2008

Evaluating ecological equivalence in created marshes

Christopher Bromley Llewellyn
Louisiana State University and Agricultural and Mechanical College

EVALUATING ECOLOGICAL EQUIVALENCE IN CREATED MARSHES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements of the degree of
Master of Science

in

The School of Renewable Natural Resources

by
Christopher B. Llewellyn
B.S., Texas A&M University, 2006
December 2008

DEDICATION

I would like to dedicate this work to my grandparents, Murton and Bertha Llewellyn and Clint and Betty Burks, as well as my parents Murton and Brenda Llewellyn. Their constant love, support, and encouragement has enabled me to pursue my interest in science and I owe them a great deal of thanks.

ACKNOWLEDGEMENTS

This project was funded by the Louisiana Department of Wildlife and Fisheries. The staff at the Sabine National Wildlife Refuge provided logistical support.

I would like to thank my major professor, Dr. Megan La Peyre, for her help and guidance through graduate school. I would also like to thank my graduate committee, Dr. Brian Fry and Dr. Ken Brown, for their input and instruction on food web ecology and stable isotope analysis. I would also like the thank Bryan Gossman, Whitney Gayle, Bryan Piazza, Mason Piehler for their tireless help in the lab and field.

Finally, I want to thank my wife, Katie Llewellyn, for all of her patience, support, and understanding, including the long hours spent in the lab helping me pick hepatopancreas tissue out of blue crabs.

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ABSTRACT

The overall goal of coastal wetland restoration is to achieve ecological equivalence through the reproduction of structural and functional characteristics. This study sought to examine ecological equivalence using a chronosequence of temporal replicates of created marshes using traditional structural measures of equivalence and tested the use of stable isotopes as a measure of functional equivalence. The objectives of this study were to: (1) compare measures of structural equivalence at created and reference marshes; (2) use stable isotope analysis of blue crab muscle and hepatopancreas tissues to compare functional equivalence at created and reference marshes; and (3) determine if there is any age effect indicative of marsh development trajectories. The study was carried out at four marshes created with dredged material (5–24 years old) that were each paired with adjacent reference marshes on the Sabine National Wildlife Refuge, Louisiana during the spring and fall of 2007. At each marsh, quantitative measures of structural equivalence and functional equivalence were collected. Paired contrasts indicated that created and reference marshes supported equivalent plant and nekton populations, but differed in soil characteristics. Specifically, created marshes had consistently lower soil organic matter compared to reference marshes with no apparent age effect. A laboratory study was conducted in order to determine blue crab tissue specific isotope turnover rates. The hepatopancreas tissue had a half-life of approximately 10 days while the muscle tissue had a half-life of approximately 22-39 days. Comparison of mean hepatopancreas and muscle $\delta^{13}C$ and $\delta^{15}N$ isotope values found that the blue crabs at the youngest created marsh (5 yrs.) occupy the lowest trophic position compared to all other marshes. Overall, the results indicate that while vegetation and nekton community characteristics suggest that structural equivalency is achieved relatively rapidly (< 5yrs), functional equivalence, as measured by trophic support, may take longer to occur (> 8 years). Stable isotope

techniques may give coastal managers an important tool to investigate the complex trophic connections within these estuarine food webs and to determine if and when these marshes will achieve functional equivalence.

CHAPTER 1: GENERAL INTRODUCTION

Coastal wetlands in Louisiana are of enormous economic, ecological and cultural importance, covering approximately 3 million acres of land. Unfortunately, due to a combination of anthropogenic and natural causes, ranging from sea level rise to levee construction, Louisiana is losing approximately 61.3 $km^2 yr^{-1}$ of its wetlands (Barras et al. 2003). In an effort to combat the loss of these critically important wetland habitats, coastal managers, scientists, and lawmakers are working together in an attempt to slow marsh loss and restore critical fishery habitat. The Coastal Wetland Planning, Protection and Restoration Act (CWPPRA) provides approximately $70 million annually, designated specifically for wetland restoration, protection, and planning along coastal Louisiana. This money is used to fund a vast array of restoration projects including marsh creation, shoreline stabilization, and barrier island creation.

Coastal restoration and marsh creation are important tools for coastal managers to offset the loss of coastal wetland habitats. The overarching goal of all marsh restoration and creation projects is to build marshes that are ecologically equivalent to unaltered, or "natural" marshes in the area, by reproducing both the structural characteristics and the functional services these marshes provide (Strange et al. 2002, West et al. 2003). However, to date, structural characteristics, such as vegetated stem density, canopy structure, and nekton (fish and decapod crustaceans) density are commonly used in monitoring projects to determine success. These indicators are used due to their ease of collection with the assumption that structural equivalency is indicative of functional equivalency. This approach has become known as the Field of Dreams hypothesis which assumes that structural equivalency begets functional equivalency, or, "if you build it, they will come" (Palmer et al. 1997).

A number of monitoring studies have examined the ability of created marshes to provide

suitable nekton habitat by measuring nekton density, abundance, and biomass along with water quality and vegetation density measurements. These studies have yielded mixed conclusions on the equivalency of the created or restored marshes assessed based on vegetation and different nekton indicators used (Minello and Zimmerman 1992, Rozas and Minello 2001, Able et al. 2004, Bush-Thom et al. 2004). As fish are highly mobile organisms that are capable of recolonizing created sites fairly rapidly, structural measures such as abundance, density, and biomass may not be truly indicative of ecological equivalence as they do not address functional equivalency. More meaningful measures of habitat value such as nutrient cycling, nekton assemblage, community ecology, and trophic support provide more insight into the approximation of functional equivalency in created marsh systems (Moy and Levin 1991, Minello and Rozas 1997, Strange et al. 2002, McCay et al. 2003).

Recent work in marshes along the Gulf of Mexico has shown that while nekton biomass and density may be similar between some created and reference marshes, (1) created marshes supported lower densities of benthic-dependent species than reference marshes (Bush-Thom et al. 2004, La Peyre et al. 2007), (2) the general health of nekton species, measured by length-weight relationships, may be lower in created marshes (La Peyre et al. 2007), and (3) mean size of grass shrimp was significantly lower in created marshes (Minello and Webb 1997). This observed reduction in nekton productivity could be caused by lower soil organic matter found in created marshes which has been tied to reduced benthic invertebrate densities, a major prey source (Moy and Levin 1991, Minello and Zimmerman 1992, Sacco et al. 1994, Edwards and Proffitt 2003) The reduced soil organic matter and benthic invertebrate densities found in created marshes provides reduced trophic support to consumers compared to their reference marshes (Moy and Levin 1991, Minello and Zimmerman 1992). Increased trophic support within a system has been suggested to be linked to more stable food web structures, and ultimately, system stability and resilience (i.e., Rooney et al. 2006). An approach that attempts to

compare trophic support between created and reference marshes could prove to be a useful tool in monitoring the success of wetland creation projects.

Stable isotope techniques have been used for determining growth rates, diet quality, site fidelity, and trophic support within a marsh ecosystem (Fantle et al. 1999, Fry et al. 2003, Dittel et al. 2006, Wozniak et al. 2006). Animals acquire their isotopic signal ($\delta^{13}C$ and $\delta^{15}N$) from their diet, which is typically habitat specific. Any differences in the isotope signals of a species found in different habitats can help elucidate differences that may exist in the trophic pathways (Fry et al. 2003). Differences in the $\delta^{13}C$ and $\delta^{15}N$ ratio indicate the primary source of the diet, but can also provide information as to the protein and energy value of the diet (Fantle et al. 1999). While many studies have examined the importance of local food sources on the scale of kilometers to hundreds of kilometers (e.g., Findlay et al. 1996, Deegan and Garritt 1997, Bouillon et al. 2000), a recent study of *Fundulus* successfully examined differences in prey items, on a scale of several hundred meters using gut contents and stable isotope analysis (McMahon et al. 2005) suggesting that discrimination within small geographic areas is possible. The use of stable isotope analysis can provide a measure of functional equivalency in created marsh habitats.

The overall objective of this project was to examine ecological equivalence in marshes created using dredge material along a chronosequence of created marshes. I examined commonly used metrics to assess structural equivalence (chapter 2) and a less commonly used approach to assess functional equivalence in created marshes using stable isotope analysis (chapter 3). The objectives of chapter 2 were to (1) determine if there are any vegetation, nekton community or soil characteristic differences between created and reference marshes and (2) to determine if there is an age effect associated with equivalence at the created marshes. The objective of chapter 3 was to explore the use of blue crabs and stable isotope analysis to evaluate functional equivalence at created and reference marshes. Chapters 2

and 3 have been written as independent manuscripts with a separate introduction, methods, results, and discussion section. Chapter 4 is a comprehensive conclusion section that ties the results from chapters 2 and 3 together.

CHAPTER 2: MEASURING EQUIVALENCY OF CREATED AND REFERENCE MARSHES: COMPARISON OF VEGETATION, SEDIMENT, and NEKTON COMMMUNITY CHARACTERISTICS

INTRODUCTION

With over 90% of commercially important species caught off the Gulf coast identified as estuarine-dependent species, the coastal marshes in the northern Gulf of Mexico clearly provide highly productive habitats which support numerous nekton species (Chambers 1992, Peterson and Turner 1994, Minello 2000). In particular, marsh edge is well known for its importance to both resident and transient species (Baltz et al. 1993, Minello et al. 1994, Peterson and Turner 1994). Nekton densities are significantly lower in shallow non-vegetated habitats compared to nearby marsh edge habitats (Minello et al. 1994, Minello and Webb 1997, Bush-Thom et al. 2004). The high edge to open water ratio characteristic of the marshes in the northern Gulf of Mexico has been proposed as a reason for the high productivity of estuarine dependant fishes throughout the region (Minello et al. 1994). However, coastal Louisiana marshes are experiencing widespread land loss due to erosion, subsidence, and other anthropogenic causes at a rate of 61.3 km^2/yr (Barras et al. 2003).

In an attempt to offset this dramatic land loss, many support the creation of marsh habitats using dredged materials (Rozas and Minello 2001). Use of dredge material to create wetlands has been widely used throughout the Chenier Plain in southwest Louisiana. Beneficial dredge use wetlands are created using dredge material that is pumped as a liquefied slurry from adjacent open water habitats into a shallow open water habitat surrounded by a containment levee (Costa-Pierce and Weinstein 2002). However, beneficial dredge use marshes typically have little topographic variability and have a reduced edge to open water ratio compared to natural marshes resulting in reduced habitat value for nekton (Minello et al. 1994, Streever 2000, Rozas and Minello 2001). Recent research has shown that the habitat value of a marsh created using beneficial dredge use can be increased through the addition

of shallow tidal creeks (Minello et al. 1994, Minello and Rozas 2002). Recently created beneficial dredge use marshes have incorporated this new knowledge, increasing heterogeneity and marsh edge and, it is believed, providing more valuable nekton habitat.

The Coastal Wetlands Planning, Protection, and Restoration Act (1990) dedicates approximately $70 million annually to restore Louisiana's wetlands. With such a significant investment in wetland restoration, there is a critical need for an accurate assessment of ecological recovery at these restoration projects. Some approaches to monitoring and assessment of wetland habitats include the Habitat Evaluation Procedure (USFWS 1980), the Wetland Evaluation Technique (WET, Adamus et al. 1987), and Wetland Value Assessments (WVA, CWPPRA). While these approaches all seek to assess ecological recovery in created wetlands, they assume that equivalence in sediment, water quality, and vegetative community will provide equivalent functional services that a natural marsh provides including support of equivalent nekton communities and food web support.

One confounding factor in evaluating marsh equivalence is time, or age of the created marshes. As newly created marshes age, it is thought that they come to approximate their reference counterparts over time, meaning that conclusions related to the success of marsh creation projects will vary over time. Ideally, created marshes will approximate their reference counterparts more closely with time. For example, while the vegetative community has been shown to establish relatively quickly (Zedler and Callaway 1999, Edwards and Proffitt 2003), soil characteristics have been shown to take up to several decades to approximate the reference marsh (Webb and Newling 1985, Craft et al. 1999, Zedler and Callaway 1999, Edwards and Proffitt 2003). Few data indicate any developmental trajectory of nekton use of these created marshes, and there is uncertainty in predicting restoration trajectories of created marshes for any parameters due to the variability inherent in adjacent natural reference marshes (Minello 2000). The use of functional equivalence trajectories has been suggested as one means to

monitor the development of created marshes to accurately track and assess marsh equivalence. This approach however involves either long-term monitoring from individual sites over time (i.e., Craft et al.1999; Simenstad and Thom 1996), or short-term monitoring of multiple sites in a chronosequence approach to examine developmental trends.

In southwest Louisiana, four beneficial dredge use marshes were created using the same source material, over a 20 year time period (1983, 1993, 1999, and 2002). Using these four marshes in a, I examined structural equivalency using vegetative, soil and nekton community indicators for marsh assessment. Specifically, I asked the questions, (1) are the created marshes similar in terms of vegetation and nekton community and soil characteristics to adjacent native marsh; and, (2) using a chronosequence of created marshes, do any site-based differences correspond to marsh age and suggest any marsh development trajectories?

METHODS

Study Site and Sampling Design

The study was conducted in spring (May, June) and fall (October) 2007 at the Sabine National Wildlife Refuge in Cameron Parish, Louisiana (Figure 1). Sabine NWR is located in southwest Louisiana, USA, in an area known as the Chenier Plain. The Sabine NWR encompasses 124,511 acres of fresh, intermediate and brackish marshes between the eastern shore of Sabine Lake to the western shore of Lake Calcasieu. Salinity is managed throughout much of the refuge inside of impoundments with control gates. Study sites were located in the brackish areas on the eastern portion of the refuge in the Hog Island area adjacent to the Lake Calcasieu ship channel. The dominant emergent species in the area was *Spartina alterniflora*. The inner marsh on our reference sites was dominated by *Spartina patens* and *Distichlis spictata* while the inner marsh on our created sites was dominated by *S. alterniflora*. Tidal range is negligible in this area and water levels in the marsh are controlled more by

wind direction than by tidal forces, typical of the northern Gulf of Mexico (Chabreck 1989).

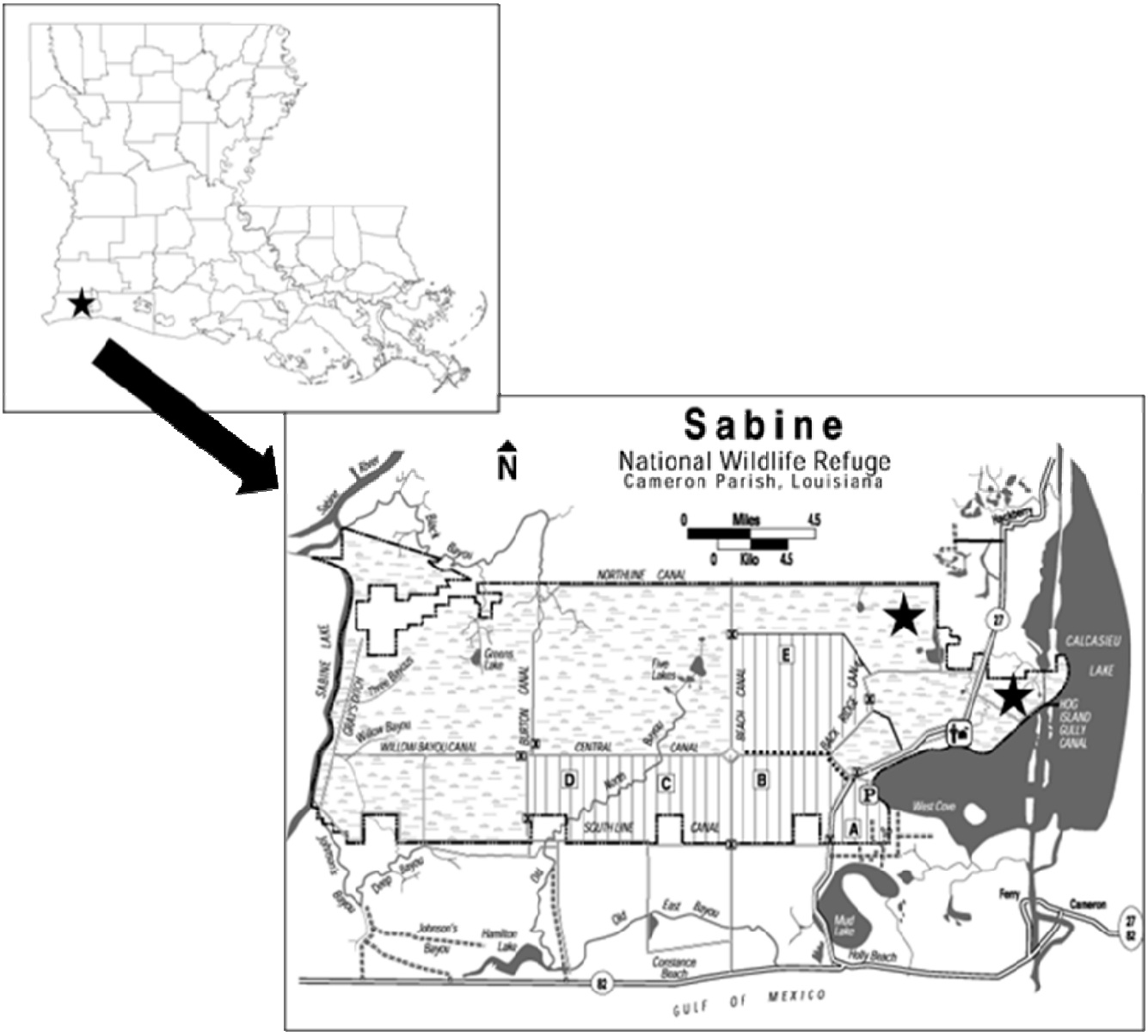

Figure 2.1. Location of study area in southwest Louisiana on the Sabine National Wildlife Refuge. Stars represent general locations of all study marshes.

This study compared four created marshes ranging in age from 5 to 24 years old created with material from the dredging of the Lake Calcasieu Ship Channel. Four marshes were created in open

water pond areas using the same source material. Marshes created in 1983, 1993 and 1999 were all allowed to revegetate naturally, while the 2002 created marsh was planted with *S. alterniflora* along its border. Each created marsh was paired with a nearby reference marsh to minimize variation of salinity, water temperature, and other environmental conditions (i.e. precipitation, flooding). Created marshes will be identified with the prefix *CS* and reference marshes will be identified with the prefix *RF*. The year the marsh was created or referenced to will be used as the suffix (i.e. CS02 represents the marsh created in 2002 and RF02 is its reference marsh). Due to difficulty in sampling and the close proximity of another reference marsh in the study, RF83 was not sampled in the fall.

There were slight variations in construction and design of the created marshes. CS02 consists of 214 acres of marsh habitat that was pumped as a sediment slurry inside a discontinuous containment levee. Some of the levee was removed following consolidation of material. CS02 also features trenasses, which are man-made bayous, created around the perimeter and bisecting the interior in a zigzag pattern. *S. alterniflora* was planted along the marsh edge of this site only and the majority of the site left to recolonize through natural processes. CS99 is approximately 160 hectares in size, is dominated by *S. alterniflora*, and was pumped inside a containment levee using dredge slurry. A long trenasse goes along the perimeter of this marsh between the marsh and containment levee. The levee surrounding CS99 has been removed recently in a few places to increase hydrologic and fishery connectivity to adjacent marsh habitats. CS93, is approximately 200 hectares in size, is also dominated by *S. alterniflora*, and was pumped inside of a containment levee in open water habitat but has had the containment levee removed in its entirety and is absent of any trenasses. Finally, CS83, approximately 40 hectares in size, was pumped inside containment dikes adjacent to a natural bayou and is located adjacent to the Lake Calcasieu Ship Channel. The containment dikes on this site have been removed as well. This site is strongly affected by the passage of large ships. *Juncus romerianus* is common in the

marsh interior at this site. The study sites thus differed in age, and in some extent in design, as marsh creation techniques and designs have progressed over the 20 year time period. However, it was hypothesized that age would override other factors in the development of marsh vegetative and nekton communities.

Within each study marsh, three sites were randomly selected for sampling. At each sampling site, water quality and nekton were collected in open water within 1 m of the vegetated edge while sediment and vegetation were collected within 3 m of the vegetated edge, on the vegetation side of the vegetation-water interface. Water quality (water temperature (ºC), salinity (ppt), and dissolved oxygen (mgL^{-1})) was measured at each site using a YSI Model 556 water quality monitor. Turbidity was measured using a secchi disk in fall 2007 samples only.

Sediment samples were collected in fall and spring 2007. Spring samples collected were one sediment core per site using a 5-cm diameter, 10 cm deep core. Triplicate samples were taken at each site in the fall in order to reduce within site variability and focus on between site variability. Cores were placed in plastic bags in a cooler and transported back to the laboratory at LSU for processing. Wet weight and length of the cores were recorded. Cores were dried to a constant weight in a drying oven at 55ºC and weighed once a constant weight had been achieved. Bulk density of the core was determined [Bulk density ($g\ cm^{-3}$) = Mass dry soil (g) / volume of dry soil (cm^3)]. Samples were homogenized and triplicate subsamples of each sample were then placed in a muffle furnace and burned at 500ºC for at least 4 hours in order to burn off all organic matter. Samples were then weighed, and percent organic matter was calculated as [% Organic matter = 100 x [1 - (final dry weight / initial dry weight)]. Mean percent organic matter of all subsamples per core were pooled for analysis.

Emergent vegetation was sampled on the vegetated side of the vegetation-water interface.

Samples were taken within 1m of the edge. Using a 0.25-m^2 quadrat, percent cover by species was visually estimated. Vegetation in quadrats was then clipped at the soil level, placed in plastic bags and returned to the laboratory for processing. Upon return to lab, all clipped vegetation was identified and sorted by species. Vegetation was then dried in a drying oven at 55ºC to a constant weight and then weighed to the nearest 0.01g dry-weight to determine biomass (g dry wt m^{-2}).

Nekton were collected using a 1-m^2 (3mm mesh diameter) sided throw trap similar to that described by Gossman (2005). The sampler was thrown within 1m of the edge of the emergent vegetation in open water less than 1m in depth. In the spring, two dip nets were used to collect nekton and in the fall a 1-m wide bar seine (3mm mesh diameter) was used to remove nekton from inside the throw trap. The trap was not considered free of nekton until 5 consecutive sweeps were made without any nekton being caught in the nets. Duffy (1997) found that using dip nets to remove nekton inside of an enclosure sampler removed 97% of grass shrimp, *Palaemonetes spp.*, when six consecutive sweeps were made that covered the entire area inside the trap. All nekton were placed on ice until return to the lab, where they were frozen until taxonomic identification could be made. All organisms were identified to the lowest feasible taxon and total length (mm) and weight (nearest 0.001g) recorded for each individual. In the event that more than 30 individuals were collected of one particular species in a sample, a subsample of 30 individuals was taken for individual length and weight measurements and then all individuals were weighed to estimate the total number of individuals. Wet weight was recorded for all nekton.

Data Analysis

Spring and fall data were analyzed separately. For all analyses, a significance level of alpha = 0.05 was used. Data were tested for normality and homogeneity of variance (Proc UNIVARIATE, SAS 9.1). Soil organic matter, nekton density and nekton biomass were log transformed (log(x+1)) to

meet assumptions of normality and homogeneity of variance. Unless otherwise stated, results are presented as mean ± standard error.

Pearson correlation analysis (Proc CORR, SAS 9.1) was performed on all habitat variables. Soil bulk density and soil organic matter were correlated (Pearson coefficient = -0.83473; $p \leq 0.0001$) in both spring and fall, so soil bulk density was excluded from statistical tests. Multivariate analysis of variance (MANOVA, Proc GLM, SAS 9.1) was used to test whether environmental variables (water temperature, salinity, and dissolved oxygen) differed significantly by season between marshes. All significant MANOVA results were further tested using a one way analysis of variance (ANOVA, Proc MIXED, SAS 9.1) of each variable to determine significant marsh effects. Significant ANOVA effects were tested using post hoc comparisons of Tukey adjusted least squared means (LSMEANS, SAS 9.1) to compare paired created and reference marshes. For variables that differed significantly between marshes, simple linear regression (Proc REG, SAS 9.1) was used to determine the relationship between marsh age and the relative equivalence of significant habitat variables, following Zedler and Callaway (1999). Relative equivalence was calculated as the ratio of the created marsh habitat variable divided by the reference marsh habitat value; a value of 1 indicates equivalence with reference marshes while a value of 0 indicates no equivalence.

For nekton analyses, rare species (those that contribute <5% total abundance) were ignored because rare species contribute little to the explanative value of the analysis (Gauch 1982). Pearson correlation analysis was also completed on nekton density and nekton biomass. Nekton density and nekton biomass were correlated in the spring (Pearson coefficient = 0.8354; $p < 0.0001$) and fall (Pearson coefficient = 0.6382; $p < 0.0008$). A univariate ANOVA (Proc Mixed, SAS 9.1) was used to determine if there were significant marsh effects in the spring and fall associated with nekton density and nekton biomass.

RESULTS

Environmental and Habitat Variables

Water temperature (°C), salinity (ppt.), and dissolved oxygen (mg L^{-1}) were typical of marshes in the area for both fall and spring (Table 2.1).

In the spring, MANOVA results indicated that water temperature, salinity, dissolved oxygen, soil organic matter (%), vegetative cover (%), and live standing aboveground biomass (g/m^2 dry weight) were significantly different among marshes (Wilks' lambda = 0.00, $F_{36, 25} = 5.58$, $p \leq 0.0001$). No statistical differences in water temperature, salinity, dissolved oxygen existed between any paired created and reference marsh. No secchi readings were taken during the spring. There were no significant differences between standing live aboveground biomass or percent vegetative cover by marsh.

In the fall, MANOVA results indicated that water temperature, salinity, dissolved oxygen, soil organic matter (%), vegetative cover (%), and live standing aboveground biomass (g/m^2 dry weight) were significantly different among marshes (Wilks' lambda = 0.00, $F_{42, 50} = 13.29$, $p \leq 0.0001$). Water temperature was not statistically significant at any created and paired reference marsh except for CS99 and its reference RF99 ($p \leq 0.0001$). No significant difference existed between any created and paired reference marsh for salinity, dissolved oxygen, or secchi depth. There were no significant differences between standing live aboveground biomass or percent vegetative cover among marshes.

In both spring and fall, soil properties were significantly different between created and reference marshes (Figure 2.2). All created marshes had significantly lower ($p \leq 0.0138$) soil organic matter when compared to their paired marshes. There was no significant relationship ($F = 2.40$; $p = 0.2612$; $r^2 = 0.5458$) between marsh age and relative equivalence of soil organic matter (Table 2.2., Figure 2.3).

Table 2.1. Environmental and habitat variables (mean ± SE) by marsh type and season.

Variable	SPRING		FALL	
	Created	Reference	Created	Reference
Temperature (°C)	29.34 (±0.66)	31.64 (±0.29)	23.82 (±0.76)	25.30 (±0.44)
Salinity (ppt)	21.29 (±0.65)	19.12 (±0.88)	14.66 (±1.08)	13.54 (±1.00)
Dissolved Oxygen (mgL^{-1})	3.76 (±0.44)	4.21 (±0.45)	2.52 (±0.42)	2.71 (±0.27)
Secchi Disk (cm)	---	---	29.3 (±3.38)	30.0 (±2.27)
Bulk Density ($g\ cm^{-3}$)	0.67 (±0.04)	0.33 (±0.02)	0.62 (±0.04)	0.28 (±0.02)
Organic Matter (%)	7.13 (±0.40)	26.74 (±2.12)	7.00 (±0.46)	24.20 (±2.29)
Vegetative Cover (%)	63.33 (±6.00)	83.00 (±6.57)	57.00 (±6.90)	65.62 (±9.52)
Aboveground Biomass (g/m^2 dry wt.)	459.70 (±87.45)	425.12 (±37.14)	446.77 (±74.44)	561.46 (±97.56)

Nekton Abundance, Density, and Biomass

In the spring, 253 organisms, representing 14 species, were collected using the throw trap. Crustaceans made up approximately 64% of the total catch and fishes made up the remaining 36% (TABLE 2.3). After rare species were removed (contribute <5% total abundance), 239 organisms, representing 4 species were used in the analysis. The most numerically abundant species, in order of most to least abundant, were *Palaemonetes spp., Brevoortia patronus, Litopenaeus setiferus,* and *Menidia beryllina* (Table 2.4). The most frequently caught species were *Palaemonetes spp., M. beryllina, L. setiferus, and Callinectes sapidus*. Total nekton biomass was 152.12g. After rare species were removed, total nekton biomass was 119.38g.

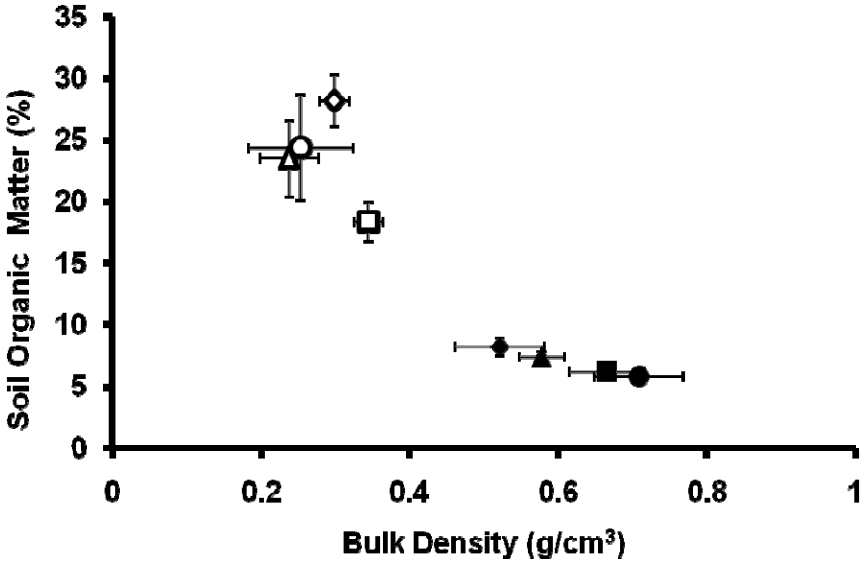

Figure 2.2. Mean soil bulk density (g/m^3) and mean soil organic matter (%) for created and reference marshes. Each created marsh and paired reference marsh share a symbol: CS02/RF02 (♦), CS99/RF99 (■), CS93/RF93 (▲) and CS83/RF83 (●). Created marshes are indicated with a filled symbol (▲) and reference marshes are indicated hollow symbol (Δ). Error bars represent standard errors.

Table 2.2. Soil bulk density (g cm^{-3}) and percent soil organic matter comparison of created marshes of different ages for actual and relativized values. Relative bulk density is calculated as a ratio of (1 - the mean soil bulk density in the created marsh) / (1 - the mean soil bulk density of the reference marshes). Relative soil organic matter is calculated as a ratio of the mean percent soil organic matter from the created marsh / the mean percent soil organic matter from the reference marsh. A ratio of 1 means that the created marsh is equivalent to the reference marsh.

Marsh	Age	Bulk Density (g cm^{-3})			Soil Organic Matter		
		Created	Reference	Relative	Created	Reference	Relative
2002	5 yrs.	0.5211	0.2988	0.6830	8.25	28.20	0.2926
1999	8 yrs.	0.6649	0.3447	0.5114	6.24	18.37	0.3395
1993	14 yrs.	0.5774	0.2382	0.5548	7.42	23.54	0.3153
1983	24 yrs.	0.7084	0.2534	0.3905	5.83	24.36	0.2391

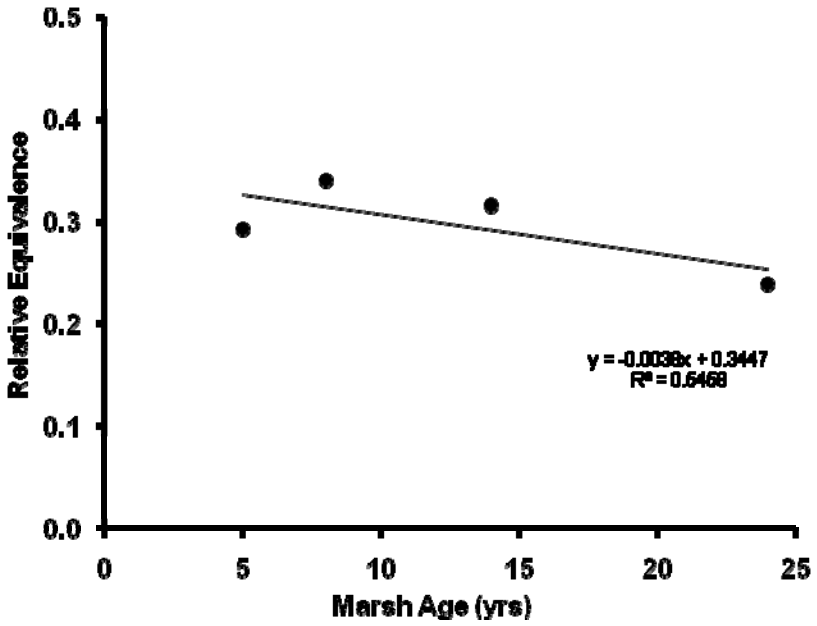

Figure 2.3. Comparison of relative equivalence for soil organic matter in created marshes of different ages. A ratio of 1 means that the created marsh is equivalent to the reference marsh.

remaining 36% (TABLE 2.3). After rare species were removed (contribute <5% total abundance), 239 organisms, representing 4 species were used in the analysis. The most numerically abundant species, in order of most to least abundant, were *Palaemonetes spp., Brevoortia patronus, Litopenaeus setiferus,* and *Menidia beryllina* (Table 2.4). The most frequently caught species were *Palaemonetes spp., M. beryllina, L. setiferus, and Callinectes sapidus*. Total nekton biomass was 152.12g. After rare species were removed, total nekton biomass was 119.38g.

In the fall, 217 organisms, representing 17 species were collected using the throw trap. Crustaceans made up approximately 39% of the total catch (Table 2.3). After rare species were removed (contribute <5% total abundance), 197 organisms, representing 7 species were used in the analysis. The most numerically abundant species, in order of most to least abundant, were *L. setiferus, Anchoa mitchilli, Gobiosoma gulosus, Gobiosoma bosc, M. beryllina, Poecilia latipinna,* and *Palaemonetes spp.*(Table 2.4). The most frequently caught species were *L. setiferus, A. mitchilli, G.*

gulosus, Farfantepenaeus aztecus, C. sapidus, M. beryllina, and Palaemonetes spp. Total nekton biomass was 181.47g. After rare species were removed, total nekton biomass was 58.00g. Six species contributed 112.77g to this difference between nekton biomass after rare species removal. Two individuals were caught from each of the following species: *Mugil cephalus* (43.68g), *Brevoortia patronus* (24.88g), and *Dorosoma cepedianum* (44.21g). All following nekton results will be reported with rare species removed from analysis.

Table 2.3. Nekton abundance, without rare species removal, grouped by created and reference marshes from the spring and the fall.

	SPRING			FALL		
	Created	Reference	TOTAL	Created	Reference	TOTAL
CRUSTACEANS						
Callinectes sapidus	2	1	3	4	0	4
Farfantepenaeus aztecus	0	2	2	1	4	5
Litopenaeus setiferus	5	16	21	52	10	62
Palaemonetes spp.	82	54	136	12	1	13
Rhithropanopeus harrissii	1	0	1	0	0	0
TOTAL	90	73	163	69	15	84
FISH						
Anchoa mitchilli	3	0	3	36	13	49
Antherina hepsetus	1	0	1	0	0	0
Bairdiella chrysoura	0	0	0	0	1	1
Brevoortia patronus	30	30	60	2	0	0
Citharichthys spilopterus	1	1	2	0	0	0
Cynoscion arenarius	1	0	1	1	0	1
Cyprinodon variegatus	0	0	0	5	0	5
Dorosoma cepedianum	0	0	0	2	0	2
Fundulus grandis	0	1	1	0	0	0
Gobiosoma bosc	0	0	0	14	0	14
Gobiosoma gulosus	0	0	0	9	18	27
Gobiosoma robustum	0	0	0	0	1	1
Leiostomus xanthurus	0	0	0	0	1	1
Menidia beryllina	11	2	13	2	12	14
Mugil cephalus	2	0	2	2	0	2
Poecilia latipinna	0	0	0	14	0	14
Pomatomus saltatrix	7	0	0	0	0	0
TOTAL	56	34	90	87	46	133

Table 2.4. Listing of species that contribute at least 5% to total nekton abundance in the spring and fall. (R) is for resident species and (T) is for transient species. The species are listed in order of most to least abundant.

	Spring	Fall
Most Abundant ↓ Least Abundant	*Palaemonetes* spp. (R) *Brevoortia patronus* (T) *Litopenaeus setiferus* (T) *Menidia beryllina* (R)	*Litopenaeus setiferus* (T) *Anchoa mitchilli* (T) *Gobiosoma gulosus* (R) *Gobiosoma bosc* (R) *Menidia beryllina* (R) *Poecilia latipinna* (R) *Palaemonetes* spp. (R)

There was no significant marsh effect for total nekton biomass ($F_{6,12} = 2.17$; $p = 0.1194$) or total nekton density ($F_{6,12} = 2.04$; $p = 0.1378$) during the spring (Table 2.5). There was also no significant marsh effect for total nekton biomass ($F_{7, 16} = 0.48$; $p = 0.8531$) or total nekton density ($F_{7, 16} = 0.90$; $p = 0.5325$) during the fall.

DISCUSSION

The created marshes were found to be similar in terms of vegetation and nekton indicators, but differed in soil properties in comparison to adjacent reference marsh. As the youngest created marsh was 5 years old, this suggests that, with the exception of soil characteristics, structural equivalence was achieved for both vegetation and nekton indicators relatively rapidly. Differences in soil properties did not demonstrate a trajectory of organic matter approaching that of their reference sites however, suggesting that soil properties may be influenced either more by localized or stochastic disturbance events, or that the dredged sediment source differed significantly from local sediment to a point that soil properties may take many decades to reach equivalence.

Table 2.5. Mean nekton density and biomass (±SE) for each marsh and overall mean nekton density and biomass for created and reference marshes. All data reported are after rare species were removed from analysis. *Only species caught were removed from analysis. **RF83 was not sampled in the fall and RF93 was used instead. ***Insufficient sample size for standard error.

MARSHES	MEAN DENSITY (fish/m^2)		MEAN BIOMASS (g/m^2)	
	Spring	Fall	Spring	Fall
2002				
Created	6.33 (±2.96)	16.33 (±14.84)	1.85 (±0.52)	2.63 (±1.64)
Reference	11.00 (±10.50)	13.33 (±6.67)	6.19 (±6.91)	2.78 (±1.80)
1999				
Created	1.67 (±0.88)	10.33 (±8.33)	1.32 (±0.89)	1.41 (±0.74)
Reference	7.09 (±1.76)	3.33 (±0.33)	7.09 (±5.32)	1.93 (±1.13)
1993				
Created	34.33 (±13.32)	6.67 (±5.70)	21.73 (±7.66)	2.46 (±2.20)
Reference	16.00 (±14.04)	1.33 (±0.88)	3.97 (±2.12)	1.77 (±1.75)
1983				
Created	0.00 (±N/A)*	13.00 (±4.04)	0.00 (N/A)*	4.59 (±0.99)
Reference	8.00 (±N/A)***	N/A**	5.41 (±N/A)***	N/A**
OVERALL				
Created	12.70 (±5.94)	11.58 (±4.06)	5.86 (±2.93)	2.77 (±0.73)
Reference	11.10 (±4.70)	4.83 (±2.08)	6.18 (±2.38)	2.06 (±0.71)

Soil characteristics, such as percent organic matter and bulk density take longer to develop as compared to vegetation characteristics (Zedler and Callaway 1999). All created sites in this study had significantly lower percent organic matter as compared to their reference sites. Interestingly, percent organic matter did not differ between created sites, although there was a trend of decreasing percent organic matter with time. This is in contrast to the conclusions of an earlier study which examined the same 1983, 1993, and 1999 created marshes, finding that percent organic matter and site age were highly correlated exponentially with age (Edwards and Proffitt 2003). However, Edwards and Proffitt (2003) also found a significant decrease in soil organic matter at two of the marshes six years after their initial sampling; the one marsh not experiencing significant decreases experienced a dieback of *S.*

alterniflora dieback. They suggest that localized disturbance events may play a significant role in increasing soil organic matter in created marshes.

Although not significant, our opposite trend, of higher percent organic matter in the youngest sites, may also be due to a stochastic event, such as Hurricane Rita which passed through the area in 2005. Turner et al. (2006) concluded that recent hurricanes were responsible for the deposition of significant amounts of inorganic sediments on coastal marshes in Louisiana. It is possible, given the location of our created marshes in the landscape, with some adjacent to open bodies of water, that the created marshes may have received a larger amount of inorganic sediments than more inland marshes, hence reducing percent organic matter in the more exposed marshes. Also, the presence or absence of a containment levee around a marsh would play a factor in protecting a site from inorganic sediment deposition. CS83 is immediately adjacent to the Lake Calcasieu Ship Channel while CS02 is the furthest away from any open water areas. CS83 lacks a containment levee while CS02 has a containment levee around most portions. This probably makes CS02 the most protected and CS83the most unprotected from inorganic sediment deposition associated with storm surge during a hurricane. This may explain why we saw the negative relationship between relative equivalence and marsh age.

Past long-term studies have found that 25 years post marsh-creation, percent organic matter was still increasing towards that of reference marshes (Lindau and Hossner 1981, Craft et al. 1999), while others found that after a few years, little increase in soil percent organic matter occurred (Zedler and Callaway 1999). The very low percent organic matter found at our dredge sites may reflect the source material which were classified as silty clay soils (Coleman and Crossley 1996) with the percentage of clay ranging from 30 to 65% (Edwards and Proffitt 2003). As this is significantly higher than in most studies of created wetlands, it is possible that some local disturbance events, or, some

organic matter enhancement is necessary to help the created marshes approach equivalency of the reference marshes.

Differences in soil bulk density and percent organic matter can be a concern in marsh creation as they have been linked to differences in plant productivity and benthic infaunal communities (Moy and Levin 1991, Sacco et al. 1994, Levin et al. 1996), which can affect the diets of fish (Moy and Levin 1991). In gut content analysis of mummichogs, *Fundulus heteroclitus,* collected in North Carolina, major differences were observed in the diets of fish collected in natural and created marshes (Moy and Levin 1991). Specifically, fish from the reference marshes consumed more plant detritus as compared to fish from the created marsh indicating that differences in the infauna community and detrital based food-web affect the diet quality of the fish. Infauna are an important component linking the primary production of the marsh to the surrounding waters, they are critical in helping a marsh reach full equivalency (Sacco et al. 1994, Craft et al. 2003).

Interestingly, despite differences in soil properties, vegetation and nekton communities did not differ between the created and reference sites. While the reference marshes typically had a more diverse plant community, they were still dominated by *S. alterniflora* with some instances of *S. robustus, S. patens,* and *D. spicata* along the edges. It appears that the dominant plant species, *S. alterniflora*, can be re-established relatively quickly (< 5 yrs) when created marshes are allowed to revegetate on their own or when *S. alterniflora* is planted along its margins. Similarly, both nekton density and abundance were similar between marshes, although catch numbers were low at all sites.

Development Trajectories

We fully expected to detect a site-based age effect due to the wide range of created marsh ages in our study, and the fact that much of the wetland restoration literature has focused on identifying development trajectories of created wetlands (i.e. Dobson et al. 1997, Zedler and Callaway 1999).

However, the only significant differences found were between created and reference sites for soil variables, and no development trajectory was evident. Given that in the search for development trajectories, few predictable trajectories have been identified or put forth using real time data (i.e., Simenstad and Thom 1996) these findings are not that surprising.

One factor that likely confounds the identification of a development trajectory that can not be controlled for in a chronosequence design with temporal replicates is the fact that significant improvements have been made in the field of wetland creation using dredge materials. As outlined earlier, increased scientific knowledge has led to changes in the design and construction of the created wetlands between 1983 and 2002. These include emphasis on the importance of achieving proper site elevation, building trenasses into the created marsh design, placement of the dredge pipes and the removal of containment levees. All of these factors can help initiate the created marsh at a closer point in terms of structural equivalence to the reference marsh. These improvements in wetland creation techniques on the created sites may have overridden any of the expected age effects. For example, the 2002 marsh has the largest edge:open water ratio due to the creation of trenasses that zigzag across the interior of the 2002 marsh. This technique only saw limited application on the 1999 marsh and was not used on the 1993 or 1983 marshes. This technique was developed after the importance of the marsh edge for nekton use was demonstrated in the literature (Baltz et al. 1993). Also, planting the edges of the 2002 marsh with *S. alterniflora* allows for more rapid re-establishment of the dominant macrophyte and increases the soil organic matter more rapidly than allowing the vegetation to colonize the area naturally. These improvements in marsh creation may have negated any time effect that we expected to see. It took less than 5 years for equivalent vegetative and nekton communities to become reestablished on the 2002 marsh. Vegetation and nekton communities appear to reach equivalence with reference marshes relatively quickly.

Implications and Future Directions for Monitoring Marsh Restoration Projects

Soil level characteristics were not equivalent between created and reference marshes but the importance of this is unknown since nekton (e.g. density, biomass) and vegetation (e.g. percent cover, aboveground biomass) were equivalent for all created marshes. While created and reference marshes of different ages supported equivalent nekton and vegetation characteristics, it is still somewhat unknown as to whether these marshes are truly ecologically equivalent. Ecological equivalence is met through the reproduction of structural and functional equivalence. Measures of vegetation only examine structural equivalence. While nekton population metrics have been used to approximate functional equivalence, it has been shown that these nekton data are not always as indicative of functional equivalence as one would hope. La Peyre et al. (2007) found that several species of fish in marsh terrace ponds were in poorer condition than the same species collected in unterraced ponds. Also, the lower soil organic matter in created marshes has been linked to reduced benthic infaunal communities (Sacco et al. 1994). These findings suggest that additional metrics that examine the differences in trophic support at created and reference marshes are needed to evaluate functional equivalence, and ultimately, full ecological equivalence.

CHAPTER 3: STABLE ISOTOPE ANALYSIS OF BLUE CRABS TO EXAMINE DIFFERENCES IN TROPHIC SUPPORT BETWEEN CREATED AND REFERENCE MARSHES

INTRODUCTION

Coastal restoration often has as a primary goal to increase fishery habitat in terms of area and quality. Despite this goal, structural characteristics of vegetation and sediments are generally measured in monitoring projects; most restoration projects have proceeded with the assumption that physical structure represents function, leading to the field of dreams hypothesis of restoration ecology where, "if you build it they will come" (Palmer et al. 1997). A number of studies have explicitly examined the ability of restored marshes to provide equivalent nekton (fish and decapod crustaceans) habitat, as measured by nekton density, biomass or abundance, with mixed results (i.e., Minello and Webb 1997; Rozas and Minello 2001; Able et al. 2004; Bush Thom et al. 2004). However, as fish can rapidly colonize new sites, species abundance may not be the most accurate gauge of habitat value; use of these general indicators may mask more meaningful measures such as individual species health, nekton assemblage structure and community ecology (Minello and Webb 1997; Weinstein et al. 2000; Callaway et al. 2001).

Furthermore, numerous studies of created and reference marshes, including a recent study at our study sites, have found significant soil differences with lower organic matter (OM) and higher bulk density in created marsh soils (Craft et al. 1999, Zedler and Callaway 1999, Edwards and Proffitt 2003; La Peyre et al., 2007). Differences in sediment characteristics may be a critical indicator of prey availability for many nekton species as sediment OM is positively correlated with infauna density (Moy and Levin 1991, Minello and Zimmerman 1992, Craft and Sacco 2003). Combined, these data suggest that differences in food web support may exist between created and reference marshes. Food web characteristics, such as heterogeneity in food web pathways, have been suggested as important to

stable food webs (Rooney et al. 2006); marsh creation efforts ultimately seek to build stable and resilient systems suggesting that the food web development or support between created and reference marshes may provide insight into functional differences, including long term system stability and resilience.

Recently, stable isotope techniques have been suggested as a possible approach for assessing ecological recovery as they can be used to trace the food web support to consumers and may provide a better assessment of the functional aspects of the marsh (Weinstein et al. 2000). Consumers acquire an isotopic signal from their diet. These isotope signals are derived from the various trophic pathways of their food items that have been integrated over time and are habitat specific (Schmidt et al. 2007). Differences in habitat and trophic diversity will be reflected as differences in the isotopic signals of consumers. Specifically, consumer $\delta^{13}C$ isotope values have been used to determine the base of the food web because $\delta^{13}C$ changes very little between trophic steps (DeNiro and Epstein 1978, Peterson and Fry 1987). In contrast, $\delta^{15}N$ isotope values can be used to assess an organism's trophic position in a food web relative to the base of that food web due to well documented $\delta^{15}N$ fractionation rates in various tissues (DeNiro and Epstein 1981, Peterson and Fry 1987, Vander Zanden and Rasmussen 1999, Post 2002). Furthermore, differences in ratios of $\delta^{13}C$ and $\delta^{15}N$ can be used to assess an organism's position within a food web (Peterson and Fry 1987, Vander Zanden and Rasmussen 1999, Post 2002), dietary niche width (Layman 2007 a,b), and the "value" of the food resources within that habitat (Fantle et al. 1999). Additionally, different tissues within an organism possess different isotope turnover rates (Tieszen et al. 1983, Hesslein et al. 1993, Guelinckx et al. 2007). Thus, when an organism experiences a change in diet, there may be differences in the isotope values in different tissues of that organism (Tieszen et al. 1983, Hesslein et al. 1993, Logan et al. 2006, Guelinckx et al. 2007). This may make certain tissues more beneficial for use to monitor differences between habitats.

A number of stable isotope studies have been undertaken in estuarine environments, including examining carbon dynamics of invertebrates in estuaries (Connolly et al. 2005), examining the influence of the Mississippi River on estuarine food webs (Wissel and Fry 2005), and examining wastewater pollution in estuarine fish (Schlacher et al. 2005), however, only recently has isotope analysis been used to examine their value as indicators of ecological change (Wozniak et al. 2006, Layman et al. 2007b). Layman et al. (2007a) proposed the use of a novel analytical approach that examines an organism's trophic niche space and trophic diversity based upon the organism's $\delta^{13}C$ and $\delta^{15}N$ isotope values plotted on a $\delta^{13}C$/ $\delta^{15}N$ biplot, referred to as convex hull testing. Trophic support provided through dietary diversity has been suggested to be important for system stability and resiliency. The methods described by Layman et al. (2007a) allow one to quantify the area and diversity of an organism's diet within an ecosystem, which can be used as indicators of functional equivalence provided through trophic support. With the pressing need to develop accurate indicators of functional equivalency of restored, rehabilitated and created marshes, the use of stable isotope ecology to explore different aspects of the tropic ecology, including primary producer support, consumer diet, and food web diversity, is an approach holds promise for coastal Louisiana.

In coastal Louisiana marshes, the blue crab, *Callinectes sapidus*, is a nekton species of special interest because it is a commercially important species that relies on coastal marshes for a good portion of its life. Furthermore, the blue crab is an ideal species for examining the effectiveness of marsh restoration, and the potential use of isotopic signatures as an indicator of marsh equivalency, as it is ubiquitous in coastal marshes, and uses marsh habitats throughout much of its benthic life (Wilson et al. 1990, Fitz and Wiegert 1991, McClintock et al. 1993). The blue crab is also a generalist feeder and opportunistic forager; blue crabs have been found to consume crustaceans, gastropods, fish, bivalves, detritus, algae, vascular plants, zooplankton and infauna (Millikin and Williamson 1980, Laughlin

1982, Perry and McIlwain 1986, Schindler et al. 1994). The ability of blue crabs to consume a wide variety of food items makes them an ideal study organism to compare the trophic diversity and support within created and reference marshes. The objectives of this study were (1) to examine turnover rates of $\delta^{13}C$ and $\delta^{15}N$ isotope values in the blue crab hepatopancreas and muscle tissues, (2) determine if there are any detectable and significant differences in blue crab $\delta^{13}C$ and $\delta^{15}N$ isotope values at paired created and reference marshes, (3) to determine if there are any differences in trophic diversity and support between paired created and reference marshes, and (4) using a chronosequence approach with temporal replicates, examine whether any site-based differences correspond to marsh age and suggest any marsh development trajectories related to trophic support.

METHODS

Study Site

The study was conducted in spring (May, June) and fall (October) 2007 at the Sabine National Wildlife Refuge in Cameron Parish, Louisiana (Figure 3.1). Sabine NWR is located in southwest Louisiana, USA, in an area known as the Chenier Plain. The Sabine NWR encompasses 124,511 acres of fresh, intermediate and brackish marshes between the eastern shore of Sabine Lake to the western shore of Lake Calcasieu. Salinity is managed throughout much of the refuge inside of impoundments. Our study focused on the brackish areas on the eastern portion of the refuge in the Hog Island area adjacent to the Lake Calcasieu Ship Channel. The dominant emergent species was *Spartina alterniflora*. The inner marsh on our reference sites was dominated by *Spartina patens* and *Distichlis spictata* while the inner marsh on our created sites were dominated by *S. alterniflora*. Salinity at all study sites ranged from 9 ppt–23 ppt in the spring and 8 ppt–17 ppt in the fall. Tidal range is negligible in this area and water levels in the marsh are controlled more by wind direction than by tidal forces which is typical for this area of the northern Gulf of Mexico (Chabreck 1989).

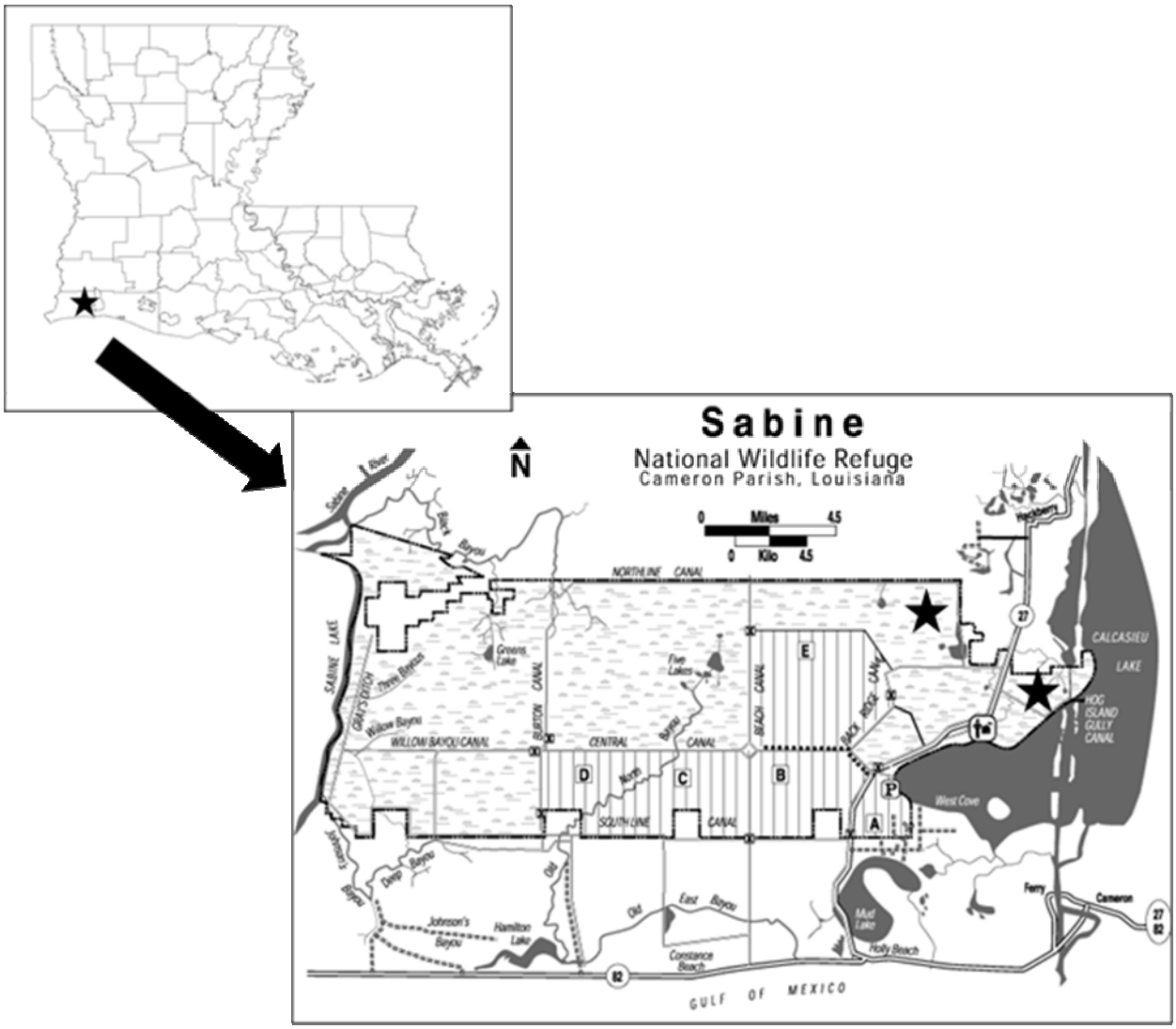

Figure 3.1. Location of study area in southwest Louisiana on the Sabine National Wildlife Refuge. Stars represent general locations of all study marshes.

This study compared created and reference marshes located in the Hog Island area of the Sabine NWR, including the area just west of Hog Island (Figure 3.1). This area is bordered on the east by Lake Calcasieu and the Lake Calcasieu Ship Channel and extends west past Hwy 27. The marshes examined were created over a span of 20 years providing a chronosequence of temporal replicates; in all cases, dredge material from the Lake Calcasieu Ship Channel was used to create marshes in areas of open water. Thus, created marshes were created with the same source material. The marshes were

created in 2002, 1999, 1993, and 1983. The 1999, 1993, and 1983 created marshes were all allowed to revegetate naturally, while the 2002 created marsh was planted along its border. A containment levee, used during marsh creation to help consolidate dredge material, was still present at the 2002 and 1999 created marsh but hydrologic connectivity was maintained through cuts in the containment levee allowing for the passage of nekton species. Each site was paired with a nearby reference marsh to minimize variance in salinity, water temperature, and other environmental conditions (i.e. precipitation, flooding). Due to difficulty in sampling and the close proximity of another reference marsh in the study (reference 1993), the reference 1983 marsh was not sampled in the fall. Created marshes will be identified with the prefix *CS* and reference marshes will be identified with the prefix *RF*. The year the marsh was created or is referenced to will be used as the suffix (i.e. CS02 represents the marsh created in 2002 and RF02 is its reference marsh).

Field Study

Within each study marsh, three points were randomly selected for sampling. Samples were collected in the spring (May, June) and fall (October) during 2007 at three sampling points within each marsh (2 seasons x 8 marshes x 3 sample points). At each sampling point, water quality, blue crabs and primary producers were collected. Water quality (water temperature (ºC), salinity (ppt), and dissolved oxygen (mgL^{-1})) was measured in open water within 1 m of the edge at each site using a YSI Model 556 water quality monitor. Blue crabs were collected using either standard baited crab traps or were dip netted using turkey necks on a string as bait. The bait in the traps was placed in mesh bags (1 mm) and wrapped in fine metal mesh (5 mm) to prevent crabs from consuming the bait. Baited traps were not left out longer than 12 hours to prevent blue crabs from ingesting the bait or cannibalizing each other inside the traps. Three crabs were collected for stable isotope analysis at each sample point, and pooled for analysis as we were interested in differences between marshes and not individual crab

variability. Only crabs between 90-150 mm carapace width were kept and immediately placed in separate, labeled mesh bags, and placed on ice until return to the lab for stable isotope analysis. Primary producers were collected at each site including several stems of the dominant C3 (*Iva frutescens, Juncus romerianus, Schoenoplectus robustus*) and C4 (*S. alterniflora, S. patens, D. spictata*) plants, in addition to any submerged aquatic vegetation, benthic algae, and detritus. All primary producers were place on ice until return to the lab and frozen.

Laboratory Isotope Turnover Study

During the summer of 2007, a laboratory experiment was conducted to determine the stable isotope ($\delta^{13}C$, $\delta^{15}N$) turnover rate of blue crab hepatopancreas and muscle tissue. Forty-one crabs were collected from Hog Island Gully, Sabine National Wildlife Refuge. The crabs were kept in coolers surrounded by wet burlap until return to the lab. Only crabs with two claws were used in this study. Upon return to the lab, crabs were placed into individual containers held in a re-circulating system holding 1000L of artificial seawater (Hawaiian Marine Imports). Water in each system was filtered through 10- and 1-um filters. Water in each system was recirculated at a rate of 4 times per hour, except during feeding, and was constantly aerated. Food was withheld for 2 days prior to initiation of experiment to allow for complete evacuation of gut contents. Water temperature was maintained at 18ºC throughout the experiment and salinity was maintained at 25 ppt throughout the experiment. Water quality was monitored weekly.

On Day 0, 5 crabs were randomly collected for isotope analysis. Remaining crabs (N=36) were randomly assigned to one of three different feeding treatments (N=12 crabs/treatment). Feeding treatments consisted of unique diets of either: (1) thin-ribbed mussel (*Geukensia demissa*), (2) detritus, or (3) smallmouth buffalo (*Ictiobus bubalus*). Crabs were fed ad libitum for 30 minutes, once a day, for the remainder of the experiment. During feeding, water bypassed each container. Any uneaten

food was removed from containers after 30 minutes. Wet weight (g) and carapace width (mm) were recorded for all crabs not yet sampled on days 0, 2, 7, 10, and 20. Following measurements, 3 randomly selected crabs from each of the three treatments were selected and placed in labeled mesh bags on ice until return to the lab for isotope analysis. Triplicate samples of each diet item used were also collected for isotope analysis.

Isotope Analysis

In preparation for isotope analysis, crabs were rinsed with distilled water to remove ectoparasites and sediment before wet weight (g) and carapace width (mm) were recorded. Hepatopancreas tissue and muscle tissue from one claw were collected from each crab. The tissue samples were rinsed with distilled water to remove any detritus or shell fragments. For the field study, similar blue crab tissues (hepatopancreas, muscle) from crabs collected from the same sample point and sample period were pooled together. This resulted in N=3 for each sample period for each marsh.

Crab tissue samples were placed in a drying oven at 55ºC for at least 48 hours. After drying, hepatopancreas tissue samples were placed in separate scintillation vials. Lipids in the hepatopancreas tissue were extracted with hexane at room temperature following Fry et al. (2003). However, this method may not have removed all lipids from the hepatopancreas tissue. This was done in two separate 24-hr decantations, pouring off hexane-lipid solution after each decantation. Once residual hexane had evaporated from hepatopancreas sample, the vials were placed back into drying oven at 55ºC for another 48 hours. All primary producer tissues were rinsed with distilled water prior to drying and were dried separately by species in a drying oven at 55ºC for at least 48 hours. Once samples were dry, they were ground using either a mortar and pestle or Wig-L-Bug into a fine powder. Animal tissue samples of 1±0.2 mg and plant tissue samples to 2-3 mg were weighed out for stable isotope analysis. Two sets of detritus samples were weighed out as a larger sample (5-6 mg) was

needed for determination of nitrogen natural abundance. Weighed samples were packaged in 5 x 9mm tin capsules (Costech Analytical Technologies). All samples were analyzed by the University of California Stable Isotope Facility for dual isotope natural abundance of $\delta^{13}C$ and $\delta^{15}N$ and all detritus samples were analyzed for dual isotope natural abundance (2-3 mg sample) and only $\delta^{15}N$ natural abundance (5-6 mg) using a PDZ Europa ANCA-GSL elemental analyzer interfaced with to a PDZ Europa 20-20 isotope ratio mass spectrometer (Secron Ltd., Cheshire, UK). A second sample of detritus was needed due to insufficient levels of nitrogen during dual isotope analysis and a CO_2 trap was used to measure $\delta^{15}N$ for detritus samples.

Data Analysis

Field Study

For all analyses, a significance level of alpha = 0.05 was used. Data were tested for normality and homogeneity of variance (Proc UNIVARIATE, SAS 9.1). Unless otherwise stated, results are presented as mean ± standard error.

Multivariate analysis of variance (MANOVA, Proc GLM, SAS 9.1) was used to test whether environmental variables (water temperature, salinity, and dissolved oxygen) differed significantly between marshes and seasons. All significant MANOVA results were further tested using a two factor analysis of variance (ANOVA, Proc MIXED, SAS 9.1) of each variable to determine significant marsh, season, and marsh by season interactions. Significant ANOVA interactions were tested using post hoc comparisons of Tukey adjusted least squared means (LSMEANS, SAS 9.1) to compare paired created and reference marshes.

Stable isotope data were analyzed by tissue using a two factor analysis of variance (Proc MIXED; SAS 9.1.3) to determine if there were differences in mean values of $\delta^{13}C$ or $\delta^{13}N$ values for crab tissues and primary producers (dominant C3, C4, SAV, benthic algae, detritus) by marsh and

season. When present, significant interactions (i.e. marsh by season) were further tested using LSMeans. When only main effects were significant, a one-factor ANOVA was run by season and a priori contrasts of paired created and reference marshes were run when marsh was significant. Simple linear regression was used to measure the relationship between the mean blue crab $\delta^{13}C$ isotope values and salinity at each marsh by tissue type and season.

Blue crab trophic position at each marsh was estimated using a simplified model using the following equation from Post (2002):

$$TP = 1 + (\delta^{15}N_{blue\ crab} - \delta^{15}N_{base}) / TEF$$

TP is trophic position, $\delta^{15}N_{blue\ crab}$ is the mean blue crab $\delta^{15}N$ value at a marsh *x*, $\delta^{15}N_{base}$ is the mean $\delta^{15}N$ for the base of the food web at marsh *x*, and TEF represents the trophic enrichment factor per trophic level. To estimate trophic position of blue crabs collected at the Aransas National Wildlife Refuge, Hoeinghaus and Davis (2007) used a TEF of +2.5‰ based on meta-analysis of $\delta^{15}N$ fractionation by Vanderklift and Ponsard (2003). Based on the assumption that $\delta^{13}C$ fractionates very little between trophic levels, $\delta^{15}N_{base}$ was chosen based on isotope data collected from primary producers at each site that most closely matched the $\delta^{13}C$ value of the blue crab muscle tissue from each site. Detritus was used for all marshes as the base of the food web. Trophic position was estimated using only muscle tissue.

Blue crab dietary niche breadth and trophic diversity at each marsh was calculated following Layman et al. (2007 a, b). Blue crab $\delta^{13}C$ and $\delta^{15}N$ isotope values were plotted together for each marsh and pooled across seasons. To measure niche breadth, the total area (TA) of the smallest convex hull polygon that contained all points was calculated using a VBA script that created a minimum convex polygon around the data in ArcMap 9.2 (ESRI, 2006). TA is a measure of overall dietary niche space occupied and can serve as an indicator as to the extent of trophic diversity for a

species at a site (Layman et al. 2007b). The centroid, or mean $\delta^{13}C$ and $\delta^{15}N$ values, at each marsh was then plotted and the euclidean distance from the centroid to each point was determined using Hawth's Tools in ArcMap 9.2 (Hawth's Analysis Tools for ArcGIS, www.spatialecology.com/htools). The mean distance to the centroid (CD) can provide a measure of the average degree of trophic diversity for a particular species or food web (Layman et al. 2007a,b). TA values were compared between paired created and reference marshes but statistical analysis were not performed because only one TA value for each marsh was available. Mean CD was analyzed using a one-way ANOVA by marsh with *a priori* contrasts between paired marshes when significant ANOVAs were found.

Laboratory Study

One-way analysis of variance (SAS, PROC GLM; factor: day) using a priori contrasts were used to test if there were significant differences in $\delta^{13}C$ and $\delta^{15}N$ isotopes at the initiation (day 0) and conclusion (day 20) of the feeding experiment, by food item, separately for hepatopancreas and muscle tissue. Crabs that were fed the smallmouth buffalo (*Ictiobus bubalus*) diet during the laboratory study were used to calculate the $\delta^{13}C$ and $\delta^{15}N$ isotope half-life in the hepatopancreas and muscle tissue as smallmouth buffalo exhibited the most unique isotope signatures as compared to the initial blue crab values. Simple linear regression was used to analyze growth of crabs which was measured as the relative growth rate (final weight / initial weight) and reported as a ratio. Isotope turnover rates can be attributed to organismal growth, tissue replacement, or a combination of the two. Over the course of this laboratory experiment, no significant growth was observed for blue crabs so isotope turnover associated with associated with only tissue replacement was calculated. Hesslein et al. (1993) used the following equation to calculate the isotope specific fractional decay for tissue replacement alone:

$$\text{Fractional decay} = \ln\left(1 - (\delta_x - \delta_{initial}) / (\delta_{final} - \delta_{initial})\right)$$

δ_x represents the blue crab isotope value at time interval *x*, $\delta_{initial}$ represents the initial blue crab isotope

value before the change in diet, and δ_{final} represents the blue crab isotope value at equilibrium with the new diet. Based on the data, it appears that the feeding experiment was not conducted long enough for the blue crab tissue isotopes to reach equilibrium with the new diet. For the purpose of this calculation, the new diet (smallmouth buffalo) isotope values were chosen to be the blue crab equilibrium values, or δ_{final}. A simple linear regression (SLR, Proc REG) was used to determine the effect of time on the calculated fractional decay. The slope of the line of regression was then used to calculate the isotope half-life using the following equation:

Isotope half-life = ln (0.5) / (slope of line of regression)

These equations were using to calculate $\delta^{13}C$ and $\delta^{15}N$ isotope half-life in the hepatopancreas and muscle tissue.

RESULTS

Field Study

Water temperature (°C), salinity (ppt.), and dissolved oxygen (mg L^{-1}) were typical of marshes in the area (Table 3.1). There was a significant marsh by season interaction for water temperature, salinity, and dissolved oxygen (Wilks' lambda = 0.1784, $F_{21, 127} = 4.97$; $p \leq 0.0001$). This interaction was largely explained by CS02 and RF02 which had significantly lower salinity than other marshes. Salinity was significantly lower in the fall than the spring as well. Dissolved oxygen was not significantly different between paired created and reference marshes in the spring and fall but dissolved oxygen was significantly lower in the fall.

Blue crab $\delta^{15}N$ values from hepatopancreas and muscle tissue differed by marsh (hepatopancreas: $p = 0.0033$; muscle: $p < 0.0001$) and by season (hepatopancreas: $p = 0.0070$; muscle: $p < 0.0001$) (Table 3.1, Figure 3.2, 3.3). Contrasts run by season indicated that CS02 and CS99 were significantly more enriched, or higher, than their references in the fall (hepatopancreas), and the spring

(hepatopancreas, muscle), and CS83 was significantly more enriched than its reference in the fall ($p < 0.05$) (Table 3.1). In most cases, blue $\delta^{15}N$ values were more enriched at created versus reference sites.

Blue crab $\delta^{13}C$ values from the hepatopancreas tissue had a significant marsh by season effect ($p = 0.0249$) (Table 3.1, Figure 3.2, 3.3). LSMeans indicated that this interaction was due to significant differences between the 2002 sites and all other marshes examined. In contrast, blue crab $\delta^{13}C$ values from muscle tissue differed significantly among marshes ($p < 0.0001$) and seasons ($p = 0.0384$). A priori contrasts found no significant differences in the fall, but significant differences were detected between marshes for the spring ($p < 0.0001$). During the spring, the mean blue crab muscle $\delta^{13}C$ value at CS02 was significantly more depleted, or lower, than the $\delta^{13}C$ value at RF02 ($p = 0.0006$). Simple linear regression showed that there was a significant positive relationship between blue crab $\delta^{13}C$ values and salinity for the muscle (Spring, $r^2=0.3933$, $p<0.0001$; Fall, $r^2=0.4876$, $p=0.0006$) and hepatopancreas tissue (Spring, $r^2=0.2590$, $p=0.0011$; Fall, $r^2=0.3682$, $p=0.0046$) during the spring and fall (Figure 3.4).

Overall, mean $\delta^{13}C$ values of all primary producers pooled by marsh did not differ significantly but mean $\delta^{15}N$ values from all primary producers pooled by marsh differed significantly by marsh ($p < 0.0001$) and season ($p = 0.0009$). A priori contrasts showed that a significant difference in the mean $\delta^{15}N$ primary producers pooled by marsh existed between CS02 and RF02 ($p < 0.0001$) and between CS99 and RF99 ($p < 0.0441$) during the spring. In the fall, the mean primary producer $\delta^{15}N$ value at CS02 was significantly higher than RF02 ($p < 0.0001$).

Comparison of mean $\delta^{13}C$ values of blue crabs to mean $\delta^{13}C$ values from various primary producers within each marsh indicates that the crabs collected in this study are primarily in a detritus based food web except for RF02 which appears to be in an algal/detritus based food web (Figure 3.5). Using detritus as the contributing primary producer, blue crab trophic position was estimated to be

Table 3.1. Mean blue crab $\delta^{13}C$ and $\delta^{15}N$ values by tissue and season for each marsh. A priori contrasts were used to compare paired created and reference marshes. Significant mean differences between paired marshes are indicated in bold. All results reported as mean (±SE). All significant differences are marked in bold.

MARSH	MUSCLE TISSUE				HEPATOPANCREAS TISSUE			
	Spring		Fall		Spring		Fall	
	δ13C	δ15N	δ13C	δ15N	δ13C	δ15N	δ13C	δ15N
2002								
Created	**-17.30 (±0.28)**	**9.38 (±0.27)**	-17.57 (±0.66)	**7.91 (±1.59)**	-19.92 (±0.47)	**8.28 (±0.41)**	-20.64 (±1.57)	**7.00 (±1.29)**
Reference	**-19.36 (±0.61)**	**7.72 (±0.34)**	-18.66 (±0.82)	**5.68 (±0.45)**	-21.41 (±0.72)	**6.86 (±0.44)**	-21.76 (±1.14)	**4.62 (±0.43)**
1999								
Created	-15.80 (±0.26)	**9.17 (±0.18)**	-15.88 (±0.71)	8.72 (±0.27)	-18.72 (±0.18)	**7.90 (±0.17)**	-20.27 (±0.76)	6.44 (±2.79)
Reference	-16.49 (±0.36)	**8.19 (±0.21)**	-16.15 (±0.60)	8.35 (±0.33)	-19.07 (±0.65)	**6.99 (±0.13)**	-19.96 (±0.53)	7.27 (±0.50)
1993								
Created	-16.41 (±0.51)	8.50 (±0.22)	-14.84 (±0.16)	8.54 (±0.14)	-18.93 (±1.15)	7.35 (±0.21)	-16.72 (±0.12)	7.31 (±0.16)
Reference	-16.18 (±0.56)	8.82 (±1.00)	-15.90 (±0.36)	7.28 (±0.05)	-17.38 (±0.32)	7.16 (±0.37)	-17.52 (±0.38)	6.63 (±0.35)
1983								
Created	-17.37 (±0.41)	9.56 (±0.25)	-15.97 (±0.49)	**8.73 (±0.35)**	-20.54 (±0.79)	8.13 (±0.22)	-17.40 (±0.50)	8.32 (±0.29)
Reference	-16.26 (±0.23)	8.66 (±0.28)	-15.90 (±0.36)	**7.28 (±0.05)**	-18.32 (±0.44)	7.31 (±0.29)	-17.52 (±0.38)	6.63 (±0.35)

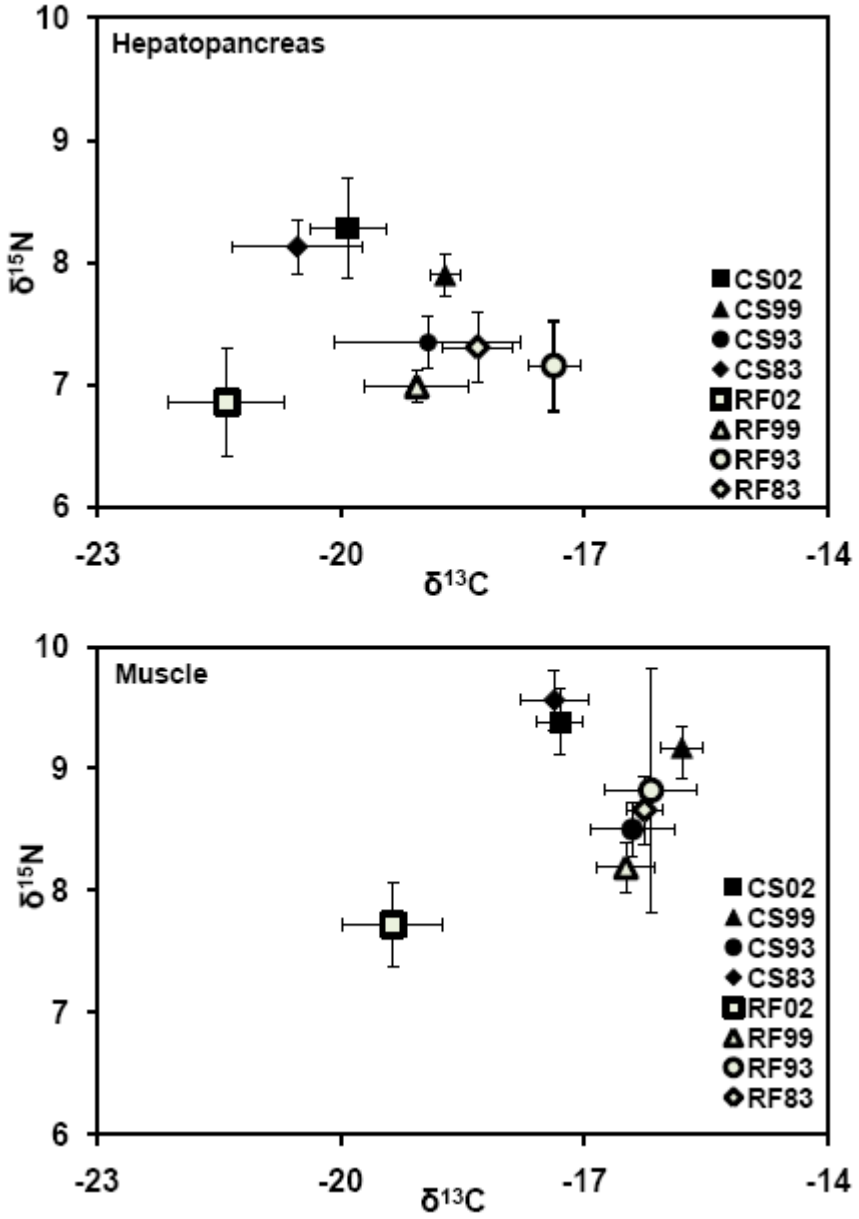

Figure 3.2. Mean blue crab $\delta^{13}C$ and $\delta^{15}N$ values from each marsh during the spring for the muscle and hepatopancreas tissue. Filled symbols represent created marshes and open symbols represent reference marshes. Square=CS02/RF02; Triangle=CS99/RF99; Circle=CS93/RF93; Diamond=CS83/RF83.

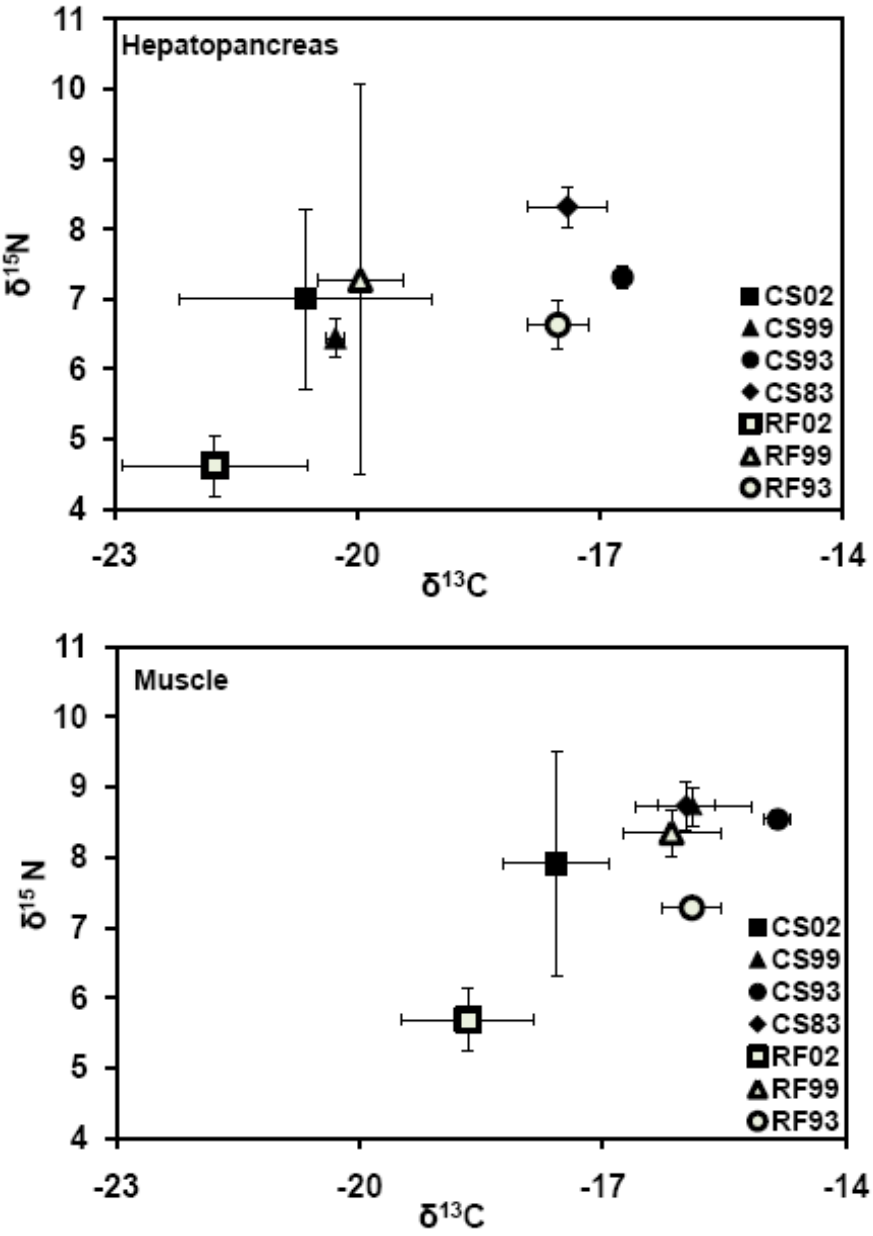

Figure 3.3. Mean blue crab $\delta^{13}C$ and $\delta^{15}N$ values from each marsh during the fall for the muscle and hepatopancreas tissue. Filled symbols represent created marshes and open symbols represent reference marshes. Square=CS02/RF02; Triangle=CS99/RF99; Circle=CS93/RF93; Diamond=CS83/RF8

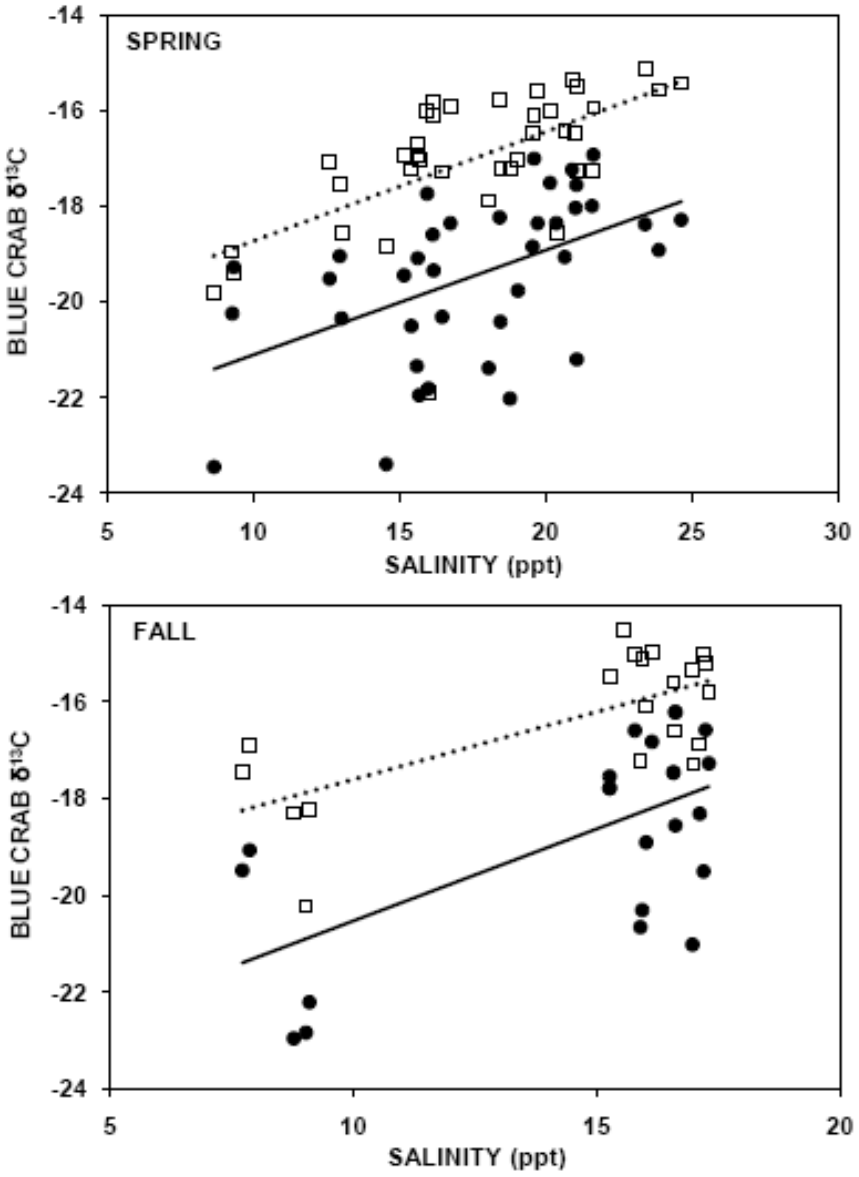

Figure 3.4. Regression analysis of blue crab $\delta^{13}C$ values versus salinity by season for hepatopancreas and muscle tissues. Muscle data are represented by empty squares and hepatopancreas data are represented by filled circles.

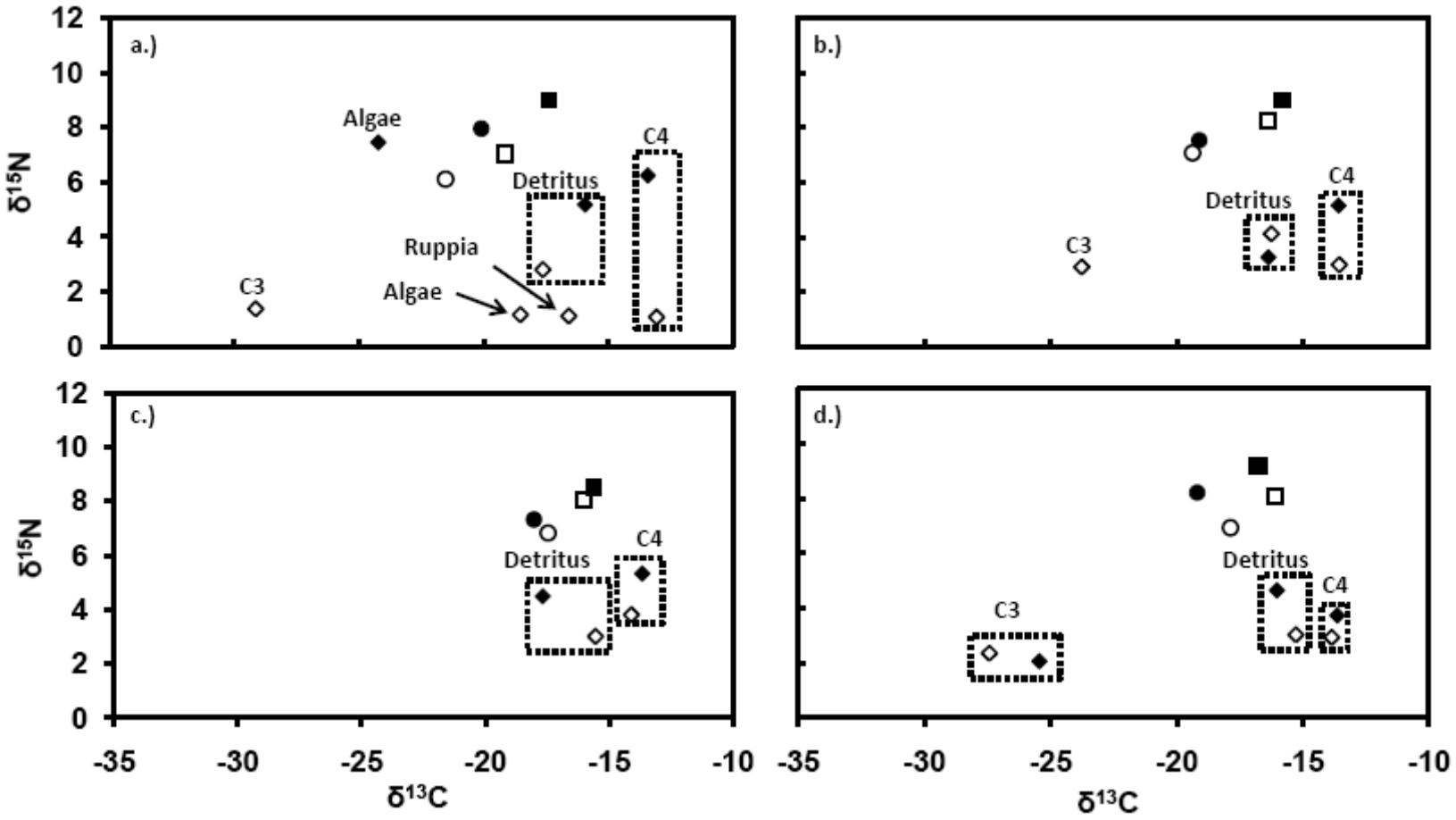

Figure 3.5. Comparison of blue crab and primary producer isotope values from paired created and reference marshes. The letter in the top right corner of each graph represents the paired comparison (a.)CS02 v. RF02; b.)CS99 v. RF99; c.)CS93 v. RF93; d.)CS83 v. RF83). Created marshes are represented with filled symbols and reference marshes are represented with empty symbols. Circles represent blue crab hepatopancreas tissue isotope values. Squares represent blue crab muscle tissue isotope values. Diamonds represent primary producers and each primary producer is labeled by type.

higher in most reference marshes. Only CS99 held a higher trophic position compared to its reference marsh. The youngest marsh, CS02, held the lowest trophic position compared to all other created marshes (Table 3.2).

Blue crab total dietary niche breadth (TA) was smaller at created marshes compared to their paired reference marshes in most instances for both the hepatopancreas and muscle tissue (Table 3.3, Figure 3.6). There was no significant marsh effect for mean centroid distance (CD) in the hepatopancreas ($p = 0.0989$) or muscle ($p = 0.1155$) tissue (Table 3.3).

Table 3.2. Mean blue crab $\delta^{15}N$, mean primary producer $\delta^{15}N$, and estimated trophic position of blue crabs from each marsh. The $\delta^{15}N$ value for the blue crabs for each marsh comes from the mean $\delta^{15}N$ found in the muscle tissue. The mean primary producer $\delta^{15}N$ (BASE $\delta^{15}N$) is from detritus samples collected at that marsh. Trophic position is calculated as $TP = 1 + (\delta^{15}N_{blue\ crab} - \delta^{15}N_{base}) / F$. A constant fractionation rate of +2.5‰ is assumed based on meta-analysis by Vanderklift and Ponsard (2003). All results are reported as mean (±SE).

MARSH	CRAB $\delta^{15}N$	BASE $\delta^{15}N$	TROPHIC POSITION (TP)
CS02	9.01 (±0.43)	5.18 (±0.62)	2.53
RF02	7.04 (±0.42)	2.80 (±0.24)	2.70
CS99	9.02 (±0.16)	3.27 (±0.66)	3.30
RF99	8.24 (±0.17)	4.13 (±0.80)	2.64
CS93	8.52 (±0.12)	4.49 (±0.65)	2.61
RF93	8.05 (±0.56)	3.01 (±0.55)	3.02
CS83	9.20 (±0.25)	4.64 (±1.01)	2.82
RF83	8.07 (±0.32)	3.03 (±0.38)	3.02

Laboratory Study

Crabs fed a diet of detritus showed no significant change in isotope ratios during the study. Crabs fed a diet of *Geukensia* only showed a significant change from day 0 to day 20 for the $\delta^{13}C$ in the hepatopancreas. $\delta^{13}C$ and/or $\delta^{15}N$ isotope values of *Geukensia* and detritus items closely matched the initial crab isotope ratios. However, for crabs fed smallmouth buffalo, there was a significant

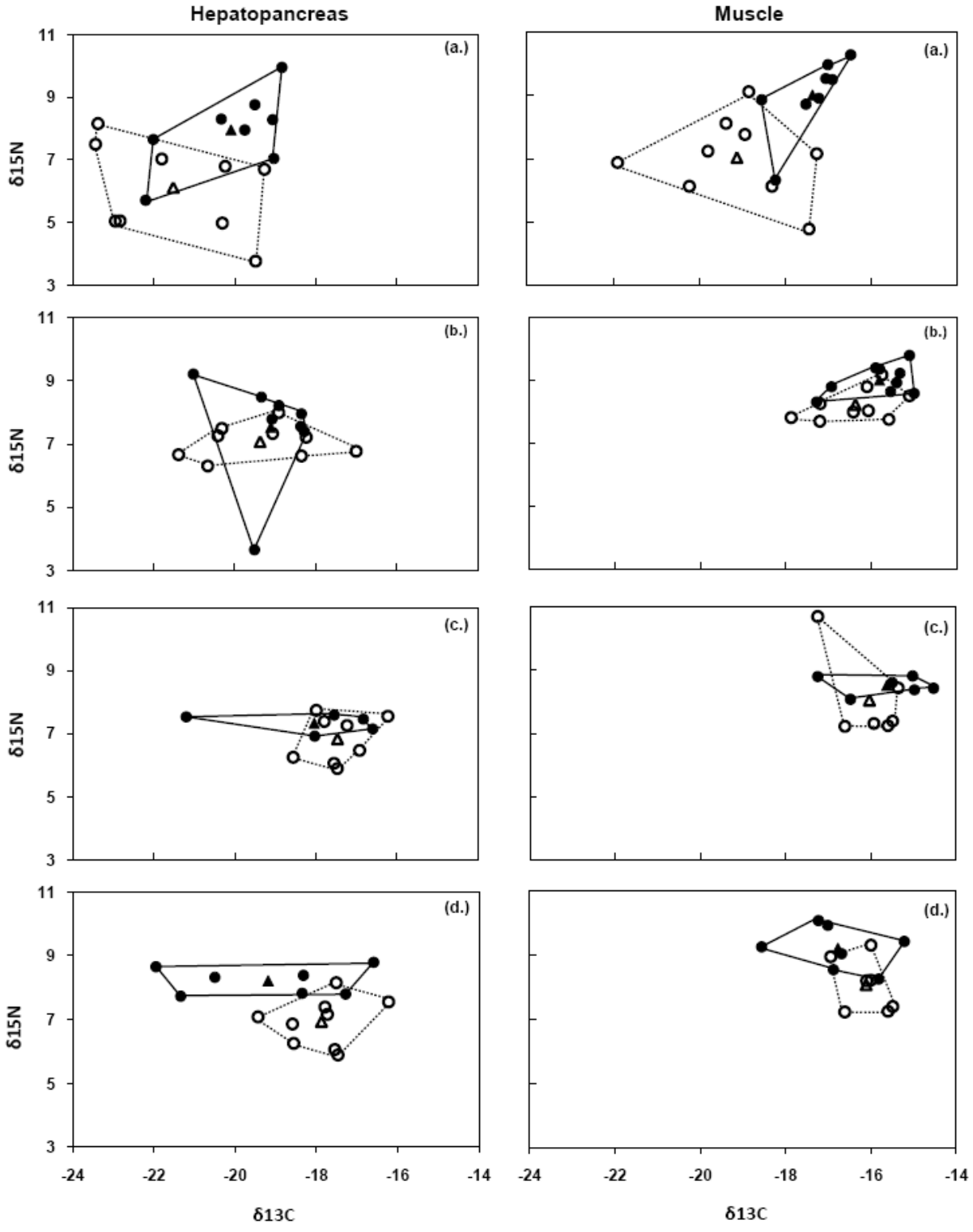

Figure 3.6. Total area of each paired created and reference marsh for hepatopancreas and muscle tissue. Filled symbols and black lines represent created marshes. Open symbols and grey lines represent reference marshes. Triangles represent the centroid, mean $\delta^{13}C$ and mean $\delta^{15}N$, for each marsh. (a)CS02/RF02, (b)CS99/RF99, (c)CS93/RF93, (d)CS83/RF83

Table 3.3. Total niche breadth, measured by total area, and trophic diversity, measured by mean centroid distance (CD) by blue crab tissue type for each marsh. Statistical analyses were only performed on Mean (CD). Results were significant at alpha=0.05. All significant results are marked in bold. All results are reported as mean (±SE).

MARSH	HEPATOPANCREAS		MUSCLE	
	Total Area	Mean (CD)	Total Area	Mean (CD)
CS02	7.35	1.45 (±0.34)	2.91	1.05 (±0.30)
RF02	11.59	2.02 (±0.23)	10.24	1.64 (±0.27)
CS99	7.04	1.36 (±0.44)	1.70	0.76 (±0.15)
RF99	3.92	1.30 (±0.20)	2.13	0.88 (±0.14)
CS93	1.73	1.36 (±0.49)	1.28	0.87 (±0.20)
RF93	2.60	0.90 (±0.11)	3.22	1.21 (±0.34)
CS83	4.49	1.82 (±0.30)	3.29	1.04 (±0.21)
RF83	3.94	1.01 (±0.16)	2.06	0.80 (±0.17)

difference in the $\delta^{13}C$ and $\delta^{15}N$ isotope values between day 0 and day 20 in the muscle ($\delta^{15}N$, $p = 0.0002$; $\delta^{13}C$, $p = 0.0158$) and hepatopancreas ($\delta^{15}N$, $p < 0.0001$; $\delta^{13}C$, $p < 0.0001$) tissues (Table 3.4, Figure 3.7). For all feeding treatments, there was no significant growth of blue crabs (Figure 3.7). In the hepatopancreas tissue, the $\delta^{13}C$ isotope half-life is approximately 10 days ($y = -0.0756x - 0.2634$, $r^2 = 0.7839$; 9.75 days) and the $\delta^{15}N$ isotope half-life is approximately 10 days ($y = -0.0722x - 0.2294$, $r^2 = 0.9133$; 9.60 days) (Figure 3.8). In the muscle tissue, the $\delta^{13}C$ isotope half-life is approximately 39 days ($y = -0.0179x - 0.1219$, $r^2 = 0.7056$; 38.72 days) and the $\delta^{15}N$ isotope half-life is approximately 22 days ($y = -0.0316x - 0.0802$, $r^2 = 0.8804$; 21.94 days).

DISCUSSION

Comparisons of $\delta^{13}C$ and $\delta^{15}N$ isotopes between created and reference marshes suggest that differences exist in trophic support with created marshes only reaching comparable trophic support after 8 years or more. Specifically, comparison of $\delta^{13}C$ and $\delta^{15}N$ isotope values from blue crab muscle and hepatopancreas tissues shows that the youngest marsh, created in 2002, differed significantly from

Table 3.4. Initial (day 0) and final (day 20) blue crab isotope values ($\delta^{13}C$ and $\delta^{15}N$) from the isotope turnover experiment. Results are reported as mean (±SE) for each feeding treatment. Significant results are marked in bold.

TREATMENT	DIET		MUSCLE		HEPATOPANCREAS	
	δ13C	**δ15N**	**δ13C**	**δ15N**	**δ13C**	**δ15N**
Geukensia	-23.64 (±0.09)	7.09 (±0.03)				
Initial			-17.95 (±0.25)	7.84 (±0.25)	**-20.84 (±0.50)**	7.79 (±0.60)
Final			-22.00 (±0.24)	7.30 (±0.16)	**-23.65 (±0.83)**	6.83 (±0.12)
Detritus	-16.54 (±0.13)	0.94 (±0.13)				
Initial			-17.95 (±0.25)	7.84 (±0.25)	-20.84 (±0.50)	7.79 (±0.60)
Final			-19.77 (±0.73)	9.26 (±0.49)	-21.74 (±0.27)	8.87 (±0.09)
Smallmouth Buffalo	-33.30 (±0.17)	13.62 (±0.10)				
Initial			**-17.95 (±0.25)**	**7.84 (±0.25)**	**-20.84 (±0.50)**	**7.79 (±0.60)**
Final			**-22.35 (±1.59)**	**10.65 (±0.39)**	**-30.92 (±1.48)**	**12.39 (±0.42)**

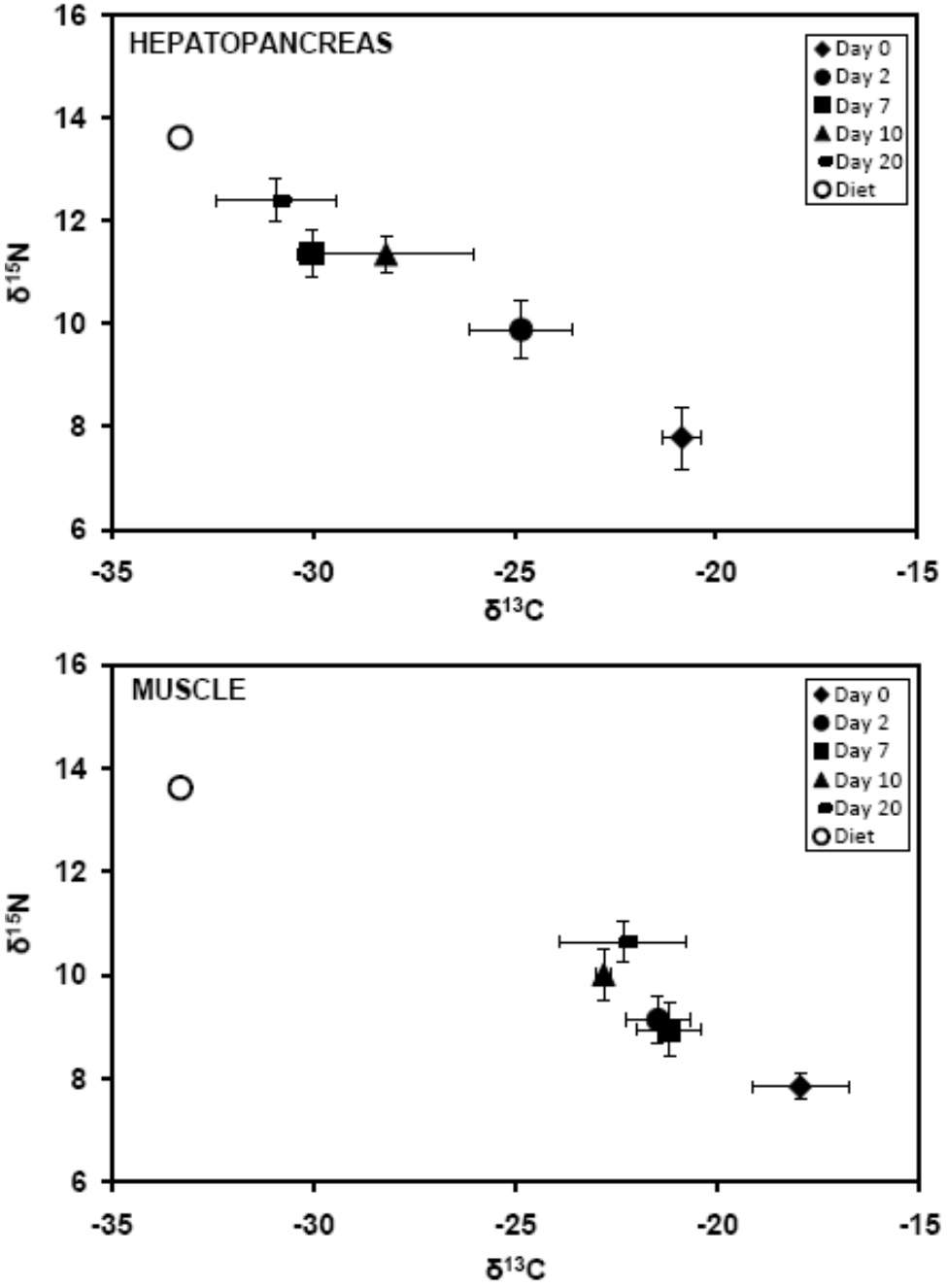

Figure 3.7. Mean blue crab isotope values by sample period during the feeding experiment from the smallmouth buffalo diet treatment. Results are presented as mean blue crab value from sample period.

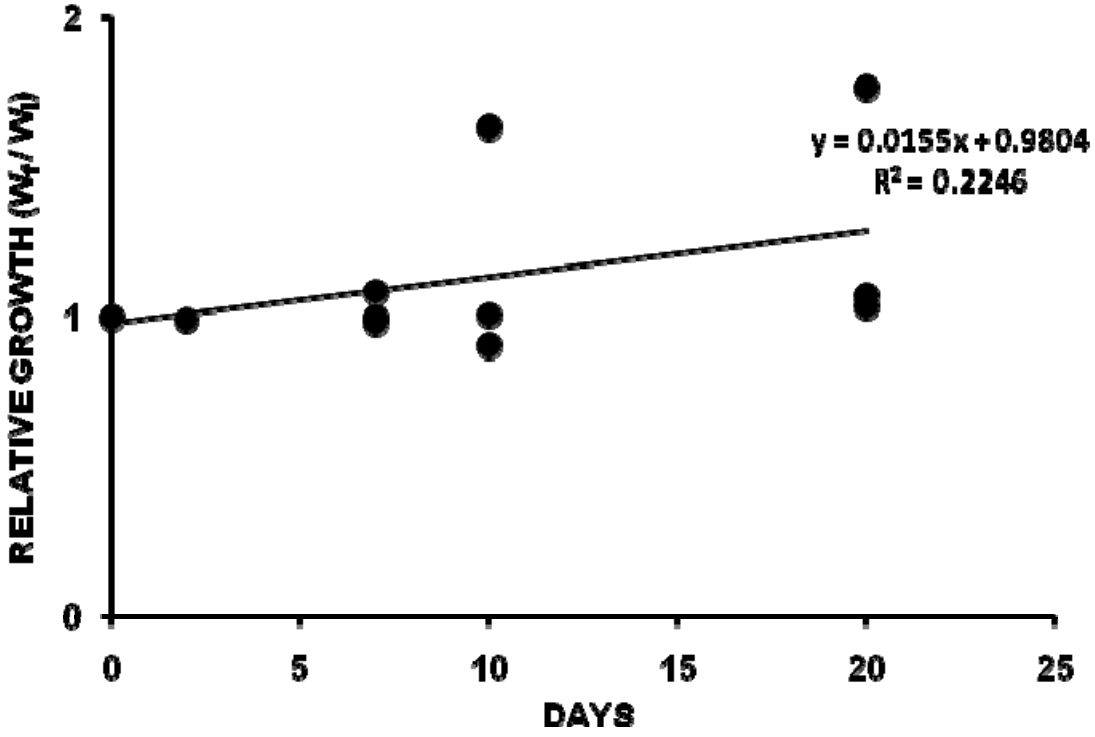

Figure 3.8. Regression analysis of relative growth rate from crabs fed a diet of smallmouth buffalo muscle tissue. Growth was measured as relative growth rate calculated as the weight at collection / weight at initiation of experiment; W_{final} / $W_{initial}$. Blue crabs did not experience significant growth during the feeding experiment.

its reference marsh with blue crabs occupying lower trophic positions in the created marsh. Furthermore, dietary niche breadth was found to be approximately half in the youngest created marsh (CS02) as compared to its reference counterpart (RF02) suggesting lower heterogeneity and diversity in the trophic support of blue crabs in the youngest created marsh. These data, coupled with a lab study which demonstrated relatively rapid turnover of the blue crab hepatopancreas tissues (~10days), suggest that the use of stable isotopes for assessment of marsh equivalence could be a useful tool that provides insight into the development of trophic support in created marshes, and may guide managers in identifying suitable time frames for achieving functionally similar and resilient marsh systems. Trophic niche breadth and dietary diversity analysis is a relatively novel technique in terms of stable isotope analysis that allows researchers to quantitatively evaluate the complexity of food webs using

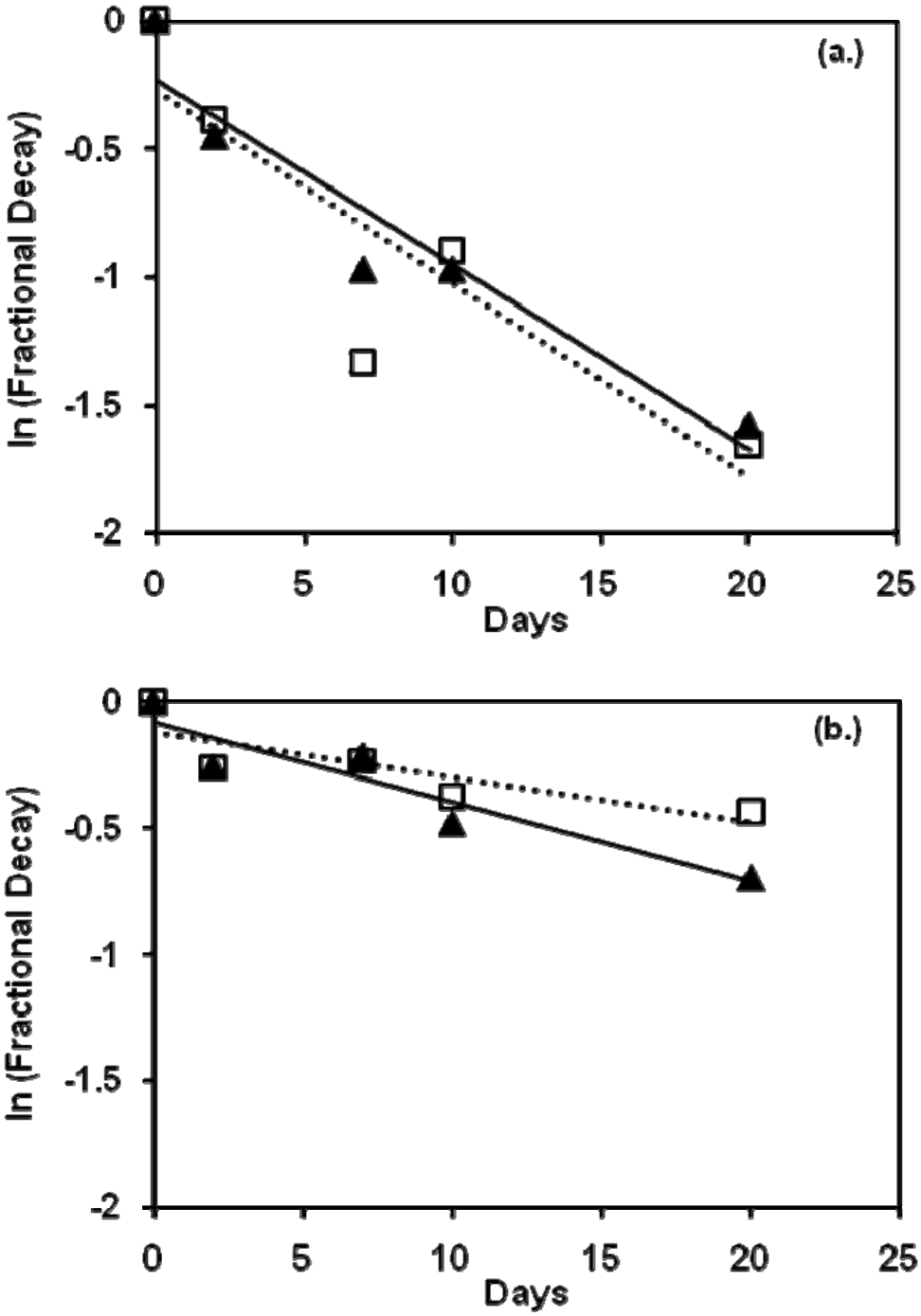

Figure 3.9. Regression analysis of blue crab $\delta^{13}C$ and $\delta^{15}N$ isotope fractional decay from the hepatopancreas (a.) and muscle (b.) tissue. The slope from these regression lines were used to calculate the turnover rate of each isotope in the muscle and hepatopancreas tissues. Filled triangles represent $\delta^{15}N$ fractional decay values and open squares represent $\delta^{13}C$ fractional decay values.

stable isotope data (Layman et al. 2007). This technique is sensitive to variation within ecosystems, in our case created and reference marshes, and may prove to be a useful tool to study intraspecific and community wide trophic structure when used in conjunction with structural habitat and nekton metrics (Layman et al. 2007b). Except in the case of 2002 paired marshes where the reference marsh had a greater TA and mean CD than its created counterpart, there were only slight differences in trophic niche breadth and dietary diversity between the remaining paired marshes. While differences between the 2002 sites could be related to site age, they could also be due to the fact that the 2002 sites differed from our other sites in having lower salinity. Lower salinity marshes tend to have a greater diversity of basal primary resources available which is lacking in many created marshes that are dominated by *S. alterniflora*. In order to achieve functional equivalence of created marshes in estuarine environments with lower salinities, it may take more active restoration of a greater diversity of primary producers, or more time for the diversity of species to recruit. Primary producers found at the 2002 reference site, but at no other references sites included submerged aquatic vegetation such as *R. maritima* as well as dense mats of benthic algae. CS02 was only planted with *S. alterniflora* and will have to rely on natural recolonization of submerged aquatic vegetation in order to expand its trophic diversity to match that of the reference.

It should be noted that while the methods proposed by Layman et al. (2007a) have been criticized for not accurately representing the trophic support in many ecological systems (see Hoeinghaus and Zeug 2008, Layman and Post 2008). Hoeinghaus and Zeug 2008 state that comparisons of isotope data are only comparable when isotope ratios from basal sources are equal or standardized. Standardization of isotope ratio data allow researchers to compare trophic support through the percent contribution of source isotope ratios through the use of mathematical models (n +1 sources (n represents the # of isotopes measured) or IsoSource (Phillips and Gregg 200?) when there

are more than $n + 1$ sources) (Newsome et al. 2007). In this study, the 1999, 1993, and 1983 marsh comparisons met the assumption that the systems must share similar basal resource isotope ratios in order to be comparable. As stated earlier, the RF02 marsh had a more diverse primary producer community than CS02 and direct comparisons of trophic support can not be made between these two marshes using the Layman et al. (2007a) method. This does not however change the results found in this study. Layman et al (2007a) agree that while this method does not provide a universal tool to compare trophic support between systems, when used in conjunction with other methods including gut content analysis, quantitative faunal and floral studies, this isotope based method is useful way to understand the ecological complexities within the systems in question.

Consumer $\delta^{13}C$ isotope values are useful in determining the use of basal resources within food webs because these isotopes seldom fractionate across trophic steps. The data from this study indicated that despite the finding that the blue crabs from the younger created marshes (1999, 2002) were in a lower trophic position as compared to their paired reference marsh, created and reference marshes appeared to have similar basal resources. Similar to findings by Hoeinghuas and Davis (2006), blue crabs in our size class (90-150mm) were found to be defined as detritus based food webs, although they may also consume food resources using other basal resources such as benthic algae or *R. maritima* if it is present. The created marshes in this study provided equivalent basal primary producer trophic support to blue crabs in *S. alterniflora* dominated systems where the crabs rely heavily on a detritus based food web but again, this trophic support was lacking in more diverse primary producer food webs, as was found in the 2002 lower salinity sites.

Consumer $\delta^{15}N$ isotope values can be used to estimate an organism's trophic position within a food web because there is approximately a 2.5-3.4‰ increase in $\delta^{15}N$ at each trophic step causing incremental enrichments in consumer $\delta^{15}N$ values (Peterson and Fry 1987, Vanderklift and Ponsard

2003). Based upon tissue specific blue crab isotopic analysis, blue crabs in created marshes were found to have more enriched $\delta^{15}N$ values as compared to their reference counterparts. However, examining only blue crab $\delta^{15}N$ is somewhat misleading without further analysis of food web resources. This $\delta^{15}N$ enrichment found in crabs from the created marshes appears to be caused by enriched $\delta^{15}N$ values of the primary producers in the created marshes compared to the reference marshes. When blue crab trophic position is calculated following Post (2002), the blue crabs from created marshes actually occupy a lower trophic position even though they have a more enriched $\delta^{15}N$ value than crabs collected from reference marshes. Furthermore, blue crabs from the youngest created marsh had the lowest trophic position of all created marshes. As the created marshes age, the blue crab trophic positions become more equivalent to their reference counterparts as the availability of prey items, or trophic support, increases.

Although more research is needed, it is possible that this $\delta^{15}N$ enrichment may be due partly to the material used for creation of the marshes. For example, a study examining nutrient enrichment associated with thin layer dredge placement on existing marshes found that this technique led to increased soil nitrate levels on marshes receiving dredged sediments (La Peyre unpublished data). Similarly, Bucci et al. (2007) showed that there is a positive correlation between blue crab $\delta^{15}N$ isotope values and nitrate concentration within the water column. This same type of reasoning may be used to explain the reverse pattern of $\delta^{15}N$ enrichment in the reference oldest site (1983) which may be due to the close proximity of the site to Lake Calcasieu. Anthropogenic inorganic nitrates originating from runoff entering Lake Calcasieu is one possible explanation for observed differences, as enriched $\delta^{15}N$ isotope values in consumers has been found in areas impacted with anthropogenically increased nitrate levels. Research in other types of created marshes might help in clarifying this question regarding $\delta^{15}N$ enrichment.

An organism's mobility and site fidelity are of concern when conducting stable isotope studies because an organism's isotope values have been integrated over time. This could present several challenges to stable isotope studies using blue crabs as study organisms to monitor functional equivalence at created marshes. To address this issue in our study, we present two lines of evidence to suggest that the data for the blue crabs we present are indeed site specific. First, a recent blue crab telemetry study examined the home ranges of male and female blue crabs and found that overall, over 8 day periods, the crabs had relatively small home ranges (male 108 m^2; immature female 157 m^2, female mature 1052 m^2) (Wrona 2004). In comparison to the size of our created and reference marshes (40-240 hectares in size), the size of these ranges are miniscule. Furthermore, during this study, 79% of the blue crabs collected during the study were males. Second, our laboratory study found that the turnover rate of the hepatopancreas tissue was rapid, and estimated to occur in approximately 10 days. Combined, these data provide fairly strong evidence that the isotope data were capturing diet information from within the defined study areas.

Similar to past studies in fishes, tissue specific isotope analysis showed that the blue crab hepatopancreas had a more rapid isotope turnover rate as compared to muscle tissue (Tieszen et al. 1983, Hesslein et al. 1993, Guelinckx et al. 2007). Aquatic ectotherm isotope turnover rates in muscle have been shown to depend largely on growth, while isotope turnover rates in liver (hepatopancreas is the equivalent for crabs) has been shown to be largely a reflection of metabolic replacement (Mac Avoy et al. 2001, Perga and Gerdeaux 2005). Given that this laboratory turnover study did not record any growth in the crabs, our isotope turnover rate of the blue crab hepatopancreas of approximately 10 days suggests that the blue crab hepatopancreas tissue may be most useful to determine functional equivalence at created marshes. However, the hepatopancreas is a lipid-rich tissue and lipids have a more depleted, or negative, $\delta^{13}C$ value than protein-rich tissues like claw muscle. Chemical lipid

removal is a useful tool to remove the effect of the more depleted $\delta^{13}C$ but this technique remains a contested issue (Sweeting et al. 2006, Post et al. 2007). We chose to remove lipids from the hepatopancreas using a hexane solution but did not analyze hepatopancreas tissue that had not undergone lipid extraction. Lipid extraction has been shown to cause significant, but low, $\delta^{15}N$ fractionation around 0.15-0.25‰ that is not much more than the typical analytical error from $\delta^{15}N$ using continuous flow techniques as well as increase $\delta^{13}C$ variability (Sweeting et al. 2006, Post et al. 2007). Comparison of studies using different techniques may confound findings, and caution should be exercised when different techniques were used.

Implications and Future Directions for Assessing Functional Equivalence with Stable Isotopes

In terms of trophic support to blue crabs, it appears that created marshes may be functionally equivalent to their reference counterparts within approximately 8 years. Comparison of mean $\delta^{13}C$ and $\delta^{15}N$ isotope values at the youngest marsh, CS02, indicate that the blue crabs at this marsh possess the lowest relative trophic position compared to all other marshes in addition to possessing lower trophic diversity and trophic niche breadth compared to its reference marsh. Quantitative measures of trophic diversity and niche breadth may serve as important indicators of functional equivalence because they provide a way to examine community wide trophic support within a food web (Layman et al. 2007b.). The greater the dietary niche breadth and the more diverse the diet within a food web, the more heterogeneous trophic support is provided to consumers. This heterogeneity has been suggested to result in a more resilient system (Rooney et al. 2006). Achieving ecological equivalence through the reproduction of structural and functional services is paramount to the success of these marsh creation projects. Identifying techniques that can help inform researchers and managers regarding marsh development and long term stability are of critical importance.

Future studies should investigate the significant impact nitrate levels in the sediment may have

on enriching $\delta^{15}N$ values in primary producers. Stable isotope ecology provides a means by which coastal managers and researchers can assess functional equivalence in a relatively rapid manner and can be particularly useful when gut content analysis is not feasible. Based on this study, seasonal effects are slight but it is recommended that a monitoring protocol utilizing isotope analysis would sample during the same season from year to year to minimize variability amongst primary producer $\delta^{15}N$ isotope values as they move through the growing season. One complaint of stable isotope analysis is the cost associated with sample analysis. It is recommended that pooling tissues from multiple individuals collected at a site at the same time in order to reduce variability between sites and analyze a mean value from each site. Once a good sampling protocol and tissue preparation protocol have been established, stable isotope techniques provide great promise to monitor functional equivalence at created marshes in terms of trophic support, and ultimately, system stability.

CHAPTER 4: GENERAL CONCLUSIONS

Ecological equivalence is achieved through the reproduction of structural and functional characteristics. This study sought to examine ecological equivalence at created marshes that were created over a broad temporal range (5-24 years old) using traditional structural measures of equivalence and stable isotope ecology as a measure of functional equivalence.

In chapter 2, structural measures including soil bulk density, soil organic matter, standing live aboveground biomass, percent vegetative cover, nekton biomass, and nekton density were measured and compared between paired created and reference marshes. Only soil organic matter differed between marshes, with soil organic matter being significantly lower in all created marshes as compared to their reference counterparts. Using the chronosequence of marshes, no development trajectory for these soil characteristics was evident. It is possible that local stochastic events may play a large role in influencing soil characteristics which may quickly mask any developmental changes. Furthermore, percent soil organic matter has been shown to be directly correlated with abundance and densities of benthic infauna, suggesting that there may be differences in the trophic ecology of these marshes that was not detectable through this part of the study

Chapter 3 examined the trophic support in these same created marshes using stable isotope data collected from blue crabs at the same marshes studied in Chapter 2. Crabs in reference marshes for the younger created sites in this study were found to have a broader dietary niche breadth and a more diverse diet than crabs in their paired created marshes while the older created sites appear to have similar dietary niche breadth and dietary diversity as their reference marshes. Comparison of $\delta^{13}C$ isotope values between paired marshes shows that in general, there is equivalent trophic support provided by basal resources but comparisons of $\delta^{15}N$ isotope values between paired marshes indicates

that there is a lack of trophic support due to reduced trophic level support in the youngest created marshes. This reduction in trophic level support is caused by reduced prey availability and can be expected in created marshes because it has been shown that there is a positive correlation between soil organic matter and benthic primary consumers at created marshes. As found in Chapter 2, soil organic matter was consistently lower at the created marshes; although as it was consistent across all marshes, soil organic matter itself can not explain the differences in trophic ecology between the younger and the older paired marshes. It appears that functional equivalence, in terms of trophic support, can be restored at the marshes in our study within approximately 8 years but this could be dependent upon the connectivity of these created marshes with adjacent reference marshes in order to allow natural recruitment of primary consumers. Further studies are needed to examine the link between benthic infauna and sediment characteristics at our field sites.

While the created marshes were found to be structurally equivalent for nekton and vegetation production, there appears to be a lack of functional equivalence, in terms of trophic support, at our youngest created marshes. Monitoring structural equivalence is important as it has been identified as an indicator of the capacity of a system (NOAA 1999); at the same time, monitoring should also seek to evaluate functional equivalence which is a measure of the capability of a system to be resilient, resist anthropogenic and natural perturbations, and lead to long term sustainability (NOAA 1999). Stable isotope techniques can be added to a list of tools that will give coastal managers the ability to investigate the complex trophic connections within these estuarine food webs and to determine if and when these marshes will achieve functional equivalence.

REFERENCES

Able, K.W., D.M. Nemerson, and T.M. Grothues. 2004. Evaluating salt marsh restoration in Delaware Bay: analysis of fish response at former salt hay farms. Estuaries 27:58-69.

Baltz, D.M., C. Rakocinski, and J.W. Fleeger. 1993. Microhabitat use by marsh-edge fish in a Louisiana estuary. Environmental Biology of Fishes 36:109-126.

Barras, J., S. Beville, D. British, S. Hartley, S. Hawes, J. Johnston, P. Kemp, Q. Kinler, A. Martucci, J. Porthouse, D. Reed, K. Roy, S. Sapkota, and J. Suhayda. 2003. Historical and projected coastal Louisiana land changes: 1978-2050. USGS Open File Report 03-334, 39p. (Revised Jan. 2004).

Bouillon, S., P.C. Mohan, N. Sreenivas, and F. Dehairs. 2000. Sources of suspended organic matter and selective feeding by zooplankton in an estuarine mangrove ecosystem as traced by stable isotopes. Marine Ecology-Progress Series 208:79-92.

Bucci, J.P., W.J. Showers, S. Rebach, D. DeMaster, and B. Genna. 2007. Stable isotope analyses (δ15N and δ13C) of the trophic relationshipos of *Callinectes sapidus* in two North Carolina estuaries. Estuaries and Coasts 30:1049-1059.

Bush-Thom, C. S., M.K. La Peyre, and J.A. Nyman. 2004. Evaluation of nekton use and habitat characteristics of restored Louisiana marsh. Ecological Engineering 23:63-75.

Callaway, J.C., J.S. Desmond, G. Sullivan, G.D. Williams, and J.B. Zedler. 2001. Assessing the progress of restored wetlands: Hydrology, soil, plants, and animals. Pp. 271-335 in. J.B. Zedler (ed) Handbook for Restoring Tidal Wetlands. CRC Press, Boca Raton, FL.

Chabreck, R.H. 1989. Creation, restoration and enhancement of marshes of the northcentral Gulf coast. p. 127-144. *In* J.A. Kusler and M.E. Kentula (eds.) Wetland Creation and Restoration: The Status of the Science. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR, USA. EPA 600/3-89/038a.

Chambers, J.R. 1992. Coastal degradation and fish population losses. In: Stroud, R.H. (Eds.), Stemming the Tide of Coastal Fish Habitat Loss. National Coalition for Marine Conservation Inc, Savannah, GA, pp. 45-51.

Coasta-Pierce, B.A. and M.P. Weinstein. 2002. Use of dredged materials for coastal restoration. Ecological Engineering 19:181-186.

Coleman, D.C. and D.A. Crossley, Jr. 1996. Fundamentals of Soil Ecology. Academic Press, New York, New York, pp.205

Connolly, R. M., D. Gorman, and M.A. Guest. 2005. Movement of carbon among estuarine habitats and its assimilation by invertebrates. Oecologia 144: 684-691.

Craft, C. and J.N. Sacco. 2003. Long-term succession of benthic infauna communities in constructed *Spartina alterniflora* marshes. Marine Ecology-Progress Series 257:45-58.

Craft, C., J. Reader, J.N. Sacco, and S.W. Broome. 1999. Twenty-five years of ecosystem development of constructed *Spartina alterniflora* (Loisel) marshes. Ecological Applications 9:1405-1419.

DeNiro, M.J. and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochemica et Cosmochimica Acta 42:495-506.

DeNiro, M.J. and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochemica et Cosmochimica Acta 45:341-351.

Deegan, L.A. and R.H. Garritt. 1997. Evidence for spatial variability in estuarine food webs. Marine Ecology-Progress Series 147:31-47.

Dittel, A. I., C. E. Epifanio, and M. L. Fogel. 2006. Trophic relationships of juvenile blue crabs (*Callinectes sapidus*) in estuarine habitats. Hydrobiologia 568:379-390.

Dobson, A.P., A.D. Bradshaw, and A.J.M. Baker. 1997. Hopes for the future: Restoration ecology and conservation biology. Science 277:515-522.

Duffy, K.C. 1997. Macrofaunal community structure in the introduced and native submerged macrophyte beds of Lake Pontchartrain estuary. Ph.D. dissertation. Louisiana State University, Baton Rouge, Louisiana, USA.

Edwards, K.R., and C.E. Proffitt. 2003. Comparison of wetland structural characteristics between created and natural salt marshes in southwest Louisiana, USA. Wetlands 23:344-356.

Fantle, M.S., A.I. Dittel, S.M. Schwalm, C.E. Epifanio, and M.L. Fogel. 1999. A food web analysis of juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120:416-426.

Findlay, S., M. Pace, and D. Fisher. 1996. Spatial and temporal variability in the lower food web of the tidal freshwater Hudson River. Estuaries 19:866-873.

Fitz, H.C. and R.G. Wiegert. 1991. Utilization of the intertidal zone of a salt marsh by the blue crab *Callinectes sapidus* density, return frequency, and feeding habits. Marine Ecology-Progress Series 76: 249-260.

Fry, B., D.M. Baltz, M.C. Benfield, J.W. Fleeger, A. Grace, H.L. Haas, and Z.J. Quiñones-Rivera. 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. Estuaries 26:82-97.

Gauch, H. G. Jr. 1982. Multivariate analysis in community ecology. Cambridge University Press, New York. 298p.

Gray, A., C.A. Simenstad, D.L. Bottom, and T.J. Cornwell. 2002. Contrasting functional performance of juvenile salmon habitat in recovering wetlands of the Salmon River estuary, Oregon, USA. Restoration Ecology 10:514-526.

Gossman, B.P. 2005. Use of terraced marsh habitats by estuarine nekton in southwestern Louisiana. M.S. thesis. Louisiana State University, Baton Rouge, Louisiana, USA.

Guelinckx, J., J. Maes, P. Van Den Driessche, B. Geysen, F. Dehairs, and F. Ollevier. 2007. Changes in $\delta^{13}C$ and $\delta^{15}N$ in different tissues of juvenile sand goby *Pomatoschistus minutus*: a laboratory diet-switch experiment. Marine Ecology-Progress Series 341:205-215.

Hesslein, R.H., K.A. Hallard, and P. Ramlal. 1993. Replacement of sulphur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a shift in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Canadian Journal Fisheries and Aquatic Science 50:2071-2076.

Hoeinghaus, D.J. and S.E. Davis. 2007. Size-based trophic shifts of saltmarsh dwelling blue crabs elucidated by dual stable C and N isotope analyses. Marine Ecology-Progress Series 334:199-204.

La Peyre, M.K., B. Gossman, and J.A. Nyman. 2007. Assessing functional equivalency of nekton habitat in enhanced habitats: Comparison of terraced and unterraced marsh ponds. Estuaries and Coasts 30:526-536.

Laughlin, R.A. 1982. Feeding habits of the blue crab, *Callinectes sapidus* Rathbun, in the Apalachicola Estuary, Florida. Bulletin of Marine Science 32:807-822.

Layman, C.A., D.A. Arrington, C.G. Montaña, and D.M. Post. 2007a. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42-48.

Layman, C.A., J.P. Quattrochi, C.M. Peyer, and J.E. Allgier. 2007b. Niche width collapse in a resilient top predator following ecosystem fragmentation. Ecology Letters 10:937-944.

Levin, L.A., D. Talley, and G. Thayer. 1996. Succession of macrobenthos in a created salt marsh. Marine Ecology-Progress Series 141:67-82.

Lindau, C.W. and L.R. Hossner. 1981. Substrate characterization of an experimental marsh and three natural marshes. Soil Science Society of America Journal 45:1171-1176.

Logan, J., H. Haas, L. Deegan, and E. Gaines. 2006. Turnover rates of nitrogen stable isotopes in the salt marsh mummichog *Fundulus heteroclitus,* following a laboratory diet switch. Oecologia 147:391-395.

Mac Avoy, S.E., S.A. Macko, and G.C. Garman. 2001. Isotopic turnover in aquatic predators: quantifying the exploitation of a migratory prey. Canadian Journal of Fisheries and Aquatic Science 58:923-932.

McCay, D.P.F., C.H. Peterson, J.T. DeAlteris, and J. Cantena. 2003. Restoration that targets function as opposed to structure: replacing lost bivalve production and filtration. Marine Ecology-Progress Series 264:197-212.

McClintock, J.B., K.R. Marion, J. Dindo, P.W. Hsueh, and R.A. Angus. 1993. Population studies of blue crabs in soft bottom, unvegetated habitats of a subestuary in the northern Gulf of Mexico. Journal of Crustacean Biology 13:551-563.

McMahon, K.W., B.J. Johnson, and W.G. Ambrose. 2005. Diet and movement of the killifish, *Fundulus heteroclitus*, in a Maine salt marsh assessed using gut contents and stable isotope analyses. Estuaries 28:966-973.

Millikin, M.R. and A.B. Williams. (1984). Synopsis of biological data on the blue crab, *Callinectes sapidus* Rathbun. NOAA Tech. Rep. NMFS 1, FAO Fisheries Synopsis No. 138 p. 1-39.

Minello, T.J. 2000. Temporal development of salt marsh value for nekton and epifauna: utilization of dredged material marshes in Galveston Bay, Texas, USA. Wetlands Ecology and Management 8:327-341.

Minello, T. J., and R. J. Zimmerman. 1992. Utilization of natural and transplanted Texas salt marshes by fish and decapod crustaceans. Marine Ecology Progress Series 90:273-285.

Minello, T.J., R.J. Zimmerman, and R. Medina. 1994. The importance of edge for natant macrofauna in a created salt marsh. Wetlands 14:184-198.

Minello, T. J., and J. W. Webb. 1997. Use of natural and created *Spartina alterniflora* salt marshes by fishery species and other aquatic fauna in Galveston Bay, Texas, USA. Marine Ecology-Progress Series 151:165-179.

Minello, T.J. and L.P. Rozas. 2002. Nekton in gulf coast wetlands: fine-scale distributions, landscape patterns, and restoration implications. Ecological Applications 12:441-455.

Morgan, P.A. and F.T. Short. 2002. Using functional trajectories to track constructed salt marsh development in the Great Bay Estuary, Maine/New Hampshire, U.S.A. Restoration Ecology 10:461-473.

Moy, L.D., and L.A. Levin. 1991. Are *Spartina* marshes a replaceable resource? A functional approach to evaluation of marsh Creation Efforts. Estuaries 14:1-16.

NOAA (National Oceanic and Atmospheric Administration). 1999. "Habitat equivalency analysis: an overview." Policy and technical paper series, No. 95-1. NOAA Damage Assessment and Restoration Program, Damage Assessment Center, Silver Spring, Maryland (Revised May 2006).

Palmer, M.A., R.F. Ambrose, and N.L. Poff. 1997. Ecological theory and community restoration ecology. Restoration Ecology 5:291-300.

Perga, M.E. and G. Gerdeaux. 2005. 'Are fish what they eat' all year round? Oecologia 144:598-606.

Perry, H. M. and T. D. McIlwain. 1986. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Gulf of Mexico)–blue crab. United States Fish and Wildlife Service, Washington, DC, USA. Biology Report 82 (11.55).

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293-320.

Peterson, G.W. and R.E. Turner. 1994. The value of salt marsh edge vs. interior as a habitat for fish and decapod crustaceans in a Louisiana tidal marsh. Estuaries 17:235-262.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718.

Post, D.M., C.A. Layman, D.A. Arrington, G. Takimoto, J. Quattrochi, and C.G. Montaña. 2007. Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179-189.

Rooney, N., K. McCann, G. Gellner, and J.C. Moore. 2006. Structural asymmetry and the stability of diverse food webs. Nature 442:265-269.

Rozas, L.P. and T.J. Minello. 2001. Marsh terracing as a wetland restoration tool for creating fishery habitat. Wetlands 21:327-341.

Sacco, J.N., E.D. Seneca and T. Wentworth. 1994. Infaunal community development of artificially established salt marshes in North Carolina. Estuaries 17:489–500.

Schindler, D. E., B. M. Johnson, N.A. Mackay, N. Bouwes, and J.F. Kitchell. 1994. Crab: snail size-structured interactions and salt marsh predation gradients. Oecologia 97: 49-61.

Schlacher, T.A., B. Liddell, T.F. Gaston, and M. Schlacher-Hoenlinger. 2005. Fish track wastewater pollution to estuaries. Oecologia 144: 570-584.

Schmidt, S.N., J.D. Olden, C.T. Solomon, and M.J. Vander Zanden. 2007. Quantitative approaches to the analysis of stable isotope food web data. Ecology 88:2793-2802.

Simenstad, C.A. and R.M. Thom. 1996. Functional equivalency trajectories of the Gog-Le-Hi-Te estuarine wetland. Ecological Applications 6:38–56.

Strange, E., H. Galbraith, S. Bickel, D. Mills, D. Beltman, and J. Lipton. 2002. Determining ecological equivalence in service-to-service scaling of salt marsh restoration. Environmental Management 29:290-300.Sweeting, C.J., N.V.C. Polunin, and S. Jennings. 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Communications in Mass Spectrometry 20:595-601.

Streever, W.J. 2000. *Spartina alterniflora* marshes on dredged material: a critical review of the ongoing debate over success. Wetlands Ecology and Management 8:295–316.

Tieszen, L.L., T.W. Boutton, K.G. Tesdahl, and N.A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32-37.

Turner, R.E., J.J. Baustian, E.M. Swenson, and J.S. Spicer. 2006. Wetland sedimentation from Hurricanes Katrina and Rita. Science 314:449-452.

Vander Zanden, M.J. and J. Rasmussen. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395-1404.

Vanderklift, M.A. and S. Ponsard. 2003. Sources of variation in consumer-diet δ15N enrichment: a meta-analysis. Oecologia 136:169-182.

Weinstein, M.P., S.Y. Litvin, K.L. Bosley, C.M. Fuller, and S.C. Wainright. 2000. The role of tidal salt marsh as an energy source for marine transient and resident finfishes: A stable isotope approach. Transactions of the American Fisheries Society 129:797-810.

West, T. L., L. M. Clough, and W.C. Ambrose. 2000. Assessment of function in an oligohaline environment: Lessons learned by comparing created and natural habitats. Ecological Engineering 15:303-321.

Wilson, K. A., K. W. Able, and K.L. Heck. 1990. Habitat use by juvenile blue crabs: a comparison among habitats in southern New Jersey. Bulletin of Marine Science 46: 105-114.

Wissel, B. and B. Fry. 2005. Tracing Mississippi River influences in estuarine food webs of coastal Louisiana. Oecologia 144: 659-672.

Wozniak, A. S., C. T. Roman, S. C. Wainright, R. A. McKinney, and M. J. James-Pirri. 2006. Monitoring food web changes in tide-restored salt marshes: A carbon stable isotope approach. Estuaries and Coasts 29:568-578.

Wrona, A.B. 2004. Determining movement patterns and habitat use of blue crabs (*Callinectes sapidus* Rathbun) in a Georgia saltmarsh estuary with the use of ultrasonic telemetry and a geographic information system (GIS). Ph.D. dissertation. University of Georgia, Athens, Georgia, USA.

Zedler J.B. and J.C. Callaway. 1999. Tracking wetland restoration: Do mitigation sites follow desired trajectories? Restoration Ecology 7:69-73.

Zhou, J., Y.Wu, J. Zhang, Q. Kang, and Z. Liu. 2006. Carbon and nitrogen composition and stable isotope as potential indicators of source and fate of organic matter in the salt marsh of the Changjiang Estuary, China. Chemosphere 65:310-317.

VITA

Christopher Bromley Llewellyn was born in Houston, Texas, to Mr. Murton and Mrs. Brenda Llewellyn. He graduated from Bellaire High School in 2002. Chris attended Texas A&M University where he earned a Bachelor of Science in wildlife and fisheries science in 2006. Chris came to Baton Rouge, Louisiana, in the fall of 2006 to pursue a Master of Science at Louisiana State University under the guidance of Dr. Megan La Peyre. Following graduation, Chris will begin a post-graduate internship with the United States Environmental Protection Agency at the Region 6 office in Dallas, Texas, working with wetland restoration planning.