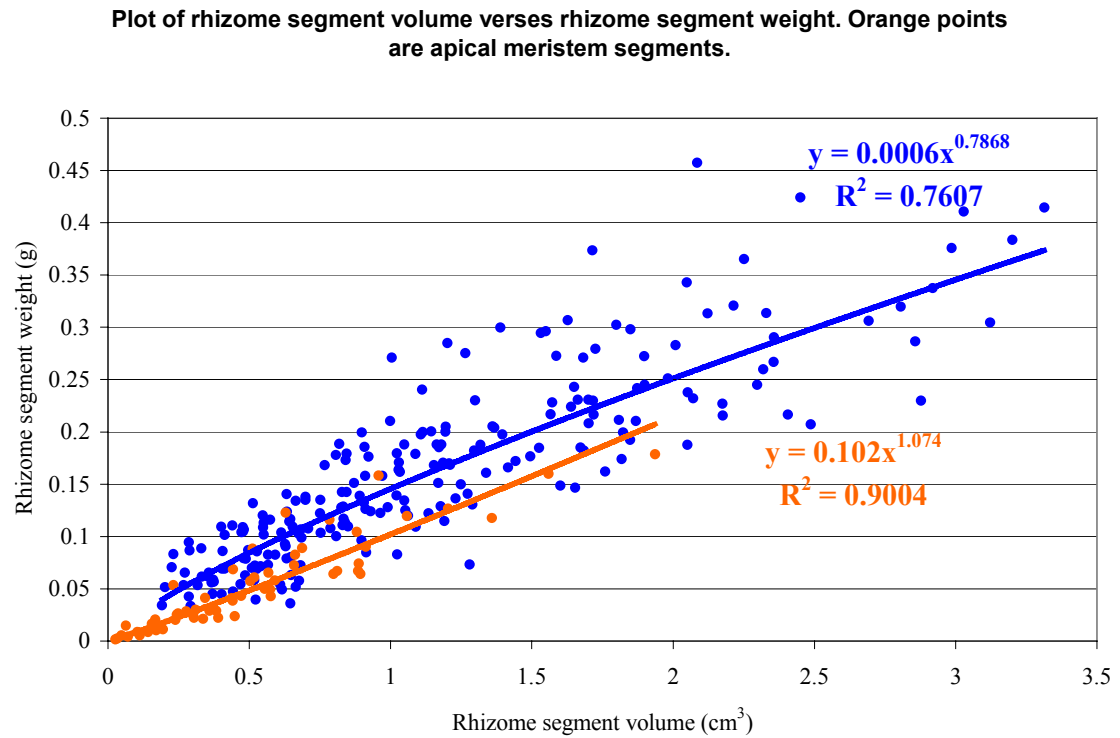
in the sediment (cm below the sediment surface).

Plot of rhizome segment volume verses rhizome segment weight. Orange points are apical meristem segments.

$y = 0.0006x^{0.7868}$

$R^2 = 0.7607$

$y = 0.102x^{1.074}$

$R^2 = 0.9004$

Rhizome segment weight (g)

Rhizome segment volume (cm^3)

Figure 17. Chart of rhizome weight plotted against rhizome segment volume. Trend lines for groups are included. Group one (orange) includes those segments from the first short shoot to the apical meristem. Group two (blue) are all rhizome segments older than the first short shoot.

Table 31. Table of percentages of nitrogen, carbon, phosphorus, and sulfur in rhizome

tissue. N=nitrogen, C=carbon, P=phosphorus, and S=sulfur.

Segment or tissue type	N % and (n)	N standard deviation	C % and (n)	C standard deviation	P % and (n)	P standard deviation	S % and (n)	S std dev
Apical meristem	2.02% (36)	0.31%	38.40% (36)	1.83%	0.17% (4)	0.02	0.00% (8)	
Segment 2	1.41% (36)	0.30%	39.37% (36)	1.14%	0.13% (4)	0.01	0.00% (8)	
Segment 3	1.50% (33)	0.26%	38.03 (33)	2.03%	0.11% (4)	0.01	0.00% (7)	
Segment 4	1.39% (20)	0.16%	37.23 (20)	1.05%			0.00% (7)	
Segment 5	1.11% (17)	0.12%	37.16% (17)	1.28%			0.0% (6)	
Segment 6	0.96% (16)	.03%	38.56% (16)	1.19%			0.34% (6)	0.17 %
Segment 7	0.98% (7)	.02%	38.91% 7)	0.31%			0.65% (6)	0.31 %
Segment not known –old rhizome tissue					0.04% 3	0.009%	1.18% (11)	0.15 %
Dead Rhziome							4.16% na (one overall sample)	0.59 %

Sulfur was not present in young rhizome tissue. The analyses found that the presence of sulfur wasn't consistent throughout the population, with some plants showing a sulfur signal much earlier than other plants in the study. By the sixth segment most samples showed a low percentage of sulfur. In dead tissue the percentage was approximately 4.1%, of the sample but much of this tissue was the outer husk of the rhizome.

Another factor affecting the weight to volume ratio is the amount of organic elements in the rhizome tissue. Table 32 shows the percentages of organic materials measured by combusting rhizome segments. The apical meristem segments have a higher average percentage of organics, but the standard deviation shows that the distribution was wider than older segments. The length of the apical meristem segment was not controlled in this analysis. Samples with higher organic percentages may represent shorter and newer segments. The analysis for older rhizome segments showed a less disperse distribution. Organics appear to constitute approximately 90% of the rhizome tissue.

Changes in rhizome structure associated with age provide evidence of increasing and then decreasing oscillation of biomass over time. This oscillation may be associated with changes in biological function. Table 33 contains data on the change in rhizome diameter with age. The diameter of a rhizome increases between the ages of 10 to 20 scars. The trend reverses in the interval between 40 and 60 scars. The length of a rhizome segment does not change with age.

Comparing the estimates from this investigation to established methods (Table 34) showed that the methodology developed by this study underestimates the productivity estimates generated using Patriquin's method (1975). The process for determining estimates is outlined in Appendix C. The estimated mean difference in number of scars

Table 32. Percentages of organic material in rhizome tissue.

Tissue Type	Average percent of organic material	Standard Deviation	N
Apical Meristem	91.9%	2.9%	11
General Rhizome	89.4%	1.4%	19

Table 33. Mean segment diameter (cm) changes with age (scars).

Age group	Mean	n	Std. deviation
Under 10 scars old	0.44	68	0.07
11 – 20 scars old	0.48	113	0.09
21 – 40 scars old	0.49	92	0.12
41 – 60 scars old	0.48	39	0.10
Over 60 scars old	0.41	17	0.11

Table 34. Data relating to productivity estimates from both the Patriquin and the methods developed by this study.

	n	Mean	Stand Dev.	Minimum	Maximum
Average rhizome segment weigh (g)	119	0.19	0.08	0.05	0.41
Days Between successive short shoots (d)	72	102.35	40.43	39	183
Age difference in scars between short shoots (scars)	72	3.86	1.36	2	8
Volume estimates (cm^3) from this study	122	1.04	0.40	0.40	2.39
Weight estimates (g) from this study	122	0.15	0.06	0.06	0.34
Estimate of days between short shoots from this study (d)	122	102.10	21.60	24.77	134.05
Rhizome productivity (mg/day) from this study	122	1.81	1.63	0.47	13.63
Patriquin rhizome productivity estimates (mg/day) using the same data	72	2.51	2.04	0.35	9.55

between successive short shoots was 3.8 scars. This value would be used in both methods. The averages in Table 34 are for a single rhizome segment. Since values were so small, weight was converted from grams to milligrams. In summary, the method from this study underestimates Patriquin's method by 0.7 mg per day. This would result in an under estimation of Patriquin's method of 0.9 grams per day per meter^2. Both methods have sizable standard deviations.

Section two discussion

Establishing the link between above-ground dynamics and static below-ground measures is dependent on documenting regularities of plant structure. In the simplest case the plant produces above and below-ground biomass with predictable regularity. The ratio of above to below-ground biomass would be equally predictable in this simplest of cases. The most complicated scenario would result from irregular patterns of growth. This would force any model of productivity to account for changeable and difficult to observe antecedent events that produce the irregularities. The data from this study provide evidence of regularities and predictability that will help develop a conceptual model of below-ground production in *Thalassia testudinum*. However, the data also have shown irregularities that will affect the robustness of the model. These irregularities concentrate the direction of future research because accounting for these irregularities will lead to a more effective model.

One of the best ways to assess the regularity of the plant was to examine differences in the length of sequential rhizome segments. This within subject comparison provides important information about the population as a whole. For example, the data suggest that this population is not increasing the rhizome segment length with age. When the plants are examined individually, there were small-scale irregularities, which means the plant may be displaying a phenotypic plasticity. Resource availability may be affecting the timing of when a short shoot is produced. The degree to which the plant is displaying deterministic growth verses environmental response is central to this discussion. Evidence for the interaction between internal and external growth control was

detected in examination of the three sequential rhizome segments (Figure 8). A change

in sequential rhizome segment length provided evidence of the influence of external

controls on growth. Evidence of genetic influences were seen in the cases where the first

and the third segments are almost identical lengths. Not enough data was collected to

suggest whether this is a regular pattern of growth for the plant, or whether these were

one time adjustments in segment length. Appendix D contains a chart where

approximately 15% of the cases presented in Figure 7 were removed. These cases

represent the points that most adversely affect the least squares equations. When the other

85% of the population is examined the value for R^2 jumps from 0.27 up to 0.69 in the

sequential length comparison. With a majority of the population displaying structural

regularities and a smaller percent displaying the long-short-long regularity, the

conclusion is that sequential rhizome segments display an overall structural regularity.

The diameter of sequential rhizome segment displayed regularity. Consistency in

diameter positively affected the regularity of sequential rhizome segment volume.

Sequential segment lengths were variable, and it was possible that this variability could

have significantly weakened the sequential segment volume relationship. It appears that

there may be an interaction between length and diameter in the rhizome segment.

Section one noted that the average length of the rhizome segment increased between

year-one and year-two of the study. However, there was a corresponding decrease in the

diameter of the rhizome segments. Thus, the average segment volume did not differ

statistically over the two years of the study. It was also noted that within individual

plants, diameter change appeared to correspond with changes in length resulting in

consistent sequential segment volumes. An interaction between length and diameter

would suggest that storage of resources is a major feature in the survival of this species. It could be hypothesized that if rhizome segment length is shortened to exploit a patch of resources, diameter is increased to provide an enhanced storage capacity.

One of the questions this study addressed was how above ground standing crop related to below-ground structure. The amount of leaf area attached to a short shoot provides limited ability to describe the below-ground structure (Table 25). Mindful of those results, the next step was to look at the productive capacity of the short shoot. This capacity was measured as the amount of leaf area the short shoot was adding per day. The data suggest that this dynamic measure is the best proxy for describing the below-ground structure of the *Thalassia testudinum* plant. The data presented in Table 27 provide an early indication that above ground dynamics might be a good proxy for below-ground measures.

It is important to note that choosing either leaf area added, or rhizome volume as the dependent measure may be misleading. The two variables are related, and there is a significant problem in determining which is the actual outcome measure. Leaf area added may be a function of the rhizome volume and the capacity of the plant to provide resources. On the other hand, rhizome volume may be responsive to resource flow from the short shoot. Finally, genetics could control both rhizome volume and short shoot production. If the system is considered as a whole, leaf area added per day and rhizome volume may be linked as multiple dependent variables, altering as a function of genetics, nutrient availability, light attenuation, water depth, and a number of other factors. The ability to successfully account for the individual influence and the interaction of these factors will improve the ability to quantify below-ground production.

The interaction between deterministic growth and phenotypic plasticity is difficult to study. A great deal of control is required to isolate the influences that affect this interaction. Propagation of *Thalassia testudinum,* as described in this study, is asexual. Short Shoots on the same rhizome are presumed to be genetically identical. Genetics regulate a certain control over the leaf structure and productive capacity of a short shoot. Factors controlling clonal integration, such as resource storage and transport, might also be under the same genetic control. If genetics completely regulated this species, the patterns observed in this investigation would have been expected to be more structured. In contrast, a purely phenotypic response resulting from changes in resource availability would have shown a less ordered pattern. An exception might occur if the ecosystem at Rabbit Key Basin had a resource base that was static and homogenized. Assuming resource patchiness, a variability in structure might be expected if the plant response was entirely phenotypic. These small scale structural modifications would likely result in a commensurate rise or fall in the need for resource storage. In other words, the plant would need to adjust structure locally to compensate for the changing metabolic demand of the short shoots along a runner. If phenotypic response was the primary factor controlling plant structure, the patterns observed likely would have been more random. Very little published research is available on this issue, and almost none as it pertains to seagrass research.

Whether genetics or environment factors control the mechanisms, resource flow and storage appear to be important to the survival of this plant. Meeting the metabolic demand of non photosynthetic tissue can be thought of as a pipe flow problem. Satisfying the metabolic demands of the entire plant during resource limitation can be seen as a

storage capacity problem. In either case, a mechanistic model where leaf area added per day is related to below-ground structure is valid. In summary, though not causal, the data collected in the course of this study do suggest a link between the productive capacity of a short shoot and the associated below-ground structure. That determination is important to this investigation.

One of the goals of this study was the applied use of above-ground data to predict below-ground biomass. The correspondence between the static measure of leaf area and the dynamic measure of leaf area added per day was high, yet these two variables had different capacities to predict the below-ground structure. Logistically, measuring leaf area is a simpler task. The researcher (or below-ground predictor) selects a site, measures short shoot leaves, and uses those observations to predict below-ground structure. Because leaf area was only moderately correlated to rhizome volume there is likely to be significant error associated with an estimate. In the case of leaf area added per day, this requires a greater effort. The researcher must select and set up a semi-permanent site. The short shoots must be tagged and the leaves marked. After an appropriate period of time the site must be revisited and growth measured. The leaf area added per day can be computed and that valued can be used to predict volume. In this the correspondence between leaf area added per day and rhizome volume is higher, so this would yield more accurate estimates of below-ground structure. A step by step discussion of this method can be found in Appendix E.

Another method for estimating productivity would utilize the relationship between the age of the short shoot and the distance of that short shoot to the apical meristem tip (Figure 13). This relationship had a strong linear relationship and power

function. When individual cases were analyzed the R^2 values often exceeded 0.90, though the growth slopes for individual plants were variable (Figure 14). The missing element in being able to determine volume is the diameter of the rhizome. This study was unable to uncover a good above-ground predictor for rhizome diameter. Leaf area added per day displayed a moderately good capacity to predict rhizome diameter. Leaf area added per day also could be used to predict the leaf plastochrone interval. In practice resolution and accuracy would increase if leaf plastochrone interval was measured directly. Volume can be used to estimate weight while leaf plastochrone interval and scars can be used to estimate the time interval. The results of those two estimates are used to compute the rhizome productivity. The findings at Rabbit Key Basin would suggest that if the equations were calibrated based on site specific plant structure, this model is able to exploit relationships between dynamic and static variables to construct a rhizome productivity estimate. There are no published papers that have addressed this approach.

The observed population variance clearly affects the precision of the rhizome productivity estimates. Factors that could possibly affect below-ground structure have to be examined with the goal of reducing the unexplained variance. One of the first considerations is depth of the rhizome. The results in Table 30 showed that there was a weak, non-significant relationship between rhizome segment volume and depth in the sediment. Depth was a stronger predictor ($R^2=.15$) of rhizome segment length (Figure 16). The core data presented in section one gave evidence of a well-defined depth limit of the rhizoshpere. Perhaps this is a result of the sediment chemistry profile. The density of the rhizome matrix, suggests that physical space in the sediment is a competitive resource. The sediment-water column boundary imposes one barrier for growth. Lower

sediment depth conditions appear to impose another. This might explain why sediment depth is not a good predictor of rhizome structure. The growth zone might be too thin to select for, or give an advantage to plants with a given volume, diameter or length. Standard residual analyses found no correlations between the variables and depth.

Sediment characteristics vary throughout Florida Bay (Fourqurean & Zieman, 1992). Differences in the rhizome distribution may correspond with sediment changes. If the factor or factors controlling the depth gradient in Rabbit Key Basin were removed, then the depth of the rhizosphere could be extended deeper into the sediment. In such a case, the metabolic demand of the plant could become more of a controlling factor. In an attempt to account for the unexplained variance, data from a subset of the population was analyzed. All cases that had a high leaf area added per day and a low rhizome volume were selected for individual analysis. These cases were selected to determine their distribution in the sediment depth continuum. A similar analysis selecting for cases with low leaf area added per day and high segment volume was also conducted. The results from both analyses showed the same general distribution as the rest of the population. A systematic decrease in rhizome volume due to depth could have been a sign of a higher metabolic demand associated with higher below-ground biomass. A higher biomass corresponds to higher respiration. Higher respiration affects the plant's ability to store resources at the same rate as those plants near the sediment surface. The data did not support this hypothesis, but as stated, the rhizosphere may be too narrow to affect changes in structure.

Measurement of sediment chemistry was not included in this study. However, future research would benefit from documenting a sediment profile. The physiological

structure of *Thalassia* is adapted to respond to changes in sediment chemistry. The results in this study provided evidence about the changing capacity of gaseous transport. The evidence is circumstantial, but the change in the density of the apical meristem segments could be related to this issue. The apical meristem segments had a lower weight to volume ratio than older rhizome segments (Figure 17). This density difference could be a result of greater gaseous space in the apical meristem segments. This trend could be associated with a changing capacity to move gases within the plant. Here again a model can be suggested that would explain the relationship of leaf area added per day to rhizome volume. This species meets some of the nutrient demand through sediment intake. The energetic return for some nutrients varies with the oxidation state.

Highly productive plants are going to need to move oxygen at greater rates than plants with lower production. This can be conceived as a pipe flow problem. The rhizome has several functions which need to be integrated into a complete system. Certain percentages of the rhizome volume are dedicated to the various functions, gaseous transport being one of those functions. The same pipe flow argument might apply as the plant works to reduce sulfide toxicity. Newer segments might be susceptible to damage from sulfide toxicity. The larger the volume of the rhizome segment, the greater the area of exposure to the sediment.

Establishing the link between above and below-ground dynamics was partially successful in this study. The differing photosynthetic yields of the individual short shoots could account for the lack of an absolute correspondence between above and below-ground dynamics. The fact that photosynthetic yield is not directly expressed in the form of leaf area added per day is a probable source of error in this relationship. This presents

another area of future study needed in order to improve the capacity to estimate below-ground production.

Another possible factor in the relationship between leaf area added per day and rhizome volume could be age of the short shoot. The results showed that age is not a good predictor of rhizome volume. When age and leaf area added per day are used in a multiple regression to predict rhizome segment volume, age has no effect on the equation. But the aging short shoot is an important proxy for age of the rhizome segment. The rhizome goes through a defined pattern of volume change, which might be associated with a change in function.

The results from this study show that the weight to volume ratio of the apical meristem segment is lower than that of segments located behind the first short shoot (Figure 17). This would indicate that in the time interval between the fixing of the rhizomatic tissue and the initiation of the following short shoot, the plant is either adding biomass or losing volume. Flow of resources must occur to sustain meristematic growth as it fixes biomass. After the biomass is fixed, resource flow must be maintained to support metabolic demands of the new rhizome and new short shoot tissue. As a short shoot becomes an energy source rather than an energy sink the plant may begin the process of storing resources which increases biomass. Seasonal changes in level of soluble carbohydrates have been noted (Dawes & Lawrence, 1980) and this suggests that the plant is adding and removing energy to meet the changing needs of the plant.

The variable weight to volume ratio implied changes the elemental make-up of the rhizome tissue. The results in Table 31 show that percentages of carbon stayed steady and then slightly decreased as the rhizome segment aged. In looking at absolute values

for carbon, they changed slightly, but generally followed the trend observed in the percentage data. These analyses did not determine the actual form of the carbon, and this would be an important future study. The carbon to nitrogen ratio increased as rhizome segments got older, but this was due primarily to the diminishing percentage of nitrogen. The high nitrogen levels observed in the early developmental stages is likely due to the nitrogen demand of the meristematic tissue. These processes may be working simultaneously to affect a change in the weight to volume. After the newest short shoot is established, the plant maybe reducing the gaseous volume. There was evidence of other changes in the percentages of elements in the established rhizome tissue. For instance, sulfur increased as the rhizome segment aged, while levels of phosphorus decreased. The phosphorus trend would be expected because this element would be highly correlated to the energy demands associated with fixing biomass. Important as phosphorus is in controlling the distribution of this species (Fourqurean & Zieman 1992), it accounts for less than 0.20% of the tissue sample. It is likely that unmeasured elements such as hydrogen, and oxygen are the constituents that are affecting the changes in biomass and volume. Of the elements studied in this project, carbon is the most abundant. Since the form and presence of carbon is important to understanding the energy balance, future studies will be useful in establishing patterns of change over time. It would be most useful to study the complete suite of elements and establish the presence and variability of the rhizome continuants. This study accounted for approximately 50% of the elemental make up of the rhizome tissue. However, as in the case of phosphorus, less abundant elements may be important to understanding the life cycle of this species. If

predictable patterns of elemental change are observed through time, the processes of productivity and respiration might be better understood.

Short shoot scar age was a plant characteristic that improved the understanding of below-ground dynamics. The age of the short shoot was found to be a good predictor of the distance to the apical meristem. As the short shoot ages, the plant continues its asexual propagation via rhizome elongation and establishment of new short shoots. The data indicated that rhizome growth was in steady state as measured by shoot age. This result means that short shoot scar age can be used to estimate the length to the apical meristem. Determination of the plastochrone interval would allow the elongation to be expressed in days. There is a question of whether the power function described (Figure 13) would provide a more appropriate estimate of length to the apical meristem. If the power function is a more appropriate tool, then its use has implications about the growth pattern of the short shoot. Speculation exists regarding variation in leaf production as a function of age (Hemminga, et al., 1999). As a reminder, Patriquin's (1975) method is based on the assumption that leaf production is in steady state. In the small scale perspective of two sequential short shoots on the same rhizome, his method may not be affected by this assumption. But the data presented in Figure 13 offers some evidence that leaf production is affected by the aging process of the short shoot.

While the primary focus of this section was new growth, long term changes the rhizome segment length, diameter, and volume were included in the study. These long term changes in structure and weight are essential to understanding biomass gain and loss in the rhizome segments.

The analysis of length to age of the rhizome segment used the short shoot to estimate the age of the rhizome tissue.. This analysis compared segments within tightly spaced age groups, making the analysis sensitive to seasonal changes. The results suggested that length of the rhizome segment does not appear to be affected by seasonal factors at Rabbit Key Basin. The data collected also suggest that the length of the segments, once fixed by the initiation of a new short shoot, remain static. If a segment did continue to elongate with age, then a pattern of older and longer segments should have been observed. The data do not support that expectation. In contrast, the diameter of the rhizome segment shows some variability.   The diameter increases associated with early developmental growth were speculated to coincide with the productive capacity of the short shoot. From that point the rhizome diameter appeared to remain static until the rhizome tissue was 50 short shoot scars old. If the leaf plastochrone interval in Rabbit Key Basin is used to estimate age, this would mean the diameter change occurs when the segment is approximately 4 years old. This affects the weight to volume ratio and has implications for the survival strategy of the plant.

When examining weight to volume ratios of the rhizome segments, it was discovered that older segments with smaller diameters had greater biomass per unit volume. This could suggest several patterns of biomass allocation.   Diameter and volume declined after the segment was older than 50 scars. One explanation for the density change, is loss of gaseous space. Movement of resources may be less critical as the plant ages.   Studies establishing the actual transport of resources forward or backwards along the rhizome have been limited. None of these studies have used tracers to determine the exact extent to which *Thalassia testudinum* will transport resources.

Unless the process is passive it can be assumed that the greater the distance of resource transport, the lower the energetic efficiency. This is going to be an important future area of study. Use of tracers may reconcile the energy budget and show the pattern of distribution for the unobservable production in the above-ground portion of the plant.

The time interval between short shoots is a critical component of the rhizome productivity estimate. The estimate of this interval was determined two ways, first by direct observation and second using predictive equation generated from the data from this study. The two time interval estimates were approximately the same. The major difference was that the standard deviation for the direct measurement estimate was twice the size of the standard deviation for the predictive estimate. It is not surprising that these estimates were close because the same time interval data was used to generate both estimates. If these estimates reflect the actual behavior of the system then the plants at Rabbit Key Basin have a widely variant pattern in the number of days between successive short shoots. The leaf PI was carefully measured in this study and the estimates are believed to be reliable. the long-term changes in growth rates of the short shoot were not measured. It is not known whether the age difference in short shoots, as estimated under a steady state assumption, is an accurate estimate, which is a shortcoming of both Patriquin's (1975) method and the method presented in this study.

Patriuin's (1975) method would estimate a rhizome productivity of 0.25 $(g/day)/m^2$. The calculations generated by this project are lower, estimating a value of 0.18 $(g/day)/m^2$. The difference in estimation values when comparing to Patriuin's (1975) method may be due to the unobserved productivity in the short shoot leaves. If photosynthetic yield could be accurately measured to determine levels of energy

designated for leaf respiration and growth as well as energy export, then a correction factor could be applied to the predictive equations. Patriquin's method may also be prone to this type of error.

In summary, above-ground production appears to provide a rough estimate of below-ground rhizome production. Two methods were proposed. The first method uses leaf area added per day to predict volume of a rhizome segment. leaf area added per day also predicts the time interval for production of the segment. Volume was used to estimate weight. Weight and the time interval were used to estimate rhizome productivity. The second method used short shoot age to estimate length of the entire rhizome runner. The diameter of the runner was estimated using leaf area added per day. Diameter and length were used to compute volume; volume used to estimate weight. The time interval was defined by the age of the short shoot. If Patriuin's (1975) method represents an accurate estimate of rhizome productivity, then the methods presented in this paper represent an underestimate. As stated, the error associated with Partriquin's method is not known. All three methods lack direct observation of rhizome productivity. Without that ground truth, the error of all three methods remains in question. The major focus of this study is to understand below-ground structure and productivity in relation to above-ground processes. Unidentified factors affect the patterns observed in these data. Future study and experimentation will help to better understand the factors controlling productivity of the *Thalassia testudinum* rhizome.

Section Three

Roots

Results

Root attachment occurs in two locations on the *Thalassia testudinum* plant, on the rhizome and on the short shoot. In the x-y-z coordinate system of sediment volume, roots were found to grow exclusively along three linear planes. Roots growing out the rhizome were attached to the plant along the axis situated at the bottom of the structure. These roots grew 180 degrees downwards into the z-plane of the sediment volume. Roots attached to the short shoots were found to be growing orthogonal to the direction of the rhizome runner. Generally, these roots were attached to the outer side of the short shoot which corresponds to the position furthest away from the rhizome. Sequential short shoots emerge alternately on opposing sides of the rhizome, and this appears to the mechanism that orients the growth of the roots away from the rhizome runner. A small percentage (3.05%) of short shoots had roots that deviated from this pattern and crossed the plane of rhizome. An even smaller percentage of short shoots (1.21%) had younger roots growing 180 degrees in the opposite direction of the older roots. There was no systematic co-occurrence that predicted this deviation.

The length of a root was strongly correlated to its dry weight (Figure 18). This was true for roots attached to the rhizome and the short shoots.   In a multiple regression with root weight as the independent variable (Table 35), segment length is the first variable entered into the model (R^2=0.87). When root diameter was included in the model there was minimal change (R^2=0.90). The remaining 10% of unexplained variance

Length of root segment verses dry weight of segment
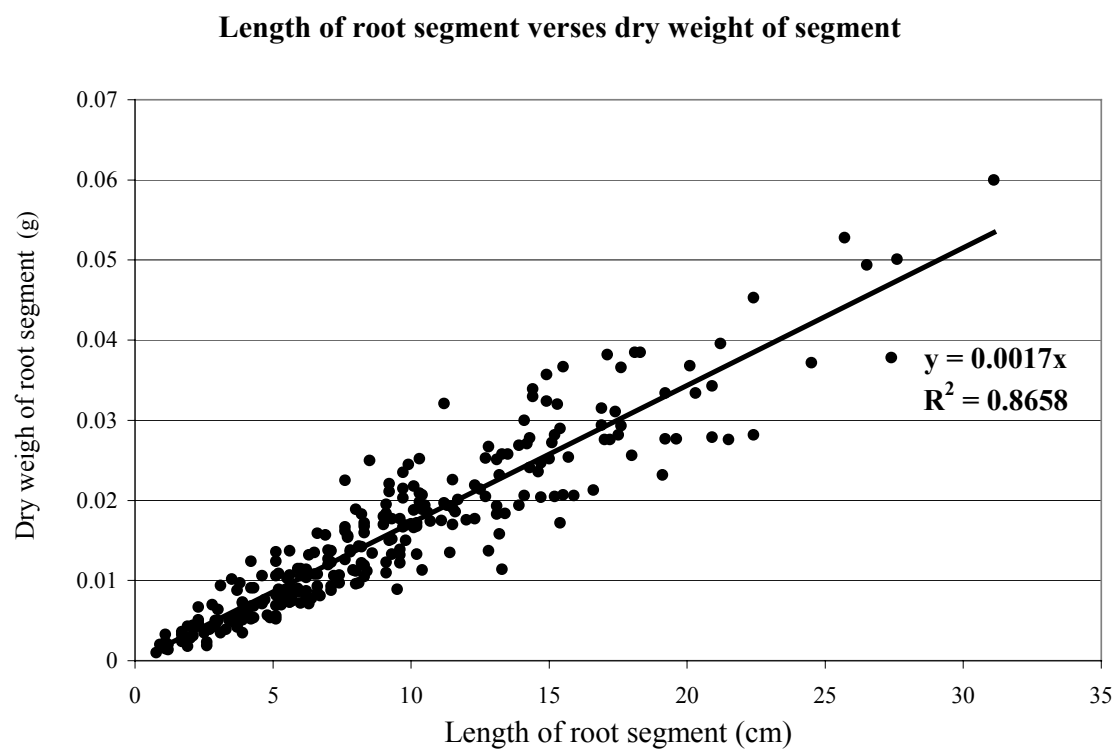
Figure 18. Chart of root segment length (cm) plotted against root segment weight (g).

Table 35. Model summary of multiple regression. Dependent variable is root segment weight (g). Independent variables are root segment length and diameter (cm).

Multiple regression model	R	R^2	Adjusted R^2	Std. Error of the estimate
Dependent: Root segment weight (g) Independent: Root length (cm)	0.93	0.87	0.87	0.004
Dependent: Root segment weight (g) Independents: Root length (cm) and root segment diameter (cm)	0.95	0.90	0.90	0.003

Table 36. Coefficients of the multiple regression where root segment weight (g) is dependent, and root segment length and diameter (cm) are independents. *=unstandardized coefficients, **=standardized coefficnets.

	*		**		
Multiple regression model Dependent: Root segment weight (g)	B	Std. Error	Beta	t	Sig.
Constant	-0.0011	0.00		-1.60	0.11
Root segment length (cm)	0.0016	0.00	0.93	26.81	0.00
Constant	-0.0085	0.00		-6.71	0.00
Root segment length (cm)Short	0.0016	0.00	0.87	28.24	0.00
Root segment diameter	0.0895	0.01	0.20	6.57	0.00

appears to be affected by density of the root segment. The weight per unit of volume

was computed so as to allow a measure of the biomass density for each segment. The

distribution of the root density was scattered more than what was expected (Figure 19).

These data show that the root segments included in this study had a range in density from

0.1 gram per cm^3 up to 0.49 g per cm^3. When weigh to volume was plotted against the

diameter of the root segment, a trend of higher density values associated with lower

diameter roots was observed (Figure 20). This issue will be addressed in greater detail in

the discussion section.

Since root segment diameter has limited predictive value and is difficult to

measure, length was used as the single predictor of weight. As the plots (Figure 18) and

the multiple regression showed (Table 35), length explains 87% of the variance in the

weight of the root segment. Predictive equations for both length and weight (Equation

3.1) will be important to the rest of this results and discussion section.

Equation 3.1

Estimated Value	Equation	R^2
Root segment weigh	Weight (g)=0.0017*length (cm)	0.87
Root segment length	Length (cm) = 557.19*weight(g)	0.87

Roots growing off rhizome

The roots growing off the rhizome appear to be formed early in the development

of the segment. Likely, roots are established by the meristematic tissue, similar to the

process of short shoot initiation. This conclusion is supported by the observation that the

rhizome does not add additional roots as the segment ages. The average number of roots

Weight to volume ratio of root sample (weight per unit of volume)
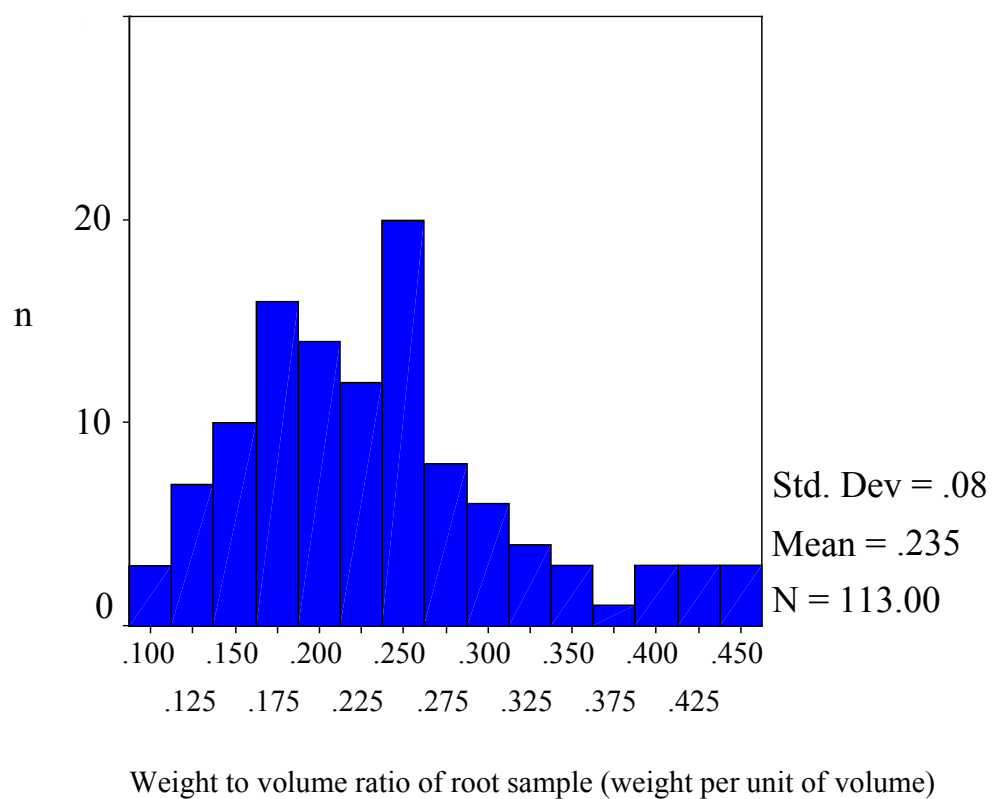
Figure 19. Histogram showing the distribution of weight to volume values. Weight to volume represents tissue density of a root segment. The y-axis represents the number of observations.

Root segment weight to volume ratio verses the diameter of the root segment
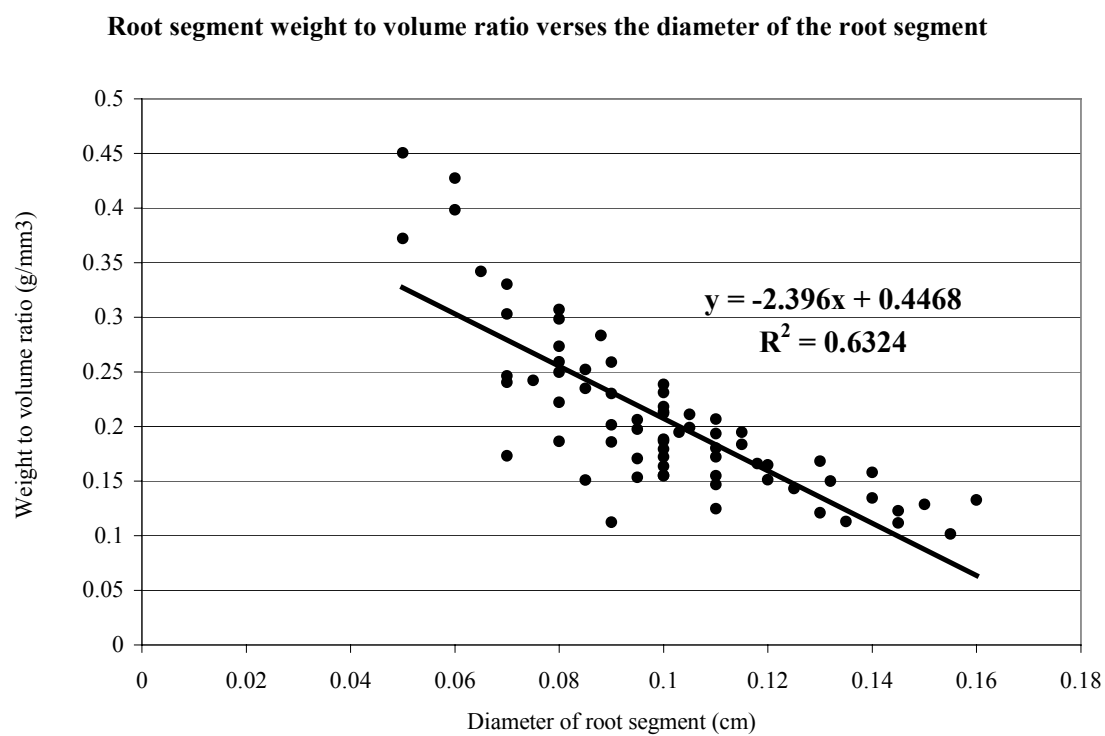
Figure 20. Chart of segment weight to volume (g/cm^3) plotted against root segment diameter (cm).

and root scars stays approximately the same for all age groups in the population (Table 37). Statistically the first group does differ from the other groups, however this group includes new rhizome segments not yet grown to full length. This would mean that these new segments have not yet established all the roots on the segment. There was one other group, 41 to 45 scars old, that differed statically, but the small group number make the group average susceptible to error or fluctuation. This data would suggest that the rhizome does not initiate new roots beyond the early stages of development. If there were roots being initiated beyond this early stage, the average number of roots and root scars would increase with greater age of the rhizome.

Once initiated, the data indicate that root growth from the rhizome continues for an extended period. Recalling the core data (Table 23), live root biomass extended down to approximately –z 65-70 cm. With the lower level of the rhizosphere being located around –z 20 cm. This would indicate a maximum root length of 50-65 cm depending on what part of the rhizosphere the plant was located. The core data show that 0.042 g of live root biomass was located in the lowest zone. The predictive equation (Equation 3.1) estimates that 24 cm of root were alive in the core section located –z 65-70 cm below the sediment surface. Since the core was segmented into 5 cm units, this likely represents four to five roots that grew to this depth. The diameter of the core was 15.29 cm, which represents a surface area of 0.018 m^2. Reviewing the short shoot density at Rabbit Key Basin, it could be expected that 24 rhizome segments would have been extracted with the core. Each segment would be estimated to have 2.19 roots attached. That means 52 roots would be estimated to be growing into the z plane of the sediment core.

Table 37. The average number of roots and root scars on rhizome segments grouped by age.

Age Grouping Variable	Mean	n	Std. Deviation
1 to 5 Short Shoot Scars Old	1.80	5	1.10
6 to 10 Scars	2.21	29	.98
11 to 15 Scars	2.30	27	1.41
16 to 20 Scars	2.57	23	1.34
21 to 25 Scars	2.27	15	1.22
26 to 30 Scars	2.70	10	1.06
31 to 35 Scars	2.00	10	1.15
36 to 40 Scars	2.60	10	1.51
41 to 45 Scars	1.86	6	1.87
46 to 50 Scars	2.50	14	1.23

This represents an estimate that approximately 10% of the roots growing off rhizomes elongated to this depth. The root weight value that was reported for the 65-70 cm depth is derived from a single core. It's noteworthy that five cores were extracted. The other four cores had no observed live root material at that depth. Reviewing the data and extrapolating to all five cores, 265 roots were growing into the –z plane of the sediment. That alters the estimated to a value of 2% rhizome root population that grew to a depth greater than –z 65 cm.

The distribution of roots through the sediment showed a decreasing biomass with sediment depth. The pattern of growth displayed by the study population (Figure 21) would be an indication of steady state root growth. As the population gets older, a greater root mass might be expected to reach the deeper sediment depths. The percentages in Figure 21 were computed using equation 3.1 where root weight was used to predict the length of all roots in each of the core segments. This total length was then divided by the 5 cm core segment length to estimate the number roots that had elongated to the various sediment depths. It was estimated that between the depths of –z 21-70 cm, Rabbit Key Basin has 137.78 g dry weight live root biomass per meter^2. This sediment depth continuum is assumed to specifically represent the roots growing off the rhizome. This is based on the assumption that roots growing off of short shoots do not redirect their growth downwards into the sediment.

The growth rate of the root is a critical component of the production estimate. Like rhizomes, direct observation was not possible. Therefore the rate must be estimated by associating the length of a root with an estimated time step. The live root tissue that was found at a depth of –z 65-70 cm allows for a range of estimates of the growth rate.

Percentage of roots growing off rhizome
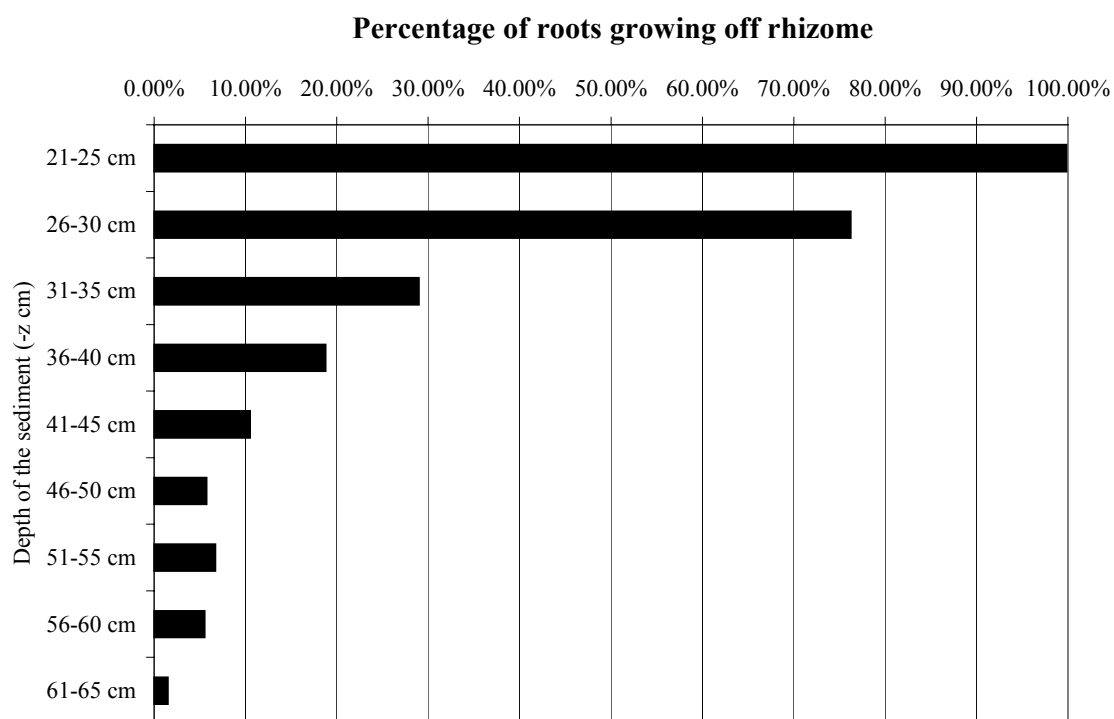
Figure 21. Bar chart of percentage of roots growing off rhizome that penetrate to various depths.

Figure 22 is a chart that contains a series of points that represent possible growth rates of the roots. The unknown value is the age of the attachment point on the rhizome. The X-axis contains theses unknown values. If the root biomass that reached a depth of 65 cm was attached to a rhizome segment that was of the average age for the population, 25.8 scars, the growth rate would be 0.08 cm/day. If the attachment point was one standard deviation older, or 38 scars old, then the growth rate would be 0.06 cm/day. Finally, roots attached to the oldest rhizome observed in the study would have grown at a rate of 0.02 cm/day. These estimates are generated under the assumption of steady state growth that continued up to the time that the core was extracted. The possibility that the root grew to a depth $-z$ 65-70 cm and stopped growing has to be acknowledged. The range of rates can help determine which value is most consistent with the population structure and the estimate of 137.78 g dry weight/m^2 of rhizome root biomass.

Early root development off the rhizome was measured to address the questions whether root growth is steady state. Examination of early root development was necessitated by the fact that the greatest percentage of complete roots were extracted from the youngest rhizome segments. A root was classified as a complete segment only when there was a clearly distinguishable tip and the segment was confirmed to be attached to the rhizome. The most useful samples were obtained from rhizome runners that had two or more complete roots.

The data from this study suggest that the early stages of root growth off the rhizome is steady state. This was determined by studying those rhizome runners that had

Projected rates of growth (cm/day)
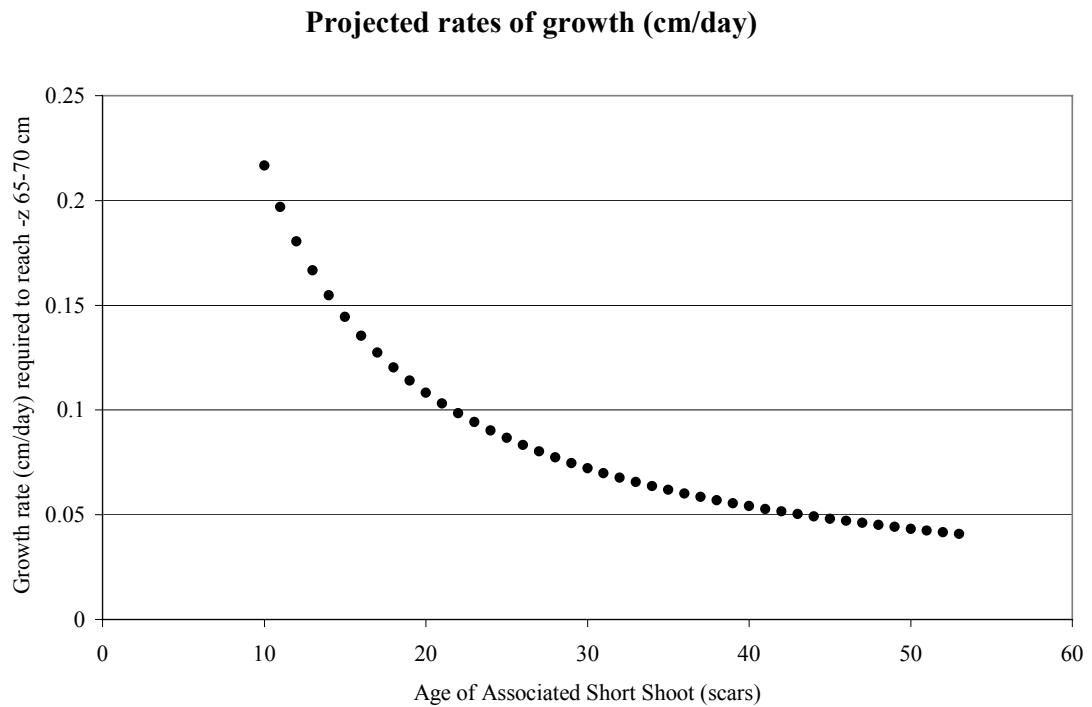
Figure 22. Chart of projected root growth rates. The points along the x-axis are scar ages.

The points on the y-axis represent the growth rate needed for the root to reach a sediment

depth of 65-70 cm.

three or more complete roots. The linear relationship between the length of the complete root and the distance of attachment point to the apical meristem tip was used to establish the growth slope. Figure 23 shows a case where five complete roots were attached to an individual runner. The regression equation shows that the growth slope was 1.23 cm of root growth for every 1 cm of rhizome growth. The $R^2=0.95$ indicates that all five of the roots on this runner are growing approximately at the same rate.

Of all the rhizome runners with three or more complete roots, 82% had R^2 values greater than 0.90, and 88% had R^2 values greater than 0.85. The 12% of remaining cases had an erratic series of R^2 values some approaching 0.00. Cases with R^2 values greater than 0.85 were selected for an analysis of the variability of individual growth slopes. Figure 24 shows the distribution for these cases. This figure shows that the growth ratio was observed to be as small as 0.13 cm, and as large as 1.63 cm of root growth to every 1 cm of rhizome growth. The scattering of growth slopes was evenly distributed across this spectrum. In summary it appears that root elongation off the rhizome, in the early stages, is in a steady state growth mode when compared to rhizome elongation. The growth rates of the individual plants is quite variable.

The relationship of root elongation to rhizome elongation appears to be strong. However, because rhizome elongation could not be directly assessed, it offers no evidence of the growth per unit of time. The best alternative in providing a time step is to estimate the age of a rhizome segment using attached short shoots. As a reminder, age is estimated using the leaf plastochrone interval and the number of scars on the short shoot.

Chart of root length (cm) plotted against distance of root attachment point to apical meristem tip (cm)
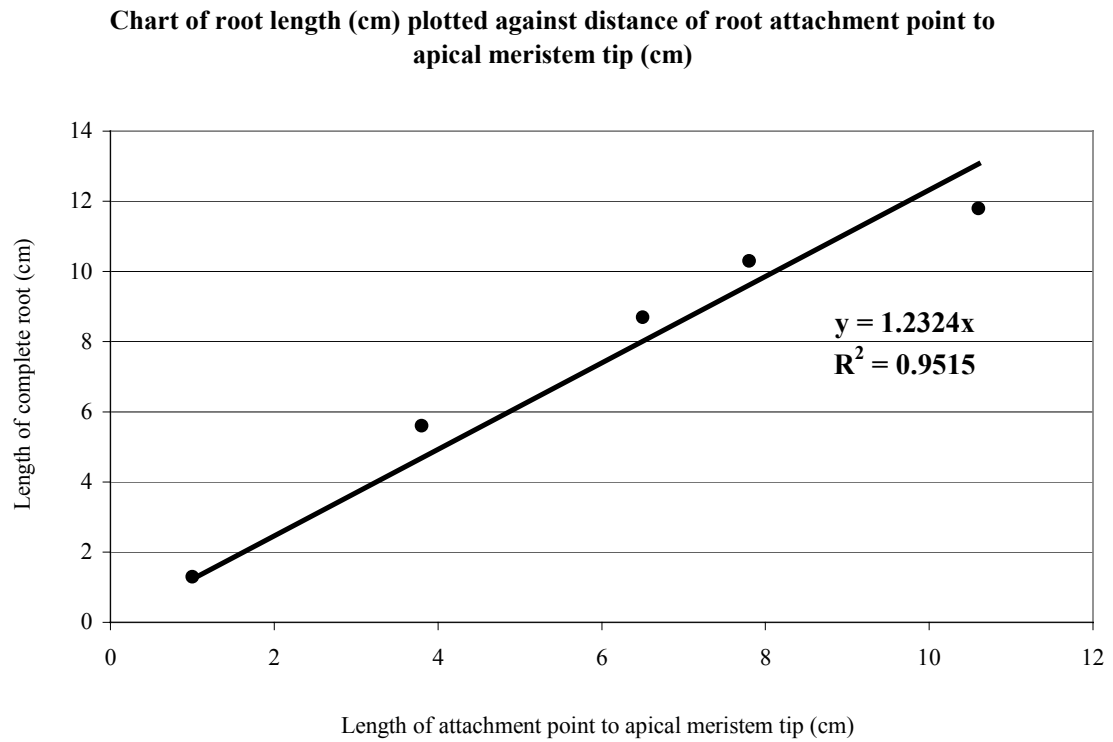
Figure 23. Relationship of complete root length (cm) to attachment point distance to apical meristem tip (cm). Regression line indicating growth slope also included.
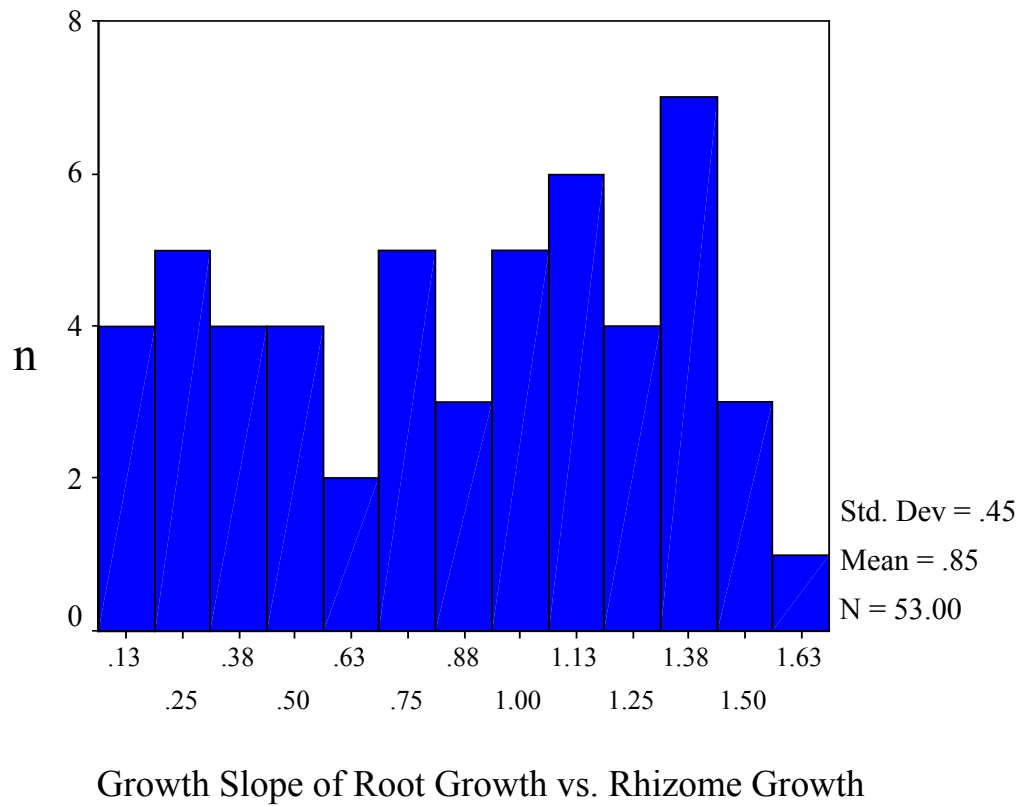
Growth Slope of Root Growth vs. Rhizome Growth

Figure 24. Histogram of growth slope for roots growing off rhizomes. Cases listed in this figure have three or more attached roots. Cases also had an R^2 value greater than 0.85 for a regression of root length verses length to apical meristem tip.

Figure 25 shows the relationship between the estimated age of the root and the length. These data show a weak to moderate linear relationship, and a moderate to strong non linear relationship. It is very important to point out that this distribution might represent a sampling bias. It is possible that the relationship is linear but many of the longer root segments were lost in the extraction process. That point acknowledged, it does appear that there is a general non linear trend in the data that is best described by a power or a natural log function.

In order to get a better indication of whether root growth is linear or non linear, a separate analysis was conducted using additional sediment sods collected to allow in-depth study of the Rabbit Key Basin population. Using these samples, the age of rhizome segments was estimated and root length was measured. The leaf plastochrone interval was not measured for these plants, therefore age of the rhizome is expressed as short shoot scars. Figure 26 shows a strong linear relationship between root length and the scar age. Error may have be introduced in this analysis due to an under sampling of older roots. In summary, the early stages of root growth appear to be linear. Beyond the early stage, sampling bias limits credibility of the hypothesis. These data offer evidence that the following equations are viable estimates for short shoots under 30 scars old.

Root length =0 .84 age of closest short shoot*   Equation 3.2

*Root weight =0 .0017*root length*   Equation 3.3

Total weight per rhizome is going to equal root weight multiplied by the average number of roots (2.19) per rhizome segment. Total weight of early growth per meter^2 will equal

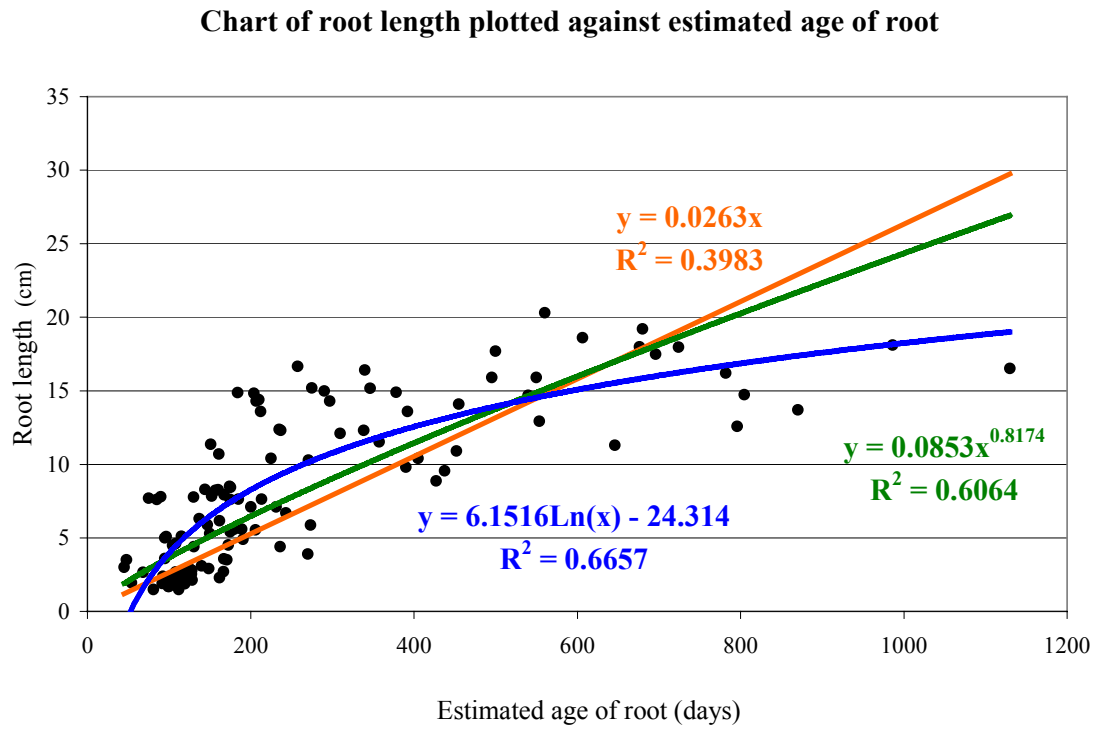**Chart of root length plotted against estimated age of root**

Figure 25. Chart of root length (cm) plotted against estimated root age (days). Root age estimated using short shoot scar and plastochrone interval data. Linear function (orange), power function (green), and natural log function (blue) are also included.
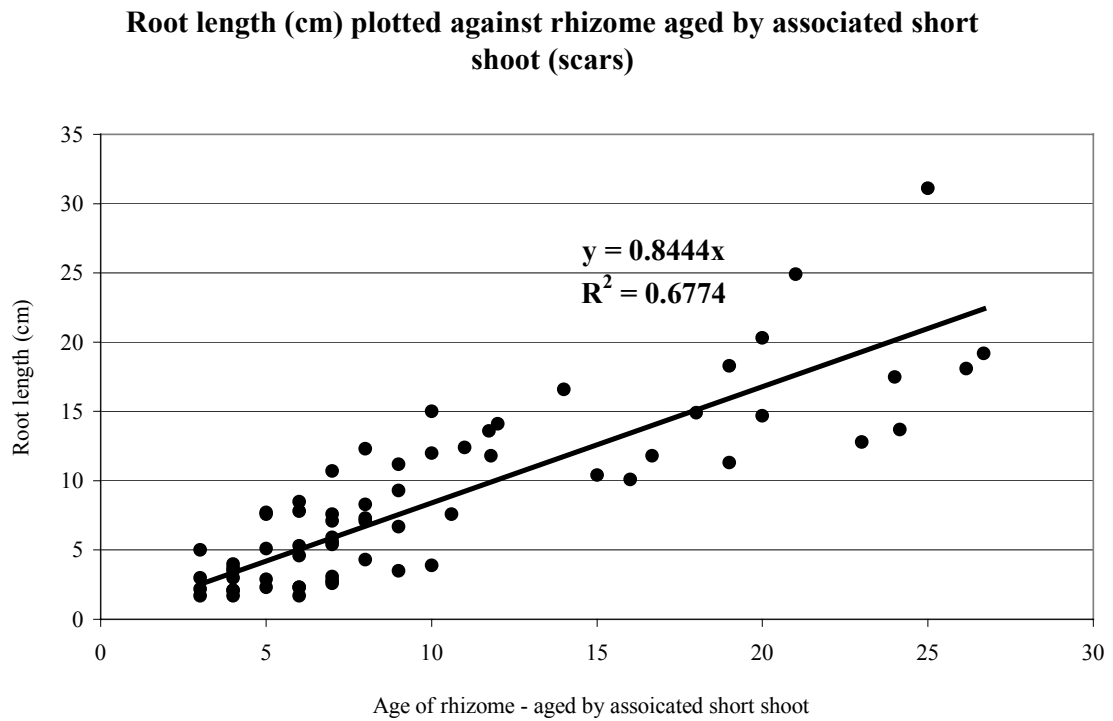
Root length (cm) plotted against rhizome aged by associated short shoot (scars)

Figure 26. Chart of root length plotted against age of attached rhizome. Age of rhiozme is estimated using number of scars observed on associated short shoot.

total weight per rhizome multiplied by the percentage of total density (n=1318 at

Rabbit Key Basin) under 30 scars in age. Productivity rate will equal the total weight of

early growth m^2 divided by the leaf plastochrone interval.

Roots growing off the short shoots

In contrast to roots growing off the rhizome, the short shoot roots displayed

pattern of growth that was more variable. When the variability was studied in depth,

three distinct classifications of growth emerged.   First were roots that were judged to be

growing in a steady state. The second classification included roots growing to common

length and common terminal end point, usually around 5 cm in length. The third

classification was fast growing roots. Before addressing these classifications, general

growth trends will be explored.

The average short shoot in the Rabbit Key Basin population had one root (or root

scar) for every 3.1 leaf scars (Figure 27). In contrast to Tomlinson's (1966) description,

the pattern of grouping for the roots was highly variable. Gaps of up to 17 leaf scars were

observed be followed by a cluster of roots separated by a single leaf scar.   Tomlinson

described a population where the short shoots had a lower number of roots occurring at

regular intervals. Figure 27 shows the distribution pattern for plants included in this

study, and what it demonstrates most clearly is that roots tend to be grouped in clusters.

Subjective judgment complicates the classification of clustering. To improve the

understanding of root clustering, 315 individual roots were classified as being, or not

being part of a cluster.   Classification was based on the number of leaf scars that

separated a root from the next root on the short shoot. The simplest cluster included two

n

100

Std. Dev = 2.68
Mean = 3.1
N = 275.00

0

2.0   4.0   6.0   8.0   10.0   12.0   14.0   16.0

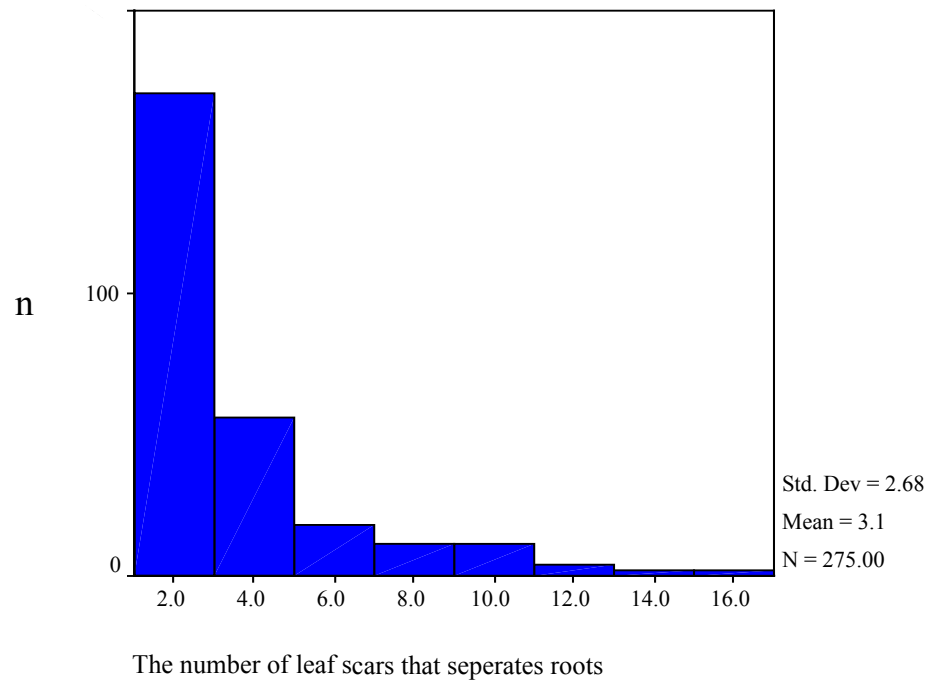The number of leaf scars that seperates roots

Figure 27. Histogram of number of scars that separate two roots in a sequence on the same short shoot.

roots separated by one leaf scar. A less strenuous criterion defined the second group, which included roots separated by one or two leaf scars. The third type of cluster included roots separated by three or less leaf scars. For clarity, all 315 cases were tested for each threshold meaning that roots in group-one would be included in groups two and three. Likewise, roots in group-two would be included in group-three. Table 38 shows that 40% of the roots were clustered within one leaf scar of another root. The percentage of clustered roots increases to 77.1 % when the threshold is expanded to include roots within two leaf scars of each other. Of the 315 cases only 12.4% did not fall into a clustering category, meaning 39 cases were four or more leaf scars away from the next root in the sequence. In summary, it appears that most short shoots in the Rabbit Key Basin population produce roots in clusters.

Roots growing off short shoots had an approximate life span of twenty leaf scars. This pattern may reflect a sampling bias as older live roots may detach more easily than younger roots. Any root tissue observed in this study attached beyond the 20th scar was darkened, necrotic, or clearly dead. It has to be noted that the bird bands used for identification caused roots on the tagged short shoots to be detached or damaged. Therefore there are no associated leaf plastochrone intervals to be associated with this 20 leaf scar life span. Using the population average of 25 days for the leaf plastochrone interval, the average life span of a short shoot root appears to be about a year and a half. If the plastochrone interval is assumed slow down in winter, then the life span of root is likely to be approximately 2 years. As stated before, these age estimates may be low if the extraction process selectively detached live roots from points older than 20 scars.

Table 38. Data for root clustering on short shoots. The same 315 samples are included for each cluster category. Threshold for inclusion defines categories.

Cluster Category	Number Included	Percent included	Number not Included	Percent not included
Group-one – separated by 1 leaf scar	126	40.0%	189	60.0%
Group-two – separated by 1 or 2 leaf scars	243	77.1%	72	22.9%
Group-three – separated by 1, 2 or 3 leaf scars	276	87.6%	39	12.4%

The average length of a complete root attached to a short shoot was 9.10 cm (Figure 28). This histogram may be showing an artifact of sampling bias. The extraction methodology may systematically damage or remove longer roots. So the possibility exists that these data do not provide a reliable estimate for the population. Given the technique for extracting the plant, a reliable estimate may not be possible. However, the length of the root did provide information about the three different growth patterns noted earlier in this section. Figure 29 shows a moderate correspondence between the length of a root length and its age. Within this figure points are clustered so as to provide the evidence for these growth patterns.

Figure 28 contains a cluster of points between with lengths of 22 and 30 cm. This subgroup appears to be normally distributed and is notable because it consists of very fast growing roots. In Figure 29 these fast growers cluster away from the main group of points and have the effect of weakening the age to length relationship. Figure 30 shows the same plot with the regression line for the fast growing roots plotted as a separate function. The roots in the fast growing group are attached to the short shoot somewhere between six and twelve scars. The regression line in Figure 30 shows that the growth rate for the fast growers is 2.6 cm for every leaf scar. In comparison, the slope for the entire group was 1.09, and 0.98 for the non-fast growers. It is not known whether these points clustered between 25-30 cm reflect the maximum growth of these roots or reflect a sampling bias. With the exception of three points, there is a noticeable absence of roots with lengths of 25-30 cm that are older than 15 scars. Since roots without a tip were not included in the study, this pattern could be an artifact of the sampling process. It is estimated that approximately 10% of roots in Rabbit Key Basin are fast growing roots.
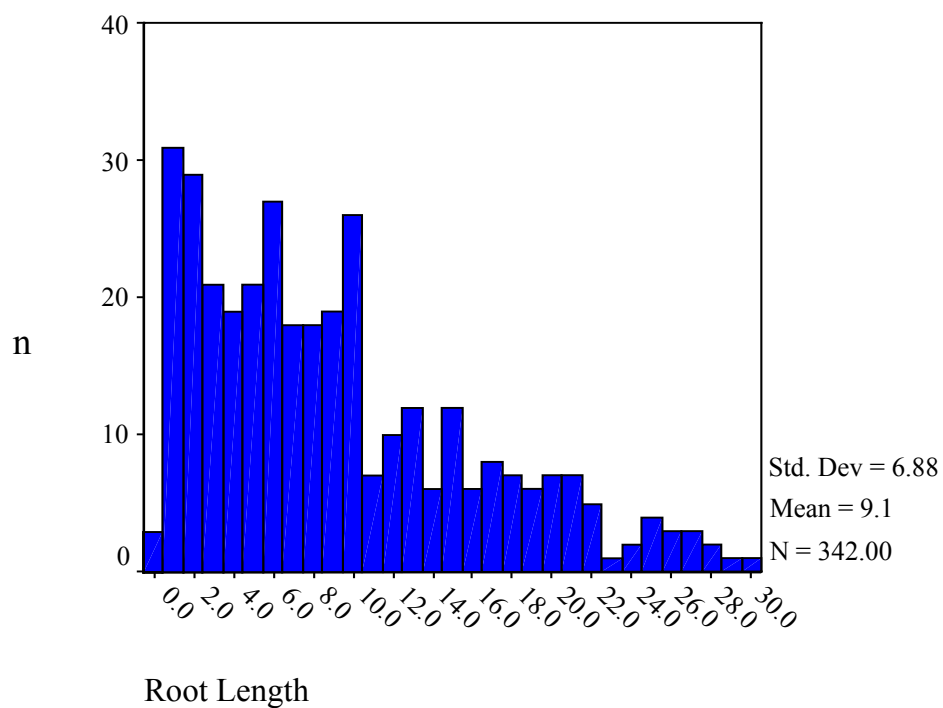
Figure 28. Histogram of root length. This distribution includes only roots attached to short shoots.

Plot of leaf scar attachment point vs. root length

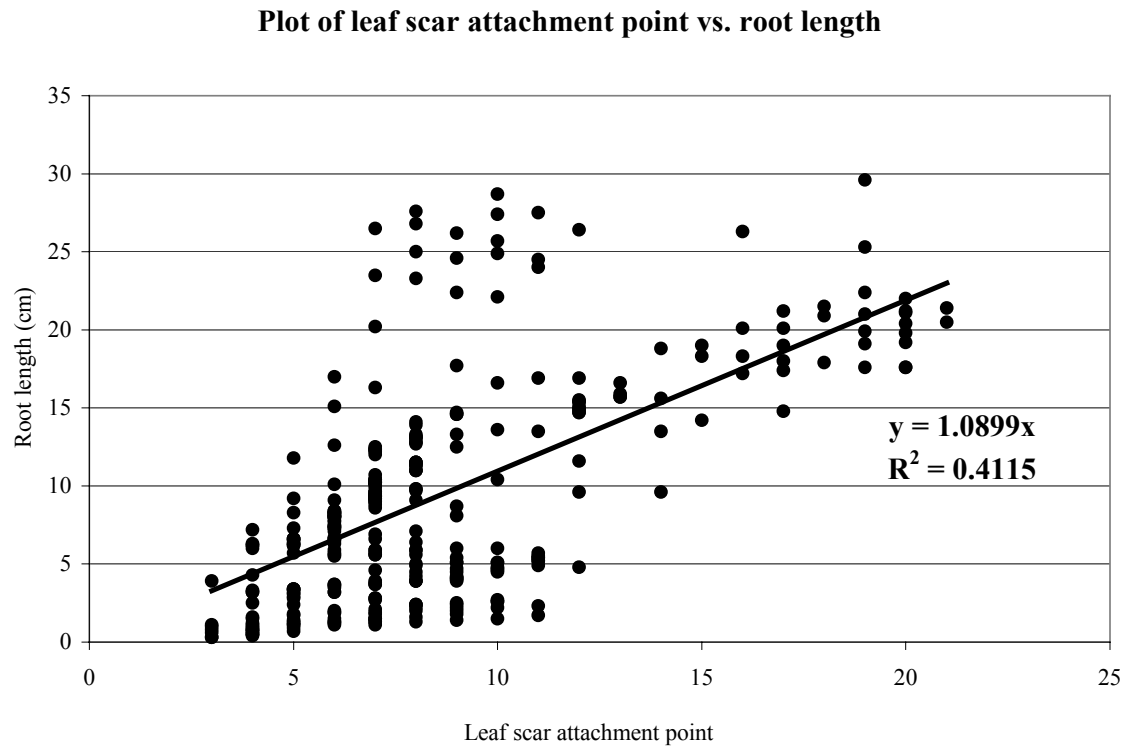$y = 1.0899x$

$R^2 = 0.4115$

Figure 29. Chart of leaf scar attachment point (leaf scars) plotted against root length or

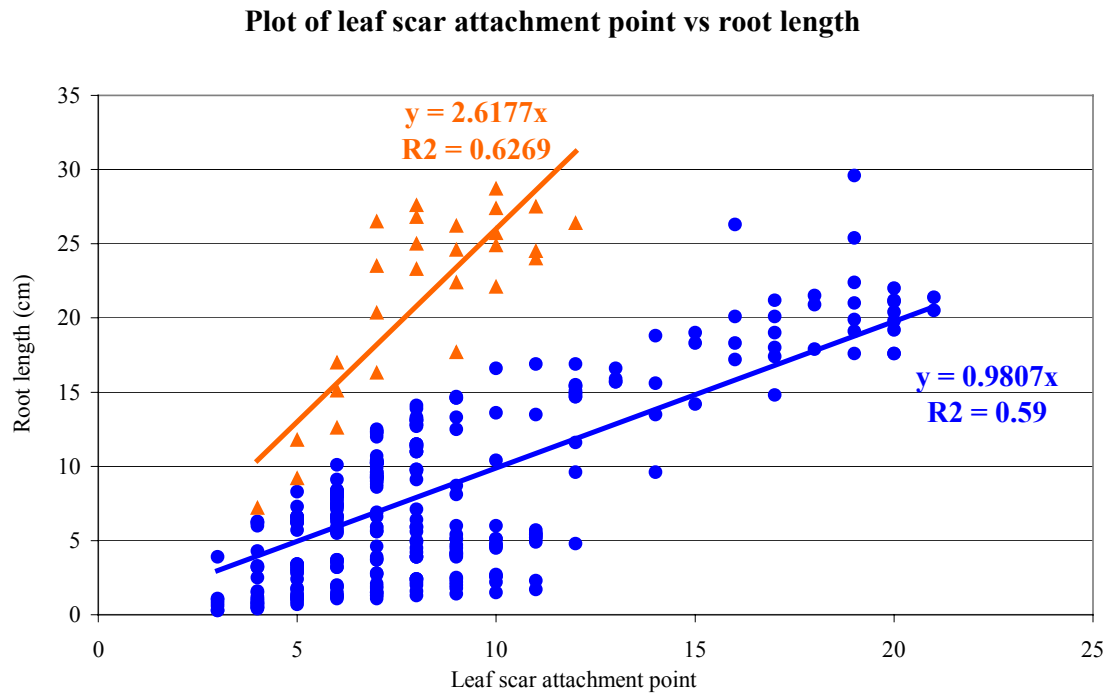root (cm). Leaf scar attachment point is a proxy for age of root.

Plot of leaf scar attachment point vs root length

Figure 30. Reprint of Figure 29. This figure separates fast growers (orange) into a separate data series.

The growth slope for the fast growers was estimated to be 2.6 cm per leaf scar. Using the average leaf plastochrone interval, an elongation rate of 0.11 cm/day is estimated. Using the length to weigh regression formula (0.0017*root length) it is estimated that each fast growing root produces 0.0002 g dry weight biomass per day. Using data presented in this study it is estimated that 510 fast growing roots would be elongating at any point in time. Therefore fast growers are adding 0.10 g dry weight biomass per meter^2 per day.

The second category of roots are those that grew to a length between one and five cm and then appear to halt their growth. This classification was based on a number of observations where two to three roots in a cluster were observed to the same approximate length. The evidence for this pattern was strengthened by younger roots observed to be longer than the older growth halted roots. In these cases the younger root did not need to be a complete root with a tip. All that was necessary was that the younger root had be attached to the short shoot and had to be a greater length than the older cluster of roots. The roots in the cluster did require a root tip in order to meet the criterion of a complete root. Unfortunately, this pattern was identified about half way through the assessment process and the actual percentage of roots that fall into this category isn't exactly known. However, the percentage can be estimated using an alternative method. These growth-halted roots seem to have reached their maximum length by time they were five to six scars old. Therefore an analysis of the distribution of lengths for roots that were seven scars old should offer insight into the percentage of roots that have halted their growth. Figure 31 contains the distribution of lengths for complete roots attached to the short shoot at leaf scar number seven. This figure shows three distributions that delineate the
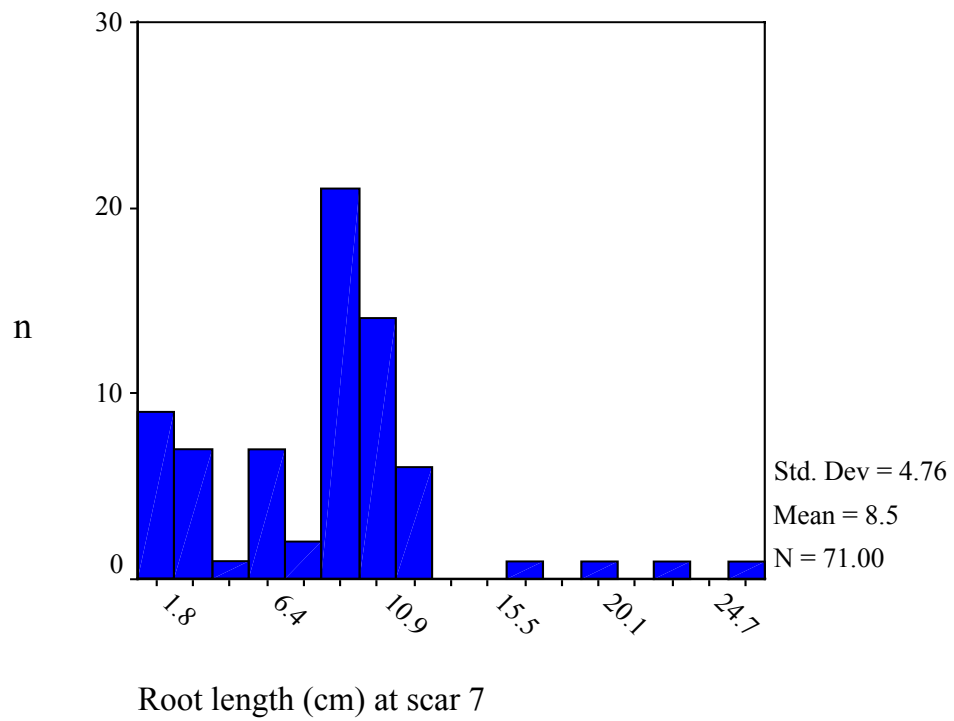
Figure 31. Histogram of lengths (cm) for complete roots attached to the short shoot at leaf scar number seven. The x-axis are root lengths (cm), and the y-axis is the count.

three growth patterns. The cases furthest to the right along the x-axis are the fast growers. The distribution of cases that peaks around 10 cm are those cases listed as steady state growers, and they will be discussed shortly. The final group, clustered on the left side of the x-axis are the growth-halted roots. There is a clear separation at length 4.8 cm that isolates this group. This cluster represents about 18 out of the 71 cases, and from that it is estimated that 25.3% of roots are classified as growth-halted. Figure 29 would suggest that these roots reach their maximum length by five scars. Separating out the 18 cases, the mean length for this group is 2.6 cm, which would yield a growth slope of 0.52 cm per scar, up to scar five. This is a rate of .02 cm/day. The plants at Rabbit Key Basin are producing 53 scars per meter^2 per day. A root is produced every 3.1 scars, so 17.1 roots are produced per meter^2 per day. Of those, 25%, or 4.3 are slow growers. This would mean that there would be approximately 537 growth-halted roots elongating per m^2. At a rate of .02 cm/day 537 roots would be elongating 10.72 cm per day yielding a dry biomass of 0.02 g dry weight per meter^2 per day.

The final category of short shoots roots are the steady state growers. These cases are presented in Figure 32. These are the same data presented in Figure 29 with the fast growers and the growth-halted cases excluded. In the early stages of growth it is impossible to differentiate which root will be steady state and which will be growth-halted so all cases were included. There may be some growth-halted cases that are weakening the relationship presented in Figure 32, but with nearly 70% of the variance explained, the growth slope will be accepted as a sound estimate for the population. Approximately 65% of short shoot roots fall into the category of steady state growers.

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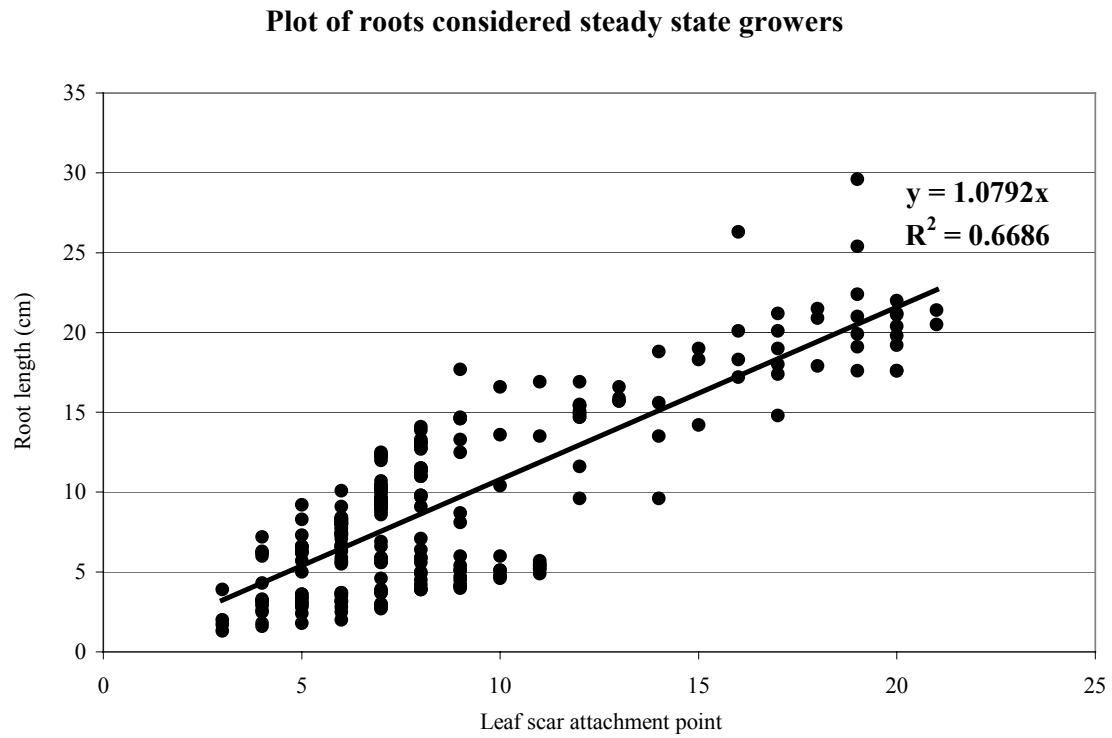**Plot of roots considered steady state growers**

Figure 32. Chart of leaf scar attachment point plotted against complete root length (cm). This is the same data presented in previous Figures with fast growers and growth-halted roots excluded from the plot.

The data might suggest length limits of 30 cm and age limits of roughly 20 leaf scars, but this may reflect a sampling bias.

The growth slope for normal growing roots is estimated at 1.08 cm per leaf scar. Using the average leaf plastochrone interval, the elongation rate is estimated to be 0.04 cm/day. Recalling that Rabbit Key Basin produces 17.1 roots per day, 65%, or 11.1 are steady state growers. The life span is 500 days and so there would be 5557.5 steady state roots elongating per meter^2 . This gives an estimate of 222.1 cm per meter^2 per day. Using the weight to length relationship, these roots add 0.38 g dry weight biomass per meter^2 per day.

Elemental constituents of root tissue

The procedure for this analysis was identical to the elemental analysis of the rhizome tissue. Roots were evaluated individually and in groups. The group analysis acted as an integration of multiple root samples. Approximately 20 complete roots were aligned at the tip and were cut at 5 cm intervals. Each group of segments was dried, ground up, and multiple replicates analyzed.

Roots appear to display the same pattern of elemental change as rhizome tissue. Higher levels of nitrogen and carbon were detected at the root tip (Table 39). The percentages of these two elements decline with age of the tissue. Distance from tip acts as a proxy for age of root tissue. It also appears that the percentage of sulfur increases with age of the root tissue. The increase in sulfur percentage with age is something that needs to be confirmed with further study. The instrument used to determine these values was a Carlo Erba elemental analyzer, and sulfur determination required a reconfiguration of the machine. This reconfiguration caused problems and a breakdown of the machine

Table 39. Break down of nitrogen, carbon, phosphorus, and sulfur percentages in root tissue. Percentages are broken down on a categorical continuum of distance to the root tip.

Location of Root Tissue sd n	Nitrogen % sd n	Carbon % sd n	Phosphorus % sd n	Sulfur % sd n
Tip-5 cm	2.19% 0.21% 43	42.13% 1.32% 43	0.07% 0.002 3	0% na 8
6-10 cm	1.56% 0.36% 43	40.03% 1.78% 43	0.05% 0.004 3	0% na 8
11-15 cm	1.17% 0.29% 37	38.19% 1.09 37	0.04% 0.001 3	0% na 8
15-20 cm	1.07% 0.27% 33	36.15% 0.99 33	No data	0.72% 0.34% 8
Old Root	0.79% 0.11% aprox 100	34.81% 1.14% aprox 100	0.02% 0.001 3	2.31% 0.49 aprox 50
Dead root	0.61% 0.09 aprox100	28.67% 2.58% aprox 100	No data	4.67% 0.37 aprox 50

occurred during one of the sample runs. As a result only eight samples were able to be analyzed. Five to ten replicates were run on each of the eight samples, so the values presented in Table 39 reflect accurate machine readings. However a sample size of eight is too small to establish these percentages as an absolute estimate of the population.

The standard deviations for the elemental analyses (Table 39) may reflect both machine drift of the Carlo Erba and natural variation of the *Thalassia testudinum* roots. The sample numbers include the runs were multiple roots were ground into a integrated mixture. These integrated mixtures provided enough tissue sample to run 10 to 15 replicates which allowed for a very stable estimate of carbon and nitrogen. In the cases of old roots and dead roots the exact number of roots wasn't known as 20 to 30 roots were ground into a integrated mixture. Again, this allowed for enough sample to run 10 to 15 replicates.

Section Three Discussion

Root production estimates of *Thalassia testudinum* are complicated by several factors. First, roots grow off both rhizomes and short shoots. Second, growth occurs within a complicated matrix of plant tissue, infauna and other sediment elements. Extraction techniques make a comprehensive study of roots logistically difficult. Extracting a complete runner with rhizome, multiple short shoots, and the root system completely in tact was unsuccessful. Only plants with the newest complete roots attached were extracted. The sediment cores provided evidence that roots at Rabbit Key Basin grow to a depth of 0.65 meters (Table 23). But a complete individual root attached to the plant, penetrating to those depths was not successfully extracted. Results reported on the complete, attached roots collected by this study would produce biased population estimates. However, there were identifiable patterns in root growth that do provide information about the population.

Roots growing at Rabbit Key Basin matched descriptions published by Tomlinson (1966). Roots connected to the rhizome were always attached to the bottom axis of the runner. This uniform orientation would direct all rhizome root growth into the z-plane of the sediment. Given the complexity and tightly defined limits of the rhizosphere, downward growth of the roots likely allows exploitation of a resource base in the unoccupied sediment volume. However there are energetic gains and losses that have to be considered. The area below the rhizosphere has more living space, and likely a nutrient resource base. However, occupation of that sediment space subjects the plant to a

changing redox potential, and to the problem of sulfide toxicity. Therefore it becomes a questions of energetic cost verses return.

Florida Bay has variable sediment types (personal observation). There may be a phenotypic response of root tissue due to sediment type. In sandy sediments the need for anchoring might be greater. Sandy sediments would also represent a less hostile environment in terms of the redox potential. Theses factors could affect the structure of the root and future study should include an investigation on how sediment type affects the root.

Most of the roots observed growing off the short shoots conformed to Tomlinson's (1966) description of root growth. He described roots growing orthogonal to the rhizome. However, he reported a consistent pattern of the root growth away from the rhizome runner. He does not report root growth that crosses the plane of the rhizome. This study observed two variants on the pattern that Tomlinson described. In both cases roots did cross the plane of the rhizome. In the first adaptation, the older roots grew normally away from the rhizome, then at a point, no lower than the 10th scar, the younger roots changed the orientation of growth by 180 degrees. In the second variant pattern, all roots on the short shoot grew across the plane of the rhizome. Tomlinson viewed the short shoot as a horizontal version of the rhizome. To this end, he viewed the single axis of root growth out of the short shoot as analogous to the downward growth of roots attached to the rhizome. The patterns observed in this study would suggest a more flexible response than Tomlinson described. The function of roots will be discussed later, but it seems clear that this growth pattern is meant to maximize resource foraging. If the short shoot produced roots to anchor the plant, attachment along a single axis will offer

minimal trussing support. If the roots serve as vector of nutrient delivery then orthogonal growth maximizes likelihood of unique exploitation of an area of resources. Root growth in the direction of the rhizome is likely to tap the resource base of the rhizome. Further, roots growing parallel to the rhizome would eventually get tangled with the roots of sequential short shoots. If roots were directed at an orientation of 45° then short shoots on the same rhizome would be competing with each other once the roots reached a certain length.

The data collected allowed the development of equations to predict root biomass. The weight of the root segment was highly correlated to the length. The diameter of the root proved to be a complicating factor as this variable did little to improve the predictive capacity of the model. Differences in the weight to volume ratio for individual samples were observed. Roots with a smaller diameters had a greater weight per unit volume (Figure 20). It is possible that the roots with smaller diameter have a corresponding loss, or lack of lacune space. Some short shoots, perhaps those in the upper sediment, may need stabilization in the form of anchoring by roots. If there is a need for some roots to act as anchors, then dense, non porous tissue is likely to offer greater support. A microscopic visual analysis of the root structure is needed to explore the possibility that roots have differentiated functions. If primary root function is extraction of nutrients from the sediment, a greater surface area would enhance the roots ability to extract those nutrient. A root with the primary function of anchoring the plant might have different structural attributes. These would be lower surface area, higher bulk density of the tissue, and limited maximum length. These attributes will be discussed later in this section. The

immediate point is that the low diameter roots weakened the weight to volume prediction equation.

As an alternative to including root diameter, length of the root was considered as a single and linear predictor for root weight. Length explains most of the variance in the weight of a root. Since diameter accounts for very little of the unexplained variance, and is difficult to measure, length was used as the predictor for weight.

Rhizome roots

The data suggest that the *Thalassia testudinum* plant establishes the roots early in the development of the rhizome. The evidence showed that the number of rhizome roots and root scars did not change as a function of segment age. Using attached short shoots as a proxy, the rhizome segments were categorized by age. The ONEWAY ANOVA verified that there were no statistical differences in the number of root scars in any of the age categories. This would indicate that the rhizome is not generating new roots as the segment ages.

Growth patterns for roots attached to the rhizome were established using complete segments, meaning they had a discernable tip. This root growth was measured two ways. First was length of a complete root verses the distance of the root attachment point to apical meristem. Second was length of root as a function of age. In this second case, the closest short shoot acted as the chronometer. The first method offers better resolution in the relative growth of the two plant parts. However, without specific information about rhizome productivity, the first measure fails to provide a time based rate of growth. However, the quantification of the relative growth is accurate. The second method

provides a time based rate of growth, but accuracy is affected by using the short shoot

as a proxy for age.

The data on root growth relative to rhizome growth and short shoot scar age

suggest that early growth, while variable plant to plant, is in a steady state of

development. The photograph presented in Figure 33 represents an optimum

representation of this growth. The data that were presented show a slightly more variable

system, but generally consistent with the photograph. The extraction process limits

measurement to the early growth patterns of the root.

The core data provides evidence that long term rhizome root growth may not be

steady state. Given the population distribution and the assumption of continual growth,

then the depth distribution of root biomass wouldn't taper off in the pattern evidenced by

the cores. The tapering off of root biomass would be consistent with the depth

distribution of the rhizome biomass and the age distribution of the population. The live

root tissue at depths of 60-70 cm are a good indication that continued growth occurs. But

the low volume of root tissue at these depths would indicate that only a few plants

manage to get roots to the deeper depths. Knowing the weight of the root tissue, and

knowing the exact volume of the core segment allowed for an estimate of the length of

root tissue. This in turn allows an estimate of the number of roots that actually reach this

depth. This was outlined in the results section, and it appears that very few of the rhizome

produce roots that reach these depths. This would provide evidence that the energetic

cost is high for whatever resource is returned. Because of the nature of the core

extraction, it is not known whether these deep roots represents those attached to oldest of

rhizome. However it seems clear that continued growth of the roots growing off the

rhizome has to be included in the below-ground production estimates. For this study, early root productivity was estimated. Long term patterns of rhizome root growth is a focus for future study. Zieman (1982) observed extended root growth into ancient mangrove stands, but no literature is available that addresses quantification of long-term growth rates.

Figure 33. Photograph of roots growing off a rhizome runner.

Short Shoot Roots

The pattern of root growth off the short shoots shows a different pattern than root attached to the rhizome. First, the attachment location on the short shoot necessitates that the roots start off growing horizontally in the sediment. It is likely that growth continues

in a horizontal direction, though this hypothesis has no direct evidence. The fact that roots grow orthogonal to the rhizome would suggest a survival strategy to systematically forage for resources away from the plant. A downward turn in the direction of growth might cause an inter-plant competition for resources.

Unlike roots attached to the rhizome, the short shoot roots were attached in an irregular and in clustered pattern. When a cluster did occur, it typically consisted of three roots within a space of three to four leaf scars. Root clusters could be evidence of the plant's adaptability to a patchy resource base. If a new root was to detect, or start drawing from new found nutrients, for instance a dead clam, the plant might initiate a sequence of roots in order to fully exploit the localized resource. It could be theorized that there exists a threshold of resource transport within the root that triggers the initiation of a sequence of roots. This hypothesis would be consistent with the short shoot roots classified as common length and common terminal end point. This was one of the two classifications of non steady state root growth.

A second classification of non steady state growth was fast growing roots (Figure 34). These were roots, which, because of their attachment point, were known to have accelerated elongation rates. This pattern of growth could be due to the fact that these roots, being near the top of the short shoot, were also near the sediment surface. The upper sediment volume may have unique chemical properties that allow accelerated growth. A second hypothesis is that the fast growing roots are acting as probes. The initiation of a new rhizome runner occurs in the same direction as root growth. The fast growing root may be part of the mechanism to trigger a new runner. The alternative theory is the root is growing until a resource pocket is located.

Figure 34. Photograph of roots growing off short shoot. Root towards the top of the short shoot is an example of a fast growing root.

Most of the roots in this study were classified as steady state grower. The data (Figure 32) show that the actual growth slope was variable from case to case. But when individual short shoots were examined, and the analysis did not include a fast grower or a halted-growth root, the linear relationship between the attachment point and the length of the root was usually very strong. This pattern makes sense from a population standpoint as variability is expected in normally distributed systems. So while there is individual variation, the growth appears to be steady state. This means predicting individual cases

might be difficult, but with adequate data, population estimates of normal root growth should have an acceptable level of precision.

The variable weight to volume ratio of the roots displayed irregularities in population that might be related to the variability of the lacunae space within the root. One of the future research initiatives will be studies that investigate the structure of the root. Seagrass roots may act as anchors, but their unidirectional growth would provide support in a single direction. The rhizome roots might provide anchoring against the upward pull of leaf movement in the water column. It could be hypothesized that the rhizomes located near the sediment surface would be the individuals in greatest need of support. This hypothesis could be tested in a future study. Density differences in the roots, based on the rhizome's depth in the sediment, could provide evidence that some roots act to anchor the plant.

It is important to note that, unlike rhizome roots, short shoot roots do not appear to grow continually. Often young root tissue was observed near the top of the short shoot while black, necrotic tissue was observed at the lower parts of the shoot. This pattern suggests the upward movement of resource extraction by the short shoot roots. Live roots were observed on older rhizome segments. Since the evidence indicates that those roots were established at the same time as the rhizome segment, there exists the possibility differential function in *Thalassia testudinum* roots. If short shoot roots provided nutrients to both above-ground and below-ground portion of the plants the distribution of root biomass on the short shoot would be evenly distributed. The senescence of root tissue on the short shoot would not be expected. Rhizome tissue has it's own roots, and root life span may be commensurate with the life span of the rhizome

segment. This may explain why live roots move up the short shoot. Theoretically the roots would stay alive based on an energetic balance. It could be theorized from the patterns observed that the short shoot roots move resources in an upward pattern. Another argument for the differential life span of a root could be horizontal competition for space and nutrients. In time the sediment volume may become depleted of resources, and thus the occupation of that sediment space becomes an energy drain.

The elemental make of the roots conformed, with slight deviations, to the patterns observed in rhizome tissue. The growing tip had the highest percentage of nitrogen to support the growing process. The only interesting deviation in elemental patterns was the higher percentage of sulfur found in old and dead root tissue. Sulfur needs to be studied in greater detail for several reasons. First, the intrusion or the presence of sulfur in the root tissue might be indicative of plant stress. If the correspondence of tissue age and sulfur level reflect a natural process then the quantification of this relationship could be used for such tasks as aging the root. This same argument could be used in consideration of carbon and nitrogen. Sulfur is different because of the complete absence of the element in the younger tissue. As for carbon and nitrogen it is not known whether the young plant tissue is extracting these elements or whether they are being translocated to the growth point of the root.

The *Thalassia testudinum* root system comprises a significant portion of the below-ground biomass. The pattern of growth would indicate that it serves a very important function in meeting the resource needs of the plant. Understanding of the structure and function of this plant component needs to be advanced. Some important research initiatives would include cross sectional microscopic studies of the root

structure. Another would be experimentation on the direction and quantity of nutrient flow. In studying elemental movement, it would be quite valuable to determine of the ultimate fate of nutrients extracted by the root system. The study of structure and function of the root with regards to of the density differences in the weight per unit volume. And finally, more exacting quantification of the productivity rates for root tissue.

Section Four

Short Shoots

Results

The clonal unit or ramet of the *Thalassia testudinum* plant is the short shoot. It connects the photosynthetically active green leaves to the rest of the plant. There was considerable variability in the structure of short shoots because the rhizomes are located at various depths (Table 40). As the short shoot grows it continually adds length, but this rate may be affected by the depth of the rhizome and the sedimentation rate of the surrounding ecosystem. An example of this variability is evidenced by a standard deviation for the length which is nearly as large as the mean.

The population at Rabbit Key Basin was observed to have approximately 0.026 g dry weight biomass per cm of short shoot length (Figure 35). Short shoots added .0026 g dry weight biomass per leaf scar (Figure 36). In both cases the power function was better able to describe the relationship. This may reflect the influence of short shoot volume, but since weight, length and scars are linear variables the linear functions will be used to describe the relationships.

The ability to predict short shoot weight using leaf scars is complicated by variation in the inter-scar length. The variation of inter-scar length is best illustrated in a plot of the length of a short shoot verses the number of scars (Figure 37). This figure shows a strong correspondence between these two variables (R^2=0.74), but it also shows an increasing variation in short shoots older than 20 leaf scars and longer than four cm. Depth of the rhizome or sedimentation may affect the inter scar distance and in turn it

Table 40. Descriptive statistics on the short shoot length (cm), average diameter (cm), volume (cm^3), dry weight (g), and number of leaf scars.

	n	Minimum	Maximum	Mean	Standard Deviation
Short Shoot Length (cm)	454	0.11	11.27	2.56	2.18
Short Shoot diameter (cm)	451	0.10	0.66	0.41	0.09
Short Shoot Volume (cm^3)	451	~0.00	3.32	0.39	0.41
Dry Weight (g)	418	~0.00	0.53	0.07	0.07
Scars	447	3	108	25.79	18.85

Plot of short shoot length verses weight
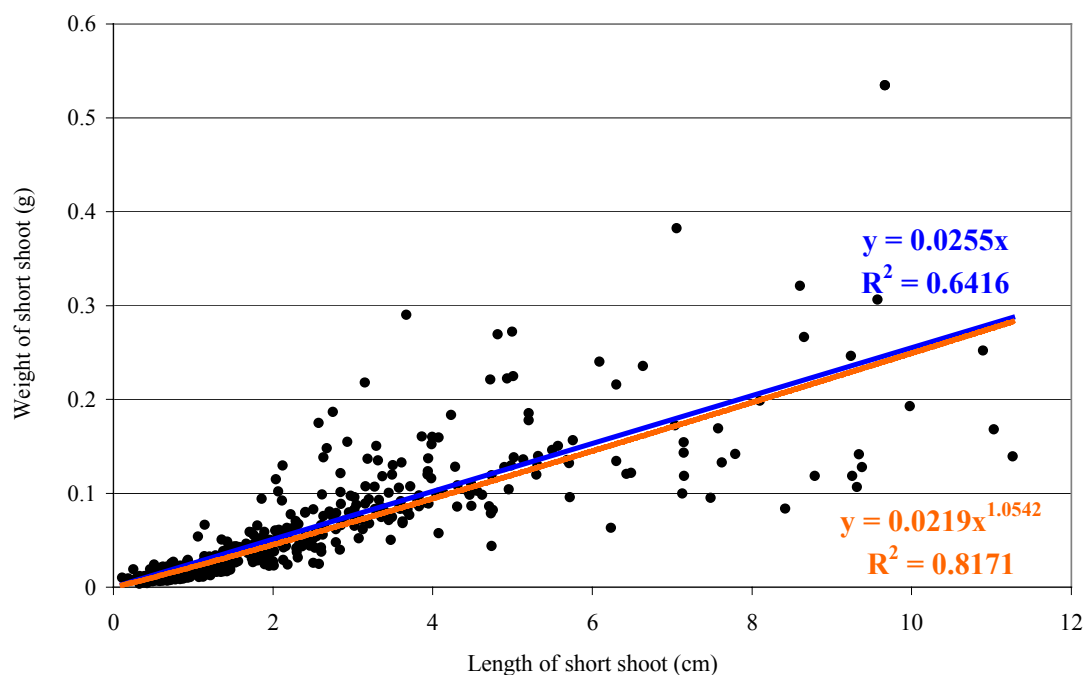
Figure 35. Chart of short shoot segment length (cm) plotted against segment weight (g). Linear and power functions are also included.

Short shoot leaf scars verses weight
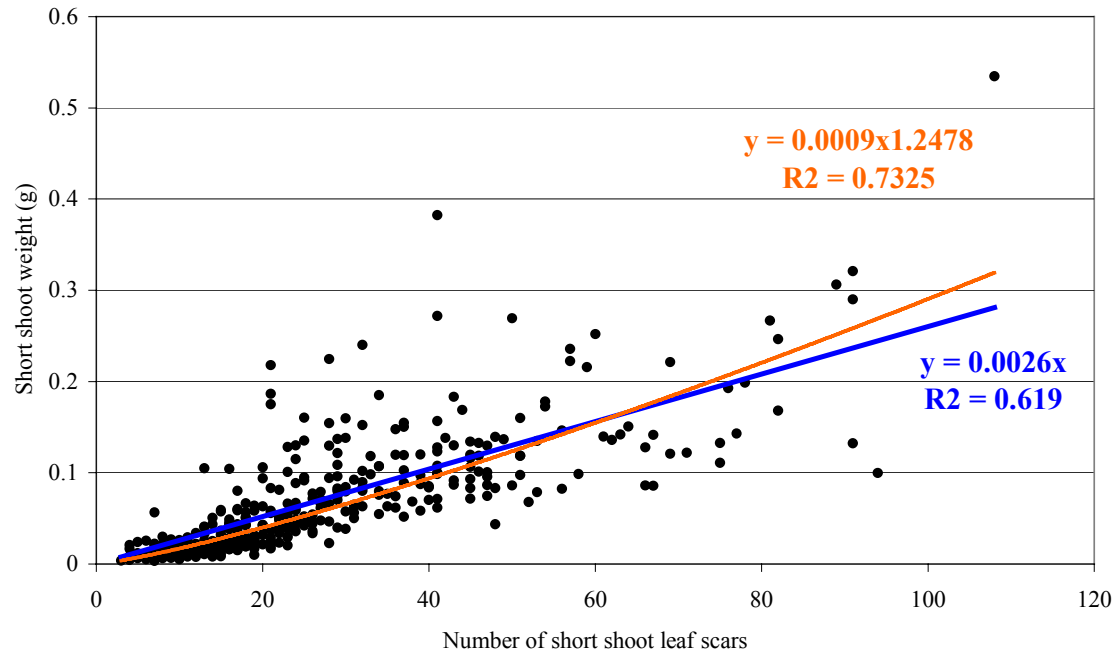
Figure 36. Chart of short shoot leaf scars plotted against segment weight (g). Linear and power functions are also included.
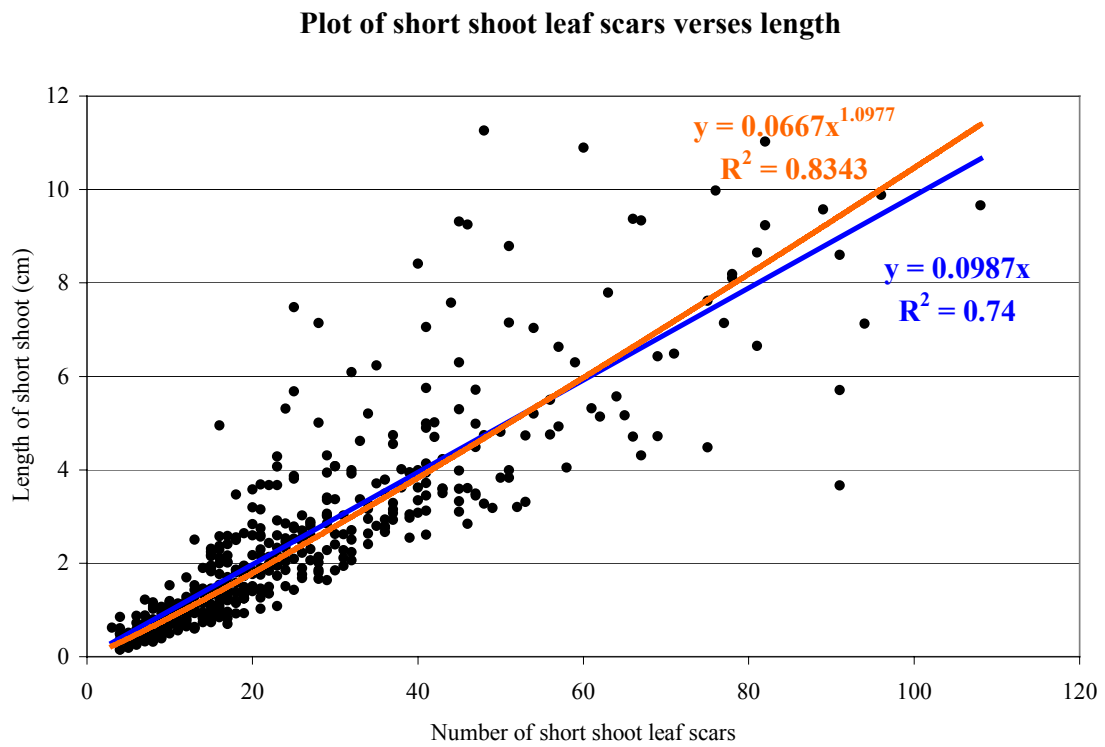
Plot of short shoot leaf scars verses length

$y = 0.0667x^{1.0977}$
$R^2 = 0.8343$

$y = 0.0987x$
$R^2 = 0.74$

Length of short shoot (cm)

Number of short shoot leaf scars

Figure 37. Chart of short shoot leaf scars plotted against length of segment (cm).

may affect the biomass. Likewise, the diameter of the short shoot affects the weight to length relationship (Figure 35).   Rather than include the short shoot diameter in a multiple regression, length and diameter were used to compute the volume of the short shoot. Short shoot segment volume has the advantage of combining length and diameter into a single measure. Figure 38 shows the relationship between short shoot volume and weight. Because volume is a square function, a non-linear power function is included in this figure.   Compared to rhizomes or roots, extraction of short shoots is easy to accomplish, and is standard procedure in Zieman's (1974) productivity determination method. Therefore, direct measurement of length, diameter and number of scars will provide most of the information needed to determine short shoot productivity. Individual leaf plastochrone intervals are the final element needed to determined the productivity estimate. If individual values are not available, population means can be substituted.

While extraction is required, establishment of short shoot depth can add to the understanding of productivity patterns. For the population at Rabbit Key Basin there was a moderate relationship between depth of short shoot and the elongation rate (Figure 39). In this figure elongation rate is expressed as the average vertical distance between sequential scars on the short shoot. Figure 39 shows that the deeper the short shoot the greater the inter scar length. It would suggest that the plants growing at the deeper depths are generating more biomass to get the crown of the short shoot closer to the sediment-water column interface.

The estimates for short shoot productivity at Rabbit Key Basin were based on the average amount of dry weight biomass added per scar. This value was 0.0023 grams dry weight per scar. The short shoots were adding one scar every 24.7 days. This means
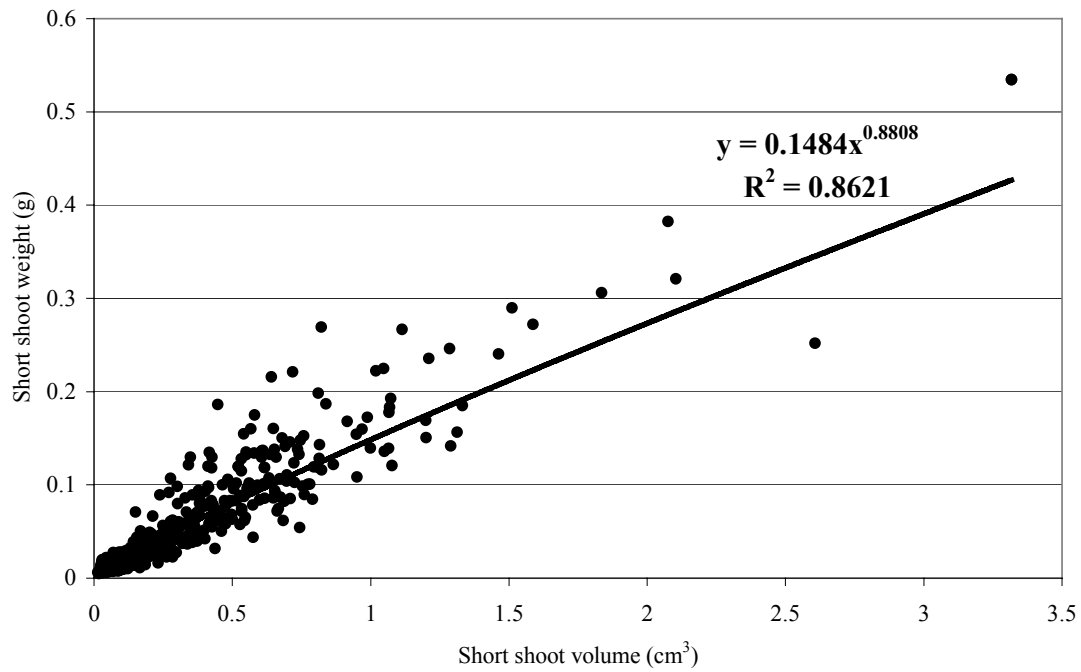
Plot of short shoot weight verses volume

$$y = 0.1484x^{0.8808}$$
$$R^2 = 0.8621$$

Figure 38. Chart of short shoot volume (cm^3) plotted against segment weight (g). The power function is also included.

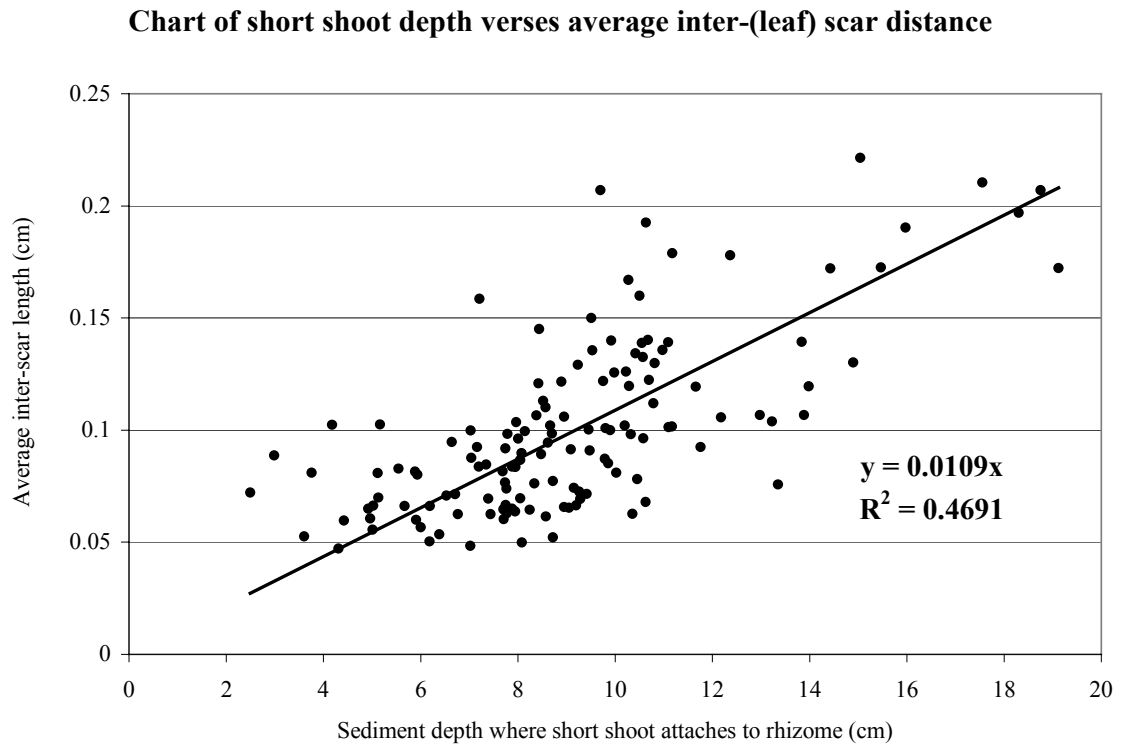**Chart of short shoot depth verses average inter-(leaf) scar distance**

Figure 39. Chart of short shoot attachment point depth (cm) plotted against the length between sequential leaf scars on the short shoot (cm). Also included is the linear function.

each shoot was adding 0.000093 g dry weight per day. There were 1318 short shoots per meter^2, therefore the population of *Thalassia* at Rabbit Key Basin was adding 0.12 grams of dry weight biomass per meter^2 per day.

Section Four Discussion

Short shoot productivity is perhaps the easiest below-ground component to quantify because the short shoot has scars which act as a chronometer. The short shoots are easy to collect and the scars are relatively easy to count. Leaf production is not assumed to be in a steady state, but it is regular enough to be a proxy, in the form of leaf scars, for age of the short shoot. The short shoot can be extracted, scars counted, weighed, and a weight per scar computed as a productivity rate. If the plastochrone interval is available the rate can be converted to biomass added per day. Resolution and error of the productivity estimate will be dependent on how accurately the leaf plastochrone interval is measured. The longer the monitoring, the better the estimate of leaf growth and plastochrone interval. If the monitoring period was long enough, seasonal fluctuation can be computed into the short shoot productivity estimates..

One of the goals of this study was to test the ability to produce below-ground production estimates based solely on above-ground measurements. In the case of the short shoot, continued elongation complicates any attempt to establish this relationship. Leaf area added per day and leaf width did explain most of the variance in the diameter of the short shoots. Without information about the length, those relationships are not very useful. Also, considering the time spent in the determination leaf area added per day, it would be a negligible time expense to extract, measure and weigh short shoots. Therefore use of above-ground measures to predict short shoot production does not seem like a defensible position to take.

In terms of the long term growth patterns of the short shoots, it appears that there is a maintained, steady state period of growth. The sediment surface imposes a ceiling on the growth of the short shoot. The plant appears to respond to this ceiling with a change in the intra leaf scar distance. When short shoots were examined for the length between leaf scars it was found that attachment to deeper rhizomes was associated with greater inter scar length (Figure 39). Future studies can examine the distance between leaf scars in greater detail to determine how depth, age, sedimentation, and other conditions affect this characteristic.

The slow down of leaf production in the winter is understood to be a byproduct of lower light availability due to shorter days and a lower solar zenith. Also, colder water temperatures change the metabolism of the plant which will affect the rate of growth. Outside of the seasonal fluctuations, stochastic, non seasonal events can also affect rates of production. A good example of which would be a sedimentation event caused by a storm. In measuring samples, many short shoots were observed to have a regular pattern of biomass added per leaf scar. There was a sizable sub-population where a change in the distance between scars was easily observable. The pattern was not consistent. In some cases larger inter-scar distances became smaller, and the opposite pattern was also observed. Sedimentation events could be hypothesized to increase the inter-scar distance to compensate for the added sediment depth. If this sort of phenotypic plasticity exists within the plant why does the plant elongate the short shoot and not the leaf. The short shoot may act as a storage device that increases the likelihood of survival during periods of resource depravation. Extending of the lower leaf would add to the respiratory demand. Since the lower leaf does not produce energy, this tissue would add to the stress

of the plant in times of resource limitation. Another possibility why the short shoot seems more responsive is that the structure of the leaf, especially the lower leaf, is less capable of a phenotypic response. This would suggest a hypothesis where short shoot elongates to position itself at the optimum sediment depth level. Future studies could document seasonal changes in growth patterns. Future studies could also experiment with shading and sedimentation to determine how these factors affect the structure of the short shoot.

The short shoot likely affects energy flow throughout the plant. Primary production occurs above the short shoot, while primary storage occurs below. There was some evidence in this study that allows the hypothesis that the short shoot acts more as a conduit rather than a storage device. First, the limited life span of the roots attached to the short shoot are an indication that roots are transferring resources in an upward direction to support primary production. If the roots were providing resources that were being moved downward into the rhizome, it would be likely that the roots would stay alive longer. Clonal integration is likely a bi-directional process in the short shoot. This makes energy transfer similar to a pipe flow problem. The short shoot likely needs to devote more of its volume to resource movement. These are areas for future studies. Likely the most meaningful studies will involve tracers that follow the flow of energy within the plant.

The results showed that number of scars is strongly related to the length of the short shoot (R^2=0.68). It was also noted that some of the unexplained variance lies in the irregular distances between the leaf scars. Earlier it was hypothesized that sedimentation events may be the cause of the increase in inter scar length. Another possibility is that the

greater inter-scar distance, which equates to greater biomass, represents localized resource storage. If the plant was working at absolute maximum photosynthetic capacity and all metabolic, growth and storage needs were met, this could be the last alternative for storage of excess energy. Future studies could use tracers to determine whether increased inter-scar length represents an increase in above-ground production. A central component of this question is whether the plant, while in these high production states, adds biomass in an even or uneven distribution throughout the plant. In an uneven distribution, the short shoot may be the last component of the plant to add biomass, and this may account for the added biomass between leaf scars.

A final alternate suggestion could be that these larger inter-scar distances represent failed attempt to establish a new rhizome runner. The added distance could represent space, and a build-up of resources to support a new rhizome runner. Many of the cases where a new rhizome runner emerged out of a short shoot had an abnormally large inter scar gap. A chemical or hormonal signal may start the process of initiating a new rhizome runner. At some point in development the signal may change or the short shoot may not have crossed a threshold of resource availability to generate the runner.

Short shoot diameter was related to the width of the leaf. This may represent a general physical requirement that a leaf with a greater width requires a greater area of attachment. Leaf movement in the water column is driven by currents. The currents in Florida Bay are often wind driven. This means that the leaf will be moved in multiple directions, and that drag force will be exerted in multiple directions. A greater leaf area will represent a greater drag force. There have been studies on drag force, but mechanical wear on the leaf is not just a question of current speed, but direction changes

and turbulence. It would be the turbulence that would impose a torque on the leaf. In the evolution of Thalassia testudinum, nature seems to have selected for a leaf that is quite flexible in its movement in the water column. This flexible waving response allows all the leaves attached to the short shoot to move in an individual and separated pattern. This may allow all the leaves on the short shoot access to the light penetrating to the canopy. In states of quiescence, or high currents this pattern is disrupted. However, in the case of slow currents or back and forth water movement, drag and torque forces will be applied unevenly to the leaf. The greater the diameter of the attachment point, the more the resistance to these forces are distributed.

Section Five

Lower Leaf

Results

The lower leaf of the *Thalassia testudinum* plant is not photosynthetically active. Tomlinson (1966) was able to differentiate the lower portion of the leaf so that it is considered separate in form and function. The lower leaf is initiated at the crown of the short shoot. In this study new leaves were observed to be a brown, thinner version of leaves already present on the short shoot. The new leaf grows in the center of the leaf cluster, enveloped by the older leaves. Tomlinson describes the early stage of leaf development as a period of elongation and widening. Once the tip of the lower leaf reaches an abscission point, elongation slows to what is essentially zero growth. Tomlinson describes a progressive change along the length of the lower leaf where the tissue becomes more vascularized near the abscission point. The leaf abscission layer, which is at the crest of the lower leaf, becomes the site of leaf elongation. The elongating green leaf tissue is the site of primary production. The top portion of the lower leaf effectively acts as a basal meristem.

The leaf plastochrone interval is an important component of the lower leaf productivity estimate. The plastochrone interval is defined by the days between the emergence of two sequential leaves. Once a new leaf crosses the abscission point, the plastochrone interval is set for that leaf and the sequence and the timing begins for the next leaf. Using the plastochrone interval for quantification of lower leaf biomass is

problematic for several reasons. The first two concerns center around the assumption

that there is only one new leaf growing at a time. A newer leaf may already be growing

before the preceding leaf reaches the abscission layer. Alternately, there may be a time

delay in the initiation of a new leaf even though the older leaf has reached the abscission

layer. The third confound is the assumption that all lower leaf biomass is fixed at the

abscission layer. As the leaf ages, there is a minimal elongation in the lower leaf. These

factors do introduce some error into the estimate, but the plastochrone interval is a

chronometer to measure growth.

The average lower leaf area for the Rabbit Key Basin population was 4.69 cm^2

(Table 41), and the average weight 0.012 (g). Lower leaf area is a good predictor of dry

weight biomass (Figure 40), however, developmental factors appear to be affecting this

relationship.

Since both the leaf plastochrone interval and the lower leaf data were collected for

individual short shoots, a productivity estimate based on actual values can be generated

(Table 41).   The weight of the lower leaf was divided by the plastochrone interval to

compute a weight added per day. The average for the group where weight and the

plastichron are known is 0.00052 g, which differs slightly from the population estimate

presented in Table 41 (0.00055 g). For the population at Rabbit Key Basin adding lower

leaf biomass at an average of 0.69 g dry weight biomass added per m^2 per day.

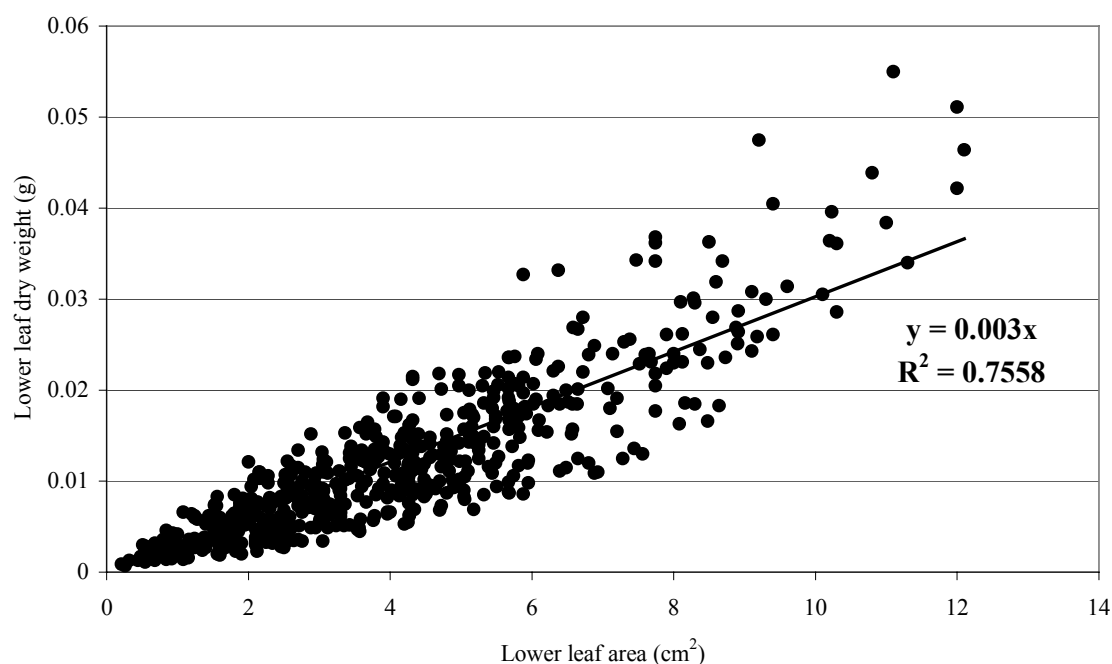**Chart of lower leaf area verses dry weight**

Figure 40. Chart of lower leaf area (cm^2) plotted against sample weight (g). Trend line for the linear function is also included.

Table 41. Estimates of lower leaf production for Rabbit Key Basin.

Measure	Population mean Std dev. n	Estimate for m^2 at Rabbit Key Basin
Lower leaf area (cm^2)	4.13 cm^2 2.03 cm^2 559	4539.69 (cm2)
Lower leaf weight (g of dry weigh biomass per day)	0.012 (g) 0.008 (g) 559	15.95 (g)
Leaf PI	24.7 days 5.23 115	
Productivity (g of dry weigh biomass per day)	0.00055 (g of dry weigh biomass per day)	0.72 (g of dry weigh biomass per day)

Section Five Discussion

The estimate of lower leaf productivity differs from the upper leaf for the reason that lower leaf elongation halts or slows once the new leaf reaches the abscission layer. At that point, the total leaf area will continue to increase, but the lower leaf area would not add biomass. The site of leaf elongation is the abscission layer that separates the upper and the lower leaf. This means the lower leaf has reached its maximum biomass early in leaf development. The simplest way to measure this growth is to modify Zieman's productivity method (1974). His method employs a needle puncture mark just slightly above the abscission layer. The plant is allowed to grow and the puncture point marks the growth on the upper leaf. In theory the same method could be used, simply moving the puncture location to immediately above the short shoot crest. There are two problems. First, sometime the exact location of the short shoot crest is difficult to determine. Second, the new leaf tissue is very delicate in the early stages. A puncture point may damage the new leaf or snag it so that growth is folded in an accordion like fashion.

Another alternative, since the lower leaf does have a discrete period of growth, would be to measure the leaf plastochrone interval and perform calculations similar to those in Table 41. The weights could be measured directly or could be computed using the predictive equation lower-leaf-weight=0.003*leaf-area. Referring back to Section One of the discussion, this equation predicts both upper and lower leaf weights. The drawbacks were discussed. There is likely to be a little error introduced by new lower leaves already growing, or delayed by an unknown time step. But this error is likely to

be small, and since it isn't practical to determine the exact moment that a new leaf emerges beyond the abscission point, it can be considered part of the overall sampling error.

The final issue to address is the minimal amount of lower leaf elongation that takes place as the leaf ages. In this study it was often observed that the oldest leaf on the short shoot was longer in length than the younger leaves. While the younger leaves seemed to joined, wrapped, or intertwined around the abscission point, the oldest leaf gave the appearance of being disassociated from the general leaf bundle. Likely the older leaf is approaching senescence and the repeated motion of the leaf may have pulled the old leaf away from the cohesive envelopment. Another possibility is that a structural change occurs that releases the older leaf from the abscission layer envelopment bundle. Once a leaf loses the added strength of the bundle there may be added wear and tare which accelerates senescence. It could be hypothesized that the short shoot increases the probability of survival by releasing old, less productive leaves, and thereby increasing the light field for the younger leaves. The appearance of extended length of the lower leaf may simply be a recycling of chlorophyll. It also could represent a late developmental elongation that accelerates the senescence of the leaf. Future studies could more accurately determine the processes that are occurring.

Section Six

Issues of clonal integration and overall summary

Results

Data from individual short shoots were compiled and used to determine above-ground structure and dynamics, for example leaf area added per day. The case presented in Figure 41 would be an illustration of the type of data that was collected. This particular figure represents a case where three short shoots on the same rhizome runner were tagged. The orange lines represent four leaves from the short shoot number 1592. The x-axis represents time and therefore the second orange line, from the left, indicates the a new leaf that emerged around day two of the study. If that line is followed through time, the figure shows that senescence took place around day 59. The blue and the black lines represent leaves on two other short shoots on the same rhizome runner.

Around day 24 the growth slope for the second leaf on the black plant changes. This is noteworthy because soon after the growth slope of the third leaf on the orange plant also alters its growth slope. The change in slope and duration are almost identical for the two leaves on different short shoots. Around day 38, the slope for both leaves on both short shoots becomes steeper. The leaves appear to return to a rate of growth similar to the early stages of its development. In the same approximate time frame both short shoots appear to have new leaves emerging. Additionally, the blue short shoot, which is the youngest of the three, has new leaves emerging in this same time frame. The same pattern appears to repeat around day 56 of the study.

These data are from a single case, however this case may represent a piece of evidence of the clonal integration and resource allocation that has often been mentioned in this paper. In this study, there were very few tagged short shoots on the same rhizome runner. If a future study methodology was to expand the sampling area to increase the probability of tagging multiple short shoots on an individual runner, more would be know about the pattern described in the previous paragraph.
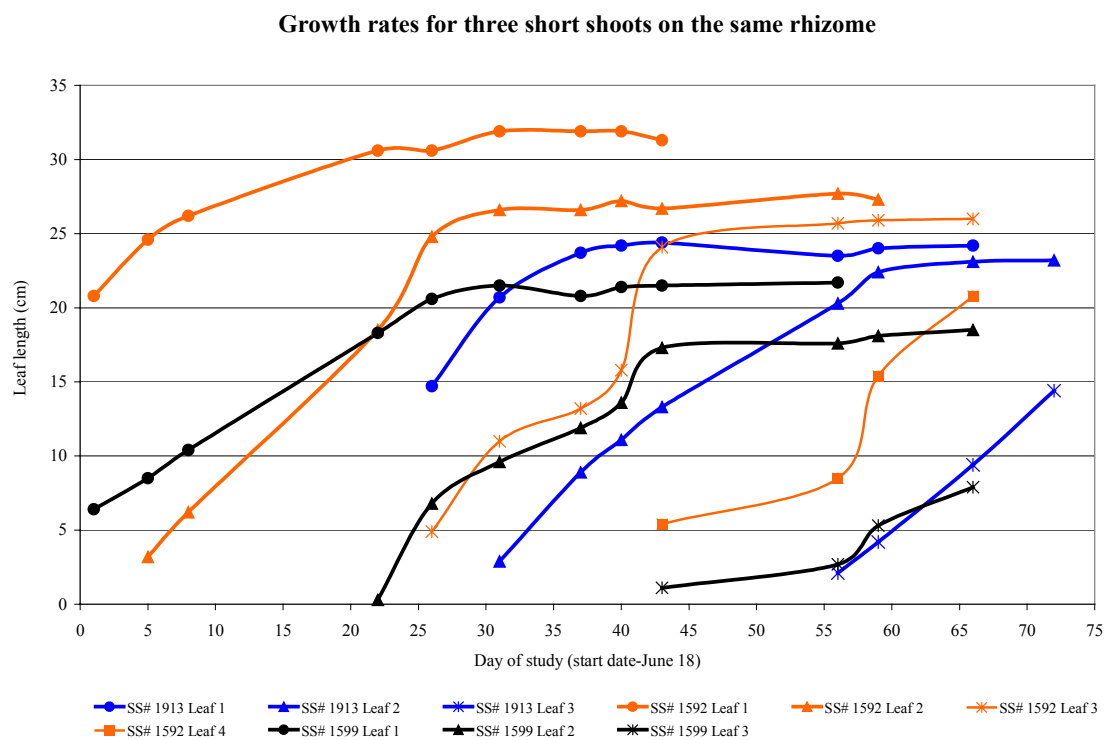
Growth rates for three short shoots on the same rhizome

Figure 41. Chart of leaf length (cm) plotted against day of study that leaf length was measured. Leaves from three short shoots on the same rhizome runner are included in the chart.

Section Six

Summary discussion and conclusions

The success of this study was dependent on structural consistencies of the *Thalassia testudinum* plant. The most meaningful results would have been obtained if patterns and ratios of biomass allocation and growth were completely predictable. This study documented moderate structural variability, but primarily it documented a high degree of correspondence between the different components of the plant. In some cases, such as age of rhizome verses distance to apical meristem tip, the relationships were strong. In other relationships much of the variance was unexplained. Each of the five sections ended with the conclusion that further study would be helpful in accounting for the unexplained variance in these relationships. This last section will utilize the explained and the unexplained variability to suggest models that describe the structure and function of this plant. This section will also draw conclusions about the usefulness of above-ground measurement as a proxy for below-ground production.

The strongest predictor of below-ground structure was leaf area added per day. In the study of biology, physiology or ecology, it is unusual that any two factors or variables have a perfect covariance, and this was true for these two variables. The interplay between individual population members and the surrounding ecosystem leads to a complex set of interactions. In light of that complexity, the variance that was explained by leaf area added per day, in this example and others, provides evidence that above-ground dynamics are a good proxy for below-ground structure. Future investigations

must move from univariate to multivariate studies to explore other factors that may be affecting these relationships.

In this study, the pattern of below-ground structure provided an indication that, population regularity can be expected and exploited. The regularity reported in this study can be confirmed in future studies. The irregularities provide a focus for future studies, but also provide a starting point to advance theoretical constructs. It is important to have a clear grasp of range of structural patterns. It is even more important to understand the factors that affect those changes. From a theoretical standpoint it is useful to consider is what a plant gains and loses by structural regularity.

Any normally distributed population has a structural regularity. Parents and their offspring may regress to and away from the mean, but most populations have a structural consistency. For example, the average rhizome segment length for this study population was approximately 6.0 cm, with a standard deviation of 2.0 cm. Therefore, most plants in Rabbit Key Basin are assumed to have segment lengths between 4-8 cm. Evolutionary theory would hold that this reflects a characteristic that maximizes survival. The same argument would be true for other characteristics such as diameter and volume. Influences within the plants (e.g. genetics) and the ecosystem (e.g. nutrients) have interacted to affect these structural features. In terms of the population at Rabbit Key Basin, it appears that the important rhizome characteristic is volume. The primary evidence of this is that while rhizome length varied segment to segment, volume was held relatively constant. It appears that holding volume constant may be a factor that helps maximize survival of the species at this site. If conditions at Rabbit Key Basin were to change, it could affect a change in population characteristics to a different structural regularity. This argument

can be applied to plants living at other sites in and outside of Florida Bay. This study demonstrated a certain regularity, but it is a regularity that may be unique to Rabbit Key Basin.

Structural regularity could be the result of several mechanisms. First possible factor could be the plant's regulation of resource flow. When considering the fundamental processes that would maximize survival in a clonal plant, resource allocation is an important issue. An individual ramet will initially be dependant on resources provided by older parts of the plant that have an energy surplus. In a viable clonal plant the development of the ramet progresses from being an energy sink to an energy source. Once the ramet has met its metabolic demand, excess energy can be allocated to new growth, storage, or it can be translocated to different parts of the plant. Translocation of resources is assumed to be directed to younger areas of the plants. These are the locations that are not yet able to meet their own metabolic demand. However, survival may also depend on backwards translocation in times of resource deprivation.

The second factor that could be controlling structural regularity is resource storage. Individual changes in segment volume may be a physiological response by the plant to increase the odds of survival. The data from this study demonstrated that the amount of attached leaf area and the overall productivity of a short shoot was individually quite variable. In the case of short shoots with greater amounts of attached leaf area, metabolic demand will be commensurate with the greater amount of tissue. If those short shoots, because of circumstantial changes, are unable to meet their metabolic demand, they becomes reliant on stored or translocated energy. To maximize survival it makes mechanistic sense to have metabolic demand proportional to the energy storage capacity.

Likewise, short shoots with a lower attached leaf area will have a lower metabolic demand and a lower requirement for energy storage in the case of resource limitation.

Another factor controlling plant structure is genetics. A major question is the degree to which genetics verses resource availability control the architectural structure of the plant. A site like Rabbit Key Basin makes this determination a little more difficult. The population is evenly distributed which likely suggests an evenly distributed resource base. In the case of an adequate and evenly distributed resource base, genetics is likely the controlling factor in plant structure. In the case of an ecosystem with a patchy resource base there is likely to be an interaction between genetics and phenotypic plasticity. As the population density increases then living space itself becomes a very important resource. Phenotypic plasticity may then become more of a controlling factor. The short shoot density in Rabbit Key Basin is high and space may be a resource that controls below-ground structure.

Corresponding changes in above and below-ground structure and productivity may be an evolutionary survival strategy. This study looked for patterns of synchronous changes in the physiological characteristics of the plant. Sequential rhizome segment lengths showed considerable variability. The segment diameters did not vary as much, but a distribution is easily observable (Figure 8). These data would suggest that plant has a basic structural framework that could be responsive to localized stimuli. This poses a question. What would be the survival advantage of changing the length of a rhizome segment? In terms of providing resources via translocation, the closer the short shoots the more efficient the transport. Also, in an ecosystem with a patchy resource base it may be a benefit to initiate a sequence of short shoots in a close area. The disadvantage would

be that two short shoots might be exploiting the same exhaustible resource base. Also, proximal short shoots are also more susceptible to localized disturbance from waves, storms, grazing, or boats. In considering longer rhizome segments, the opposite line of reasoning applies. In this case, sequential short shoots are less likely to be competing with each other, but translocation occurs across a greater distance. Similar arguments can be used when considering changing rhizome diameter in terms of a survival strategy. A greater diameter equates to a greater capacity to store resources. It also is associated with a greater metabolic demand.

This brings up the issue of metabolic demand. With its substantial rhizome volume *Thalassia testudinum* has the capacity to survive periods of resource limitation. Rhizome volume was seen to be invariant with depth. Theoretically, plants with low rhizome volume located at greater depths are likely to be the first to show signs of stress in times of resource limitation. This could be tested in future studies where resource limitation was imposed upon a sub-population of plants. In the simplest case, light could be attenuated at a much greater rate, or even totally shaded for extended periods of time. Once the plants start to show signs of stress or die, the area can be extracted and analyzed to determine which plants where showing the greatest signs of stress or determine which were the first to die. This proposed future study is governed by the assumption that all plants are equally stressed by the lack of a necessary resource, light for example. The research question is based on the assumption that all shaded plants will be depleting stored resources. If the study found that a higher percentage of plants at greater depth were to die off first, it would suggest that the rhizome storage is critical to survival during periods of stress.

Determining how the above and below-ground metabolic demand would make the plant more or less susceptible to stress and die-off would provide valuable information. When primary production is reduced or eliminated, plants with greater reparatory demands are going to draw on reserves more dramatically. Long term survival of the plant is dependent on how the plant budgets its energy. A plant that budgets all energy to growth may die in times of resource limitation. There may be an energetic efficiency that is linked to the survival of the species.

An example of this energy efficiency is the life cycle of the short shoot. *Thalassia testudinum* short shoots develop through stages of energy dependence, energy equilibrium, and energy excess and export. As the short shoot gets very old it may return to a state of energy equilibrium or ultimately it may renew its draw of resources until it dies. Natural selection has created a system where the life span of a species is related to the length of time it takes to bring the offspring to viable self sufficiency. Beyond that point the parent no longer contributes to the survival of the species. Therefore, nature will not select for biological processes that extend the life of the parent. A diminished capacity for tissue regeneration, and cellular breakdown lead to the eventual death of the organism. While *Thalassia testudinum* does reproduce sexually, survival of the species is also accomplished by clonal growth. This appears to be the primary method of propagation in Rabbit Key Basin. Survival of the plant is predicated on keeping the apical meristem alive. It is this merstimatic tissue that initiates all asexual reproduction. Another important survival strategy is keeping new short shoots alive. Loss of a new or young short shoot would not doom the plant. However, the system is dependent on new

short shoots eventually assuming the energy burden to support below-ground production. So continued loss of young shoots would jeopardize the survival of the plant.

Within the conceptual framework of the evolutionary model, plant components may change their function with time. The rhizome runner is vector for resource transport that will diminish as individual segments get older. The point where an individual rhizome segment is no longer critical to the clonal integration, it functionally becomes a localized storage unit. This may explain the shrinking diameter of the rhizome with age, and the greater density per unit volume. It remains unclear what exactly the rhizome is storing or transporting. Future studies need to address the weight to volume ratio change. Understanding in the pattern of constituent dynamics, such as changes in percentages of soluble carbohydrates, will further the understanding of this species survival.

One of the undetermined factors controlling below-ground production is the degree to which meristimatic tissue is responding to localized resource availability or genetic forces. It is also not clear whether the meristematic tissue is responding to the level of productivity of mature short shoots. The apical meristem may be fixing biomass in response to translocation of resources from short shoots with energy surpluses. This energy or resource flow may signal the productive capacity of the plant. Theoretically, the volume of the new rhizome segment is under genetic control. The actual volume being fixed could be viewed as the degree to which the plant is reaching its genetic maximum potential. Variation in volume may be occurring due to the productive capacity of older short shoots. This may allow a similar potential productive capacity of new short shoots. In theory, the plant may be fixing a rhizome volume that will allow the maximum productive capacity of the next short shoot to be initiated. These theoretical maximum

productive capacities are under the control of resource availability. In summary a plant may have fixed the volume of rhizome based on antecedent, rather than immediate stimuli. This theoretical construct could account for some of the unexplained variance in the leaf area added per day to volume relationship.

The sediment chemistry is another issue closely related to metabolic demand of the *Thalassia testudinum* plant. One of the adaptive abilities of this plant is the capacity to move oxygen into the sediment. There are several beneficial outcomes of this oxygen transport. First is alteration of the oxidation state which changes the species of a nutrient and allows greater energetic yield. Second is facilitation of decomposition which allows the quicker recycling of nutrients. The sediment cores showed a distribution of decreasing amounts of dead tissue, or dead tissue recognizable as a particular plant part. This would suggest that there is enough oxygen throughout the sediment to facilitate decomposition. This is an important point, especially at Rabbit Key Basin. If the sediment was in a steady state of anoxia, then very little decomposition of tissue would take place. This would affect the rhizome and root growth because competition for space in the growth matrix appears to be significant. The complexity of the growth matrix appears to affect the pattern of an individual small-scale growth. The extracted plants exhibited twits and turns in their structure. These small scale changes in growth patterns provide an area of future research. Studies on how the complexity of the growth matrix affects the length, diameter and volume of this species would improve the understanding of below-ground dynamics. In the most extreme cases, demand for space is high enough that younger rhizome and roots were observed growing into the husk of dead rhizome segments. In summary, the transport of oxygen to the sediment is important as it alters

sediment chemistry which allows decomposition which increases the availability of growing space.

Another advantage to oxygen transport to the sediment is the reduction of sulfide toxicity. The trend in the elemental constituents showed an increasing presence of sulfur as the rhizome tissue aged. If sulfur was essential for growth it would have been detected in the apical meristem segments. So the trend of increasing sulfur may represent an intrusion of this element. Intrusion may correspond with a decreased capacity to pump oxygen.

One of the clear deficiencies of this study was the single study site. Site specific elements of this study have to eventually be addressed. *Thalassia testduinum* is a seagrass that shows a variable morphology corresponding with sediment type, sediment depth, and the physio-chemical characteristics of the sediment and water column. An important follow up to this study would be to test some of these relationship in various basins around Florida Bay. These basins have a broad range of sediment types and depths. To get a comprehensive understanding of this species, similar study methodologies can be employed for *Thalassia* populations throughout Florida, the Gulf of Mexico, and the Caribbean.

Energy and resource flow were often central to the discussions in this paper. They were hypothesized to be a factor in the form and function of this plant. The basic hypothesis of this study was that above ground production, by necessity, must describe below-ground production. Primary production only occurs in the above-ground portion of the plant. Above-ground photosynthesis is the sole source of energy to meet all the respiratory demands of the non photosynthetic tissue that reside below the sediment

surface. If this study was not able to find clear, well defined relationships between local production (leaf area added on short shoot) and local biomass characteristics (the volume and weight of the associated rhizome), then future studies have to be expanded to a broader scale. The most intriguing future research will attempt to determine the pattern in which the plant distributes energy and resources.

Though it is a single case, one finding that may be early evidence of energy distribution throughout the plant is presented in Figure 41. The syncopated change displayed in this figure could be caused by multiple antecedents such as a shading event, or a rise or fall in temperature or salinity. The alternative to this disturbance hypothesis is that these two short shoot diverted energy from leaf elongation to some other purpose. This hypothesis is supported by the occurrence, around day 56, of another syncopated change in growth slope. The final point to make is that these growth slope changes correspond to the emergence of new leaves on the blue short shoot. The blue short shoot is the youngest of the short shoots in the sequence and may still be dependent on resources from the older short shoots.

There isn't sufficient data to support the hypothesis that clonal integration explains the patterns observed in Figure 42. But if the Thalassia testudinum plant does have a pattern of resource allocation that occurs beyond a local scale, then photosynthetic activity might not be manifested in a steady state fixing of biomass at predictable points. This is the area of research that could resolve much of the unexplained variance presented in this study.

The key points of this study are as follows:

- Below-ground structure has an exploitable regularity

- Univariate measures of leaf dynamics have a moderate correspondence with below-ground structure. Future multivariate, and experimental studies may account for sizable percentages of unexplained variance in the relationship between above-ground dynamics and below-ground structure.

- Other, static, above-ground measures such as short shoot age also displayed moderate to strong covariance with below-ground structure and estimated productivity.

- Roots growing off rhizome displayed patterns of extended growth. This pattern of growth is not consistent with roots attached to short shoots.

- Roots attached to short shoots displayed three distinct patterns of growth which may indicate a differentiated root productivity based on function.

- Short shoot productivity based on sample weight and scar age can provide productivity estimates. However, uncertainty in the conversion of scar age to age expressed in days affects the accuracy of the estimate.

- Clonal integration may be affecting the observable correspondence of above-ground dynamics to below-ground structure and productivity. Early evidence of an integrated physiological response provided some evidence to support this assumption. It also demonstrates the need to better understand resource flow and energy transport within the plant.

In summary, this study has presented, in its early stages, information about the structure of the buried portions of the *Thalassia testudinum* plant. This study has also proposed improvements for estimating below-ground production. The data that were presented provide objectives for future research. The first aim of future research would be

studies that address site specific differences in the patterns and relationships described in this study. Second, multivariate experimental studies need to be conducted to determine if above-ground production can better describe below-ground productivity. Third, and most important, future studies need to advance the understanding of energy production and resource distribution in *Thalassia testudinum*.

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Appendix A

Process for determining leaf biomass estimates

Using the leaf area estimates and the following computations, the dry weight biomass per short shoot can be estimated. These estimates are for the population at Rabbit Key Basin.

Dry Weight Leaf Biomass Per Short Shoot=(0.003381*51.95)   Equation A1

Dry Weight Green Leaf Biomass =(0.003381*38.94)   Equation A2

Dry Weight Lower Leaf Biomass=(0.003381*13.25)   Equation A3

These computations give the following values:

Dry Weight Leaf Biomass Per Short Shoot=0.177 grams   Equation A4

Dry Weight Green Leaf Biomass Per Short Shoot =0.132 grams   Equation A5

Dry Weight Lower Leaf Biomass =0 Per Short Shoot=0.045 grams Equation A6

Using those estimates along with the short shoot density estimates, leaf biomass per meter^2 can be estimated:

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Dry Weight Leaf Biomass Per Meter^2=0.177*1318 = 233.29 grams

Equation A7

Dry Weight Green Leaf Biomass Per Meter^2=0.132*1318 = 173.98 grams

<div align="right">Equation A8</div>

Dry Weight Lower Leaf Biomass Per Meter^2=0.045 *1318 = 59.31 grams

<div align="right">Equation A9</div>

This process of determining biomass was repeated for short shoots and rhizome tissue. The results for all estimates are reported in the results and discussion sections of this paper.

Appendix B

This is a graphic representation of data that was presented in the main body of the results section. Theses values were reported in Table 27 in section two.
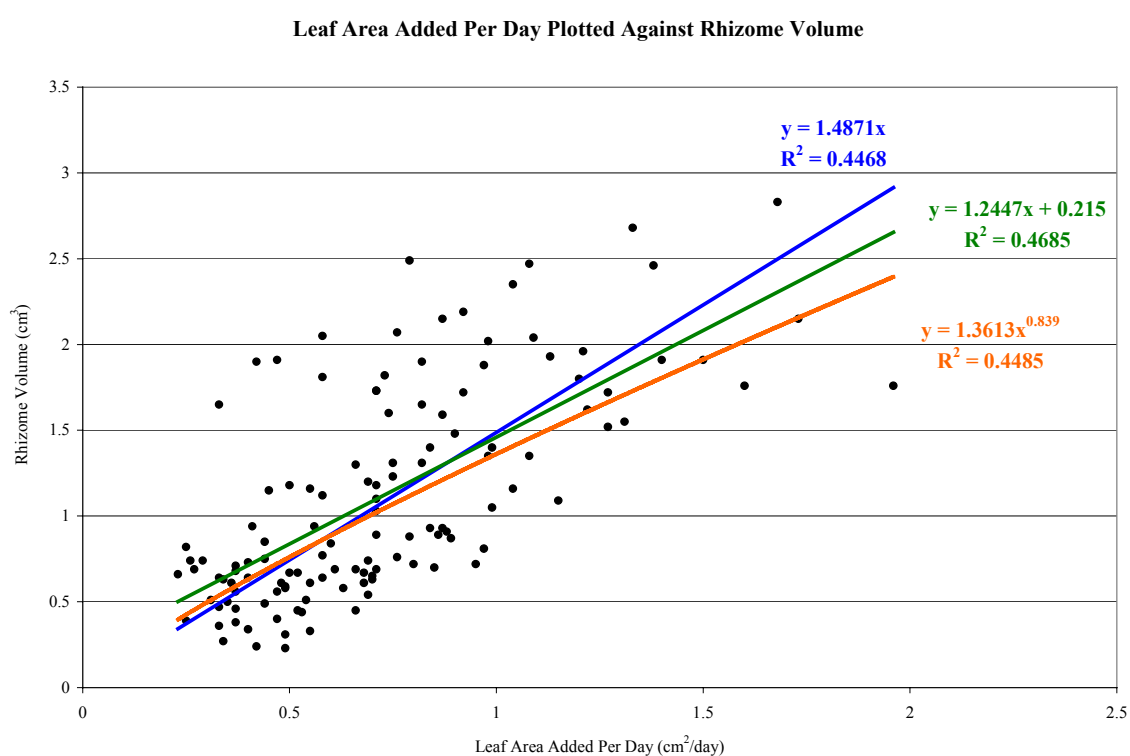
Leaf Area Added Per Day Plotted Against Rhizome Volume

$y = 1.4871x$
$R^2 = 0.4468$

$y = 1.2447x + 0.215$
$R^2 = 0.4685$

$y = 1.3613x^{0.839}$
$R^2 = 0.4485$

Figure 1-Appendix B.   Chart of leaf area added per day plotted against associated rhizome segment volume. Trend lines for linear and power functions are included.

Appendix C

Methodology used to determine estimates of rhizome productivity. Two cases are presented. The first is the methodology presented by this study, the second is Patriquin's method.

1) Leaf area added per day predicts rhizome volume using the power function presented in Table 27 or Figure 1 in Appendix B.

2) The volume was used to predict the weight using the function derived from the weight to volume relationship presented in Figure 11 Section 2

3) Leaf area added per day was used to predict the time interval (days) between successive short shoots (Figure 15 Section 2)

4) Weight was divided by the time interval to generate the productivity estimate.

5) Mean of individual cases was computed.

The time interval between short shoots for Patriquin's method was computed by multiplying actual leaf plastochrone interval values by the scar difference between successive short shoots The weights used for the Patriquin method determination were the actual weights of the segments. Patriquin's estimates used the following steps:

1) Time interval (days) was computed.

2) Weight was determined

3) Weight was divided by the time interval

The mean for all individual cases was computed.

Appendix D

This is a chart of an incomplete data set. This chart shows the length of sequential rhizome segments plotted against each other. Cases from the overall data set have been selectively excluded if they displayed a pattern of long-short-long, or short-long-short. These data are meant to be complementary to the data presented in Figure 7. The cases in that Figure presented patterns of long-short-long where the length of the long segments we approximately the same lengths.

Plot of sequential segment lengths with 30% of the cases removed from Figure 7
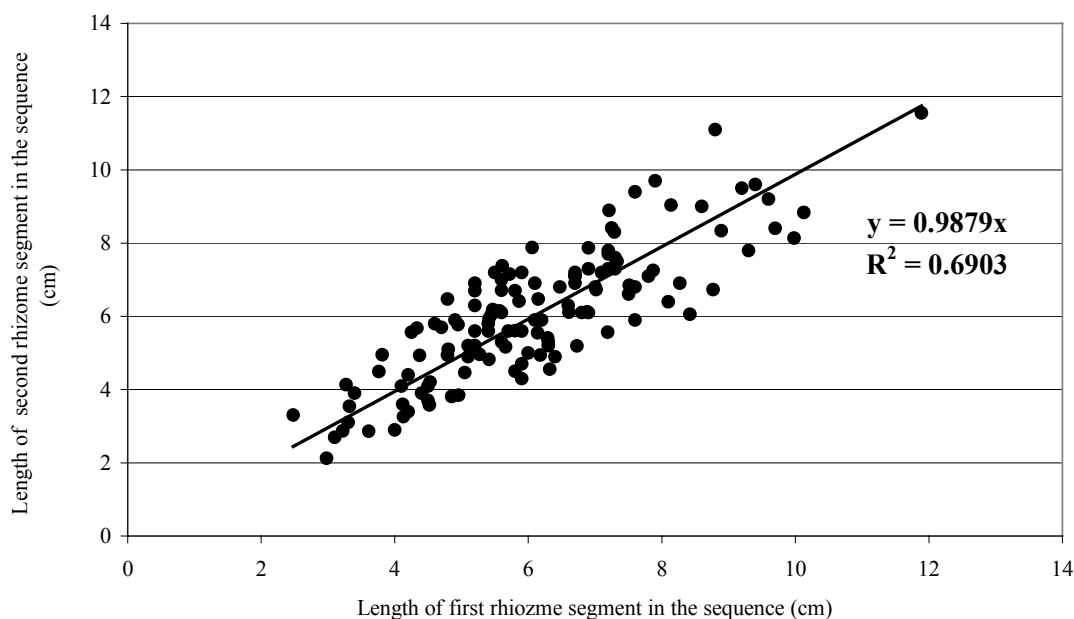

$y = 0.9879x$

$R^2 = 0.6903$

Figure 2-Appendix D. Chart of sequential rhizome segment lengths. These data are selected cases from a larger data set.

Appendix E

The following equations can be used to estimate population parameters for *Thalassia testudinum* living at Rabbit Key Basin:

Rhizome Volume (cm^3) = 1.3613*leaf area added per day$^{0.839}$ (r^2=.47)   Equation A10

Rhizome Volume (cm^3) = 0.1251*LeafArea$^{0.562}$ (r^2=.27)   Equation A11

The following equation will be used to convert volume to a dry weight biomass:

Rhizome Biomass (g) = 0.1492*Rhizome Segement Volume$^{0.9389}$ Equation A12

The final requirement is determination of a rate. This calculation uses the fundamentals of Patriquin's (1974) method. The plastochrone interval and the number of scars between sequential short shoots are used to estimate the number of days the rhizome grew between short shoots. Those data were then regressed against leaf area added per day to produce the following formula:

Time interval between short shoots = 148.73 + (-63.31*leaf area added per day).

Equation A13