

GEOGRAPHIC VARIATION IN DIET COMPOSITION OF THE GRASS SNAKE (*NATRIX NATRIX*) ALONG THE MAINLAND AND AN ISLAND OF ITALY: THE EFFECTS OF HABITAT TYPE AND INTERFERENCE WITH POTENTIAL COMPETITORS

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The diet of grass snakes (*Natrix natrix*) on the mainland and an island of Italy was compared by pooling literature data and original data. A total of 535 prey items were recorded (444 prey items from specimens >40 cm SVL), but the number of items was very variable between sites. Body lengths (both sexes) varied between geographical areas, and females were larger than males in all study areas. Specimens from the island (central Sardinia) and from one mainland mountainous locality (Duchessa Mountains) were significantly smaller than those from all the other localities. Amphibians were the main prey for both sexes, but females ate more toads and fewer frogs or tadpoles than males; females also consumed more rodents than males. There was a strong effect of locality on diet composition i.e. newts/salamanders were found only in two montane areas; hylids were found only in the single island area; and rodents were commonly preyed upon only at a single mainland locality. Two lizard corpses (*Podarcis muralis*) were scavenged by grass snakes at a mainland locality. The presence of the piscivorous snake *Natrix tessellata*, a potential competitor for food, did not have any apparent effect on the food types eaten by grass snakes because grass snakes consumed fish when sympatric with *N. tessellata*, but not at other sites. The dietary variation exhibited by grass snakes suggests that, by shifting their diets to other prey, they might be able to persist in areas where their usual natural prey has declined drastically, but this remains to be demonstrated.

Key words: body sizes, Colubridae, competition, food habits

INTRODUCTION

It is widely recognized that our understanding of the evolutionary ecology of snakes (e.g. habitat use, activity patterns, etc.) can be aided by detailed data on feeding ecology (e.g. Arnold, 1993; Cundall & Greene, 2000). Diet variation associated with differences in geography and habitat may be particularly useful in this respect (Gregory, 1984; Shine, 1987; Luiselli & Angelici, 2000; Henderson, 2002; Luiselli *et al.*, 2002; Luiselli, 2003). In addition, it has been recently demonstrated in some colubrids from tropical Africa (*Natriciteres* spp.) that the presence of a potential competitor for food (*N. variegata*) may produce a shift in the diet composition of the target species (*N. fuliginoides*). The result is that where species are sympatric, there is a partitioning of food resources between the two predator species and a reduction in the diversity of prey taken by the target species (Luiselli, 2003). The generality of this ecological phenomenon for snakes cannot be tested at present because, for most species, we do not have enough data to compare diets from areas where they live alone and areas where they coexist with potential competitors. However, food resource partitioning has been observed in several communities of snakes from both temperate and tropical regions (for a review, see Mushinsky, 1987), suggesting that the

mechanism of dietary shifts observed in Afrotropical *Natriciteres* (see Luiselli, 2003) may be relatively widespread.

Data on these issues are scarce. In Europe, geographic variation in diets has been studied only for *Coronella girondica* (Luiselli *et al.*, 2001), and microhabitat variation in diets has been studied only for *Elaphe longissima* (Capula & Luiselli, 2002; Gomille, 2002), *Elaphe quatuorlineata* (Filippi *et al.*, 2005) and *Vipera berus* (Luiselli & Anibaldi, 1991; Völkl & Thiesmeier, 2002). To date, the effects of potential competitors on diet composition of any European snake species has not been tested.

The diet of the grass snake (*Natrix natrix*) has been carefully studied in Italy over the last 15 years, but detailed data also are available for other conspecific populations from elsewhere (e.g. see Kabisch, 1974; Kratzer, 1974; Beschov & Dushkov, 1981; Madsen, 1983; Drobenkov, 1995; Reading & Davies, 1996; Gregory & Isaac, 2004). In this paper, we present detailed data on the diet of Italian grass snakes on one island and in several mainland habitats. We report on variations in grass snake diet that can be related to differences in geography, habitat and the effects of the presence of a potential competitor (*Natrix tessellata*).

In particular, we attempt to answer the following questions. Firstly, are there any significant differences in the diet composition of male and female grass snakes in areas with different climate and habitat con-

ditions on the mainland and an island of Italy? The rationale behind this question is based on the fact that, in natricine snakes, females are usually bigger than the males (e.g., Madsen, 1983; Madsen & Shine, 1993a; Luiselli *et al.*, 1997; Luiselli, 2003; Shine, 2003; Shine *et al.*, 2003; Gregory & Isaac, 2004). Such body size differences may parallel differences in prey size and type. These parallels have been shown in other snake species, e.g. *Acrochordus arafurae* (Shine, 1986; Camilleri & Shine, 1990; Houston & Shine, 1993) and *Python regius* (Luiselli & Angelici, 1998), and we may therefore expect them to occur in *N. natrix*. Another reason for dietary differences between the sexes is the higher nutritional requirements of reproductive females compared to males (Bonnet *et al.*, 1998, 2001a; Gregory & Skebo, 1998; Shine, 2003): males often do not feed during the breeding season, whereas females are likely to select high-energy foods. Secondly, inter-population differences in diet may be related to weather/climate/habitat conditions, which in turn may affect energetic requirements (Capula & Luiselli, 2002). Secondly, do grass snakes exhibit shifts in feeding ecology associated with the presence or absence of *Natrix tessellata*, a closely related potential competitor which is also abundant and widespread in Italy (see Bruno & Maugeri, 1990; Luiselli & Rugiero, 1991; Filippi *et al.*, 1995)? This type of pattern may result in an apparent food partitioning between coexisting snakes, and has been demonstrated in another genus of natricine snakes, i.e. the small-sized *Natriciteres* species from tropical Africa (Luiselli, 2003).

We also address some general management implications that can be derived from the comparative data of the present study.

MATERIALS AND METHODS

The study is based on original field research conducted from March 1985 to May 2003 by us and our associates (primarily Drs U. Agrimi, C. Anibaldi, D. Capizzi, and L. Rugiero), with additional datasets from our own published research on *N. natrix* (e.g. Luiselli & Rugiero, 1991; Capula *et al.*, 1994; Luiselli *et al.*, 1997; Filippi & Luiselli, 2002).

STUDY AREAS

In total, six study areas (five on mainland Italy and one on the island of Sardinia), representing a range of different habitats and climatic conditions, are compared in this paper (Table 1). Although there is little genetic or morphological differentiation among mainland populations, Sardinian populations are extremely divergent and are classified as a different subspecies (*Natrix natrix cetti*; see Thorpe, 1975, 1979 for morphological evidence; Capula, unpublished data for genetic evidence).

METHODS

Field methods were nearly identical at all study areas, and are detailed in the original literature sources (see Table 1). Here, we summarize the main methodological points.

Fieldwork was conducted under all climatic conditions. We searched for grass snakes along standardized routes in the various microhabitats frequented by snakes at the study areas. We captured snakes by hand, but additional free-ranging specimens were captured by pitfall traps with drift fences. We always recorded the site of capture and the habitat at each capture site. Each snake

TABLE 1. List of study areas, including details of general habitats, presence or absence of the potential competitor (*Natrix tessellata*), and pertinent literature source.

| Study area | Habitat features | Reference |
|---------------------------|-----------------------------------------------------------------------------------------------------------------|-------------------------------|
| MAINLAND ITALY | | |
| La Marcigliana (Rome) | cultivated land; 50 m a.s.l.; Mediterranean climate; <i>Natrix tessellata</i> not present. | this paper |
| Palidoro (Rome) | artificial canal, 10 m a.s.l.; Mediterranean climate <i>Natrix tessellata</i> present. | Luiselli & Rugiero, 1991 |
| Tolfa Mountains (Rome) | permanent stream; 350 m a.s.l.; Mediterranean climate <i>Natrix tessellata</i> present. | Filippi <i>et al.</i> , 1996 |
| Duchessa Lake (Apennines) | glacial lake; 1800 m a.s.l. cold climate <i>Natrix tessellata</i> not present. | Filippi & Luiselli, 2002 |
| Sella Nevea (Alps) | detrital cone along a Swiss pinewood; 1100 m a.s.l.; alpine climate <i>Natrix tessellata</i> not present. | Luiselli <i>et al.</i> , 1997 |
| ISLAND AREA | | |
| Oliena (Nuoro, Sardinia) | Mountain stream; 800 m a.s.l.; cool Mediterranean climate <i>Natrix tessellata</i> not present. | Capula <i>et al.</i> , 1994 |

was sexed, measured for snout-vent length (SVL, to the nearest ± 1.0 mm), weighed with an electronic balance, and individually marked by ventral scale clipping for future identification. Then, the snakes were palpated in the abdomen until regurgitation of ingested food or defaecation occurred. In addition, specimens found already dead during our surveys (e.g. snakes killed by farmers, or by cars, etc.) were dissected to determine if prey was present. We identified prey items to the lowest taxonomic level possible. We estimated the mass of prey items at the time of ingestion, when possible, by comparing the item to intact conspecifics of various sizes from our own personal collection, or measuring the fresh biomass in perfectly preserved items. We utilized data collected from both stomachs and faeces. Generally, each specimen contributed data from either only stomach contents or faeces but not both (i.e. to avoid dual counts of the same food item), unless faecal and stomach samples contained obviously different material (e.g. mammal hair vs. an amphibian), in which case both sources were used in the data set. In the case of stomach contents, we always counted number of prey items rather than number of snakes with a particular prey type.

Vouchers (of both prey and predators) were deposited in the herpetological collections of the Centre of Environmental Studies 'Demetra' (Rome), F.I.Z.V. (Rome), and Municipal Museum of Zoology (Rome).

All statistical tests were done with alpha set at 0.05. Means are followed by ± 1 SD. For all analyses we separated small specimens (<40cm SVL) from large specimens based on two criteria: (1) they were certainly immature; (2) due to the fact that juveniles are more elusive than adults, our samples of small snakes were strongly biased, i.e. they varied considerably from study area to study area, and did not allow for robust statistical comparisons at some areas.

TABLE 2. Composition of the diet of grass snakes in a cultivated area in Mediterranean central Italy (Marcigliana, Rome). These data are based on examination of 71 adult males and 54 adult females, out of which 33 males and 20 females contained identifiable prey in either their stomach or faeces. Numbers represent the number of individual prey, not the number of snakes containing a given prey type.

| Prey types | No. in males | No. in females |
|-----------------------|--------------|----------------|
| AMPHIBIANS | | |
| <i>Bufo bufo</i> | 8 | 6 |
| <i>Bufo viridis</i> | 2 | 3 |
| <i>Rana bergeri</i> | 6 | 1 |
| MAMMALS | | |
| <i>Microtus savii</i> | 10 | 11 |
| <i>Rattus rattus</i> | 3 | - |
| <i>Mus domesticus</i> | 1 | - |
| <i>Crocidura</i> sp. | 4 | - |

RESULTS

DIET OF GRASS SNAKES IN AN AGRICULTURAL AREA

The only original data set presented in this paper concerns a grass snake population from an agricultural area in Mediterranean central Italy (La Marcigliana, see Table 1). The composition of the diet of this grass snake population is summarized in Table 2. These data are based on an examination of 71 adult males and 54 adult females, out of which 33 males (46.5%) and 20 females (37%) contained identifiable prey in either their stomachs or faeces. A total of 34 prey items were collected from males, and 21 prey items from females. Small mammals were eaten slightly more often than amphibians in both males (53% versus 47% of the dietary spectrum) and females (52.4% versus 47.6% of the dietary spectrum). In both sexes, the most common prey items were Savi's voles, *Microtus savii*, (accounting for 29.4% of the prey items found in males, and 52.4% of those found in females) and common toads *Bufo bufo* (23.5% of prey items in males, and 28.6% in females). Savi's voles were by far the commonest rodents in the agricultural lands of the study area (Capizzi & Luiselli, 1996a,b), whereas common toads were the most abundant amphibians in the same habitat (Capizzi & Luiselli, unpublished).

COMPARING THE STUDY AREAS

Mean body sizes. Mean SVL of adult (i.e. >40 cm) grass snakes varied between sites (Table 3). Females were significantly longer than males at all study areas (one-way ANOVA, significance is always at least $P < 0.001$). Male SVL varied significantly between geographical regions (one-way ANOVA, $F_{5,207} = 38.6$, $P < 0.01$), and a Tukey HSD post-hoc test demonstrated that specimens from the island locality (Oliena) and from one mainland mountainous locality (Duchessa Mountains) were not significantly different from each other, but were significantly smaller than those from all the other localities, whose male body sizes were similar. An identical pattern was found for females (overall one-way ANOVA, $F_{5,141} = 44.3$, $P < 0.01$).

TABLE 3. Mean length (SVL) \pm SD for grass snakes at all the study areas. Detailed data are presented in the original literature sources (see the text). In bold are the populations that differed significantly in mean body length from all other populations, but not from each other (for statistical details, see the text).

| Study area | Body length (cm) males | Body length (cm) females |
|----------------------|---------------------------------------|---------------------------------------|
| La Marcigliana | 71.9 \pm 10.4 (n=71) | 87.5 \pm 3.6 (n=54) |
| Palidoro | 69.3 \pm 15.6 (n=57) | 84.8 \pm 14.3 (n=39) |
| Tolfa Mountains | 72.3 \pm 12.8 (n=21) | 89.7 \pm 18.2 (n=19) |
| Duchessa Lake | 51.4\pm17.6 (n=8) | 62.2\pm28.9 (n=4) |
| Oliena | 47.3\pm4.7 (n=10) | 62.7\pm11.6 (n=8) |
| Sella Nevea | 71.3 \pm 10.2 (n=43) | 82.1 \pm 13.7 (n=20) |

TABLE 4. Summary of diet data of grass snakes from the various study areas. Data are pooled from all localities, and only grass snake specimens longer than 40 cm SVL are considered.

| Prey types | No. in females | % in females | No. in males | % in males |
|---------------------------|----------------|--------------|--------------|------------|
| FISHES | | | | |
| Gobiidae | 1 | 0.4 | 3 | 1.4 |
| Cyprinidae (unidentified) | 4 | 1.8 | 3 | 1.4 |
| <i>Anguilla anguilla</i> | 1 | 0.4 | 3 | 1.4 |
| AMPHIBIANS | | | | |
| <i>Bufo</i> adults | 41 | 18.3 | 12 | 5.4 |
| <i>Bufo</i> metamorphs | 51 | 22.8 | 57 | 25.9 |
| <i>Bufo</i> tadpoles | 23 | 10.3 | 36 | 16.4 |
| <i>Rana</i> adults | 43 | 19.2 | 59 | 26.8 |
| <i>Rana</i> metamorphs | 4 | 1.8 | 9 | 4.1 |
| <i>Rana</i> tadpoles | 6 | 2.7 | 2 | 0.9 |
| <i>Hyla</i> adults | 3 | 1.3 | 3 | 1.4 |
| <i>Hyla</i> tadpoles | 2 | 0.9 | 4 | 1.8 |
| Newts/salamanders | 15 | 6.6 | 14 | 6.4 |
| Amphibia (unidentified) | 0 | 0 | 1 | 0.4 |
| REPTILES | | | | |
| Lizards | 3 | 1.3 | 2 | 0.9 |
| BIRDS | | | | |
| Passeriformes | 2 | 0.9 | 0 | 0 |
| MAMMALS | | | | |
| Rodents | 25 | 11.2 | 12 | 5.4 |
| TOTAL | 224 | 100.0 | 220 | 100.0 |

Diets. Detailed data on the diet composition of the various grass snake populations compared here are presented in the original bibliographic sources, but a list of the various prey items is reported in Appendix 1. When we pooled all the studied populations, there was a total of 535 recorded prey items, although the number of items was very variable between sites due to different lengths of study period, and differences in the abundance of grass snakes between sites.

When we excluded small specimens (those <40 cm SVL) for which we had good data from only a single locality (i.e. Palidoro; see Luiselli & Rugiero, 1991, where they fed mainly on anuran tadpoles), we were left with a total of 224 prey items from adult females and

220 prey items from adult males (Table 4). Amphibian prey accounted for the greatest part of the diet in both females (83.9 % of the total number of prey items) and males (89.5 %). The sexes did not differ significantly in terms of frequency of consumption of amphibians (contingency table χ^2 , with comparisons of each prey type, one at a time, against all others: $\chi^2=1.61$, $df=1$, $P=0.20$), fish prey ($\chi^2=3.12$, $df=1$, $P=0.08$), reptile prey ($\chi^2=0.01$, $df=1$, $P=0.93$), or bird prey ($\chi^2=1.98$, $df=1$, $P=0.16$). However, the sexes did differ significantly in terms of frequency of consumption of mammals ($\chi^2=20.21$, $df=1$, $P<0.001$), with males taking fewer rodents than females. In addition, when we looked in more detail at the various types of amphibians consumed some significant intersexual differences emerged i.e. females took more adult *Bufo* than males ($\chi^2=88.86$, $df=1$, $P<0.001$), but fewer *Bufo* tadpoles ($\chi^2=12.99$, $df=1$, $P<0.001$) and adult *Rana* ($\chi^2=11.48$, $df=1$, $P<0.001$) than males.

In addition to differences between the sexes, there was a strong effect of locality on grass snake diet as (1) newts/salamanders were found only in two mountain areas (Duchessa Lake and Sella Nevea); (2) treefrogs were found only in a single area (Oliena); and (3) rodents were commonly preyed upon only at a single locality (La Marcigliana).

Amphibian prey were dominant at almost every study area apart from La Marcigliana (Fig. 1), and the percentage of amphibians in the diet was not significantly

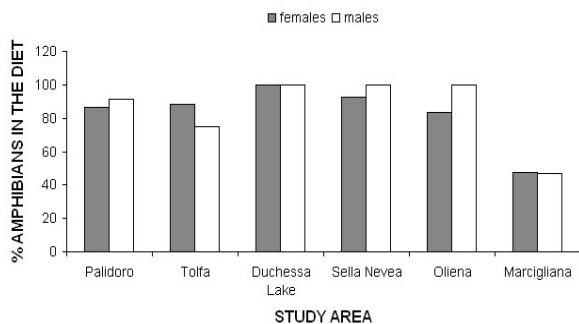


FIG. 1. Variation between sites in the percentages of amphibians in the diet of grass snakes of both sexes. For the total number of prey items at each study area, see Appendix 1.

related to the altitude of the various study areas in either females ($r^2=0.42$, regression: $F_{1,4}=2.94$, $P=0.16$) or males ($r^2=0.42$, regression: $F_{1,4}=2.91$, $P=0.16$). The two regression lines did not differ significantly in terms of slope (heterogeneity of slopes test: $F_{1,8}=0.03$, $P=0.87$) or y-intercepts (heterogeneity of intercepts: $F_{1,8}=0.09$, $P=0.62$).

Feeding on carrion was documented in two cases, where one adult male and one adult female from the Tolfa Mountains site ingested lizard corpses (*Podarcis muralis*). Evidence of carrion-feeding by grass snakes in these cases was proved by (1) the direct observation of a snake collecting and ingesting a lizard squashed on the road, and (2) by a lizard head, in perfect conditions (so clearly ingested within a short time), but without its body, regurgitated by another grass snake. In this latter case, it is likely that this lizard was killed by a bird of prey, which ingested the body of the lizard after having removed the head (which was subsequently ingested by the grass snake).

MONTHLY VARIATION IN DIETS

For reasons of climatic homogeneity, we considered the monthly variation in prey consumption by grass snakes from three areas of Mediterranean central Italy, i.e. Palidoro, Tolfa Mountains; and La Marcigliana (Table 5). The data collected from some other localities (Duchessa Lake, Oliena) were too few to be analysed in terms of monthly intervals, and data from Sella Nevea cannot be compared directly because the climate of this region is much harsher than that of the above areas. For both sexes, more prey were consumed during the spring months, particularly in May (Fig. 2). This seasonal pattern is partly explained by the presence of multiple prey items of a small size (e.g. anuran tadpoles) in the guts of some spring-captured grass snakes, but it also perhaps reflects a need for intense foraging after the hibernation period in this species. Indeed, if we consider the proportion of fed specimens (after having pooled data into two-month intervals in order to reach an adequate sam-

TABLE 5. Summary of the monthly distribution of diet data of grass snakes at three study areas in Mediterranean central Italy. Numbers of prey items are indicated in italics for females and boldface for males.

| Prey types | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov |
|------------------------------|--------------|------------|--------------|------------|------------|----------|----------|------------|--------------|
| PALIDORO | | | | | | | | | |
| <i>Anguilla anguilla</i> | | | 1 | <i>1,1</i> | | | | | |
| <i>Cyprinidae</i> sp. | | | <i>1</i> | 2 | <i>2</i> | | | | |
| <i>Rana bergeri</i> | | | <i>1,2</i> | 2,1 | <i>1,2</i> | <i>1</i> | | | |
| <i>Bufo bufo</i> tadpoles | <i>19,28</i> | <i>4,7</i> | | | | | | | |
| <i>Bufo bufo</i> metamorphs | | 6,3 | <i>12,10</i> | | | | | | |
| Amphibia (unidentified) | | | | | 1 | | | | |
| <i>Chalcides chalcides</i> | | | 1 | | | | | | |
| <i>Rattus</i> sp. | | | | | | | | <i>1</i> | |
| <i>Turdus merula</i> | <i>1</i> | | | | | | | | |
| <i>Passer</i> sp. | | <i>1</i> | | | | | | | |
| TOLFA MOUNTAINS | | | | | | | | | |
| <i>Anguilla anguilla</i> | | | | 1 | | | | | |
| <i>Cyprinidae</i> | | | | <i>1</i> | | | | | |
| Gobiidae | | | | <i>1,1</i> | <i>1,1</i> | 1 | 1 | | |
| <i>Bufo bufo</i> adults | | <i>1</i> | <i>4</i> | <i>3</i> | <i>1</i> | | <i>1</i> | | |
| <i>Bufo bufo</i> metamorphs | | 9,6 | 1 | | | | | | |
| <i>Rana italica</i> | | | | 1 | 1 | 1 | | <i>1</i> | |
| <i>Rana bergeri</i> | | <i>1,1</i> | <i>3,5</i> | <i>1,2</i> | 1 | | | | |
| <i>Rana bergeri</i> tadpoles | | | <i>1</i> | <i>4,1</i> | <i>1,1</i> | | | | |
| <i>Podarcis muralis</i> | | <i>1</i> | | | | | | 1 | |
| <i>Apodemus sylvaticus</i> | | | | | | | 1 | | |
| MARCIGLIANA | | | | | | | | | |
| <i>Bufo bufo</i> | | <i>1</i> | <i>2,4</i> | <i>1,3</i> | | | <i>1</i> | <i>1,1</i> | |
| <i>Bufo viridis</i> | | <i>3,1</i> | 1 | | | | | | |
| <i>Rana bergeri</i> | | | <i>1,3</i> | 1 | 1 | 1 | | | |
| <i>Microtus savii</i> | <i>1</i> | <i>2,1</i> | <i>4,1</i> | 2 | | | | <i>2,5</i> | <i>1,1 1</i> |
| <i>Rattus rattus</i> | | 1 | | | | | | 2 | |
| <i>Mus domesticus</i> | | | | | | | | 1 | |
| <i>Crocidura</i> sp. | 1 | | 1 | | | | | 1 | 1 |

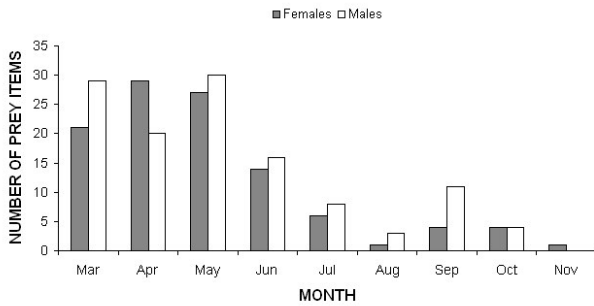


FIG. 2. Monthly numbers of prey items found in adult grass snakes of both sexes after pooling data from three localities of Mediterranean central Italy (Palidoro, Tolfa Mountains, and Marcigliana) that were carefully surveyed throughout the various periods of the year. Note the large number of prey items found during the spring months.

ple size for statistical comparisons), it also resulted in a peak of specimens with food in the stomach in the spring months (Fig. 3; March-April and May-June being the major peaks; at least $P < 0.05$: χ^2 test comparisons with all other two-month-intervals; differences not significant between the two above-mentioned intervals). Moreover, the feeding peak in spring (Figs. 2 and 3) mirrors a peak in above-ground grass snake activity that is seen in the same months at the Tolfa Mountains' site (Fig. 4; and see Filippi, 1995, page 102).

POTENTIAL COMPETITORS AND GRASS SNAKE DIET

Fish prey were found in only two out of six populations of grass snakes, and in both cases *Natrix tessellata*, a potential competitor feeding on fish (see Luiselli & Rugiero, 1991), was present at the site. Surprisingly, grass snakes did not eat fish in the four areas where *N. tessellata* was absent, although fish were present at one of these sites (see Discussion). This result was not an artifact of the sample sizes, as there were no significant relationships between the total number of prey items taken by grass snakes in each area and the

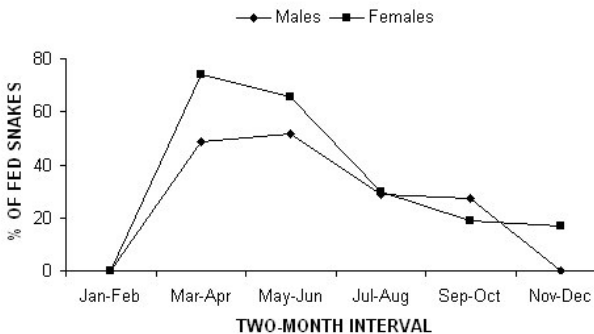


FIG. 3. Variation in the percentage of grass snakes with prey in the stomach in relation to period of capture. Data are included for three Mediterranean localities (La Marcigliana, Palidoro, and Tolfa Mountains). Data were grouped into two-month-intervals in order to reach an adequate sample size for statistical comparisons. Sample sizes: Jan-Feb, No. males=10, No. females=8; Mar-Apr, No. males=41, No. females=23; May-Jun, No. males=31, No. females=32; Jul-Aug, No. males=31, No. females=27; Sep-Oct, No. males=33, No. females=16; Nov-Dec, No. males=3, No. females=6.

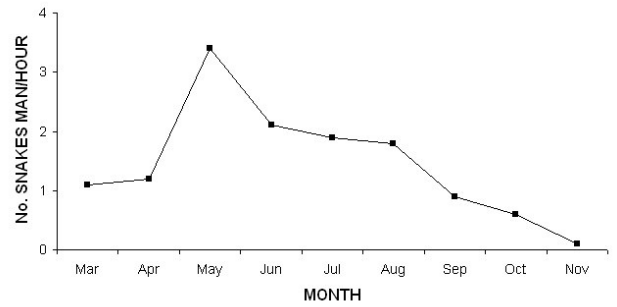


FIG. 4. Monthly patterns of above-ground activity of grass snakes at Tolfa Mountains evaluated in terms of mean number of specimens observed active per three hours of field effort made by two researchers working independently (for more details, see Filippi, 1995). Male and female data are combined.

number of fish eaten ($r=0.52$, regression: $F_{1,4}=1.50$, $P=0.29$). Also, the proportion of amphibians eaten was not much different between areas with or without *N. tessellata* (see Fig. 1).

DISCUSSION

COMPARING THE VARIOUS STUDY AREAS

Although it is generally accepted that grass snakes frequently feed upon anuran amphibians (e.g. see Kabisch, 1974; Arnold & Burton, 1978; Beshkov & Dushkov, 1981; Bruno & Maugeri, 1990) no previous study has examined local variation in the diet of the grass snake over a well defined geographical region, such as Italy. Thus, this study may be useful for discovering patterns of diet variation that have been masked by studies conducted at single sites. Furthermore, the information gained may be important from a conservation perspective.

Our study shows that the dietary variation of grass snakes is high. This is demonstrated by the preponderance of alternative prey to anurans in areas with particular habitat and climate characteristics. For instance, newts and salamanders are the main prey in high mountain regions with a cold climate, and voles are the main prey in an agricultural zone without pristine habitat patches. We interpret these dietary differences to be a consequence of differences in prey abundance (and perhaps relative availability of prey of different sizes) because all the studied mainland populations are genetically very close (M. Capula, unpublished data) and there is thus probably little influence of phylogeny on dietary variation. The smaller body sizes of grass snakes from Duchessa mountains in comparison to the other mainland localities (see Table 3) may be a consequence of limited snake activity in a very cold climate (i.e. short feeding period). If this is the case, it is another instance of the effects of plasticity in body sizes and life history traits of snakes (Madsen & Shine, 1993b; Forsman, 1996; Forsman & Shine, 1997; Queral-Regil & King, 1998; Bonnet *et al.*, 2001b). In this regard, it is noteworthy that in Swedish grass snakes, both the growth rates and the asymptotic body sizes were reduced because of low food availability, without any genetic modifications

of the populations (Madsen & Shine, 1993b). By contrast, the small body size of Sardinian grass snakes is more likely to be genetic, as (1) the average body lengths of the individuals of the subspecies *cetti* are known to be smaller than those of mainland Italy (Thorpe, 1975, 1979; Stefani, 1983), and (2) other insular snake populations offer some dramatic examples of geographic variation in body size (e.g. Shine, 1987; Schwaner & Sarre, 1988; King, 1989).

The effects of phylogenetic distance on food variations may, however, be relevant in the case of the genetically isolated and diverging populations from Sardinia (M. Capula, unpublished data), which showed a dietary spectrum remarkably different from that of their mainland conspecifics. Their diet consists only of hylids and lizards, which are rarely eaten on the mainland, and this may also be related to (1) the smaller size of Sardinian grass snakes compared to mainland populations (Stefani, 1983; Capula *et al.*, 1994); (2) the different prey base available in Sardinia compared with mainland Italy; and (3) the drier climate of the Sardinian mountains compared to the Apennines and the Alps.

MONTHLY VARIATION IN DIETS

Our data indicate that there is a higher probability of finding food in the stomachs of grass snakes captured in spring than in summer or autumn, at least in three Mediterranean study areas (Table 5). These results are in agreement with data on British grass snakes (Gregory & Isaac, 2004), which also showed a higher probability of having food in the stomachs during spring than in summer. However, British grass snakes also had a high probability of having food in stomachs during autumn (Gregory & Isaac, 2004), whereas the same pattern was not found in Italian conspecifics. We suggest that these differences are strongly linked to the different climates and, consequently, the likelihood of snakes finding amphibians active in the open. Indeed, in Mediterranean areas the weather in early autumn (September) is still very hot and dry, many ponds and streams are desiccated, and it is very hard to find frogs and toads active in the open. Hence, there is little reason for grass snakes to move around in search of prey. Conversely, the British weather is much wetter, and it is likely that the same problems in finding amphibians do not apply to British grass snakes. In European *Vipera* species, it has also been demonstrated that feeding rates tend to be lower in summer than in spring or autumn in the dry Mediterranean climate (Luiselli & Agrimi, 1991), whereas they are higher in summer than in spring or autumn in cooler and wetter regions (Brito, 2004). Thus, it seems evident that the foraging periods of European snakes are strongly related to the proximate climatic conditions of the various study areas.

POTENTIAL COMPETITORS AND GRASS SNAKE DIET

Interactions with a potential food competitor, i.e. the piscivorous *N. tessellata*, did not have any of the expected effects on the food types consumed by grass

snakes because grass snakes also consumed fish, which were generally not consumed in the absence of *N. tessellata*. We believe that grass snakes fed on fish in the two areas where *N. tessellata* is present merely because fish are an abundant food resource in these areas, and high dietary overlap can therefore be tolerated between the two snake species. Fish were also abundant at Duchessa Lake, but this is a high mountain site with cold water year-round and so it is likely that grass snakes cannot spend a long time foraging aquatically, and consequently cannot prey efficiently on fast-swimming animals such as fish. The snakes at Duchessa Lake are therefore possibly forced to feed upon newts, which are much easier to capture in cold water because they are slower than fish of a comparable size. Additionally, newts are found in shallower water (< 30 cm depth) than fish (generally > 90 cm depth), i.e. in sites where the water temperature is higher (E. Filippi & L. Luiselli, unpublished data).

The dynamics of coexistence of *N. natrix* and *N. tessellata* apparently differ from those of the Afrotropical marsh colubrids *Natriciteres variegata* and *Natriciteres fuliginoides* (see Luiselli, 2003), although in both cases a pattern of food resource partitioning seems to be present. The mechanism of food resource partitioning in *Natriciteres* species appears to be induced by the competition between two species which, when in separate locations, have generalist dietary preferences. In our study sites where the two *Natrix* species coexist, however, diet partitioning between the species seems to occur as *N. tessellata* seems to specialise on fish, and *N. natrix* on amphibians. Thus, in this latter case, it seems very unlikely that present day competition can explain the coexistence of these two species. Moreover, whereas the two *Natriciteres* species are nearly identical in terms of both body size and habitat preferences (Luiselli, 2003), *N. tessellata* is considerably smaller and more aquatic than its congener (Bruno & Mauderi, 1990). Perhaps *N. tessellata* has undergone a previous evolutionary character displacement for specialized feeding on fish. By contrast, *N. natrix* is not a specialist, but simply an adaptable species that usually prefers anurans, but feed on fish (or other prey types) if conditions allow. Concerning the island grass snake population, its potential competitor was not *N. tessellata* (which is absent from Sardinia, see Bruno & Mauderi, 1990), but the viperine snake *Natrix maura*, which is widespread and abundant in Sardinia (Bruno & Mauderi, 1990), and has been suspected to be a major competitor for *N. natrix cetti* (Stefani, 1983). Indeed, *N. maura* also has a dietary spectrum similar to that of *N. natrix*, with anuran amphibians and fish being the main prey (Santos & Llorente, 1998), and in Sardinia its main prey species were *Hyla sarda* and *Discoglossus sardus* (Rugiero *et al.*, 2000). Although it is possible that the two species may indeed compete for food at our island study area given their similar dietary spectrums, the absence of other control areas in Sardinia prevents us from stressing any conclusion on this issue. At the moment we

are still collecting data on the coexistence of these two species at several study areas in Sardinia, and perhaps we will present the data in a forthcoming article.

CONCLUSIONS

Our review shows that grass snakes consume a wide variety of vertebrate prey. The grass snake also can be added to the list of species that will occasionally eat carrion (for a review, see DeVault & Krochmal, 2002). In addition, we have shown that grass snake populations differ in their dietary spectrums. As further evidence of the dietary variability of these snakes, we note that populations in Kent (southern England) are known to feed intensively on *Rana ridibunda*, which is an introduced species to Great Britain (Gregory & Isaac, 2004). Such data may have implications for the management strategies used in grass snake conservation, especially given that their main prey (amphibians) are declining globally (see Gardner, 2001, for a review), which suggests that grass snakes may become threatened in areas with vulnerable amphibian populations. However, the variable diet composition exhibited by grass snakes suggests that these snakes possibly can be preserved in areas where their usual prey has declined drastically, because they presumably can switch to alternative prey. How – and how fast – dietary shifts occur remains to be seen because evidence for such shifts so far is based only on differences between populations (this study, and see also Gregory & Isaac, 2004), not on observed changes within populations. Indeed, it is possible, but still unverified, that grass snakes may be plastic in diet, i.e. that the individuals change diet in response to changing conditions (Ford & Seigel, 1994), but it is also possible that the observed variation in diet arises from local adaptation and is actually non-plastic. By contrast, the decline of amphibians (mainly due to the introduction of non-native trout) has apparently caused a decline of the amphibian-eating natricine snake *Thamnophis elegans elegans* in the Sierra Nevada (Matthews *et al.*, 2002), and so it is more than likely that the responses of the various species of natricine snakes to changes in amphibian prey availability may vary substantially.

In conclusion, *N. natrix* is not a specialist on anurans (e.g. see Bruno & Mauder, 1990) but an adaptable generalist with a preference for amphibians (e.g. Gregory & Isaac, 2004), and this information could be important for conservation if diet change can occur rapidly within a population.

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APPENDIX 1

- ecosystems: is there a link with declining amphibian populations and nonnative trout introductions? *Journal of Herpetology* **36**, 16-22.
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- PALIDORO (data from Luiselli & Rugiero, 1991): total number of prey items: 205; juveniles (shorter than 40 cm SVL) – *Anguilla anguilla* (n=1), Cyprinidae indetermined (n=1), *Rana bergeri* (n=1), *Bufo bufo* tadpoles (n=74), *Bufo bufo* metamorphs (n=16); females – *Anguilla anguilla* (n=1), Cyprinidae indetermined (n=3), *Rana bergeri* (n=4), *Bufo bufo* tadpoles (n=23), *Bufo bufo* metamorphs (n=18), *Rattus* sp. (n=1), *Turdus merula* (n=1), *Passer* sp. (n=1); males – *Anguilla anguilla* (n=2), Cyprinidae indetermined (n=2), *Rana bergeri* (n=5), *Bufo bufo* tadpoles (n=35), *Bufo bufo* metamorphs (n=13), Amphibia unidentified (n=1), *Chalcides chalcides* (n=1).
- TOLFA MOUNTAINS (data from Filippi *et al.*, 1996): total number of prey items: 63; females – Cyprinidae unidentified (n=1), Gobiidae unidentified (n=2), *Bufo bufo* adults (n=10), *Bufo bufo* metamorphs (n=9), *Rana italica* (n=1), *Rana bergeri* (n=5), *Rana bergeri* tadpoles (n=6), *Podarcis muralis* (n=1); males – Cyprinidae indetermined (n=1), Gobiidae unidentified (n=3), *Anguilla anguilla* (n=1), *Bufo bufo* metamorphs (n=7), *Rana italica* (n=3), *Rana bergeri* (n=9), *Rana bergeri* tadpoles (n=2), *Podarcis muralis* (n=1), *Apodemus sylvaticus* (n=1).
- DUCHESSA LAKE (data from Filippi & Luiselli, 2002): total number of prey items: 9; females – *Triturus carnifex* (n=2); males – *Bufo bufo* tadpoles (n=1), *Triturus carnifex* (n=6).
- SELLA NEVEA (data from Luiselli *et al.*, 1997): total number of prey items: 190; females – *Rana temporaria* adults (n=26), *Rana temporaria* metamorphs (n=4), *Bufo bufo* adults (n=22), *Bufo bufo* metamorphs (n=26), *Salamandra atra* (n=6), *Triturus alpestris* (n=7), *Lacerta vivipara* (n=1), *Apodemus sylvaticus* (n=6); males – *Rana temporaria* adults (n= 6), *Rana temporaria* metamorphs (n=9), *Bufo bufo* adults (n=2), *Bufo bufo* metamorphs (n=37), *Salamandra atra* (n=5), *Triturus alpestris* (n=3).
- OLIENA (data from Capula *et al.*, 1994): total number of prey items: 13; females – *Hyla sarda* adults (n=3), *Hyla sarda* tadpoles (n=2), *Podarcis tiliguerta* (n=1); males – *Hyla sarda* adults (n=3), *Hyla sarda* tadpoles (n=4).