MODERN CALIBRATIONS OF TEMPERATURE AND NUTRIENT PROXIES FOR

PALEOENVIRONMENTAL RECONSTRUCTIONS IN TROPICAL MOLLUSKS

A Thesis

by

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ABSTRACT

Molluscan shell O- and C-isotope values have been shown to be useful indicators of

upwelling and freshwater input (and thus nutrient status) in nearshore marine environments, but

few studies have had the accompanying long term measurements of water $\delta^{18}O$ (δ_w) and

dissolved inorganic carbon (DIC) $\delta^{13}C$ needed to best test the fidelity of shell isotope values as

environmental indicators. Previously measured seawater δ_w and $\delta^{13}C_{DIC}$ values collected

biweekly from 2011-2012 allow us to determine if shell $\delta^{18}O$ and $\delta^{13}C$ values record

temperature, δ_w, $\delta^{13}C_{DIC}$, and ultimately nutrient status in tropical waters. *Conus*, *Vasum*, and

Strombus shell $\delta^{18}O$ values reflect predicted seasonal upwelling and freshening signals in the

Pacific and seasonal freshening with minimal upwelling in the Caribbean. Both Pacific and

Caribbean shell $\delta^{13}C$ profiles show cyclicity, but only *Conus* samples from Veracruz Beach

(Pacific) record seasonal changes in $\delta^{13}C_{DIC}$. This observation likely results from: (1) more

distinct seasonal $\delta^{13}C_{DIC}$ variation in Pacific waters compared with Caribbean waters and (2)

greater availability of metabolic CO_2 for shell growth in *Strombus*, which we hypothesize is

related to greater activity associated with an herbivorous feeding habit.

To examine N-isotope ratios ($^{15}N/^{14}N$) in tissues and shell organic matrix of bivalves as a

proxy for natural and anthropogenic nutrient fluxes in coastal environments, *Pinctada imbricata*,

Isognomon alatus, and *Brachidontes exustus* bivalves were live-collected and analyzed from

eight sites in Bocas del Toro, Panama. Sites include a variety of coastal environments, including

more urbanized, uninhabited, riverine, and oceanic sites. At all sites there is no single dominant

source of organic matter contributing to bivalve $\delta^{15}N$ and $\delta^{13}C$ values. Bivalve $\delta^{15}N$ and $\delta^{13}C$

values likely represent a mixture of mangrove and seagrass N and C, although terrestrial sources

cannot be ruled out. Despite hydrographic differences between end-members, we see minimal

δ^{15}N and δ^{13}C difference between bivalves from the river-influenced Rio Guarumo site and those from the oceanic Escudo de Veraguas site, with no evidence for N from open-ocean phytoplankton in the latter. Lastly, δ^{15}N values of tissue and shell organic matrix correlate significantly for pterioideans *P. imbricata* and *I. alatus*. Thus for these species, N isotope studies of historical and fossil shells may provide records of ecology of past environments.

For my gramps.

ACKNOWLEDGMENTS

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CHAPTER I

INTRODUCTION

Chapter II of this study compares $\delta^{18}O$ and $\delta^{13}C$ profiles recorded in live-collected gastropod shells to measured water temperature, salinity, δ_w, and $\delta^{13}C_{DIC}$ data from the Caribbean and Pacific coasts of Panama. Comparing expected and measured shell profiles tests how well modern mollusk shell carbonates record temperature, δ_w, and $\delta^{13}C_{DIC}$. These results provide validation and refinement of the use of fossil shells for intrannual paleoenvironmental reconstruction in the SWC and TEP, and are the first direct test of how well tropical mollusk shell $\delta^{13}C$ profiles reflect annual variation in $\delta^{13}C_{DIC}$.

In Chapter III, N-isotope ratios in tissues and shells of modern bivalves (*Isognomon alatus, Pinctada imbricata*, and *Brachidontes exustus*), supplemented by stable carbon isotope ratios were examined as an indicator of terrestrial, marine, and anthropogenic nutrient sources in coastal environments in the Bocas del Toro Archipelago, Panama. Spatial variation in shell and tissue $\delta^{15}N$ values was determined in three mollusk species. Since bivalves are primary consumers, temporal variation in $\delta^{15}N$ values more likely reflect changing N source than trophic level changes (Carmichael et al., 2004, 2008). This research aids in application of $\delta^{15}N$ in mollusk shells to understand nutrient sources in past environments where measured environmental data are lacking. To my knowledge, this is the first nitrogen isotope study of bivalves in the southwest Caribbean.

CHAPTER II

MODERN GASTROPOD SHELL $\delta^{18}O$ AND $\delta^{13}C$ VALUES AS A PROXY OF

TEMPERATURE, δ_W, $\delta^{13}C_{DIC}$ AND NUTRIENTS IN TROPICAL WATERS

2.1 Introduction

For decades, oxygen and carbon isotopes in mollusk shells have been used as proxies for sea surface temperature (SST), salinity, and $\delta^{13}C_{DIC}$ in ancient environments (e.g. Lowenstam and Epstein, 1954; Anderson et al., 1994; Kobashi and Grossman, 2003; Grossman, 2012; Robbins et al., 2012, in prep.). To understand factors influencing isotopic signals in fossil mollusks, researchers have conducted modern calibrations to determine how shells record modern environmental conditions. One shortcoming of many previous studies is the lack of coeval water $\delta^{18}O$ (δ_W) and dissolved inorganic carbon $\delta^{13}C$ ($\delta^{13}C_{DIC}$) measurements to compare with shell $\delta^{18}O$ and $\delta^{13}C$ values. In this study, we take advantage of measured δ_W, $\delta^{13}C_{DIC}$, and salinity (Robbins et al., 2012) to evaluate modern $\delta^{18}O$ and $\delta^{13}C$ profiles in gastropod shells as proxies for seasonal variation in temperature, δ_W, $\delta^{13}C_{DIC}$, and nutrients in waters off the Pacific and Caribbean coasts of Panama.

The study of stable isotopes in modern gastropods from Panama have direct application to isotopic studies of environmental change related to the closure of the Central American Seaway (CAS) ~3.5 Ma ago. This event resulted in a decreased mean annual water temperature range (MART), increased salinity, and decreased upwelling in the southwest Caribbean (SWC), in contrast to strong upwelling of nutrient-rich waters in the tropical eastern Pacific (TEP; Schmidt, 2007; O'Dea et al., 2007; Leonard-Pingel et al., 2012). Decreased upwelling in the SWC after closure of the CAS resulted in decreased productivity and a major extinction of reef corals and mollusks, while continued upwelling sustained high phytoplankton productivity in the

TEP (O'Dea et al., 2007). Curiously, the Caribbean Plio-Pleistocene extinction event lagged the

closure of the CAS by about 2 Ma (O'Dea et al., 2007). This lag may be related to rates of

environmental change or stochastic processes. Understanding the reasons for the lag and the
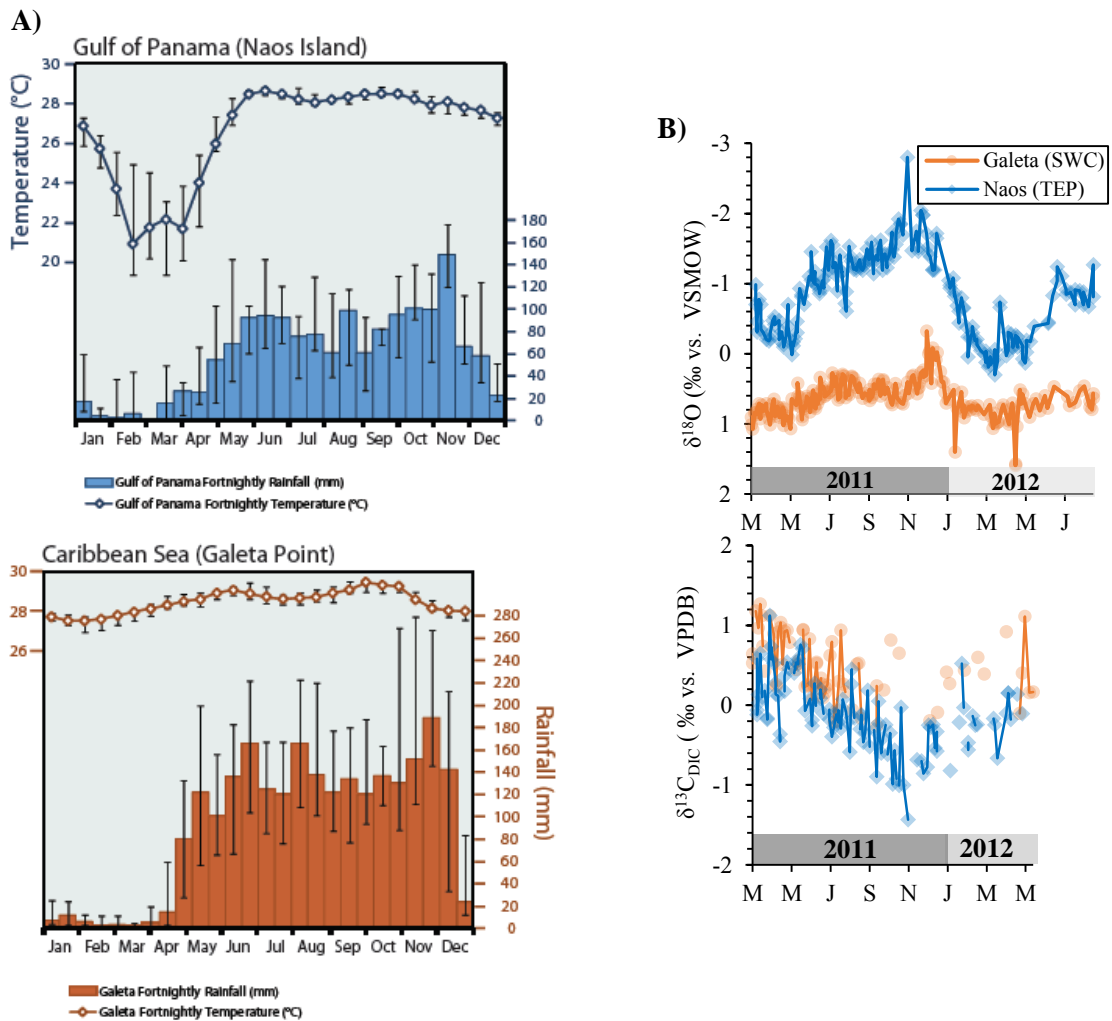
Figure 2.1 (A) Gulf of Panama and Caribbean rainfall and water temperature averaged form 1974 to 2008. From Robbins et al., 2012. (B) Water $\delta^{18}O$ (‰ vs. VSMOW) and $\delta^{13}C_{DIC}$ (‰ vs. VPDB) measurements collected biweekly from the STRI Galeta Marine Station and Naos Marine Station from March 2011 to August 2012 by Robbins et al. (2012). Note $\delta^{18}O$ axis is reversed.

causes of the marine extinction have been the subject of recent studies (e.g. O'Dea et al., 2007; Jackson and O'Dea, 2013). High resolution shell isotopic profiles generated in this study may be used to better understand upwelling and freshening in Plio-Pleistocene shells (Robbins et al., 2012, in prep.) and consequently Plio-Pleistocene extinction mechanisms.

Several studies have examined isotopic records of serially-sampled mollusk shells in the SWC and TEP. Specifically, they tested the efficacy of modern shell $\delta^{18}O$ and $\delta^{13}C$ values as indicators of upwelling and/or freshening in the SWC and TEP (Geary et al., 1992; Bemis and Geary, 1996; Tao et al., 2013). Geary et al. (1992) found that a modern Pacific *Strombus* shell profile had higher $\delta^{18}O$ amplitudes than a Caribbean shell due to high seasonal fluctuations in temperature and salinity in the Pacific and low seasonality in the Caribbean (Figure 2.1A). Similar results were observed in modern venerid bivalves from the SWC and TEP by Bemis and Geary (1996). These high amplitude temperature and salinity variations in the Pacific result from seasonal upwelling from January to May (Robbins et al., 2012; Geary et al., 1992; Bemis and Geary, 1996) derived from migration of the Intertropical Convergence Zone, southward shift of the trade winds, and subsequent movement of waters off the Pacific coast (e.g. Donguy and Henin, 1980, O'Dea et al., 2012). The rainy season occurs from roughly May-November in both the SWC and TEP (Figure 2.1A). Bemis and Geary (1996) noted that the correlation between shell $\delta^{18}O$ and $\delta^{13}C$ values may help identify upwelling and non-upwelling environments. The upwelling of cool (more positive $\delta^{18}O$), nutrient rich (more negative $\delta^{13}C$) waters can cause a negative $\delta^{18}O$-$\delta^{13}C$ (O-C) correlation (Killingley and Berger, 1979; Tao et al., 2013). In contrast,

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freshwater influence introduces water with low $\delta^{18}O$ and low $\delta^{13}C$, resulting in a positive O-C correlation (Killingley and Berger, 1979; Tao et al., 2013).

This study compares $\delta^{18}O$ and $\delta^{13}C$ profiles recorded in live-collected gastropod shells to measured water temperature, salinity, δ_w, and $\delta^{13}C_{DIC}$ data from the Caribbean and Pacific coasts of Panama. Samples for salinity, δ_w, and $\delta^{13}C_{DIC}$ measurements were collected biweekly from March 2011 to August 2012 at the Galeta Marine Laboratory (Caribbean) and Naos Island Marine Laboratory (Pacific) by personnel of the Smithsonian Tropical Research Institute's Marine Environmental Science Program (Figure 2.1B; Robbins et al., 2012). Gastropods (*Conus, Vasum, Strombus*) were collected live from nearby locations in 2013. Comparing expected and measured shell profiles tests how well modern mollusk shell carbonates record temperature, δ_w, and $\delta^{13}C_{DIC}$. These results provide validation and refinement of the use of fossil shells for

Table 2.1. Specimen and collection information. Shell age and growth rate are derived from $\delta^{18}O$ measurements. All shells were collected July 24-26, 2013.

Location	Lat	Long	ID	Species	Depth (m)	Shell diameter (mm)	Shell height (mm)	Age (yrs)	Mean growth rate (cm/yr)
Caribbean									
Galeta	9°24'12"N	79°51'41"W	PG01-4	*Vasum muricatum*	<1	60	80	5	4.3
			PG01-6	*Vasum muricatum*	<1	90	100	3.5	7.3
			PG01-7	*Vasum muricatum*	<1	75	95	4.5	4.3
Pacific									
Veracruz Beach	8°53'00"N	79°35'49W	VC04-5	*Conus mahogani*	3	14	29	1.5	4.5
			VC04-6	*Conus mahogani*	3	15	35	3	2.0
			VC04-7	*Conus mahogani*	3	15	34	2.5	2.3
			VC04-8	*Conus mahogani*	3	15	33	3.5	1.8
			VC04-11	*Strombus gracilior*	3	10	24	0.5	7.2
Isla Uraba	8°46'53"N	79°32'16"W	IU01-9	*Vasum muricatum*	3	53	65	8	2.2
Isla Taboga	8°48'17"N	79°33'47"W	IT02-1	*Vasum muricatum*	3	73	90	8.5	2.8
			IT02-2	*Vasum muricatum*	3	65	83	7.5	2.7

intrannual paleoenvironmental reconstruction in the SWC and TEP, and are the first direct test of how well tropical mollusk shell $\delta^{13}C$ profiles reflect annual variation in $\delta^{13}C_{DIC}$.

2.2 Samples and Methods

Study specimens include four *Conus*, one *Strombus*, and six *Vasum muricatum* shell(s) from the southwest Caribbean (Punta Galeta) and eastern equatorial Pacific (Veracruz Beach, Isla Uraba, Isla Taboga; Table 2.1). In the Caribbean, samples were hand collected in a shallow (~1 m) lagoon at the Galeta Marine Laboratory within 70 m of the intake where water samples were collected at 1 m depth (Robbins et al., in prep; Figure 2.2). In the Pacific, Veracruz samples
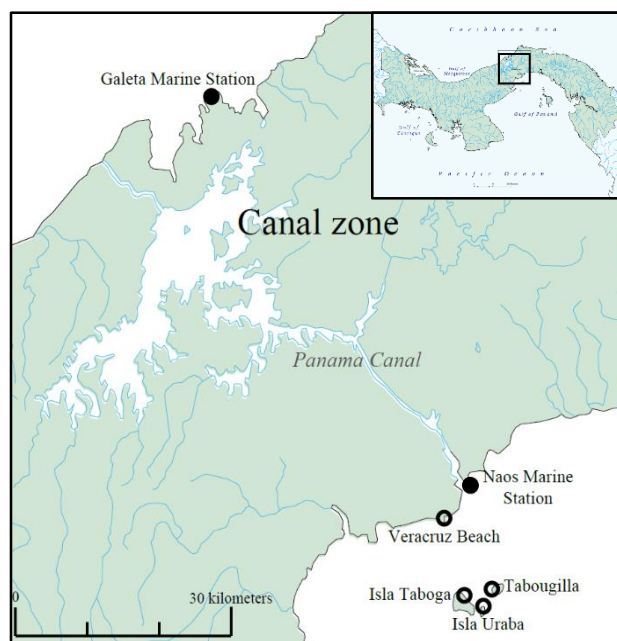
Figure 2.2. Map of study area in Panama. Sample sites are labeled with black circles. STRI Marine Laboratories noted by black circles. Inset map shows study area within the map of Panama.

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were beach-collected at lowest low tide (spring tide), while remaining samples were collected at 3 m depth off the coasts of Isla Uraba and Isla Taboga. Isla Uraba and Isla Taboga shell samples are within 15 km of the water collection site. Water collection sites in both the Pacific and Caribbean were ≤ 100 m from shore.

Sample handling and analysis was as follows. After collection, gastropod bodies were removed and shells were cleaned to remove surface contaminants by lightly sanding and scrubbing with dilute soap and water. *Vasum* shells were soaked in dilute bleach (1/3 bleach, 2/3 water) to remove encrustations and persistent surface dirt. Approximately 760 samples for C and O isotopic analyses were drilled around the spire at <0.5 mm depth using a 0.5 mm dental bur and a dental drill at low speed. Samples were drilled at 2-3 mm intervals. Powdered shell carbonate (40-80 µg) was analyzed for $\delta^{18}O$ and $\delta^{13}C$ on a Thermo Scientific MAT 253 IRMS/Kiel IV instrument at the Stable Isotope Geosciences Facility at Texas A&M University. At least every 5th sample was run in duplicate. Precision was 0.07‰ for $\delta^{18}O$ and 0.03‰ for $\delta^{13}C$ based on replicates of standards.

2.3 Results and Discussion

2.3.1 Oxygen Isotopes

Measured shell $\delta^{18}O$ profiles from the TEP show more variability than those from the SWC (Figure 2.3). The average $\delta^{18}O$ range ($\Delta^{18}O$) in the Pacific (Veracruz Beach = 3.1‰, Isla Uraba = 2.8‰, Isla Taboga = 2.5‰) is about 1-2‰ greater than that for the Caribbean (1.3‰; Table 2.2). Reduced $\delta^{18}O$ seasonality in SWC shells compared with TEP shells can be partially attributed to the little to no upwelling in this region of the SWC (D'Croz et al., 1991; D'Croz et al., 1999). Average shell $\delta^{18}O$ values from Veracruz Beach, Isla Uraba, and Isla Taboga in the TEP (-2.1‰) are about 1‰ lower than those from Punta Galeta in the SWC (-1.3‰). Shell $\delta^{18}O$

Table 2.2. Sample information, shell and environmental isotopic data and relationships, and predicted $\delta^{18}O$ values and ranges. Predicted shell $\Delta^{18}O$ range and average $\delta^{18}O$ were calculated using the previously described methods.

Location	Shell ID	Species	n	O-C R	O-C slope	$\Delta^{18}O$ (‰)	$\Delta^{13}C$ (‰)	Mean $\delta^{18}O$ (‰)	Mean $\delta^{13}C$ (‰)	C_M (%)
Tropical Eastern Pacific (TEP):										
δ_w & $\delta^{13}C_{DIC}$ (Robbins et al., 2012)						3.1	2.6	-0.8	-0.1	
Predicted shell				0.4	1.0	5.1	2.6	-2.6	2.6	
Veracruz Beach	VC04-5	*Conus*	34	0.5	1.6	3.3	1.6	-2.1	1.3	9
Veracruz Beach	VC04-6	*Conus*	30	0.7	2.0	3.2	1.5	-2.3	0.9	11
Veracruz Beach	VC04-7	*Conus*	30	0.7	1.9	3.4	1.2	-2.6	0.7	13
Veracruz Beach	VC04-8	*Conus*	32	0.7	1.6	3.4	1.3	-2.4	1.2	9
Veracruz Beach	VC04-11	*Strombus*	18	0.8	1.6	2.6	1.4	-1.8	-0.7	22
Isla Uraba	IU01-9	*Vasum*	59	0.2	-0.3	2.8	2.1	-2.0	1.3	8
Isla Taboga	IT02-1	*Vasum*	80	0.1	0.2	2.6	2.4	-1.9	1.4	8
Isla Taboga	IT02-2	*Vasum*	65	0.3	-0.3	2.3	2.0	-2.1	1.2	9
Shell mean				**0.5**	**1.0**	**2.9**	**1.6**	**-2.1**	**0.9**	**10**
Southwest Caribbean (SWC):										
δ_w & $\delta^{13}C_{DIC}$ (Robbins et al., 2012)						1.9	1.5	0.6	0.5	
Predicted shell				0.4	0.4	1.7	1.5	-0.7	3.2	
Punta Galeta	PG01-7	*Vasum*	58	0.3	0.2	1.3	2.2	-1.3	1.6	10
Punta Galeta	PG01-4	*Vasum*	71	0.3	0.4	1.3	1.1	-1.3	1.7	10
Punta Galeta	PG01-6	*Vasum*	87	0.1	0.1	1.4	1.1	-1.3	1.8	9
Shell mean				**0.2**	**0.2**	**1.3**	**1.5**	**-1.3**	**1.7**	**10**

versus length (Figure 2.3) highlights the more pronounced cyclicity in the TEP compared with the SWC. Counting peaks and troughs, the Veracruz Beach (TEP) samples show 0.5 to 3.5 cycles, the Isla Uraba (TEP) sample shows 8 cycles, the Isla Taboga (TEP) samples show 7.5 to 8.5 cycles, and the Galeta (SWC) samples show 3.5 to 5 cycles (Figure 2.3).
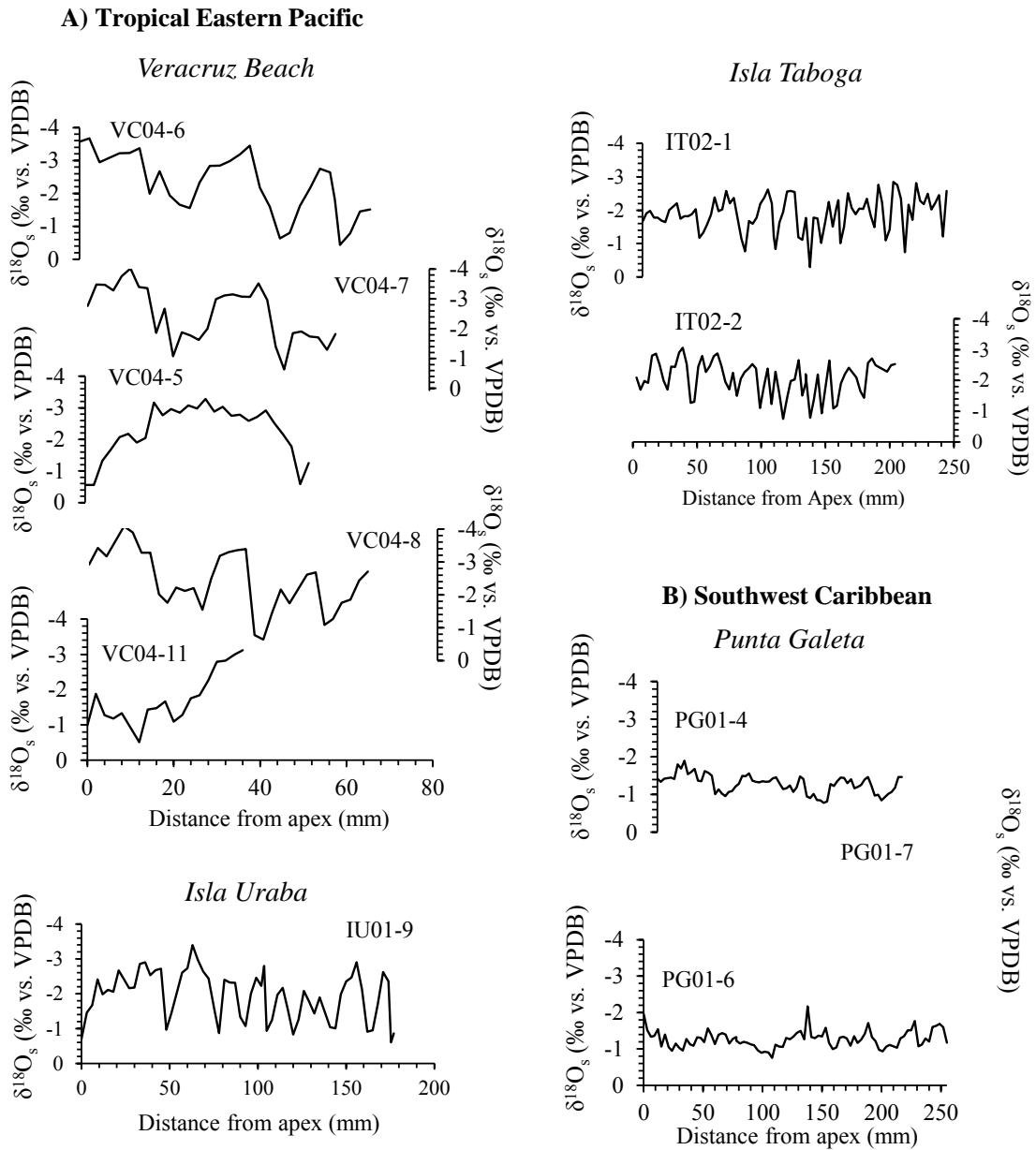
Figure 2.3. Shell oxygen isotope profiles versus distance in (A) Pacific and (B) Caribbean samples.

To determine whether these are annual cycles, we must first calculate predicted shell aragonite $\delta^{18}O$ values ($\delta^{18}O_{ar}$ versus VPDB) from water temperatures (T), seawater $\delta^{18}O$ values (δ_w versus VSMOW), and the Grossman and Ku (1986) equation, as modified by Hudson and Anderson (1989):

$$T\ (°C) = 19.7 - 4.34\ (\delta^{18}O_{ar} - \delta_w) \qquad (1).$$

Water temperatures were provided by STRI's Physical Monitoring Program. For the years lacking water data, expected $\delta^{18}O_{ar}$ was determined based on 2011-12 δ_w values and daily temperatures (see Figure 2.4, 2.5). Measured isotope profiles are compared to predicted profiles in Figures 2.4 and 2.5.

Dates were assigned to shell length by matching predicted and measured $\delta^{18}O$ minima and maxima as anchor points (Figure 2.4, 2.5). The collection date was also chosen as an anchor point. Between anchor points, dates were assigned based on linear interpolation. The predicted $\delta^{18}O_{ar}$ values show an annual cyclicity for both coasts. Thus, each measured $\delta^{18}O_{ar}$ cycle represents one year of growth.

The larger *Vasum* shells from both the Pacific and Caribbean typically have longer records (3.5-8.5 years) than the smaller *Conus* shells from the Pacific. Interestingly, the average growth rate for *Vasum* specimens in the Pacific (2.6±0.3 cm/yr) is half that in the Caribbean (5.3±1.4 cm/yr). *Vasum* and *Conus* from the Pacific have comparable average growth rates (2.6 and 2.7 cm/yr, respectively), whereas the one *Strombus* sample has a growth rate of 7.2 cm/yr (Table 2.1).
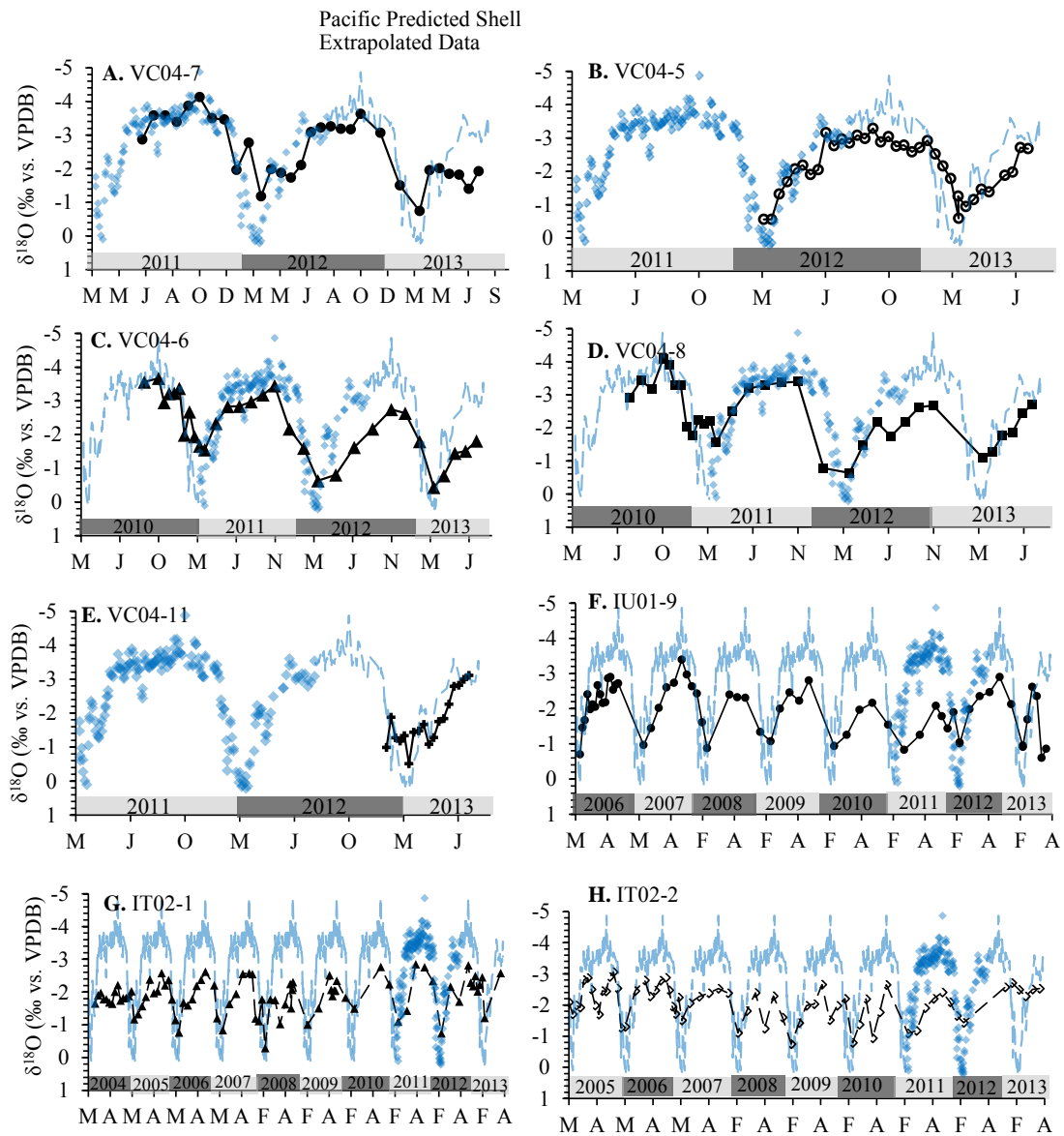
Figure 2.4. Measured versus predicted δ18O profiles for tropical eastern Pacific (TEP) specimens. Figures 1-4A-E show data for *Conus* (A-D) and *Strombus* (E) specimens from Veracruz Beach. Figures 1-4F-H show data for *Vasum* specimens from islands in the Gulf of Panama (F, Isla Uraba, *Vasum*; G,H, Isla Taboga). The predicted shell profile for the TEP is represented by transparent blue diamonds. Predicted δ18O values for extrapolated years (dashed blue lines) are based on 2011 and 2012 water temperatures provided by STRI's Physical Monitoring Program and δw values. Curves were fit by matching maximum and minimum values from the measured curve to the predicted curve, beginning at the date of collection in July 2013 and going back through time. Between these anchor points, dates were assigned based on linear interpolation.

For individual shells, the average measured $\delta^{18}O$ value is statistically identical to the predicted value (TEP= -2.6‰, SWC= -0.7‰; Figure 2.4, 2.5). In contrast, the average measured $\Delta^{18}O$ ranges for the TEP (2.9‰) and for the SWC (1.3‰) shells are less than the predicted shell ranges, 5.1‰ and 1.7‰, respectively.

At Punta Galeta (SWC), measured shell profiles (e.g. PG01-7, PG01-4, PG01-6) reflect the irregular pattern similar to the predicted shell profiles, including multiple sub-peaks (Figure 2.5). In the Pacific, Veracruz Beach shell profiles typically exhibit an asymmetrical shark-tooth pattern slightly offset from the shape of the predicted profile (e.g. VC04-6, IU01-9; Figure 2.4). This asymmetrical shark-tooth pattern may indicate faster growth during upwelling, characteristic in the Gulf of Panama (Tao et al., 2013). Isla Uraba and Isla Taboga samples (TEP) exhibit a more cuspate to irregular shape.

To test the fidelity of shell $\delta^{18}O$ values as recorders of the aforementioned δ_w values, measured shell $\delta^{18}O$ profiles were compared to predicted $\delta^{18}O_{ar}$ values (Figures 2.4, 2.5). Measured shell $\delta^{18}O$ profiles exhibit the same general patterns observed in predicted $\delta^{18}O$ shell profiles, with some differences. Measured $\Delta^{18}O$ ranges are 1.7 to 2.8‰ less than predicted shell $\Delta^{18}O$ ranges in the TEP and 0.3 to 0.4‰ less than predicted shell $\delta^{18}O$ ranges in the SWC (Figures 2.4, 2.5). In general, measured TEP shell $\delta^{18}O$ profiles do not appear to record $\delta^{18}O$ minima or maxima (Figure 2.4). *Conus* and *Strombus* specimens from Veracruz Beach appear to record these values within about 1‰ in contrast with *Vasum* samples from Isla Uraba and Isla Taboga which record these values within about 2‰ (Figure 2.4). These sites are more offshore and appear to be less influenced by upwelling and freshening than nearshore samples. This is comparable to results of Tao et al. (2013) who attribute the inability to record upwelling events ($\delta^{18}O$ maxima) to the isolated and short-lived nature of upwelling events in the Gulf of Panama.
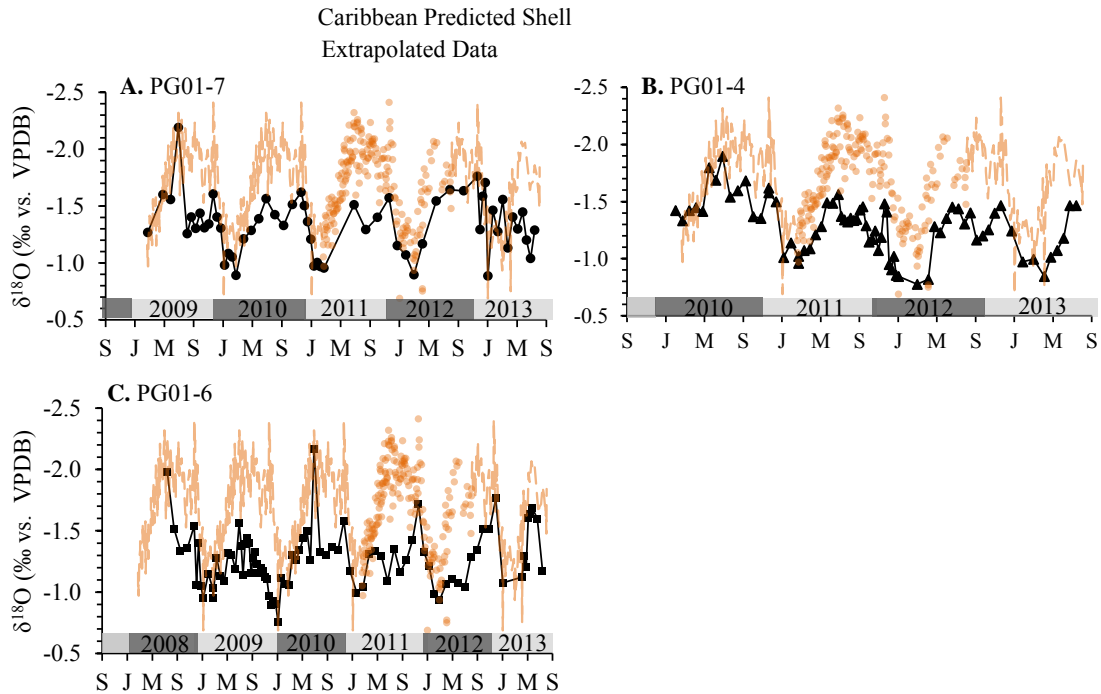
Figure 2.5. Measured versus predicted δ^{18}O profiles for Punta Galeta (Caribbean) *Vasum muricatum* (PG01-7, PG01-4, PG01-6) specimens. Predicted shell profile for the SWC is represented by transparent orange circles. Predicted δ^{18}O values for extrapolated years (dashed orange lines) are based on 2011 and 2012 water temperatures provided by STRI's Physical Monitoring Program and δ$_w$ values. Curves were fit by matching maximum and minimum values from the measured curve to the predicted curve, beginning at the date of collection in July 2013 and going back through time. Between these anchor points, dates were assigned based on linear interpolation.

The same might be said for freshening events. Shells from Punta Galeta in the SWC show a different trend. Measured SWC shell δ^{18}O profiles appear to capture δ^{18}O maxima, but not δ^{18}O minima (Figure 2.5). At Punta Galeta, freshwater influence at the water intake location may explain why predicted δ^{18}O values are more negative than measured δ^{18}O values from shells collected closer to shore.

2.3.2 Carbon Isotopes

Although more subtle than δ^{18}O profiles, both Pacific and Caribbean shell δ^{13}C profiles show cyclicity (Figure 2.6A, B). Average shell and δ^{13}C$_{DIC}$ values from the SWC (1.7‰ and

0.5‰, respectively) are greater than those from the TEP (0.9‰ and -0.1‰, respectively). The range in shell $\delta^{13}C$ values ($\Delta^{13}C$) in the TEP (1.6‰) is roughly equivalent to the SWC (1.5‰).

Mollusk shell $\delta^{13}C$ values commonly decrease with ontogeny likely reflecting increased metabolism with body size as shell growth rates slow with age (Lorrain et al., 2004; Gillikin et al., 2007, 2009). However, no *Conus* shell $\delta^{13}C$ values show this decreasing trend with ontogeny. There is a decrease in $\delta^{13}C$ with ontogeny in one *Strombus* sample (VC04-11; R^2=0.76) and four *Vasum* specimens (PG01-7, R^2= 0.42; IT02-1, R^2= 0.35; IT02-2, R^2= 0.59; IU01-9, R^2= 0.59). The Veracruz Beach *Strombus* $\delta^{13}C$ averages 1.8‰ lower than *Conus* and *Vasum* values for the same area.

The oxygen isotope chronologies previously discussed are used to compare $\delta^{13}C_{DIC}$ (predicted shell $\delta^{13}C$) to measured shell $\delta^{13}C$ values. Predicted shell $\delta^{13}C$ curves for the TEP and SWC are approximated using measured $\delta^{13}C_{DIC}$ and the aragonite-bicarbonate fractionation relation of Romanek et al. (1992). Average measured shell values are ~1-3‰ lower than average predicted shell values (Figure 2.6A, B), reflecting the incorporation of light metabolic C into the shell carbonate. In the TEP samples, some $\delta^{13}C$ values are upwards of 3.0‰ lower than predicted shell $\delta^{13}C$ values (i.e. VC04-11, VC04-7).

Veracruz Beach, Pacific

Isla Taboga (IT) & Isla Uraba (IU), Pacific

Punta Galeta, Caribbean
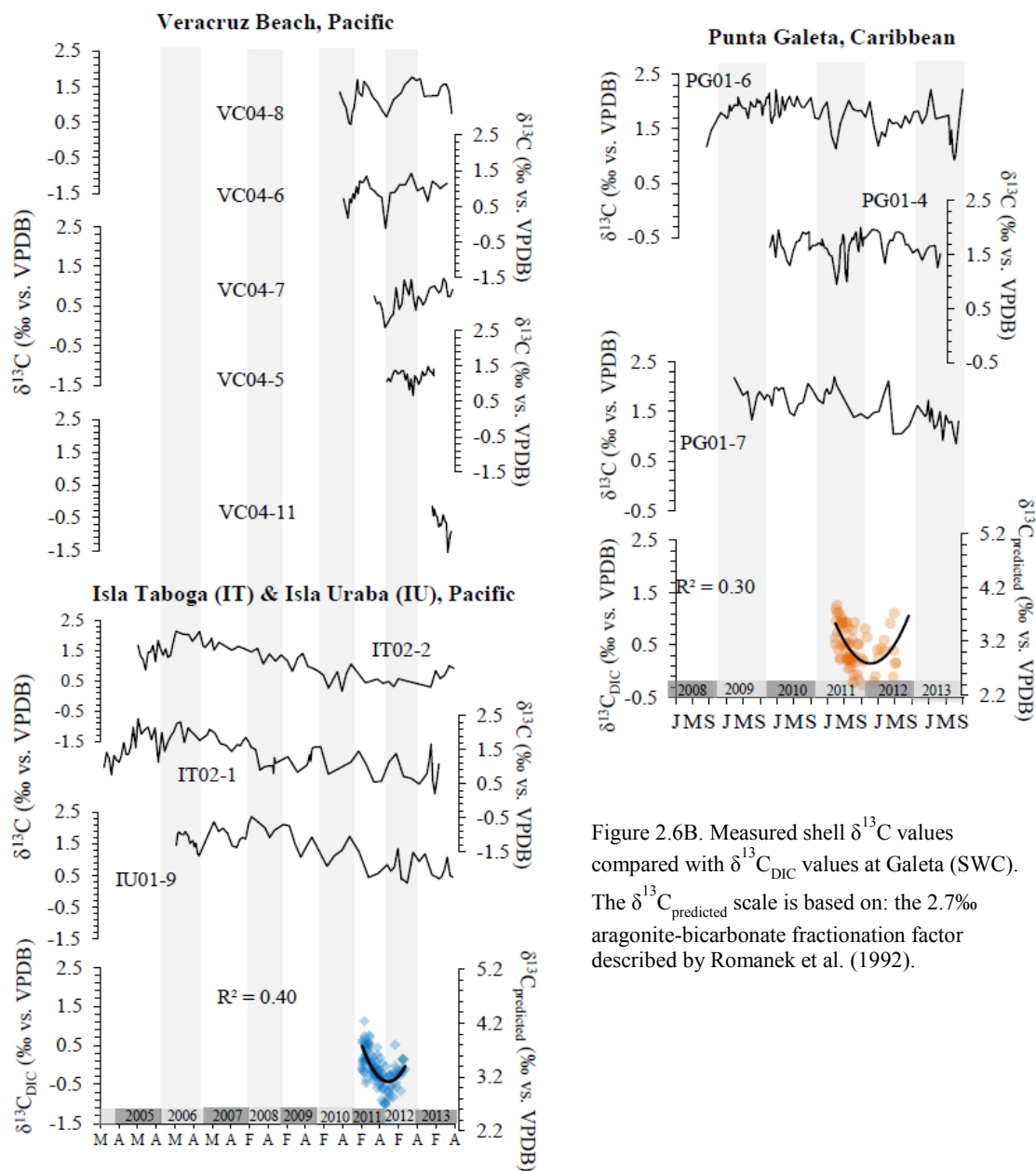
Figure 2.6A. Measured shell δ^{13}C values compared with δ^{13}C$_{DIC}$ values at Naos (TEP). The δ^{13}C$_{predicted}$ scale is based on: the 2.7‰ aragonite-bicarbonate fractionation factor described by Romanek et al. (1992).

Figure 2.6B. Measured shell δ^{13}C values compared with δ^{13}C$_{DIC}$ values at Galeta (SWC). The δ^{13}C$_{predicted}$ scale is based on: the 2.7‰ aragonite-bicarbonate fractionation factor described by Romanek et al. (1992).

Conus carbon isotope shell profiles from Veracruz Beach (TEP) appear to reflect changes in $\delta^{13}C_{DIC}$ (Figure 2.6A). Veracruz Beach (*Conus*) samples record the negative trend as $\delta^{13}C_{DIC}$ decreases from March to September 2011 primarily during the rainy season, and the positive trend as $\delta^{13}C_{DIC}$ increases from October 2011 to March 2012 primarily during the dry season. *Vasum* shells do not appear to record the same trends in $\delta^{13}C_{DIC}$. During the rainy season, ^{13}C-depleted DIC enters these coastal environments due to increased runoff (Robbins et al., 2012). During the dry season, Gulf of Panama waters are also influenced by the upwelling of ^{13}C-depleted DIC during upwelling events (Robbins et al., 2012). Though upwelling advects ^{13}C-depleted water, its effect on seawater $\delta^{13}C_{DIC}$ will be less than freshwater input. This is because the $\delta^{13}C_{DIC}$ of upwelled waters from 60-80 m depth (D'Croz and O'Dea, 2007) will be greater than >1‰ (Quay et al., 2003) and much higher than $\delta^{13}C_{DIC}$ of freshwater discharge from tropical rivers (e.g., -10‰; Moyer et al., 2013). While Galeta (SWC) $\delta^{13}C_{DIC}$ values decrease during the rainy season (May-December), biweekly variability is substantial (Figure 2.6B; Robbins et al., 2012). *Vasum* shell $\delta^{13}C$ values from Punta Galeta (SWC) do not appear to record $\delta^{13}C_{DIC}$ variability, except for sample PG01-6 (Figure 2.6B). This is likely due to more poorly resolved seasonal variation in Punta Galeta $\delta^{13}C_{DIC}$ ($R^2 = 0.30$) compared with Naos $\delta^{13}C_{DIC}$ ($R^2 = 0.40$).

The $\delta^{13}C$ variations between species from the same site can be explained by non-equilibrium incorporation of C resulting from kinetic effects or metabolic effects (i.e. vital effects; Beirne et al., 2012). Specifically, greater metabolic carbon (C_M) availability in internal fluids perhaps due to decreasing growth rates (high relative respired to precipitated carbon ratio) causes the incorporation of more ^{12}C into shell carbonate (Lorrain et al., 2004; Gillikin et al., 2007; Gillikin et al., 2009). For each shell, C_M can be calculated using the equation described by Gillikin et al. (2009),

$$C_M = (\delta^{13}C_{ar} - \varepsilon_{ar\text{-}b} - \delta^{13}C_{DIC})/(\delta^{13}C_M - \delta^{13}C_{DIC}) \qquad (2)$$

where $\delta^{13}C_{ar}$ is the shell $\delta^{13}C$ value (Table 2.2), $\varepsilon_{ar\text{-}b}$ is the fractionation factor between aragonite and HCO_3^- (2.7‰; Romanek et al., 1992), $\delta^{13}C_{DIC}$ is the water dissolved inorganic carbon value (Table 2.2), and $\delta^{13}C_M$ is the $\delta^{13}C$ of metabolic carbon (-15.3‰ coeval *Conus* tissues, unpublished data) under the assumption that $\delta^{13}C_b \approx \delta^{13}C_{DIC}$. The *Strombus* shell incorporates roughly 22% C_M compared with an average of 10% for *Conus* and 9% for *Vasum* specimens.

Feeding habit differences between *Conus*, *Strombus*, and *Vasum* gastropods may impact the incorporation of C_M. The offset between $\delta^{13}C_{DIC}$ and shell values are within ranges observed by others (~1‰; Beirne et al., 2012). Using %C_M values and $\delta^{13}C_{DIC}$ values, the relationship between average $\delta^{13}C_s$ and $\delta^{13}C_{DIC}$ values for *Conus* (eq. 3), *Strombus* (eq. 4), and *Vasum* (eq. 5) can be described as,

$$\delta^{13}C_{ar} = \delta^{13}C_{DIC} + 1.1‰\ (\pm 0.2) \qquad (3)$$

$$\delta^{13}C_{ar} = \delta^{13}C_{DIC} - 0.6‰ \qquad (4)$$

$$\delta^{13}C_{ar} = \delta^{13}C_{DIC} + 1.3‰\ (\pm 0.1) \qquad (5).$$

Interestingly, *Conus* and *Vasum* are both predatory carnivores (Todd, 2001) and have similar $\delta^{13}C_{ar}$ -$\delta^{13}C_{DIC}$ offsets, whereas *Strombus* are herbivore-detritivores (Aranda et al., 2003) and have a lower $\delta^{13}C_{ar}$ -$\delta^{13}C_{DIC}$ offset. This may be related to mobility differences between species. For instance, *Strombus*' greater metabolic CO_2 availability in shell growth may reflect continual "leaping" and grazing in search of macroalgae, requiring an active metabolism (Berg, 1975). Although *Conus* are also mobile, they feed slowly, only at night, and remain immobile during digestion of their prey (Kohn, 1959, 1968).

2.3.3 Isotope profiles as environmental proxies

Shell δ^{18}O- δ^{13}C (O-C) correlations and δ^{18}O range (Δ^{18}O) can help identify upwelling and freshening environments in the SWC and TEP. Previous studies indicate the expected O-C correlation is negative during the dry season and positive during the rainy season in both Naos (TEP) and Galeta (SWC) (Robbins et al., 2012). The O-C correlations in Galeta (minimal upwelling) are weaker than those from Naos, which are influenced by upwelling and freshening. Therefore the Gulf of Panama is characterized by a large Δ^{18}O range and little O-C correlation, whereas the SWC is characterized by a low Δ^{18}O range and a non-significant to positive O-C correlation (Robbins et al., 2012).

Oxygen and carbon isotopic compositions from Veracruz Beach (TEP) shells in this study are similar to shallow (10-15 m) *Conus* shells from the Gulf of Panama (TEP) from Tao et al. (2013), but they record more positive O-C correlations indicating more freshwater influence (Figure 2.7). Veracruz Beach samples, collected in the intertidal zone, are more nearshore than any samples collected in the aforementioned study. These samples in Figure 2.7 plot within or above the zone "substantial seasonal upwelling and freshening" identified as a eutrophic environment based on hydrographic information (e.g. D'Croz et al. 1991). The Veracruz Beach samples show more freshwater influence than offshore Isla Taboga and Isla Uraba samples.

The TEP shell Δ^{18}O ranges from this study are comparable to those found in shallow environments by Tao et al. (2013), but shells from this study showed more positive δ^{18}O- δ^{13}C (O-C) correlations suggestive of more freshwater influence. This study also suggests Punta Galeta in the SWC experiences "seasonal freshening and minimal upwelling" in contrast with "minimal seasonal upwelling and freshening" found in other SWC localities based on models produced by Tao et al. (2013). Again, this is likely due to the shallow occurrence of our shells and their close proximity to shore. Specimens from the Punta Galeta (SWC) record mesotrophic

values in agreement with hydrographic data from nearby San Blas Archipelago, Panama (SWC;

D'Croz et al., 1999). This area may be subject to episodic eutrophication resultant from the

removal of mangrove forests at nearby Rio Coco Solo that act as a natural barrier between

terrestrial nutrients and the coastal ocean (Lin and Dushoff 2004).
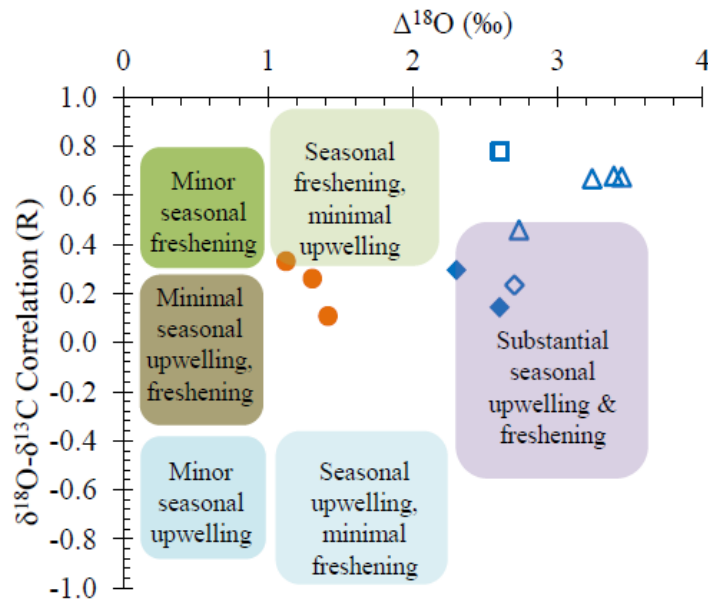
Figure 2.7. Shell O-C correlation and $\delta^{18}O$ range ($\Delta^{18}O$) for all samples. Filled blue diamonds = *Vasum* from Isla Uraba, unfilled blue diamonds = *Vasum* from Isla Taboga, blue 'x' = *Strombus* from Veracruz beach and unfilled triangles = *Conus* from Veracruz Beach. The orange filled circles = *Vasum* from Punta Galeta. Predicted shells are black squares (Naos (TEP) = filled, Galeta (SWC) = unfilled). Labeled fields are based on models of environments described by Tao et al. 2013.

2.3.4 Application to Plio-Pleistocene environmental reconstruction

When applying fossil shell $\delta^{18}O$ and $\delta^{13}C$ measurements for marine paleoenvironmental reconstructions of the Plio-Pleistocene, one must proceed with caution. Fossil shell $\delta^{18}O$ minima and maxima may underestimate variations in paleotemperature and salinity in both the TEP and SWC. Therefore, measures of quantified upwelling and freshening may be skewed.

Average shell $\delta^{18}O$ values in this study (TEP = -2.1‰, SWC = -1.3‰) and Tao et al. (2013) (TEP = -1.9‰, SWC = -1.2‰) indicate modern habitats in the SWC and TEP are similar on average. However, fossil specimens chosen for paleoenvironmental reconstructions should have well constrained paleodepths considering modern environments at shallower depths (3 m from this study versus 10-15 m in Tao et al., 2013) produce slightly higher average C-O correlations (TEP = 0.3, SWC = 0.2) and $\Delta^{18}O$ ranges (TEP, SWC = 0.2) (Tao et al., 2013). Furthermore, proximity to freshwater influence must be well understood since the Gulf of Panama (TEP) samples from this data set are more nearshore ($\leq$ 20 km) and record more freshening (^{18}O depletion) than those offshore (~120 km; Tao et al., 2013).

Finally, shell $\delta^{13}C$ of *Conus*, *Vasum*, and *Strombus* gastropods incorporate about 10-20% C_M, thus do not represent $\delta^{13}C_{DIC}$ values alone. Nevertheless, *Conus* $\delta^{13}C$ profiles record seasonal $\delta^{13}C_{DIC}$ variations in the Gulf of Panama, where seasonality is distinct. Fossil *Conus* shell $\delta^{13}C$ records appear to be most useful for O-C correlations, especially in identifying freshwater input.

2.4 Conclusions

Measured shell $\delta^{18}O$ and $\delta^{13}C$ values reflect predicted seasonal upwelling and freshening signals in the Pacific and seasonal freshening with minimal upwelling in the Caribbean, in agreement with previous studies. Consequently, the Caribbean $\delta^{18}O$ and $\delta^{13}C$ profiles show

reduced seasonality compared with Pacific profiles. However, shell $\delta^{18}O$ minima and maxima often underestimate variations in paleotemperature and salinity in both the TEP and SWC.

Both Pacific and Caribbean shell $\delta^{13}C$ profiles show cyclicity. *Conus* samples from Veracruz Beach (TEP) record seasonal changes in $\delta^{13}C_{DIC}$. The decrease in $\delta^{13}C_{DIC}$ is detectable during the rainy season due to freshwater influence, while the increase in $\delta^{13}C_{DIC}$ is detectable during the dry season despite the fact that upwelling is occurring. Galeta (SWC) samples do not appear to record overall trends in $\delta^{13}C_{DIC}$ due to variability in $\delta^{13}C_{DIC}$.

Measured $\delta^{13}C$ profiles are ~1-3‰ less than predicted $\delta^{13}C$ profiles calculated based on measured $\delta^{13}C_{DIC}$ values and $\varepsilon_{ar-b} = 2.7$‰. This can be attributed to the incorporation of ^{13}C-depleted metabolic carbon into the shell carbonate. The percent C_M content of *Conus* and *Vasum* shells is about 10% in comparison with one *Strombus* specimen that has 22% C_M incorporation. The taxonomic differences in C_M likely reflects availability of metabolic CO_2 for shell growth, which we hypothesize is related to feeding habit and activity. *Strombus* are herbivore-detritivores and very active, whereas *Conus* and *Vasum* are both predatory carnivores and, at least in the case of *Conus*, are relatively inactive.

CHAPTER III

CONCLUSIONS: NITROGEN ISOTOPES IN BIVALVES AS AN INDICATOR OF

NUTRIENT SOURCE IN COASTAL WATERS IN THE BOCAS DEL TORO

ARCHIPELAGO, PANAMA

3.1 Introduction

Both natural and anthropogenic factors contribute to the increased eutrophication,

pollution, and sedimentation that threaten the Bocas del Toro Archipelago, Panama (Klaus et al.,

2011; Aronson et al., 2014). Dredging for shipping in Bocas del Toro results in physical damage

to these ecosystems (Seemann et al., 2014). Indirect consequences include the removal of

mangrove forests, destabilization of soil, and increased erosion (Lin and Dushoff, 2004).

Mangrove destruction removes a natural barrier that limits influx of terrestrial nutrients (NO_3^-,

NO_2^-, PO_4^{3-}, NH_3) reaching coastal waters (Lin and Dushoff, 2004). Without mangrove buffers,

coastal environments are more susceptible to eutrophication promoting, faunal turnover and loss

of biodiversity in the southwest Caribbean (Jackson et al., 2001; Seemann et al., 2014).

In this study, N-isotope ratios in tissues and shells of modern bivalves (*Isognomon*

alatus, Pinctada imbricata, and *Brachidontes exustus*), supplemented by stable carbon isotope

ratios were examined as an indicator of terrestrial, marine, and anthropogenic nutrient sources in

coastal environments in the Bocas del Toro Archipelago, Panama. Spatial variation in shell and

tissue $\delta^{15}N$ values was determined in three mollusk species since coastal habitat heterogeneity

and species offsets can be unpredictable (Carmichael et al., 2008; Carmichael, 2010). These

epifaunal bivalves are byssally attached to dock supports or mangrove roots, filtering water for

organic matter through their gills (Todd, 2001). Since bivalves are primary consumers, temporal

variation in $\delta^{15}N$ values more likely reflect changing N source than trophic level changes

(Carmichael et al., 2004, 2008). In contrast, predators are enriched in ^{15}N by 3.4‰ compared with their prey (Minigawa and Wada, 1984; Peterson and Fry, 1987). This research aids in application of $\delta^{15}N$ in mollusk shells to understand nutrient sources in past environments where measured environmental data are lacking. To my knowledge, this is the first nitrogen isotope study of bivalves in the southwest Caribbean.

3.2 Nitrogen Isotopes as a Nutrient Tracer

Nitrogen is transferred between reservoirs by nitrification and denitrification. Nitrification oxidizes NH_3 to produce NO_3^-, biologically useable N, whereas denitrification refers to the reduction of NO_3^-, typically by bacteria, a process used to remove biologically-active nitrogen from wastewaters. These reactions impart isotope fractionations that can be traced by $^{15}N/^{14}N$ ratios (reported as $\delta^{15}N$ relative to air). For instance, NO_3^- in human and animal waste is enriched in ^{15}N ($\delta^{15}N$ = 10 to 20‰; McClelland et al., 1997; Savage, 2005) by the volatilization of ammonia from microbial degradation of wastewaters (e.g. McClelland et al., 1997; Montoya, 2007). Specifically, an undeveloped tropical environment from Playa Limon, Panama, had groundwater NO_3^- $\delta^{15}N$ values of around -3‰, whereas a similar but more urbanized site susceptible to wastewater input had groundwater values closer to 12‰ (Bowen and Valiela, 2008). The $\delta^{15}N$ of terrestrial organic matter from a shallow marine environment in Brazil is on average lower (3.7‰) than that of marine organic matter (7.5‰) on average (Corbisier et al., 2006); however nitrification and denitrification often enrich terrestrial inputs in $\delta^{15}N$ before they reach the ocean (Montoya, 2007).

Carbon isotopes are used to describe sources of organic input to coastal ecosystems. The Brazilian environment discussed previously has average terrestrial $\delta^{13}C$ values (-29.4±0.3‰) that are substantially lower than average marine particulate organic matter (POM) values from the

same site (-18.6±0.5‰, respectively; Corbisier et al., 2006). Estuarine phytoplankton δ^{13}C values from Jamaica's Hunts Bay are intermediate at -23.1‰ (Andrews et al., 1998). In Almirante Bay, Panama, marine and terrestrial organic matter sources have δ^{15}N and δ^{13}C values of respectively 2.5‰ and -12.1‰ for terrestrial grass, 1.5‰ and -8.5‰ for seagrass *Thalassia testudinum*, and 1.7‰ and -26.5‰ for mangrove (*Rhizophora*) peat (Hilbun, 2009). A nearby tropical site at Barro Colorado Island, Panama has forest floor litter δ^{13}C values of -30.1±0.5‰ and grassland values of -16.2±4.2‰ (Schwendenmann and Pendall, 2006). These values provide guidelines for interpreting the relative contributions these varied organic matter sources to bivalve δ^{15}N and δ^{13}C values.

Analyses of organic matrix preserved in shells can extend this approach into the historical and fossil record. Tissue-shell offsets in δ^{15}N (Δ^{15}N$_{\text{tissue-shell}}$) can be variable depending on tissue composition, turnover rate, or other physiological factors (Lorrain et al., 2002; Carmichael et al., 2008). Thus, to confirm shell δ^{15}N values as an indicator of tissue values, one

Table 3.1. Physical characteristics of the eight sample sites in Bocas del Toro, Panama at the time of sampling. Dashes occur where data was not collected. I= *I. alatus*, P= *P. imbricata*, Br= *B. exustus*.

	Escudo de Veraguas	Cayo Adriana	Isla Popa	Rio Guarumo	Boca del Drago	STRI Facility	Bocas Town	Bocas Town Marina	Avg.	Standard Deviation
DO (mg/L)	5.3	5.5	3.9	4.4	5.2	6.7	5.1	4.4	5.0	0.8
DO %	83	80	57	68	78	104	78	67	77	13
T (°C)	28.5	28.1	28.7	29.3	28.2	30.0	28.8	28.7	28.8	0.6
SPC (mS/cm)	51.8	47.9	48.5	-	48.4	48.1	49.2	49.7	49.1	1.3
Sal (ppt)	34.0	31.1	31.5	31.6	31.5	31.3	32.0	32.4	31.9	0.9
Mangroves (Y/N)	Y	Y	Y	Y	Y	Y	N	Y		
Seagrass (Y/N)	Y	N	Y	-	Y	-	N	-		
Algae (Y/N)	Y	N	Y	-	Y	Y	Y	Y		
Species Available (I, P, Br)	P	I, P	I, P, Br	P, Br	I, P, Br	I, P, Br	I, P	I, P		
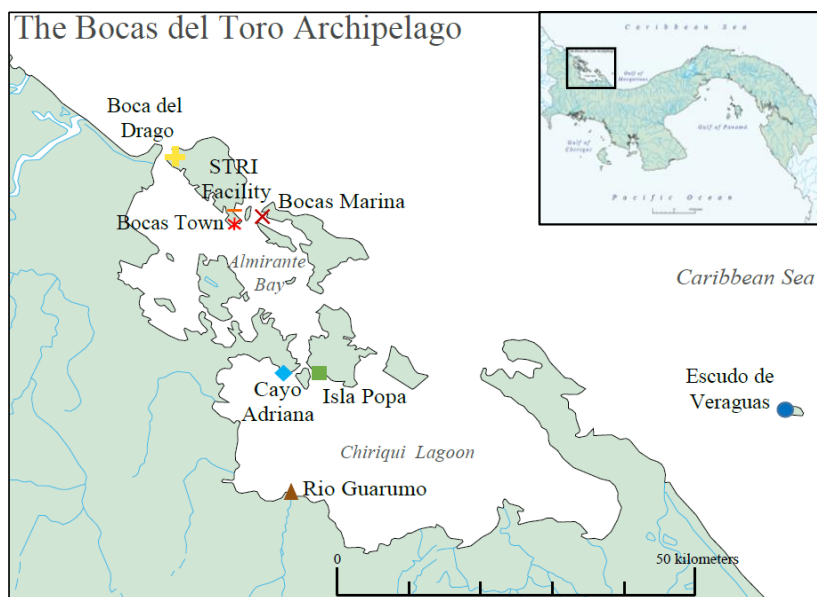
Figure 3.1. Map of the Bocas del Toro Archipelago, Panama. Sample sites are labeled with location symbols used in future figures.

must consider various bivalve species and tissues from the same environment (Cabana and Rasmussen, 1996; Lorrain et al., 2002; Carmichael et al., 2008; O'Donnell et al., 2003). Establishing baseline $\delta^{15}N$ values for modern primary consumers such as bivalves will make stable isotope ecology and paleoecology studies more accessible in the southwest Caribbean.

3.3 Hydrologic Setting

The Bocas del Toro Archipelago is comprised of two oceanographic features, Almirante Bay and Chiriquí Lagoon (Figure 3.1). These semi-enclosed areas are bordered by mangroves and have restricted exchange with Caribbean waters (Quiroz et al., 2011). Seasonality in Almirante Bay and Chiriquí Lagoon is dominated by runoff and rainfall regimes controlled mainly by the migration of the Intertropical Convergence Zone (Kaufmann and Thompson,

2005). Almirante Bay is exposed to the Caribbean Sea near Boca del Drago. Almirante Bay also has small outlets near Isla Colón and Isla Popa. Chiriquí Lagoon is exposed to the Caribbean Sea near Cayo Agua, however the lagoon receives significant freshwater from the Cricamola River, Rio Guarumo, and numerous others (Quiroz et al., 2011). Thus, Chiriquí Lagoon is more turbid and nutrient-rich (N, P, Si) than Almirante Bay (D'Croz et al., 2005; Kaufmann and Thompson, 2005).

River discharge is the primary control on nutrient content in Almirante Bay and Chiriquí Lagoon. Increased N influence from runoff has been detected in nearshore Chiriquí Lagoon compared with Almirante Bay and marine environments (D'Croz et al., 2005). Nutrient-rich waters favor the expansion of macroalgae over seagrass and coral reef habitats, decreasing overall biodiversity. In Bocas del Toro especially, there is a correlation between high runoff and high chlorophyll-a concentrations (D'Croz et al., 2005). Changes in habitat and nutrient conditions may have significant detrimental impacts on coastal marine biodiversity, fisheries, and tourism (e.g. Jackson 2001; Jackson et al., 2001; Lin and Dushoff, 2004; Seemann et al., 2014).

3.4 Samples and Methods

Live specimens of the bivalves *Pinctada imbricata*, *Isognomon alatus*, and *Brachidontes exustus* were hand-collected in July 2013 from mangrove roots or docks at eight locations in the Bocas del Toro Archipelago, Panama (Figure 3.1). Study sites represent coastal environments with varying human influence, including more urbanized, less urbanized, uninhabited, and oceanic sites. Escudo de Veraguas serves as an open-ocean end-member, whereas the Rio Guarumo site is most affected by freshwater. Bocas Marina and Bocas Town sites are considered more urbanized and are known to be affected by high sedimentation rates and urban runoff (e.g.

Hilbun, 2009). Intermediate sites include by the Smithsonian Tropical Research Institute (STRI) Facility, Boca del Drago, Cayo Adriana, and Isla Popa, representing intermediate degrees of oceanic and anthropogenic influence.

3.4.1 Water Measurements

Water temperature, conductivity, and dissolved oxygen (DO) were measured at the time of collection using a YSI Pro 2030 multiparametric sonde from each bivalve sample site at roughly 0.5 m water depth (Table 3.1). The presence of mangroves, seagrass, and algae was also noted.

3.4.2 Tissues

The bivalves were dissected and tissues were separated by gill, muscle, mantle, and stomach, and subsequently dried overnight at 60-65°C. Samples were then sealed in airtight microcentrifuge tubes and crushed and homogenized using a mortar and pestle. Roughly 1 mg of the powdered sample was analyzed for C and N isotopes using a Thermo Finnigan Delta EA-IRMS at the Stable Isotope Geosciences Facility at Texas A&M University. At least every 5th sample was run in duplicate. Analytical precision was 0.08‰ for $\delta^{15}N$ and 0.13‰ for $\delta^{13}C$ based on replicates of standards.

3.4.3 Shells

Bivalve shells were cleaned by lightly sanding and scrubbing with dilute soap and water to remove surface contaminants. Using the lowest speed (5000/min) on a Dremel 3000 tool carbonate powder was milled on the shell exterior, parallel to the direction of growth, to obtain an average $\delta^{15}N$ value for each shell. For small shells, the entire outer layer of the shell was

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removed and homogenized with a mortar and pestle. Care was taken to avoid the inner aragonitic (nacreous) shell layer. Powders were analyzed on a Thermo Scientific MAT 253 IRMS/Kiel IV for $\delta^{13}C$ and $\delta^{18}O$ (40-100 µg) and a Thermo Finnigan Delta EA-IRMS for $\delta^{15}N$ (5 mg) at the Stable Isotope Geosciences Facility at Texas A&M University. Sample gas was passed through a CO_2 trap to remove CO_2 due to the relatively small amount of nitrogen in the shell compared to carbon. On average, *P. imbricata* and *I. alatus* shell contained 0.01% N, while *B. exustus* contained 0.06% N. At least every 5th sample was run in duplicate. Analytical precision was 0.11‰ for $\delta^{15}N$, 0.06‰ for $\delta^{13}C$, and 0.08‰ for $\delta^{18}O$ based on replicates of standards.

3.5 Results

3.5.1 Shell $\delta^{18}O$ and $\delta^{13}C$ values

The average $\delta^{18}O$ shell values for all specimens from all sites range from -1.0 to -2.4‰ (Figure 3.2). The open-ocean site at Escudo de Veraguas has the highest average $\delta^{18}O$ value (-1.0‰), whereas the Rio Guarumo site yields the lowest (-1.7‰; Figure 3.2A, C). The Isla Popa site (-1.4‰ *P. imbricata*; -1.5‰ *I. alatus*), enclosed within Chiriquí Lagoon, exhibits intermediate $\delta^{18}O$ values. Bocas Marina shells differ in $\delta^{18}O$ according to taxon and show intermediate values, with *I. alatus* values averaging -1.5‰ and *P. imbricata* values averaging -1.2‰. Sites Bocas Town, Boca del Drago, STRI Facility, and Cayo Adriana are all intermediate and similar in value (average -1.1 to -1.3‰). *Isognomon alatus* and *P. imbricata* species from the same site yielded statistically identical $\delta^{18}O$ values except at Bocas Marina (Figure 3.2). Escudo de Veraguas and Rio Guarumo did not have any *I. alatus* specimens present. Mytilid *B. exustus* specimens were not significantly different from pterioideans *P. imbricata* or *I. alatus* in $\delta^{18}O$ values at any site.
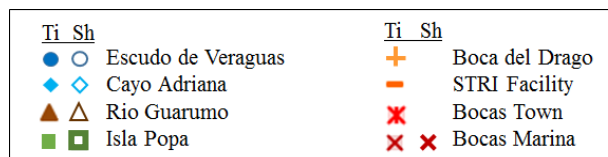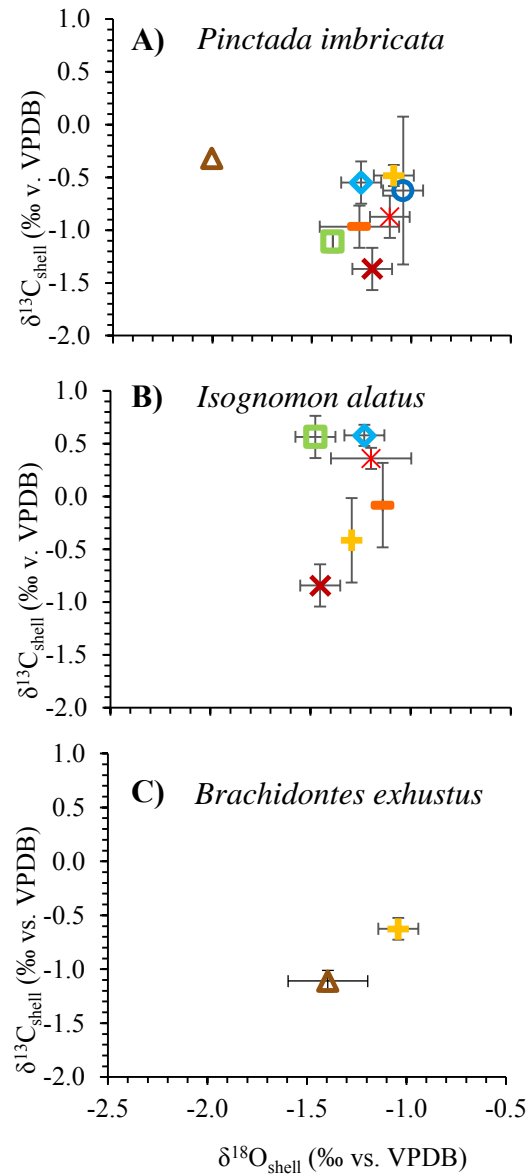
Figure 3.2. Average shell δ^{13}C versus δ^{18}O values (‰ vs. VPDB) shell carbonate values for each location including (A) *Pinctada imbricata*, (B) *Isognomon alatus*, and (C) *Brachidontes exhustus* (n= 73). Location symbols are the same for remaining figures unless stated otherwise. Ti = tissue, Sh = shell. Error bars shown are ± 2 SE.

Overall, shell $\delta^{13}C$ values from the Bocas del Toro region range from -1.7 to 1.8‰ and do not covary with $\delta^{18}O$. Shell $\delta^{13}C$ values vary by up to ~2‰ when comparing *Pinctada imbricata* and *Isognomon alatus* specimens from the same site (Figure 3.2). Shell $\delta^{13}C$ values are significantly different between *P. imbricata* and *I. alatus* at all sites except Boca del Drago, where no significant difference exists between *P. imbricata*, *I. alatus*, and *B. exustus*. Generally, *I. alatus* shell $\delta^{13}C$ values are enriched in ^{13}C compared with *P. imbricata* specimens from the same site.

3.5.2 Tissue $\delta^{13}C$ and $\delta^{15}N$ values

There is some variability in gill, stomach, mantle, and muscle tissue $\delta^{13}C$ and $\delta^{15}N$ values within a specimen and species. Muscle tissues have heavier $\delta^{13}C$ values on average (*P. imbricata* -17.5‰, *I. alatus* -18.0‰, *B. exustus* -18.3‰) than mantle tissues (*P. imbricata* -18.3‰, *I. alatus* -18.8‰, *B. exustus* -19.9‰) for all species (Figure 3.3).
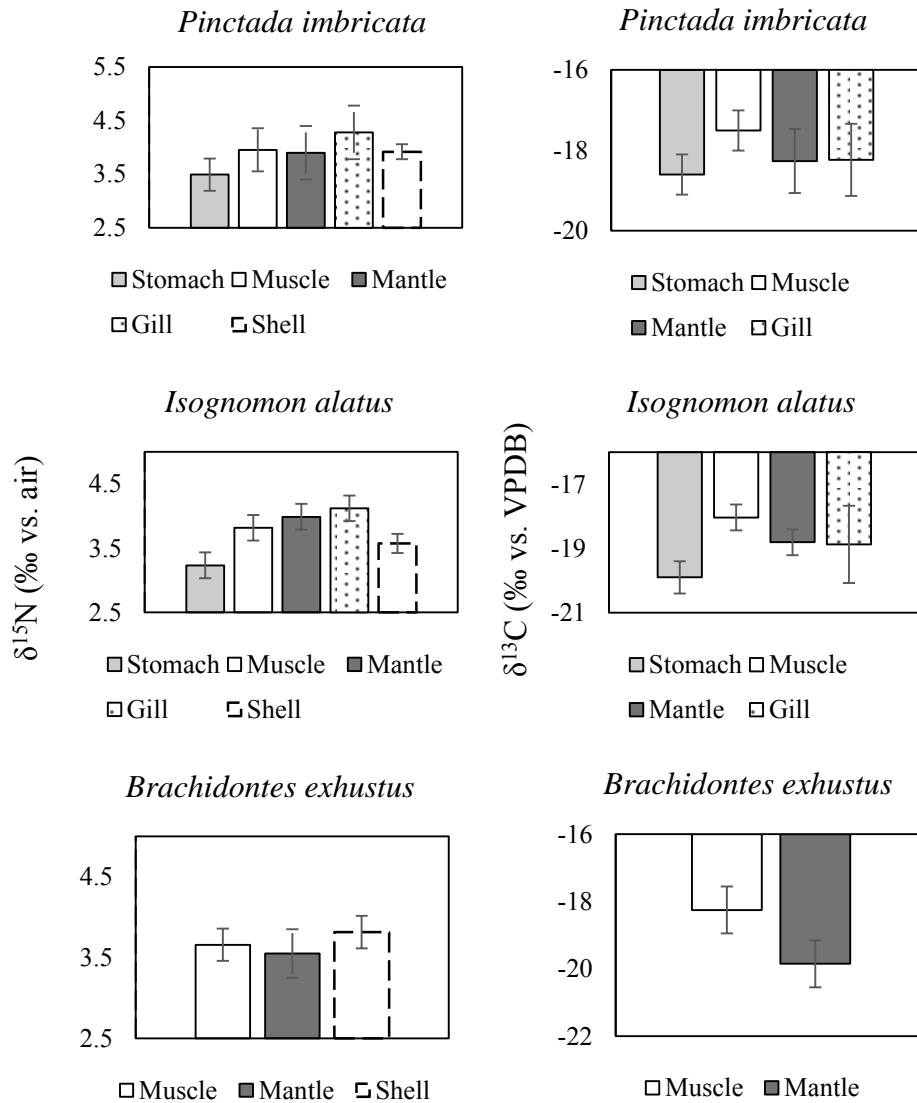
Figure 3.3. Muscle, mantle, stomach, and gill tissue δ^{15}N and δ^{13}C values including all locations for *Pinctada imbricata*, *Isognomon alatus*, and *Brachidontes exhustus*. Shell δ^{15}N values are denoted by the box outlined by a dashed line in each δ^{15}N panel.

Gill δ^{13}C values are comparable to mantle δ^{13}C values, whereas stomach values are the lightest among the tissues measured. δ^{15}N values do not show a consistent relationship between different tissue types.

Considering all species and tissue types, Bocas Marina tissue δ^{15}N values range from 2.5 to 6‰, Boca del Drago values range from 3 to 4.5‰, and STRI Facility values range from
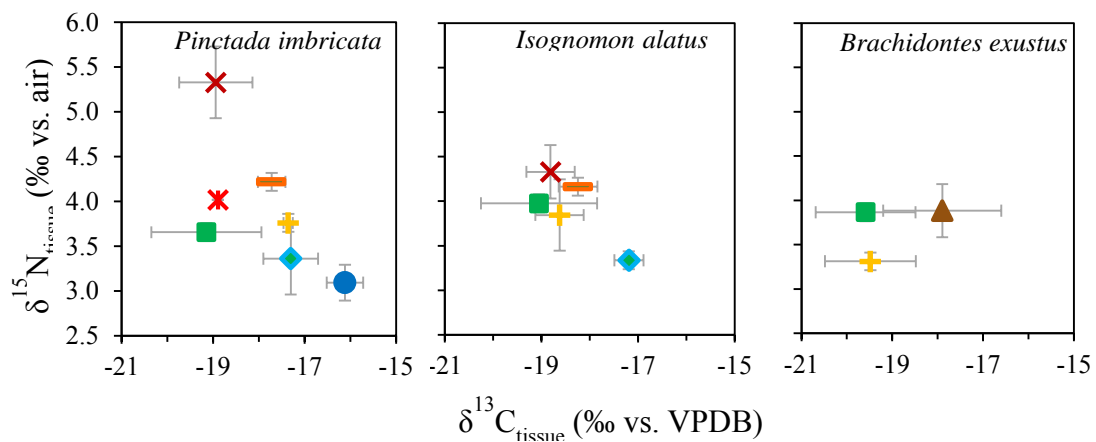
Figure 3.4. Location averages for muscle and mantle tissue δ^{15}N (‰ vs. air) versus δ^{13}C (‰ vs. VPDB) for each respective location. Error bars shown are ± 2 SE.

3.5 to 5.3‰ (Appendix I). In the case of Bocas Marina, this range is roughly equivalent to an increase in trophic level (~3‰). Narrowing the focus to muscle and mantle tissues, variability decreases significantly.

In general, the data show no consistent taxonomic differences with respect to δ^{13}C$_{tissue}$ or δ^{15}N$_{tissue}$ (Figure 3.4). For δ^{15}N, some sites show significant differences (P<0.05) between *P. imbricata* and *I. alatus* species (Isla Popa, Boas Marina), but not others (Cayo Adriana, STRI

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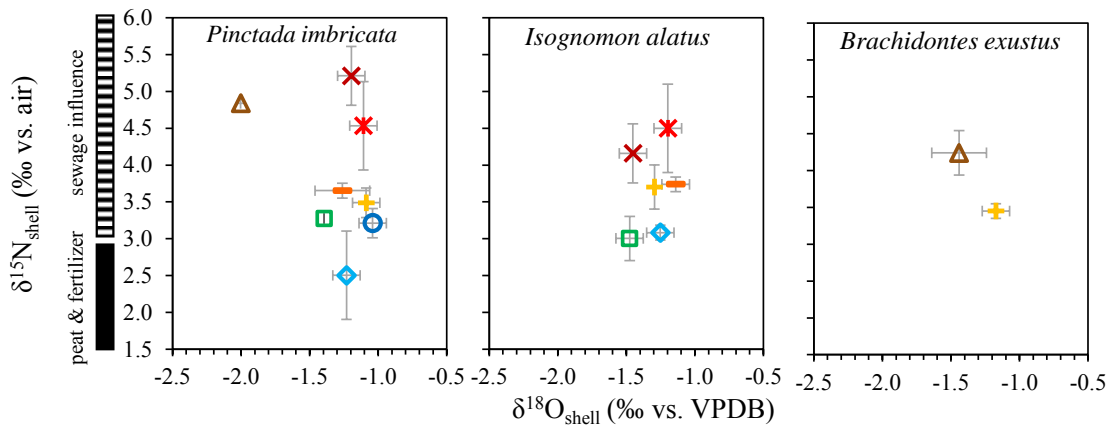
δ15N value of 3.7‰ for terrestrial primary producers from a shallow marine environment in southwestern Brazil (Corbisier et al., 2006)

Figure 3.5. Location averages for $\delta^{15}N$ (‰ vs. air) versus $\delta^{18}O$ (‰ vs. VPDB) for shells including *Pinctada*, *Isognomon*, and *Brachidontes* species. Error bars shown are ± 2 SE. Sewage and peat and fertilizer $\delta^{15}N$ ranges measured in macroalgae from the lower Florida Keys by Lapointe et al. (2004).

Facility, Boca del Drago) (Appendix II). Open-ocean end-member Escudo de Veraguas has a distinctly low average $\delta^{15}N_{tissue}$ value (~3‰), in contrast with human occupied sites which have high $\delta^{15}N_{tissue}$ values (≥4‰).

Organic matter $\delta^{13}C$ values from all sites range from about -21 to -16‰ (Figure 3.4). Escudo de Veraguas specimens have a high $\delta^{13}C$ value (~-16‰) compared with human occupied sites (<-17.5‰) (Figure 3.4). The $\delta^{15}N$ and $\delta^{13}C$ differences between the open-ocean Escudo de Veraguas site and the human occupied sites drive a negative correlation between $\delta^{15}N_{tissue}$ and $\delta^{13}C_{tissue}$ values in *P. imbricata* (R^2= 0.40) and *I. alatus* (R^2 = 0.60), the taxa best represented in the sample set (Figure 3.4).

3.5.3 Shell $\delta^{15}N$ values and tissue-shell comparisons

Bivalve shells analyzed from urban sites (Bocas Town and Bocas Town Marina) have the highest $\delta^{15}N_{shell}$ values on average (all species), 4.6‰ and 4.8‰, respectively (Figure 3.5). The river-influenced Rio Guarumo site had the next highest average $\delta^{15}N$ values (4.4‰). STRI

Facility and Boca del Drago have intermediate $\delta^{15}N$ values of 3.7‰ and 3.5‰, respectively. These sites are susceptible to human influence, but are not heavily urbanized. Isla Popa has a low average $\delta^{15}N$ value of 3.3‰, similar to open-ocean end-member Escudo de Veraguas (3.2‰). The site with the lowest $\delta^{15}N$ value is Cayo Adriana (2.7‰). There are no significant differences in shell $\delta^{15}N$ values between *P. imbricata* and *I. alatus* at all sites except Bocas Marina (P= 0.005).

Average $\delta^{15}N$ values in shells co-vary with, and are statistically identical to, those in tissues for pterioideans *P. imbricata* and *I. alatus* (Figure 3.6). Muscle and mantle tissue $\delta^{15}N$ values were not significantly different from *B. exustus* shell $\delta^{15}N$ values. Conventionally, studies measure the $\delta^{15}N$ of muscle and/or mantle tissue, therefore these values are averaged for $\delta^{15}N_{tissue}$ versus $\delta^{15}N_{shell}$ comparisons in this paper. Correlations between $\delta^{15}N_{tissue}$ and $\delta^{15}N_{shell}$ values are significant for *P. imbricata*, *I. alatus*, and combined *P. imbricata* and *I. alatus* (Figure 3.6).

3.6 Discussion

Shell $\delta^{18}O$ values indicate Chiriquí Lagoon receives more freshwater input than Almirante Bay, in agreement with hydrographic studies (D'Croz et al., 2005; Kaufmann and Thompson, 2005). As discussed previously, the open-ocean end-member Escudo de Veraguas has the highest average $\delta^{18}O$ (-1.0‰) value and the freshwater end-member Rio Guarumo (Chiriquí Lagoon) shows the lowest $\delta^{18}O$ (-1.7‰; average of *Pinctada* and *Brachidontes*). These results are consistent with the recently updated $\delta^{18}O$-salinity relationship for the southwest Caribbean which suggests that Escudo de Veraguas seawater (salinity 34) would be 0.25-0.5‰ heavier in $\delta^{18}O$ than Rio Guarumo seawater (salinity 31.6) (Robbins et al., 2012). Isla Popa (-1.4‰) is enclosed within Chiriquí Lagoon showing characteristic freshwater influence similar to Rio Guarumo (D'Croz et al., 2005). Bocas Town, Boca del Drago, STRI Facility, and Bocas
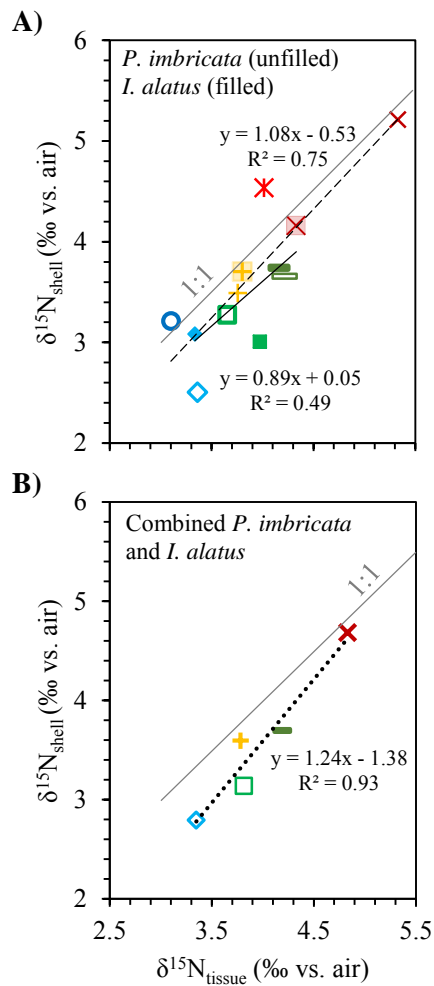
Figure 3.6. $\delta^{15}N$ comparison for muscle and mantle tissues and corresponding species average shell value for (**A**) *P. imbricata, I. alatus,* and (**B**) combined (compared to average shell values for both species). *Brachidontes exustus* were not available in enough abundance for statistical analysis. Each point represents the average value of specimens at one location.

Town Marina are semi-enclosed and have shell $\delta^{18}O$ values that lie closer to the marine end-member than the freshwater end-member. These transitional coastal sites are indistinguishable based on shell $\delta^{18}O$ values.

The range of bivalve tissue $\delta^{13}C$ values, about -20 to -16‰, includes average $\delta^{13}C$ values for marine suspended POM (-18.6‰; Corbisier et al., 2006) and grassland values from Panama (-16.2‰; Schwendenmann and Pendall, 2006). The bivalve tissues are enriched in $\delta^{13}C$ relative

to marine phytoplankton values (-23.1‰; Andrews et al., 1998), terrestrial organic matter values (-29.4‰; Corbisier et al., 2006), banana leaf (δ^{13}C= -26.5‰; Hilbun, 2009), and *Rhizophora mangle* peat (δ^{13}C= -26.5‰; Hilbun, 2009) from coastal tropical environments (Figure 3.7). Conversely, they are depleted in ^{13}C with respect to terrestrial grass (δ^{13}C= -12.1‰) and *Thalassia testudinum* (δ^{13}C= -8.5‰) values from Almirante Bay, Panama (Hilbun, 2009). The range of organic matter δ^{13}C values from this study indicates a mixture of contributing sources, with no single source dominating the signal. In general, terrestrially influenced sites such as Bocas Town and Bocas Marina are depleted in ^{13}C relative to open-ocean end-member Escudo de Veraguas. A previous study measuring δ^{13}C signatures in seagrasses from Florida suggests this may be due to the mineralization of mangrove organic matter, or to increased terrestrial organic matter influence (Anderson and Fourqurean, 2003).

Variations in δ^{15}N values cannot be simply be explained by the degree of freshwater, or terrestrial, influence as measured by average δ^{18}O values. The average δ^{15}N values measured in Almirante Bay (Bocas Town, STRI Facility, Bocas Marina) are higher than those from river-influenced Chiriquí Lagoon. These sites are also more urbanized and may be subject to anthropogenic nutrient influx in the form of sewage or septic waste, which would introduce enriched δ^{15}N values to these sites.

Our contention that high $\delta^{15}N$ values from urban sites Bocas Town Marina and Bocas

Town partially reflect influx of anthropogenically-derived nutrients is consistent with previous

reports of high relative urban runoff and erosion in sediment cores examined near the Bocas

Town airport (Hilbun, 2009). Additional evidence of high chlorophyll-a concentrations in
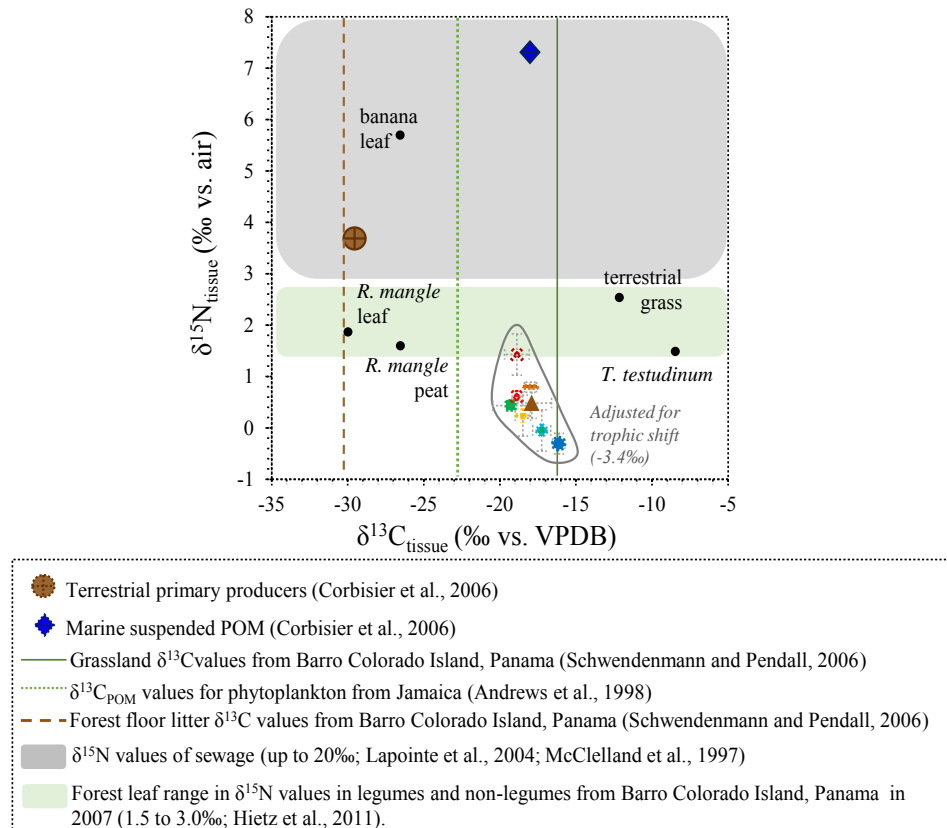
Figure 3.7. Location averages for $\delta^{15}N$ (‰ v. air) versus $\delta^{13}C$ (‰ v. VPDB) including all species. The key the values above were corrected for a trophic enrichment of 3.4‰ (Minigawa and Wada, 1984). Error bars shown are ± 2 SE. The gray rounded rectangle indicates $\delta^{15}N$ values of sewage influence (up to 20‰; Lapointe et al., 2004; McClelland et al., 1997). The green rounded rectangle estimates the leaf range in $\delta^{15}N$ values in legumes and non-legumes from Barro Colorado Island, Panama in 2007 (1.5 to 3.0‰; Hietz et al., 2011).

sediment cores near Bocas Town confirms that nutrient input has increased in recent years (Hilbun, 2009). Increased erosion, runoff, and nutrient influx has been attributed to deforestation in the area (Seemann et al., 2014).

Previous studies have used high $\delta^{15}N$ values (10 to 20‰; McClelland et al., 1997; Cabana and Rasmussen, 1996; Savage, 2005) as an indicator of wastewater influence. When trophic enrichment is considered (-3.4‰; Minigawa and Wada, 1984), $\delta^{15}N$ values for Bocas del Toro bivalve tissue suggest a N source of <1.5‰, similar to or lower than the $\delta^{15}N$ of natural N sources. Even considering lower $\delta^{15}N$ values for septic-influenced sites, like those found in a study of lower Florida Keys macroalgae (3 to 5‰; Lapointe et al., 2004), no support is found for input of sewage nitrogen. Thus, considering trophic enrichment, there is no N-isotopic evidence that populated sites, Bocas Marina, Bocas Town, and the STRI Facility, are influenced by sewage input (Figure 3.7). Although $\delta^{15}N$ values at human-influenced sites are higher than those at uninfluenced sites, there appear to be many sources contributing to shell and tissue $\delta^{15}N$ values at all locations. In agreement with the wide variety of organic $\delta^{13}C$ sources in the area, it is obvious that no single source dominates the $\delta^{15}N$ signal in bivalve shells and tissues from the Bocas del Toro Archipelago, Panama.

Bivalve $\delta^{15}N$ values for Escudo de Veraguas, the marine end-member, do not show any evidence for N from open ocean phytoplankton (7.5‰; Corbisier et al., 2006), but instead appear to indicate a mixture of coastal marine source (e.g. *T. testudinum* and *R. mangle*), though terrestrial sources (e.g. terrestrial grass and leaves) cannot be ruled out. The Rio Guarumo site, on the other hand, should be most influenced by river processes and terrestrial N and C sources, being located within 50 m of the river mouth. Riverine sources of organic matter typically include vegetation from the catchment area (Bouillon and Connolly, 2009). Therefore, one would expect greater N contributions from terrestrial primary producers (3.7‰; Corbisier et al.,

2006), banana leaf fragments (5.7‰; Hilbun, 2009), terrestrial grass (2.5‰; Hilbun, 2009), and

forest leaf fragments (1.5 to 3‰; Hietz et al., 2011) at this locality than at other sites. When

comparing average $\delta^{15}N$ values from the two sites however, there is only a small (0.8‰)

difference. Thus, despite hydrographic differences between these end-members, they suggest a

similar mixture of N sources, probably reflecting the dominance of mangrove and seagrass in the

bivalve's nearshore (< 5 m) habitat.

Lastly, there is a significant correlation between shell and tissue $\delta^{15}N$ values in tropical

pterioideans *P. imbricata* and *I. alatus* (Figure 3.6), supporting the use of shell $\delta^{15}N$ values as a

proxy for tissue $\delta^{15}N$ values. Small shell-tissue offsets ($\Delta^{15}N_{tissue-shell} \leq 1.2$‰) may be partially

attributed to the averaging of seasonal signal in shell organic matrix compared to the short-term

Table 3.2. Average tissue-species offset ($\Delta\delta^{15}N_{tissue-shell}$ (‰)) for various bivalve
species. Table modified from Versteegh et al. (2011).

Species	$\Delta\delta^{15}N_{tissue-shell}$ (‰)	Tissue Type	Study
Ruditapes philippinarum	1.1±0.4	Whole	Watanabe et al. 2009
Mercenaria mercenaria	2.4±0.3	Whole	Carmichael et al. 2004
Mercenaria mercenaria	0.2±0.7	Mantle	O'Donnell et al. 2003
Arctica islandica	2.7	Whole	LeBlanc 1989
Mytilus edulis	-0.1±0.2	Whole	LeBlanc 1989
Mytilus edulis	-2.2 to -1.5	Mantle	Versteegh et al. 2011
Pinctada imbricata	0±0.5	Muscle	This study
Pinctada imbricata	0±0.6	Mantle	This study
Isognomon alatus	-0.2±0.4	Muscle	This study
Isognomon alatus	-0.4±0.4	Mantle	This study
Brachidontes exustus	0.2±0.2	Muscle	This study
Brachidontes exustus	0.3±0.3	Mantle	This study

δ^{15}N recorded in tissues (Table 3.2). Variance in Δ^{15}N$_{tissue-shell}$ values between studies may be due to irregular tissue turnover rates, seasonal variations in metabolism, and overall inconsistencies in methodology.

3.7 Conclusions

Shell δ^{15}N values are a reliable proxy for organic matter δ^{15}N values in bivalves in tropical environments. Nitrogen isotopic compositions of tissue and shell organic matrix correlate significantly for *Pinctada imbricata* data (Pearson's R= 0.75), *Isognomon alatus* data (Pearson's R= 0.49), and combined *Pinctada* and *Isognomon* data (Pearson's R= 0.93). Thus, for these species, N isotope studies of historical and fossil shells may provide records of ecology of past environments.

There does not appear to be any one dominant source of organic matter contributing to δ^{15}N and δ^{13}C values in mollusks. δ^{15}N and δ^{13}C values can be explained by a mixture of mangrove and seagrass N and C, but contributions from a mixture of C$_3$ and C$_4$ terrestrial N and C cannot be ruled out. Despite hydrographic differences between these end-members, there is only minor difference between river-influenced end-member Rio Guarumo and open-ocean end-member Escudo de Veraguas. This suggests there is a dominance of local C and N from mangrove and seagrass in the bivalve's nearshore habitat.

CHAPTER IV

CONCLUSIONS

Chapter II concludes that measured shell $\delta^{18}O$ and $\delta^{13}C$ values reflect predicted seasonal upwelling and freshening signals in the Pacific and seasonal freshening with minimal upwelling in the Caribbean, in agreement with previous studies. However, shell $\delta^{18}O$ minima and maxima often underestimate variations in paleotemperature and salinity in both the TEP and SWC.

Both Pacific and Caribbean shell $\delta^{13}C$ profiles show cyclicity. *Conus* samples from Veracruz Beach (TEP) record seasonal changes in $\delta^{13}C_{DIC}$. The decrease in $\delta^{13}C_{DIC}$ is detectable during the rainy season due to freshwater influence, while the increase in $\delta^{13}C_{DIC}$ is detectable during the dry season despite the fact that upwelling is occurring. Measured $\delta^{13}C$ profiles are ~1-3‰ less than predicted $\delta^{13}C$ profiles due to the incorporation of ^{13}C-depleted metabolic carbon (C_M) into the shell carbonate. Taxonomic differences in C_M likely reflects availability of metabolic CO_2 for shell growth, which we hypothesize is related to feeding habit and activity.

Chapter III concludes that shell $\delta^{15}N$ values are a reliable proxy for organic matter $\delta^{15}N$ values in bivalves in tropical environments. Nitrogen isotopic compositions of tissue and shell organic matrix correlate significantly for *Pinctada imbricata* data (Pearson's R= 0.75), *Isognomon alatus* data (Pearson's R= 0.49), and combined *Pinctada* and *Isognomon* data (Pearson's R= 0.93). Thus, for these species, N isotope studies of historical and fossil shells may provide records of ecology of past environments.

There does not appear to be any one dominant source of organic matter contributing to $\delta^{15}N$ and $\delta^{13}C$ values in mollusks. $\delta^{15}N$ and $\delta^{13}C$ values can be explained by a mixture of mangrove and seagrass N and C, but contributions from a mixture of C_3 and C_4 terrestrial N and C cannot be ruled out. This suggests there is a dominance of local C and N from mangrove and seagrass in the bivalve's nearshore habitat.

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Appendix I. Tissue oxygen and carbon isotope values at each location. *P. imbricata* symbols are filled, *I. alatus* symbols are unfilled, and *B. exustus* samples are light gray and filled.

Legend:
■ muscle
● mantle
◆ stomach
▲ gill

Pinctada imbricata (filled symbols)
Isognomon alatus (unfilled symbols)
Brachidontes exustus (grey, filled symbols)

$\delta^{15}N$ (‰ vs. air)

$\delta^{13}C$ (‰ vs. VPDB)