SEXUAL AND SIZE-RELATED DIFFERENCES IN THE DIET OF THE SNAKE *NATRIX MAURA* FROM THE EBRO DELTA, SPAIN

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The Ebro Delta is a wetland area partially covered by rice fields. The viperine snake *Natrix maura*, is a common colubrid snake in this ecosystem. Males delayed feeding activity by one month, and foraged for a shorter time, compared to females. Diet consisted mainly of fish and frogs, although size-related and sexual differences were found: immature snakes had a more diverse diet than adults, and adult females ate more frogs than adult males. Males captured larger numbers of smaller fish than females. Like other water snakes, large viperine snakes avoid small prey. The biomass ingested is higher in large females, providing supplementary resources for reproduction. These dietary differences may be related to the ecological needs of each category of snakes and reflect the influence of the rice cycle on diet dynamics.

INTRODUCTION

Aquatic snakes of the genus *Natrix* are mainly found in temperate climates and often occur at high densities, allowing the collection of large enough samples for carrying out very detailed dietary studies. Indeed, the diet of the viperine snake, *Natrix maura*, has been described in several previous studies (e.g. Duguy & Saint Girons, 1966; Valverde, 1967; Alberch & González, 1973; Hopkins, 1974; Vericad & Escarre, 1976; Schätti, 1982; Hailey & Davies, 1986; Galan, 1988; Jaén, 1988; Pleguezuelos & Moreno, 1989).

Wetlands in the Ebro Delta Natural Park are highly productive areas with a high diversity and biomass of vertebrate fauna (Martínez-Vilalta, 1989). The natural vegetation has partially been supplanted by rice fields, which constitute temporary aquatic and highly dynamic ecosystems as a result of rapid changes in water levels (Fasola & Ruiz, 1996). These unusual environmental characteristics cause seasonal disturbances to the biology of semi-aquatic species, such as the viperine snake, which is the most common snake in the Ebro Delta ecosystem. Here we provide detailed data on the trophic ecology of *N. maura* in this area, highlighting sexual and size-related differences as well as the influence of the seasonal flooding of rice fields on the diet of this species.

MATERIAL AND METHODS

STUDY AREA

The Ebro Delta (40° 42'N, 0° 51'E) is a coastal plain of about 28 000 ha located in the north-east of the Iberian Peninsula. It is one of the most important wetland areas in the Mediterranean basin, and is protected by Spanish law.

More than 40% of the Ebro Delta surface is covered by rice fields, with a dense network of channels. The rest of the surface consists of natural wetlands and other field crops. The rice growing cycle is seasonal: the channels and rice fields are dry from December to April. By April the channels overflow and become colonized by a varied fauna including *Gambusia holbrooki*, *Cyprinus carpio* and *Rana perezi*. The rice grows until September, when it is harvested. After that, the fields and channels are drained again.

SAMPLING AND DATA RECORDING

Rice fields were searched for viperine snakes on at least two consecutive days per month between February 1990 and October 1991, by two observers. Every snake collected was induced to regurgitate following the method of Fitch (1987), and the prey items obtained in this way were preserved in 70% alcohol. No specimens were induced to regurgitate more than once during the study. Additional food samples consisted of the stomach contents of fresh specimens found killed on roads and of specimens sampled for histological studies. All the snakes were measured (snout-vent length, SVL in mm) and weighed. Adults were sexed by observation of gonads in dead specimens, and by tail shape and the number of subcaudal scales in live snakes (Feriche, Pleguezuelos & Cerro, 1993).

Prey items were measured (furcal length in fish and snout-vent length in frogs and tadpoles) and identified to species level, distinguishing between adult and larval forms of amphibians. Dry weight (biomass) of prey was estimated using linear regression of length against dry weight established using individuals collected in the Ebro Delta (González-Solís, Bernardí & Ruiz, 1996).

STATISTICAL PROCEDURES

The diet descriptors used were abundance (%P), occurrence (%N) and the resource use index (IU) (Jover, 1989). IU is a good descriptor of the diet because it combines in a single index (standardized as a percentage) the three diet components: (1) abundance, (2) occurrence and (3) homogeneity, which is estimated by calculating the diversity as a measure of variance of the animals consuming a resource.

To establish sexual and size-related differences in the diet, comparisons of (1) number of prey, (2) prey size, and (3) prey biomass were performed by ANCOVA analysis using SVL as a covariate. Sheffé *post hoc* tests were used after obtaining a significant result in the ANCOVA. Normality was assessed in all variables and log transformation was performed on the variables prior to ANCOVA tests. All means are reported ± 1 SE. All tests were two-tailed, and α was set at 5%.

Margalef's diversity index (Brillouin's index for diet) was used according to Pielou (1966, 1975) and Hurtubia (1973). Mean individual diversity (*Hi*), population diversity (*Hp*) (estimated by the jack-knife technique, see Jover, 1989) and total accumulated diversity (*Hz*) were calculated. Population diversities were compared by *t*-tests, instead of using the analysis of variance, because of their non-additivity. Evenness (*E*) was calculated using the Shannon-Weaver Diversity Index (Pielou, 1969; Magurran, 1989).

RESULTS

A total of 343 snakes were examined for food. Prey items were found only in 13.4% of individuals, all belonging to samples collected from March to October: 15 males (SVL= 322.3±13.4 mm), 15 females (SVL= 411.2±27.8 mm), 15 immatures (SVL= 163.7±8.2 mm) and one undetermined adult. The proportion of stomachs containing prey did not vary between sexes (χ^2=0.07, df=1, *P*=0.79). Adults (15.0%) contained food more frequently than immatures (10.9%), though differences were not significant (χ^2=0.92, df=1, *P*=0.34). Males only fed from April to July, while females fed for a longer period, i.e. from March to October. During August both sexes avoided feeding. Immatures showed the same foraging activity period as females.

NUMBER OF PREY PER STOMACH

The mean number of prey per stomach was 2.44±0.74 (mean±SE). Sixty-three percent of the examined snakes had only one prey item in the stomach, but the maximum number of prey found in a single stomach was 33 fish (a male, 392 mm SVL, captured in May 1990).

Male stomachs contained a higher number of prey (4.87±2.12) than females (1.46±0.17). Immatures contained only one prey item per stomach (Table 1). The differences in the number of consumed prey were significant among males, females and immatures (Kruskal-Wallis test: $H_{2,45}$ = 15.36, *P*<0.001). Dunn's *post hoc* test showed significant differences only between males and immatures.

There was no correlation between SVL and the number of prey items when all snakes were pooled (r_s=0.27, *n*=45, *P*=0.07), or when males and females were considered separately (males: r_s=-0.18, *n*=15, *P*=0.52; females: r_s=-0.11, *n*=15, *P*=0.68).

TABLE 1. Mean and standard error of the number of prey per stomach and of the prey size. Ranges are given in parentheses.

	Prey per stomach	Prey size (mm)
Males	4.87±2.12 (1-33)	19.91±0.66 (11-37)
Females	1.46±0.17 (1-3)	36.70±3.34 (18.7-84)
Adults	3.17±1.09 (1-33)	23.18±1.11 (11-84)
Immatures	1±0.00 (1)	26.34±2.36 (15-42)
Total	2.44±0.74 (1-33)	23.51±1.02 (11-84)

The mean number of fish per stomach did not differ from the mean number of frogs per stomach (Mann-Whitney *U*=136.5, *P*=0.15). The mean number of frogs per stomach did not differ between males and females (Mann-Whitney *U*=12.0, *P*=0.3), whilst the mean number of fish per stomach was significantly higher in males than in females (Mann-Whitney *U*=19.5, *P*= 0.04).

PREY TYPE AND SIZE

Only five prey types were detected in the stomachs: two fish (*Cyprinus carpio* and *Gambusia holbrooki*), adults and tadpoles of *Rana perezi*, and earthworms. None of the stomachs contained more than one kind of prey. Table 2 shows the diet descriptors for male, female and immature snakes. Frogs and carp were the prey most frequently eaten by adults. Mosquito fish, tadpoles and earthworms were sporadically preyed upon and consumed mainly by young snakes.

There were sexual differences in the prey type: males fed mainly on carp, with frogs as a secondary prey (*IU*=96.2% and 3.8%, respectively); whereas females fed on both these prey types without significant differences (*IU*=55.2% and 44.8%, respectively).

The total accumulated diversity was 0.43 in males, 0.88 in females and 1.61 in immatures. The population
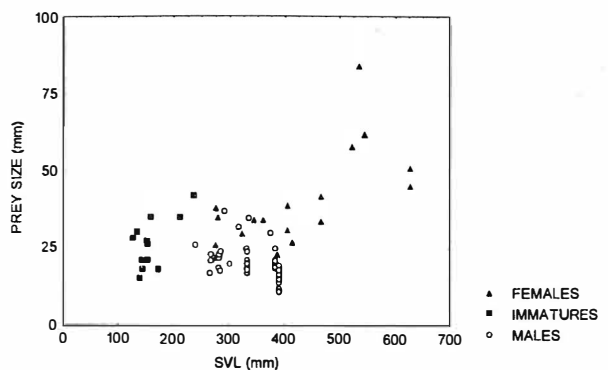

FIG. 1. Relationship between prey size and SVL for male, female and immature *Natrix maura* in the Ebro Delta.

TABLE 2. Diet descriptors. *N*, snake sample size; *n*, number of prey captured, %*P*, occurrence of each prey type; %*N*, abundance of each prey type. *IU*, Resource Use Index (Jover, 1989). *C.c, Cyprinus carpio, Gh. Gambusia holbrooki, Rp, Rana perezi* adults (ad) and larvae (l). Ew, earthworms. *One unsexed adult is included.

| | N | n | %P | | | | | %N | | | | | IU | | | | |
			Cc	Gh	Rp ad	Rp l	Ew	Cc	Gh	Rp ad	Rp l	Ew	Cc	Gh	Rp ad	Rp l	Ew
Males	15	73	73.3	-	20.0	6.7	-	91.8	-	4.1	4.1	-	96.2	-	3.8	-	-
Females	15	22	53.3	-	46.7	-	-	54.5	-	45.5	-	-	55.2	-	44.8	-	-
Adults*	31	104	64.5	-	32.3	3.2	-	84.6	-	12.5	2.9	-	84.2	-	15.8	-	-
Immatures	15	15	13.3	40.0	26.7	13.3	6.7	13.3	40.0	26.7	13.3	6.7	7.3	56.4	29.1	7.3	-
Total*	46	119	47.8	13.0	30.4	6.5	2.2	75.6	5.0	14.3	4.2	0.8	73.2	5.0	19.9	1.9	-

diversity and variance calculated by the jack-knife technique was 0.44 (v=1.50) in males, 1.02 (v=0.03) in females and 2.07 (v=0.69) in immatures. The comparisons between groups showed that the population diversity was higher in immatures than in both males (t=4.28, df=28, P=0.0002) and females (t=4.81, df=28, P<0.0001). There were no differences between males and females (t=1.82, df=28, P=0.08). Although the accumulated diversity cannot be compared between groups, the similar tendency of accumulated and population diversity values corroborates evidence that the samples used are representative of the diet spectrum (Jover, 1989). The evenness of diet was E=0.218 in males, E=0.994 in females and E=0.892 in immatures.

The larger snakes selected against smaller prey sizes (Fig. 1), as the minimum prey size increases directly with snake size (r_s=0.33, n=41, P=0.03). However, this correlation was significant only for females (r_s=0.63, n=15, P=0.01), but not for males (r_s=-0.05, n=14, P=0.87) or immatures (r_s=0.38, n=12, P=0.22).

Females fed on larger prey than males and immatures (Table 1; ANCOVA: $F_{2,37}$= 6.04, P=0.005). Moreover, females fed on larger fish than males when only fish-eating snakes were compared (Mann-Whitney U=13.0, P=0.01), but both sexes fed on similarly sized frogs (Mann-Whitney U=7.0, P=1.0).

There was a positive correlation between snake size and biomass eaten (r_s= 0.63, n = 41, P<0.001), especially in the case of females (r_s= 0.75, n = 15, P = 0.001). The contribution of frogs to biomass was higher than the other prey types (ANCOVA: $F_{3,36}$ = 6.56, P< 0.001).

DISCUSSION

The percentage of snakes containing prey in the stomach was higher when rice fields were flooded (19.3% wet and 6.4% dry, χ^2=9.56, df=1, P=0.002), suggesting that feeding frequency increased with prey availability. These results suggest that the seasonal flooding of this ecosystem (and the resulting dramatic shifts in water availability) can be an important factor regulating the trophic ecology of *Natrix maura* in the Ebro Delta.

In August, the feeding frequency was very low (4.3%). A reduction in feeding activity during the warmest month of the year has already been reported for *Vipera aspis* in Mediterranean central Italy (Luiselli & Agrimi, 1991). In both cases high temperatures during summer could be the determining factor.

Based on the proportions of specimens containing food in the stomachs, immature viperine snakes fed less frequently than adults, as in conspecific populations (Hailey & Davies, 1987; Pleguezuelos & Moreno, 1989) and in other species (e.g. Shine, 1987; Luiselli & Agrimi, 1991; Agrimi & Luiselli, 1994; Luiselli, 1996).

Males foraged from April to July, while females showed a longer feeding period, starting their foraging activity in March and finishing it in October. A similar delay in the commencement of feeding activity in males has been reported for other conspecific populations (Duguy & Saint-Girons, 1966; Pleguezuelos & Moreno, 1989), as well as for other snakes, such as *Nerodia sipedon* (Feaver, 1977; King, 1986) and *Morelia s. spilota* (Slip & Shine, 1988). This suggests that sexual activity reduces male feeding activity, as reported in vipers (i.e. Luiselli & Agrimi, 1991).

Gravid females were found in the field from June to August and, except for August, they fed even more frequently than in the other months. Females with very enlarged follicles contained prey in the stomach, while females with oviductal eggs did not. This contrasts with other studies where a reduction in food intake during gestation has been observed (Keenlyne, 1972; Keenlyne & Beer, 1974; Shine, 1979, 1980, 1986, 1987). A reduction in locomotive ability of gravid females could increase the risk of predation and could decrease foraging success in some snake populations (Shine, 1980; Seigel, Huggins & Ford, 1987). However, we hypothesize that the coincidence between gestation and flooding, when prey biomass abundance in the field is very high (González-Solís *et al.*, 1996), might induce females to maintain their feeding activity, even if it could involve a reduction in activity and an increase in predation risk.

The fact that males consumed more fish relative to females or immatures is similar to the pattern observed in *Acrochordus arafurae* (Houston & Shine, 1993). Indeed, only the males and the small females frequently contained more than one prey item in the stomachs. The low number of fish found in the stomachs of the largest females suggests that they spent less time foraging.

If only May-July is considered, sexual differences in the diet decreased (*IU*: 98.4% carp and 1.6% frogs for males; 77.4% carp and 22.6% frogs for females), and in both cases the diet consisted mainly of fish. The increase in frogs in the diet of females occurred in spring (March-April) when rice fields were dry, and autumn (September-October), after rice harvesting. In spring, drought led to a lack of fish, while in autumn, the fish population declined (although drought was not so marked as in spring), probably due to the influence of harvesting on the availability of this prey (González-Solís *et al.*, 1996).

Despite an increase in frogs in the diet of the largest snakes, a size-related change in the type of fish consumed was observed with increases in snake size (from *Gambusia* to *Cyprinus*). Furthermore, immatures eat more earthworms and tadpoles than adults, as previously reported in other studies (Schätti, 1982; Hailey & Davies, 1986; Pleguezuelos & Moreno, 1989). Size-related changes in diet composition have also been observed in other water snakes: e.g. *Natrix natrix* (Luiselli, Capula & Shine, 1997), *Nerodia rhombifera*, *N. cyclopion*, *N. fasciata* and *N. erythrogaster* (Mushinsky, Hebrard & Vodopich, 1982), and *N. rhombifera* studied in a fish hatchery in Arkansas (Plummer & Goy, 1984).

The evenness values of the diet showed that males fed on a dominant prey type (carp) whereas female diet consists of two different and equally abundant prey (carp and green frogs). Immatures fed on a wider range of prey (five taxa) than adults, and none of them were dominant as shown by the evenness value.

A size-related shift in the lower size limit of prey was observed when all the individuals were considered. However, only snakes larger than 400 mm SVL (all females) tended to select against smaller prey sizes. This pattern, characteristic of fish-eating snakes (see Plummer & Goy, 1984; Jayne, Voris & Heang, 1988), corresponds to the concept of "ontogenetic shift in lower size limit" and has been qualified as "enigmatic" by Arnold (1993). In the range of prey sizes consumed, only the smaller females overlap with males and immatures. This apparent resource partitioning might be relevant for reducing intraspecific competition.

Only females showed a positive correlation between biomass ingested and SVL. In many snake species, including *N. maura* (Santos & Llorente, unpublished data), large females have a higher clutch mass (Seigel & Ford, 1987). If females need more food for reproductive output (Houston & Shine, 1993; Shine, 1989), sexual dietary differences in food consumption can be explained in terms of the additional food needed for reproduction, as has been previously shown in *Natrix*

natrix (Luiselli *et al.*, 1997) and *Coronella austriaca* (Luiselli, Capula & Shine, 1996).

ACKNOWLEDGMENTS

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