INDIVIDUAL AND SEASONAL VARIATION IN THE DIET OF THE BARTON SPRINGS SALAMANDER (*EURYCEA SOSORUM*)

AN APPLICATION OF STABLE ISOTOPE ANALYSIS TO THE CONSERVATION OF AN ENDANGERED SPECIES

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Introduction

Short-term variation in food resources or prey selection may be crucial for understanding population dynamics in poorly understood (e.g. endangered) species. Long-term dietary trends may be important in assessing ecological effects of environmental disturbances such as changes in global climate, land use, or introduction of invasive species. This critical information can be used to predict the impacts of enhanced or depleted resource availability, predator removal and species introductions on endangered species and communities. This study investigates such interactions in a species that is both endangered and an indicator of water quality in rapidly urbanizing central Texas.

This study uses stable isotope analysis of diet to investigate how:

The dietary composition of the Barton Springs Salamander (*Eurycea sosorum*) responds to temporal changes in prey availability in field populations

The degree to which *intra*population diet variation influences overall population diet estimates.

While little is known about its foraging ecology, *E. sosorum* is assumed to be a generalist predator that relies heavily on the amphipod *Hyallela azteca* for food (Bogart 1967, USFWS 1998, USFWS 2006); there is little hard evidence to support this claim. The salamander's secretive behavior and obscure microhabitat make direct foraging observations impossible, and they are too small and fragile for stomach pumping for gut content analysis. Thus, stable isotope analysis may be the only feasible means of estimating diet in these animals.

You are what you eat: how stable isotopes are used to study diet.

Stable isotope analyses of diet use ratios of naturally occurring carbon and nitrogen isotopes to infer dietary trends by comparing consumer isotope values with those of potential food sources. Consumers take up C and N from food use them to build tissue, inheriting and combining the isotope values from multiple food sources.

- **Carbon** isotope ratios (**$^{13}C/^{12}C$**) change little from prey to predator, thus reflecting carbon (diet) sources.
- **Nitrogen** isotope ratios (**$^{15}N/^{14}N$**) enrich (become heavier in ^{15}N) from prey to predator, thus reflecting trophic level.
- Ratios are reported as **differences** (using the symbol delta, **δ**) between observed ratios in tissues and international lab standards in units of 'permil' or per thousand (using the symbol **‰**).
- **Mixing models** use mass balance to solve for a all possible prey item isotope value contributions to consumers.

Figure 1. Adult *Eurycea sosorum* from Eliza Spring. Aerial photograph shows Barton Springs Pool and Eliza Spring, two of the four small springs where *E. sosorum* exists. This is the smallest range of any endangered species. Note heavy physical modification to habitat and small size of Eliza Spring.

Natural History

- The Barton Springs Salamander (*Eurycea sosorum*) was listed as an endangered species in 1998 and is endemic to four small spring outflows near downtown Austin, Texas (USFWS 1998). Populations are threatened by physical habitat modifications and degradation of water quality and quantity in the karstic Edwards Aquifer.
- This study takes place at Eliza spring, which currently hosts the largest salamander population (monthly population counts range between 67-1294 individuals since March 2007).
- Individual salamanders are small (50-70mm total length as adults), and ***neotenic*** (they retain larval characteristics and remain aquatic as adults). Salamanders live underneath benthic rocky substrate. The spring is also inhabited by a variety of freshwater invertebrates (see Figure 4), fish and native plants.
- The City of Austin maintains a captive breeding population of *E. sosorum* as well as long-term datasets on wild population size, invertebrate abundance and water quality.

Methods

- Measurements of $\delta^{13}C$ and $\delta^{15}N$ for adult (<2") *Eurycea sosorum* and invertebrate prey were taken in Fall (November) 2007, Winter (February) 2008, Spring (May) 2008 and Summer (August) 2008 from Eliza spring.
- Prey items were selected on the basis of **size** (<7mm, the min. salamander adult head length) and **ability to be removed from rocky substrate** using a small pipette (simulating suction feeding by salamanders).
- Salamander tissue was obtained from 5mm tail clips (muscle and bone tissue) from animals at Eliza Spring.
- Samples were frozen in the field and dried at 50ºC for seven days. One milligram of homogenized tissue was weighed into tin capsules and analyzed at The University of California at Davis Stable Isotope Facility.
- For Fall 2007, Winter 2008, and Spring 2008, the multi-source mixing model IsoSource (Phillips & Gregg 2003) was applied to mean isotope data from salamanders and prey to obtain a distribution of likely prey item contributions to salamander diet. All increments=1%, tolerance= 0.7‰ (largest SE for $\delta^{13}C \pm 0.2$ ‰ instrument precision range).
- For Summer 2008, the following two-source mixing model was applied to mean nitrogen isotope data (from Fry 2006):

$$Proportion_{planarian} = (\delta^{15}N_{salamander} - \delta^{15}N_{amphipod})/(\delta^{15}N_{planarian} - \delta^{15}N_{amphipod}); \quad Proportion_{amphipod} = 1 - Proportion_{planarian}$$

- Nitrogen enrichment estimates from a previous study (Gillespie, unpublished data) were used to correct salamander isotope values in IsoSource by subtracting 2.31‰ in nitrogen; carbon enrichment was not significant.

Stable Isotope Analysis

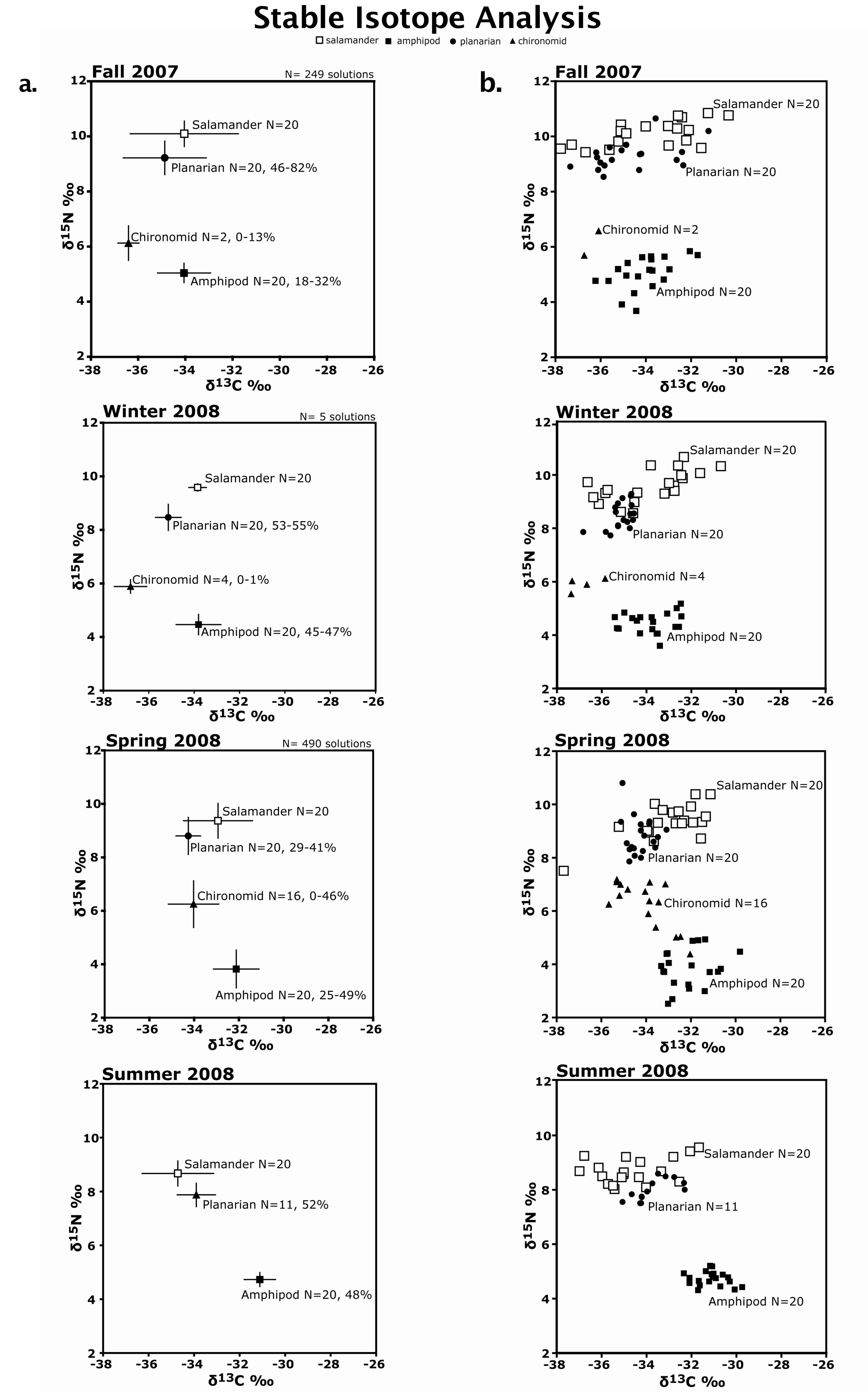

Figure 2. Seasonal Isotope Analysis of Diet for *Eurycea sosorum*.

a. Mean Isotope Values ± 1SD. Range of feasible percent contributions to salamander diet of each prey item as estimated by the mixing model are shown next to sample size of prey.

b. Individual Isotope Values. Plots show individual salamanders, planarian flatworms (symbols represent a composite of 15 individuals), chironomid larvae (symbols represent individual larvae) and amphipods (symbols represent a composite of five individuals) for each season.

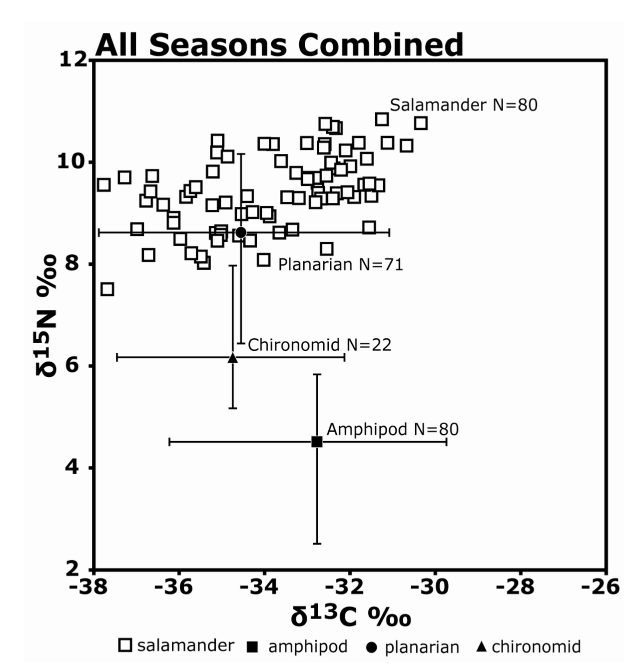

Figure 3. Individual isotope values for all seasons combined (Fall 2007-Summer 2008) illustrate the range of isotopic variation in salamanders and prey over the time period of the entire study. Individual salamanders are shown as well as mean values for planarians, chironomids and amphipods. **Bars represent the *range* of isotopic values for the prey items** over the course of the study.

Results

- There is **large variation between individual salamanders** in carbon isotope values (Fig 2b; range of up to 7.9‰).
- The relative isotopic relationships between amphipods, planarians and chironomids remain similar over time.
- Mixing model results using average isotope values show **planarians and amphipods contribute the most to salamander diet** in all months observed, with planarians contributing slightly more.
- **Chironomids contribute little, if any, to salamander diet** in all months using average isotope values. Distribution of possible solutions for Spring 2008 (0-46%) is highly skewed towards zero.
- Over the course of the study (all seasons combined) **all salamander isotope values fall within the range of prey isotope values** (Figure 3).

Discussion

- **Results suggest that amphipods are not the primary food source as was previously thought. In fact, salamanders may feed on amphipods and planarians nearly equally.**
  - **Amphipods** are abundant in most seasons. These are some of the only free-living (not attached to substrate) prey items in Eliza spring. Amphipods have been found in fecal contents, but remain in-tact because of their hard exoskeleton. Though encounter rates with amphipods may be high, they may provide relatively less nutritional value than other prey because of poor digestibility.
  - **Planarians** (flatworms) are abundant in most seasons, live on the surface of rocks as scavengers and predators (thus high $\delta^{15}N$) and are easily removed from substrate. Planarians have a highly clumped distribution in Eliza spring and do not appear in fecal contents because they are soft-bodied. Being soft-bodied may also make planarians more nutritionally valuable, requiring less handling and digestion to process.
  - **Chironomid larvae** are sometimes, but rarely, found free from substrate-bound cases and are a less likely source in any season because of their relative rarity. This may explain their low ranges in the average mixing model solutions, though the *individual* plots in Figure 2b show that some individual salamanders may feed on chironomids.
- **Diet composition among individual salamanders is *much more variable than previously thought*.** If amphipods were the primary food source (as previously assumed), then all salamanders would have very similar isotope values in all seasons within the carbon range for amphipods, which is *not* the case.
- **Prey tissue may turn over more quickly than salamander tail clips** (which contain bone, a tissue with slow turnover rate). While concurrent prey isotope values may not explain diet in a few individual salamanders, the longer turnover rate for salamander tail clips dictates that prey in previous months may explain diet in these individuals. Experiments are ongoing to determine the exact turnover time of salamander tail tissue.

Figure 4. Barton Springs Salamander & Prey from Eliza Spring (L-R): *E. sosorum*, planarian, amphipod, chironomid, *E. sosorum*

Future Study & Broader Impacts

- Isotope data from Fall 2008, Winter 2009 and Spring 2009 are currently being analyzed to understand salamander diet over a longer time scale.
- Monthly quantitative invertebrate censuses are tracking relative abundance of invertebrates to assess whether salamanders forage in proportion to prey availability.
- Results provide conservation biologists at the City of Austin with essential information on the diet and ecology of *E. sosorum*. Much survey effort is spent estimating presence/absence of all invertebrates in Eliza spring. Results from this study can help to narrow down taxa (such as amphipods and planarians) that should be quantitatively monitored, freeing survey efforts for other purposes.
- This research adds to the growing body of research (1) on amphibians using stable isotopes, a group that is *extremely* underrepresented in the stable isotope literature compared to other vertebrates and (2) that applies stable isotope techniques to conservation of rare, secretive and endangered species, taxa that are often difficult to study with traditional methods of analysis.

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