Stable Isotopic Enrichment Among Soma and Liver Tissues in *Poecilia latipinna* and *Gambusia holbrooki* : Implications for Analyzing Field Samples

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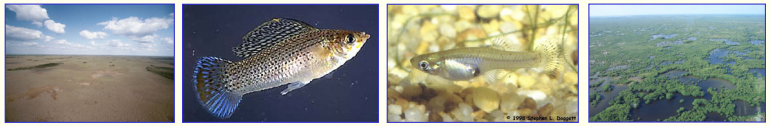
Abstract

We studied fish community dynamics along two oligohaline gradients in the southern Everglades National Park to document patterns of standing crops, species composition, and food-web relationships. We report spatial and temporal patterns observed in stable isotopic signatures from a five-year study of fish communities. We found that $\delta^{13}C$ and $\delta^{15}N$ values of key oligohaline fish species varied considerably, but the trends are not consistent with any seasonal or hydrologic fluctuations. Trophic fractionation is the enrichment or depletion of $\delta^{13}C$ or $\delta^{15}N$ values resulting from biochemical processes between prey and its predator. $\delta^{13}C$ shows weak enrichment, allowing it to be a useful determinant for energy inputs, while $\delta^{15}N$ is typically enriched by 3.4‰ in consumer tissue relative to prey tissue, which defines trophic levels. Energetic demands vary along the salinity gradient associated with physiological stress from osmoregulation, and this may affect isotopic fractionation. We used aquarium and field mesocosm studies to estimate the effect of salinity gradients on fractionation of carbon and nitrogen isotopes in tissues of two species of fish (*Poecilia latipinna* and *Gambusia holbrooki*) by calibrating their rate of turnover in muscle and liver tissue. Liver tissues reflected the $\delta^{13}C$ value of the bulk diet by Day 20 for sailfin mollys and by Day 5 for mosquitofish. Soma tissues display a much longer turnover rate and did not completely reflect their predicted values by the end of the experiment for either fish species. Lipids were extracted and showed no difference in sailfin molly soma tissues with and without lipids. However, liver tissues with lipids extracted were consistently enriched by 1‰ relative to liver tissues with lipids present. These results add to the understanding of differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues and provide an important link between physiological and field food-web studies. These data will be applied to future field studies to ensure correct interpretation, when using $\delta^{13}C$ stable isotope analyses to determine primary energy inputs and $\delta^{15}N$ stable isotope analyses to illustrate the among-slough variation in food-chain length.

Keywords: $\delta^{13}C$ and $\delta^{15}N$; oligohaline gradients; tissue turnover; Everglades food webs.

RESEARCH OBJECTIVE

To determine rates of change in stable isotopic signatures of soma and liver tissue in two species of Everglades oligohaline region fish residents, as a preliminary step for field applications of $\delta^{13}C$ and $\delta^{15}N$ stable isotope techniques



Fractionation

Trophic fractionation is the enrichment or depletion of $\delta^{13}C$ or $\delta^{15}N$ values resulting from biochemical processes between prey and predator.

- $\delta^{13}C$ shows weak enrichment
  - useful determinant for energy inputs
- $\delta^{15}N$ typically enriched by 3.4‰ in consumer tissue relative to prey tissue
  - defines trophic levels

Physiological stress associated with osmoregulation is likely to affect fractionation. When interpreting stable isotopic signatures from oligohaline zone fish samples correctly, we must completely understand fractionation that occurs with the anabolism and catabolism of specific tissues. Tissue-specific rates of isotopic enrichment need to be calibrated. This provides an excellent opportunity for laboratory studies to provide a link between physiological and field food-web studies

Results

Long-term analysis

$\delta^{13}C$ and $\delta^{15}N$ signatures, used to track temporal changes in food-web relationships of key oligohaline ecotone fish species, indicate that $\delta^{13}C$ and $\delta^{15}N$ values varied considerably from 2001 – 2004, but not in patterns consistent with simple seasonal or hydrological fluctuations (Figure 1).

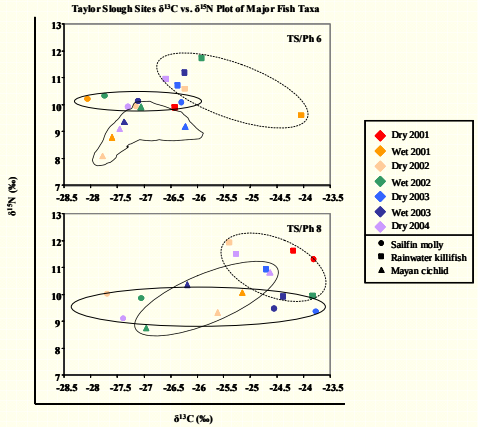


Figure 1. $\delta^{13}C$ and $\delta^{15}N$ signatures from muscle tissues of three fish species collected at two FCE estuarine sites from dry season 2001 through dry season 2004. Sample sizes were generally 5. Variability among individuals of the same species within a sample was generally small (CV=0.10) and is not shown to reduce clutter.

Freshwater Aquarium Studies

Using a controlled diet in aquariums and an uncontrolled diet in field cages along a salinity gradient in the oligohaline zone of the Everglades, we are estimating fractionation differences in tissues by calibrating the rate at which muscle and liver tissues turnover in their $\delta^{13}C$ and $\delta^{15}N$ values. Two fish species, *Poecilia latipinna* (herbivore) and *Gambusia holbrooki* (carnivore) are being used to determine the length of time before the various tissues reflect the values of the bulk diets.

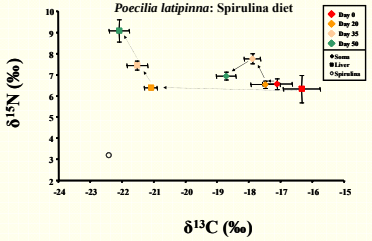


Figure 2. Sailfin molly tissue turnover times obtained from a *Spirulina* diet aquarium study. Mean values and SE bars for soma and liver tissues are displayed. Symbol color changes track the progression of the days of the experiment. The open circle represents the value of the bulk diet.

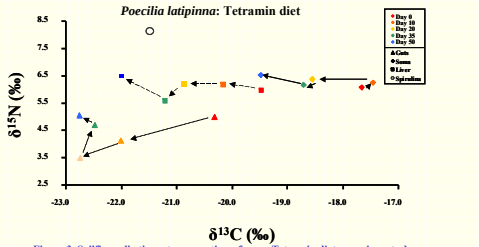


Figure 3. Sailfin molly tissue turnover times from a Tetramin diet aquarium study. Mean values for soma, liver, and hind gut contents are displayed. SE bars are absent to reduce clutter. The symbol color changes track the progression of the days of the experiment. The open circle represents the value of the bulk diet.

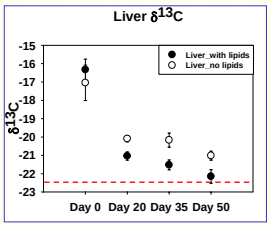


Figure 4. Sailfin molly liver tissues comparing tissue samples with lipids present vs. tissues with lipids extracted. Red line indicates value of the *Spirulina* diet.

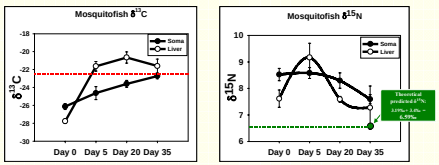


Figure 5. Mosquitofish tissue turnover times from a Spirulina diet aquarium study. A. Mean $\delta^{13}C$ values and SE bars for soma and liver tissues are displayed. The red line is the stable isotopic value of the bulk diet. B. Mean $\delta^{15}N$ values and SE bars for soma and liver tissues are displayed. The theoretical predicted $\delta^{15}N$ represents the accepted average trophic shift of 3.4‰ plus the value of the bulk diet ("You are what you eat plus 3.4‰" – or so they say!)

Conclusions

Ongoing work: Energetic demands are expected to differ among populations at opposite ends of the salinity gradient due to physiological stresses associated with osmoregulation, and this is likely to affect fractionation. The aquarium study is currently being replicated at a salinity of 15-ppt. We are also harvesting fish from field mesocosms at opposite ends of a salinity gradient to compare our aquarium results with results from a more natural diet.

Implications from preliminary results:

- Poorly-understood results arise from spatial and temporal variability in SIA
- Appears that liver tissue provides a better indication of more recent dietary activity than does soma
- Lipid extraction from liver tissues could be an important step for true interpretation of stable isotopic signatures from wild-caught fish

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