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SNAKES: INTRIGUING ORGANISMS AND THEIR DIET. THE CASE OF THE EUROPEAN WHIP SNAKE, *HIEROPHIS VIRIDIFLAVUS*

Abstract - Interest toward snake biology and ecology is nowadays a matter of fact. Despite dozen and dozen papers on snake biology and diet, it is paradoxical that virtually nothing is available on energetic of natural preys in the wild, nor if geographically intra-specific differences may exist. We have been aimed at testing if prey energetic estimated values compared to actually observed prey profiles from wild snakes, may lead to a better interpretation of the diet spectrum, diet range, diet overlapping found in most of the studied snakes. As model species we used the European Whip snake, *Hierophis viridiflavus*, the commonest Italian snake species. Two contrasted areas and populations (Western France vs Central Italy) show marked differences in both prey percentage as well as in prey energetic, suggesting a strong relationship climate-habitat-metabolic requirements.

Key-words - European Whip snake, diet composition, prey energetics, comparative analysis.

Riassunto - *I serpenti: organismi «intriganti» e la loro dieta. Il caso particolare del biacco maggiore,* Hierophis viridiflavus. L'interesse per i serpenti come organismo modello è un dato consolidato ormai da diversi decenni. Nonostante un numero elevato di lavori che si occupano anche della dieta, di fatto non si hanno informazioni sul valore energetico della dieta dei serpenti né su eventuali differenze complessive a carico di una specie nell'ambito della sua distribuzione.
Abbiamo considerato preliminarmente l'analisi dei valori in kcalorie di diverse tipologie di prede, comparate con i gruppi frequentemente predati dai serpenti, in particolare dal biacco maggiore, *Hierophis viridiflavus.* Nel confronto tra due popolazioni con valori molto diversi nella percentuale delle prede (soprattutto a carico di rettili e di mammiferi) (Francia occidentale-Italia centrale) corrisponde un diverso valore di kcalorie stimate, tanto da supporre una stretta relazione tra clima/habitat e necessità metaboliche.

Parole chiave - Biacco maggiore, composizione della dieta, valore energetico delle prede, analisi comparativa.

Introduction

Snakes represent excellent model organisms for a variety of biological and ecological aspects: due to their elongated cylindrical and simplified structure, natural and sexual selection have strongly forced adaptive and selective responses actually on a limited number of anatomical structures or behavioural aspects (e.g. Shine, 2000; Shine *et al.,* 2000; Shine *et al.,* 2002; Boback, 2003; Aubret *et al.*, 2004). Among other morphological aspects, body size, including total length and body mass, attains the major source of variability, ranging from the few centimetres of the Leptotyphloid *Leptotyphlops carlae* to more than 7 m total length of Boids (*Python sebae*, *Eunectes murinus*) (Coborn, 1999).
Similarly to several other vertebrates, snakes do not chew: they are predators constrained by their maximum mouth gaping. Differently from many other vertebrates, snakes have a kinetic skull, that gives an extraordinary ability to enlarge when capturing and swallowing preys much larger than expected.
Maintenance of different dimensions has been favored throughout times as adaptive mechanism from specialized (e.g. Cobb, 2004) to opportunistic dietary regimes (e.g. Capula *et al.*, 2006).

We have selected the European Whip snake, *Hierophis viridiflavus* (Fig. 1), for its wide distribution in Southern Europe (Naulleau, 1997), in all Italy, comprising fairly all the small to large islands. Nevertheless, most papers regarding food preference or food ecology of the European Whip snake, deal with data and behaviours of a restricted part of the whole distributive range of the species (central Mediterranean Italy: Rugiero & Luiselli, 1995; Capula *et al.*, 1997; Zuffi 2001), thus reducing to a narrow window the comprehension of feeding rate and food preference patterns.
Data on the energetic value of ingested food (prey quality) and its relationship to body size and geographic distribution of some European species have been studied only recently (Zuffi *et al.*, 2010).
This preliminary report analyses the prey quality, in terms of energetic values, between a continental population of the European whip snake of N Europe and a Mediterranean one, exploring body size relationships to diet energetics.

Materials and methods

Basically, the ingested preys have been determined at the lower *taxon* possible: in wild snakes regurgitation has been induced, and in preserved specimens, food has been taken directly from the stomach or studied as fecal pellets from the intestine. This allowed us to indicate the prey composition at each site on an average snake (percentages relate to central to north Italy: insects, 18.2%; reptiles, 45.45%; small mammals, 4.37%; see Zuffi, 2007; Zuffi *et al.*, 2010 for

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Fig. 1 - European Whip snake, *Hierophis viridiflavus.*

a detailed description of materials and methods and procedures herein).
The energetic value of comparative matters has been determined with standard procedure. Immature house mice (*Mus domesticus*, *N* = 10), adult lizards (Italian wall lizard, *Podarcis sicula*, *N* = 6 males, *N* = 4 females) and crickets (10 samples of about 7 g each, with about 21 crickets per sample, males and females in random proportions), were used to determine the kilocalories and main nutrients of each food category. It was not possible to consider single crickets for each analysis, due to their extremely low body mass (less, on average, than 0.30 g each, range 0.14-0.41 g, male lower, female higher). The analyses were then performed on at least 7 g of crickets per sample: this mass was the smallest suitable to analyse in our laboratory. Crickets and house mice (frozen specimens) were obtained from a pet shop; adult lizards were captured in the field and were sacrificed (permission «Ministero dell'Ambiente e della Tutela del territorio. Direzione per la Protezione della Natura»; #N DPN/IID/2005/28177 to ZMAL), within 24 hours from capture. All samples were freshly weighed, then dehidrated (in a ventilated oven at 103°C for 18-24 hrs until a constant weight was reached) and subsequently ground. From each sample (e.g. mammals) a sub-unit (e.g. 0.1 g for proteins, 2-2.5 g for ether extracts; remaining parts for ashes) was collected from the homogeneous sample, and prey net weight, humidity, total nitrogens (proteins), ether extracts (lipids), ashes, and kilocalories (kcal) were calculated. Birds, often present in this species diet, have not been considered, due to technical bias in the used procedure (see Zuffi *et al.*, 2010 and explanation therein). Amphibians, being marginal in the Whip snake diet, were excluded. From Zuffi (2007) and unpublished personal data, it has been possible to state that an adult *Hierophis viridiflavus* feeds on average six preys/month per nine months of activity, for a total of 54 meals a year.
This amount of total preys has been distributed among the known percent of insects, reptiles and mammals and their calculated energetic values. Due to a general description of diet energetics in the species and not between sexes, we shall not discuss the Sexual Dimorphism of the species, nor will trait body size measurements for sex separately.
Statistical analyses were performed with SPSS release 13.0

Results

Northwestern France snakes had average body size of 862 ± 109 mm snout to vent length and 223 ± 88 g body mass; the Central Italy snakes had average body size of 916 ± 187 mm snout to vent length and 244 ± 108 g body mass. Snakes from northern France were significantly shorter than Central Italy ones (Student t-test = -2.019, P = 0.046), while no significant

Tab. 1 - Energetic estimation of differential diet composition in *Hierophis viridiflavus*.

	Insects	Reptiles	Mammals	Total Kcalories
N Europe	0.00	86.38	3699.50	3785.88
C Italy	29.69	704.90	1511.78	2246.37

difference has been found when considering body mass (Student t-test = -1.118, P = 0.266). We thus consider the two related variables as quite similar between two localities. However, in Northwestern France, the European Whip snake preyed upon 5.66% reptiles, 5.66% birds, 88.68% mammals (target groups cover 94.34%) (courtesy of X. Bonnet, CNRS Chizé); in Central Italy it preyed upon 8.75% insects, 5.00% amphibians, 46.25% reptiles and 36.25% mammals (target groups cover 91.95%). Maximum variation has shown in reptile and mammal fractions: the energetic values of relative categories [insects 417 ± 47.83 Kcal; lizards 390.97 ± 48.91 Kcal, mammals 425.62 ± 25.92 Kcal (as in Zuffi, 2007)], applied to the above mentioned proportions, is much different between the two localities (Tab. 1), being highly significant (χ^2 test = 353.92, 2 df, $P < 0.001$).

Discussion

Despite information on morphological and biometrical variation in the European Whip snake is relatively recent (see Fornasiero *et al.*, 2007; Zuffi 2007; 2008; Zuffi *et al.*, 2007), data on prey-energetic differences and, if any, food preferences, has not still studied in depth (see Zuffi *et al.*, 2010) or not studied at all.

Thus, some traits of our results should be widely introduced. Typically, small mammals represent the best dietary choice for terrestrial snakes (Shine, 1993; Forsman & Lindell, 1993; Capula & Luiselli, 2002; Brito, 2004; Luiselli, 2006a) and, in terms of the total amount of the produced nutrients, an optimal item for most terrestrial and avian predators (e.g. Stangl *et al.*, 2005; Sidorovich *et al.*, 2008). Lipid composition of our target prey groups was different. Insects and mice were quite similar, and both were more energetic than lizards. Notably, ashes are an additional important nutrient, metabolized in order to maintain the turnover of bones' mineral components (Davis & Jackson, 2007). Lizards that are much richer in ashes than the other prey, may contribute, in principle, in a lesser way to the nutritional energetic income for a given snake, but could however represent an important source of minerals.

Summarising, since a rodent or an insectivore is on average heavier than a lizard and much heavier than an insect, in order to reach the equivalent content in lipids and proteins (hence the related energetic gain) of 100 g mouse body mass, a predator could be forced to prey on approximately three times more lizards and sixty times more crickets. This appears to be a relatively compulsory strategy for a small-sized predator, whose possibilities of feeding on prey of a certain size are limited by catching and handling capabilities.

Snakes are, more than other vertebrates, mouth-gape limited, and the predator-prey size relationship is, within this class, much stronger (Arnold, 1993). The relatively high content of proteins, lipids, and kilocalories of small-sized prey is therefore fundamental for small sized snakes (see Cobb, 2004).

We are confident that the recorded differences, easily understandable due to the different percentage of the target prey group (i.e. mammals), may reflect a different selection in prey preference, perhaps associated to the different climate (Continental vs Mediterranean) that could involve a different time-lag of daily and seasonal activity duration. This, in turn, may adaptively select a differential predation system, devoted to more caloric and energetic prey types in Continental climate with respect to a less caloric profile in Mediterranean areas. In snakes, food niche is usually the partitioned resource (Luiselli, 2006b), and perhaps Italian snakes do feed more often on lizards to minimize competition with an higher number of species found sympatric.

To date, incoming research should focus on the energetic of small versus large island snake populations, to test for area and food supplies constraints and adaptive morphology (and physiology) of targeted individuals.

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