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Stable Isotopes in the Eye Lenses of *Doryteuthis plei*: Exploring Natal Origins and Migratory Patterns in the Eastern Gulf of Mexico

by

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of the requirements for the degree of
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Abstract:

Stable isotope analysis is an emerging tool to examine trophic pathways and migratory patterns of marine organisms. Squid are widely distributed in coastal and deep water regions of the Gulf of Mexico. Stable isotope ratios of carbon and nitrogen found within cephalopod tissues can provide information on both trophic level and habitat of their food sources. More recently, ontogenetic changes in stable isotope ratios within squid eye lenses have been documented. Concentric layers of crystallin proteins are added to the lens as the squid ages; the center of the lens contains the oldest layer and the youngest layers are on the outermost surface. The crystallin proteins are rich in carbon and nitrogen, providing suitable sources for isotopic analysis of both $\delta^{15}N$ and $\delta^{13}C$. *Doryteuthis plei* is a common inshore squid in coastal waters of the western Atlantic region. This study identifies the geographic movements of *D. plei* in the eastern Gulf of Mexico using changes in isotope ratios in eye-lens layers. Isotopic analyses suggest that these squid begin their lives in the deep chlorophyll maximum of the outer shelf and move inshore as they age.

1. Introduction

1.1 Objectives

Doryteuthis plei (Blaineville, 1823;Cephalopoda: Teuthoidea: Loliginidae), commonly called the slender inshore squid or the arrow squid, is an ecologically and commercially important species in the Gulf of Mexico. However, little is known about its populations sizes and movements. *D. plei* makes diel vertical migrations and are commonly found near the bottom during the day and move up into the water column at night over their 200 meter depth range (Judkins & Vecchione 2013). Their horizontal distribution is less understood and there are conflicting reports in the literature regarding latitudinal, ontogenic and on-shore/offshore migrations (Whitaker 1980, Voss & Brakoniecki 1985, Rodrigues & Gasalla 2008, Judkins & Vecchione 2013).

The goal of the present study was to learn more about the possible migration patterns of this species in the eastern Gulf of Mexico by applying the novel approach of stable isotope analysis of eye lenses. The isotopic histories over the lifespan of *D. plei* can be obtained from eye lens layers. This investigation addresses the following questions: Can stable isotope analysis be used to trace migratory patterns of squid in the eastern Gulf of Mexico? Where are the natal origins of this species? Do these squid migrate after hatching? Are there any localized spawning grounds?

1.2 *Doryteuthis plei* background information

This neritic species is distributed throughout the inshore coastal waters of the Western Atlantic, including the Gulf of Mexico and the Caribbean Sea. It is widely distributed from 36°N to 35°S in warm, tropical and subtropical waters (Judkins & Vecchione 2013). *D. plei* is a muscular species of squid that ranges up to a maximum dorsal mantle length of 370 mm in males and 260 mm in females (Jereb et al. 2011); males are substantially longer than females. This species can occur in large schools that are targeted by commercial fisheries.

D. plei exhibits a paralarval stage that feeds mainly on crustaceans, whereas later developmental stages shift to small fishes as dominant prey. Although mostly piscivorous as adults, *D. plei* also commonly preys upon crustaceans, cephalopods and polychaetes (Gasalla et al. 2010). These squid are most likely opportunistic feeders that feed throughout the water column with ontogenetic and seasonal shifts in diet depending on prey availability.

Cephalopod ecology research has utilized stomach-content analysis as well stable isotope analysis to obtain trophic information. Stomach analysis offers a qualitative approach to dietary studies yet there are limitaions when examining cephalopods. Cephalopods tear prey into small pieces using the radula before moving food through a narrow esophagus, which is encircled by their brain (Rodhouse and Nigmatullin 1996). Stomach contents only reveal the most recent feeding, with no long-term feeding information (Jackson et al. 2007). Another problem that arises from using stomach contents is that squid may feed unnaturally in the presence of sampling gear (Rodhouse and Nigmatullin 1996). While this approach can provide insight on recent feeding patterns, stable isotope analysis offers a more quantitative approach in which information on the entire lifetime of the diet can be obtained.

D. plei is abundant in its range and therefore is an important predator and prey link in the food webs of their distribution. This link can be so strong that the species can influence the ecosystem dynamics as a keystone species. Research on this species in South Brazil determined that *D. plei* plays a “keystone” role in the South Brazil Bight region (Gasalla et al. 2010). Squid are also an important fisheries resource, utilized as food and bait. Directed fisheries are concentrated in southeastern United States and the southern Caribbean region of *D. plei’s* distribution, and include artisanal and larger-scale commercial catches (Judkins & Vecchione 2013). In the Gulf of Mexico, there is a small-scale fishery (Voss & Brakoniecki 1985). The total catch of fisheries tends to target spawning grounds. The very short life cycle, dense spawning aggregations, and fishing techniques that could remove certain sexes or sizes could lead to recruitment issues in *D. plei* (Hanlon 1998).

Cephalopod research is becoming increasingly important as new threats have emerged in recent decades. These threats include global climate change, ocean warming, sea-level rise, biodiversity loss, overfishing, ocean acidification and expanding hypoxia (Xavier et al. 2014). *Doryteuthis plei*, as mentioned earlier, is an important link within coastal marine ecosystems, serving as both predator and prey. Due to the short life cycles and rapid turnover of populations, squids can respond quickly to environmental or ecosystem changes. Elevated temperatures can accelerate the life histories of squid, and inshore species could respond in complex ways as the ocean temperatures rise (Judkins & Vecchione 2013).

1.3 Current knowledge of migrations

In southeast Florida, small *D. plei* was reported moving to the deeper continental slope during summer and fall while larger *D. plei* was found offshore during all seasons except spring (Judkins & Vecchione 2013). *D. plei* off the southeastern United States coast seem to migrate south with the seasonal drop in temperature due to their temperature range of 12-30°C (Whitaker 1980). In southeast Brazil, a high proportion of immature squid found offshore indicates that the juveniles develop offshore and then migrate inshore to spawn when they have matured in the spring and summer (Rodrigues & Gasalla 2008). In the Gulf of Mexico, it has been reported that arrow squid found off southwestern Florida moves westward offshore in the northern Gulf into warmer waters during April as well as southward along the Mexican coast in November and December (Voss & Brakoniecki 1985). It has been suggested that larger *D. plei* migrate from the northern Gulf of Mexico and the northwest Atlantic to warmer waters in southern Florida, the Caribbean or the southern Gulf of Mexico during autumn to overwinter (Herke and Foltz 2002).

1.4 The use of stable isotope analysis

Stable isotope analysis is emerging as a powerful tool in ecological studies to examine trophic positions and migratory patterns of various marine groups (Fry 1981, Hobson 1999). Fractionation is a process that causes stable isotope ratios to change between diet and consumer during assimilation and metabolic processing. Due to trophic fractionation, values of $\delta^{15}N$ and $\delta^{13}C$ become enriched between prey and predator (McCutchen et al. 2003). In ecological studies, it is assumed that consumers become enriched in $\delta^{15}N$ by 3.4‰ and in $\delta^{13}C$ by 0.4-1.3‰ (Minagawa & Waga 1984, Vander Zanden and Rasmussen 2001, Post 2002, McCutchan et al. 2003).

Stable isotope ratios can be used to trace pathways of of carbon and nitrogen within foodwebs. The variation of nitrogen isotopes ($\delta^{15}N$) is an indicator of the animals' trophic level as consumers become enriched in $\delta\ ^{15}N$ relative to their food. $\delta^{15}N$ also provides insight into nutrient sources (like sewage, fertilizer or soils), and there is often a transition from eutrophic to oligotrophic waters (Hansson et al. 1997). The carbon isotopes ($\delta^{13}C$) can be used to determine the primary producer that supports these food chains (the basal resource). In the marine environment, $\delta^{13}C$ values can indicate whether the food intake is from pelagic phytoplankton or benthic algae (Cherel & Hobson 2005). Along continental shelves, $\delta^{13}C$ gradient commonly exist in which values are higher in shallow inshore waters compared to deeper offshore water due to the presence of benthic algae which is about 5‰ more enriched than phytoplankton (Fry 1981).

Most animal tissue undergoes metabolic turnover which makes it difficult to observe the isotopic trends throughout the lifespan of an organism. Turnover times vary by tissue, from days in blood to months in muscle. It is therefore beneficial to use archival tissues, tissues that are metabolically inert, to observe lifelong trends. Archival structures that have been successfully studied include whiskers, dentin in teeth, fish otoliths, shark vertebrae and more recently eye lenses (Cherel et al. 2009, Hobson and Sease 1998, Mendes et al. 2007, Schwarcz et al. 1998, Estrada et al. 2006, Wallace et al. 2014). There are four hard structures in a squid's body that grow continuously with no turnover; the crystallin lens, the statolith, the gladius and the beak.

1.5 Crystallin proteins and the eye lens

Crystallin and other lens proteins are rich in carbon and nitrogen, unlike the beak and gladius which are depleted in nitrogen due to the presence of chitin. The addition of concentric layers to eye lenses as they grow provides the potential to observe lifelong isotopic histories of $\delta^{15}N$ and $\delta^{13}C$. Wallace et al. (2014) demonstrated this application in bony fishes and proposed that it could be used in sharks and cephalopods based on similarities in eye structure. Coleoid cephalopods have well-developed eyes with an iris, nearly circular lens, a vitreous cavity and photoreceptor cells that form a retina. Most coastal cephalopod eyes possess a cornea (Budelmann 1994).

The cephalopod eye lens is very similar to the teleost lens and is considered an example of convergent evolution (Packard 1972). The squid eye lens is comprised of a posterior and anterior segment. Squid crystallin proteins accumulate in both segments of the lens (West et al. 1994). Among cephalopods, only one major class of water-soluble crystallin proteins exists, called *S-crystallins*, where the *S* stands for *squid* (Tomarev and Piatigorsky 1996). S-crystallins are continually synthesized as the lens grows. Chronological deposition of concentric layers to the lens occurs as the squid ages (Hunsicker et al. 2010). The center of the lens contains the oldest layer and the youngest layers are on the outermost surface.

This method of stable isotope analysis of eye lenses has been evaluated on squid in two dissertations and one published paper (Parry 2003, Hunsicker et al. 2010, Onthank 2013). Parry (2003) examined the stable isotopes in the eye lenses of 14 *Ommastrephes bartamii* individuals and 14 *Sthenotheuthis oualaniensis* individuals from the North Pacific. Parry found that the δ^{15}N of the *O. bartamii* squids showed a sigmoidal relationship with the mantle length. The majority of the *S. oualaniensis* individuals exhibited an exponential relationship with mantle length. Overall, the eye lens tissue, along with mantle tissue and blood, supported the general trends of increasing δ^{15}N with size for both species in the study. These increases were attributed to ontogenetic trophic-level increases (Parry 2003). A study was conducted on the commander squid, *Berryteuthis magister*, using stable isotopes analysis of eye lens layers to examine their trophic role in the Bering Sea. The isotopic analysis of the eye lenses indicated squid diets may not simply increase from lower to higher trophic levels as body size increases. The eye lenses showed finer scale changes in diet that the isotopic analysis of muscle tissue did not (Hunsicker et al. 2010). Onthank (2013) used the carbon and nitrogen isotopes of the eye lenses of *Dosidicus gigas*, the Humbolt squid, to explore diet and migratory histories. It was found that the squid in this study shifted their diet at various times in their lives between consuming prey at two trophic levels, one level being mostly euphausiids and the second, one level higher, containing small fish based a comparison of isotopic values from common prey. This finding highlights the opportunistic foraging behavior of this species. This study also suggests that this species has short-term shoaling behavior based on the migratory histories of the individuals. Onthank was also able to suggest where the squid originated by interpreting the values of the innermost eye lens layers. *Dosidicus gigas*, is an oegopsid squid which lacks corneas while the other two studies examined myopsid squid. It appears that stable isotope analysis of eye lenses is useful in both suborders of Teuthoidea. These three studies took place in the Pacific Ocean, the present study is the first to examine the eye lenses of squid from the Gulf of Mexico.

1.6 Isoscapes

Maps of geographic variability in stable isotopes of the West Florida Shelf were developed by Radabaugh et al. (2013) and are called *isoscapes*. These isoscapes were based on fish muscle from the Florida Fish and Wildlife Conservation Commission's Southeast Area Monitoring and Assessment Program (SEAMAP) cruises. In the northern region of the West Florida Shelf (WFS), δ^{15}N values are higher as a result of discharge from the Mississippi and other rivers (Radabaugh & Peebles 2014). The northern, more eutrophic waters of the WFS differ from the southern region where nitrogen fixation is more prevalent; the diazotroph *Trichodesmium* prevails in the nutrient-poor waters of the southern WFS and lowers the values of δ^{15}N in the system (Holl et al. 2007). Predators on the WFS, like other continental shelves, have generally higher values of $\delta^{13}C$ in the shallow inshore region compared to the deeper offshore region likely due to greater dependence on benthic primary production (Radabaugh & Peebles 2014). Benthic algae are approximately 5‰ higher than phytoplankton (France 1995).

The average isotopic values from the WFS were consistent interannually and seasonally, which provides reliable isoscape information (Radabaugh et al. 2013). The migratory patterns of individual animals can be re-created using these isoscapes. The present study will examine the changes in isotopic values of the eye lenses of squid over entire lifetimes, inferring geographic movement from these published isoscapes (Radabaugh et al. 2013, Radabaugh & Peebles 2014).

2. Materials and Methods

Doryteuthis plei samples were obtained from summer and fall, 2015, research cruises of the Florida Fish and Wildlife Conservation Commission's Southeast Area Monitoring and Assessment Program (SEAMAP) cruises. The first cruise was conducted from October 8th to October 30th, while the second cruise was conducted from June 8th to June 30th. These cruises occurred in the eastern Gulf of Mexico, extending from offshore of Pensacola southward to the Dry Tortugas of Florida (Figure 1), with two legs for each cruise. The summer cruise legs were conducted from June 8th to June 18th and June 20th to June 30th. The fall cruise legs were conducted from October 8th to October 18th and October 20th to October 30th. Each cruise visited randomized stations within National Marine Fisheries Service (NMFS) statistical zones (i.e., it was a stratified-random survey design). Squid were collected from 13 stations. To examine isotopic differences along the geographic range of these surveys, the NMFS statistical zones were separated into four regions from north to south, identified here as North, Upper Middle, Lower Middle, and South. At each station, a 42-foot semi-balloon groundfish trawl was towed at three knots for thirty minutes. Water-column data were collected from a CTD cast at each station**.** During the daytime stations, turbidity data were collected using a Secchi disk.

Squid collected from SEAMAP cruises were frozen as whole specimens and sent to the University of South Florida St. Petersburg Invertebrate Laboratory. The squid samples were thawed and measured before each dissection. The species identification, mantle length, weight and sex were recorded for each squid (Table 1). A tissue sample, the gladius, statolith, beak and both eyes were dissected and frozen for each specimen.

The preparation and analysis of the eye lenses followed the methods outlined in Wallace et al. (2014). To prepare the eye lens for the mass spectrometer, the lens was dissected from the eye. The anterior lens was discarded and the posterior lens was isolated for analysis. The lens diameter was measured using an ocular micrometer under an Olympus microscope model SZX-ILLD100. Then the outermost lamina (layer) of the lens was excised and set aside. Two methods of excising the lens layers were tested; a wet method was performed on the left eye of each squid, in which excess amounts of deionized water were used, keeping the lens saturated during the entire dissection, and a dry method that was performed on the right lens, in which DI water was used very sparingly, only to assist delamination (Figure 2). Figure 2 presents a comparison of the two delamination methods. The left eye was tested under the wet method and right eye was tested under the dry method for one male and one female. The average differences were calculated for each of the resulting, four plots. The average differences for $\delta^{15}N$ values of the male was 0.10 and 0.03‰ for the female. The average differences for $\delta^{13}C$ values were 0.03 for the male and 0.31‰ for the female. These differences were low relative to the total amount of variation observed within the lens, and thus there does not appear to be a substantial difference in data obtained using the wet vs. dry method or left vs. right eye. The wet method allows for easier delamination and therefore was selected for stable isotope analysis on one lens per specimen, from the left eye.

Delamination was repeated until there were no more laminae to be removed. The diameter was measured to the nearest 0.1 mm after each lamina was removed. The laminae were then air dried, ground into powder, and weighed on an analytical balance. A dry weight of 300-600 µg was then placed into tin capsules for combustion and isotopic analysis.

The ratios ^{13}C /^{12}C, $^{15}N/^{14}N$ and C/N were measured using a Carlo-Ebra NA2500 Series II elemental analyzer coupled to a continuous-flow ThermoFinnigan Delta+XL isotope ratio mass spectrometer at the University of South Florida College of Marine Science. Calibration standards were NIST 8573 and NIST 8574 L-glutamic acid standard reference materials. Analytical precision was obtained by replicate measurements of NIST 1577b bovine liver. The results are presented in standard delta notation defined by the following equation: $\delta = [(Rsample/Rstandard)-1] \times 1000$; delta values are expressed in per mil (‰) relative to international standards Pee Dee Belemnite (PDB) and air.

Replicate isotope measurements were averaged and plotted for descriptive purposes. Isotope measurements of $\delta^{15}N$ and $\delta^{13}C$ were plotted for each individual squid. Lamina position is the radial midpoint of the lamina (mm), where the midpoint is the lens radius after lamina removal. STATGRAPHICS Centurion (v. 17) software was used for all statistical analyses. Linear regressions were conducted to model $\delta^{15}N$ and $\delta^{13}C$ on lamina midpoint and to predict $\delta^{15}N$ based on $\delta^{13}C$ (Table 2, Figures 1A-2A). Slope *p*-value and R^2 value were recorded for each regression.

3. Results

Stable-isotope analysis was performed using the eye lens of 21 male and 21 female specimens. Nine to 19 laminae were excised from each eye. Linear regression results are summarized in Table 2. Of the 42 specimens, 26 squid had significant increases in δ^{13}C, while only 5 had a significant decreases. It was also found that 28 squid had a significant increases in δ^{15}N, while none exhibited a decrease in δ^{15}N.

Linear regressions were also performed on the ratios of δ^{15}N/δ^{13}C over the lifetime of each squid in order to determine if typical trophic fractionation slopes were present (Figures 1A-2A). Trophic fractionation slopes were also examined using absolute (maximum) difference values. The absolute differences, wherein the lowest isotopic values were subtracted from the highest isotopic values, were plotted relative to a 3:1 slope. A 3:1 slope for δ^{15}N/δ^{13}C approximates the variable isotopic shift associated with changes in trophic position (Table 3, Figure 3, McCutchan et al. 2003). This figure indicated there was not a 3:1 trend in the absolute lifetime differences.

The isotopic values of the innermost laminae (core values) were plotted to compare natal origins (Figure 4). The core values from each of the 42 eye lenses had an average δ^{15}N value of 6.2‰ and an average δ^{13}C value of -20.05‰. Box and whisker plots of the core values were created to compare differences in δ^{13}C and δ^{15}N between the four regions (Figures 5-6). To compare isotopic patterns throughout the lives of the squid, a first-order grouping of the linear regression results was performed (Figure 7). An analysis of the first-order regression trends was then performed to compare males and females (Table 4). Note that this male-female comparison did not preserve individual identities, but instead pooled all $\delta^{15}N$ and $\delta^{13}C$ trends.

One-way ANOVA (Figure 8) identified a gradual increase in $\delta^{13}C$ with increasing laminar midpoint (F-ratio = 29.65, $p<0.0000$). The corresponding ANOVA for $\delta^{15}N$ was not significant.

4. Discussion

4.1 Isotopic changes over lifetime are not trophic

Consumers can be expected to become enriched in $\delta^{15}N$ by 3.4‰ with each trophic step (Minagawa & Waga 1984), whereas trophic enrichment of $\delta^{13}C$ for consumers is between 0‰ 1.3‰ (Vander Zanden and Rasmussen 2001, Post 2002, McCutchan et al. 2003). These values are variable, but the global generalization of $\delta^{15}N$: $\delta^{13}C$ trophic fractionation is often described as 3:1 (McCutchan et al 2003). If the squid did not exhibit any geographic movement during their lifespans, then only the theoretical slope of a 3:1 trophic increase (or possibly an asymptotic variant of this increase) would be expected. Squid are carnivorous throughout life and therefore start life at a trophic level of least 3.0. *Doryteuthis plei* likely increase by at last one trophic level as they shift their diet from crustaceans to fish during growth (Gasalla et al. 2010). After performing a linear regression on the ratio of $\delta^{15}N$: $\delta^{13}C$ for each individual squid, only two specimens (< 5% of specimens) appeared to approximate the theoretical 3:1 slope (Table 2). Specimen BM80 had a significant slope of 2.76 and specimen BM69 had a significant slope of 3.23. In the absence of correlation between $\delta^{15}N$ and $\delta^{13}C$, isotopic changes cannot be explained solely by trophic growth and therefore may be due to geographic movement. The isotopic changes over life did not appear to solely reflect increases in trophic level, except perhaps in two individuals. Many of the regression slopes had remarkably low slopes, suggesting more variation in $\delta^{13}C$ than $\delta^{15}N$.

Figure 3 presents a scatterplot of the absolute difference values, indicating a general lack of agreement with the 3:1 slope. However, in this comparison, there were seven individuals (17% of total individuals) that had slopes near 3:1. Regardless of the type of analysis considered, most individuals had patterns that were consistent with geographic movement as a cause of isotopic variation during life.

4.2 Core lens values suggest natal origins may be associated with the deep chlorophyll maximum

The deep chlorophyll maximum (DCM) is a subsurface layer of elevated chlorophyll *a* concentration (Cullen 1982). The DCM is not detected by satellites and often has negative $\delta^{15}N$. Autonomous profiling floats determined the DCM in the Gulf of Mexico to range from 30–120 m depth, with chlorophyll concentrations averaging 10 times higher than the surface, with some locations ranging as much as 30–40 times higher than the surface (Green et al. 2014). On average, the DCM occurs at 35–50 m along the outer half of the West Florida Shelf. The DCM becomes shallower when upwelling events occur along the continental shelf; these upwelling events also cause the DCM to extend shoreward, reaching the shore in some cases (Weisberg et al. 2000). Concentrations of phytoplankton carbon in the DCM in the Gulf of Mexico is often dominated by dinoflagellates, while the zooplankton carbon is largely composed of copepods (Hobson & Lorenzen 1972), which are known to be important prey for early juvenile squid (Chen et al. 1996).

In the western Gulf of Mexico, vertical profiles of the δ^{15}N and δ^{13}C of particulate organic matter indicate the deep chlorophyll maximum δ^{15}N ranges from approximately -2–2‰ and δ^{13}C ranges from -24 to -21‰ (Holl et al. 2007). These data were obtained from shelf-break to off-shelf locations. The Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND) consortium of the Gulf of Mexico Research Initiative has also collected offshore isotopic data, and found a general trend of enrichment in δ^{15}N with increasing depth. The average from these results is -24.10 for δ^{13}C and 2.78 for δ^{15}N at the deep chlorophyll maximum (T. Richards, pers. comm.).

The absolute isotopic values of the lens cores were very low, yet the squid must have been at a trophic level of at least 3.0 because they are not known to be herbivorous. Thus, the δ^{15}N baseline must have been close to zero or negative, assuming trophic fractionation of 3‰ per trophic step. Due to the low isotopic values of δ^{13}C, the basal resource appears to be mostly phytoplankton, which is in low abundance in shallow, oligotrophic waters. Therefore, the deep chlorophyll maximum (DCM) is likely the basal resource. The lens core data from the squid appears to be most consistent with dependence on the DCM, and agrees with isotopes from DCM-dependent fishes from the WFS (Huelster 2015).

4.3 Spawning takes places throughout the eastern Gulf of Mexico

It is likely that *D. plei* spawning is distributed along the outer West Florida Shelf and not exclusively in the south or north based on the fact that both δ^{15}N and δ^{13}C cores differed by region (Figure 5–6). Core δ^{15}N varied widely and was highest in the north. The differences in the average δ^{15}N among the four groups suggest the squid started their life along different regions of the latitudinal δ^{15}N gradient.

Squids in the genus *Doryteuthis* are demersal spawners that lay eggs in large communal masses, and large spawning aggregations of hundreds of thousands of adult squids sometimes occur (Hanlon 1998). These egg masses are attached to hard substrate or are anchored in the sand. The entire Florida peninsula has the potential to act as spawning grounds for this species (Herke & Foltz 2002). Populations of this species have been found to spawn year-round with two seasonal peaks. In southern Brazil, *D. plei* were found to breed and spawn throughout the year, with a significant reproduction event occurring during the summer and a secondary event occurring during winter (Perez et al. 2002). Between these two reproductive events, microcohorts were produced continually, resulting in an protracted recruitment season that is thought to improve recruitment success (Perez et al. 2002). In the northeastern Venezuelan population, spawning peaks occurred during late spring-early summer and fall, while the Caribbean Sea population had spawning peaks during spring and fall (Jereb et al. 2011).

4.4 There was a general trend of shoreward movement during life

Juvenile *D. plei* appear to associate with the DCM on the outer shelf, and individual lifetime patterns are likely dependent on which region of the δ^{13}C and δ^{15}N gradients they move to later in life. Increases in δ^{13}C would indicate a movement inshore due to the higher values of δ^{13}C inshore (Radabaugh & Peebles 2013). If there no increases or decreases in δ^{13}C were observed, then the squid would most likely remained associated with the DCM, which upwells to the coast on occasion (Weisberg et al. 2000).

Overall, there was a general increase in δ^{13}C during life, suggesting these squid moved inshore. Figure 8 presents δ^{13}C binned into five groups of laminar midpoints, indicating a general increase in δ^{13}C. If squid were moving inshore from the DCM, then δ^{13}C values should have remained relatively similar for squid caught in the northern region, but should have increased over time for squid caught in the southern region where there is more benthic productivity. This variation in trends is evident in the linear regression slopes of $\delta^{13}C$ (Table 2).

However, there were five individual squid that had a significant decrease in $\delta^{13}C$ during life. This could be explained by increasing dependence on a less complex food web, which would have the effect of decreasing trophic level (Jennings et al. 2001), or movement into deeper offshore waters. This pattern was observed in only a small fraction of the 42 specimens (n = 5), whereas the vast majority were consistent with the tendency to move inshore during life.

Females appeared to be more likely to move inshore than males (Table 4). Females experienced a more frequent increase in $\delta^{13}C$ during their lives than males, which could mean that the females went closer to shore. Inshore migration also occurs in *Doryteuhis pealeii*, which co-occurs in the Gulf of Mexico and can be difficult to distinguish from *D. plei* (Roper et al, 1984). *D. pealeii*, the long-finned inshore squid, is a neritic species that occurrs throughout the Western Atlantic. In the northeastern USA, *D. pealeii* migrates inshore to spawn from the edge of the continental shelf (Jacobson 2005). It has been reported that *D. pealeii* in the mid-Atlantic Bight migrates inshore during late spring to spawn and then returns to deeper waters during late fall (Summers 1969). In late fall to early winter, *D. pealeii* in the northwest Atlantic migrates offshore and southward into deeper water towards the edge of the continental shelf, and during the late spring to early summer migrates inshore and northward into shallow coastal waters (Black et al. 1987). These migrations may allow the squid to avoid unfavorable water temperatures and to occupy spawning grounds. It is believed that this species migrates inshore during the spring to feed and spawn close to juvenile nursery areas in coastal waters (Black et al. 1987).

While many individuals followed the same patterns, there were several that did not (Figure 7). This is consistent with evidence that individuals within any given population can be highly variable in their utilization of habitats and resources (Bolnick et al. 2003, Bearhop et al. 2004).

Increases in $\delta^{15}N$ suggest movement northward, where higher baseline values of $\delta^{15}N$ occur (Radabaugh & Peebles 2013). Absence of such changes in $\delta^{15}N$ suggest certain individuals were not likely undergoing latitudinal movements. Decreases in $\delta^{15}N$ over their lifetime would indicate movement south towards the oligotrophic region of the WFS.

Many of the squid (n = 28) experienced an increase in $\delta^{15}N$. Only 14 individuals had neither a significant increase or decrease in $\delta^{15}N$ during life. Males and females were very similar in their $\delta^{15}N$ patterns (Table 4), suggesting some northward movement and no southward movement. This is contradictory to previous findings that this species moved south during winter in the southeastern United States (Whitaker 1978). This is also contracticory to Herke and Foltz (2002) who suggested a southern migration during the autumn from the northern Gulf of Mexico to southern Florida, the Carribean, or the southern Gulf of Mexico to overwinter.

4.5 Direction for future work

Animal movement can often be confounded with trophic growth. Future research using this approach could attempt to decouple these two factors using compound-specific isotopic analysis of amino acids (CSIA-AA), which would identify basal-resource dependence with much more certainty. This method of CSIA-AA was performed on squid gladii and muscle for the first time in 2013 (Ruiz-Cooley et al. 2013). The method was successful in providing direct information on the distinct geographic origins and ontogenetic changes in habitat use of the jumbo squid, *Dosidicus gigas,* in the Northern California Current System (Ruiz-Cooley et al. 2013).

Analyzing oxygen isotopes could also be used as a confirmatory step. Oxygen isotope values have an inverse relationship with temperature and a direct relationship with salinity. Measurements of $\delta^{18}O$ isotopes have been taken from fish otoliths to describe temperature histories (Patterson et al. 1993). Oxygen isotope analysis of squid eye lenses could be investigated, although the oxygen in eye lenses is organic in origin and thus may not be comparable to the oxygen in otoliths. If the $\delta^{18}O$ in eye lenses was found to be comparable, then $\delta^{18}O$ analysis could be used to establish environmental histories, as there is less thermal seasonality in deep offshore ocean waters and thus dampened seasonal cycles in $\delta^{18}O$.

It would also be beneficial to determine the age of each squid from their statoliths. The statoliths of cephalopods are paired, calcareous structures within the statocyst organs. The statocysts are equilibrium organs found within the cranial cartilage that have receptor systems for gravity and angular acceleration (Stephens and Young 1982). Statoliths have been used as a method for age and growth estimation (Ceriola & Milone 2007). Statolith studies on *D. plei* have identified daily deposits of growth increments. In the northwestern region of the Gulf of Mexico, daily statolith increments of *D. plei* revealed a rapid, non-asymptotic growth and a short lifespan of five months during the warmer spring-to-summer months (Jackson & Forsythe 2002). A study of *D. plei* statoliths combined with gladii from southern Brazil inferred that increments were likely deposited daily, and that *D. plei* may live up to nine months (Perez et al. 2006). Perez et al. (2006) found the growth of *D. plei* is likely to be determined by the environment and geographic range. Eye-lens layers appear to have a sub-monthly resolution, but it could prove useful to better understand how often these layers are deposited, and then compare this information to the precise age of the individuals being studied.

Future work could also include stable isotope analysis of the beak and gladius. These hard structures have the potential to show lifelong trends in isotopic variation, and could be compared to the eye lens results presented in the present study. The beak is a chitinous structure that resists digestion and contains isotopic records. Cherel and Hobson were the first to measure the stable isotopic signature in Giant and Colossal squid beaks from the stomachs of predators, and found this method to be useful at the community and population level (Cherel & Hobson 2005). Due to the presence of chitin, caution must be taken when comparing the $\delta^{15}N$ values of the beak to other cephalopod tissues because chitin and protein synthesis differ in their metabolic pathways, and this can result in different isotopic signatures. However, $\delta^{15}N$ values of the beak may be compared among different cephalopods because there are likely little differences among the metabolic pathways of chitin synthesis among species (Cherel & Hobson 2005). Since this preliminary study, the beak has been confirmed in several studies as a useful tool for re-creating long-term isotopic histories (Ruiz-Cooley et al. 2006, Hobson & Cherel 2006, Guerra et a.l 2010, Fang et al. 2016).

The gladius, or pen, is an internal chitinous shell within the mantle that is continually synthesized throughout the squids' life with no turnover. Its stable isotope composition can be analyzed by sectioning the prostracum (Ruiz-Cooley et al. 2010). This method has proven to be a powerful tool in depicting ontogenetic variation in individual foraging strategies of squids (Lorrain et al. 2011). Stable isotope analysis of the gladius has also been used as a tool for identifying migratory patterns of male and female Neon Flying squids (Kato et al. 2016).

4.6 Conclusions

The results from this study suggest *D. plei* begins life at the deep chlorophyll maximum (DCM) along the West Florida Shelf. It appears that *D. plei* tend to move inshore from the DCM during life. There did not appear to be any movement to the west or south in this region of the Gulf of Mexico, as previously reported by Whitaker (1980) and Voss and Brakonieki (1985).

The novel approach of stable isotope analysis using eye-lenses as lifetime isotopic recorders appears to be useful for describing certain aspects of the histories of individual squid. Reporting suggests this species may live up to one year, therefore the eye lenses appear to provide a sub-monthly temporal resolution, which is a resolution that cannot be obtained from other tissues.

This study has outlined the spawning patterns and migrations of *D. plei* in the eastern Gulf of Mexico, which is a central component in the management of commercially valuable squids. The results of this research have provided information on the distribution of this squid so that we can become more aware of the impacts of its fishery in the future.

Tables

Table 1. Summary of individual squid specimens including the station, zone, region and season in which they were caught, sex, mantle length, weight and eye lens diameter.

Sample ID	Station	NMFS Zone	Region	Season	Sex	Mantle Length (mm)	Weight (g)	Eye Lens (mm)
BM20	SMP171505014	8	North	Fall	M	130	45.4	3.3
BM21	SMP171505014	8	North	Fall	M	112	30.4	2.9
BM22	SMP171505014	8	North	Fall	F	110	31.8	2.5
BM23	SMP171505014	8	North	Fall	F	95	25.9	2.5
BM77	SMP171505013	8	North	Fall	M	117	33.3	2.8
BM78	SMP171505013	8	North	Fall	F	87	20.9	2.5
BM79	SMP171505013	8	North	Fall	M	94	24.2	2.8
BM80	SMP171505013	8	North	Fall	F	110	26.9	2.9
BM73	SMP171505015	8	North	Fall	F	112	23.6	3.1
BM74	SMP171505015	8	North	Fall	M	103	26.9	2.7
BM75	SMP171505015	8	North	Fall	F	117	30.1	2.7
BM76	SMP171505015	8	North	Fall	M	138	27.6	3.7
BM10	SMP171503086	5	UpMid	Summer	F	132	66.4	3.3
BM15	SMP171503086	5	UpMid	Summer	F	118	32	2.9
BM161	SMP171503086	5	UpMid	Summer	F	115	33.5	2.9
BM11	SMP171503086	5	UpMid	Summer	M	164	65.8	3.4
BM12	SMP171503086	5	UpMid	Summer	M	172	53.6	3.2
BM14	SMP171503086	5	UpMid	Summer	M	166	45.8	3
BM29	SMP171503085	5	UpMid	Summer	M	272	163.5	3.9
BM31	SMP171503085	5	UpMid	Summer	M	198	111.5	4.2
BM24	SMP171505024	6	UpMid	Fall	F	153	76	4.3
BM27	SMP171505024	6	UpMid	Fall	F	119	36.4	3.1
BM85	SMP171503040	4	LowMid	Summer	F	152	98.8	3.9
BM86	SMP171503040	4	LowMid	Summer	M	97	21.7	2.5
BM87	SMP171503040	4	LowMid	Summer	F	87	25	2.5
BM89	SMP171503040	4	LowMid	Summer	M	78	17.4	2.3
BM40	SMP171503033	4	LowMid	Summer	F	138	77.3	3.9
BM41	SMP171503033	4	LowMid	Summer	F	88	20.5	2.6
BM69	SMP171505053	4	LowMid	Fall	M	136	55.1	3.4
BM72	SMP171505053	4	LowMid	Fall	M	136	54.6	4.5
BM50	SMP171503051	3	LowMid	Summer	F	185	91.6	3.2

Table 1 (Continued)

Sample ID	Station	NMFS Zone	Region	Season	Sex	Mantle Length (mm)	Weight (g)	Eye Lens (mm)
BM54	SMP171503051	3	LowMid	Summer	F	190	112.9	3.6
BM55	SMP171503051	3	LowMid	Summer	M	163	81.3	3.1
BM19	SMP171505069	2	South	Fall	M	235	186.9	5.5
BM178	SMP171503110	2	South	Summer	M	188	58.3	2.8
BM181	SMP171503110	2	South	Summer	F	126	42.1	2.9
BM182	SMP171503110	2	South	Summer	M	131	44.8	3.2
BM183	SMP171503110	2	South	Summer	F	127	29.4	3
BM143	SMP171505065	2	South	Fall	F	120	45	3.8
BM144	SMP171505065	2	South	Fall	M	127	38.6	4
BM146	SMP171505065	2	South	Fall	M	119	28.7	2.9
BM147	SMP171505065	2	South	Fall	F	119	48.7	3.6

Table 2. Linear regressions of δ^{13}C versus lamina midpoint, δ^{15}N versus lamina midpoint and δ^{15}N vs. δ^{13}C, with slope *p*-values and R² values.

Sample ID	*n*	δ^{13}C Slope	δ^{13}C *p*-value	δ^{13}C R^2	δ^{15}N slope	δ^{15}N *p*-value	δ^{15}N R^2	δ^{15}N/δ^{13}C slope	δ^{15}N/δ^{13}C *p*-value	δ^{15}N/δ^{13}C R^2
BM20	12	-0.07	0.05	22.82	0.44	0.0004	54.97	-2.44	0.006	38.16
BM21	12	0.39	0.0001	81.48	0.50	< 0.0001	92.25	1.09	0.0001	81.16
BM77	11	0.16	0.12	24.58	0.18	0.15	21.78	1.11	< 0.0001	91.59
BM79	9	0.18	0.10	34.45	-0.03	0.42	9.52	0.17	0.15	26.88
BM74	10	0.12	0.06	38.18	0.44	0.0007	78.10	1.13	0.21	18.94
BM76	11	-0.08	0.02	53.54	0.23	0.001	75.48	-1.10	0.19	20.80
BM22	10	0.29	0.004	67.03	0.40	0.0006	79.09	1.01	0.005	64.35
BM23	9	0.47	0.02	56.56	0.19	0.04	46.37	0.27	0.08	37.72
BM78	9	0.28	0.007	67.42	0.54	0.002	77.67	1.10	0.08	38.26
BM80	13	0.13	0.003	55.70	0.82	< 0.0001	90.74	2.76	0.04	32.58
BM73	13	0.12	0.008	49.00	0.26	0.0004	70.12	1.23	0.009	48.18
BM75	12	0.22	0.008	52.21	0.28	0.0003	75.04	0.74	0.02	45.23
BM11	12	0.43	< 0.0001	89.55	0.13	0.005	56.41	0.26	0.01	46.85
BM12	13	0.08	0.50	4.22	0.07	0.25	12.21	-0.37	0.003	56.46
BM14	12	0.34	0.03	38.08	0.30	0.001	66.53	0.20	0.34	9.28
BM29	17	-0.24	0.004	43.67	0.09	0.007	39.67	-0.26	0.003	44.57
BM31	15	0.29	0.009	42.03	0.21	0.02	37.57	0.28	0.18	13.62
BM10	15	0.68	0.0005	76.14	0.03	0.61	0.67	0.00	0.98	0.00
BM15	10	0.61	< 0.0001	94.90	0.02	0.82	0.68	0.05	0.62	3.17
BM24	12	0.72	0.00	51.11	0.52	0.004	57.52	0.14	0.52	4.33
BM161	10	0.43	< 0.0001	91.23	0.34	0.001	75.14	0.81	0.0003	82.56
BM27	11	-0.48	0.002	68.47	0.63	< 0.0001	88.74	-0.75	0.03	42.21
BM86	10	0.76	0.0001	87.07	0.61	0.0002	83.70	0.67	0.004	66.62

Table 2 (Continued)

Sample ID	*n*	$\delta^{13}C$ Slope	$\delta^{13}C$ *p*-value	$\delta^{13}C$ R^2	$\delta^{15}N$ slope	$\delta^{15}N$ *p*-value	$\delta^{15}N$ R^2	$\delta^{15}N/\delta^{13}C$ slope	$\delta^{15}N/\delta^{13}C$ *p*-value	$\delta^{15}N/\delta^{13}C$ R^2
BM89	9	0.04	0.49	6.99	0.44	0.005	69.49	0.04	0.98	0.01
BM69	14	0.12	0.02	38.01	0.83	< 0.0001	95.71	3.23	0.003	53.02
BM72	16	0.03	0.23	10.24	0.23	0.0001	66.00	0.55	0.49	3.54
BM85	14	0.79	< 0.0001	93.78	0.13	0.02	37.47	0.14	0.07	25.39
BM40	13	0.89	< 0.0001	79.07	0.05	0.17	16.57	0.03	0.45	5.37
BM41	9	1.42	0.0001	89.17	-0.07	0.50	6.81	-0.10	0.16	25.58
BM87	9	0.45	0.004	72.48	0.17	0.02	55.40	0.21	0.18	24.54
BM55	13	0.15	0.08	25.16	0.24	0.0002	74.16	0.22	0.44	5.49
BM19	19	0.28	0.0007	50.18	0.06	0.27	7.19	0.23	0.07	18.16
BM178	14	0.12	0.26	10.40	0.23	0.002	56.30	0.07	0.78	0.68
BM182	11	0.48	0.0003	77.54	0.09	0.45	6.36	-0.02	0.94	0.07
BM144	15	-0.24	0.009	41.72	0.11	0.17	13.96	0.09	0.69	1.23
BM146	13	0.11	0.25	11.65	0.26	0.004	53.66	0.48	0.15	17.87
BM50	12	0.41	0.0002	77.03	0.08	0.07	29.40	0.05	0.61	2.77
BM54	14	0.80	< 0.0001	92.03	0.00	0.94	0.03	-0.07	0.26	5.19
BM183	12	0.21	0.02	41.61	0.09	0.52	4.19	-0.2	0.65	2.10
BM181	14	0.60	0.0001	75.65	-0.07	0.25	10.67	-0.16	0.08	22.99
BM143	14	0.15	0.07	24.78	0.64	< 0.0001	87.73	0.49	0.44	5.01
BM147	11	0.21	0.07	31.73	0.59	0.0005	76.03	0.21	0.74	1.25

Table 3. Absolute difference values of $\delta^{15}N$ and $\delta^{13}C$ for each individual squid.

Sample ID	$\delta^{13}C$ AD	$\delta^{15}N$ AD
BM20	0.49	1.85
BM21	0.94	1.22
BM22	0.74	0.79
BM23	1.01	0.51
BM77	0.72	1.18
BM78	0.79	1.19
BM79	0.52	0.21
BM80	0.42	1.89
BM73	0.45	0.7
BM74	0.35	1.03
BM75	0.57	0.84
BM76	0.33	0.85
BM10	1.34	0.39
BM15	1.44	0.47

Table 3 (Continued)

Sample ID	**δ^{13}C AD**	**δ^{15}N AD**
BM161	1.03	0.71
BM11	1.06	0.5
BM12	0.95	0.57
BM14	1.26	1.01
BM29	1.14	0.43
BM31	1.16	1.1
BM24	2.69	2.2
BM27	1.47	1.69
BM85	2.3	0.98
BM86	1.85	1.18
BM87	0.95	0.52
BM89	0.29	0.77
BM40	2.98	0.35
BM41	2.83	0.55
BM69	0.66	2.2
BM72	0.32	0.73
BM50	1.21	0.48
BM54	2.37	0.77
BM55	0.86	0.76
BM19	1.11	1.12
BM178	1.11	1.12
BM181	1.27	0.6
BM182	1.55	0.84
BM183	0.88	0.92
BM143	0.93	1.31
BM144	0.95	0.79
BM146	0.79	0.78
BM147	1.39	2.05

Table 4. Tally of significant trends based on linear regression for males and females (numbers are numbers of individuals).

	δ^{13}C Increases	**δ^{13}C Decreases**	**δ^{13}C Flat**	**δ^{15}N Increases**	**δ^{15}N Decreases**	**δ^{15}N Flat**
Males	8	4	9	15	0	6
Females	18	1	2	13	0	8

Figures

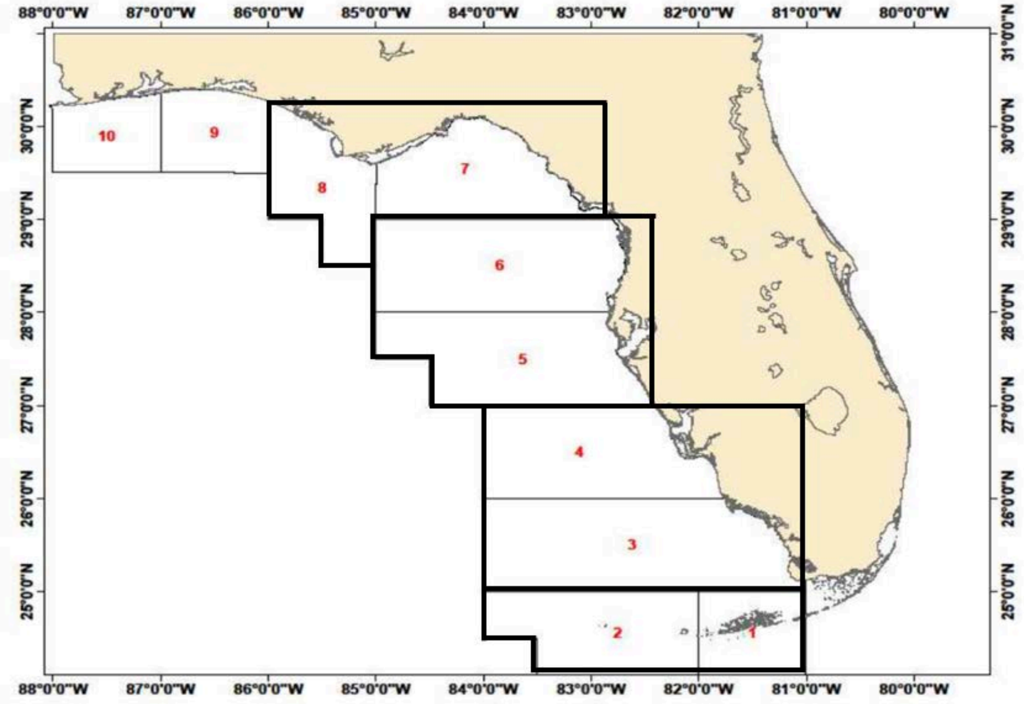

Figure 1. Map of the western coast of Florida displaying the 2015 SEAMAP NMFS zones. The four regions from N to S are outlined in black (North, Upper Middle, Lower Middle, South).

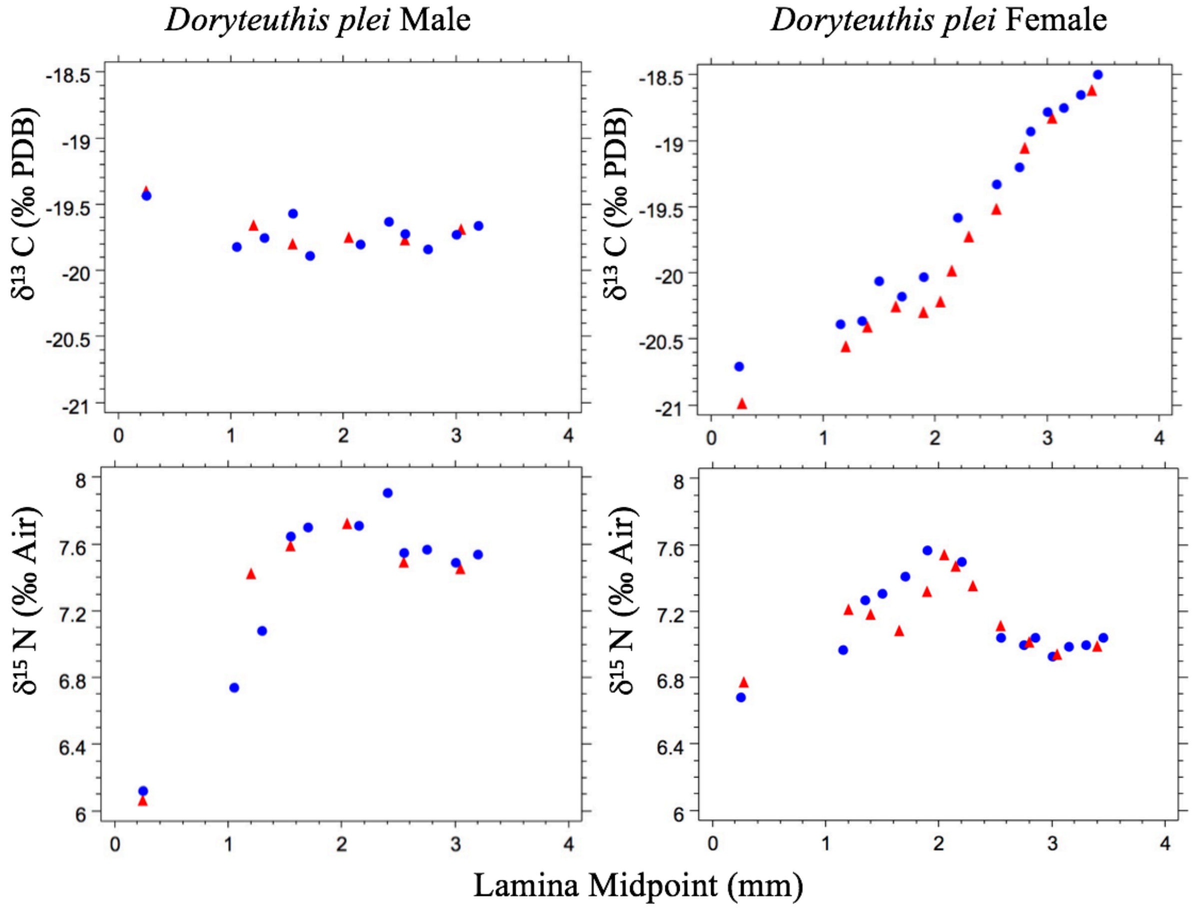

Figure 2. Comparison of wet and dry methods of δ^{13}C and δ^{15}N with lamina midpoint for left (wet method) and right (dry method) eyes of a male specimen (left panels, specimen BM20) and a female specimen (right panels, specimen BM54). Red triangles indicate right eye, blue circles indicate left eye.

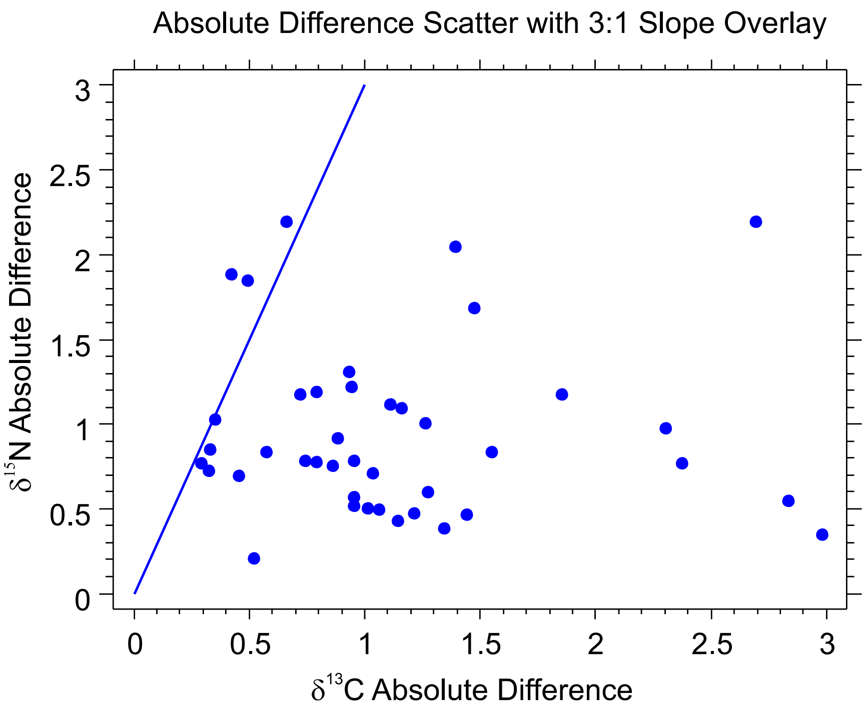

Figure 3. Plot of observed lifetime range of isotopic values for individual *D. plei*, compared with the 3:1 slope that might be expected from lifetime changes in trophic level.

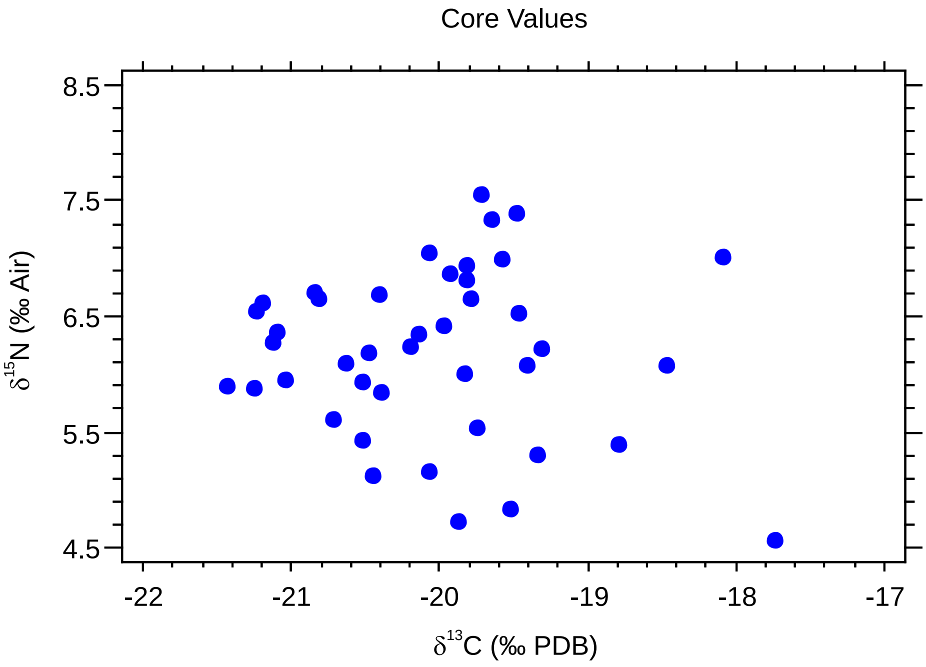

Figure 4. Plot of δ^{13}C and δ^{15}N from the center of the eye lens (core) for individual *D. plei* indicating wide isotopic variation during very early life.

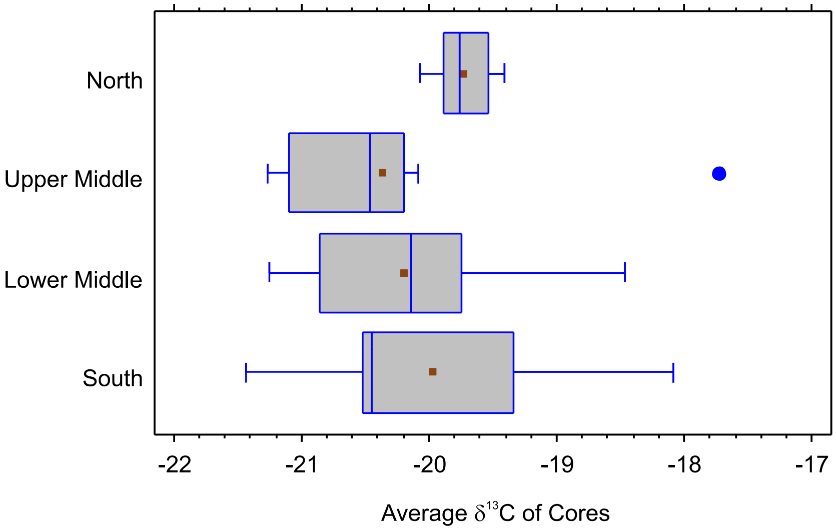

Figure 5. Comparison of δ^{13}C of eye-lens cores by region.

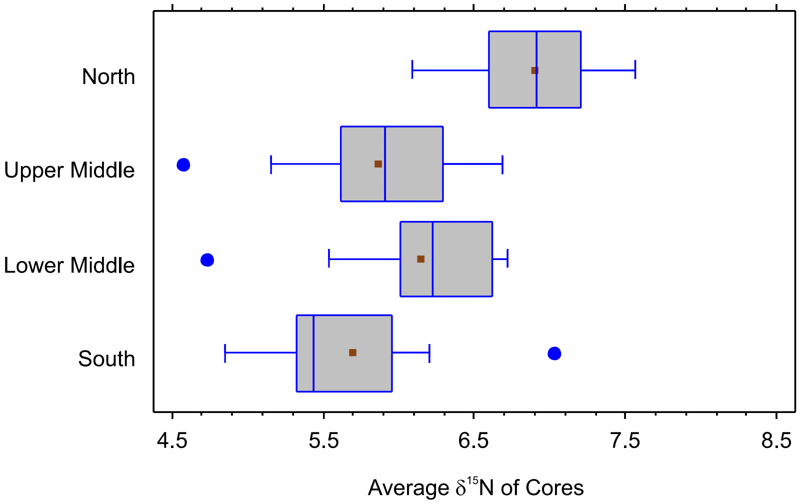

Figure 6. Comparison of δ^{15}N of eye-lens cores by region.

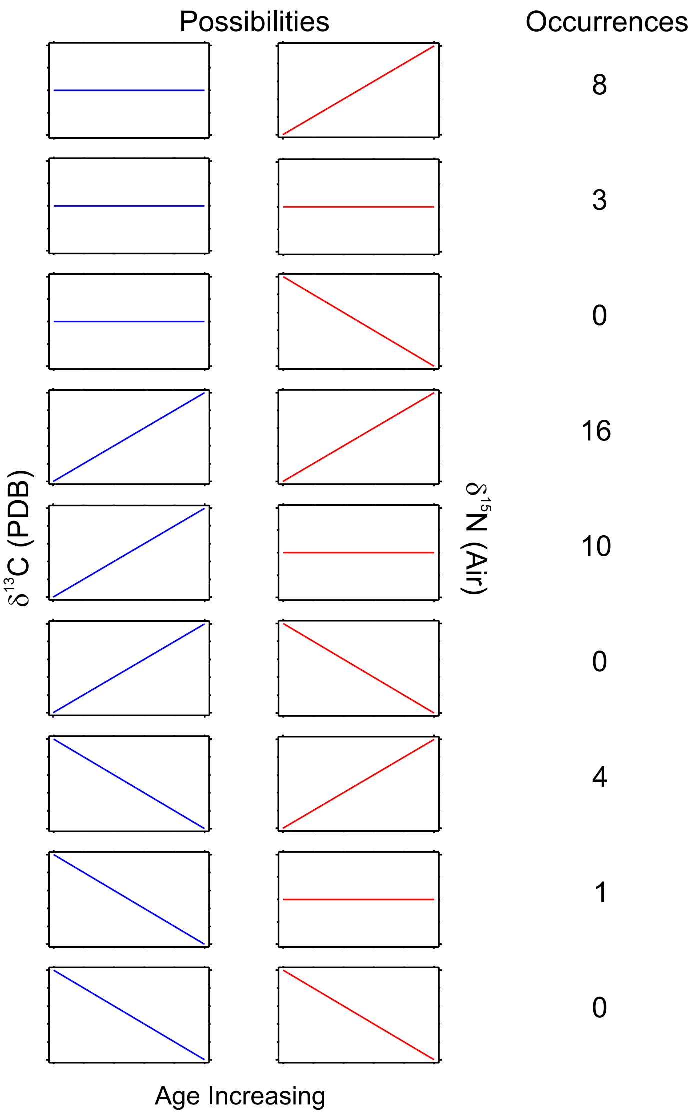

Figure 7. Tally of significant trends based on linear regression for all individuals (n = 42).

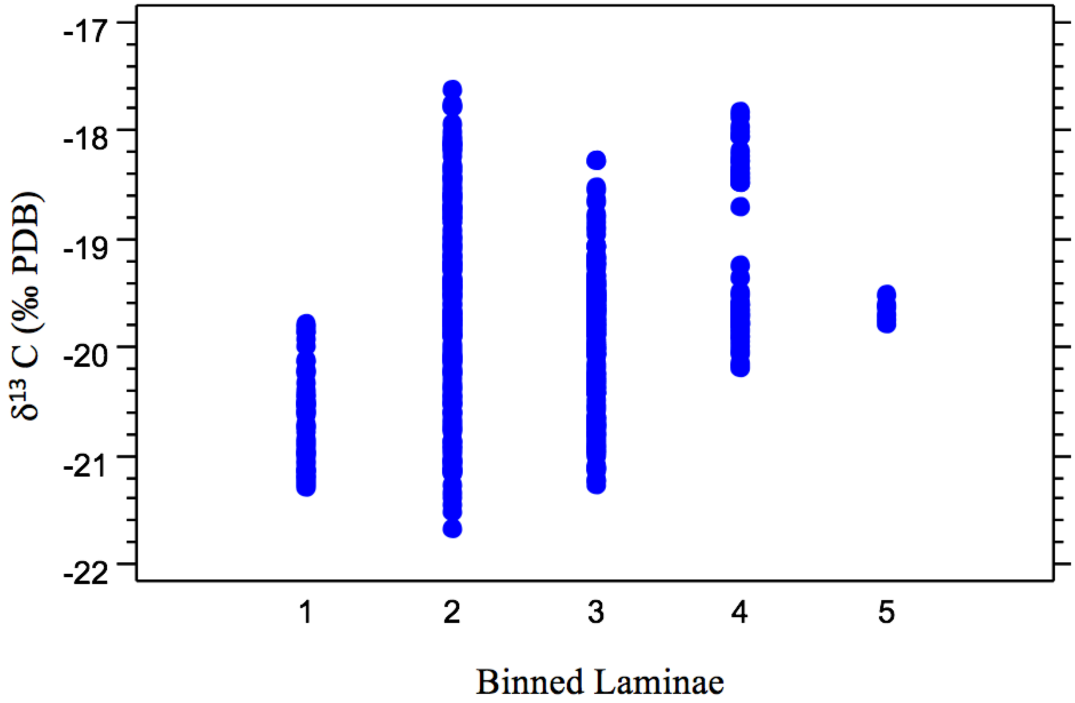

Figure 8. One-way ANOVA of δ^{13}C values compared among all lamina midpoints. Lamina midpoints were binned into five groups.

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Appendices

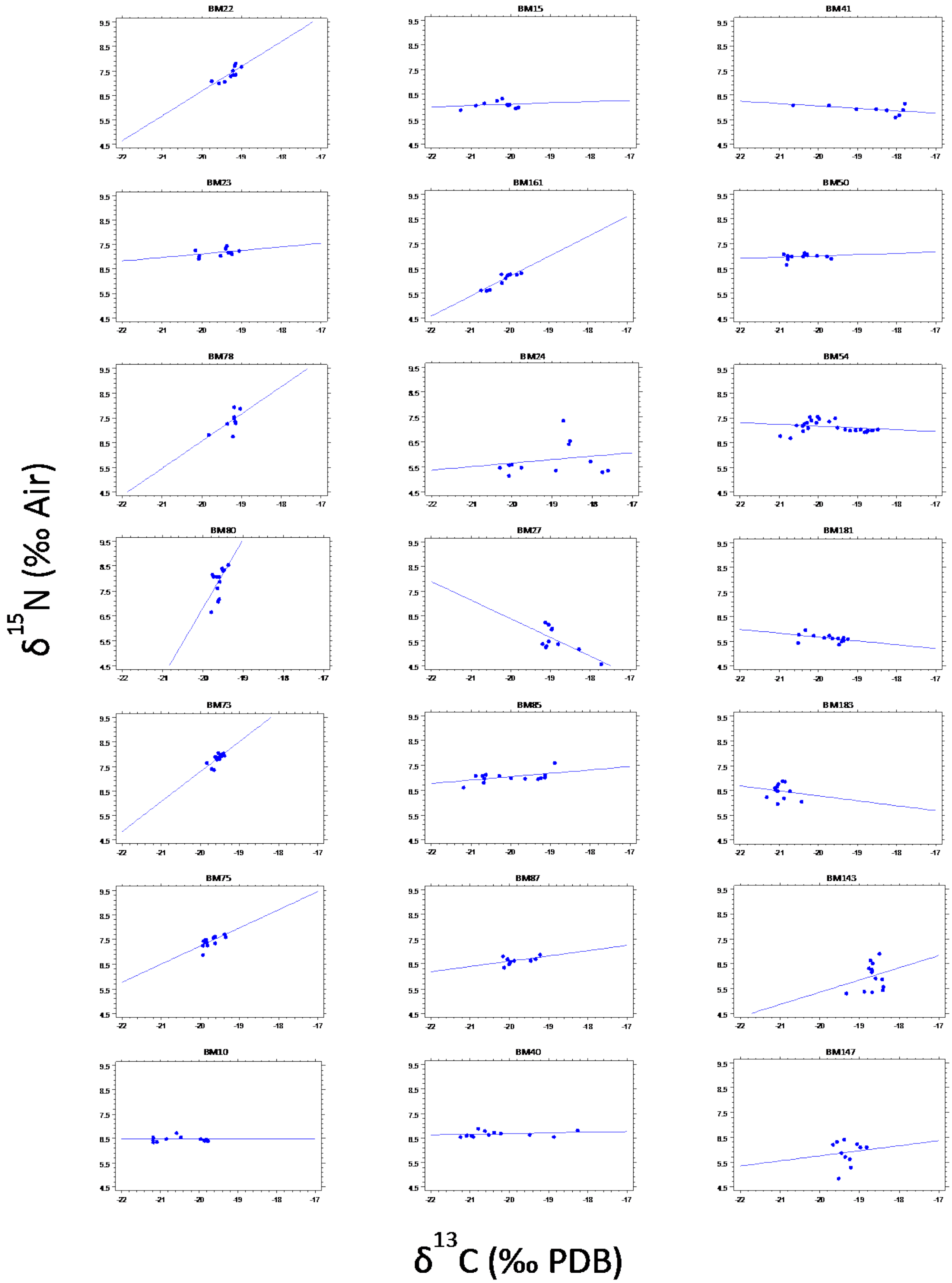

Figure A1. Linear regressions of δ^{15}N vs. δ^{13}C for female specimens.

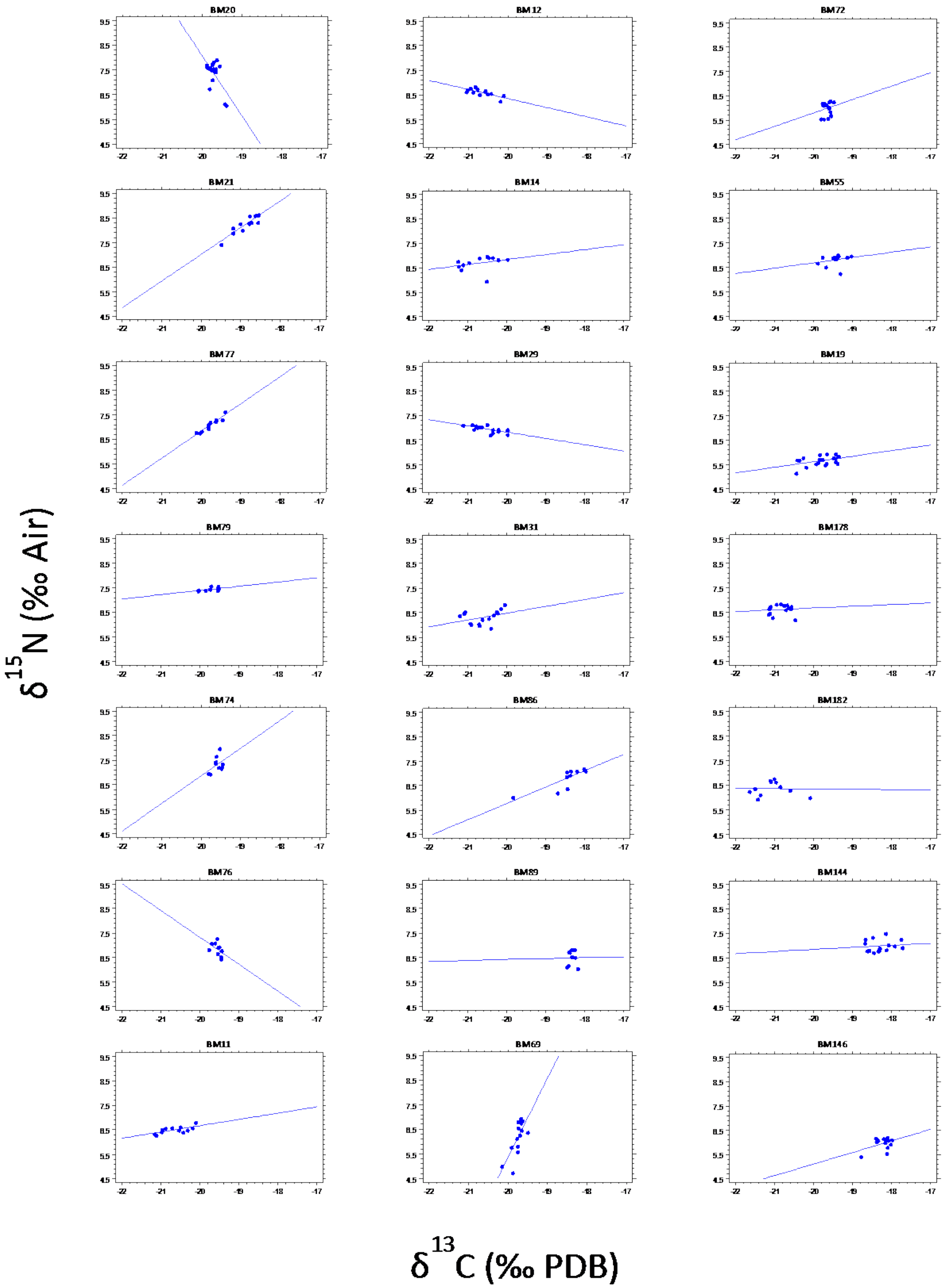

Figure A2. Linear regressions of δ^{15}N vs. δ^{13}C for male specimens.