

More Discoveries with Fewer Deaths:
Substituting Dragonfly Exuviae for Living Insects
in Stable Isotope Studies of Food Webs

By
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**More Discoveries with Fewer Deaths:
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ABSTRACT

The stable carbon and nitrogen isotopic signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of consumers' tissues are used to infer their trophic roles in communities, but stable isotope analysis often requires the undesirable sacrifice of small-bodied animals. My objectives were to determine if the differences between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of whole dragonflies and their nonliving exuviae (final-instar larval exoskeletons) are similar enough to enable their substitution, and to characterize spatial, taxonomic, and temporal variability in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and adult-exuviae isotopic fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$). In New Mexico, I compared two coexisting species of emerging adults and their exuviae, conspecifics emerging from two different sinkholes, conspecifics from the same sinkhole on different dates, and conspecific larvae. In Kansas, I compared four species of adult-exuviae pairs and the exuviae of three additional species present in various combinations at a constructed pond, a reservoir, and a small artificial pond complex. There were spatial and taxonomic differences in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the direction of $\Delta^{13}\text{C}$, but all mean absolute $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ values were less than 3.3‰ and 1.5‰ respectively, regardless of state, waterbody, taxon, or collection date. Furthermore, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of two species of larvae were intermediate between the signatures of conspecific adults and exuviae. This study demonstrates that exuviae can be used instead of entire dragonflies in stable isotope studies of food web structure because the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of exuviae very closely correspond to adult and larval signatures. This correspondence will enable trophic research to proceed in a manner that reduces negative impacts on sensitive dragonfly populations and their fragile aquatic habitats.

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CHAPTER 1: INTRODUCTION

Food web structures of natural communities represent the movement of energy and other resources into, within, and out of the ecosystems in question, and often drive other phenomena of interest to ecologists such as biodiversity patterns, behavioral ecology, population demographics, and community assembly dynamics. From an applied perspective, effective management of fragile habitats or sensitive populations of organisms requires an understanding of the complex trophic interactions of cohabiting species, but how can ecologists obtain meaningful information about these trophic interactions without destroying the very organisms and habitats that they wish to understand through their investigations?

Traditional methods of establishing dietary connections between various components of food webs include field observations of behavior, experimental predation studies staged in laboratories or the field, and examination of the contents of consumers' digestive tracts to determine what they have recently ingested. Unfortunately, these methods may require the sacrifice of dozens if not hundreds of individual organisms to obtain sufficient data for analysis yet may reveal less about generalizable ecological patterns than intended. Unnatural environmental conditions, the presentation of atypical prey items, or unusual predator densities can confound interpretations of behavior in the laboratory (Relyea 2003), and gut content analysis provides only a momentary glimpse into the final and idiosyncratic and recent dietary choices of the individual under the dissecting scope (including potentially indigestible material; Baker 2014) and is thus not necessarily an accurate depiction of the species' typical diet over time. Furthermore, collecting large quantities of these organisms for experiments or

gut content analysis may negatively impact small populations and damage the habitats where they occur.

Since the 1980s, analyzing the stable isotopic compositions of organisms' tissues in conjunction with (or instead of) traditional techniques has led to many astonishing revelations about long- and short-term dietary patterns, community structures, and long-range migratory movements while avoiding the drawbacks of gut content analysis or behavioral studies. Stable isotopes of common elements such as carbon, hydrogen, oxygen, nitrogen, and sulfur can provide information about the integration of nutrients or other compounds into body tissues over the long term because consumers preferentially excrete the more metabolically active, or "light" isotopic form of an element (*e.g.*, ^{12}C , ^{14}N) and preferentially retain the heavier isotopic form (*e.g.*, ^{13}C or ^{15}N) in their tissues during metabolism (Peterson and Fry 1987). The ratio of the heavier to the lighter isotope (*e.g.*, $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) relative to a laboratory standard is referred to as an isotopic "signature" (*e.g.*, " $\delta^{13}\text{C}$ " or " $\delta^{15}\text{N}$ ") and is measured in parts per mil, or ‰. The metabolic process by which a consumer's tissues become "enriched" in the heavier isotopes (and its isotopic signature increases) is referred to as "dietary fractionation." Stable isotope analyses can be used to characterize trophic relationships because the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of consumer tissues increase in a stepwise fashion with each corresponding increase in trophic level, and the typical amounts by which isotopic signatures increase have been extensively studied and quantified for many pairs of organisms.

Only a minor degree of dietary carbon isotopic fractionation occurs during metabolism (DeNiro and Epstein 1978), with estimates of 0.5-0.8‰ made by Vander Zanden and Rasmussen (1999) and 0.4‰ by Post (2002) in aquatic systems. This small difference between the $\delta^{13}\text{C}$

signature of a consumer and its diet enables the basal carbon source(s) in its diet to be inferred if the $\delta^{13}\text{C}$ signatures of the potential sources of carbon such as photosynthetic plants or algae are separated by at least several ‰, as is the case with C_4 and C_3 plants. The differences in photosynthetic pathways used by C_4 and C_3 plants result in distinct and characteristic $\delta^{13}\text{C}$ signatures in their tissues (O'Leary 1981, Farquhar *et al.* 1989). Terrestrial C_4 plants exhibit relatively enriched leaf $\delta^{13}\text{C}$ signatures ranging from -10 to -16‰ and terrestrial C_3 plants with relatively depleted values in the range of -25 to -30‰ (Collister *et al.* 1994). In semiaquatic systems such as saltmarshes, Cloern *et al.* (2002) discovered a similar distinction in $\delta^{13}\text{C}$ signatures between C_4 and C_3 vascular plants of roughly -17 to -12‰ and -30 to -23‰, respectively. In freshwater lentic ecosystems such as lakes, the differences in photosynthetic organisms' locations within the system are often reflected in differences in their $\delta^{13}\text{C}$ signatures, with littoral vegetation often less depleted than pelagic producers by roughly 6.7‰ depending on the size of the lake (Post 2002).

Although consumers fractionate carbon isotopes to only a minimal degree during metabolism, many organisms preferentially excrete the lighter isotope of nitrogen (^{14}N) while retaining the heavier form (^{15}N) in their tissues. This metabolic fractionation of nitrogen can result in a stepwise increase of approximately 3-5‰ in consumer $\delta^{15}\text{N}$ values with each increase in trophic level (Minagawa and Wada 1984, DeNiro and Epstein 1981, Vander Zanden and Rasmussen 2001, Post 2002). Therefore, comparing the relative $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of community members has long been acknowledged as an excellent way to infer trophic relationships between individual species as well as the overall food web structure of their communities (Peterson and Fry 1987).

Original methods of collecting tissues for stable isotope analyses had often required the sacrifice of entire organisms, but more recent studies have demonstrated that nonlethal tissue sampling in the form of small plugs or clips may suffice. Schielke and Post (2010) determined that the isotopic signatures of small muscle tissue plugs taken from fish (sometimes a nonlethal sampling technique) were similar to signatures of whole sacrificed fish larger than 9 cm long, and many authors since then have demonstrated that small fin clips can substitute for fish muscle tissue for a wide variety of fish taxa (*e.g.*, Maitland and Rahel 2021). A recent study by Bélouard *et al.* (2019) on several species of anuran tadpoles showed that tissue clips removed from the tadpoles' tails have similar isotopic signatures to muscle tissue and obviate the need to sacrifice these organisms.

Muscle tissues have long been standard in stable isotope studies, but organisms also possess other types of tissue that might be suitable for analysis. Auerswald *et al.* (2010) cautioned against using keratin, chitin, or other metabolically inert tissues in stable isotope analyses of food webs because these tissues exhibit relatively low turnover rates when compared to muscle and other active tissues with high rates of turnover, but inert tissues have been employed with great success depending on the question. Hobson *et al.* (1999) used the carbon and hydrogen isotopic signatures of keratin to determine the natal origins of migratory monarch butterflies, and DeLong and Thorp (2009), in a study on long-term trends in food webs, found a close correspondence between the stable carbon and nitrogen isotopic signatures of mollusc shell periostracum and the soft tissues from preserved museum specimens.

The relative ease of harvesting inert tissues in a non-invasive manner from vertebrates, in addition to these tissues' long-term chemical stabilities, may represent distinct advantages when investigating ecological questions that are related to the integration of the organism's diet over its entire lifetime, during a particular ontogenetic period, or during a migration event. An increasing number of ecological studies now use stable isotope analyses of inert tissues such as bird feathers (Hobson and Clark 1992; Ruhl *et al.* 2020), brown bear guard hairs (Rogers *et al.* 2020), infant chimpanzee hair (Bădescu *et al.* 2017), terrapin claws (Balzani *et al.* 2016; Suriyamongkol *et al.* 2022), eider duck claws (Steenweg *et al.* 2022) and even the feces of migratory whales (Silva *et al.* 2019) to address questions such as these.

These studies and many others have shown that it is not always necessary to sacrifice vertebrates to obtain dietary information, but similar nonlethal initiatives for invertebrates such as insects are much less common in the literature. The small body sizes of many invertebrates and the technical limitations of analytical instruments often requires that whole animals must be used, and for the smallest taxa, many individuals may need to be pooled into one sample to obtain enough tissue mass to analyze. Although stable isotope analyses can often be conducted on relatively few individuals with little loss of statistical power depending on the question of interest and the degree of individual variability in the population studied (Lancaster and Waldron 2001), the need to collect so many invertebrate specimens can hamper a study's progress, especially if the species in question is especially small, difficult to find, or protected by law. Furthermore, standard methods of collecting aquatic macroinvertebrates – long recognized as valuable sources of information about the structure and interspecific dynamics of their local communities -- often entail dredging, suction, kick-netting, or other

mechanical extraction methods that can severely damage the substrate of fragile waterbodies and/or result in unintended and catastrophic mortality of non-target organisms such as larval fish (Griffith and Andrews 1981).

Despite the negative aspects of specimen collection, larval dragonflies and damselflies (Order Odonata) are often included in aquatic food web studies because they are present in the benthos of many types of waterbodies and are relatively long-lived, generalist predators that attempt to consume any organisms that they can catch and manipulate during the months or years that they spend as aquatic larvae (Corbet 1999). The wide variety of prey taxa consumed by odonate larvae led France and Schlaepfer (2000) to posit that their stable isotope signatures are likely to reflect the overall values of their habitats and are thus especially informative. Furthermore, teneral (newly emerged) odonates function as living bridges between the aquatic habitat in which they lived as larvae and the terrestrial realm that they occupy after ecdysis (May 2019), transporting energy and other resources or contaminants such as heavy metals (Fletcher *et al.* 2022) out of their natal waterbodies when they emerge and fly away. Teneral odonates are preyed upon by a wide variety of terrestrial organisms including birds (da Silva *et al.* 2021), jumping spiders (personal observation), and larger odonates (personal observation), and have been identified as a rich source of highly unsaturated omega-3 fatty acids (HUFAs) for local birds such as eastern phoebes (Twining *et al.* 2019).

A nonlethal means of investigating the trophic positions of odonate larvae that could be employed to great effect involves the use of exuviae, the final-instar larval exoskeletons left behind by adult dragonflies and damselflies when their larvae emerge from their natal water bodies and complete ecdysis. Collecting exuviae is a useful inventory method for surveying

sensitive odonate populations because exuviae often retain distinct larval morphological characteristics that enable species-level identification, their presence is incontrovertible evidence of local breeding success (Raebel *et al.* 2010), they often persist for at least a few days after emergence (Lubertazzi and Ginsberg 2009), and their removal does not harm living members of local populations. Exuviae can sometimes be found in great numbers, even at small ponds; Benke and Benke (1975) collected more than 5,500 exuviae in 1970 and more than 4,100 exuviae in 1971 from just a 40-meter stretch of shoreline. Furthermore, they can easily be removed by hand from emergence substrates such as vegetation and rock outcrops, minimizing negative impact on fragile aquatic habitats and coexisting organisms (Corbet 1999, Foster and Soluk 2004).

Given its many advantages, it is not surprising that collecting odonate exuviae during surveys is a popular technique for investigating ecological phenomena. Korkeamäki and Suhonen (2002) used exuviae and larvae to assess local extinction processes in Finnish streams, recognizing that these immature forms represent the best evidence of the continued presence of breeding populations at their sites. Other authors have monitored the presence of exuviae to evaluate ecosystem effects of pollution (Fletcher *et al.* 2022), restoration activities (D'Amico *et al.* 2004, Maezono and Miyashita 2004), alternate farming methods (Baba *et al.* 2019), and recreational fishing (Müller *et al.* 2003) on aquatic habitats. Moreover, Golfieria *et al.* (2016) developed the Odonate River Index using exuviae, larvae, and adults as a means of measuring ecological integrity in flowing waters. The collection of exuviae has also been employed to great effect in sensitive species inventories where the removal of living material is prohibited by law, as in the case of the U.S. federally endangered dragonfly *Somatochlora hineana* (Foster and

Soluk 2004), or when the species is otherwise of great conservation concern (Zouaimia *et al.* 2022). DNA can successfully be extracted from exuviae for use in genetics studies (Watts *et al.* 2005; Landmann *et al.* 2021), and examination of nonlethal damage recorded in exuvial structures offers insight into the behavioral ecology of damselfly larvae (Stoks 1998). The discovery of exuviae with unusual morphological features well before their adult forms were recognized as distinctive led to the description of two gomphid species previously unknown to science: *Ophiogomphus susbehcha* (Vogt and Smith 1993) and *O. smithi* (Tennesen and Vogt 2004; Smith, *personal communication*).

The chemical composition and physical structure of exuviae might also be particularly advantageous for stable isotope analysis because insect exoskeletons contain large amounts of chitin, a carbon- and nitrogen-rich polysaccharide that is a polymer of N-acetylglucosamine ($[C_8H_{13}NO_5]_n$). In combination with protein, chitin makes up a large percentage of insect exoskeletal structures (Sugumaran 1998) and can represent as much as 40% of the dry mass of insect exuviae depending on the species (Kramer *et al.* 1995). Chitin is a major repository of nitrogen in arthropods (Schimmelman *et al.* 1998) and is the primary structural polysaccharide in the insect cuticle, gut lining, and muscle attachment points (Muthukrishnan *et al.* 2012). The remarkable chemical stability of chitin has been demonstrated in palaeoecological studies, in which chitin was discovered in the cuticles of beetles and crickets in the Pleistocene deposits of the La Brea tar pits (Stankiewicz *et al.* 1997a) and in fossilized beetles preserved in shale dating to 24.7 mya (Stankiewicz *et al.* 1997b).

Although it is chemically stable after an organism's death, chitin has been shown to be extensively reworked during repeated molting and growth cycles (Merzendorfer and Zimoch

2003, Muthukrishnan *et al.* 2011) and therefore might be more appropriate for stable isotope analysis than Auerwald *et al.* (2010) believed. Schimmelmann *et al.* (1998) demonstrated that chitin reflects the changes in $\delta^{15}\text{N}$ signatures that occur with increasing trophic positions in marine crustaceans. More recently, Quinby *et al.* (2020b) used nonlethal clippings from burying beetle elytra to estimate dietary fractionation between the beetles and their food sources, and Marker *et al.* (2022) showed that spider molts have $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures that resemble whole-body signatures. The results of these studies suggest that examining chitin-rich exoskeletal tissues such as odonate exuviae instead of whole insects may be useful in stable isotope studies. Furthermore, unlike fresh tissues which must immediately be frozen or desiccated to prevent decay, the structural and chemical stability of the chitin in discarded odonate exuviae could prevent extensive degradation and the resultant alteration of natural isotopic ratios for at least several days, allowing increased flexibility in sampling schedules and sample storage prior to analysis.

Using exuviae instead of living tissue offers an additional advantage: avoiding potentially confounding isotopic information derived from ingested but unassimilated (and potentially indigestible) prey items stored in the guts of consumers at the time of collection. Some authors have advocated maintaining predatory invertebrates in captivity for several days to allow their alimentary tracts to clear prior to analysis (*e.g.*, Quinn *et al.* 2003), removing predators' guts altogether before analysis (Jardine *et al.* 2005), or using only appendages instead of whole bodies to prevent gut contents from being included in the analysis (Marker *et al.* 2022) – but none of these precautions would be needed if only exoskeletal materials are used. Quinby *et al.* (2020a) noted that insect exoskeletons may reflect the isotopic signatures of dietary intake over

a much longer time scale than do tissues with faster turnover rates, a phenomenon that was observed by Belivanov and Hambäck (2015) in spiders, whose molts display the isotopic signatures of their dietary intake at the time that the shed exoskeleton was being formed. Because last instar odonate larvae have been observed to stop eating for about a week to 11 days (Carvalho 1987, Fincke 1994) prior to emergence and ecdysis, the isotopic signature of their exuviae should reflect the signatures of prey items that were consumed and fully assimilated well before emergence takes place.

Using stable isotope analysis in concert with odonate exuvial surveys – two minimally invasive yet informative techniques – represents a potentially powerful and environmentally sensitive way to investigate trophic interactions of odonates with other organisms in fragile aquatic ecosystems. Hershey *et al.* (1993) assumed (but did not test) that the exuviae and adult tissues of *Baetis* mayflies possess similar isotopic signatures, but this assumption has rarely been tested in other insects and to my knowledge has never been explicitly tested in odonates.

Therefore, the goals of this study were two-fold: 1) to determine if the stable isotopic carbon and nitrogen signatures of dragonfly exuviae and their respective adults or conspecific larvae differ by such a small amount (*i.e.*, less than 1.0‰ for $\delta^{13}\text{C}$ and less than 3.5‰ for $\delta^{15}\text{N}$) that exuviae can be substituted for whole insects in food web studies; and 2) to characterize the variability in carbon and nitrogen isotopic signatures among conspecific individuals and between different taxa, locations, and collection periods. To accomplish these goals, I compared the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of adults and their respective exuviae for several dragonfly species at small sinkholes and ponds in New Mexico and Kansas. For one species in each state, I compared whole larval odonate tissue to exuvial and teneral (newly emerged)

adult tissue. The studies were also designed to assess the degree of spatial variability by comparing adult and exuvial tissues from conspecifics emerging from multiple sites, and to assess the degree of temporal variability by comparing conspecifics emerging from the same sinkhole in New Mexico on three different dates. In Chapter 2, I will present the results of the study that I conducted in 2001 and 2003 at two saline sinkholes located on the Bitter Lake National Wildlife Refuge in southeastern New Mexico. In Chapter 3, I will present the results of the study that I conducted in 2021 at three sites in Lawrence, Kansas.

CHAPTER 2: ANISOPTERA OF NEW MEXICO

METHODS

Study site

Sampling for this study took place in May and June 2001 and July 2003 on the Bitter Lake National Wildlife Refuge in Chaves County, New Mexico, USA. The refuge is located in the Pecos River floodplain and is underlain by limestone karst with alluvial fill and carbonate rock aquifers containing saline groundwater (Robson and Banta 1995). Present on the refuge are dozens of water-filled subsidence sinkholes of varying sizes, geomorphologies, and water chemistries, surrounded by a matrix of desert scrub and grassland. Two sinkholes located approximately 1.6 km apart were selected as study sites: Lake Saint Francis and Sinkhole 32 (Figure 2.1). Lake Saint Francis is the largest and deepest sinkhole on the refuge. It is surrounded by bare soil with scattered stands of *Distichlis* sp., *Tamarix* sp., and *Allenrolfea occidentalis*, and supports populations of Pecos pupfish (*Cyprinodon pecosensis*), plains killifish (*Fundulus zebrinus*), and *Gambusia* sp. in addition to many invertebrates. A notable feature is the presence of a muddy beach several meters wide along its western edge which slopes gently into the water. Sinkhole 32 is a smaller, shallow sinkhole with narrow strips of exposed soil and clumps of emergent *Scirpus* sp., *Distichlis* sp., and *Allenrolfea occidentalis* around its perimeter. Sinkhole 32 also contains Pecos pupfish. Site locations and water quality parameters measured in July 2001 are presented in Table 2.1.

Study design and sample collection

The sampling scheme was designed to determine the degree of intraspecific, interspecific, spatial, and temporal variability in carbon and nitrogen stable isotopic compositions of teneral adult, exuvial, and larval dragonfly tissues. I collected samples from: (a) two species from different families emerging from the same sinkhole; (b) one species emerging from two different sinkholes on the same day; and (c) the same species emerging from the same sinkhole two years later.

The focal species was *Phanogomphus militaris* (Hagen in Selys 1858; family Gomphidae), a fairly abundant, medium-sized species with a distinctive dorsoventrally compressed larval morphology. The larvae of this species burrow just beneath the muddy substrate of several sinkholes on the refuge and emerge upon the bare soil or rocks on the perimeters of their natal sinkholes, usually just above the water line. I selected *P. militaris* for the study because I had observed large groups of its larvae emerging synchronously over several weeks during the previous summer, which would enable me to easily collect at least ten individuals during just a few hours on multiple dates. To assess family-level taxonomic differences in isotopic signatures while controlling for spatial variability, I also collected *Libellula composita* (Hagen 1873; Libellulidae) because its larvae coexist with *P. militaris* larvae in Sinkhole 32 and are of a similar size. Members of this genus tend to sprawl on top of muddy substrates, later leaving those microhabitats to crawl up the vertical stems and leaves of macrophytes at the water's edge prior to beginning ecdysis (Needham *et al.* 2000).

Sampling took place in May and June of 2001 and July of 2003. Ten *P. militaris* adults and their exuviae were collected at Sinkhole 32 on 27 May 2001 and at Lake Saint Francis on 27

May 2001 and 20 June 2001. Ten *L. composita* adults and their exuviae were collected at Sinkhole 32 on 22 June 2001 (Figure 2.2). This species had not yet begun to emerge from Sinkhole 32 in May 2001, so only the June 2001 collection was possible. On 6 July 2003, I collected 13 emerging *P. militaris* adults with their exuviae and 11 last instar *P. militaris* larvae from Sinkhole 32 to check for correspondence among whole larval, exuvial, and adult tissue stable isotope signatures, and determine the degree of temporal variability between the June 2001 and July 2003 collection dates.

Surveys for emerging larvae were conducted by walking along the perimeter of each sinkhole and searching suitable emergence substrate for each species: sloping beaches and bare sediment outcrops for *P. militaris* and emergent vegetation next to the water for *L. composita*. Once located, individuals were monitored as ecdysis proceeded. After each teneral adult had completely emerged from its larval exoskeleton (now referred to as an exuviae) and had begun to pump blood into its wings, the adult and its exuviae were placed in a polyethylene box. The adult's tissues were allowed to harden before the boxes were placed on dry ice. Larvae were placed in individual boxes and immediately placed on dry ice to prevent ecdysis from occurring. At the end of the sampling day, the boxes containing teneral adults, exuviae, and larvae were placed in a freezer. After death, all specimens were individually wrapped in aluminum foil packets and stored in a freezer at -20°C to prevent tissue degradation prior to preparation for stable isotope analysis, following the recommendations of Ponsard and Amlou (1999).

Sample preparation

Larvae and exuviae were removed from their foil packets and gently agitated by hand in 25 ml distilled water for 2 min to remove sediment adhering to their surfaces (following the recommendation from S.E. Bunn, *personal communication*). Acidification of the larvae and exuviae to remove surficial carbonates was not performed, as Bunn *et al.* (1995) discovered that acid washing may change tissue stable isotope signatures enough to confound analytical results, making it impossible to use the data in food web studies.

Because freeze-drying has been shown to have no effect on stable carbon and nitrogen isotope ratios in prepared fish and shrimp tissues (Bosley and Wainright 1999), I used this method to desiccate the specimens. After rinsing in distilled water, larvae and exuviae were placed in new aluminum foil packets that were perforated to allow moisture to escape; adults were not rinsed prior to placement in perforated foil packets. The foil packets were placed in a lyophilizer, and all specimens were freeze-dried to a constant weight. Finally, the desiccated tissue from each specimen was individually pulverized by hand using a mortar and pestle.

After processing, a 1.00 ± 0.01 mg sample from each specimen collected in 2001 was packaged in a tin capsule and sent to the Environmental Isotope Laboratory at the University of Waterloo in Ontario, Canada for analysis. Tissues collected in 2003 were rinsed, freeze-dried, and pulverized as described above. The bulk pulverized material was then sent to the Department of Geosciences Isotope Geochemistry Laboratory at the University of Arizona in Tucson for weighing into tin capsules and subsequent analysis at that facility.

Stable isotope analysis

At the University of Waterloo, the samples collected in 2001 were measured for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, carbon content, and nitrogen content on an Isochrom continuous flow stable isotope mass spectrometer (Micromass) coupled to a Carlo Erba elemental analyzer (CHNS-O EA1108). Results in this facility were corrected to carbon standards IAEA-CH6 (sugar), EIL-72 (cellulose) and EIL-32 (graphite), and nitrogen standards IAEA-N-1 and IAEA-N-2 (both ammonium sulfate). Analytical precision was about $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. Carbon and nitrogen compositions were calculated based on Carlo Erba elemental standards B2005, B2035 and B2036, with an error of $\pm 1\%$. Samples sent to the University of Arizona were measured for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, carbon content, and nitrogen content on a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL) coupled to an elemental analyzer (Costech). Standardization was based on acetanilide for elemental concentration, NBS-22 and USGS-24 for $\delta^{13}\text{C}$, and IAEA-N-1 and IAEA-N-2 for $\delta^{15}\text{N}$. Analytical precision was $\pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 0.2\text{‰}$ for $\delta^{15}\text{N}$, based on repeated internal standards. The results are reported using standard delta notation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} \text{ (in parts per mil, ‰)} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000,$$

where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively.

Statistical analysis

Paired *t*-tests were performed on sets of adults and their exuviae to test for differences between tissue types originating from the same individual. Two-sample *t*-tests or one-way analysis of variance (ANOVA) were used to test for differences among groups as appropriate. After ANOVA, multiple comparison Games-Howell *post hoc* tests were used to identify the pairs

of groups that were significantly different at the $\alpha = 0.05$ level. All statistical analyses were performed in Minitab 21.

RESULTS

Relationships between adult and exuvial tissues

The results of stable isotope analyses and statistical analyses are summarized in Tables 2.3 through 2.8 and depicted in Figures 2.3-5. The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of adults and their respective exuviae were broadly related for all collection groups. Paired *t*-tests revealed that except for $\delta^{13}\text{C}$ values in the 13 *P. militaris* adults and their exuviae from Lake Saint Francis in July 2003, slight but significant differences existed between the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for all groups of emergent adults and their respective exuviae (otherwise known as “fractionation”). For *L. composita*, the mean carbon fractionation between adults and their exuviae ($\Delta^{13}\text{C}$) was 0.94‰, while the mean $\Delta^{13}\text{C}$ in groups of *P. militaris* ranged from -0.46 to -1.40‰ (Table 2.3; Fig. 2.5). The mean $\Delta^{15}\text{N}$ for *L. composita* was 0.74‰ and ranged from 0.59 to 1.41‰ for *P. militaris* (Table 2.4; Fig. 2.5). The relationships between adult and exuvial stable isotopic signatures varied in their strength and resemblance to a 1:1 relationship (Figs. 2.6 and 2.7; Tables 2.5 and 2.6). Simple linear regressions performed on $\delta^{13}\text{C}$ signature data revealed that the only statistically significant relationships were between sets of *L. composita* exuviae and adults collected at Sinkhole 32 in June 2001 ($R^2 = 0.73$, $F(1, 8) = 22.13$, $p = 0.002$) and *P. militaris* exuviae and adults collected at Lake Saint Francis in July 2003 ($R^2 = 0.63$, $F(1, 11) = 18.44$, $p = 0.001$). For $\delta^{15}\text{N}$, the only statistically significant relationships were between sets of *P. militaris* exuviae and adults collected at Lake Saint Francis in June 2001 ($R^2 = 0.64$, $F(1, 8) = 14.53$, $p = 0.005$) and July 2003 ($R^2 = 0.80$, $F(1, 11) = 45.01$, $p < 0.001$).

Variation between larval tissues and adult or exuvial tissues

Phanogomphus militaris adults, their exuviae, and conspecific larvae were collected at Lake Saint Francis on 6 July 2003, and the results of stable isotope analyses and two-sample *t*-tests comparing these tissues are summarized in Table 2.7. The mean larval $\delta^{13}\text{C}$ signature of -15.20‰ did not significantly differ from either the mean adult $\delta^{13}\text{C}$ signature (-15.47‰ ; $t(16) = -0.54$, $p = 0.594$) or mean exuvial $\delta^{13}\text{C}$ signature (-15.00‰ ; $t(21) = 0.30$, $p = 0.764$) for conspecifics collected on the same day. The mean larval $\delta^{15}\text{N}$ signature (8.70‰) was also not significantly different from the mean adult $\delta^{15}\text{N}$ signature (8.74‰ ; $t(18) = 0.31$, $p = 0.759$). The mean larval $\delta^{15}\text{N}$ signature was significantly higher than the mean exuvial $\delta^{15}\text{N}$ signature (7.89‰), but there was only a mean difference of 0.81‰ between the two tissue types ($t(19) = -5.44$, $p < 0.001$).

Spatial variation between conspecifics

Two-sample *t*-tests revealed no significant differences in the mean adult $\delta^{13}\text{C}$ signatures (-16.39 vs. -15.86‰ ; $t(13) = -1.35$, $p = 0.201$), exuvial $\delta^{13}\text{C}$ signatures (-15.12 vs. -14.46‰ ; $t(15) = -1.69$, $p = 0.112$), or $\Delta\delta^{13}\text{C}$ values (-1.28 vs. -1.40‰ ; $t(12) = 0.28$, $p = 0.787$), for *P. militaris* collected at Sinkhole 32 and at Lake Saint Francis on 27 May 2001 (Table 2.8). However, all parameters related to ^{15}N had significantly higher values at Lake Saint Francis than at Sinkhole 32, suggesting that significant spatial variation exists with respect to adult $\delta^{15}\text{N}$ signatures (6.31 vs. 9.63‰ ; $t(16) = -20.47$, $p < 0.001$), exuvial $\delta^{15}\text{N}$ signatures (4.90 vs. 8.91‰ ; $t(17) = -26.10$, $p < 0.001$), and $\Delta\delta^{15}\text{N}$ values (1.41 vs. 0.72‰ ; $t(17) = 4.33$, $p < 0.001$).

Temporal variation among conspecifics

Temporal variation in mean stable isotope signatures and fractionations between adults and their exuviae was minimal among the groups of *P. militaris* collected on three dates at Lake Saint Francis (Table 2.9). 1-way ANOVA revealed no significant differences in any parameters between any of the tissues collected at Lake Saint Francis three weeks apart in May and June 2001. Furthermore, no significant differences existed between the material collected in 2001 and that collected in July 2003 with respect to $\Delta^{15}\text{N}$ or any parameter related to ^{13}C . The only temporal differences were that the mean adult $\delta^{15}\text{N}$ signature in July 2003 ($8.74 \pm 0.29\text{‰}$) was significantly lower than the May and June 2001 signatures ($9.63 \pm 0.40\text{‰}$ and $9.64 \pm 0.31\text{‰}$, respectively; $F(2, 30) = [30.34]$, $p < 0.001$), and the mean exuvial $\delta^{15}\text{N}$ signature in July 2003 ($7.89 \pm 0.33\text{‰}$) was significantly lower than the May and June 2001 signatures ($8.91 \pm 0.34\text{‰}$ and $9.05 \pm 0.31\text{‰}$, respectively; $F(2, 30) = [34.87]$, $p < 0.001$).

Taxonomic variation between the two species at Sinkhole 32

Two-sample *t*-tests revealed significant taxonomic variation between *L. composita* and *P. militaris* at Sinkhole 32, with the exception of their adult $\delta^{13}\text{C}$ signatures (*L. composita*: $-16.07 \pm 0.67\text{‰}$, *P. militaris*: $-16.39 \pm 0.59\text{‰}$; $t(17) = 1.14$, $p = 0.270$) and exuvial $\delta^{15}\text{N}$ signatures (*L. composita*: $4.70 \pm 0.44\text{‰}$, *P. militaris*: $4.90 \pm 0.35\text{‰}$; $t(16) = -1.14$, $p = 0.271$; Table 2.10). The most striking difference was in the way in which the two species fractionated carbon: every *L. composita* adult was enriched in ^{13}C (had a less negative $\delta^{13}\text{C}$ value) by a mean of $0.95 \pm 0.37\text{‰}$ compared to its exuviae, whereas the reverse was true of *P. militaris*, in which adults were more depleted than their exuviae and thus the mean $\Delta^{13}\text{C}$ value was negative ($-1.28 \pm 0.58\text{‰}$).

Furthermore, *P. militaris* in Lake Saint Francis showed a similar pattern of adults being depleted relative to their exuviae (mean $\Delta^{13}\text{C}$ values of -0.46 to -1.40‰, depending on collection date; Fig. 2.3). Other significant taxonomic differences were observed in exuvial $\delta^{13}\text{C}$ signatures (*L. composita*: $-17.02 \pm 0.71\text{‰}$, *P. militaris*: $-15.12 \pm 0.67\text{‰}$; $t(17) = -6.16$, $p < 0.001$), adult $\delta^{15}\text{N}$ signatures (*L. composita*: $5.44 \pm 0.26\text{‰}$, *P. militaris*: $6.31 \pm 0.31\text{‰}$; $t(17) = -6.81$, $p < 0.001$), and adult-exuviae ^{15}N fractionation (*L. composita*: $0.74 \pm 0.50\text{‰}$, *P. militaris*: $1.41 \pm 0.34\text{‰}$; $t(15) = -3.53$, $p = 0.003$).

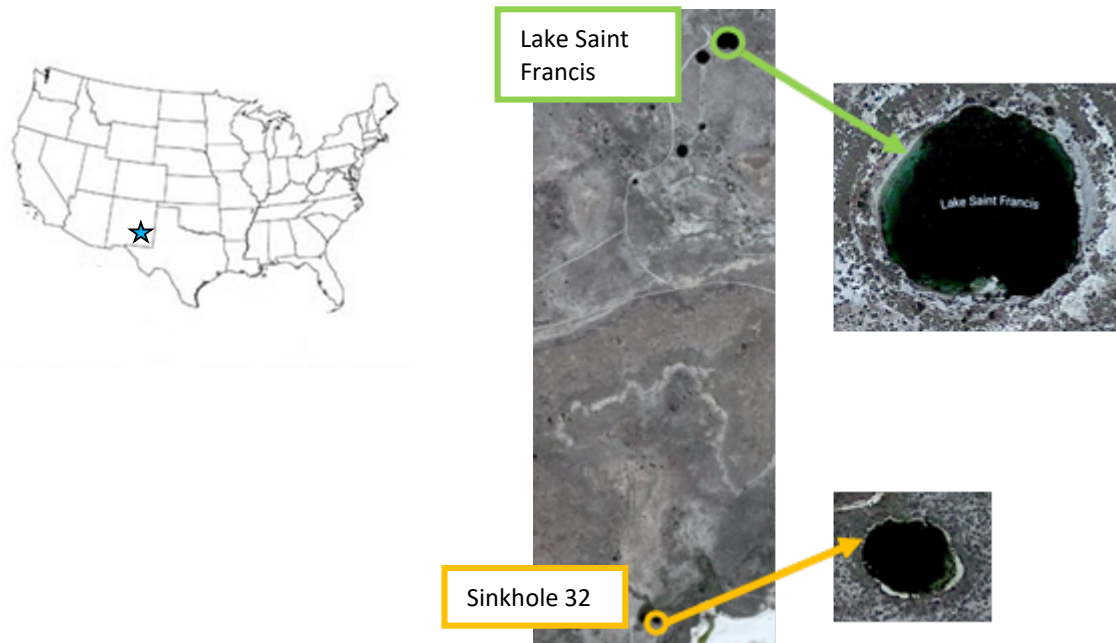


Figure 2.1. General location of the study area (blue star) and aerial views of the Bitter Lake National Wildlife Refuge sinkhole complex in Chaves County, New Mexico, USA. Collection sites were at Lake Saint Francis (green circle) and Sinkhole 32 (orange circle), which are approximately 1.6 km apart. Modified from Google Earth.

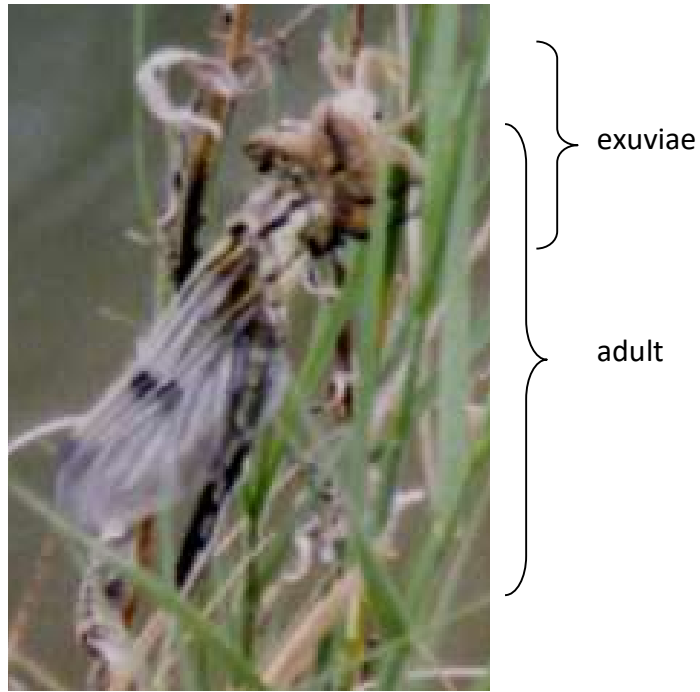


Figure 2.2. Newly emerged *Libellula composita* adult (center) hanging from its exuvia (upper right) at Sinkhole 32, Bitter Lake National Wildlife Refuge.

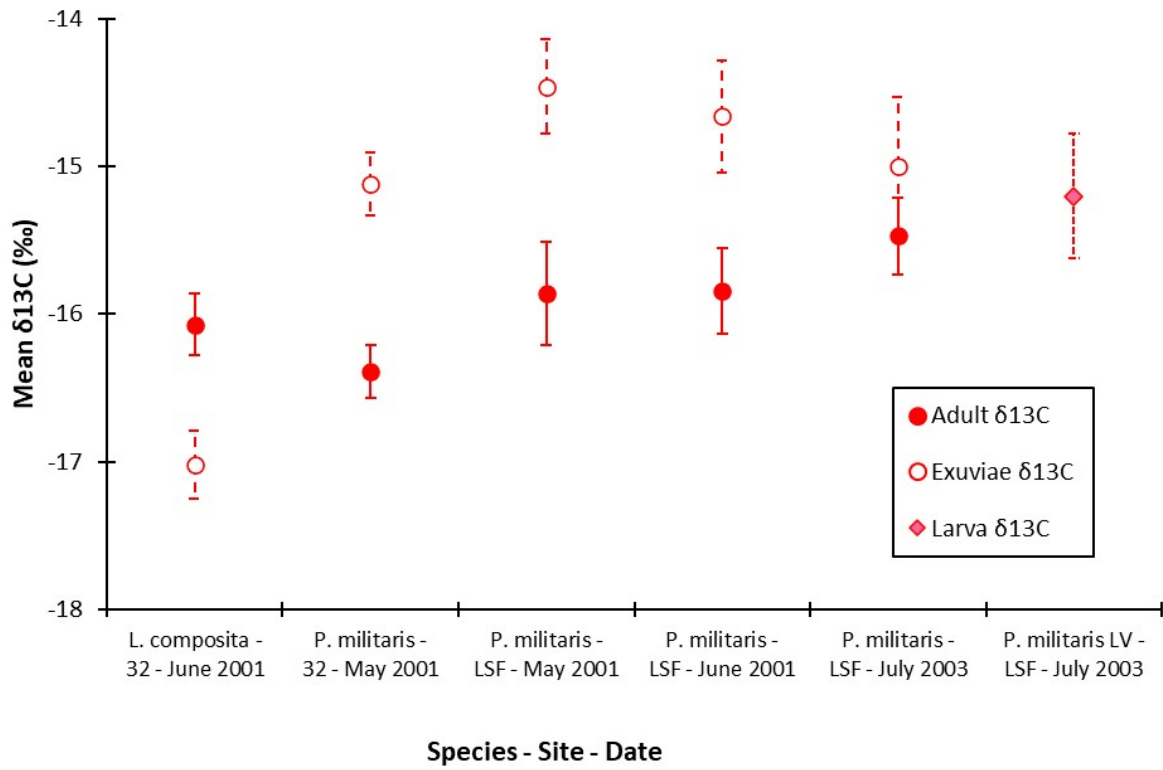


Figure 2.3. Mean (in ‰, \pm SE) $\delta^{13}C$ signatures of adults, exuviae, and larvae (LV). 32 = Sinkhole 32, LSF = Lake Saint Francis.

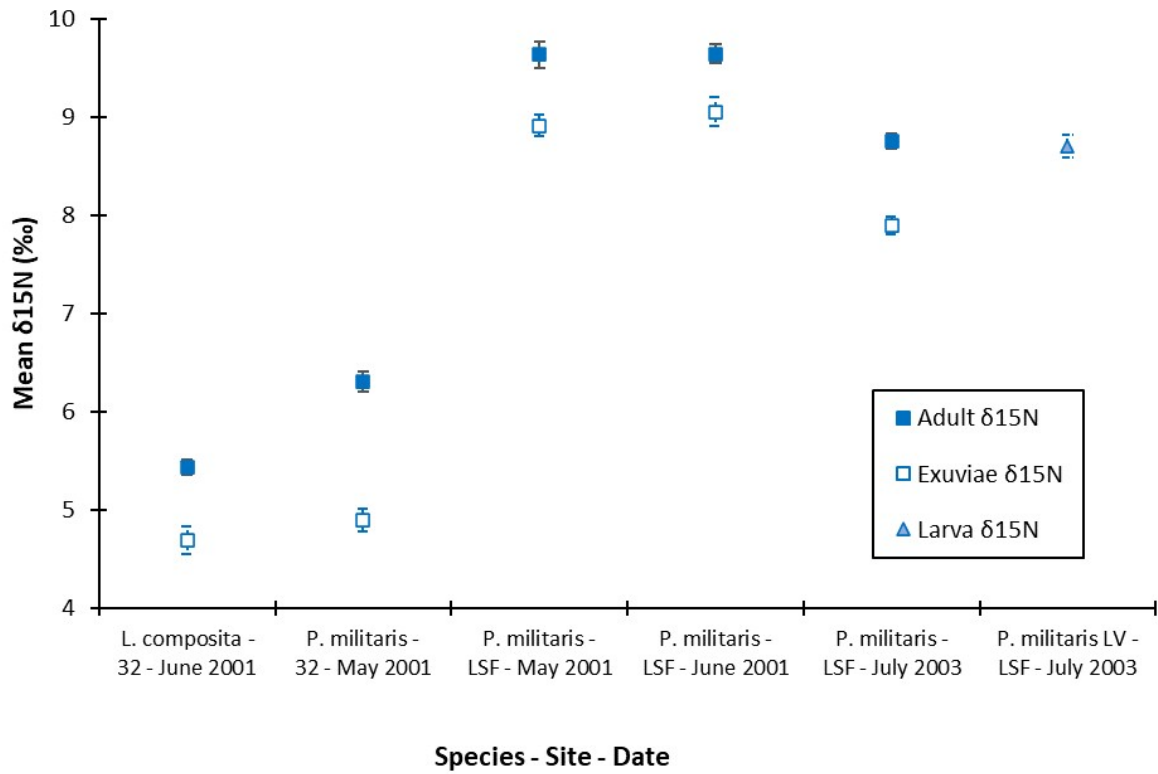


Figure 2.4. Mean (in ‰, \pm SE) $\delta^{15}\text{N}$ signatures of adults, exuviae, and larvae (LV). 32 = Sinkhole 32, LSF = Lake Saint Francis.

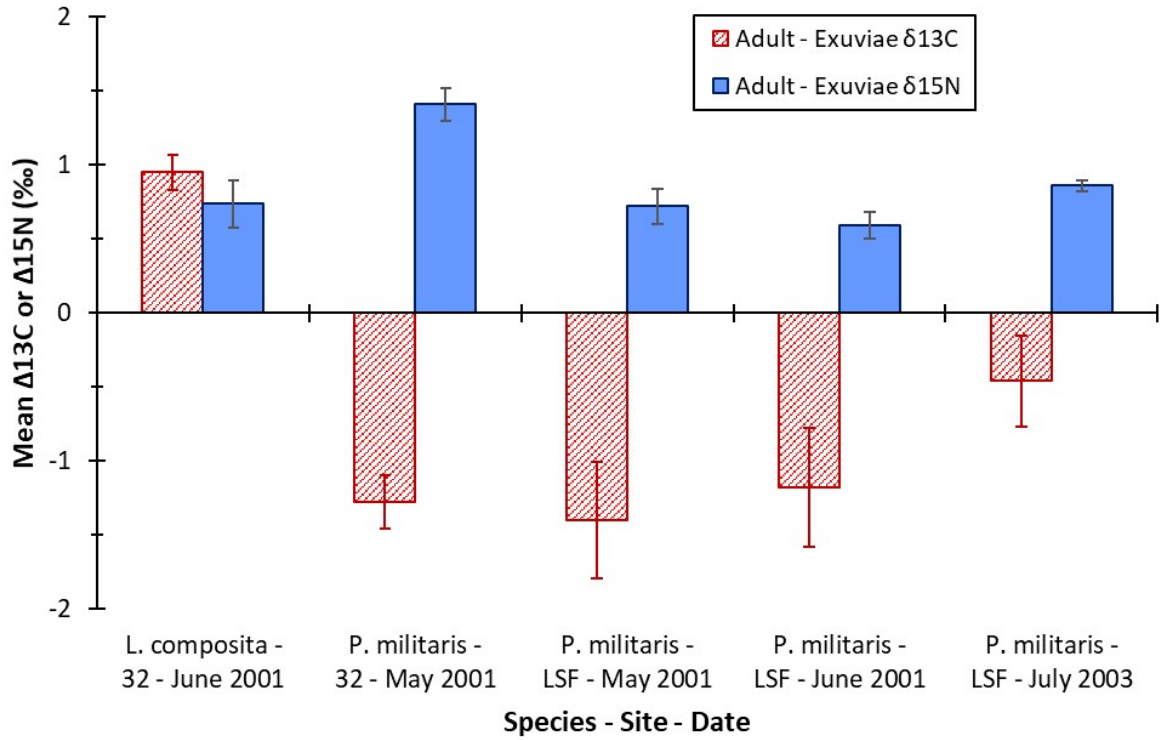


Figure 2.5. Mean (in ‰, \pm SE) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ adult-exuviae fractionation values ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$). 32 = Sinkhole 32, LSF = Lake Saint Francis.

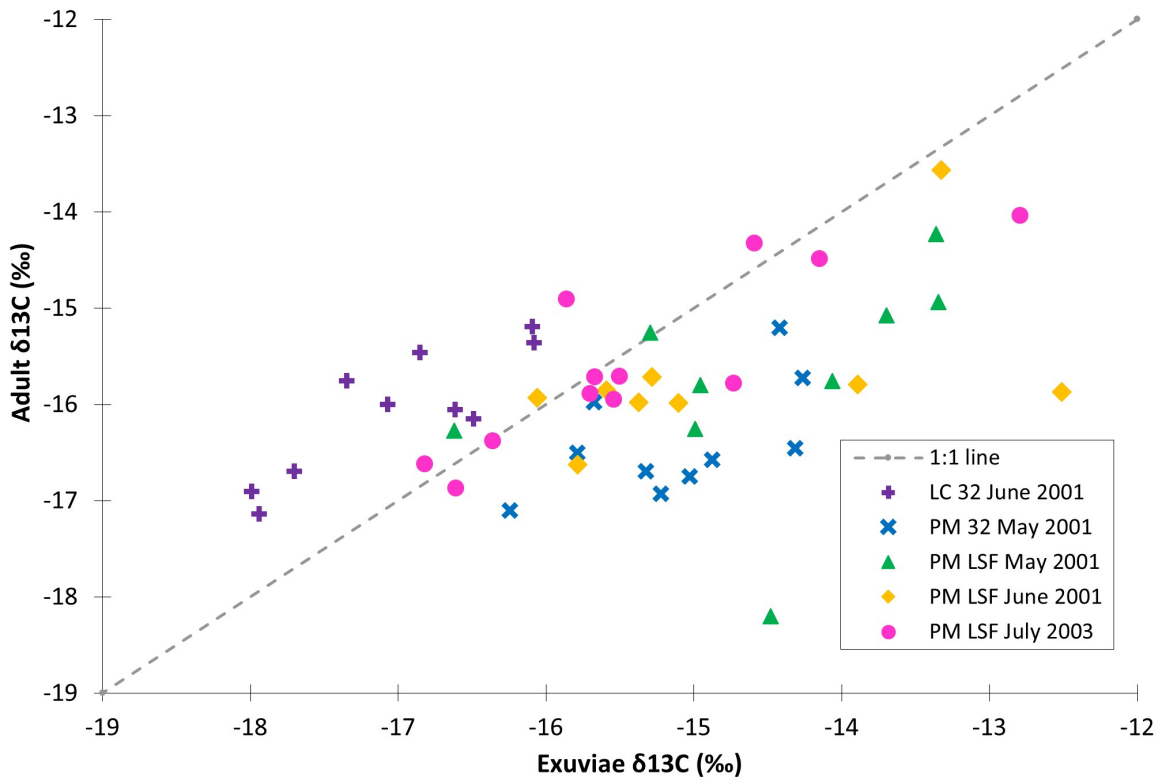


Figure 2.6. Correspondence between the $\delta^{13}\text{C}$ signatures of individual adults and their associated exuviae. Presence on the dashed 1:1 line indicates exact correspondence between adult and exuvial stable isotope signatures. LC = *Libellula composita*, PM = *Phanogomphus militaris*, 32 = Sinkhole 32, LSF = Lake Saint Francis.

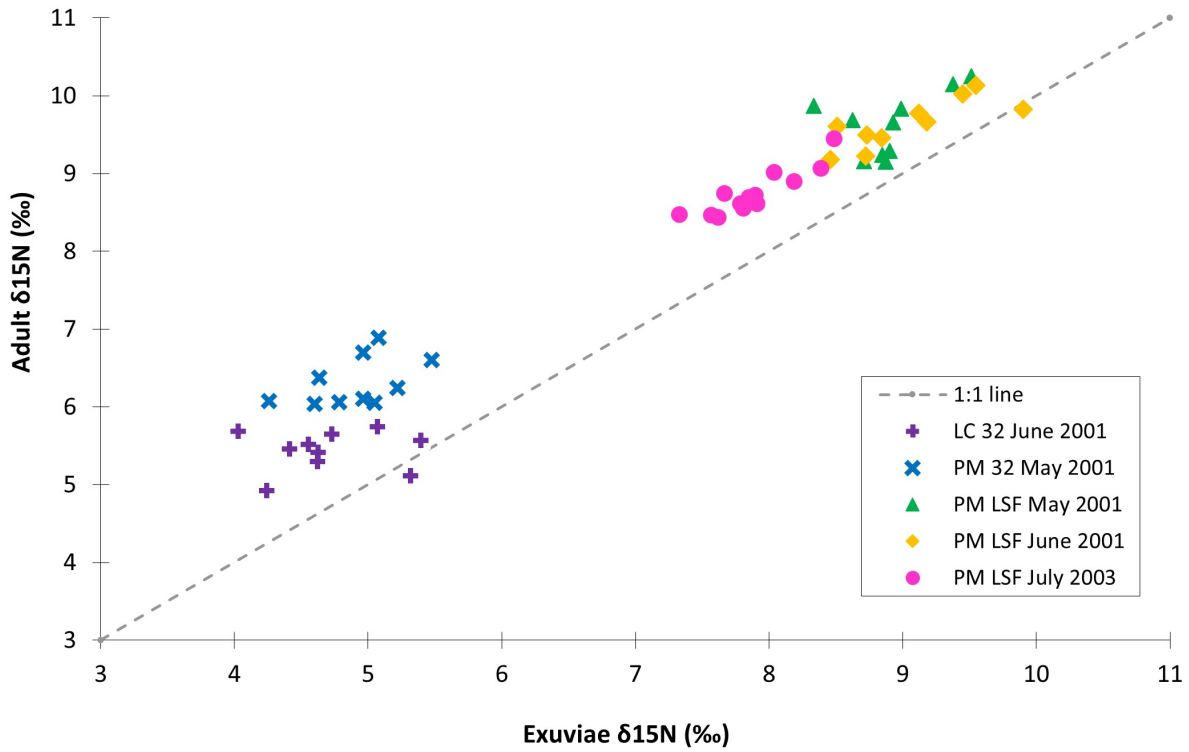


Figure 2.7. Correspondence between the $\delta^{15}\text{N}$ signatures of individual adults and their associated exuviae. Presence on the dashed 1:1 line indicates exact correspondence between adult and exuvial stable isotope signatures. LC = *Libellula composita*, PM = *Phanogomphus militaris*, 32 = Sinkhole 32, LSF = Lake Saint Francis.

Table 2.1. Site locations, approximate surface areas, and water quality parameters measured in July 2001.

Site Name	Lat / Long Coordinates	Elevation (m)	Surface Area (m ²)	pH	Conductivity (μS)	DO (mg/L)	Temp (°C)
Sinkhole 32	33°28'37" N, 104°25'07" W	1,066	380	8.86	18	8.08	30.1
Lake Saint Francis	33°29'30" N, 104°25'00" W	1,072	3,300	8.02	14	7.12	27.5

Table 2.2. Species, collection sites, collection dates, and number of specimens analyzed.

Species	Family	Location	Date	Adults (n)	Exuviae (n)	Larvae (n)
<i>Libellula composita</i>	Libellulidae	Sinkhole 32	06/22/01	10	10	0
<i>Phanogomphus militaris</i>	Gomphidae	Sinkhole 32	05/27/01	10	10	0
<i>Phanogomphus militaris</i>	Gomphidae	Lake Saint Francis	05/27/01	10	10	0
<i>Phanogomphus militaris</i>	Gomphidae	Lake Saint Francis	06/20/01	10	10	0
<i>Phanogomphus militaris</i>	Gomphidae	Lake Saint Francis	07/06/03	13	13	11

Table 2.3. Mean $\delta^{13}\text{C}$ signatures and adult-exuviae fractionation ($\Delta^{13}\text{C}$) values (in ‰, \pm SE) and results of paired *t*-tests on adult vs. exuviae $\delta^{13}\text{C}$ signatures for collection groups. 32 = Sinkhole 32, LSF = Lake Saint Francis. The mean $\delta^{13}\text{C}$ signatures of all adults and their respective exuviae are significantly different except for *Phanogomphus militaris* from Lake Saint Francis in July 2003.

Species	Site	Date	n	Adult $\delta^{13}\text{C}$	Exuviae $\delta^{13}\text{C}$	$\Delta^{13}\text{C}$ (Adult - Exuviae)	Larva $\delta^{13}\text{C}$	<i>p</i> -value, paired <i>t</i> -test: Adult vs. Exuviae
<i>L. composita</i>	32	06/22/01	10	-16.07 \pm 0.21	-17.02 \pm 0.23	0.95 \pm 0.12	-	<i>p</i> < 0.001
<i>P. militaris</i>	32	05/27/01	10	-16.39 \pm 0.19	-15.12 \pm 0.21	-1.27 \pm 0.18	-	<i>p</i> < 0.001
<i>P. militaris</i>	LSF	05/27/01	10	-15.86 \pm 0.35	-14.46 \pm 0.32	-1.40 \pm 0.39	-	0.006
<i>P. militaris</i>	LSF	06/20/01	10	-15.84 \pm 0.29	-14.66 \pm 0.38	-1.18 \pm 0.40	-	0.017
<i>P. militaris</i>	LSF	07/06/03	13	-15.47 \pm 0.26	-15.00 \pm 0.47	-0.46 \pm 0.31	-	0.157
<i>P. militaris</i>	LSF	07/06/03	11	-	-	-	-15.20 \pm 0.42	-

Table 2.4. Mean $\delta^{15}\text{N}$ signatures and adult-exuviae fractionation ($\Delta^{15}\text{N}$) values (in ‰, \pm SE) and results of paired *t*-tests on adult vs. exuviae signatures for collection groups. The mean $\delta^{15}\text{N}$ signatures of all adults and their respective exuviae are significantly different.

Species	Site	Date	n	Adult $\delta^{15}\text{N}$	Exuviae $\delta^{15}\text{N}$	$\Delta^{15}\text{N}$ (Adult - Exuviae)	Larva $\delta^{15}\text{N}$	<i>p</i> -value, paired <i>t</i> -test: Adult vs. Exuviae
<i>L. composita</i>	32	06/22/01	10	5.44 \pm 0.08	4.70 \pm 0.14	0.74 \pm 0.16	-	0.001
<i>P. militaris</i>	32	05/27/01	10	6.31 \pm 0.10	4.90 \pm 0.11	1.41 \pm 0.11	-	<i>p</i> < 0.001
<i>P. militaris</i>	LSF	05/27/01	10	9.63 \pm 0.13	8.91 \pm 0.11	0.72 \pm 0.12	-	<i>p</i> < 0.001
<i>P. militaris</i>	LSF	06/20/01	10	9.64 \pm 0.10	9.05 \pm 0.15	0.59 \pm 0.09	-	<i>p</i> < 0.001
<i>P. militaris</i>	LSF	07/06/03	13	8.74 \pm 0.08	7.89 \pm 0.09	0.86 \pm 0.04	-	<i>p</i> < 0.001
<i>P. militaris</i>	LSF	07/06/03	11	-	-	-	8.70 \pm 0.12	-

Table 2.5. Linear regression models describing the relationship between $\delta^{13}\text{C}$ signatures of exuviae and their associated adults. The only statistically significant relationships are between *Libellula composita* exuviae and adults from Sinkhole 32 in June 2001 and *Phanogomphus militaris* exuviae and adults from Lake Saint Francis in July 2003.

Species	Site	Date	Regression Model	R ²	d.f.	F-value	p-value
<i>L. composita</i>	32	06/22/01	Adult $\delta^{13}\text{C} = -2.46 + 0.80$ (Exuviae $\delta^{13}\text{C}$)	0.73	1, 8	22.13	0.002
<i>P. militaris</i>	32	05/27/01	Adult $\delta^{13}\text{C} = -8.74 + 0.51$ (Exuviae $\delta^{13}\text{C}$)	0.33	1, 8	3.98	0.081
<i>P. militaris</i>	LSF	05/27/01	Adult $\delta^{13}\text{C} = -10.57 + 0.37$ (Exuviae $\delta^{13}\text{C}$)	0.11	1, 8	1.03	0.339
<i>P. militaris</i>	LSF	06/20/01	Adult $\delta^{13}\text{C} = -12.55 + 0.22$ (Exuviae $\delta^{13}\text{C}$)	0.09	1, 8	0.79	0.401
<i>P. militaris</i>	LSF	07/06/03	Adult $\delta^{13}\text{C} = -8.86 + 0.44$ (Exuviae $\delta^{13}\text{C}$)	0.63	1, 11	18.44	0.001

Table 2.6. Linear regression models describing the relationship between $\delta^{15}\text{N}$ signatures of exuviae and their associated adults. The only statistically significant relationships are between sets of *Phanogomphus militaris* exuviae and adults from Lake Saint Francis in June 2001 and July 2003.

Species	Site	Date	Regression Model	R ²	d.f.	F-value	p-value
<i>L. composita</i>	32	06/22/01	Adult $\delta^{15}\text{N} = 5.25 + 0.04$ (Exuviae $\delta^{15}\text{N}$)	0.05	1, 8	0.04	0.852
<i>P. militaris</i>	32	05/27/01	Adult $\delta^{15}\text{N} = 4.19 + 0.43$ (Exuviae $\delta^{15}\text{N}$)	0.23	1, 8	2.39	0.161
<i>P. militaris</i>	LSF	05/27/01	Adult $\delta^{15}\text{N} = 4.35 + 0.59$ (Exuviae $\delta^{15}\text{N}$)	0.25	1, 8	2.63	0.143
<i>P. militaris</i>	LSF	06/20/01	Adult $\delta^{15}\text{N} = 4.88 + 0.53$ (Exuviae $\delta^{15}\text{N}$)	0.64	1, 8	14.53	0.005
<i>P. militaris</i>	LSF	07/06/03	Adult $\delta^{15}\text{N} = 2.46 + 0.80$ (Exuviae $\delta^{15}\text{N}$)	0.80	1, 11	45.01	$p < 0.001$

Table 2.7. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (in ‰, \pm SE) of *Phanogomphus militaris* adult, exuviae, and larvae from Lake Saint Francis in July 2003. The only significant difference is that the mean larval $\delta^{15}\text{N}$ signature is significantly higher than the mean exuvial $\delta^{15}\text{N}$ signature.

Parameter	Adult (n = 13)	Exuviae (n = 13)	Larva (n = 11)	d.f.	t-value	p-value
$\delta^{13}\text{C}$	-15.47 \pm 0.26	-	-15.20 \pm 0.42	16	-0.54	0.594
$\delta^{13}\text{C}$	-	-15.00 \pm 0.47	-15.20 \pm 0.42	21	0.30	0.764
$\delta^{15}\text{N}$	8.74 \pm 0.08	-	8.70 \pm 0.12	18	0.31	0.759
$\delta^{15}\text{N}$	-	7.89 \pm 0.09	8.70 \pm 0.12	19	-5.44	$p < 0.001$

Table 2.8. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuviae fractionation values (in ‰, \pm SE) of *Phanogomphus militaris* collected on 27 May 2001 from Sinkhole 32 and Lake Saint Francis. Only the three ^{15}N parameters are significantly different between the two sites.

Parameter	Sinkhole 32 (n = 10)	Lake Saint Francis (n = 10)	d.f.	t-value	p-value
Adult $\delta^{13}\text{C}$	-16.39 \pm 0.18	-15.86 \pm 0.35	13	-1.35	0.201
Exuviae $\delta^{13}\text{C}$	-15.12 \pm 0.21	-14.46 \pm 0.32	15	-1.69	0.112
$\Delta^{13}\text{C}$ (Adult - Exuviae)	-1.28 \pm 0.18	-1.40 \pm 0.39	12	0.28	0.787
Adult $\delta^{15}\text{N}$	6.31 \pm 0.10	9.63 \pm 0.13	16	-20.47	$p < 0.001$
Exuviae $\delta^{15}\text{N}$	4.90 \pm 0.11	8.91 \pm 0.11	17	-26.10	$p < 0.001$
$\Delta^{15}\text{N}$ (Adult - Exuviae)	1.41 \pm 0.11	0.72 \pm 0.12	17	4.33	$p < 0.001$

Table 2.9. Results of 1-way ANOVAs comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuvia fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) values (in ‰, \pm SE) of *Phanogomphus militaris* from Lake Saint Francis in May 2001, June 2001, and July 2003. Values in the same row that do not share a letter are significantly different according to a Games-Howell *post hoc* test ($\alpha = 0.05$). The only parameter values that are significantly different are the adult $\delta^{15}\text{N}$ and exuvia $\delta^{15}\text{N}$ of tissues collected in July 2003, which are both significantly lower than the signatures of corresponding tissues collected in May and June 2001.

Parameter	May 2001 (n = 10)	June 2001 (n = 10)	July 2003 (n = 13)	d.f.	F-value	p-value
Adult $\delta^{13}\text{C}$	-15.86 \pm 0.35 a	-15.84 \pm 0.29 a	-15.47 \pm 0.26 a	2	0.60	0.560
Exuvia $\delta^{13}\text{C}$	-14.46 \pm 0.32 a	-14.66 \pm 0.38 a	-15.00 \pm 0.47 a	2	0.44	0.651
$\Delta^{13}\text{C}$ (Adult - Exuvia)	-1.40 \pm 0.39 a	-1.18 \pm 0.40 a	-0.46 \pm 0.31 a	2	1.99	0.165
Adult $\delta^{15}\text{N}$	9.63 \pm 0.13 a	9.64 \pm 0.10 a	8.74 \pm 0.08 b	2	30.34	$p < 0.001$
Exuvia $\delta^{15}\text{N}$	8.91 \pm 0.11 a	9.05 \pm 0.15 a	7.89 \pm 0.09 b	2	34.87	$p < 0.001$
$\Delta^{15}\text{N}$ (Adult - Exuvia)	0.72 \pm 0.12 a	0.59 \pm 0.09 a	0.86 \pm 0.04 a	2	3.56	0.054

Table 2.10. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuviae fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) values (in ‰, \pm SE) of *Libellula composita* and *Phanogomphus militaris* from Sinkhole 32 in 2001. All parameter values are significantly different except for adult $\delta^{13}\text{C}$ and exuviae $\delta^{15}\text{N}$.

Parameter	<i>L. composita</i> (n = 10)	<i>P. militaris</i> (n = 10)	d.f.	<i>t</i> -value	<i>p</i> -value
Adult $\delta^{13}\text{C}$	-16.07 \pm 0.21	-16.39 \pm 0.18	17	1.14	0.270
Exuviae $\delta^{13}\text{C}$	-17.02 \pm 0.23	-15.12 \pm 0.21	17	-6.16	<i>p</i> < 0.001
$\Delta^{13}\text{C}$ (Adult - Exuviae)	0.95 \pm 0.12	-1.28 \pm 0.18	15	10.18	<i>p</i> < 0.001
Adult $\delta^{15}\text{N}$	5.44 \pm 0.08	6.31 \pm 0.10	17	-6.81	<i>p</i> < 0.001
Exuviae $\delta^{15}\text{N}$	4.70 \pm 0.14	4.90 \pm 0.11	16	-1.14	0.271
$\Delta^{15}\text{N}$ (Adult - Exuviae)	0.74 \pm 0.16	1.41 \pm 0.11	15	-3.53	0.003

CHAPTER 3: ANISOPTERA OF KANSAS

INTRODUCTION

In Chapter 2, I described the study that I undertook at two sinkholes in southeastern New Mexico to characterize the relationships between the stable carbon and nitrogen isotopic signatures of emergent adults, their exuviae, and conspecific larvae. I observed some intriguing spatial and taxonomic patterns, and I established that little to no temporal variation existed in mean isotopic signatures over gaps of three weeks or two years between collecting dates. However, the scope of my New Mexico study was limited both spatially (only two sites separated by roughly 1.5 km) and taxonomically (only two species, each representing one family). Furthermore, only one of the New Mexico sites supported both species, which limited my ability to draw broad conclusions about within-site taxonomic variation. I designed the Kansas study to investigate a broader range of spatial and taxonomic variability in three important ways by collecting material from: (1) three sites (one located more than 11.5 km from the other two sites); (2) two to four species at each site; and (3) a total of seven species representing three families.

METHODS

Study site

Sampling occurred at three sites in Douglas County, Kansas, USA: (1-2) a constructed reservoir and several small artificial ponds at the University of Kansas Field Station; and (3) a small pond about 11.3 km to the southwest on property owned by The Land Institute (Fig. 3.1). These three sites occur in an ecotone between tallgrass prairie and eastern deciduous forest (NEON, no date), while the Land Institute property also includes experimental agricultural fields planted with perennial grain crops (The Land Institute, no date). This region is in the Lower Kansas River watershed (USGS, no date), and the underlying geology is of the Admire Group Formation, which is composed primarily of shale with some thin layers of limestone (Kansas Geological Survey, no date and 2009). Water quality parameters were measured on October 21, 2021 using an Oakton PC 450 handheld meter, and pond surface areas were calculated using Google Earth maps (Table 3.1).

Study design and sample collection

The sampling scheme was designed to determine the degree of spatial and taxonomic variability in the ^{13}C and ^{15}N isotopic compositions of bulk tissues derived from newly emerged adult dragonflies and their associated exuviae.

To assess spatial differences in isotopic signatures while controlling for taxonomic variability, I compared values for *Libellula luctuosa* (Burmeister, 1839; family Libellulidae) emerging from Ponds 5E-8E with their conspecifics from Reservoir 2. I also compared values for *Plathemis lydia* (Drury, 1773; Libellulidae; Fig. 3.2) from Ponds 5E-8E with their conspecifics

from Biculture Pond. To assess species-level taxonomic differences within the genus *Libellula* while controlling for spatial variability, I compared the congeners *L. luctuosa* and *L. pulchella* (Drury, 1773) emerging from Ponds 5E-8E. I assessed genus-level taxonomic differences in isotopic signatures while controlling for spatial variability and family by comparing two libellulid species (*L. luctuosa* and *Tramea lacerata* (Hagen, 1861)) from Reservoir 2.

Although these four species belong to the Libellulidae, other families of odonates also breed at these sites. Adults and exuviae of *Anax longipes* (Hagen, 1861; Aeshnidae), *Arigomphus submedianus* (Williamson, 1914; Gomphidae), and *Phanogomphus militaris* (Hagen in Selys, 1858; Gomphidae) were observed at one or more of the sites, but I did not find adults of these species in the process of emerging from their exuviae so I could not conduct paired adult vs. exuvial tissue analyses. However, in the interest of adding family-level taxonomic breadth to this study, I analyzed these species' exuviae when present at Biculture Pond or Reservoir 2.

Sampling took place at the KU Field Station sites between May 28 and July 14 and at the Land Institute's Biculture Pond on June 8 and 10, 2021. Collecting efforts at the KU Field Station were extended for several weeks because very few individuals of any one species were found emerging on any given day, requiring several visits to collect a sufficiently large sample size for analysis. However, because no significant differences were observed in the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for conspecifics collected 3 weeks apart at Lake Saint Francis in New Mexico (see Chapter 2), I was confident that collecting samples over a similar timespan in Kansas would not introduce an unduly large degree of variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Surveys for emerging adults and specimen collections were conducted as described in Chapter 2.

A total of 152 specimens were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures as noted in Table 3.2.

Sample preparation

Larvae and exuviae were rinsed in distilled water and placed in perforated aluminum foil packets as described in Chapter 2. The foil packets were placed in a drying oven for at least 48 hours, then stored in desiccating cabinets for at least 24 hours. Finally, each specimen's desiccated tissue was individually pulverized in a plastic vial using a Wig-L-Bug[®] amalgamator.

After processing, the bulk pulverized material was delivered to the KECK-NSF Paleoenvironmental and Environmental Laboratory at the University of Kansas for further processing and isotope analyses.

Stable isotope analysis

The samples were weighed and packaged in tin capsules as described in Chapter 2 and were measured for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, carbon content, and nitrogen content on a Thermo Finnigan MAT 253 Stable Isotope Mass Spectrometer. Samples were analyzed with primary and secondary (laboratory) stable isotope standards that included USGS-25, USGS-26 ANU, and IAEA-600. Analytical precision was less than $\pm 0.2\text{‰}$ for ^{13}C and less than $\pm 0.3\text{‰}$ for ^{15}N . The results are reported using standard delta notation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} \text{ (in parts per mil, ‰)} = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000,$$

where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively.

Statistical analysis

Paired *t*-tests were performed on sets of adults and their exuviae to test for differences between tissue types originating from the same individual. Two-sample *t*-tests or one-way analysis of variance (ANOVA) tests were used to detect any differences between or among groups as appropriate. Because sample sizes and variances were unequal, multiple comparison Games-Howell *post hoc* tests were performed after ANOVAs to identify the pairs of groups that were significantly different at the $\alpha = 0.05$ level. All statistical analyses were performed in Minitab 21.

RESULTS

The results of the stable carbon and nitrogen isotopic analyses have been plotted in Figs. 3.3-7. The stable isotopic data and results of the paired *t*-tests for differences between adults and their exuviae are summarized in Tables 3.3-4, and the results of statistical analyses comparing sets of groups are summarized in Tables 3.5-12.

Relationships between adult and exuvial tissues

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of adults and their respective exuviae were broadly related for all collection groups at the Kansas sites but paired two-sample *t*-tests revealed that for almost all groups regardless of taxon or site, slight but significant mean differences of between 0.36 – 3.26‰ for ^{13}C and 0.62 – 1.15‰ for ^{15}N existed between adults and their exuviae (Tables 3.3-4; Figs. 3.3-5). The one exception was seen in the sample of four *L. pulchella* individuals, in which the mean adult $\delta^{13}\text{C}$ signature of -25.15‰ was not significantly different from the mean exuvial value of -25.96‰; ($t(3) = 3.06$, $p = 0.055$). Adults in almost all groups had a less negative mean $\delta^{13}\text{C}$ signature than their exuviae, resulting in positive adult-exuvial fractionation values ($\Delta^{13}\text{C}$). The only exception was the sample of eighteen *P. lydia* individuals collected at Biculture Pond, in which the mean $\Delta^{13}\text{C}$ value was negative (-1.28‰). The adults were more enriched in ^{15}N than their exuviae for all groups, resulting in positive nitrogen fractionation values ($\Delta^{15}\text{N}$) regardless of site or species (Fig. 3.5). The relationships between adult and exuvial stable isotope signatures varied in their strength and resemblance to a 1:1 relationship (Figs. 3.6 and 3.7; Tables 3.5 and 3.6). Simple linear regressions performed on $\delta^{13}\text{C}$ signature data revealed that the only statistically significant relationship was between sets of *P. lydia* exuviae and adults

collected at Ponds 5E-8E ($R^2 = 0.51$, $F(1, 13) = 13.31$, $p = 0.003$). Simple linear regressions performed on $\delta^{15}\text{N}$ signature data revealed that the only statistically significant relationships were between sets of exuviae and adults of *T. lacerata* collected at Reservoir 2 ($R^2 = 0.81$, $F(1, 8) = 33.81$, $p < 0.001$), *L. luctuosa* at Ponds 5E-8E ($R^2 = 0.87$, $F(1, 8) = 52.19$, $p < 0.001$), and *P. lydia* at Ponds 5E-8E ($R^2 = 0.54$, $F(1, 13) = 15.24$, $p = 0.002$).

Relationships between adult, exuvial, and larval tissues

Two-sample *t*-tests were performed on *T. lacerata* data to determine whether the isotopic signatures of adults and larvae or exuviae and larvae were significantly different (Table 3.7). No significant differences existed between the $\delta^{13}\text{C}$ signatures of adults and larvae or exuviae and larvae, nor was there a significant difference in the $\delta^{15}\text{N}$ signatures of exuviae and larvae. The only statistically significant difference appeared between mean adult (2.68‰) and larval (1.98‰) $\delta^{15}\text{N}$ signatures ($t(9) = 3.36$, $p = 0.008$).

Spatial variation between conspecifics emerging from two different sites

Two-sample *t*-tests performed on data derived from *L. luctuosa* collected at Ponds 5E-8E and Reservoir 2 revealed that for almost all parameters, slight but significant differences existed between the mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and adult-exuvial $\Delta^{13}\text{C}$ (fractionation) values (Table 3.8). The only parameter for which no statistically significant difference was detected between the two sites was in the $\Delta^{15}\text{N}$ values ($t(6) = 1.58$, $p = 0.165$). Similarly, $\Delta^{15}\text{N}$ was the only parameter that was not significantly different for *P. lydia* emerging from Ponds 5E-8E and from Biculture Pond ($t(29) = 0.53$, $p = 0.598$; Table 3.9).

Variation between coexisting congeners at Ponds 5E-8E

Two-sample *t*-tests performed on data from adults and their exuviae from *L. luctuosa* and *L. pulchella* collected at Ponds 5E-8E revealed no significant taxonomic differences between the species for any of the measured parameters (Table 3.10).

Variation among coexisting libellulids at Ponds 5E-8E

One-way ANOVA and Games-Howell *post hoc* tests on data from the three libellulids (*L. luctuosa*, *L. pulchella*, and *P. lydia*) that coexist at Ponds 5E-8E revealed that for almost all parameters, slight but significant taxonomic differences exist between the mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and adult-exuvial fractionation values; differences between taxa are indicated by unique lower-case letters following mean values in Table 3.11. The only parameters for which no statistically significant differences were detected between the three taxa were in adult $\delta^{15}\text{N}$ values ($F(2, 26) = 2.37$, $p = 0.144$) and adult-exuvial $\delta^{15}\text{N}$ fractionation ($\Delta^{15}\text{N}$) values ($F(2, 26) = 0.94$, $p = 0.432$); all other parameters were different between at least two of the three species.

Variation between coexisting libellulids at Reservoir 2

Two-sample *t*-tests performed on adult and exuvial data from two coexisting libellulids at Reservoir 2 revealed that the mean adult $\delta^{13}\text{C}$ signature for *L. luctuosa* (-19.79‰) was significantly higher than that of *T. lacerata* (-22.38‰; $t(10) = 3.28$, $p = 0.008$). The mean adult-exuvial $\delta^{13}\text{C}$ fractionation ($\Delta^{13}\text{C}$) value for *L. luctuosa* was also significantly higher than for *T. lacerata* (1.86‰; $t(12) = 2.34$, $p = 0.037$). All other parameters were statistically similar (Table 3.12).

Variation between exuviae of coexisting libellulids and gomphids at Biculture Pond

Two-sample *t*-tests performed on exuvial data from *P. lydia* (a libellulid) and *A. submedianus* (a gomphid) at Biculture Pond revealed that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were both significantly different for these two species (Table 3.13). The mean $\delta^{13}\text{C}$ signature for *P. lydia* exuviae (-22.04‰) was higher than that of *A. submedianus* (-24.00‰; $t(13) = 10.53$, $p < 0.001$), but its $\delta^{15}\text{N}$ signature was lower (3.61‰ vs. 4.62‰; $t(17) = 7.59$, $p < 0.001$).

Variation among the exuviae of four coexisting taxa from three families at Reservoir 2

Significant taxonomic differences for exuvial $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were revealed by 1-way ANOVA and Games-Howell *post hoc* tests performed on data from the two libellulids (*T. lacerata* and *L. luctuosa*), one aeshnid (*A. longipes*), and one gomphid (*P. militaris*) that coexist in Reservoir 2 (Table 3.14). The most pronounced difference in mean exuvial $\delta^{13}\text{C}$ signatures was between *T. lacerata* (-24.24‰) and *P. militaris* (-19.70‰), with the means for *L. luctuosa* (-23.06‰) and *A. longipes* (-22.82‰) intermediate between the two. The mean $\delta^{15}\text{N}$ signature for the exuviae of *A. longipes*, a relatively large aeshnid, was significantly higher than the same parameter for the libellulids and gomphids with which it coexists ($F(3, 26) = [28.14]$, $p < 0.001$), which suggests that the large *Anax* larvae might occupy a higher trophic level than at least one of these species (possibly *T. lacerata*, 1.98‰). Analysis of emergent adult or larval tissue in addition to exuviae would have provided additional evidence to either support or refute that suggestion.

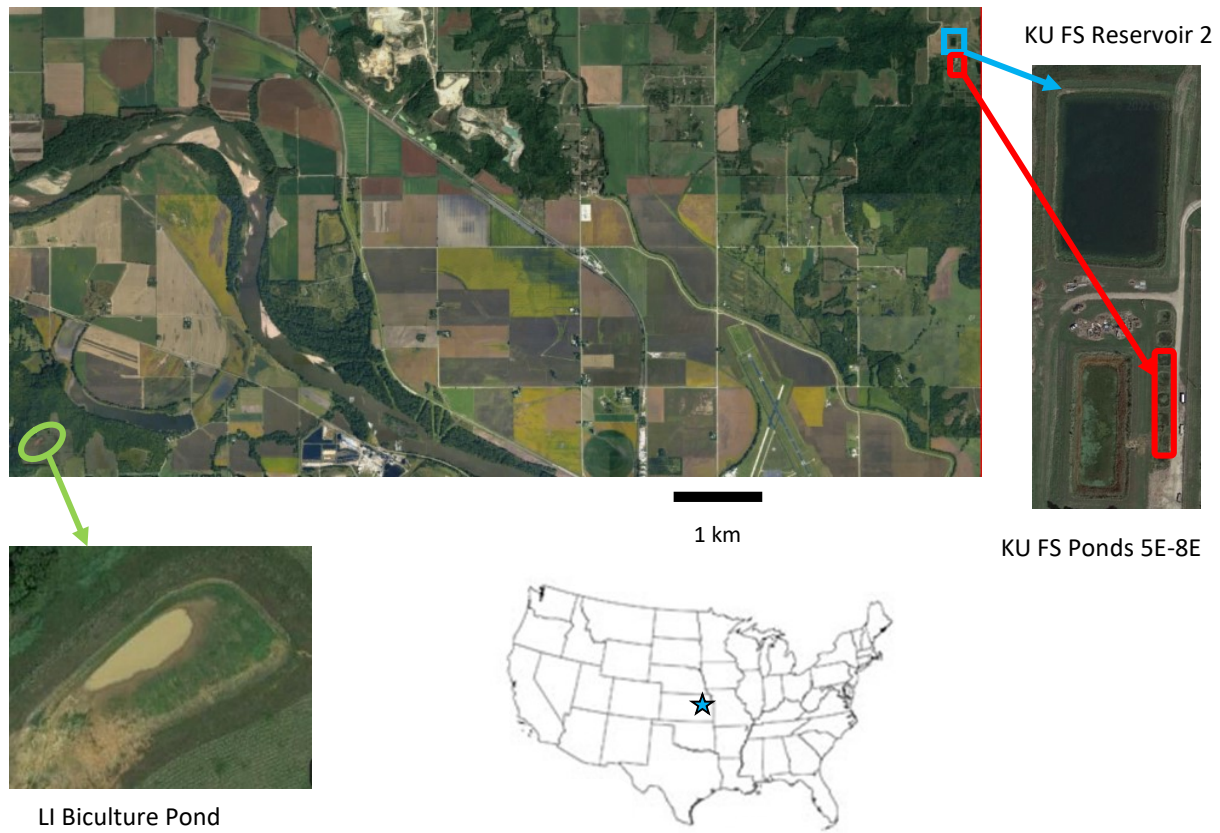


Figure 3.1. General location of the study area (blue star) and aerial views of collection sites in Lawrence, Douglas County, KS. The Land Institute Biculture Pond (LI, green oval) is approximately 11.6 km to the southwest of the University of Kansas Field Station (KU FS) Reservoir 2 (blue square) and Ponds 5E-8E (red oblong). Modified from Google Earth.

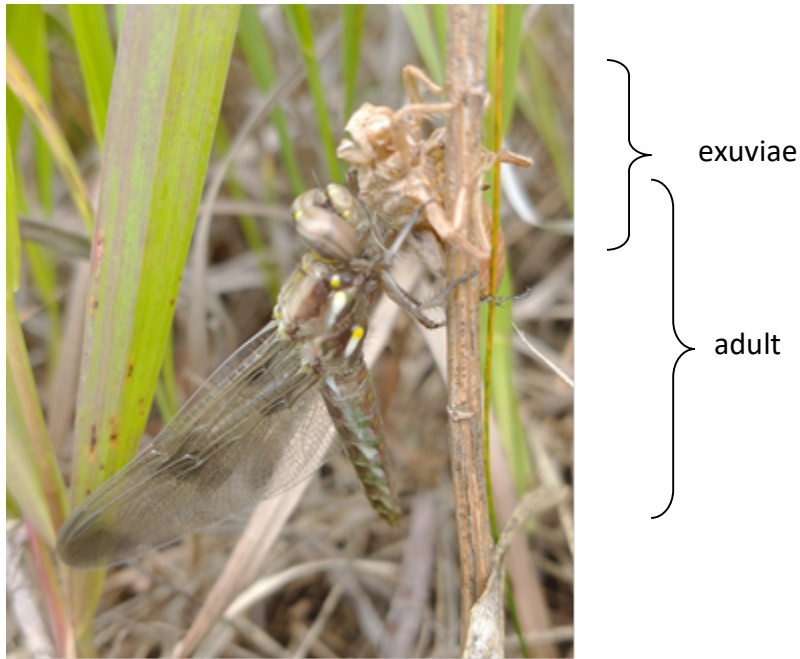


Figure 3.2. Newly emerged female *Plathemis lydia* adult (center) hanging from its exuvia (upper right) at Pond 6E, University of Kansas Field Station.

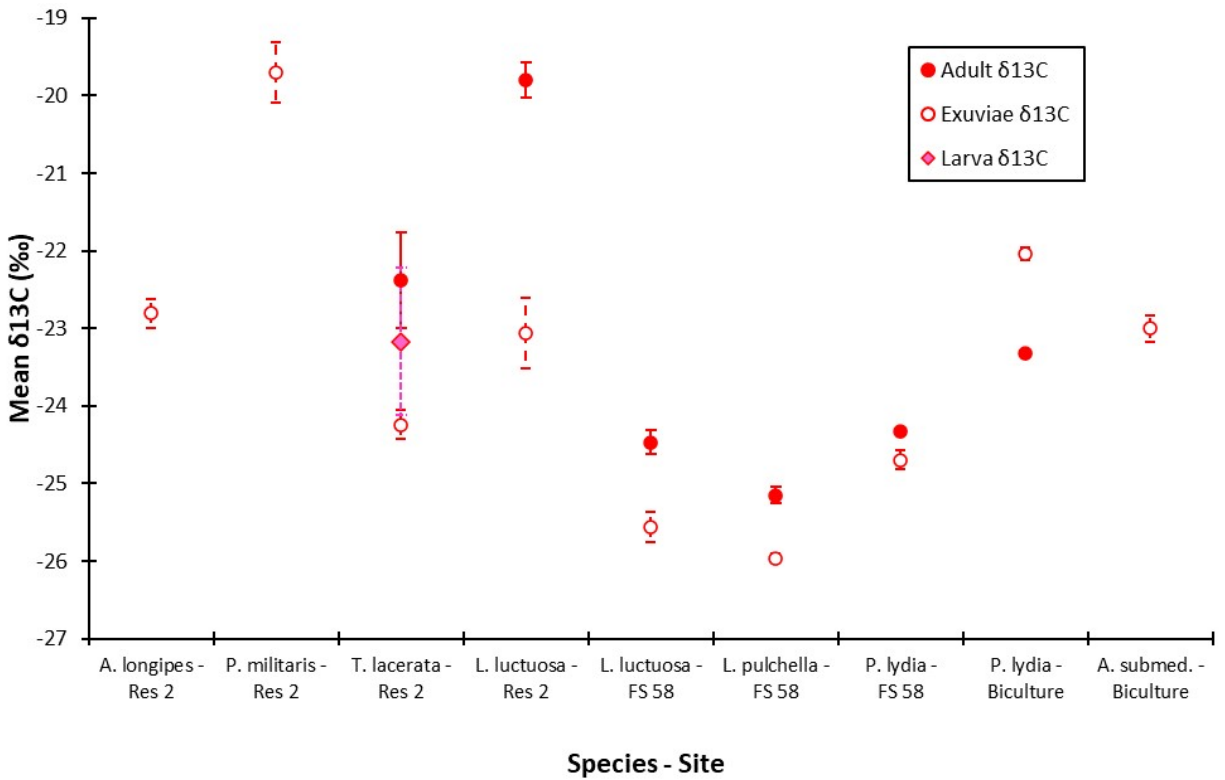


Figure 3.3. Mean (in ‰, \pm SE) $\delta^{13}\text{C}$ signatures of adults, exuviae, and larvae. FS 58 = University of Kansas Field Station Ponds 5E-8E, FS Res 2 = University of Kansas Field Station Reservoir 2, Biculture = Land Institute Biculture Pond.

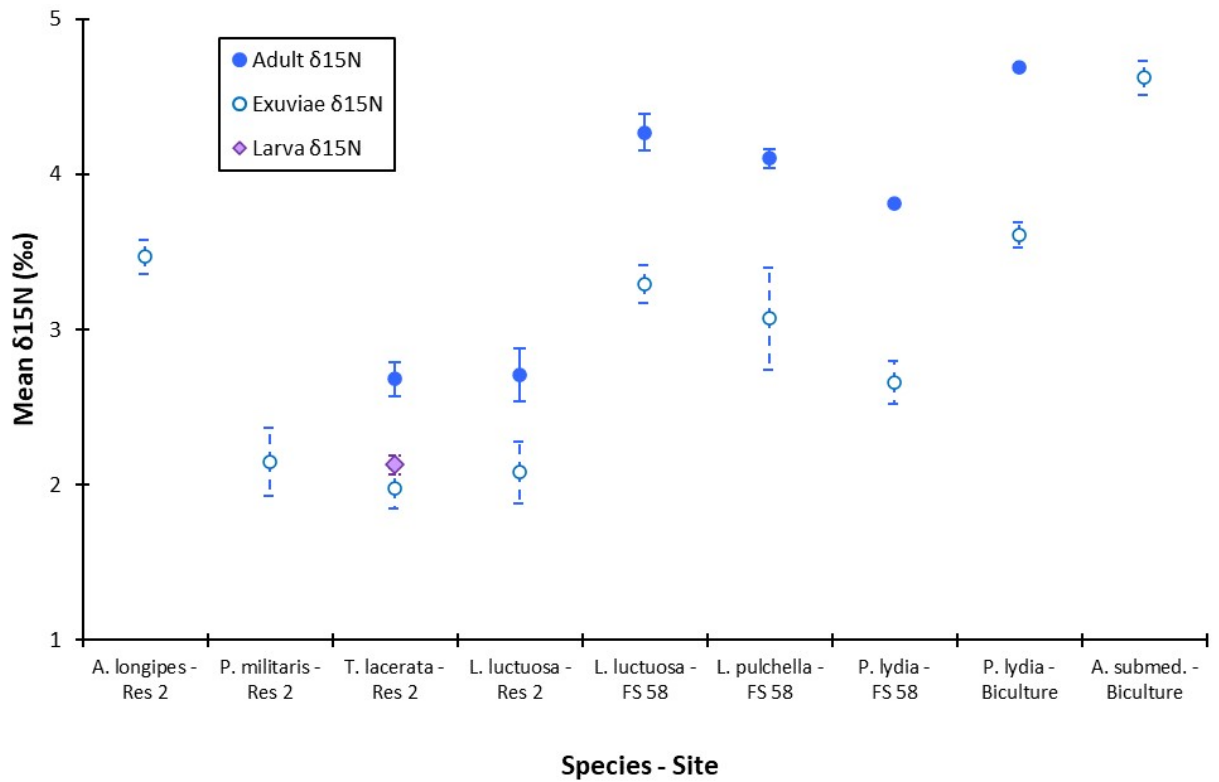


Figure 3.4. Mean (in ‰, \pm SE) $\delta^{15}\text{N}$ signatures of adults, exuviae, and larvae. FS 58 = University of Kansas Field Station Ponds 5E-8E, FS Res 2 = University of Kansas Field Station Reservoir 2, Biculture = Land Institute Biculture Pond.

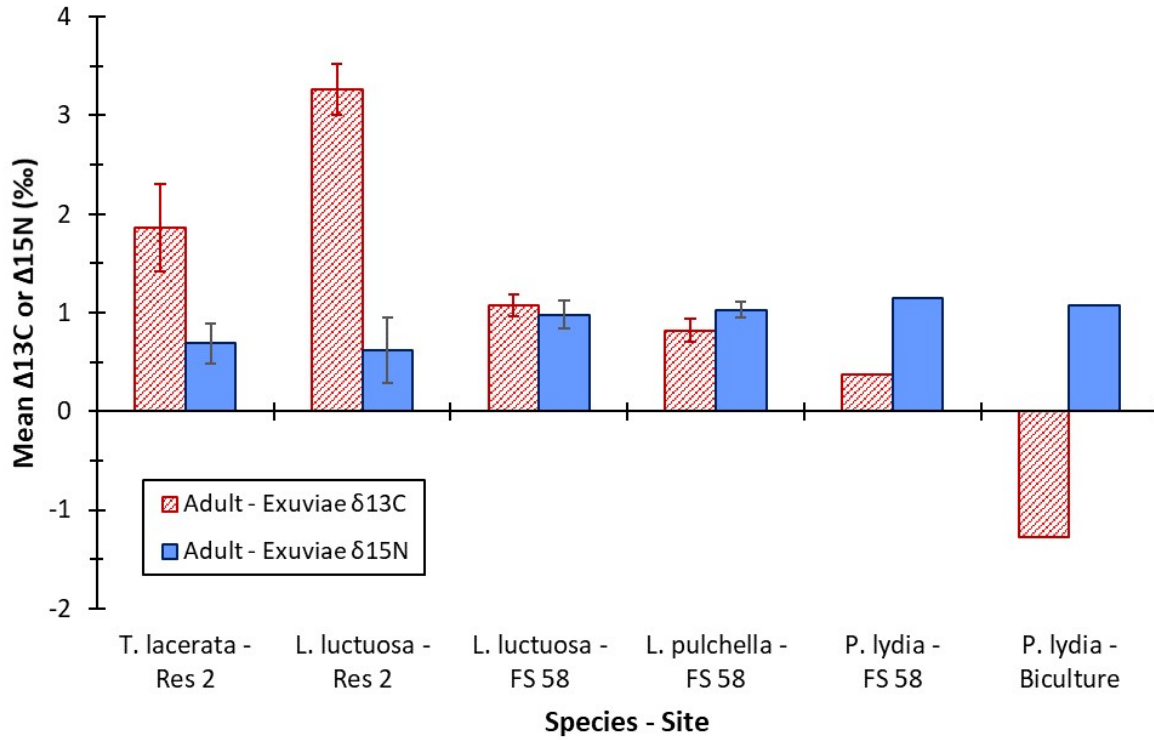


Figure 3.5. Mean (in ‰, \pm SE) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ adult-exuviae fractionation values ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$). FS 58 = University of Kansas Field Station Ponds 5E-8E, FS Res 2 = University of Kansas Field Station Reservoir 2, Biculture = Land Institute Biculture Pond.

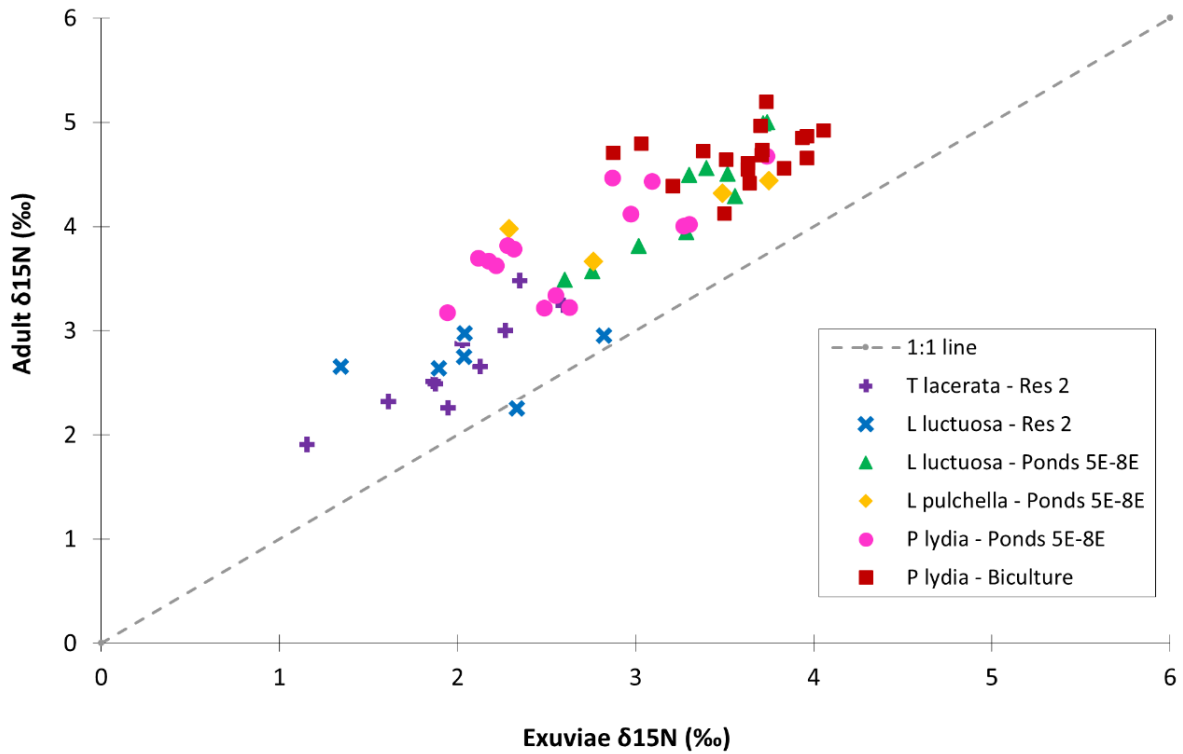


Figure 3.7. Correspondence between the $\delta^{15}\text{N}$ signatures of individual adults and their associated exuviae. Presence on the dashed 1:1 line indicates exact correspondence between adult and exuvial stable isotope signatures. Res 2 = University of Kansas Field Station Reservoir 2, Biculture = Land Institute Biculture Pond.

Table 3.1. Site locations, approximate surface areas, and water quality parameters measured on October 21, 2021. Biculture Pond = Land Institute Biculture Pond, Ponds 5E-8E = University of Kansas Field Station Ponds 5E-8E, Reservoir 2 = University of Kansas Field Station Reservoir 2.

Site Name	Lat / Long Coordinates	Elevation (m)	Surface Area (m ²)	pH	Conductivity (μS)	DO (mg/L)	Temp (°C)
Biculture Pond	39°00'35" N, 95°18'47" W	297	300	6.67	34.41	10.94	11.8
Pond 5E	39°02'58" N, 95°11'35" W	334	40	8.05	176.7	10.34	14.4
Pond 6E	39°02'58" N, 95°11'35" W	334	40	7.87	132.1	8.57	13.6
Pond 7E	39°02'58" N, 95°11'35" W	334	40	8.26	165.6	6.52	13.5
Pond 8E	39°02'58" N, 95°11'35" W	334	40	8.53	162.5	10.62	12.9
Reservoir 2	39°03'02" N, 95°11'36" W	334	6,700	8.79	186.9	13.06	15.5

Table 3.2. Species, collection sites, collection dates in 2021, and number of specimens analyzed. Ponds 5E-8E = University of Kansas Field Station Ponds 5E-8E, Reservoir 2 = University of Kansas Field Station Reservoir 2, Biculture Pond = Land Institute Biculture Pond.

Species	Family	Site	Dates	Adults (n)	Exuviae (n)	Larvae (n)
<i>Anax longipes</i>	Aeshnidae	Reservoir 2	July 2	0	10	0
<i>Arigomphus submedianus</i>	Gomphidae	Biculture Pond	June 8	0	10	0
<i>Libellula luctuosa</i>	Libellulidae	Ponds 5E-8E	June 1–22	10	10	0
<i>Libellula luctuosa</i>	Libellulidae	Reservoir 2	June 27 – July 13	6	6	0
<i>Libellula pulchella</i>	Libellulidae	Ponds 5E-8E	May 28 – June 1	4	4	0
<i>Phanogomphus militaris</i>	Gomphidae	Reservoir 2	June 19	0	4	0
<i>Plathemis lydia</i>	Libellulidae	Biculture Pond	June 8 & 10	18	18	0
<i>Plathemis lydia</i>	Libellulidae	Ponds 5E-8E	May 28 – June 12	15	15	0
<i>Tramea lacerata</i>	Libellulidae	Reservoir 2	June 21 – July 14	10	10	2

Table 3.3. Mean $\delta^{13}\text{C}$ signatures and adult-exuviae fractionation ($\Delta^{13}\text{C}$) values (in ‰, \pm SE) and results of paired *t*-tests on adult vs. exuviae $\delta^{13}\text{C}$ signatures for collection groups. Res 2 = University of Kansas Field Station Reservoir 2, 5E-8E = University of Kansas Field Station Ponds 5E-8E, Biculture = Land Institute Biculture Pond. The mean $\delta^{13}\text{C}$ signatures of all adults and their respective exuviae are significantly different except for *L. pulchella* from Ponds 5E-8E.

Species	Site	n	Adult $\delta^{13}\text{C}$	Exuviae $\delta^{13}\text{C}$	$\Delta^{13}\text{C}$ (Adult - Exuviae)	Larva $\delta^{13}\text{C}$	<i>p</i> -value, paired <i>t</i> -test: Adult vs. Exuviae
<i>A. longipes</i>	Res 2	10	-	-22.82 ± 0.19	-	-	-
<i>P. militaris</i>	Res 2	4	-	-19.70 ± 0.39	-	-	-
<i>T. lacerata</i>	Res 2	10	-22.38 ± 0.49	-24.24 ± 0.18	1.86 ± 0.41	-	0.001
<i>T. lacerata</i>	Res 2	2	-	-	-	-23.17 ± 0.95	-
<i>L. luctuosa</i>	Res 2	6	-19.80 ± 0.62	-23.06 ± 0.45	3.26 ± 0.44	-	0.001
<i>L. luctuosa</i>	5E-8E	10	-24.47 ± 0.24	-25.56 ± 0.19	1.08 ± 0.20	-	$p < 0.001$
<i>L. pulchella</i>	5E-8E	4	-25.15 ± 0.22	-25.96 ± 0.05	0.82 ± 0.26	-	0.055
<i>P. lydia</i>	5E-8E	15	-24.33 ± 0.15	-24.70 ± 0.12	0.36 ± 0.11	-	0.004
<i>P. lydia</i>	Biculture	18	-23.32 ± 0.11	-22.04 ± 0.08	-1.28 ± 0.12	-	$p < 0.001$
<i>A. submedianus</i>	Biculture	10	-	-24.00 ± 0.17	-	-	-

Table 3.4. Mean $\delta^{15}\text{N}$ signatures and adult-exuviae fractionation ($\Delta^{15}\text{N}$) values (in ‰, \pm SE) and results of paired *t*-tests on adult vs. exuviae $\delta^{15}\text{N}$ signatures for collection groups. The mean $\delta^{15}\text{N}$ signatures of all adults and their respective exuviae are significantly different.

Species	Site	n	Adult $\delta^{15}\text{N}$	Exuviae $\delta^{15}\text{N}$	$\Delta^{15}\text{N}$ (Adult - Exuviae)	Larva $\delta^{15}\text{N}$	<i>p</i> -value, paired <i>t</i> -test: Adult vs. Exuviae
<i>A. longipes</i>	Res 2	10	-	3.47 \pm 0.11	-	-	-
<i>P. militaris</i>	Res 2	4	-	2.15 \pm 0.22	-	-	-
<i>T. lacerata</i>	Res 2	10	2.68 \pm 0.15	1.98 \pm 0.13	0.69 \pm 0.07	-	<i>p</i> < 0.001
<i>T. lacerata</i>	Res 2	2	-	-	-	2.13 \pm 0.06	-
<i>L. luctuosa</i>	Res 2	6	2.71 \pm 0.11	2.08 \pm 0.20	0.62 \pm 0.21	-	0.031
<i>L. luctuosa</i>	5E-8E	10	4.27 \pm 0.17	3.29 \pm 0.12	0.98 \pm 0.07	-	<i>p</i> < 0.001
<i>L. pulchella</i>	5E-8E	4	4.10 \pm 0.17	3.07 \pm 0.33	1.03 \pm 0.22	-	0.019
<i>P. lydia</i>	5E-8E	15	3.81 \pm 0.12	2.67 \pm 0.14	1.15 \pm 0.09	-	<i>p</i> < 0.001
<i>P. lydia</i>	Biculture	18	4.69 \pm 0.06	3.61 \pm 0.08	1.08 \pm 0.08	-	<i>p</i> < 0.001
<i>A. submedianus</i>	Biculture	10	-	4.62 \pm 0.11	-	-	-

Table 3.5. Linear regression models describing the relationship between $\delta^{13}\text{C}$ signatures of adults and their associated exuviae. The only statistically significant relationship is between sets of *Plathemis lydia* exuviae and adults from Ponds 5E-8E.

Species	Site	Regression Model	R ²	d.f.	F-value	p-value
<i>T. lacerata</i>	Reservoir 2	Adult $\delta^{13}\text{C} = 13.20 + 1.47$ (Exuviae $\delta^{13}\text{C}$)	0.31	1, 8	3.61	0.094
<i>L. luctuosa</i>	Reservoir 2	Adult $\delta^{13}\text{C} = 2.90 + 0.98$ (Exuviae $\delta^{13}\text{C}$)	0.51	1, 4	4.21	0.110
<i>L. luctuosa</i>	Ponds 5E-8E	Adult $\delta^{13}\text{C} = -5.55 + 0.74$ (Exuviae $\delta^{13}\text{C}$)	0.33	1, 8	3.95	0.082
<i>L. pulchella</i>	Ponds 5E-8E	Adult $\delta^{13}\text{C} = -116.40 - 3.51$ (Exuviae $\delta^{13}\text{C}$)	0.80	1, 2	3.23	0.107
<i>P. lydia</i>	Ponds 5E-8E	Adult $\delta^{13}\text{C} = -1.48 + 0.92$ (Exuviae $\delta^{13}\text{C}$)	0.51	1, 13	13.31	0.003
<i>P. lydia</i>	Biculture	Adult $\delta^{13}\text{C} = -14.72 + 0.39$ (Exuviae $\delta^{13}\text{C}$)	0.09	1, 16	1.57	0.229

Table 3.6. Linear regression models describing the relationship between $\delta^{15}\text{N}$ signatures of adults and their associated exuviae. The only statistically significant relationships are between sets of *T. lacerata* exuviae and adults from Reservoir 2, *L. luctuosa* from Ponds 5E-8E, and *P. lydia* from Ponds 5E-8E.

Species	Site	Regression Model	R ²	d.f.	F-value	p-value
<i>T. lacerata</i>	Reservoir 2	Adult $\delta^{15}\text{N} = 0.56 + 1.07$ (Exuviae $\delta^{15}\text{N}$)	0.81	1, 8	33.81	$p < 0.001$
<i>L. luctuosa</i>	Reservoir 2	Adult $\delta^{15}\text{N} = 2.52 + 0.09$ (Exuviae $\delta^{15}\text{N}$)	0.03	1, 4	0.11	0.761
<i>L. luctuosa</i>	Ponds 5E-8E	Adult $\delta^{15}\text{N} = -0.02 + 1.30$ (Exuviae $\delta^{15}\text{N}$)	0.87	1, 8	52.19	$p < 0.001$
<i>L. pulchella</i>	Ponds 5E-8E	Adult $\delta^{15}\text{N} = 2.84 + 0.41$ (Exuviae $\delta^{15}\text{N}$)	0.62	1, 2	3.23	0.214
<i>P. lydia</i>	Ponds 5E-8E	Adult $\delta^{15}\text{N} = 2.04 + 0.67$ (Exuviae $\delta^{15}\text{N}$)	0.54	1, 13	15.24	0.002
<i>P. lydia</i>	Biculture	Adult $\delta^{15}\text{N} = 3.94 + 0.21$ (Exuviae $\delta^{15}\text{N}$)	0.08	1, 16	1.34	0.265

Table 3.7. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (in ‰, \pm SE) of *Tramea lacerata* adult, exuvial, and larval tissues from Reservoir 2. The only significant difference is that the mean adult $\delta^{15}\text{N}$ signature is significantly higher than the mean larval $\delta^{15}\text{N}$ signature.

Parameter	Adult (n = 10)	Exuviae (n = 10)	Larva (n = 2)	d.f.	<i>t</i> -value	<i>p</i> -value
$\delta^{13}\text{C}$	-22.38 \pm 0.49	-	-23.17 \pm 0.95	1	0.74	0.593
$\delta^{13}\text{C}$	-	-24.24 \pm 0.18	-23.17 \pm 0.95	1	-1.11	0.466
$\delta^{15}\text{N}$	2.68 \pm 0.15	-	2.13 \pm 0.06	9	3.36	0.008
$\delta^{15}\text{N}$	-	1.98 \pm 0.13	2.13 \pm 0.06	9	-1.05	0.321

Table 3.8. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuviae isotopic fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) values (in ‰, \pm SE) of *Libellula luctuosa* from Ponds 5E-8E and Reservoir 2. All parameter values are significantly different except for adult-exuviae ^{15}N fractionation.

Parameter	Ponds 5E-8E (n = 10)	Reservoir 2 (n = 6)	d.f.	t-value	p-value
Adult $\delta^{13}\text{C}$	-24.47 \pm 0.24	-19.79 \pm 0.62	6	-7.01	$p < 0.001$
Exuviae $\delta^{13}\text{C}$	-25.56 \pm 0.19	-23.06 \pm 0.45	6	-5.09	0.002
$\Delta^{13}\text{C}$ (Adult - Exuviae)	1.08 \pm 0.20	3.27 \pm 0.44	7	-4.55	0.003
Adult $\delta^{15}\text{N}$	4.27 \pm 0.17	2.70 \pm 0.11	13	7.71	$p < 0.001$
Exuviae $\delta^{15}\text{N}$	3.29 \pm 0.12	2.08 \pm 0.20	8	5.17	0.001
$\Delta^{15}\text{N}$ (Adult - Exuviae)	0.98 \pm 0.07	0.63 \pm 0.21	6	1.58	0.165

Table 3.9. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuviae isotopic fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) values (in ‰, \pm SE) of *Plathemis lydia* from Ponds 5E-8E and Biculture Pond. All parameter values are significantly different except for adult-exuviae ^{15}N fractionation.

Parameter	Ponds 5E-8E (n = 15)	Biculture (n = 18)	d.f.	t-value	p-value
Adult $\delta^{13}\text{C}$	-24.33 \pm 0.15	-23.32 \pm 0.11	26	-5.37	$p < 0.001$
Exuviae $\delta^{13}\text{C}$	-24.70 \pm 0.12	-22.04 \pm 0.08	25	-18.39	$p < 0.001$
$\Delta^{13}\text{C}$ (Adult - Exuviae)	0.37 \pm 0.11	-1.28 \pm 0.12	30	10.41	$p < 0.001$
Adult $\delta^{15}\text{N}$	3.81 \pm 0.12	4.69 \pm 0.06	19	-6.50	$p < 0.001$
Exuviae $\delta^{15}\text{N}$	2.67 \pm 0.14	3.61 \pm 0.08	22	-6.10	$p < 0.001$
$\Delta^{15}\text{N}$ (Adult - Exuviae)	1.15 \pm 0.09	1.08 \pm 0.08	29	0.53	0.598

Table 3.10. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuviae isotopic fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) values (in ‰, \pm SE) of two species in the genus *Libellula* from Ponds 5E-8E. There were no significant differences between the species for any of the parameter values.

Parameter	<i>L. luctuosa</i> (n = 10)	<i>L. pulchella</i> (n = 4)	d.f.	<i>t</i> -value	<i>p</i> -value
Adult $\delta^{13}\text{C}$	-24.47 \pm 0.24	-25.15 \pm 0.22	9	2.11	0.064
Exuviae $\delta^{13}\text{C}$	-25.56 \pm 0.19	-25.97 \pm 0.05	10	2.11	0.061
$\Delta^{13}\text{C}$ (Adult - Exuviae)	1.08 \pm 0.20	0.81 \pm 0.26	6	0.81	0.447
Adult $\delta^{15}\text{N}$	4.27 \pm 0.17	4.10 \pm 0.17	8	0.68	0.517
Exuviae $\delta^{15}\text{N}$	3.29 \pm 0.12	3.07 \pm 0.33	3	0.61	0.584
$\Delta^{15}\text{N}$ (Adult - Exuviae)	0.98 \pm 0.07	1.03 \pm 0.22	3	-0.22	0.842

Table 3.11. Results of 1-way ANOVAs comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuviae isotopic fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) values (in ‰, \pm SE) of three libellulid species from Ponds 5E-8E. Values in the same row that do not share a letter are significantly different from one another according to a Games-Howell *post hoc* test ($\alpha = 0.05$). There are no significant differences among the three species in adult $\delta^{15}\text{N}$ and adult-exuviae ^{15}N fractionation ($\Delta^{15}\text{N}$).

Parameter	<i>L. luctuosa</i> (n = 10)	<i>L. pulchella</i> (n = 4)	<i>P. lydia</i> (n = 15)	d.f.	F-value	p-value
Adult $\delta^{13}\text{C}$	-24.47 \pm 0.24 ab	-25.15 \pm 0.22 b	-24.33 \pm 0.15 a	2	4.66	0.037
Exuviae $\delta^{13}\text{C}$	-25.56 \pm 0.19 b	-25.97 \pm 0.05 b	-24.70 \pm 0.12 a	2	45.95	$p < 0.001$
$\Delta^{13}\text{C}$ (Adult - Exuviae)	1.08 \pm 0.20 a	0.81 \pm 0.26 ab	0.37 \pm 0.11 b	2	4.99	0.040
Adult $\delta^{15}\text{N}$	4.27 \pm 0.17 a	4.10 \pm 0.17 a	3.81 \pm 0.12 a	2	2.37	0.144
Exuviae $\delta^{15}\text{N}$	3.29 \pm 0.12 a	3.07 \pm 0.33 ab	2.67 \pm 0.14 b	2	5.40	0.033
$\Delta^{15}\text{N}$ (Adult - Exuviae)	0.98 \pm 0.07 a	1.03 \pm 0.22 a	1.15 \pm 0.09 a	2	0.94	0.432

Table 3.12. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures and adult-exuviae isotopic fractionation ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$) values (in ‰, \pm SE) of *Libellula luctuosa* and *Tamea lacerata* from Reservoir 2. There are no significant differences between the species except that *T. lacerata* adult $\delta^{13}\text{C}$ is significantly lower and *L. luctuosa* adult-exuviae ^{13}C fractionation ($\Delta^{13}\text{C}$) is significantly higher.

Parameter	<i>T. lacerata</i> (n = 10)	<i>L. luctuosa</i> (n = 6)	d.f.	<i>t</i> -value	<i>p</i> -value
Adult $\delta^{13}\text{C}$	-22.38 \pm 0.49	-19.79 \pm 0.62	10	3.28	0.008
Exuviae $\delta^{13}\text{C}$	-24.24 \pm 0.18	-23.06 \pm 0.45	6	2.42	0.052
$\Delta^{13}\text{C}$ (Adult - Exuviae)	1.86 \pm 0.41	3.27 \pm 0.44	12	2.34	0.037
Adult $\delta^{15}\text{N}$	2.68 \pm 0.15	2.70 \pm 0.11	13	0.16	0.872
Exuviae $\delta^{15}\text{N}$	1.98 \pm 0.13	2.08 \pm 0.20	9	0.41	0.691
$\Delta^{15}\text{N}$ (Adult - Exuviae)	0.69 \pm 0.07	0.63 \pm 0.21	6	-0.30	0.774

Table 3.13. Results of two-sample *t*-tests comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (in ‰, \pm SE) of *Plathemis lydia* and *Arigomphus submedianus* exuviae from Biculture Pond. Both parameter values are significantly different.

Parameter	<i>P. lydia</i> (n = 18)	<i>A. submedianus</i> (n = 10)	d.f.	t-value	p-value
Exuviae $\delta^{13}\text{C}$	-22.04 \pm 0.08	-24.00 \pm 0.17	13	10.53	$p < 0.001$
Exuviae $\delta^{15}\text{N}$	3.61 \pm 0.08	4.62 \pm 0.11	17	7.59	$p < 0.001$

Table 3.14. Results of 1-way ANOVAs comparing mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (in ‰, \pm SE) of exuviae of four species from three families from Reservoir 2. Values in the same row that do not share a letter are significantly different from one another according to a Games-Howell *post hoc* test ($\alpha = 0.05$). The mean $\delta^{13}\text{C}$ signature of *Phanogomphus militaris* (Gomphidae) is significantly less depleted than that of the other species and the mean $\delta^{15}\text{N}$ signature of *Anax longipes* (Aeshnidae) is significantly higher than that of the other species.

Parameter	<i>T. lacerata</i> (n = 10)	<i>L. luctuosa</i> (n = 6)	<i>A. longipes</i> (n = 10)	<i>P. militaris</i> (n = 4)	d.f.	F-value	p-value
Exuviae $\delta^{13}\text{C}$	-24.24 \pm 0.18 c	-23.06 \pm 0.45 bc	-22.82 \pm 0.19 b	-19.70 \pm 0.39 a	3	34.08	$p < 0.001$
Exuviae $\delta^{15}\text{N}$	1.98 \pm 0.13 b	2.08 \pm 0.20 b	3.47 \pm 0.11 a	2.15 \pm 0.22 b	3	28.14	$p < 0.001$

CHAPTER 4: DISCUSSION AND SYNTHESIS

This study sought to determine whether anisopteran exuviae could be substituted for whole larval or adult tissues in stable isotope studies of aquatic food web structure, and the results demonstrate that exuviae, larvae, and adult dragonflies do exhibit very similar isotopic signatures. All mean absolute differences in $\delta^{13}\text{C}$ signatures between adults and their respective exuviae were less than 1.41‰ in New Mexico, which is slightly higher than but still consistent with widely cited experimental results showing a very low degree of carbon fractionation between trophic levels (DeNiro and Epstein 1978, Post 2002). A wider range of carbon fractionation values were observed in Kansas (0.37 to 3.26‰), but these values are still probably small enough to differentiate between potential basal carbon sources that employ different photosynthetic pathways (*e.g.*, C_3 vs. C_4) or reside in different locations within a waterbody (*e.g.*, pelagic vs. littoral). A few taxonomic and site differences were observed in the mean $\delta^{15}\text{N}$ signatures for conspecific adults, exuviae, and larvae emerging from the same site (noted below) but were always less than 1.42‰ in New Mexico and 1.16‰ in Kansas -- well below the roughly 3.4‰ enrichment in $\delta^{15}\text{N}$ signatures observed between successively higher trophic levels in aquatic ecosystems by Post (2002), Vander Zanden and Rasmussen (2001), and others.

The observed differences in mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures between adults and their respective exuviae may arise because an exuviae is essentially the final larval exoskeleton and it was thus formed and solidified during the last stadial molt. As such, the exuviae would likely reflect the isotopic signature of the larva's diet prior to the last molt, but the living tissues which comprise the interior of the larva (and eventually, the emergent adult's body) are

continually being renewed for as long as the final stadium larva continues to eat prior to its eventual emergence. For example, Carvalho (1987) found that the final larval stadium for two *Gynacantha bifida* individuals lasted 20 and 27 days but the larvae only stopped feeding 8 and 11 days before emergence, respectively.

A primary objective of this study was to determine if exuviae can serve as proxies for larval odonates in stable isotope studies, and evidence from New Mexico and Kansas support that conclusion although the samples were limited to two species: *P. militaris* (11 individuals) and *T. lacerata* (only 2 individuals). The results for the sample of eleven *P. militaris* individuals at Lake Saint Francis are encouraging, as the mean larval *P. militaris* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures (-15.20‰ and 8.70‰, respectively) were intermediate between the mean exuvial (-15.00‰, 7.89‰) and adult (-15.47‰, 8.74‰) tissue signatures. Although the sample size was very small at only two individuals, mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures for *T. lacerata* larvae collected at Reservoir 2 (-23.17‰ and 2.13‰, respectively) were also intermediate between the mean signatures for adults (-22.38‰) and their exuviae (-24.30‰; Figs. 3.3 and 3.4) which suggests that the pattern observed in *P. militaris* may extend to other odonate species and families. These findings are consistent with the fact that larvae are composed of both adult and exuvial tissues. With respect to the thirteen *P. militaris* emergent adults, their exuviae represented approximately 20% (mean $21 \pm 4\%$, range 16–28%) of the total combined dry weight of each emergent adult and its exuviae, and by extension, about one-fifth of the total dry weight of a larva just prior to emergence.

Taxonomic variability: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures

The differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures among the coexisting species in the New Mexico and Kansas ponds may be the result of different larval habitat associations, the effects of body surface textures on sediment attachment, and/or idiosyncratic prey bases. For example, members of the Aeshnidae (here, represented by *Anax longipes* exuviae, the largest species collected in Kansas) are elongated ambush predators with smooth bodies that tend to live within submerged vegetation and are classified as “claspers” (Corbet 1999). Its exuviae were at least 1.32‰ more enriched in ^{15}N (on average) than the exuviae of any of the three coexisting odonates in Reservoir 2, so it is possible that *A. longipes* occupies the highest trophic position in that system and may even prey upon the other, smaller odonate larvae.

In contrast, the larvae of many gomphids such as *P. militaris* and *A. submedianus* are shallow burrowers (Westfall and Tennessen 1996) that conceal themselves just beneath the surface of mud and detritus substrates in the littoral zone of ponds and streams (Landwer and Sites 2003; *personal observation*) to attack benthic prey from below (Westfall and Tennessen 1996). The dorsal surface of *P. militaris* larvae is densely covered with granular structures from which fine setae emerge; the ventral surface lacks these granular structures but is also covered with setae (Landwer and Sites 2003). This surface texture is so rough that sediment was often observed to fully coat the emerging larvae (*personal observation*); as a result, some sediment may have remained on the exuviae even after vigorous agitation in distilled water prior to analysis. Because all *P. militaris* larvae were collected as they crawled out of the water onto muddy beaches, their exuvial signatures may more closely resemble the isotopic composition of the substrate (not measured in this study) than did the signatures of the teneral adults that

pulled themselves out of the exoskeletons. In Sinkhole 32, the $\delta^{13}\text{C}$ signatures of *L. composita* exuviae were approximately 2‰ more negative on average than coexisting *P. militaris*. In Reservoir 2, the mean $\delta^{13}\text{C}$ signatures of all coexisting odonates were also more negative than *P. militaris* by 3.12 to 4.54‰ (Figs. 2.3 and 3.3).

The best represented family in this two-state study is the Libellulidae, with one species from a New Mexican sinkhole and four species from the three Kansas sites. Members of the genus *Libellula* are classified as “sprawlers” (Westfall and Tennesen 1996) and inhabit muddy substrates with detritus (Tennesen 2019). As with many of its congeners, some fine setae are present on *L. composita* larvae (Musser 1962), but their exuviae were covered with far less sediment than were the *P. militaris* exuviae from the same sinkhole. This may be because the larval microhabitats are different and/or the vertical emergence sites on grass or shrubs typically used by *L. composita* were located well above the muddy substrate in Sinkhole 32 and were thus less likely to be inundated with turbid water while the larvae were emerging. Therefore, the mean difference in exuvial $\delta^{13}\text{C}$ signatures of approximately 2‰ in that sinkhole may be due to the differing amounts of adhered sediment to each species’ exuviae. In contrast, only minor differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were noted among the three coexisting libellulid species in the Kansas Ponds 5E-8E (Figs. 3.3 and 3.2). These similarities in isotopic composition may have arisen because the three species are closely related and/or their larvae are of similar size and morphology, inhabit similar microhabitats, and exploit the same prey base.

Taxonomic variability: Adult – exuvial fractionation of ¹³C and ¹⁵N

Frossard *et al.* (2013) hypothesized that arthropod exoskeletons may be depleted in ¹³C relative to whole organisms as a result of metabolic fractionation or differential isotopic routing during tissue development if the lighter form of carbon (¹²C) is preferentially used to create the complex macromolecules typically found in exoskeletons. The four libellulid species collected in Kansas followed this predicted pattern of adult-exuvial ¹³C fractionation for the most part, as adults were on average less negative than their exuviae in five of the six collection groups by approximately 0.37 – 3.26‰ depending on the species. There was one notable exception: *P. lydia* in Biculture Pond showed the opposite ¹³C fractionation direction, as adults were more negative than their exuviae by a mean of 1.28‰ (Figs. 3.3 and 3.5).

Two species (*P. militaris* and *L. composita*) representing families Gomphidae and Libellulidae, respectively, were included in the New Mexico study. Although the mean absolute value of ¹³C fractionation between adults and their exuviae was less than 1.40‰ for each species, there was a consistent taxonomic difference in the direction of ¹³C fractionation. The exuviae of *L. composita* had more negative $\delta^{13}\text{C}$ signatures than their adults by a mean of 0.95‰ (positive fractionation), whereas the opposite was true for *P. militaris* in New Mexico regardless of collection site or date. The mean adult-exuvial fractionation values for all groups of *P. militaris* were negative (meaning that the exuviae had more positive $\delta^{13}\text{C}$ signatures) by between -0.46 to -1.40‰ depending on the collection event (Figs. 2.5 and 2.6).

It is remarkable that *L. composita* at Sinkhole 32 and the four libellulid species at two of the three sites in Kansas fractionated ¹³C in a manner consistent with that observed in other aquatic arthropods such as chironomid midges (Frossard *et al.* 2013), tiger shrimp (Macko *et al.*

1990), and lobsters (Macko *et al.* 1990), but the *P. militaris* populations in New Mexico did not. The fact that, contrary to the expected pattern, *P. militaris* adults in New Mexico were depleted (more negative) in ^{13}C relative to their exuviae in every collection event regardless of the site is intriguing and merits further investigation. Unfortunately, no emergent *P. militaris* adults were found in Kansas, so it could not be determined if the Kansas population also fractionates carbon in this apparently idiosyncratic fashion.

No such taxonomic differences in ^{15}N fractionation were observed among the eleven samples in New Mexico or in Kansas, as the mean exuvial $\delta^{15}\text{N}$ signatures for all collection groups were lower than the paired mean adult signatures regardless of species, location, or date (Figs. 2.5 and 3.5). This result was consistent with predictions of Minagawa and Wada (1984) and Schimmelmann and DeNiro (1986). However, Tibbets *et al.* (2008) observed that this is not a universal phenomenon among insects, as the direction of larvae-exuviae fractionation in *Manduca sexta* (tobacco hornworm, order Lepidoptera) and *Tenebrio molitor* (mealworm, order Coleoptera) was reversed and larvae were depleted in ^{15}N relative to their respective exuviae.

Spatial variability

Because variability between spatially distinct conspecific populations can be significant, the environmental factors affecting baseline isotopic values should be considered when attempting to compare similar yet distinct systems.

No large site-specific differences were evident in the mean adult or exuvial *P. militaris* $\delta^{13}\text{C}$ signatures in New Mexico. However, the mean $\delta^{13}\text{C}$ signatures for taxa in the Kansas sites

did appear to vary as a function of their emergence site, with the three species emerging from Ponds 5E-8E consistently more depleted in ^{13}C relative to all species emerging from either Reservoir 2 or Biculture Pond.

In New Mexico, *P. militaris* exuvial and adult $\delta^{15}\text{N}$ signatures in Lake Saint Francis were approximately 3–5‰ higher than the signatures for their conspecifics and *L. composita* in Sinkhole 32 (Fig. 2.4). A smaller but still noteworthy difference was observed between the mean $\delta^{15}\text{N}$ signatures of the libellulid populations in Ponds 5E-8E (*L. luctuosa*, *L. pulchella*, and *P. lydia*) and Reservoir 2 (*L. luctuosa* and *T. lacerata*) despite their close proximity. Adult libellulids in Ponds 5E-8E had mean $\delta^{15}\text{N}$ signatures roughly 1.6‰ higher than adult libellulids (including a conspecific) in Reservoir 2, with similar site-specific differences in their exuviae. Additionally, the mean *P. lydia* adult and exuvial $\delta^{15}\text{N}$ signatures were approximately 1.0‰ higher than the signatures for its conspecifics in Ponds 5E-8E (Fig. 3.4).

Although the differences in observed $\delta^{15}\text{N}$ values for *P. militaris* emerging from the two sinkholes in New Mexico were in the range frequently observed between adjacent trophic levels (*i.e.*, 3.4‰, Post 2002), it cannot be assumed that the differences seen in this study are due solely to trophic organization. Sediment and basal organisms were not analyzed during this study, so the source of this difference in nitrogen signatures remains an open question. Post (2002) noted that basal $\delta^{15}\text{N}$ signatures in primary consumers ranged between 4.5-13.6‰ (roughly equivalent to three trophic levels) in the more than 20 lakes in his study, and thus the spatial variations in $\delta^{15}\text{N}$ signatures observed in New Mexico and Kansas were not entirely unexpected.

Temporal variability

The lack of temporal variability in isotopic ratios in *P. militaris* dragonflies collected from the same sinkhole on dates that were three weeks and two years apart implies that the environmental influences on odonate tissue signatures are relatively stable and suggests that collection efforts need not be concentrated by a temporal gap of a few hours or even a week or two. This flexibility will allow researchers to collect sufficient samples over time for species that do not emerge synchronously or in great numbers at a particular site.

Future directions

Quinby *et al.* (2020) noted that there is still much to discover about the relationship between dietary intake and the stable isotopic signatures of inert tissues such as those found in the insect exoskeleton, and this study provides new and useful information. Although the patterns were consistent for representatives of two anisopteran families (Libellulidae and Gomphidae) in multiple locations, the sample sizes were small and only two species were tested. Therefore, these results do not imply that a close correspondence between adult, exuvial, and larval tissues will be observed across the entire order Odonata or in other types of aquatic ecosystems such as rivers or peatlands. As Fletcher *et al.* (2017) noted in their study of trace element bioaccumulation in several genera of larval odonates, taxonomic differences in larval habitats, prey bases, and metabolic activity may result in different tissue chemical compositions (in their words: the “genus matters”). Therefore, future studies will be needed to ascertain the degree to which the isotopic signatures of larvae and exuviae are similar across broader taxonomic categories and in different types of habitats. If the patterns revealed in this

study are observed in additional taxa, then substituting odonate exuviae for larvae or adults in food web studies will add another promising tool to the expanding suite of nonlethal and environmentally sensitive techniques used to study ecological interactions in aquatic systems while minimizing harm to dragonfly populations and their habitats.

Overall conclusion

Quinby *et al.* (2020a) believed that inert tissues such as insect exoskeletons “remain understudied” in trophic ecology studies. Furthermore, Quinby *et al.* (2020b) called for increased research on the suitability of using nonlethal methods to explore trophic interactions in arthropods, especially those species that are of conservation concern. This study was an effort to address those knowledge gaps by investigating the suitability of exuviae as proxies for adult and larval dragonflies. The observed differences in the isotopic signatures of adult, larval, and exuvial odonate tissues were small, usually predictable in direction, and usually consistent across tissue types, species, and collection dates in New Mexico and Kansas. Even when the direction of fractionation was unusual compared to all other species (*e.g.*, *P. militaris* in Lake Saint Francis; *P. lydia* in Biculture Pond), the mean magnitude of isotopic fractionations never exceeded 3.26‰ for ^{13}C or 1.41‰ for ^{15}N , thus allowing for reliable discrimination between carbon sources and inference of trophic levels, respectively. Furthermore, because larval $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were intermediate between adult and exuvial signatures in the two species for which larval data was available, it follows that exuviae can be confidently substituted for larvae or adults in aquatic food web investigations with little loss of information and greatly reduced negative impacts on sensitive populations and their fragile aquatic habitats.

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