

Non-lethal Methodology for Stable Isotope Analysis of Spiny Lobsters (*Panulirus argus*)

ROBERT P. CREED JR.¹ and ROBERT C. THIGPEN III^{1,2,3}

¹ Department of Biology Appalachian State University, Boone North Carolina, 28608 USA

² Department of Anthropology, Appalachian State University, Boone North Carolina, 28608 USA

³ Hugh Parkey Foundation for Marine Awareness and Education, Belize City, Belize, Central America

ABSTRACT

Stable isotope analysis (SIA) is used to determine animal diets and construct food webs. Tissue sampling methodology for SIA is usually lethal to the study organism. As various species become endangered worldwide, it is important to develop non-lethal methods of sampling tissues for SIA. In crustaceans, animals are usually killed and abdominal tissue is used for SIA. We sampled tissues from other body parts (walking legs, antennae) of the Caribbean spiny lobster (*Panulirus argus*) that can be removed without killing the animal to determine if they would produce SI signatures similar to abdominal tissue. Lobsters were collected from seagrass meadows in the vicinity of Caye Caulker, Belize during July and August of 2006. Tissue samples were taken from 10 adult lobsters, 5 males and 5 females. Leg and abdominal $\delta^{13}\text{C}$ values were not significantly different; antennal $\delta^{13}\text{C}$ signatures were significantly different from those of abdominal tissue. Regression analysis demonstrated that isotopic carbon ratios from leg tissue were better predictors of abdominal carbon ratios ($r^2=0.918$). Leg tissues had significantly higher $\delta^{15}\text{N}$ signatures than abdominal tissue and antennal $\delta^{15}\text{N}$ values were significantly lower than abdominal values. We conducted a regression analysis to determine if either leg or antennal tissue SI values would be a good predictor of abdominal $\delta^{15}\text{N}$ values. The r^2 for the relationship between abdominal tissue and antennal tissue ($r^2=0.677$) was higher than that for leg and abdominal tissue ($r^2=0.476$). Despite the poorer relationship between leg and abdominal N signatures we feel that walking legs are the preferred tissue to remove since the loss of one walking leg probably has less of an effect on lobsters than the loss of an antenna. Our results suggest that tissue removed from other body parts of crustaceans can be used to assess diet and determine trophic level assignment.

KEYWORDS: autotomy, Belize, seagrass communities

Metodología No Letal para el Análisis de Isótopos Estables de Invertebrados Marinos

Los isótopos estables de carbono y nitrógeno han sido inestimables en el rastreo de movimientos de animales, en la determinación de dietas de animales, y se están utilizando cada vez más para evaluar disturbios ecológicos y ambientales en el ecosistema marino. Sin embargo, la metodología de muestreo de tejido para el análisis de isótopos estables (AIE) es generalmente letal. A medida que un número mayor de especies se encuentra en peligro alrededor del mundo, es importante determinar métodos no letales de muestreo para dar un buen ejemplo a las personas no científicas. Si queremos ser tomados en serio como directores de pesquerías, es importante llevar a cabo la colección de tejidos para el análisis de isótopos estables, así como otras formas de investigación, sin dañar nuestra reputación, así como también ayudar a proteger las especies estudiadas. En el caso de los crustáceos, usualmente se matan los animales y se utiliza tejido abdominal para el AIE. Nos preguntamos si los tejidos de otras partes del cuerpo que pueden ser removidos sin matar al animal producirían cocientes similares en el AIE. Comparamos la firma de los isótopos estables de una pata caminadora, una antena y el abdomen de dos invertebrados marinos comercialmente importantes: la langosta espinosa del Caribe (*Panulirus argus*). Las muestras del tejido fino fueron tomadas a partir de 10 langostas del adulto, de 5 varones y de 5 hembras. La pierna y los valores abdominales de $\delta^{13}\text{C}$ no eran perceptiblemente diferentes; las firmas antennal de $\delta^{13}\text{C}$ eran perceptiblemente diferentes de las del tejido fino abdominal. El análisis de la regresión demostró que los cocientes isotópicos del carbón del tejido fino de la pierna eran predictores mejores de los cocientes abdominales del carbón ($r^2=0.918$). Los tejidos finos de la pierna tenían firmas perceptiblemente más altas de $\delta^{15}\text{N}$ que el tejido fino abdominal y los valores antennal de $\delta^{15}\text{N}$ eran perceptiblemente más bajos que los valores abdominales. Condujimos un análisis de la regresión para determinar si la pierna o los valores antennal del SI del tejido fino sería un buen predictor de los valores abdominales de $\delta^{15}\text{N}$. El r^2 para la relación entre el tejido fino abdominal y el tejido fino antennal ($r^2=0.677$) era más alto que éste para la pierna y el tejido fino abdominal ($r^2=0.476$). A pesar de la relación más pobre entre la pierna y las firmas abdominales de N nos sentimos que las piernas que caminan son el tejido fino preferido a quitar puesto que la pérdida de una pierna que camina tiene probablemente menos de un efecto sobre langostas que la pérdida de una antena. Nuestros resultados sugieren que el tejido fino quitado de otras piezas de cuerpo de crustáceos se pueda utilizar para determinar dieta y para determinar la asignación llana trophic.

PALABRAS CLAVES: Belice, Comunidad de Yerbas Marinas

INTRODUCTION

Stable isotope analysis (SIA) is a popular method for evaluating animal diets and constructing food webs for marine communities (Peterson *et al.* 1985, Melville and

Connolly 2005, Hays 2005, Smit *et al.* 2005). Collection of tissue for SIA is frequently lethal to the study animal, however. Researchers either use entire animals for analysis or particular tissues (e.g., white caudal muscle in fish

(Melville and Connolly 2005, Smit et al. 2005), abdominal muscle in crustaceans (Whitledge and Rabeni 1997), the removal of which are lethal. Sacrificing individuals of an abundant species for SIA will not have an effect on a population. However, if tissue collection is lethal then SIA should not be used for species that are rare, endangered or threatened. An alternative to SIA is gut content analysis. Non-lethal methods for obtaining stomach contents of fish have been developed (Kamler and Pope 2001). To our knowledge, successful development of comparable non-lethal gut content analysis techniques for macroinvertebrates have not been developed. Gut content analysis in macroinvertebrates requires killing the animal in order to dissect out the foregut. At present, with no non-lethal techniques available researchers appear to have few options for ascertaining the diet of many rare species of macroinvertebrate.

Loss of certain tissues is not fatal in various invertebrates. For example, crustaceans are capable of autotomy or self-amputation of limbs (Andrews 1890, Cooper 1998, Wilhelm *et al.* 2003). Animals will autotomize limbs in response to an attack by a predator or if a limb is damaged. The limb separates along a fracture plane and a new limb will regenerate at this location with successive molts (Andrews 1890, Cooper 1998). Since limb loss is not fatal collection of limb tissue is one non-lethal alternative for collecting tissue samples for stable isotope analysis in crustaceans. However, this method will only be of value if limb tissue isotopic signatures are comparable to those of abdominal tissues.

Our goal in this study was to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures from abdominal tissue, walking leg tissue and antennal tissue of the Caribbean spiny lobster (*Panulirus argus*). Specifically, we wanted to see if tissue from appendages that could be removed without killing the lobster (walking legs and antennae) produced isotopic signatures that were similar to abdominal tissue. While the spiny lobster is not a rare species this methodological approach has to be evaluated on an abundant species first to determine its feasibility.

METHODS

Lobsters were collected from commercial lobster traps in July and August 2006. The traps were placed in the sea-grass meadows in the vicinity of Caye Caulker, Belize. Caye Caulker is located approximately 33 km northeast of Belize City and about a km west of the Mesoamerican Barrier Reef System. Ten adult lobsters (5 males and 5 females) were selected. Carapace lengths (measured from the base of the supraorbital horns to the posterior margin of the carapace) for the lobsters ranged from 95-107 mm. We then removed the base of the right antenna, the entire right first walking leg and the abdomen of *P. argus*. Each tissue sample was placed in individual, labeled sealable plastic bags. All tissue samples were immediately frozen and kept frozen until the samples were transported to the United

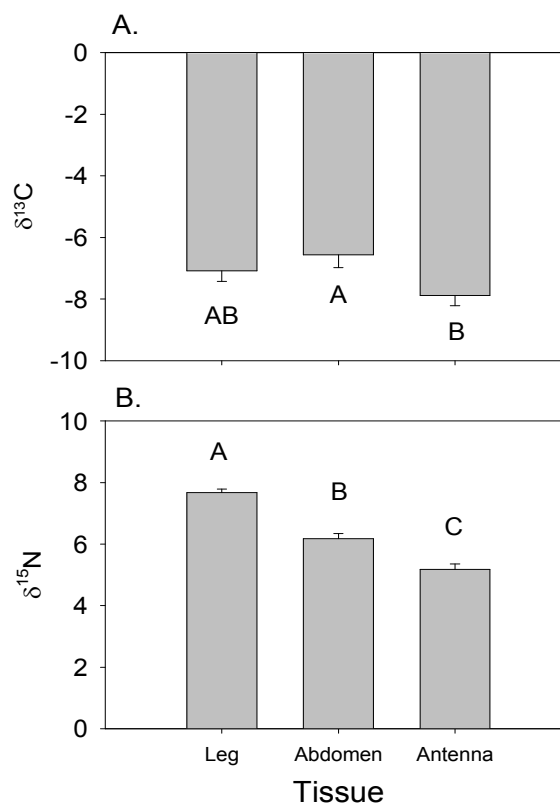


Figure 1. Carbon ($\delta^{13}\text{C}$) (A) and nitrogen ($\delta^{15}\text{N}$) (B) isotopic ratios for leg, abdominal and antennal tissue from Caribbean spiny lobsters. Units for the y axes are in parts per thousand (‰). Bars represent means; error bars are 1 standard error. Letters above or below bars represent the results of Tukey's test; bars with different letters are significantly different. Sample sizes for leg and abdominal tissue

States. In the United States, tissues were stored at $-20\text{ }^{\circ}\text{C}$ until they were processed. Tissues were thawed and then dried for at least 4 days in a $60\text{ }^{\circ}\text{C}$ drying oven. We used tissue from the fourth leg segment, the merus, of the walking leg. For antennal tissue, we used the tissue from the first 6.5 cm of the flagellum adjacent to the peduncle. The dried tissues were ground with a mortar and pestle and weighed to the nearest 0.1 mg. The carbon and nitrogen in these samples was analyzed for $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotopic ratios by mass spectrometry. The analysis was performed by the Colorado Plateau Stable Isotope Laboratory at the University of Northern Arizona, Flagstaff, Arizona. The data are expressed as δ values (‰), which is a measure of the amount by which the samples deviate from standard reference materials.

Isotopic ratios for carbon and nitrogen from the three tissues were compared using a one-way analysis of variance. Treatment means were compared using a Tukey's test. We also analyzed the data using linear regression analysis to determine how well isotopic signatures from leg and antennal tissue predicted abdominal isotopic signa-

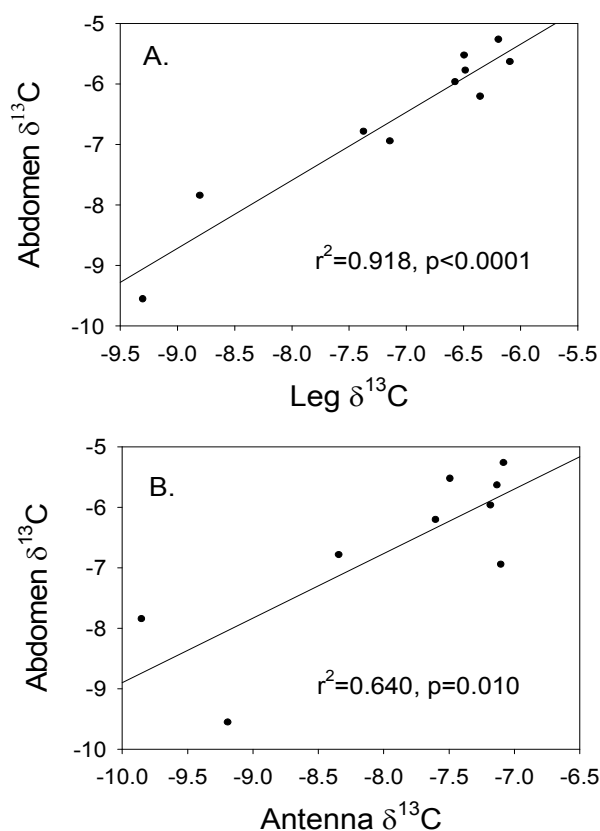


Figure 2. Plots of the relationship between leg (A) and antennal (B) ($\delta^{13}\text{C}$) signatures and abdominal ($\delta^{13}\text{C}$) signature. Units for both axes are in parts per thousand (‰). The equation for the leg and abdomen regression is $\text{Abdomen}(\delta^{13}\text{C})=1.412 + 1.126\text{Leg}(\delta^{13}\text{C})$. The equation for the antenna and abdomen regression is $\text{Abdomen}(\delta^{13}\text{C})=1.777$

tures. One antennal sample was lost so $n=9$ for antennae; $n=10$ for both leg and abdominal tissues.

RESULTS

No significant differences in isotopic signatures from any of the tissues collected were observed for male and female lobsters so data were pooled. There was a marginally significant difference ($F_{2,26} = 3.13$, $p = 0.061$) in the $\delta^{13}\text{C}$ signatures from the three tissues. When the means for the three tissues were compared antennal tissue was significantly different from abdominal tissue based on Tukey's test (Figure 1A). Leg tissue $\delta^{13}\text{C}$ signatures were not significantly different from those of either abdominal or antennal tissue. Regression analysis found that walking leg tissue was a much better predictor of abdominal carbon isotopic signature ($r^2=0.918$) than antennal tissue ($r^2=0.640$) (Figure 2).

The $\delta^{15}\text{N}$ signatures for all three tissues were significantly different ($F_{2,26} = 67.64$, $p<0.0001$) (Figure 1B).

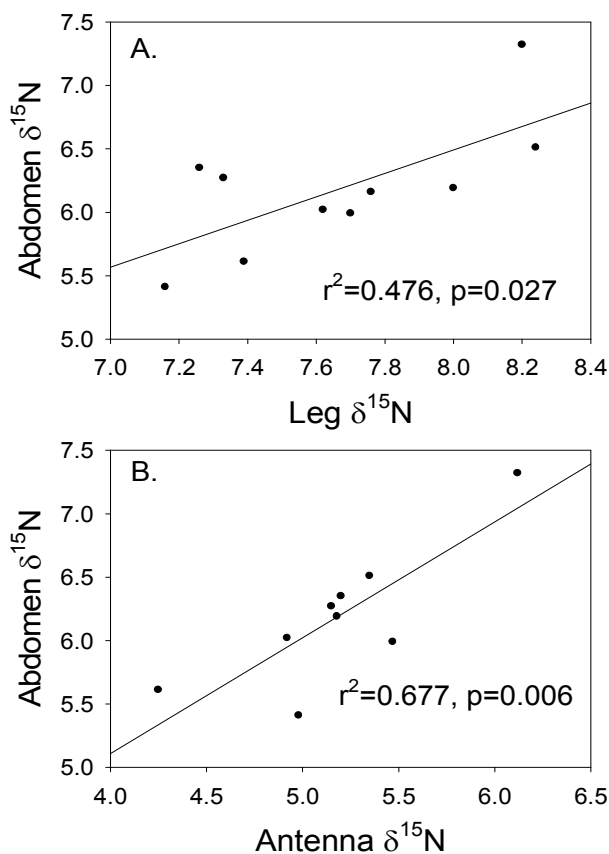


Figure 3. Plots of the relationship between leg (A) and antennal (B) ($\delta^{15}\text{N}$) signatures and abdominal ($\delta^{15}\text{N}$) signature. Units for both axes are in parts per thousand (‰). The equation for the leg and abdomen regression is $\text{Abdomen}(\delta^{15}\text{N})=-0.914 + 0.9258\text{Leg}(\delta^{15}\text{N})$. The equation for the antenna and abdomen regression is $\text{Abdomen}(\delta^{15}\text{N})=1.456$

Walking leg tissue had the highest $\delta^{15}\text{N}$ signature while antennal tissue had the lowest. Antennal $\delta^{15}\text{N}$ was a better predictor of abdominal $\delta^{15}\text{N}$ ($r^2=0.677$) than leg tissue ($r^2=0.476$) (Figure 3).

DISCUSSION

Our results demonstrate that alternative tissues can be used in *P. argus* to determine stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic ratios. Since leg $\delta^{13}\text{C}$ values were not significantly different from abdominal $\delta^{13}\text{C}$ values walking leg tissue should be an excellent surrogate for abdominal tissue when one is trying to determine the carbon source in the diet of a lobster population. Since antennal $\delta^{13}\text{C}$ values were significantly different than abdominal tissues we do not recommend their use for determining the source of carbon in a lobster's diet.

Leg and antennal $\delta^{15}\text{N}$ values were both significantly different from abdominal $\delta^{15}\text{N}$ values. The mean for leg $\delta^{15}\text{N}$ was 1.49 ‰ higher than the value for abdominal tis-

sue. The mean for antennal $\delta^{15}\text{N}$ tissue was 1.00 ‰ less than that of abdominal tissue. Thus, neither tissue will produce a highly accurate representation of abdominal tissue $\delta^{15}\text{N}$ signature. Regression analysis suggests that antennal $\delta^{15}\text{N}$ is a better predictor of abdominal $\delta^{15}\text{N}$. Nevertheless, we recommend that leg tissue be used instead of antennal tissue because we feel that the loss of one walking leg will have less of an effect on the subsequent survival of a lobster than the loss of an antenna. Antennae are used defensively by spiny lobsters so the loss of an antenna could increase an individual's susceptibility to predators. Regression equations can be used to estimate abdominal $\delta^{15}\text{N}$ from leg $\delta^{15}\text{N}$ values. Alternatively, it is possible that tissue could be removed from the distal end of an antenna without having as great an impact on lobster survival. We did not investigate this and do not know how much tissue would need to be removed.

The variation in leg and antennal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was very similar to that observed in abdominal tissues. This result suggests that use of either of these tissues will provide an investigator with an estimate of the variation in isotopic signatures that is comparable to that obtained from abdominal tissue.

Our results are similar to those of Creed and Burpee (in preparation) who compared $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures from chelae tissues in freshwater crayfish with those from abdominal tissues. Creed and Burpee found that chela tissue was a good predictor of abdominal stable isotopic signatures. Taken together, these results suggest that alternative tissues can be used in place of abdominal tissues for stable isotope analysis in a variety of crustaceans. We plan to conduct a similar comparison with the stone crab. We encourage investigators to evaluate alternative tissues from a range of crustacean species to determine how broadly applicable this technique is.

The primary impetus for this project was to see if using alternative tissues for stable isotope analysis was feasible. As we stated in the introduction, using tissues that would not kill the study animal when they are removed would be of value to scientists who wish to perform stable isotope analysis on a rare, endangered or threatened species. We believe we have demonstrated a reasonable alternative to the standard lethal technique of obtaining tissue samples for analysis. Our approach is similar to that employed by Wilhelm *et al.* (2003) for obtaining tissue for genetic analysis from rare and endangered species of crustacean. We wish to point out that this method is also of value to scientists studying abundant organisms. Scientists should endeavor to minimize their effect on the species they study, regardless of the abundance of a species. The image we project to the public and, especially to commercial fisherman, is important. Demonstrating that we do not want to kill specimens if at all possible should enhance our reputation among these groups. This is one of the benefits of the non-lethal methods employed to evaluate fish stomach contents (Kamler and Pope 2001). We feel that there

would be similar benefits to employing non-lethal techniques for SIA for crustaceans. This may be especially true for a species that is as culturally and economically important as the Caribbean spiny lobster.

ACKNOWLEDGEMENTS

We thank Jamie Castillo, Celeste Castillo and Alyssa Majil for their assistance with the data collection. We are grateful to the fishermen of Caye Caulker and the Northern Fisherman's Cooperative Society for all their support and assistance. Funding was provided by Sigma Xi, the Appalachian State University (ASU) Office of Student Research, and the ASU Prestigious Scholars Program. Rob Thigpen thanks the staff at Albert's Guest House, Mrs. Elida y especialmente de familia de Mercedes Castillo for their hospitality.

REFERENCES

- Andrews, E. A. 1890. Autotomy in the crab. *The American Naturalist* **24**:138-142.
- Cooper, R.L. 1998. Development of sensory processes during limb regeneration in adult crayfish. *Journal of Experimental Biology* **201**:1745-1752.
- Hays, C. G. 2005. Effect of nutrient availability, grazer assemblage and seagrass source population on the interaction between *Thalassia testudinum* (turtle grass) and its algal epiphytes. *Journal of Experimental Marine Biology and Ecology* **314**:53-68.
- Kamler, J.F., and K. L. Pope. 2001. Nonlethal methods of examining fish stomach contents. *Reviews in Fisheries Science* **9**:1-11.
- Melville, A. J., and R. M. Connolly. 2005. Food webs supporting fish over subtropical mudflats are based on transported organic matter not in situ microalgae. *Marine Biology (Berlin)* **148**:363-371.
- Peterson, B.J., R.W. Howarth and R.H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* **227**:1361-1363.
- Smit, A.J., A. Brearley, G.A. Hyndes, P.S. Lavery and D.I. Walker. 2005. Carbon and nitrogen stable isotope analysis of an *Amphibolis griffithi* seagrass bed. *Estuarine, Coastal and Shelf Science* **65**:545-556.
- Whitledge, G.W., and C.F. Rabeni. 1997. Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **54**:2555-2563.
- Wilhelm, F. M., M. P. Venarsky, S. J. Taylor, and F. E. Anderson. 2003. Survival of *Gammarus troglophilus* (Gammaridae) after leg removal: Evaluation of a procedure to obtain tissue for genetic analysis of rare and endangered amphipods. *Invertebrate Biology* **122**:369-374.