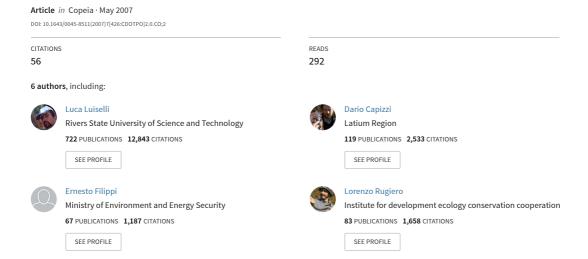# Comparative Diets of Three Populations of an Aquatic Snake (Natrix Tessellata, Colubridae) from Mediterranean Streams with Different Hydric Regimes

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Comparative Diets of Three Populations of an Aquatic Snake (*Natrix tessellata*, Colubridae) from Mediterranean Streams with Different Hydric Regimes

Luca Luiselli, Dario Capizzi, Ernesto Filippi, Claudio Anibaldi, Lorenzo Rugiero, and Massimo Capula

The Dice Snake (*Natrix tessellata*) is an aquatic colubrid that is known for having a basically piscivorous diet and a female-biased sexual size dimorphism (larger females). The feeding habits of three populations of *N. tessellata* from Mediterranean streams in central Italy were studied. The three streams differed in terms of their water regimes, one being seasonal and two being perennial. More than 2,000 snake individuals were handled for food items, and 1,200+ prey items were collected. The percent of fed snakes was significantly influenced by the study area and by the interaction term between study area and season (i.e., by the hydric regime). Fishes accounted for over 90% of the snake diets at all study areas, the most common prey species being cyprinids. Overall, the three snake populations had similar taxonomic diet composition. There was a significant ontogenetic change in diets in all populations. There were also significant intersexual differences in prey composition, with the females taking more *Scardinius erythrophtalmus* fishes and more anurans, and the males more *Leuciscus s. muticellus*. The various fish species found at the three study areas (15 species) were classified in terms of six independent ecological characteristics in order to investigate what factors make a given prey species suited or not for Dice Snakes. It appeared that *N. tessellata* selected mainly fish species that were gregarious, small to medium sized, active foraging, diurnal, and not using mud as preferred substratum. We also found that the seasonal desiccation of the stream-bed of one study area caused a shift in the diet composition of these snakes, with males and juveniles being the categories where the seasonal dietary shift was more evident. Males preyed especially on the fishes *S. erythrophtalmus* and *Leuciscus cephalus*, and the juveniles preyed upon *Leuciscus s. muticellus*, whereas in spring their primary prey was *Alburnus a. alborella*.

S TUDIES on the feeding ecology of snakes have in general focused on the list of prey eaten, the correlates of prey size, the relationships between predator size and prey size, and their potential relevance to optimal foraging theories (Mushinsky, 1987; Arnold, 1993, and references therein). Compared to other reptiles, the dietary habits of snakes are generally less well known (Greene, 2001). The main complicating factor for the research on snake feeding ecology is that these animals are in general secretive and usually difficult to observe while foraging in the wild. Thus, although in the current literature there is a great deal of information concerning snake dietary habits (Greene, 2001), field studies focusing on the food habits of large samples of snakes are still generally scarce, although they include species from Europe (Luiselli et al., 1997; Filippi et al., 2005), North America (Gregory, 1978, 1984; Gregory and Nelson, 1991), Africa (Luiselli and Akani, 2003; Luiselli et al., 2003), and Australia (Shine, 1991).

In this study we present data on the food habits of a large number of free-ranging individuals of the Dice Snake (*Natrix tessellata*). We focus on the interpopulation, intersexual, and ontogenetic variation of this species' diet, as well as on the potential factors that may orientate these snakes towards predation on specific prey. These aquatic colubrid snakes are medium sized (up to 140 cm long; Bruno and Maugeri, 1990), oviparous, mainly piscivorous (Luiselli and Rugiero, 1991), and are very abundant in the freshwater habitats of central Italy (Filippi, 2000). Although the general diet composition of this species has already been studied (Luiselli and Rugiero, 1991; Filippi et al., 1996), nothing is known of the interpopulation variation in diet composition, especially the diet variations in relation to the hydric properties of the water bodies inhabited by this species. These data are necessary to fully understand the feeding ecology and the foraging strategies of *N. tessellata,* especially since the Mediterranean climate is strongly seasonal (dry and hot in summer, cool and rainy in spring and autumn), and seasonal variations in the water regimes of streams are well known (Tomaselli et al., 1973).

In our study, the following questions are asked: Are there intersexual dietary differences in our

study species (Shine, 1986; Houston and Shine, 1993), considering that in species of the genus *Natrix* there is a female-biased sexual size dimorphism (SSD; larger female; Madsen, 1983; Luiselli et al., 1997; Gregory, 2004), including in *N. tessellata* populations (Bruno and Maugeri, 1990; Filippi, 1995; Luiselli and Rugiero, 2005)? Is there any significant ontogenetic change in *N. tessellata* diet? Does *N. tessellata* diet composition vary between different types of freshwater habitats (seasonal vs. permanent streams)? Are there any specific ecological characteristic of fishes (e.g., gregarious vs. solitary habits, benthonic vs. active swimming habits) preyed upon by *N. tessellata*?

Materials and Methods

Study areas.—The field study was carried out at three study areas in the Tolfa Mountains (250–350 m elevation) situated from about 50 to 80 km north of Rome, Italy. The Tolfa Mountains have been intensively surveyed by us for long-term studies on snake ecology (Luiselli and Agrimi, 1991; Filippi et al., 1996, 2005). The three study areas were: Rota (ROT in the following text), Ponte Bernascone (BER), and Rio Fiume valley (RIO). All study areas were under essentially identical climatic conditions: Mediterranean-temperate with cold winter, rainy spring and autumn, and dry and hot summer (hypomesaxeric subregion [type B] according to Tomaselli et al., 1973). All study areas consisted of a freshwater stream ('Fosso Verginese' in ROT, 'Fiume Mignone' in BER, and 'Rio Fiume' in RIO) and its banks. However, streams of the study areas varied substantially in terms of stream regime: at ROT the stream is perennial, although the water depth tends to decline during the summer (July to September); at BER much of the stream is perennial, but there are some small spots where the stream desiccates as a response to the summer drought (on average, about 10% of the stream desiccates); at RIO the water regime is strongly seasonal, and most of the stream desiccates during the summer. At RIO, during the summer the whole stream is fragmented into a series of small, stagnant ponds interspersed inside a stony dry river-bed. The streams were similar in terms of water depth, current speed, and type of vegetation during the spring (on average, 80–150 cm of water depth). The data sets collected in each area were subdivided into two samples: spring (April to June) and summer (July to September).

Protocol.—Data were collected at each study area from April 1985 to September 2004. Each field day lasted 7–8 hours, with fieldwork conducted under all climatic conditions. Every effort was made to maintain an equal effort during the various diel phases. Data from one study area (ROT) were already presented in part elsewhere (Filippi, 1995; Filippi et al., 1996). Overall field effort was similar among study areas (166 field days at ROT, 159 at BER, and 171 at RIO).

Field methods were identical at all study areas. In each study area, a 2-km tract of stream (and its banks) was surveyed on each field day by three people during both spring and summer months. We searched for snakes by walking slowly along the stream and their banks. On each field day, the whole stream transect was surveyed in a standardized way: we walked once in the river-current direction and once in the opposite direction. In both directions, a person walked along each bank, and the third person walked inside the river. We captured snakes by hand, recording the site of capture and the micro-habitat at each site. Each snake was sexed by examining the shape of the cloacal region, measured for snout–vent length (SVL, to the nearest ± 1.0 mm), weighed with an electronic balance (to the nearest ± 0.1 g), and individually marked by ventral scale clipping for future identification. The snakes were then palpated in the abdomen until regurgitation of ingested food. Only stomach contents were used. We did not collect feces because *N. tessellata* can feed regularly on amphibians (Beshkov and Dushkov, 1981), but it is almost impossible to quantify the number of amphibians eaten by fecal analysis (Luiselli et al., unpubl. data). Hence, the use of feces would have necessarily underestimated the presence of amphibian-prey versus fish-prey in the diets of Dice Snakes, thus seriously biasing our conclusions. Individuals found dead during our surveys were dissected to determine if prey were present. We identified prey items to the lowest taxonomic level possible and estimated the mass of prey items at the time of ingestion 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 always counted number of prey items rather than number of snakes with a particular prey type.

For the analyses of ontogenetic diet variation, food data coming from juveniles were compared with food data from adults (males and females pooled), because several juveniles were not sexed (due to the possibility of incorrect sex identification in juvenile *N. tessellata* by cloacal region examination; Filippi, 1995). For all analyses we separated juveniles from adult males and adult females. Adults were discriminated from juve-

niles using the minimum SVL of males that courted a female (48 cm) and the minimum SVL of females carrying eggs (55 cm) in the study areas (Luiselli and Rugiero, 2005). To avoid non-independence of data (Hurlbert, 1984), body size and prey type were recorded only once from each individual (i.e., it was not recorded in recaptured individuals). Fish ecological characteristics and body size scores were attributed by following Betti (1999) and Zerunian (2002). The list of fish species potentially available to *N. tessellata* at the study areas is presented in Cataudella (1977). Seven ecological characteristics of the fish species found in the three study areas were compiled, as they may be potentially important for determining their frequency of being preyed upon by the snakes. These characteristics were: (1) fish body size; (2) gregarious or solitary behavior; (3) preferred substratum type; (4) swimming depth; (5) sit-and-wait or active forager; (6) diurnal, nocturnal, or both; and (7) introduced or native in the study areas. Three body size categories were used for fishes: small (<13 cm in length from head to the basis of the tail, scored 1), medium (13 to 25 cm, scored 2), and large fishes (>25 cm, scored 3). Data on whether a fish species is exotic or native was included because an exotic fish species may be potentially less adapted to seasonally-variable water regimes of the Mediterranean streams (Zerunian, 2002), and hence perhaps easier to catch by snakes.

Statistical analyses were done using Statistica 6.0 and SPSS 8.0 for Windows PC, with all tests being two-tailed. When multiple tests were used, the Bonferroni adjustment was used to correct the alpha level depending on the number of tests performed. When χ^2 test had df = 1, the Yates' correction was applied (Zar, 1984). General loglinear models (GLM) were used to test for the separate effects of snake category (juveniles, males, females), study area (BER, RIO, ROT), and survey period (spring, summer) on the percentage of fed snakes, as well as for the interaction terms between these variables. Linear regression analysis was performed to compare (log) predator size (SVL) and (log) prey size (body length and mass of the prey items), with the Pearson's correlation coefficient being used. Two-way ANOVA of SVL (study site × sex) was used to evaluate SVL differences between sexes and across study areas. Since ANCOVA is used to compare mean values of a dependent variable among treatment levels while adjusting for the value of a potentially confounding variable with which the dependent variable is (usually linearly) related, an assumption of ANCOVA is that the slope of a given relationship is the same for each treatment level. Hence, we compared slopes between two different regression lines as a test of an assumption of ANCOVA (Zar, 1984).

RESULTS

Effects of snake category, area, and period.—Pooling the three study areas, 2255 snakes were captured, with 53.8% containing identifiable prey in the stomachs (Table 1). One-way ANOVAs indicated that neither snake category ($F_{2,1210} = 0.059$, $P = 0.812$) nor season had an effect on the percentage of fed snakes ($F_{1,1210} = 0.649$, $P = 0.505$), whereas study area had a significant effect ($F_{2,1210} = 28.021$, $P < 0.01$). The interaction of study area × season was highly significant ($F_{2,1210} = 46.099$, $P < 0.00001$).

Overall diet composition.—A total of 1533 prey items were collected from the three study populations (458 from juveniles, 526 from adult males, and 549 from adult females; Table 2). Fish accounted for the great majority of the total prey items (96.9%, $n = 1,533$), with the remaining prey being anurans. The proportion of anuran prey (range 1.1–4.7%, depending on the snake population) did not vary significantly among study areas (χ^2 test: $P = 0.341$, df = 2). Among prey species, cyprinids accounted for the highest proportion of prey items (87.5% of 391 identified fishes at ROT, 94.5% of 421 identified fishes at BER, and 95.2% of 611 identified fishes at RIO). There was no relative difference in the abundance of the Cyprinidae as prey among the three snake populations (χ^2 test: $P = 0.247$, df = 2). Other fish families (Gobiidae, Cobitidae, and Anguillidae) were preyed upon significantly less frequently than cyprinids in each study area (χ^2 test: in all cases $P < 0.0001$, df = 1), and salmonids were never preyed upon by snakes.

The most common prey species in the snake diets at all study areas were the cyprinids *Alburnus alburnus alborella, Leuciscus souffia muticellus,* and *Scardinius erythrophtalmus* (Table 2). Other fish species were common prey for snakes at a particular study area but not in others (e.g., *Rutilus rubilio* at BER; *Leuciscus cephalus* at ROT).

Ontogenetic diet variation.—There were significant diet differences between juveniles and adults at all study areas (ROT, observed vs. expected $\chi^2 = 70.66$, df = 7, $P < 0.0001$; BER, $\chi^2 = 116.14$, df = 7, $P < 0.0001$; RIO, $\chi^2 = 94.19$, df = 7, $P < 0.0001$). The main ontogenetic differences were that adults snakes preyed significantly more often on *S. erythrophtalmus, R. rubilio, L. cephalus,* and *G. nigricans* at all study areas (χ^2 test: in all cases, at

TABLE 1. NUMBER OF INDIVIDUALS EXAMINED AND PERCENTAGE OF FED SPECIMENS OF *Natrix tessellata* FROM THREE STUDY AREAS IN CENTRAL ITALY.

	Spring juveniles	Spring males	Spring females	Summer juveniles	Summer males	Summer females
ROTA						
No. captures	93	121	89	129	108	111
% of fed snakes	63.4	38.8	43.8	47.3	45.4	56.7
PONTE BERNASCONE						
No. captures	68	157	137	68	155	161
% of fed snakes	42.6	46.5	57.7	32.3	63.2	47.2
RIO FIUME						
No. Captures	94	133	120	136	188	187
% of fed snakes	62.8	48.1	65.8	72.8	56.4	59.3

least $P < 0.001$), *Barbus plebejus* at ROT and BER, and *S. fluviatilis* at ROT (χ^2 test: $P < 0.001$), and that juveniles preyed significantly more often on *A. a. alborella* at RIO ($\chi^2 = 17.38$, $P < 0.001$). The number of prey categories eaten was always less in juveniles than in adults of either sexes (ROT: 6 vs. 10 [males] and 11 [females]; BER: 8 vs. 9 and 9; RIO: 8 vs. 11 and 12; Table 2). Hence, overall the dietary diversity increased from subadult to adult age.

Sexual size dimorphism and intersexual diet variation.—The mean SVL of adult females (ROT, x = 76.3 ± 33.1 cm, $n = 192$; BER, x = 78.2 ± 31.8 cm, $n = 298$; RIO, x = 73.0 ± 27.5 cm, $n = 307$) was significantly larger than that of adult males (ROT, x = 64.3 ± 18.7 cm, $n = 229$; BER, x = 61.2 ± 19.3 cm, $n = 312$; RIO, x = 63.3 ± 19.6 cm, $n = 321$; t-test: df = 1657, $P < 0.0001$). A two-way ANOVA (SVL × study area + sex) revealed that there were significant differences in (ln-transformed) SVL between sexes at each study area (= female larger; $F_{1,1658} = 41.39$, $P < 0.0001$) but not among study areas ($F_{2,1657} = 0.13$, $P = 0.082$), and the interaction term in the ANOVA between sex and study area was not significant ($F_{2,1657} = 0.09$, $P = 0.198$). Repeating the analyses with the largest 20% of each sex in the sample (in order to overcome problems of one sex maturing at a smaller size) resulted in females being significantly larger than males (x = 88.7 ± 14.6 cm, $n = 162$ vs. 73.1 ± 8.6 cm, $n = 192$; one-way ANOVA: $F_{1,351} = 44.16$, $P < 0.0001$).

TABLE 2. LIST OF FOOD DATA OBTAINED FROM *Natrix tessellata* AT THREE STUDY AREAS IN CENTRAL ITALY. Symbols: Sp = Spring, Su = Summer, J = Juveniles, M = Males, F = Females, BER = Ponte Bernascone, RIO = Rio Fiume, ROT = Rota.

Prey	J (Sp) ROT; BER; RIO	M (Sp) ROT; BER; RIO	F (Sp) ROT; BER; RIO	J (Su) ROT; BER; RIO	M (Su) ROT; BER; RIO	F (Su) ROT; BER; RIO
Fishes						
Alburnus a. alborella	20; 2; 41	8; 8; 10	4; 6; 9	21; 3; 62	9; 18; 11	2; 10; 4
Leuciscus s. muticellus	31; 17; 27	38; 20; 36	14; 31; 24	49; 19; 71	36; 29; 58	11; 14; 59
Leuciscus cephalus	0; 0; 0	5; 6; 9	3; 5; 23	0; 1; 0	10; 8; 31	5; 7; 26
Scardinius erythrophtalmus	0; 0; 0	4; 6; 6	8; 28; 12	0; 0; 0	3; 8; 8	31; 23; 29
Rutilus rubilio	0; 6; 0	3; 21; 5	4; 15; 1	0; 4; 0	5; 21; 7	3; 9; 4
Barbus plebejus	0; 3; 1	2; 8; 0	5; 11; 0	0; 4; 1	6; 14; 6	2; 13; 1
Gobius nigricans	2; 0; 2	0; 10; 1	11; 0; 10	1; 1; 2	2; 5; 1	13; 6; 7
Salaria fluviatilis	0; 0; 0	0; 0; 0	8; 0; 0	0; 0; 0	2; 0; 0	7; 0; 0
Cobitis taenia	0; 0; 0	0; 0; 0	1; 0; 0	0; 0; 0	2; 0; 0	0; 0; 0
Anguilla anguilla	0; 0; 0	0; 0; 2	0; 0; 1	0; 0; 1	0; 1; 2	0; 0; 0
Undet. Fish	4; 3; 7	0; 3; 3	0; 1; 5	2; 3; 9	1; 6; 4	1; 6; 4
Amphibians						
Rana italica	0; 0; 0	0; 0; 0	1; 0; 0	0; 0; 0	0; 0; 3	0; 1; 5
Bufo bufo froglets	3; 0; 3	0; 0; 2	0; 0; 0	0; 0; 0	0; 0; 0	0; 0; 0
Bufo bufo tadpoles	7; 4; 7	0; 0; 8	0; 0; 4	0; 0; 0	0; 0; 0	0; 0; 0

There were significant diet differences between males and females at all study areas (ROT, observed vs. expected $\chi^2 = 41.29$, df $= 7$, $P < 0.0001$; BER, $\chi^2 = 16.25$, df $= 6$, $P < 0.013$; RIO, $\chi^2 = 17.51$, df $= 6$, $P < 0.008$). The main intersexual differences were a significant excess of *L. s. muticellus* at ROT in male snakes ($\chi^2 = 12.12$, $P < 0.001$), and in female snakes, a significant excess of *S. erythrophtalmus* at both ROT ($\chi^2 = 11.13$, $P < 0.001$) and RIO ($\chi^2 = 10.53$, $P < 0.02$), *Gobius nigricans* at both ROT ($\chi^2 = 9.3$, $P < 0.01$) and RIO ($\chi^2 = 5.92$, $P < 0.05$), and *Salaria fluviatilis* at ROT ($\chi^2 = 4.98$, $P < 0.05$). Pooling the data on anuran-eating by snakes from the three populations in order to increase the sample size, we found that adult females fed significantly more often upon anurans than males ($\chi^2 = 33.62$, df $= 1$, $P < 0.0001$). Overall, it is concluded that the diet composition of the two sexes of *N. tessellata*, although qualitatively similar (fishes representing more than 90%), was significantly different at all study areas when a finer (= identification to species level) analysis of prey items was performed.

In order to explore whether intersexual differences in diet composition depended on intersexual body size differences, we compared diets of the two sexes over the same body size range (categories: 55–59.9 cm [$n = 182$], 60–64.9 cm [$n = 207$], 65–69.9 cm [$n = 211$], 70–74.9 cm [$n = 203$]; > 75 cm [$n = 81$]). The two sexes did not differ significantly when belonging to the first three body size categories (55–69.9 cm; χ^2 test: at least $P > 0.09$), but they differed significantly when belonging to the fourth ($\chi^2 = 24.11$, $P < 0.0001$) and fifth categories ($\chi^2 = 8.74$, $P < 0.01$). The main differences for both size categories was the higher frequency of consumption of *S. erythrophtalmus* and anurans by females.

Prey and predator size relationships.—The larger snakes selected against smaller prey sizes at all study areas, as the minimum prey size (g) increased directly with snake size (ROT, $r = 0.41$, $n = 116$, $P < 0.01$; BER, $r = 0.36$, $n = 124$, $P < 0.01$; RIO, $r = 0.39$, $n = 126$, $P < 0.01$). The differences between slopes of the regression lines were not significant between ROT and BER ($P = 0.506$), ROT and RIO ($P = 0.224$), and BER and RIO ($P = 0.184$). This correlation was significant for females ($r = 0.66$, $n = 127$, $P < 0.0001$), but not for males ($r = 0.18$, $n = 120$, $P = 0.11$) or juveniles ($r = 0.12$, $n = 119$, $P = 0.14$). The differences between slopes of the regression lines were statistically significant between females and males ($P < 0.0001$), females and juveniles ($P <$

0.0001), but not between males and juveniles ($P = 0.584$).

Seasonal diet variation.—Overall, adult snakes exhibited similar taxonomic dietary preferences in both spring and summer in the two study areas with perennial and semi-perennial hydric regimes (ROT, males: $\chi^2 = 4.21$, df $= 7$, $P > 0.75$; females: $\chi^2 = 8.38$, df $= 7$, $P > 0.30$; BER, males: $\chi^2 = 4.69$, df $= 6$, $P = 0.584$; females: $\chi^2 = 7.96$, df $= 6$, $P = 0.241$). On the other hand, at the study area with seasonal hydric regime (RIO) the females did not differ significantly ($\chi^2 = 10.40$, df $= 6$, $P = 0.109$) whereas the males did ($\chi^2 = 17.86$, df $= 6$, $P = 0.006$).

There were significant interseasonal differences in the frequency of consumption of some specific prey types. First, at both ROT and RIO, *S. erythrophtalmus* was eaten significantly more often in summer by female snakes ($\chi^2 = 6.78$, df $= 1$, $P < 0.01$ for ROT; $\chi^2 = 7.38$, df $= 1$, $P < 0.001$ for RIO). Second, at RIO, *S. erythrophtalmus* ($\chi^2 = 6.05$, df $= 1$, $P < 0.001$) and *L. cephalus* ($\chi^2 = 6.05$, df $= 1$, $P < 0.001$) were eaten significantly more often in summer by male snakes. As in adults, the spring and summer diets of juveniles differed significantly at RIO ($\chi^2 = 12.02$, df $= 3$, $P < 0.0074$) but not at the other two study areas (ROT, $\chi^2 = 4.90$, df $= 2$, $P = 0.386$; BER, $\chi^2 = 1.93$, df $= 5$, $P = 0.859$). At RIO, the statistical difference depended only on the excess of *L. s. muticellus* in summer ($\chi^2 = 9.88$, df $= 1$, $P < 0.001$).

Selection of fish-prey by snakes.—The ecological characteristics of the various fish species (whether or not preyed upon by snakes) are summarized in Table 3. Fifteen fish species were potentially available to snakes, but only ten were consumed (Table 3).

The frequency of occurrence of the various fish-prey in the snake diets was significantly affected (after Bonferroni adjustment of significance level for multiple tests, with $P < 0.007$ being the significance threshold) by some fish-prey ecological characteristics. In particular, the significant ecological characteristics were: gregarious/solitary habits (one-way ANOVA; $F_{1,268} = 115.36$, $P < 0.0001$; gregarious fishes being more preyed upon), fish body size ($F_{2,267} = 25.38$, $P < 0.0001$; larger fishes being significantly less preyed upon), sit-and-wait or active foragers $F_{1,268} = 108.39$, $P < 0.0001$; active foraging fishes being more preyed upon), diurnal habits ($F_{2,267} = 11.93$, $P < 0.0001$), and type of substratum ($F_{2,267} = 7.88$, $P < 0.0005$). Tukey HSD *post hoc* test revealed that, as regards to substratum type, mud fishes were significantly less preyed than

TABLE 3. ECOLOGICAL CHARACTERISTICS AND BODY SIZE OF THE FISH SPECIES AVAILABLE IN THE THREE STUDY AREAS AS POTENTIAL PREY FOR *Natrix tessellata*. Categories: body size of adults—1 = small (up to 13 cm); 2 = medium (13 to 25 cm); 3 = large (more than 25 cm).

Fish species	Preyed upon by snakes?	Body size category	Native or exotic	Solitary or gregarious	Preferred type of river-bed substratum	Swimming depth	Sit-and-wait or active forager	Diurnal or nocturnal
Salmonidae								
Salmo trutta fario	no	3	exotic	solitary	stony	surface-swimmer	sit-and-wait	diurnal
Oncorhynchus mykiss	no	3	exotic	solitary	stony	surface-swimmer	sit-and-wait	diurnal
Cyprinidae								
Alburnus alburnus alborella	yes	1	exotic	gregarious	generalist	surface-swimmer	active	diurnal
Leuciscus souffia muticellus	yes	2	native	gregarious	stony	benthonic	sit-and-wait	diurnal
Leuciscus cephalus	yes	3	native	solitary	generalist	surface-swimmer	active	diurnal
Scardinius erythrophtalmus	yes	3	exotic	gregarious	mud	surface-swimmer	active	diurnal
Rutilus rubilio	yes	2	native	gregarious	stony	benthonic	active	diurnal
Barbus plebejus	yes	3	native	gregarious	stony	benthonic	active	diurnal
Cyprinus carpio	no	3	exotic	solitary	mud	benthonic	sit-and-wait	both
Tinca tinca	no	3	native	solitary	mud	benthonic	sit-and-wait	both
Gobiidae								
Gobius nigricans	yes	1	native	solitary	stony	benthonic	sit-and-wait	nocturnal
Salaria fluviatilis	yes	1	native	solitary	generalist	benthonic	sit-and-wait	both
Cobitidae								
Cobitis taenia	yes	1	native	solitary	mud	benthonic	sit-and-wait	nocturnal
Anguillidae								
Anguilla anguilla	yes	3	native	solitary	mud	benthonic	sit-and-wait	nocturnal
Ciclostomata								
Lampetra planeri	no	2	native	solitary	stony	benthonic	sit-and-wait	nocturnal

those using other substratum types (at least $P < 0.008$). On the other hand, swimming depth ($F_{1,268} = 0.23$, $P = 0.632$) and exotic/native origin ($F_{1,268} = 1.10$, $P = 0.294$) did not have any significant effect. Overall, we conclude that the combination of ecological characteristics of fishes presented in Table 3 reliably classified the groups of fish species that were eaten by *N. tessellata*.

DISCUSSION

The very large dataset collected in this study allowed us to identify several patterns of the foraging ecology of *N. tessellata* that remained undetected in previous studies of conspecifics based on smaller sample sizes (Luiselli and Rugiero, 1991; Filippi, 1995; Filippi et al., 1996). The only significant effects revealed by our loglinear models were the study area plus the interaction term between study area and season. Although *N. tessellata* showed a feeding cycle less regular than that observed in other European snakes (Saint Girons, 1979), it is likely that there was an effect of hydric regime on the percentage of fed individuals (given that hydric regime is precisely a 'physical outcome' of the interaction between a study area's morphological characteristics and its seasonally variable climatic conditions).

Ontogenetic dietary changes.—The three populations of *N. tessellata* studied here showed an ontogenetic change in diet composition. Diet diversity increased with body size, with adults having a broader dietary spectrum than juveniles. In particular, the adults fed on some fish species that were never eaten by juveniles and foraged significantly more frequently than juveniles on several other fish species. These patterns are

widespread in natricine snakes (*Afronatrix anoscopus,* Luiselli et al., 2003; *Natrix natrix,* Luiselli and Rugiero, 1991). However, there are snake species where the opposite pattern seems to be true (*Natrix maura,* Santos and Llorente, 1998). In other species of aquatic snakes (*Nerodia rhombifera* from North America), the smaller individuals had less diverse diets than intermediate- and large-size individuals, but more diverse diets than very large individuals (Plummer and Goy, 1984; Gibbons and Dorcas, 2004).

In this study, ontogenetic dietary changes seem to be correlated with the absolute sizes of their prey types. Ontogenetic diet changes have also been observed in other aquatic colubrids: for instance in *N. maura* (Hailey and Davies, 1986a; Pleguezuelos and Moreno, 1989; Santos and Llorente, 1998) and *N. natrix* (Luiselli et al., 1997) from Europe, *Nerodia rhombifera, Nerodia cyclopion, Nerodia fasciata, Nerodia erythrogaster* (Mushinsky et al., 1982; Plummer and Goy, 1984), *Nerodia sipedon* (Lacy, 1995), and *Nerodia clarkii* (Miller and Mushinsky, 1990) from North America, and *A. anoscopus* and *Grayia smythii* from tropical Africa (Luiselli, 2006a). In addition, ontogenetic dietary changes were described also in several terrestrial species of snakes, including non-natricine species like several viperids (Saint Girons, 1980; Luiselli and Agrimi, 1991) and elapids (Luiselli, 2006b).

Sexual size dimorphism and intersexual dietary divergence.—As expected, females were significantly larger than males both in terms of average SVL and in terms of maximum size at all study areas. Therefore, this pattern introduces the potential for this species to show divergent adaptations to foraging in each sex (Shine, 1986, 1991; Houston and Shine, 1993). Indeed, *N. tessellata* males and females differed significantly in terms of their dietary composition, in all three study populations. Thus, it is very likely that this pattern is widespread among the various European populations of *N. tessellata.* Females fed on different prey compared to males likely because they differ in sex *per se* and not because they differ in size. This is demonstrated by the significant intersexual differences in prey composition that occurred at the same body size range intervals (at least in the larger classes of individuals). However, snakes, like other organisms, exhibit allometric growth and, given the frequent observation that, as a result, adult female natricines have relatively larger heads than males of the same SVL (Gregory, 2004), it is possible that the difference between diets of males and females of the same SVL is still a function of size, but size of the head instead of the body length. In addition, our

analyses may be partially biased by the fact that females attained much larger sizes than males. Thus, this profound sexual size dimorphism meant that very few males attained a SVL of the largest size class, and furthermore, the vast majority of the 70–74.9 cm size class consisted of females.

The main differences in dietary composition between sexes were the higher occurrence of: (1) *S. erythrophtalmus* as prey for females (pattern found in all study areas), (2) anurans for females, and (3) *L. s. muticellus* for males. It is likely that these differences depended on energy intake reasons as *S. erythrophtalmus* is a relatively large cyprinid and is mainly eaten in summer. Thus, this fish species was eaten mainly immediately after the oviposition period for the female snakes (Luiselli and Rugiero, 2005) when the emaciated females need to restore their body fat reserves for the next year's reproduction. Gravid females of another closely related fish-eating colubrid (*N. maura* from Ebro Delta, Spain) also tended to select larger prey items (Santos and Llorente, 1998), and the females fed on anuran amphibians significantly more often than males (Santos and Llorente, 1998). Both of these aquatic snakes are primarily 'sentinel' predators (Hailey et al., 1982; Hailey and Davies, 1986b for *N. maura;* Filippi, 1995 for *N. tessellata*), thus their similarities in terms of general foraging ecology are expected given their common ancestry. Overall, the recent addition of new species with significant SSD to the list of those species exhibiting significant intersexual dietary differences (*Python regius* in Luiselli and Angelici, 1998; *Elaphe quatuorlineata* in Filippi et al., 2005; and *N. tessellata* in this study) not only reinforces the hypothesis of Shine (1991), but also suggests that the pattern of a simultaneous occurrence of SSD and intersexual dietary divergence in snakes is likely more widespread than previously hypothesized.

Prey and predator size relationships.—Another pattern that clearly emerged from our study is that there was a size-related shift in the lower limit of prey, which was particularly evident for female snakes (likely as a consequence of the energy intake needs of this category of snakes). *Natrix tessellata* in general, and the females in particular, tended to select against smaller prey items (although foraging on relatively small fish species, see below). The same pattern ('ontogenetic shift in lower size limit', *sensu* Arnold, 1993) has also been observed in other species of piscivorous aquatic snakes in North America (Plummer and Goy, 1984) and in Asia (Jayne et al., 1988), as well as in Europe (Santos and

Llorente, 1998). This pattern has been classified by Arnold (1993) as 'enigmatic', and to some extent may depend on statistical artifacts (Arnold, 1993). However, a deletion trend of smaller prey from the diets of larger snakes has been observed in a great variety of snake species, including *Enhydrina schistosa* (Voris and Moffett, 1981), *Tantilla gracilis* (Cobb, 1989), and *Drymobius margaritiferus* (Seib, 1984).

Selection of fish-prey by snakes.—Although we did not study the relative availability of the various fish species in the environment, there is some evidence that the snakes tended to forage on the common species and just occasionally consumed the rarer species. Indeed, the main prey species for these snakes (i.e. *Alburnus alburnus alborella, Leuciscus souffia muticellus, Leuciscus cephalus, Rutilus rubilio,* and *Scardinius erythrophtalmus*) were abundant or very abundant in the three streams (Cataudella, 1977; Luiselli et al., unpubl. obs.), whereas the few species that were not preyed upon by snakes were very rare (Cataudella, 1977) or, even if recorded by Cataudella (1977), never seen by us (e.g., *Lampetra planeri, Salmo trutta*).

Apart from the relative frequency of fishes in the field (which is likely an important factor for prey selection, but that remained unstudied by us), the snakes in this study showed a preference for fish species that were gregarious, small to medium sized, active foraging, diurnal, and not using mud as preferred substratum. It is likely that *N. tessellata* selected gregarious over solitary fish species because their chances of successful predation increased (Bertram, 1978). This was also seen by Hailey and Davies (1986c) experimentally on the piscivorous *N. maura,* which can maximize energy intake while catching fish (*Rutilus rutilus*) from groups. In our study, the concomitant presence of several fish species complicates the conclusions, because each snake may choose between different prey species (Voris et al., 1978). However, it seems likely that the same 'foraging criteria' governed the decisions of, at least, the gravid females during summertime (which focused their predatory activity upon the cyprinid *S. erythrophtalmus*).

Diurnal fishes were preferentially preyed upon by snakes probably as a consequence of the strictly diurnal habits of *N. tessellata* for most of the active period (i.e., from March–June and in September–October; Filippi, 1995), and the fact that, also during the peak of the summer season (July–August) when these snakes may be active at night (Filippi, 1995), diurnal fishes may be much easier to catch at night as they sleep during the night. The relative avoidance of fishes living on mud may just depend on the fact that fishes may be more alert in this relatively open substratum. Hence, the snakes may find it more difficult to use 'sentinel predation' (i.e., an extreme sit-and-wait strategy for catching fish; Hailey and Davies, 1986a) in this type of substratum. Some species of aquatic snakes are known to form search images for prey color (Czaplicki and Porter, 1974; Porter and Czaplicki, 1977), and perhaps *N. tessellata* may be less able to form good search images for fishes in the mud compared to other substrata, because their prey are more cryptic in this substratum.

Natrix tessellata is, as mentioned before, a sit-and-wait ('sentinel') predator. According to Huey and Pianka (1981) there are some ecological correlates of foraging mode relevant to the case of predators. One prediction is that prey type should alternate between trophic levels; hence, sit-and-wait predators should forage primarily on active, widely foraging prey animals. Overall, *N. tessellata* meets this prediction since these snakes generally feed on active widely foraging fishes, whereas sit-and-wait foragers (i.e., *Salaria fluviatilis, Cobitis taenia, Anguilla anguilla*) are rarely preyed upon or not preyed on at all (*Tinca tinca, Cyprinus carpio*). The same tendency was also documented for another sit-and-wait aquatic snake, *N. maura* (Hailey and Davies, 1986b).

Foraging responses of snakes to different water regimes.—A potential problem of our study is that, despite the large sample sizes, we had only one example of each stream type (although ROT and BER are both perennial) without replicates; hence, the generality of our results may be limited. Overall, there were no seasonal differences in the diets of the two sexes or the juveniles in the study areas with perennial or almost-perennial water regime (i.e., ROT and BER). However, at RIO, where there were strongest seasonal variations in water regime, the diets of both males and juveniles varied significantly, and the diet of the females varied seasonally in at least one prey species, i.e., *S. erythrophtalmus,* which was preyed upon significantly more frequently during summer.

Civile (protocols no. 44MC-LL-84bis and 2000-EF-LL-329bis), the Natural Reserve of Canale Monterano (authorization to EF and LL made by F. Mantero), and RomaNatura (authorization to LR made by R. De Filippis). No animals have been damaged or killed during the present project, and all were handled according to the standards of the Italian Ministry for Scientific Research and Technology.

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