Baltic J. Coleopterol. 12(1) 2012
ISSN 1407 - 8619

Stable isotopic research on ground beetles. Review of methods

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Zalewski M., Dudek D., Godeau J.-F., Maruszkiewicz M. 2012. Stable isotopic research on ground beetles. Review of methods. *Baltic J. Coleopterol., 12(1): 91 - 98.*

Stable isotope signals of nitrogen ($\delta^{15}N$) or carbon ($\delta^{13}C$) are becoming very important tools in ecology allowing investigations of animal diets and food webs. The method proved to be particularly useful in investigating secretive animals e.g. insects. Here we review methods used in stable isotope studies on the model taxon - ground beetles. A review of 50 publications quoting stable isotopes and Carabidae (only 18 publications contained relevant information) shows that stable isotopes are used to solve a variety of ecological questions and that the method is particularly popular among Japanese ecologists. While some of laboratory procedures are becoming standards, other important issues e.g. desired sample size or way of measuring isotopic baselines, remain unsolved. We additionally present results of a limited experiment on the effect of preservatives on isotopic signals. Our experiment shows that a seven-day storage in monoethylene glycol followed by a seven-day storage in 96% ethanol does not affect the $\delta^{15}N$ and $\delta^{13}C$ of ground beetles. All together, the review of the methods and experiments point to the conclusion that standard methods used in research on ground beetles are well-suited to stable isotope methodology. We conclude that the stable isotope method is likely to solve key questions that used to capture the attention of ecologists studying ground beetles.

Key words: $\delta^{13}C$, $\delta^{15}N$, Carabidae, ethanol, monoethylene glycol, preservation method, review, stable isotopes

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INTRODUCTION

Applications of stable isotope ratios of carbon or nitrogen are becoming very important tools in ecology, allowing for insight into trophic webs and animal diets (Tiunov 2007, Michener and Lajtha 2007). This method takes advantage of natural variation in stable isotope ratios and subtle changes of the proportion of heavy to light

isotopes in metabolic processes. For nitrogen, ratios of ^{15}N to ^{14}N (expressed as $\delta^{15}N$) exhibit stepwise enrichment with trophic transfers, and allow for estimating the trophic position of organisms (Ponsard and Arditi 2000). Ratios of carbon isotopes (expressed as $\delta^{13}C$) vary substantially among primary producers with different photosynthetic pathways (e.g. C3 vs. C4 plants), but change little with trophic transfers. Therefore, $\delta^{13}C$ can be used to differentiate sources of dietary carbon (DeNiro and Epstein 1981). Proper interpretation of the isotopic signal of an organism also requires some information on isotopic baseline: isotopic signal of the base of the food chain, such as primary producers or litter (Ponsard and Arditi 2000, Post 2002). Together, $\delta^{15}N$ and $\delta^{13}C$ isotopic signals of animal and signals of baseline locate organisms within a food web and give key information on their food habits. Applications of stable isotope method go well beyond web description and measuring food niche breadth (Paetzold et al. 2005, Okuzaki et al. 2009). For instance it allows for finding subtle differences in trophic niches of co-occurring species (Okuzaki et al. 2010) or drawing conclusions about influence of the feeding habits of larvae on the size of adults (Sasakawa et al. 2010). The method is applied to studies on ecological invasions (Tillberg et al. 2007), competition (Grams and Matysseka 2010) or dispersal (Schallhart et al. 2009). Stable isotope ratios of oxygen, sulfur and hydrogen are useful too (Martinez del Rio et al. 2009) and this method as a whole is one of the most important advances in ecology (Tiunov 2007). The usefulness of stable isotopes is particularly obvious in research on animals that are important elements of biocenosis but lead secretive lives and whose food habits are only partly known (Sasakawa et al. 2010). An example of such an important group of species are ground beetles. They are seen as the main predators of the temperate forest floor yet trophic position of particular species is usually not well known and the scale of omnivory is a subject to open debate (Thiele 1977). Additionally, due to a great variation of life histories and research convenience (ease to obtain large and diversified material that is easy to identify) they are a model group in terrestrial ecology of temperate habitats (Rainio and Niemelä 2003, Zalewski et al 2012). Nonetheless our knowledge on different aspects of carabid ecology is well established, recent findings suggest that the diet and in consequence trophic position of most Carabidae might be very different than presently recognized. Lundgren (2009) reviewing available data suggests that almost all ground beetles are phytophagous at some stage. It contradicts classical perspective considering carabids as strict predators and assigning phytophagy mostly to Harpalinae subfamily. The real importance of different sources of food in diet and therefore role of Carabidae in biocenosis could be most precisely verified using stable isotopes analysis.

The aim of this mini review is to pool together information on methods applied to stable isotopes analysis of ground beetles. We additionally present the results of our small experiment on the effects of standard preservative agents (monoethylene glycol and ethanol) on $\delta^{15}N$ and $\delta^{13}C$ signatures of Carabidae. In general, our paper hopes to advise researchers tempted by the opportunities given by both stable isotopes and ground beetles.

METHODS

Review

In order to recognize the current state of stable isotope research on ground beetles, we conducted a literature review. This study is based on a review of the first 50 papers that were shown by scholar.google.pl while search for “carabidae” + “stable isotopes” on 26 September 2010. This type of publication choice minimizes numerous publication biases and allows for reliable picture of state of art (Gurevitch et al. 2001).

Experiment

In order to investigate whether standard trapping methods of carabids can affect isotope signature of captured beetles, we conducted a preliminary analysis. We checked if a seven-day storage in

monoethylene glycol (preservative solution widely used in Barber traps) followed by seven-day storage in 96% ethanol, affected the $\delta^{15}N$ and $\delta^{13}C$ of ground beetles. 10 Barber traps with glycol - 'glycol' traps (emptied after one week), and 10 'live' traps (Barber traps filled only with litter to avoid predation and emptied every second day) were installed during 12-19.04.2010 in a *Pino-Quercetum* forest in Dziekanów Leśny, Central Poland. Beetles from 'live' traps were frozen for a week; beetles from 'glycol' traps were immersed in 96% alcohol for a week. After the storage period, beetles from both types of traps were dried in 60 °C for 48 h, and ground into a fine powder in the mortar. Each powdered sample was wrapped in a tin crucible and was burned in Elemental Analyzer FlashEA1112. Subsequently, nitrogen and carbon were analyzed under a continuous flow system in an Isotope Ratio Mass Spectrometer (Thermo Finnigan MAT253, Thermo Electron Corporation) at the Isotope Laboratory, Institute of Geological Sciences of the Polish Academy of Sciences. The isotopic contents were recalculated into δ units based on the relative difference (in parts per thousand) between the sample and conventional standards (atmospheric N_2 for nitrogen; Pee Dee Belemnite carbonate for carbon), according to the formula $\delta\,^{13}C$ or $\delta\,^{15}N$ (‰) = $(R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of heavy/light isotope content for the considered element. Effects of treatments were log(x+100) transformed and compared with ANOVA.

RESULTS

Review

Out of 50 papers, two were unattainable e.g. a conference report from 1980. Twenty-six works quoted Carabidae but provided no information on stable isotopes of ground beetles. Four works presented data on hydrogen information that were used for tracing past climates, so they were also excluded . The articles which did not fit the subject were removed and in total 18 papers were thoroughly reviewed. All of these papers presented data on $\delta^{15}N$ and 89% of them on $\delta^{13}C$. In most cases, sample size and standard deviation were also given. In most of the studies, Carabidae were not the only species studied. Quite often they served as possible food items for species of main interest (e.g. Stapp 2002) or they were included as background information on food web (e.g. Hyodo et al. 2010).

Stable isotope research on Carabidae clearly suffers from diverse biases. 55% of studied species originated from Japan, 22% from Europe and 20% from North America. There was one species studied from New Zealand and one from Malaysia. Most of studies were carried out in forests (39%), 33% in riparian habitats and 28% in agrocenoses and other open habitats. There was a wide range of variation in methodological approaches. It is well highlighted in case of sample size. While a majority (62%) of signatures was based on measurements of 5 or less individuals (Figure 1), there are also numerous works where sample size was around 10 individuals. On average, species stable isotope signals are based on 5.2 individuals, median is 3 (n=71).

Some methods are becoming standard procedures in studying stable isotopes in Carabidae. Thirty % of studies use 70% ethanol as a preservative for short or long term storage of insects, which was often (27%) proceeded by storing in freezer (–30 °C), and in one case, the beetles were kept dried. The common preparation procedure for analysis (55% of studies) consists of drying the insects in 50-60 °C for 24-72 h, then homogenizing the material and analyzing it. In 22% of studies, beetles were starved for 48-96 h to clean gut contents and to avoid material contamination. Pee Dee Belemnite and atmospheric nitrogen were used as the carbon and nitrogen isotope standards in five out of six studies, where isotopic standard was reported.

Measuring isotopic baseline of habitat is not such a frequent research habit in the reviewed publications. In 30% of studies, there was no baseline measurement. In the studies where a baseline was evaluated, there was large variation in approaches. In two out of 18 (11%) studies, a thorough baseline study was conducted, including

litter, soil and some plant species. In 40% of studies, possible prey species were studied, and in 16%, only plants were used as baseline assessment.

Experiment

We captured 22 carabids from 'live' and 10 individuals from the 'glycol' traps. Due to a small sample size, we could not compare effects of treatment on separate species, but we compared identical pooled samples from 'live traps and 'glycol' traps. Samples were of equal species composition: three individuals of *Amara makolski*, two of *Pterostichus oblongopunctatus*, two of *Carabus nemoralis* and one of *Carabus arvensis.* ANOVA showed that there was no significant difference between isotopic signals ($\delta^{15}N$ and $\delta^{13}C$) of frozen vs. preserved in glycol and alcohol beetles (One Way ANOVA; df=14; F=0.005; F=3.07; p=0.9; p=0.1 for $\delta^{15}N$ and $\delta^{13}C$ respectively;).

DISCUSSION

Stable isotope research on ground beetles is at a stage of fast development, which is reflected by the widespread use of the method in many branches of ecology (Martinez del Rio et al .2009). While use of the method poses a significant potential for studying the ecology of carabids, there is still some uncertainty regarding methodology. It seems that each research group follows its own laboratory practices. There is some sort of consensus with regard to preservation, way of dry-

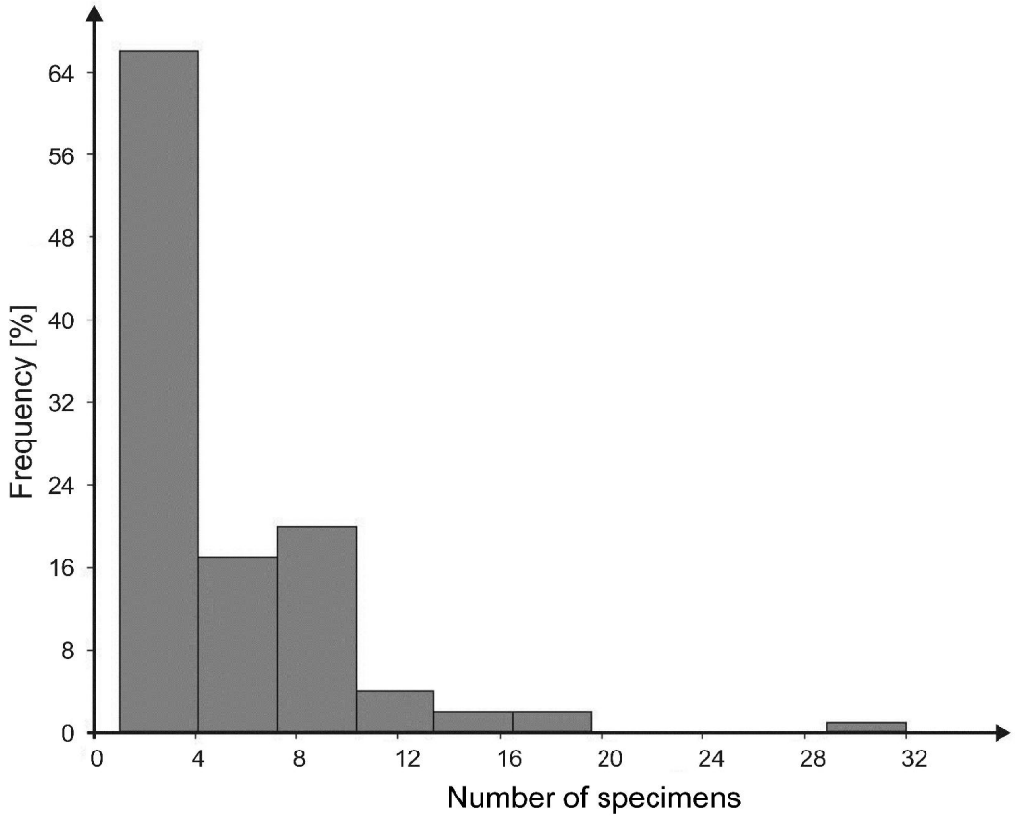

Fig.1 Sample sizes used for estimation of $\delta^{15}N$ and $\delta^{13}C$ in studies on ground beetles.

ing, powdering or using nitrogen and carbon standards, but on the other hand, there is large arbitrariness with respect to sample size and ways of measuring baseline. Variation of ways of measuring a baseline (e.g. plants, soil, litter, and diverse prey species) prevents comparisons between particular studies. Such comparisons would allow for drawing general conclusions with respect to diet, trophic position or ecological specialization of carabids. It might be of great scientific potential. The choice of baseline depends on a particular research question and the type of studied habitats, but we argue that measuring the isotopic signal of litter should be the minimum and standard requirement for most studies. Whereas data on isotopic baselines are not available, we suggest autocorrelation analysis (Legendre 1993) to account for some of the variation in baselines when comparing different communities.

The second important and unresolved issue is sample size. Sample size that is desired to properly measure isotope signature depends on the variability of stable isotope signal in the studied population. Therefore, if possible, an estimation of standard deviation of isotopic signal in population should proceed the decision on sample size. This has never been applied nor investigated in carabid studies. This important issue is a subject of a separate investigation (in preparation), but it can be noted that stable isotope studies on ground beetles seem not to deviate from studies on other groups of animals with respect to applied sample size. Sample size used in these analysis does rarely exceed 5-15 individuals (eg. Bluthgen et al 2003, Dehn el al 2006) and is constrained by the availability of material, rather than requirements of precise estimation of stable isotopes signals.

The convenient technique of collecting epigeic fauna including Carabidae is the use of Barber traps. They are usually filled with preservatives: mostly monoethylene glycol, formalin or saturated NaCl (Zalewski 1999). Collected material is then stored in ethanol or formalin. Effects of these preservatives on isotope signatures of different animals received considerable attention (reviewed e.g. in Sarakinos et al. 2002 and Barrow et al. 2008). In general, ethanol preservation of tissues of fish, quails, sheep, turtles, caddisflies, termites (Florencio et al. 2011), collembolans (Sticht et al. 2006) does not seriously modify carbon or nitrogen isotopic signatures. On the other hand, alcohol affected $\delta^{13}C$ of nematodes (Sticht et al. 2006) and Drosophila (Ponsard and Amlou 1999). Formalin affected both $\delta^{15}N$ and $\delta^{13}C$ of diverse taxa (Sarakinos et al. 2002), but in a predictable way, so this bias can be corrected. NaCl did not affect $\delta^{13}C$ nor $\delta^{15}N$ values of termites, but changed their C/N ratio (Florencio et al. 2011). Monoethylene glycol did not significantly affect the $\delta^{13}C$ values of collembolans and nematodes (Sticht et al. 2006). Despite this interest, there are no studies on the effects of preservation methods on isotope signatures of any species of Coleoptera. In our limited experiment, we found no difference between isotopic signals ($\delta^{15}N$ and $\delta^{13}C$) of frozen vs. ethanol- preserved beetles. This result has to be considered as preliminary and experiment using larger sample is needed. Anyhow, it seems than that the conventional technique of capturing Carabidae is well suited to stable isotope methodology.

Ground beetles are one of model taxons in ecology, and various research methods are developed in order to study all aspects of their life. Basic and simple methods of capturing animals were established almost century ago (Barber 1931) and has not changed. However development of diverse research questions fruited in advancement of study techniques. Here we want to highlight just some of more important achievements. Dispersal is seen as the key to understanding of carabid ecology (Zalewski and Ulrich 2006), therefore variety of methods were used to investigate movements of beetles including window traps (Meier 1974), CMR (Sklodowski 1994, Hagler and Jackson. 2001), telemetry (Hamon et. al. 1990, Riecken and Raths 1996) and tracking radioactive beetles (Baars 1979). Interests in population dynamics resulted in application of genetic techniques used for instance for investigating consequences of habitat fragmentation (Brouat et al. 2003). Finally stable isotope method is the most recent advancement in carabidology

(Paetzold et. al. 2005, Moulton 2012). Simple use of stable isotope method provides insight into trophic webs but details of diet of particular species should rather be exposed using mixing models as was recently applied to assembly of Japanese *Harpalus* by Ikeda et. al. (2010). While even mixing models give only approximation of diet, the most precise methods for studding diets are application of monoclonal antibodies (Harwood et. al. 2001) and DNA analysis of gut contents (Juen and Traugott 2006). All these advanced methods are becoming less expensive and they are powerful tools in hands of carabidologists in XXI century.

Conclusions. Stable isotope signals of nitrogen and carbon provide information that has not been available before the development of the method. Still, however, numerous issues that interest ecologists working on carabids are not investigated using this powerful method. Among many others, we can mention competition (Brandl and Topp 1985, Currie at al. 1996), species invasions (Niemelä and Spence 1999) or macroecological patterns related to dispersal or body mass (Zalewski 2000, Kotze et al. 2003). Surely it is just a matter of time until carabidologists recognize the opportunities given by this unique method, particularly the routine methods of capturing and preserving ground beetles suited well to the demands of this new ecological tool.

ACKNOWLEDGEMENTS

We thank Tadeusz Plewka who helped with species identification and two referees for their helpful comments. Work was supported by grant NN 304 354538 from the Polish Science Committee.

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Received: 12.06.2012.
Accepted:25.08.2009.