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INFLUENCE OF DIET COMPOSITION ON THE DYNAMICS OF STABLE ISOTOPES IN DIFFERENT TISSUES OF GROUND BEETLES

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During the last two decades, stable isotope analysis has been widely used for studies on food webs (Ponsard, Arditi, 2000; Pollierer et al., 2009; Girard et al., 2011), trophic niche differentiation (Schneider et al., 2004; Ikeda et al., 2010; Okuzaki et al., 2010; Sasakawa et al., 2010; Semenina, Tiunov, 2011a-b; Zalewski et al., 2013), the diet composition of individual species (Moulton, 2011; Sasakawa, 2011), as well as below- and aboveground animal community structure (Tiunov et al., 2013).

Due to technical constraints, various tissues or body parts are routinely used in isotope analyses. In particular, several conspecifics have to be bulked for the analysis of mites and collembolans while for larger invertebrates either homogenized whole specimens or certain body parts are often taken. However, different tissues and body parts have different chemical compositions, also differing in metabolic activity. Therefore, their isotopic balance may vary considerably. Changes in the isotopic composition of various tissues of model animals have been addressed in a few studies only (Webb et al., 1998; Gratton, Forbes, 2006; Phillips, Eldridge, 2006).

During several last years, interesting data about the features of isotopic balance in ground beetles have been accumulated by different researchers (Ikeda et al., 2010; Okuzaki et al., 2010; Sasakawa et al., 2010; Moulton, 2011; Sasakawa, 2011; Girard et al., 2011; Zalewski et al., 2012, 2013). However, to interpret the results obtained, not only quantitative characteristics of changes in the isotope composition of the whole body, but also of their various tissues are required. As regards Carabid beetles, such data are absent.

In our laboratory experiment, the dynamics of stable carbon and nitrogen isotope ratios in the tissues and body parts of two common European forest carabids have been studied, *Pterostichus oblongopunctatus* (F., 1787) and *Platynus assimilis* (Payk., 1790).

Pterostichus oblongopunctatus (F., 1787)
number of specimens: 824

Platynus assimilis (Payk., 1790)
number of specimens: 398

Data of trapping: October 2012

Locality: Sokolniki Park (Moscow)

Habitat: mixed forest

Start of the hibernation: October 2012

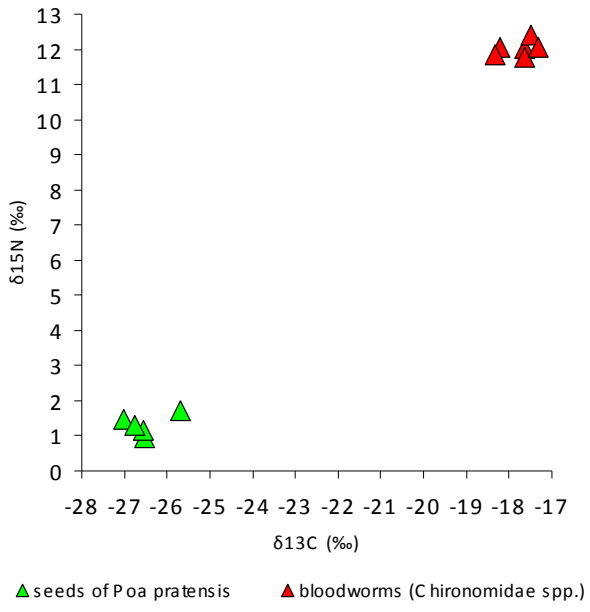
Conditions of the hibernation:

SD; T = +2°C (♂, ♀ - separately)

End of the hibernation: January 2013

Conditions of keeping after the hibernation: LD; T = +14°···+15°C (♂, ♀ - separately)

In the case of *P. oblongopunctatus* four different diets were used: bloodworms, seeds of *Poa pratensis*, mixed diet, without food, but only bloodworms and seeds in the case of *P. assimilis*.



The isotope composition of food items varied slightly and failed to change during the experiment.

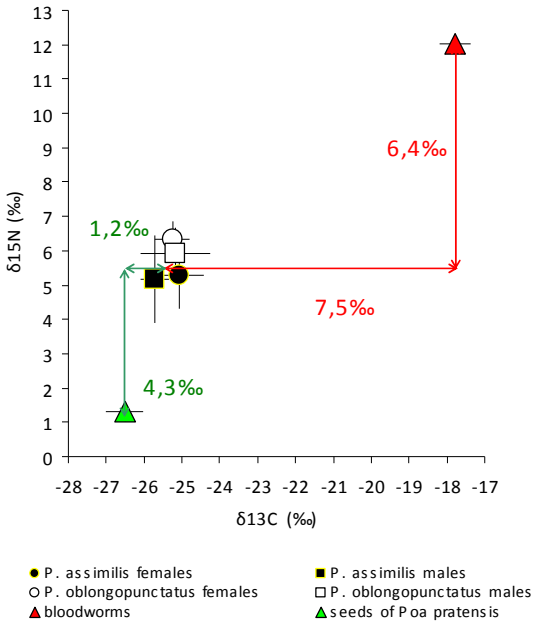
The experiment lasted 80 days from January 28 until April 10. Beetles were sampled either every ten days or 20 days in some cases. Each time, five females and five males were sampled and dissected in each species on each diet. The following tissues or body parts were taken: muscles of the prothorax, fat-body, gonads; elytra, hindgut with excrements. All material was oven-dried about 48 hours.

Isotope ratios were analyzed using a Thermo-Finnigan Delta V Plus Isotope Ratio Mass Spectrometer at the Joint Usage Center, Institute of Ecology and Evolution of the Russian Academy of Sciences in Moscow.

Stable isotope composition was expressed in delta units ("δ") as a deviation from the international standards and recalculated per mile (‰), according to the following formula: δE (‰) = (R_{sample} / $R_{standard}$ - 1) × 1000, where E - is an element (C or N), R - is the molar ratio of heavy / light isotopes. The analytical error for both heavy isotopes was no more than ±0.2‰.

The isotope ratios were compared using three-way ANOVA followed by Tukey's HSD *post hoc* test with species, sex, diets, and tissues as factors as well as Kruskal-Wallis test for multiple comparisons of mean ranks for all groups ($\alpha = 0.05$).

The differences in isotopic ratios in the muscles of model species and in food before the experiment

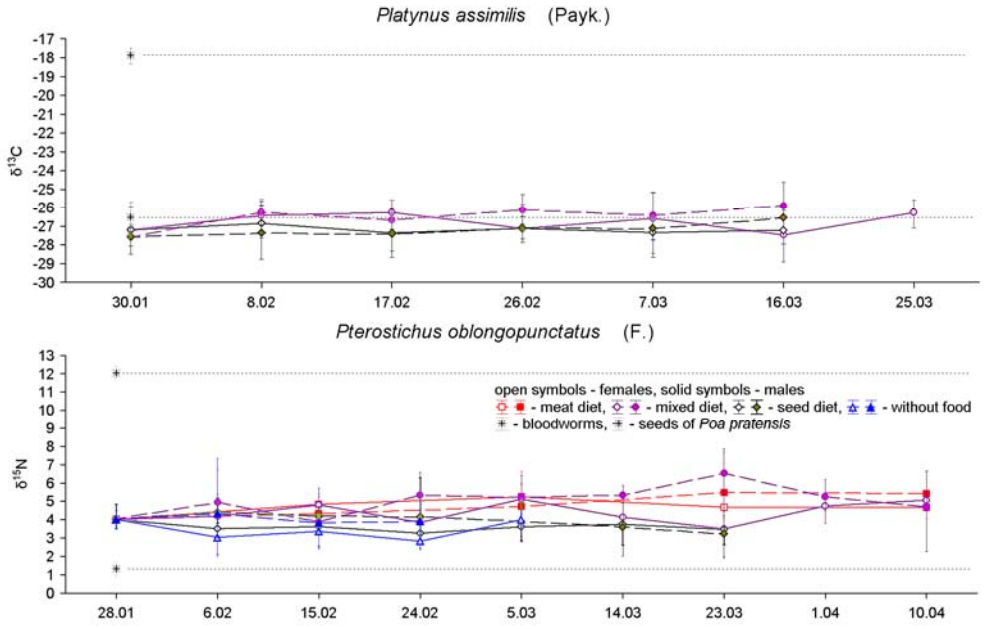


After hibernation, the different tissues or body parts of both species showed that the relative amount of both heavy isotopes was considerably less as compared to animal food, but distinctly greater as compared to vegetable food.

As a result of our experiment, considerable variability in isotopic signatures and significant differences of isotope composition in the tissues of both species were revealed.

The elytra were characterized by the most metabolic passivity. In both species, its isotopic signature was only barely dependent on the isotopic composition of food and changed only to a few degrees.

Changes in $\delta^{13}C$ and $\delta^{15}N$ values in chitin of model species on the different diets



points - means; whiskers – min - max

However, in several cases the interspecific differences, as well as those between the sexes, were significant.

General linear modeling (three-way ANOVA) of $\delta^{13}C$ and $\delta^{15}N$ ratios in elytra as dependent on species, sex, and diets as predictors

Variable	$\delta^{13}C$	$\delta^{15}N$
species	3.1 *0.08*	**27.3** *0.0*
sex	**8.4** *0.004*	0.2 0.6
diet	**29.5** *0.0*	**27.7** *0.0*
species - sex	1.1 *0.3*	**14.1** *0.0002*
species - diet	0.2 0.7	0.5 0.5
species - sex – diet	2.2 0.1	0.2 0.6

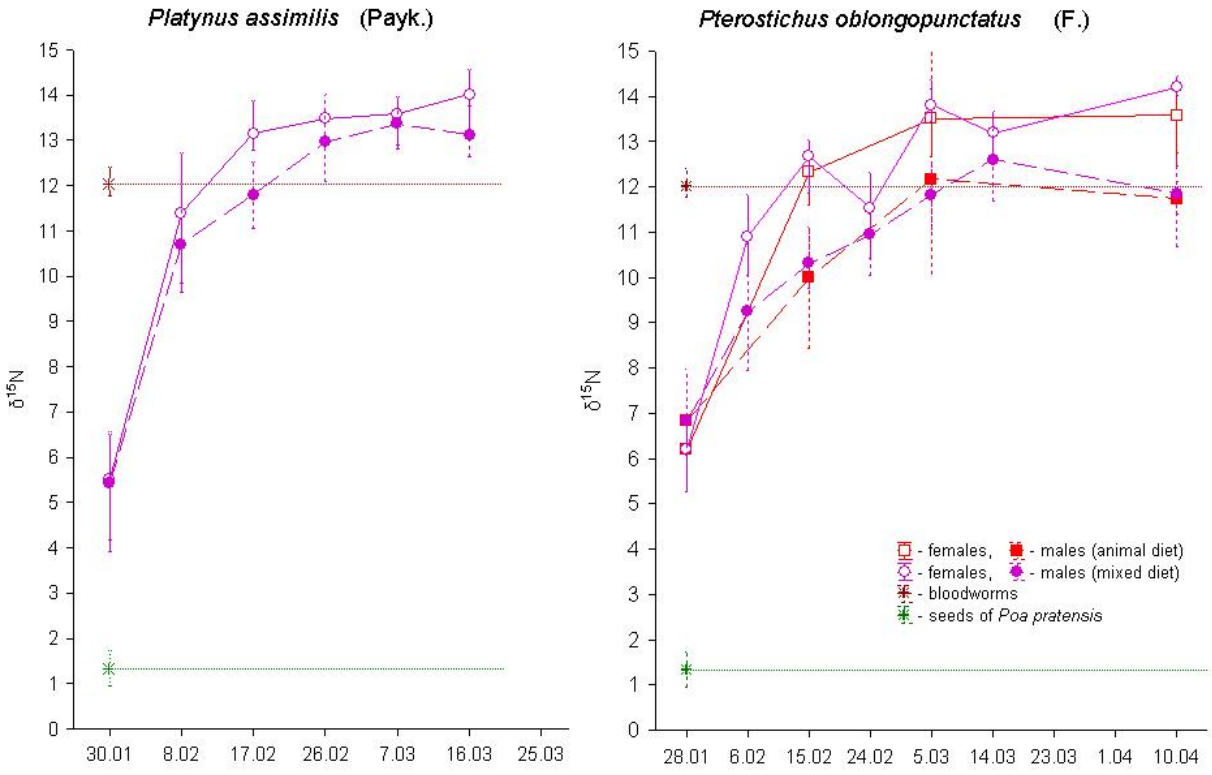
in numerator – F-ratios, in denominator – p-values

The isotopic composition of gonads changed more quickly. The rate of these changes in *P. oblongopunctatus* and *P. assimilis* differed significantly.

In females of both species, the relative amount of heavy N virtually reached the level of its animal food by the tenth day of the experiment. The difference in $\delta^{15}N$ values in gonads and bloodworms was no more than 1‰. Starting from the twentieth day, the relative amount of heavy N in gonads exceeded that in the food. On the last day, the mean $\delta^{15}N$ values in gonads of both species were more than 2‰ higher as compared to the food. On the contrary, the enrichment of male gonads with heavy N was species-specific.

The relative amount of heavy N reached the food level in *P. assimilis* by the twentieth day, versus only by the fortieth day in *P. oblongopunctatus*. Moreover, at the end of the experiment, the mean $\delta^{15}N$ values in gonads of males of *P. assimilis* were even by 1‰ higher as compared to the food, but in *P. oblongopunctatus* it was not so.

Changes in $\delta^{15}N$ values in gonads of the model species on diets with animal food



points - means; whiskers – min - max

Thus, significant variation in isotopic composition of gonads of both species and sexes was established in most cases in contrast to the elytra both in $\delta^{15}N$ and $\delta^{13}C$ values.

General linear modeling (three-way ANOVA) of $\delta^{13}C$ and $\delta^{15}N$ ratios in gonads as dependent on species, sex, and diets as predictors

Variable	$\delta^{13}C$	$\delta^{15}N$
species	**35.05** *<0.001*	1.1 *0.3*
sex	**13.0** *<0.001*	**24.7** *<0.001*
diet	**1071.7** *<0.001*	**1170.7** *<0.001*
species - sex	**6.6** *0.01*	1.9 *0.2*
species - diet	**10.8** *0.001*	**14.03** *<0.001*
species - sex – diet	**9.99** *0.002*	**12.04** *<0.001*

in numerator – F-value, in denominator – p-value

The features observed could probably be explained by differences in the regulation both of activation or reactivation of the gonads and the termination of an imaginal diapause. According to Thiele, both these species are spring breeders, but *P. oblongopunctatus* has an obligatory dormancy in the adults (parapause), while *P. assimilis* has a facultative dormancy (quiescence). In both cases, dormancy is governed by the photoperiod. However, in *P. assimilis* the dormancy of both sexes is terminated by one and the same factor (a long day), but in *P. oblongopunctatus* by different factors: in males by a short day, in females by a short day changing into a long one.

Types of dormancy and dormancy control in carabids (according to Thiele, 1977)

Adults			*Larvae*			Examples
Season of dormancy (if any)	Type of dormancy	Terminated by	Season of dormancy (if any)	Type of dormancy	Terminated by	
Hibernation	Photoperiodic Parapause	short day (with temperature optimum at 15°C) → long day	No dormancy			*Pterostichus oblongopunctatus* females
	Photoperiodic Quiescence	short day				*Pterostichus oblongopunctatus* males
	Photoperiodic Quiescence	long day				*Platynus assimilis*

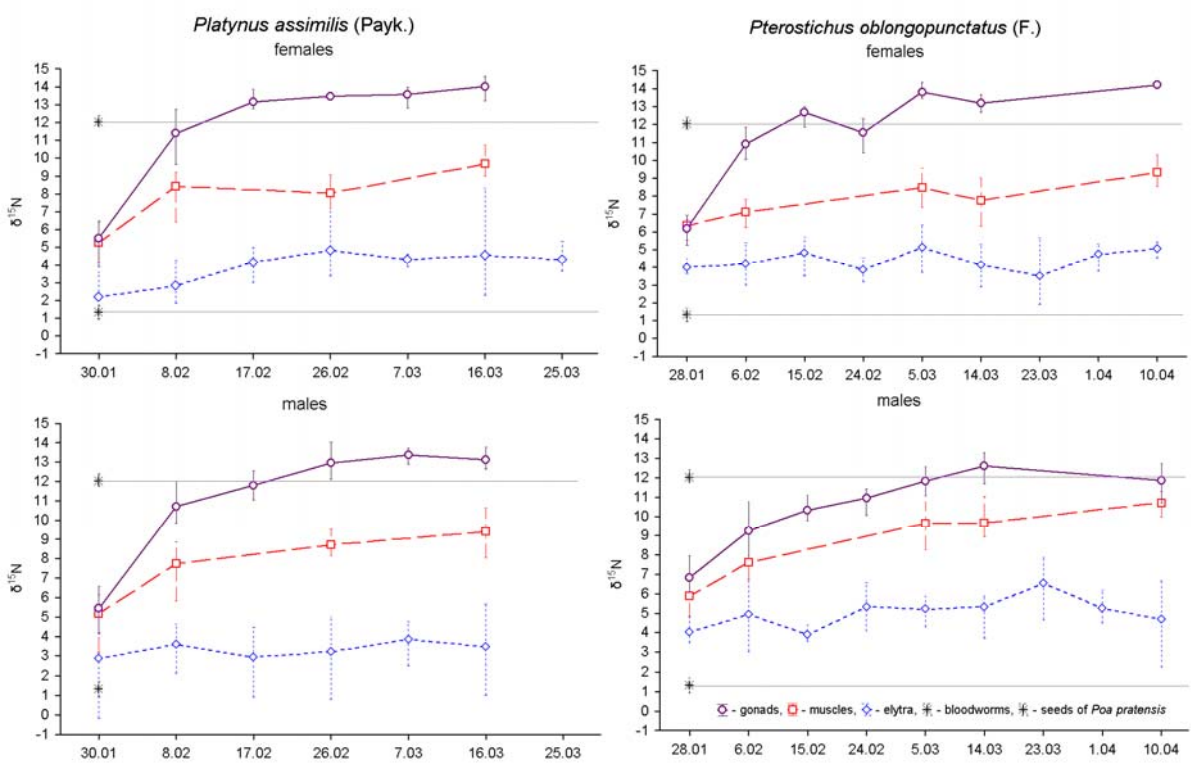
As soon as the size and weight of the gonads increased dramatically during the breeding period, they *tend to be enriched in heavy isotopes*. Without any doubts, ***it must have affected the isotope composition*** of the whole specimen. Thus, we must consider this feature when we design ecological studies in which stable isotope analysis is to be used.

Our results suggest that the isotopic composition of different tissues or body parts in Carabid beetles can vary over a wide range.

At the beginning of the experiment, we did not find any significant differences in the mean values of both $\delta^{13}C$ and $\delta^{15}N$ in gonads, muscles, fat-body, and excrements of each species. However, starting from the tenth day until the end of the experiment, in all study tissues and body parts, except the elytra, changes in isotope composition in favor of the heavy isotopes were observed.

For example, during the experiment, the differences between the mean $\delta^{15}N$ values of the elytra and gonads reached 7.0‰ or 9.5‰ in *P. assimilis*, and 4.5‰ or 9.0‰ in *P. oblongopunctatus*. Starting from the tenth day and up to the end of the experiment, the relative amounts of heavy N in the muscles were significantly, by 3.0‰ to 6.0‰, higher as compared to the elytra. However, the rate of change in isotopic composition in the muscles was 1.5 to 2.0 times less as compared to the gonads.

Changes in $\delta^{15}N$ values in different tissues and body parts of the model species on a mixed diet



points - means; whiskers – min - max

Apparently, the great distinctions in the isotopic signatures were caused by the $\delta^{13}C$ and $\delta^{15}N$ values in the animal food two or even three times exceeding as compared to the different tissues or body parts of the beetles.

On the other hand, the isotopic signatures of the tissues and body parts in the beetles living on the diets without animal food were relatively stable, as their differences from the isotope composition of the food were minor.

It is important that the newly established (stabilized) isotopic balance can vary significantly.

For example, in *P. oblongopunctatus*, significant differences in the isotopic signatures of the elytra, muscles, as well as gonads of males and females were established.

Differences in $\delta^{13}C$ and $\delta^{15}N$ values in males and females of Pterostichus oblongopunctatus (F.) on a mixed diet (after Kruskal-Wallis test)

$\delta^{15}N$

	Elytra		*Muscles*		*Gonads*	
♀	4.39		8,46		13.14	
♂	**2.97** *0.017*	5.45	**3.27** *0.007*	9,90	**3.84** *<0.001*	11.81

$\delta^{13}C$

	Elytra		*Muscles*		*Gonads*	
♀	-26.22		-21.99		-19.69	
♂	1.22 *1.0*	-25.82	1.14 *1.0*	-21.68	**4.07** *<0.001*	-21.42

in numerator – z-values, in denominator – p-values

In addition, similar variations in isotopic signatures of the elytra, muscles, and gonads of males of *P. oblongopunctatus* and in both sexes of *P. assimilis* were revealed.

In both cases, both elytra and muscles differed considerably only in $\delta^{15}N$ values while the gonads also in $\delta^{13}C$ values.

Differences in $\delta^{13}C$ and $\delta^{15}N$ values

in the model species in a mixed diet (after Kruskal-Wallis test)

species	sex	$\delta^{13}C$				$\delta^{15}N$			
				Elytra					
Pterostichus oblongopunctatus (F.)	♀	-26.22				4.39			
	♂	1.22 *1.0*	-25.82			**2.97** *0.017*	5.45		
Platynus assimilis (Payk.)	♀	2.26 *0,14*	**3.31** *0.006*	-26.89		0.35 *1.0*	**2.92** *0.02*	4.53	
	♂	0.16 *1.0*	0.86 *1.0*	2.11 *0.21*	-26.13	1.71 *0.52*	**4.17** *<0.001*	1.24 *1.0*	3.49
				Muscles					
Pterostichus oblongopunctatus (F.)	♀	-21,99				8,46			
	♂	1.14 *1.0*	-21,68			**3.27** *0.007*	9,90		
Platynus assimilis (Payk.)	♀	2.05 *0.24*	**3.13** *0.01*	-22,53		0.35 *1.0*	**2.87** *0.03*	8,57	
	♂	0.91 *1.0*	2.1 *0.24*	1.19 *1.0*	-22,25	0.31 *1.0*	**3.02** *0.02*	0.05 *1.0*	8,62
				Gonads					
Pterostichus oblongopunctatus (F.)	♀	-19.69				13.14			
	♂	**4.07** *<0.001*	-21.42			**3.84** *<0.001*	11.81		
Platynus assimilis (Payk.)	♀	1.4 *0.96*	**4.79** *<0.001*	-19.06		1.52 *0.78*	**4.71** *<0.001*	13.75	
	♂	1.5 *0.8*	**5.24** *<0.001*	0.05 *1.0*	-19.06	0.41 *1.0*	**3.14** *0.01*	1.78 *0.45*	13.13

in numerator – z-values, in denominator – p-values

According to our data, the isotope balance of food only slightly affects the isotopic signature of the elytra. However, in the beginning of the experiment the $\delta^{13}C$ and $\delta^{15}N$ values of the elytra and other tissues in most cases failed to differ significantly. Evidently, variation in isotopic signatures of the elytra, on the one hand, and muscles, fat-body and gonads, on the other, were caused by dramatic changes in diet.

A similar situation can be observed in natural conditions, for example, when the numbers of accessible prey or the habitat are changed. It seems logical to suggest that the isotope characteristics of the elytra are determined by the isotopic balance of larval food. Therefore, the differences in isotopic signatures of the elytra and gonads or muscles can imply that a particular specimen could have lived and fed in a habitat different from where its larva developed.

So we can see *not only a high level of variation* in carbon and nitrogen isotope ratios in study species, but also *significant differences in their isotopic signatures* when ***consuming the same food***.

It is generally considered that, in terrestrial ecosystems, the $\delta^{15}N$ values in the body of arthropod consumers increase by 2-4‰ per trophic level. On the contrary, the ratio of the carbon isotopes is more stable and increases usually only slightly, about 1‰ per trophic level.

However, according to the results of our experiment, the variations of $\delta^{13}C$ and $\delta^{15}N$ values in different tissues in Carabid beetles are high and can ***two or even three times*** exceed the limits of *a formal "trophic level"*.

Ignoring these facts can cause ***misinterpretations of the results*** of isotopic analyses, especially in studies on food webs and the differentiation of trophic niches.

Based on the results of the present study, we can formulate some preliminary conclusions:

1. The isotopic signatures of different tissues or body parts in Carabid beetles differ significantly and depend not only on the isotope composition of food, but also on species and the sex of the individual.
2. The isotope composition of tissues or body parts is species-specific, as a rule, but is characterized by high individual variability. For example, changes in $\delta^{15}N$ values can reach 3.0‰ or even 8.5‰.
3. The isotope composition of the gonads is altered most quickly as compared to the other tissues or body parts. Thus, the isotopic signatures of certain specimens during the breeding period would change more readily than at any other time.

The most important conclusion of our experiment can be reduced to stating high variations in isotope signatures in Carabid beetles.

Certainly, a highly artificial model situation with stable external conditions was created this time, with very high differences observed between isotope compositions in tissues or body parts of beetles and in the food. But even this has permitted to reveal that individual features of metabolism in carabids greatly affect their isotopic signature. In natural conditions, such differences are not likely to occur, but this does not mean there is no individual variability. Since the isotopic signatures of ground beetles and their food living in the same habitat can be presumed as being close, the range of individual differences is obviously small. In addition, species-specific features of metabolism appear clearly, so that various species consuming the same food show significantly different isotopic signatures.

Therefore, *the varied signatures* likely to be observed in nature in certain carabid species ***far from always imply different diets***.

To summarize, we can propose some recommendations:

1. In ecological studies, the isotopic signatures of individual tissues or body parts, but not of the whole beetle, are to be preferred.
2. Sampling for isotope analyses is to be performed regularly during the entire vegetation season.
3. In studies on food webs or community structure, the formal estimate of a "trophic level" must be used with due care, because individual variation in isotopic signatures may two or three times exceed this level of 2‰ to 4‰.
4. Differences in isotope composition of metabolic inert (elytra) and metabolic active (gonads, muscles or fat-body) tissues or body parts could potentially be used as a criterion for detecting the migrants and/or a recent change in diet.

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