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Quantification and analysis of carbon-13 and nitrogen-15 stable isotope variation in brood V cicada nymph exoskeletons

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ABSTRACT: Endemic to eastern North America, the periodical cicada of the genus *Magicicada* is an insect that has captured people's curiosity for centuries. Feeding on root xylem for 13 or 17 years, these insects emerge from the earth by the millions and shed their exoskeletons before maturing into reproductive adults. The short 2-3 month window in which periodical cicadas are active provides a short yet large resource pulse to both consumers and soil. Capitalizing on the 2016 emergence of Brood V, this study examined the $\delta^{13}C$ and $\delta^{15}N$ values of cicada nymph exoskeletons, seeking to identify patterns of stable isotope variation based on tree species and collection site. Positive findings would implicate possible utility in mapping cicada ranges and mobility. Furthermore, improved understanding of stable isotope variation may be applied to future investigations of cicada predators, trophic interactions and resource pulses. Five sites within Summit County, Ohio were used in the collection of Brood V cicadas from May through August of 2016. The sampled nymph exuviae were analyzed on an Isoprime 100 stable isotope mass spectrometer interfaced to an elemental analyzer. Minitab statistical software was used to interpret the data. Using a General Linear Model ANOVA, a relationship was found between collection site and exoskeletal $\delta^{13}C$ and $\delta^{15}N$ values ($p < 0.00$ were observed for both). A relationship was also found between tree species and $\delta^{13}C$ and $\delta^{15}N$ values ($p = 0.036$ and 0.004 for $\delta^{13}C$ and $\delta^{15}N$ respectively). Model summaries explained 96.44% of site-specific variance and 97.74% of tree species-specific variance.

Introduction:

The emergence of the periodical cicada is a spectacular phenomenon that draws the attention of both the general public and scientists alike. Upon emergence from the earth, these insects inundate fields and forests, filling the air with their distinct breeding songs. Dating back to the colonial era, people have been fascinated with the nature of these insects. Though there certainly is nothing discreet about the grand arrival of the periodical cicada, there is still much to be discovered about its tendencies and characteristics.

Belonging to the genus *Magicicada,* perhaps the most defining characteristic of this insect is its distinct emergence pattern. In the early days of the United States, settlers throughout the colonies wrote of sudden influxes of noisy swarms. Forests that had been silent for years suddenly rang with the unmistakable mating call of the periodical cicada. Once the noise died down, the forests might remain silent for over a decade. These seemingly random and mysterious patterns led to folklore and superstition. Cicadas came to be known as omens of war and illness. This paired with the overwhelming and sudden swaths that emerged drew comparisons to the story of Moses and the plague of locusts. For this reason, cicadas are often incorrectly referred to as locusts(1).

It wasn't until the beginning of the nineteenth century that this seemingly prophetic phenomenon was revealed to have a predictable pattern. Thanks to carefully recorded accounts, Dr. S.P. Hildreth discovered that cicadas in his town of Marietta, Ohio had emerged on a 17 year cycle: 1795, 1812, and 1829. Soon after, Dr. D.L. Phares of Mississippi noted a 13 year pattern of emergence in his region. With the two known emergence patterns having been identified, further studies sought to characterize the isolated populations within these two divisions. This meant gathering information on their distributions and specific emergence years. It was discovered that 17-year populations are generally found in the northern and plains states of the US, while the 13-year populations are concentrated in the South and along the Mississippi River. A system of organization was developed which referred to the distinct populations as broods. The 17-year populations were designated as Broods I-XVII, while 13-year populations were distinguished as Broods XVIII-XXX. This numbering system left room for the discovery of many more populations. However, only 15 distinct broods are known today: twelve 17 year populations and three 13 year populations(1).

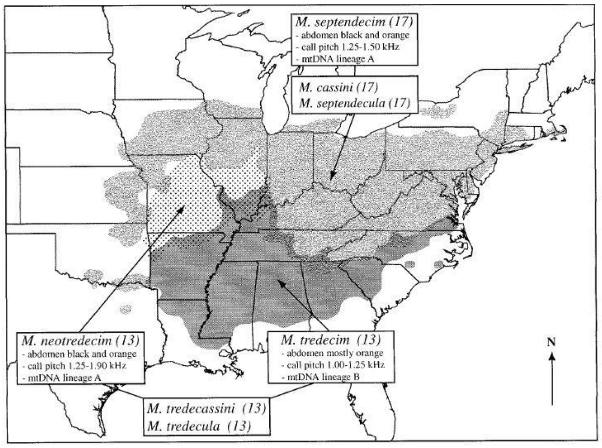

Figure 1: The apparent ranges of the 17-year and 13-year periodical cicadas broken down by species. Generally distinct, there are some areas of overlap[2].

Figure 2: These distinct emergence holes were observed at O'Neil's Woods MetroPark. Each hole is roughly one centimeter in diameter

There are three species of 17-year periodical cicada: *Magicicada septendicum, Magicicada cassini, and Magicicada septendecula*. The 13-year cicada is composed of four species: *Magicicada tredecim, Magicicada tredecassini, Magicicada tredecula* and the most recently characterized *Magicicada neotredecim*[2][3]. Broods may be composed of several species emerging simultaneously. The offset emergence years create reproductive isolation amongst broods, keeping them distinct[3].

The periodical cicada has one of the longest known life cycles of any insect. It begins with the eggs laid by the previous generation. Tiny cicada nymphs emerge from these eggs and find their way to the ground where they burrow 6-24 inches into the earth[4]. Feeding on the xylem of roots, the nymphs live a life of tunneling and growth. The number of nymphs feeding on a given tree can be staggering. In fact, periodical cicadas exhibit the highest biomass concentration of any terrestrial consumer, with up to 300 nymphs in any given square yard and potentially over 1,500,000 per acre[4].

As the cicadas grow, they employ a unique mechanism to discern the passage of time. Studies by Karban and colleagues reveal that cicada nymphs track the passage of time by seasonal cycling of the plant on which they feed. By inducing trees to accelerate their annual cycles, Karban was able to prompt premature emergence of cicadas[5]. This was further supported by observations in 2007 of Brood XIV cicadas. These cicadas emerged a year early after a warm winter spell caused trees to briefly bloom before returning to dormancy[6]. It's been theorized that timing delays and premature emergences throughout history are responsible for the development of the many broods known today[6].

When a periodical cicada has detected 17 or 13 cycles, it will begin to prepare for emergence. When the soil reaches 65°F, the nymph will burrow from the earth, leaving behind a telltale hole that is roughly a centimeter in diameter[1]. These holes can serve as a means of quantifying cicada populations in an area (**Fig 2**).

The nymph, unable to fly, will make its way to a sunny and open area. Here it will begin the final step of its maturation process. The exoskeleton dries and splits, allowing the adult cicada to emerge. Over the course of several hours, the adult cicada's wings unfurl and the body hardens, changing from cream colored to a characteristic black[1]. The nymph exoskeleton is left behind. From here, the cicada turns its attention to the 3-4 week mating cycle[4].

Perhaps the most noticeable behavior of the periodical cicada is its mating ritual. Male cicadas use specialized organs known as timbals to sing a mating song. Each species exhibits subtle nuances in their breeding calls, which range from pitch, to frequency, to light sensitive factors which affect the time of day singing occurs. Cicadas employ three particular calls: a congregation call, meant to draw males and females, a courtship call and a distress call[3]. While it may seem counterintuitive to utilize a call that congregates females as well as competing males, the congregation call is useful in clustering cicadas of a given species. This clustering creates what are known as "chorus trees" where almost all mating occurs[3]. Cumulatively, the cicada songs in these chorus trees can reach volumes of 120 decibels[7].

Figure 3: Flagging of trees due to egg deposits as observed at Hudson Springs City Park.

Once mating has been completed, females select a site to lay their eggs. This must be carefully considered, as the tree where she lays her eggs should be poised to survive at least 17 years. Generally, younger, fairly isolated trees are preferred since they are likely to receive more sunlight and nutrients than those that must compete with neighboring trees. The female cuts V-shaped slits into twigs and soft stems where she will lay up to 30 eggs. This process is repeated until all 400-600 eggs have been deposited across a range of twigs(1). This process often damages the tender plant material, killing leaves and resulting in an identifiable pattern known as flagging(1) (**Fig 3**). Once the cicada eggs have been laid, the adults have accomplished their mission and eventually die. The eggs hatch and the tiny nymphs fall to the earth to start the process anew(1).

With the massive amount of eggs being laid, it's no surprise that periodical cicadas emerge in such great numbers. Periodical cicadas rely on a strategy of predator satiation(8). The sheer volume by which they emerge virtually guarantees that many will successfully breed. Studies by Williams and colleagues examined the effectiveness of predator satiation of avian predators with respect to the periodical cicada over a 16 hectare plot of land. Utilizing the 1985 emergence of Brood XIX she found that avian predators were sufficiently satiated, having consumed only 15% of the population throughout the entire summer. This strategy works effectively for the otherwise defenseless insect(8).

Periodical cicadas are also responsible for providing a signature resource pulse. A resource pulse is a massive influx of nutrients that occurs both rapidly and infrequently(9). As might be expected with an organism that relies on overwhelming its predators by sheer volume, massive amounts of adult cicadas provide nutrients to the ecosystem. The resource pulse provided by cicadas benefits both plants and animals. Birds and other predators receive a bountiful supply of prey, while cicadas that escape predation eventually die, their carcasses fertilizing the soil(9).

A 2012 study by Louie Yang found that the addition of cicada carcasses to samples of American bellflower rosettes increased plant biomass by 61% with respect to control samples(9). The discarded nymph exoskeletons are composed of keratin and also contribute to the resource pulse(10). The emergence of the periodical cicada is an excellent example of a resource pulse that brings nutrients such as nitrogen and carbon from below ground to above-ground consumers. Additionally, cicadas serve to return nutrients to the soil for use by primary producers.

Representing one of the largest nongaseous releases of nitrogen from below ground, it is worth gathering a better understanding of the cicadas' nitrogen uptake tendencies. Furthermore, studies of $\delta^{13}C$ values in annual cicadas have suggested resource partitioning amongst cicada species in respect to their feeding on C3 or C4 plants(11). In a similar way, it may be possible to identify relationships between periodical cicadas and their host trees.

This study seeks to characterize and quantify $\delta^{13}C$ and $\delta^{15}N$ variations of Brood V periodical cicadas, seeking to identify distinguishable patterns regarding the tree species and sites on which the nymphs developed.

If stable isotope values of cicadas can be used to infer host tree species, future studies could potentially inform efforts to control or preserve cicada populations in an area through manipulation of favorable tree species. Micro-scale cicada mobility might also be observed isotopically based on tree-to-tree movement. Site-specific isotope patterns could yield insight into the effects of fertilizers, soil quality, and runoff. Furthermore, by characterizing cicadas of a certain site using stable isotopes, it may be possible to analyze the impact of the resultant resource pulse by tracking nutrient flow through trophic levels during emergence years. If the stable isotope signal is distinct enough to identify the origin site of a cicada, it may be possible to use these patterns to track broad geographic ranges and mobility of both the insect and its predators.

Methods and Materials:

Permission to collect both discarded exoskeletons and live adults was obtained from the Summit County MetroParks and the Hudson City Parks Department in Ohio. Though permission was obtained to sample both adults and exoskeletons, the experiment only utilized the latter due to the certainty of their origination site. (Due to the mobility of the adult cicadas, it was difficult to reliably discern their host trees.)

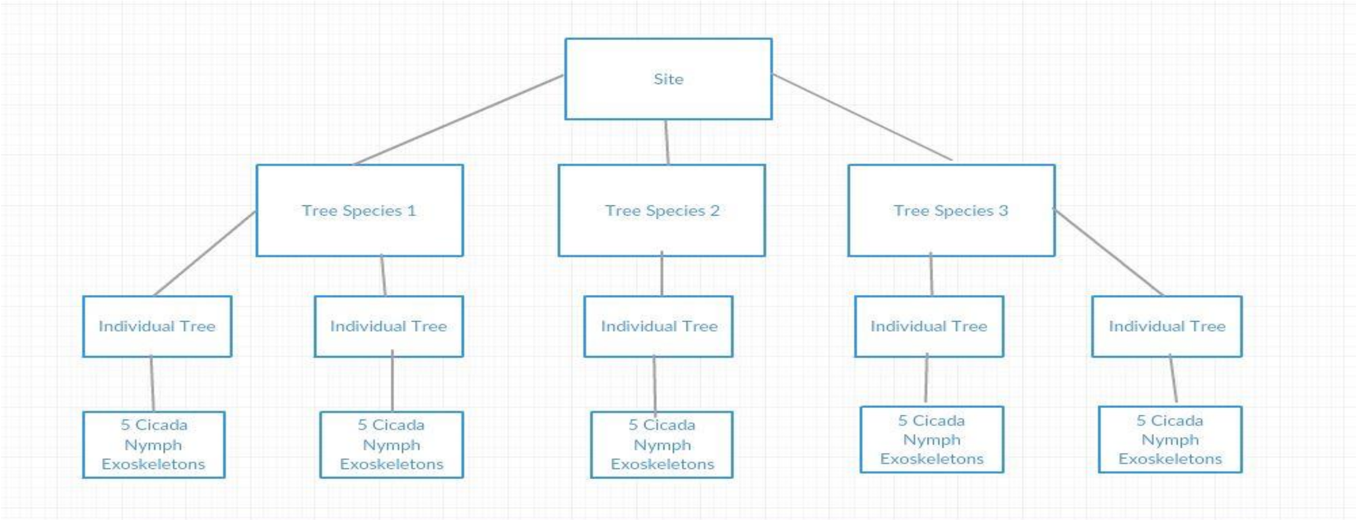

Figure 4: The nested design of the experiment is organized by site and tree species, which are further broken down into individual trees within a species and the sampled cicadas collected from the individual trees. Five cicadas were homogenized into a powder and analyzed for $\delta^{13}C$ and $\delta^{15}N$.

Collection efforts began in late May and continued through August of 2016. Five different sites were utilized: O'Neil's Woods MetroPark (ONW), Furnace Run MetroPark (FR), a park located at 34 North Oviatt St in Downtown Hudson, Ohio (HD), Hudson Springs Park (HS) and a private property on Harmony Road in Bath, Ohio (HR).

Site	Collection Date
O'Neil's Woods MetroPark (ONW)	5/25/2016
Harmony Road (HR)	6/8/2016
Furnace Run MetroPark (FR)	6/11/2016
Hudson Downtown (HD)	6/17/2016
Hudson Springs (HS)	7/5/2016

Table 1: The five sites utilized were collected throughout the summer of 2016.

A nested design was employed to facilitate organized data collection. The sampled sites were the first level of the nested design. Within each site, tree species were catalogued. Multiple samples of a particular tree species were sometimes (but not always) present within a site. As such, the individual tree samples are nested within "tree species". Finally, cicadas were collected and labeled based on the tree species and individual tree where they were collected (**Fig 4**).

In order to discern the tree from which a cicada originated, isolated trees and clusters of one tree species were used for collection. Isolation was determined by considering the tree's critical root zone. The critical root zone is the radius set by the point on the ground directly under the tree's furthest reaching branch, or "drip line"[12]. Samples were collected when no other tree or shrub was within double the radius of the critical root zone.

Samples were collected based on the nested hierarchy described above and were promptly frozen. Once collection was completed, all samples were catalogued along with the host tree species.

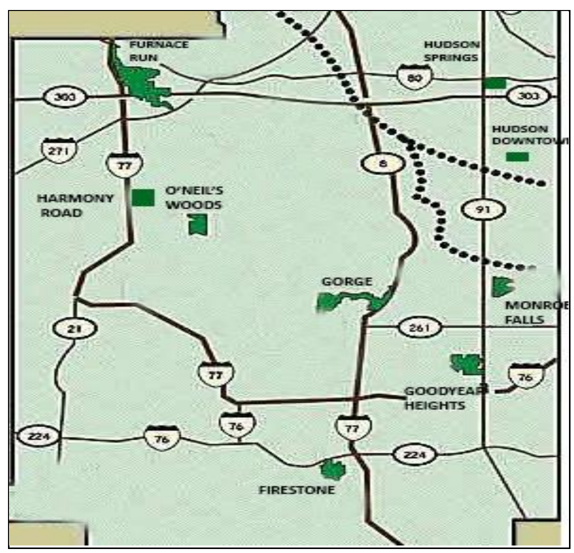

Figure 6: This map of Summit County represents all sites visited. Cicadas were not found in Munroe Falls, Gorge, Firestone and Goodyear Heights, but were abundant at Furnace Run, Harmony Road, O'Neil's Woods, Downtown Hudson, and Hudson Springs. Cicadas were abundant in northern Summit County but difficult to find in southern Summit County[13].

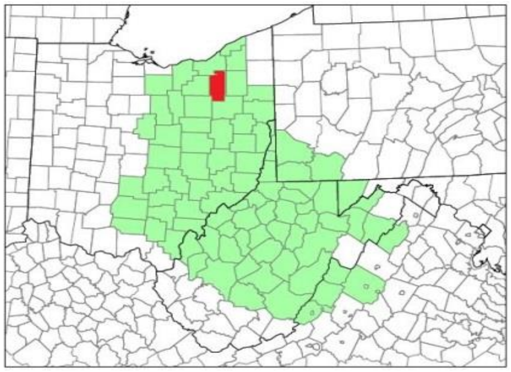

Figure 7: Location of Summit County within the range of Brood V[14].

Site	Tree Species Sampled
O'Neil's Woods MetroPark	*Aesculus glabra*
	Fagus grandifolia
	Tilia americana
	Acer rubrum
Furnace Run MetroPark	*Acer rubrum*
	Aesculus glabra
	Quercus rubra
	Carya ovata
Hudson Downtown	*Pinus virginiana*
	Juglans nigra
Hudson Springs	*Quercus coccinea*
	Quercus palustris
Harmony Road	*Acer rubrum*
	Tilia cordata
	Castanea mollissima
	Fraxinus americana

Table 2: There were 13 different tree species sampled across the five sites.

Upon returning to the lab, samples were prepared for analysis. Five random exoskeletons from each individual tree were selected and pooled together. Each sampling of exoskeletons was cleaned using 87: 13 chloroform-methanol solution. Though the exuviae are composed of keratin, this technique served as a precautionary measure to remove any trace lipids which might serve as a confounding variable when considering $\delta^{13}C$ and $\delta^{15}N$ values. Based on their strong influence on $\delta^{13}C$, lipids are typically removed from stable isotope samples[15]. The chloroform-methanol wash also served to remove dirt and debris. Each wash lasted three minutes and was followed by a one-minute wash using deionized water. Samples were transferred to an ashed vial and covered with foil to prevent contamination. Holes were punched in the foil and the vials were placed in a vacuum dryer overnight to draw off any remaining moisture.

After drying was complete, each sampling of five exoskeletons was homogenized into a powder using a mortar and pestle. Following each homogenization, the mortar and pestle were cleaned using acetone. Powders were placed back into the ashed vials from which they originated.

The final step before data collection was to mass the samples into tin capsules. An interval of 1.000 mg +/- 0.100 mg was used. The powder from each sampling was run on the Isoprime 100 stable isotope mass spectrometer interfaced to an Elementar Vario PYRO Cube Elemental Analyzer. Each sample was run once, with the exception of the samples from ONW, which were run in triplicate to measure repeatability of isotope measurements. USGS standards 40, 41 and 43 were employed to serve as house standards used to correct data. All isotope data are reported in delta notation where $\delta^{13}C$ and $\delta^{15}N$ are determined based on sample data corrected against the LSR line of the USGS standards.

Simple statistics (mean and standard deviation) were calculated in Excel. These calculations gave a brief illustration of variability between tree species as well as between sites. To further the groundwork laid by these calculations, Minitab statistical software was used to perform a general linear model ANOVA. General linear model ANOVA allows for calculation of p value and R^2 when data sets are unbalanced within a nested design. Two GLM ANOVAs were performed. The first used $\delta^{13}C$ as a response and $\delta^{15}N$ as a covariate while the second used $\delta^{15}N$ as the response and $\delta^{13}C$ as a covariate. In both cases, tree species and site were fixed independent variables, with tree species being nested into site and individual tree being nested into tree species.

Results and Discussion:

Preliminary analysis suggested possible site-specific variation of $\delta^{13}C$ and $\delta^{15}N$, while possible tree-specific variation of $\delta^{13}C$ and $\delta^{15}N$ was not as easily discernible. To gain further insight, Minitab was utilized to conduct a general linear model ANOVA.

$\delta^{13}C$ and $\delta^{15}N$ were considered to be covariates for the sake of the GLM ANOVA based on preliminary analysis. Plotting $\delta^{15}N$ against $\delta^{13}C$ for all collected data points, an R^2 of 0.4709 was observed (**Fig 11**). This suggested that $\delta^{13}C$ and $\delta^{15}N$ values are potentially covariates. Treating them as such in the GLM ANOVA model, $\delta^{13}C$ and $\delta^{15}N$ significantly affected each other, with p-values of 0.004 for each model.

Using an alpha-value of 0.05, the results of the GLM ANOVA calculations showed a significant relationship with p-values of 0.00 for site with respect to both δ13C and δ15N. There was also a significant relationship between stable isotope composition and tree species with a p-value of 0.036 for δ13C and a p-value of 0.004 for δ15N. Model summaries explained 96.44% and 97.74% of the variance.

These significant findings suggest that δ13C and δ15N vary based on the tree species a cicada fed on and the site in which it developed. With respect to tree species, isotope variation could be attributed to differences in tree metabolism and relationships with mycorrhizal and nitrogen-fixing organisms.

Variation by site might be explained by soil quality, fertilizers, runoff, and other location-related factors such as proximity to farms and rivers. It should be noted that Hudson City Parks do not use any fertilizer treatments. Therefore, the higher δ15N values of these locations cannot be attributed to animal-based fertilizers.

While collecting samples in the field, no distinction was made between the three possible species of cicada that characterize Brood V. According to the studies by Callaham, data regarding annual cicadas suggests that different species partition resources amongst themselves and emerge at different points throughout the summer. Callaham's cicadas showed trends in δ13C and δ15N that were characteristic to each species. It is possible that the trends observed in the current study were actually due to partitioning and metabolic differences between the three species of Brood V. In other words, cicada species may vary isotopically, and different cicada species may be found on different tree species and different locations.

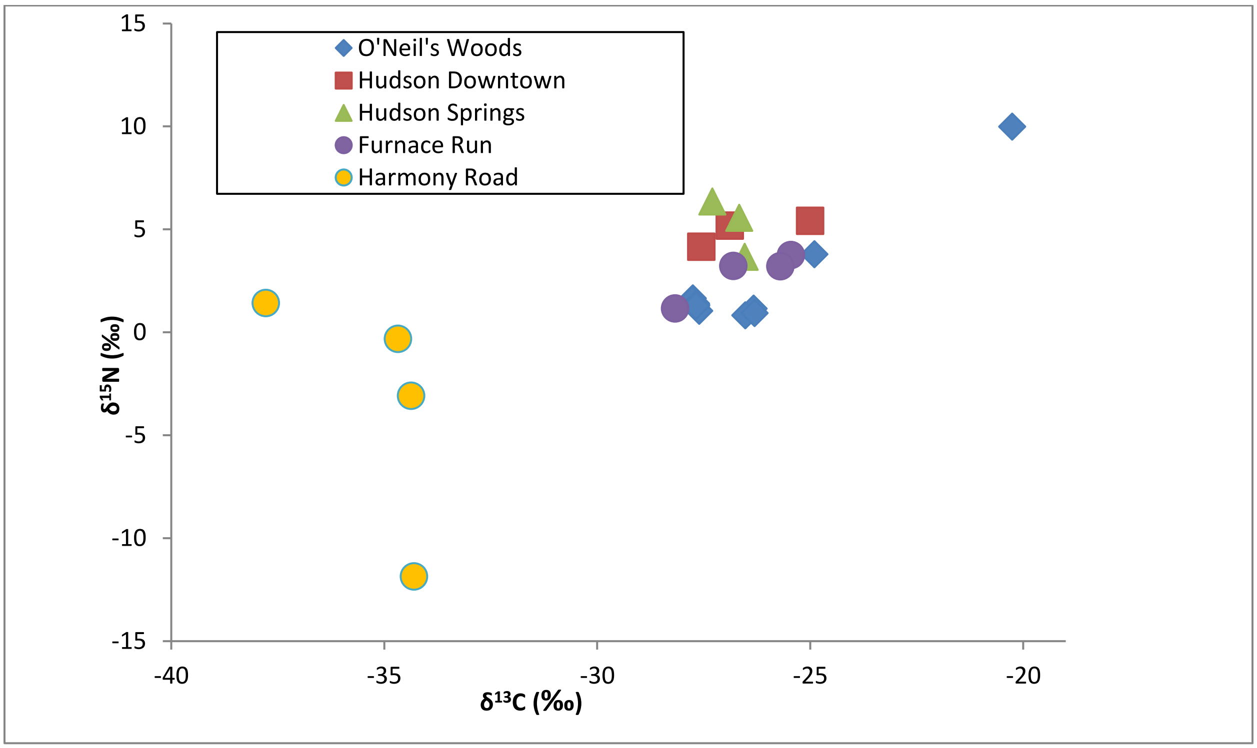

Figure 8: Preliminary analysis suggested δ13C and δ15N variability based on site. Harmony Road was a clear outlier, while both Hudson parks seemed to have higher δ15N values. Each dot represents a tree within a given site.

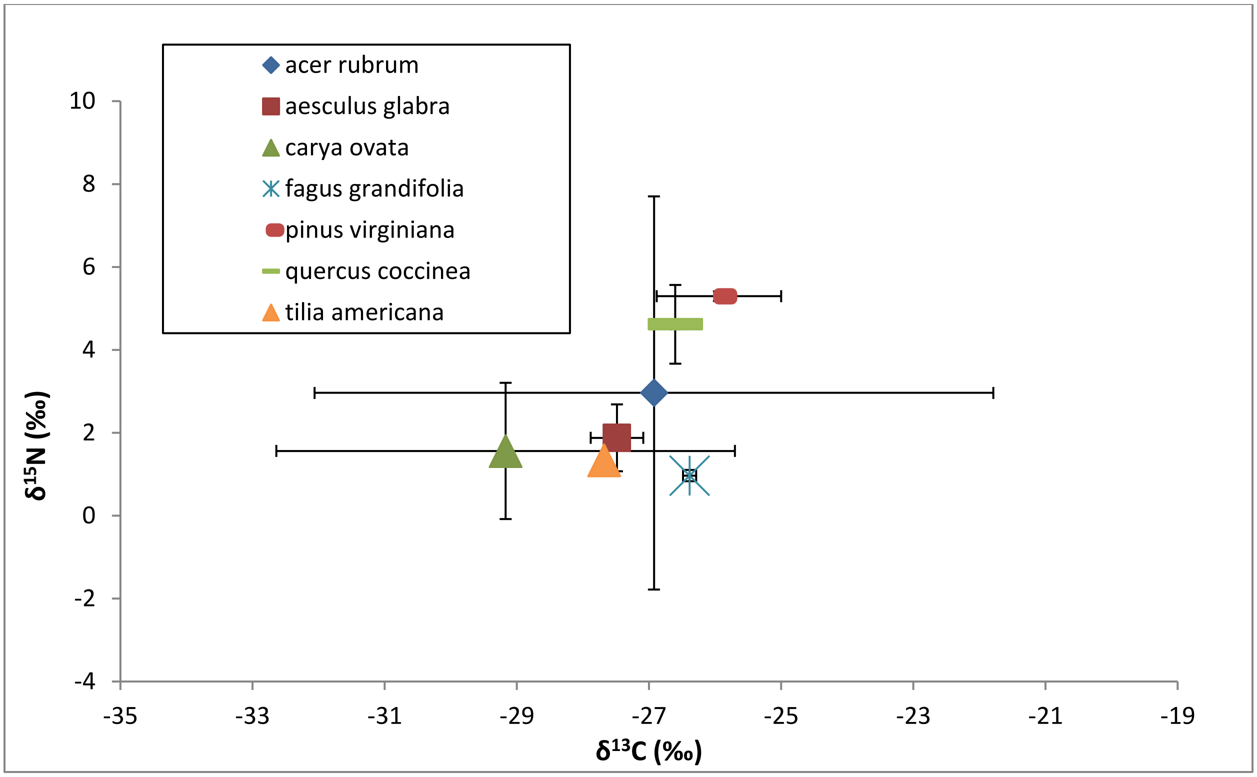

Figure 9: $\delta^{13}C$ and $\delta^{15}N$ for tree species in which multiple samples were taken. Patterns were not easily discernable.

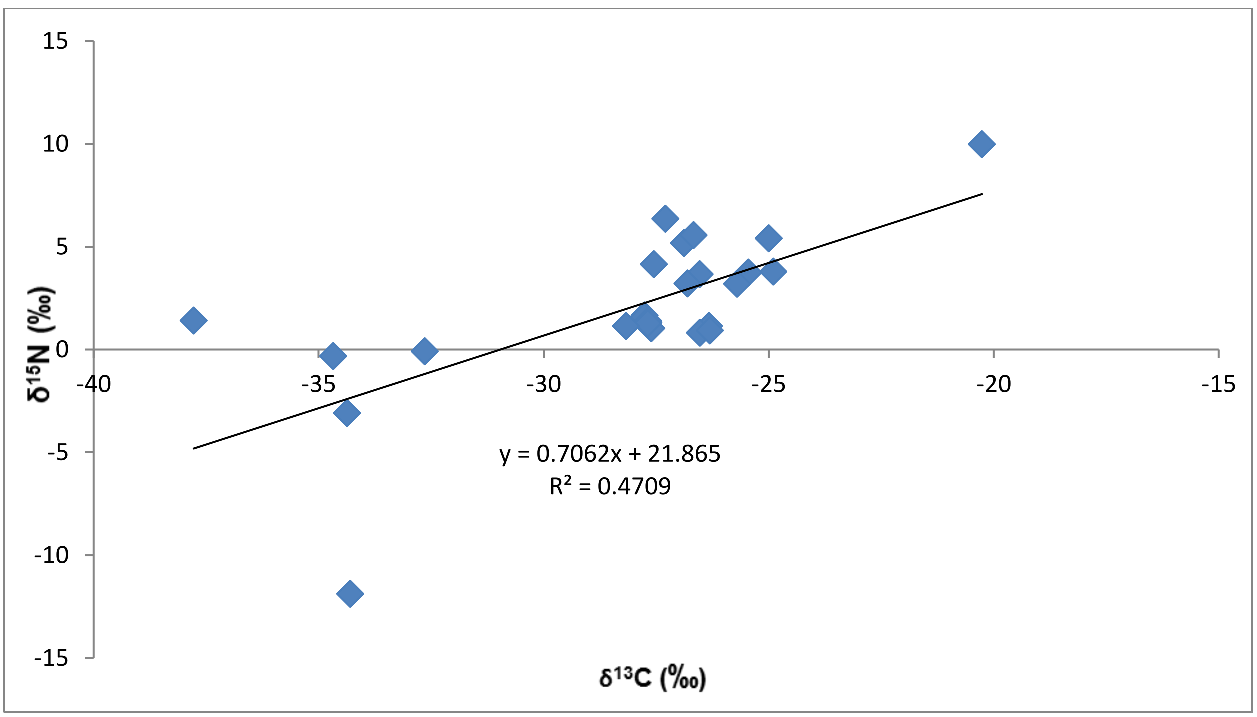

Figure 10: When $\delta^{15}N$ was plotted against $\delta^{13}C$, the R^2 was observed to be 0.4709, suggesting that the stable isotopes were covariates. A p value of 0.001 was calculated using an orthogonal linear regression, with $\delta^{13}C$ as a predictor, $\delta^{15}N$ as the response, and 5.59 as the Error Variance Ratio (Y/X). Error Variance Ratio was determined based on the triplicate data from ONW. Standard deviations based on each tree's $\delta^{13}C$ and $\delta^{15}N$ values were determined. $\delta^{15}N$ was divided by $\delta^{13}C$ for each tree species and these values were averaged, equaling 5.59.

The findings of this study could inform future experiments. Knowledge of tree-species specific isotope values could be used to investigate interactions between periodical cicadas and host trees. Micro-scale mobility of cicadas could also be studied. Comparisons between the isotopic composition of exoskeletons and adult cicadas found in a particular tree could be used to assess the tree species adults fed on as nymphs. It would also be interesting to relate this study's findings to selection of chorus trees. However, this kind of assessment would depend upon the tree species in question yielding isotopically dissimilar cicadas. This was not always the case with this study's results. For example, *Pinus virginiana* and *Juglans nigra* both exhibited similar $\delta^{13}C$ values (-26.88355249 and -27.55320564 respectively) and δ15N values (4.14852 and 5.178785 respectively). Both tree species were sampled from Hudson Downtown Park, meaning that micro-scale mobility would be difficult to assess at this location based on the collected data.

Considering site-specific variance, the possibilities are even wider. One of the first observations made in this study was the distribution of Brood V cicadas throughout Summit County Ohio. According to personal observations there was a high frequency of periodical cicadas in northern Summit County and relatively few in southern parts of the county. As seen in **Figure 6**, O'Neil's Woods, Furnace Run, Harmony Road, Hudson Downtown and Hudson Springs are all within the northern half of the county. Visits to Firestone, Goodyear Heights, Munroe Falls and Gorge in May and June of 2016 yielded no cicadas.

Comparisons of environmental factors as well as land usage in the northern versus the southern half of the county might provide insight into how to bring back struggling cicada populations(1). Perturbation of forests and soil could be to blame, since construction and deforestation cause damage to the roots that cicadas depend on. Perhaps the construction history of northern Summit County is different than the southern half. It would also be interesting to consider runoff effects based on the route of the Cuyahoga River as it travels from the northwest to southeast parts of the county.

Based on the statistically significant findings that $\delta^{13}C$ and $\delta^{15}N$ do in fact vary by site, measurement of stable isotopes in cicada populations could be used to broadly identify macro-scale mobility patterns. However, isotopic overlap between some sites shows that only a portion of sites could be differentiated based on stable C and N analysis alone.

If cicadas exhibit site-specific variation of $\delta^{13}C$ and $\delta^{15}N$, it is possible that their predators do too. Using $\delta^{13}C$ and $\delta^{15}N$ as indicators of foraging location, it may be possible to draw broad conclusions about avian predator ranges. By isotopically characterizing sites, it may be possible to predict characteristic isotope values of a predator feeding in a particular site or collection of sites. However, several sites may be isotopically similar. As a result, further investigations would need to be conducted to isotopically characterize sites within a region and to determine at what spatial scale (if any) inferences could be drawn about mobility.

By broadly mapping a particular avian predator's range, it may be possible to identify isolated populations of said predator in the process. This information could be considered when developing a region for human use, so as to optimize wildlife mobility, prevent isolation and maintain threshold populations of a predator of interest.

Finally, characterization of the $\delta^{13}C$ and $\delta^{15}N$ values in cicada exoskeletons lends itself to studies of resource pulses. The massive influx of organic material at the bases of trees provides a large amount of nutrients, which may be reflected in the tree's rings. By utilizing stable isotopes, it may be possible to analyze tree rings corresponding with emergence years to quantify and characterize trends in cicada populations over time. Again, this may find utility in identifying and encouraging struggling cicada populations. The data collected in the current study could be applied to future isotope data collected from local trees as a means of predicting the expected impact of cicada fertilization on a site to site basis.

Conclusion:

The periodical cicada is a marvel of nature, unique in its combination of lifecycle, survival strategy and mating behaviors. Living most of its life feeding on underground roots, the relationship between cicada, tree species, and site holds significant importance. Using $\delta^{13}C$ and $\delta^{15}N$ values as markers, this study supported the hypotheses that $\delta^{13}C$ and $\delta^{15}N$ vary by tree species and by site. These findings could be used to discern cicada ranges on a micro and macro scale by further assessing tree-to-tree and site-to-site patterns. Use of these findings could be taken a step higher and applied to trophic studies relating to behavior of cicada predators. Characterization of a site and the ranges of its inhabitants – both predators and prey – might allow for improved conservation by allowing identification of isolated populations and therefore improving efforts to maintain threshold population sizes. Lastly, characterization of stable isotopes in cicada nymph exoskeletons can be used to better understand the impact of the resource pulse they provide.

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Appended Data:

Sample	Location	Tree species	Corr $\delta^{13}C$	Corr $\delta^{15}N$
ONW 11.raw	O'Neil's Woods MetroPark	*aesculus glabra*	-27.75593352	1.658404095
ONW 12.raw	O'Neil's Woods MetroPark	*aesculus glabra*	-27.60792152	1.047766066
ONW 13.raw	O'Neil's Woods MetroPark	*aesculus glabra*	-27.77091168	1.593939069
ONW 21.raw	O'Neil's Woods MetroPark	*fagus grandifolia*	-26.3249997	1.150954013
ONW 22.raw	O'Neil's Woods MetroPark	*fagus grandifolia*	-26.5262775	0.822866631
ONW 23.raw	O'Neil's Woods MetroPark	*fagus grandifolia*	-26.30654119	0.930244189
ONW 31.raw	O'Neil's Woods MetroPark	*tilia americana*	-27.67319342	1.332321871
ONW 32.raw	O'Neil's Woods MetroPark	*tilia americana*	-27.67783578	1.403649075
ONW 33.raw	O'Neil's Woods MetroPark	*tilia americana*	-27.6835921	1.282506123
ONW 51.raw	O'Neil's Woods MetroPark	*acer rubrum*	-24.89897675	3.789097621
ONW 53.raw	O'Neil's Woods MetroPark	*acer rubrum*	-20.26050796	9.990886363
HD 1A1.raw	Hudson Downtown Park	*pinus virginiana*	-26.88355249	5.178784932
HD 2A1.raw	Hudson Downtown Park	*pinus virginiana*	-25.00044298	5.410924653
HD 3A1.raw	Hudson Downtown Park	*juglans nigra*	-27.55320564	4.148520479
HS 2.raw	Hudson Springs Park	*quercus coccinea*	-26.53575099	3.66645166
HS 3.raw	Hudson Springs Park	*quercus coccinea*	-26.66788415	5.565666133
HS 5.raw	Hudson Springs Park	*quercus palustris*	-27.29396234	6.357697421
FR 1.raw	Furnace Run	*acer rubrum*	-28.17256049	1.14972664
FR 2.raw	Furnace Run	*aesculus glabra*	-26.80487519	3.213888481
FR 3.raw	Furnace Run	*quercus rubra*	-25.44994184	3.746437436
FR 4.raw	Furnace Run	*carya ovata*	-25.69988501	3.205448873
FR 5.raw	Furnace Run MetroPark	*carya ovata*	-32.64037243	-0.081270077
HR 1.raw	Harmony Road	*acer rubrum*	-34.37029344	-3.086309902
HR 2.raw	Harmony Road	*tilia cordata*	-37.77754759	1.421310198
HR 3.raw	Harmony Road	*castanea mollissima*	-34.67767572	-0.314480521
HR 4.raw	Harmony Road	*fraxinus americana*	-34.30091322	-11.86386348

General Linear Model: d13C versus d15N, Site, Tree Species

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Method

Factor coding  (-1, 0, +1)


Factor Information

Factor               Type   Levels  Values
Site                 Fixed       5  FR, HD, HR, HS, ONW
Tree Species(Site)   Fixed      14  acer rubrum(FR), aesculus glabra(FR), carya
ovata(FR),

                                    quercus rubra(FR), juglans nigra(HD), pinus
                                    virginiana(HD), acer rubrum(HR), castanea

mollissima(HR),
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tilia cordata(HR), quercus coccinea(HS), quercus palustris(HS), aesculus glabra(ONW), fagus grandifolia(ONW), tilia americana(ONW)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
d15N	1	17.489	17.489	16.61	0.004
Site	4	111.806	27.952	26.54	0.000
Tree Species(Site)	9	36.400	4.044	3.84	0.036
Error	8	8.424	1.053		
Total	22	236.796			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.02617	96.44%	90.22%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-32.82	1.02	-32.22	0.000	
d15N	1.525	0.374	4.08	0.004	14.39
Site					
FR	1.736	0.453	3.84	0.005	2.59
HD	-1.126	0.950	-1.19	0.270	8.93
HR	-1.78	1.34	-1.33	0.221	17.81
HS	-2.49	1.20	-2.09	0.071	14.16
Tree Species(Site)					
acer rubrum(FR)	1.161	0.991	1.17	0.275	1.87
aesculus glabra(FR)	-0.619	0.920	-0.67	0.520	1.61
carya ovata(FR)	-0.465	0.772	-0.60	0.563	1.67
juglans nigra(HD)	0.068	0.664	0.10	0.920	1.24
acer rubrum(HR)	4.94	1.24	4.00	0.004	2.90
castanea mollissima(HR)	0.404	0.848	0.48	0.646	1.37
quercus coccinea(HS)	1.674	0.708	2.36	0.046	1.41
aesculus glabra(ONW)	-0.737	0.489	-1.51	0.170	1.36
fagus grandifolia(ONW)	1.298	0.495	2.62	0.031	1.40

Regression Equation

Site	Tree Species(Site)	
FR	acer rubrum	d13C = -29.93 + 1.525 d15N
FR	aesculus glabra	d13C = -31.71 + 1.525 d15N
FR	carya ovata	d13C = -31.552 + 1.525 d15N
FR	quercus rubra	d13C = -31.16 + 1.525 d15N
HD	juglans nigra	d13C = -33.88 + 1.525 d15N
HD	pinus virginiana	d13C = -34.02 + 1.525 d15N
HR	acer rubrum	d13C = -29.66 + 1.525 d15N
HR	castanea mollissima	d13C = -34.20 + 1.525 d15N
HR	tilia cordata	d13C = -39.95 + 1.525 d15N

HS	quercus coccinea	d13C = -33.64 + 1.525 d15N
HS	quercus palustris	d13C = -36.99 + 1.525 d15N
ONW	aesculus glabra	d13C = -29.897 + 1.525 d15N
ONW	fagus grandifolia	d13C = -27.862 + 1.525 d15N
ONW	tilia americana	d13C = -29.721 + 1.525 d15N

Fits and Diagnostics for Unusual Observations

Obs	d13C	Fit	Resid	Std Resid		
10	-28.173	-28.173	0.000	*		X
11	-26.805	-26.805	-0.000	*		X
12	-25.450	-25.450	-0.000	*		X
13	-25.700	-26.664	0.964	2.50	R	
14	-32.640	-31.676	-0.964	-2.50	R	
17	-27.553	-27.553	-0.000	*		X
18	-26.536	-28.050	1.514	2.39	R	
19	-26.668	-25.154	-1.514	-2.39	R	
20	-27.294	-27.294	0.000	*		X
21	-34.370	-34.370	0.000	*		X
22	-37.778	-37.778	-0.000	*		X
23	-34.678	-34.678	-0.000	*		X

R Large residual
X Unusual X

General Linear Model: d15N versus d13C, Site, Tree Species

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Site	Fixed	5	FR, HD, HR, HS, ONW
Tree Species(Site)	Fixed	14	acer rubrum(FR), aesculus glabra(FR), carya ovata(FR), quercus rubra(FR), juglans nigra(HD), pinus virginiana(HD), acer rubrum(HR), castanea mollissima(HR), tilia cordata(HR), quercus coccinea(HS), quercus palustris(HS), aesculus glabra(ONW), fagus grandifolia(ONW), tilia americana(ONW)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
d13C	1	5.075	5.0754	16.61	0.004
Site	4	46.692	11.6730	38.20	0.000
Tree Species(Site)	9	21.801	2.4223	7.93	0.004
Error	8	2.445	0.3056		
Total	22	108.181			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.552812	97.74%	93.79%	*

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	15.39	3.13	4.92	0.001	
d13C	0.443	0.109	4.08	0.004	9.14
Site					
FR	-0.841	0.283	-2.97	0.018	3.49
HD	1.174	0.368	3.19	0.013	4.62
HR	-0.286	0.791	-0.36	0.727	21.39
HS	2.028	0.355	5.71	0.000	4.31
Tree Species(Site)					
acer rubrum(FR)	-0.926	0.476	-1.95	0.088	1.48
aesculus glabra(FR)	0.533	0.473	1.13	0.293	1.46
carya ovata(FR)	-0.072	0.425	-0.17	0.869	1.74
juglans nigra(HD)	-0.217	0.350	-0.62	0.553	1.18
acer rubrum(HR)	-2.974	0.471	-6.32	0.000	1.45
castanea mollissima(HR)	-0.067	0.463	-0.14	0.889	1.40
quercus coccinea(HS)	-1.024	0.341	-3.01	0.017	1.12
aesculus glabra(ONW)	0.387	0.265	1.46	0.183	1.38
fagus grandifolia(ONW)	-0.665	0.277	-2.40	0.043	1.51

Regression Equation

Site	Tree Species(Site)	
FR	acer rubrum	d15N = 13.62 + 0.443 d13C
FR	aesculus glabra	d15N = 15.08 + 0.443 d13C
FR	carya ovata	d15N = 14.47 + 0.443 d13C
FR	quercus rubra	d15N = 15.01 + 0.443 d13C
HD	juglans nigra	d15N = 16.34 + 0.443 d13C
HD	pinus virginiana	d15N = 16.78 + 0.443 d13C
HR	acer rubrum	d15N = 12.12 + 0.443 d13C
HR	castanea mollissima	d15N = 15.03 + 0.443 d13C
HR	tilia cordata	d15N = 18.14 + 0.443 d13C
HS	quercus coccinea	d15N = 16.39 + 0.443 d13C
HS	quercus palustris	d15N = 18.44 + 0.443 d13C
ONW	aesculus glabra	d15N = 13.70 + 0.443 d13C
ONW	fagus grandifolia	d15N = 12.65 + 0.443 d13C
ONW	tilia americana	d15N = 13.59 + 0.443 d13C

Fits and Diagnostics for Unusual Observations

Obs	d15N	Fit	Resid	Std Resid		
10	1.150	1.150	0.000	*		X
11	3.214	3.214	0.000	*		X
12	3.746	3.746	0.000	*		X
17	4.149	4.149	0.000	*		X
18	3.666	4.645	-0.979	-2.50	R	
19	5.566	4.587	0.979	2.50	R	
20	6.358	6.358	0.000	*		X
21	-3.086	-3.086	-0.000	*		X
22	1.421	1.421	-0.000	*		X
23	-0.314	-0.314	-0.000	*		X

R Large residual
X Unusual X