DELAYED REPRODUCTION IN SNAKES SUBJECTED TO HUMAN TRADITIONAL RITUALS IN CENTRAL ITALY

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ABSTRACT. – A mountainous site in central Italy (Cocullo, Abruzzi region) offers an exceptional opportunity to test whether the effects of several centuries of “manipulations” on snakes by humans (for traditional religion-linked cultural events) may have altered the reproductive phenology of free-living snakes, which is strongly seasonal in these regions. The Saint Domenico’s procession ritual takes place at Cocullo village on the first Thursday of May of each year, and has done for well over 300 years. For this ritual, local snake-hunters search for snakes throughout the woodlands around the village during April, and capture many snake specimens just before the onset of their spring mating season. The hunters set them free by mid May, after the end of the procession rituals. The species used are mainly Elaphe quatuorlineata, E. longissima, and Coluber viridiflavus. Due to the large number of specimens captured, it is likely that the pressure of the snake-hunters is very high on the local population of snakes, and that most of the adults are indeed captured each year for the procession. Reproductive seasonality of the three species targeted for the procession and of a non-target sympatric species (Vipera aspis) was studied in both the woodlands around Cocullo and in the woodland around another village, with similar eco-climatic characteristics, where no such snake hunting occurs. There was a remarkable delay in the reproductive seasonality of target species, especially of the two Elaphe (E. quatuorlineata and E. longissima), whereas no such effect was observed in the species which is not used for the procession (V. aspis). These effects (delaying of oviposition period of 20-40 days on average) were so evident, that they probably may have important consequences for other aspects of the reproductive biology of these animals (e.g., length of the reproductive cycle, reproductive frequency, etc). On the other hand, clutch size was not influenced by hunting in any species.

INTRODUCTION

There are many ways in which human pressure has influenced the ecology and behaviour of living animals, e.g. producing range depressions or new colonizations, shifts in behaviour, changes in some eco-physiological attributes, etc (e.g. see Pimm 1991, Brandon 1995, Williamson 1996, Luiselli 2001, 2002). It is also possible that specific cultural traditions may produce alterations to the behaviour and life-history traits of specific target animals as a direct response to that cultural tradition, or as an indirect response to the changes in proxi-
mate external conditions originated by that cultural tradition (Luiselli 2003).

Snakes have attracted the cultural attention of humans in Mediterranean Europe for thousands of years. For example, they were worshipped in many regions (e.g., Harrison 1907, Bruno 1971, Bruno & Maugeri 1990), and live snakes have also been used for therapeutic purposes and healing rituals at the Roman Empire age (Angeletti et al. 1992). However, to our knowledge, it has not been previously demonstrated whether specific anthropological traditions may have profoundly altered the activity and reproductive cycle of snakes. A geographic region of central Italy (Cocullo, Abruzzo) offers an exceptional opportunity to test whether the effects of several centuries of “manipulations” on snakes by humans (for traditional religious and cultural reasons) may have altered the seasonal rhythm of the wild local population of snakes. For example, they were worshipped in many regions (e.g., Harrison 1907, Bruno 1971, Bruno & Maugeri 1990), and live snakes have also been used for therapeutic purposes and healing rituals at the Roman Empire age (Angeletti et al. 1992). However, to our knowledge, it has not been previously demonstrated whether specific anthropological traditions may have profoundly altered the activity and reproductive cycle of snakes. A geographic region of central Italy (Cocullo, Abruzzo) offers an exceptional opportunity to test whether the effects of several centuries of “manipulations” on snakes by humans (for traditional religious and cultural reasons) may have altered the seasonal rhythm of the wild local population of snakes.

To test whether the long term human hunting may have altered the seasonal rhythms of the wild populations of snakes, we address the following questions:

(1) Which species are the main target for the S. Domenico’s procession rituals?

(2) Is there evidence that the effect of the snake-hunters is really strong on the local population of snakes?

(3) Does the monthly occurrence of gravid females around Cocullo vary in respect to what happens in other neighbouring areas not subjected to the same religious rituals?

(4) Do the egg-laying dates of Cocullo snakes shift in comparison with conspecifics from other neighbouring localities not subjected to the same rituals?

(5) Are clutch sizes and mean body lengths of gravid females of Cocullo snakes different in comparison with conspecifics from other neighbouring localities not subjected to the same rituals?

MATERIALS AND METHODS

1. Study area: The study was carried out in the surroundings of the village of Cocullo. Cocullo is a small mountainous village, situated about 1000 m a.s.l. in the province of L’Aquila (Abruzzo, central Italy). At Cocullo village, on the first Thursday of May of each year since at least the 17th century, the Saint Domenico’s procession ritual (locally named ‘Festa dei Serpari’) has taken place. The origins of this ritual are very ancient, and date to the Roman age when Cocullo was sacred to Hercules (De Nino 1889, Harrison 1907, Van Wonterghen 1973). Subsequently, the ancient tradition was kept by Christians, and became a traditional catholic ritual, albeit confined to just two villages (Cocullo and Pretoro) (Bruno & Maugeri 1990). During this ritual, the statue representing Saint Domenico is taken out from the Church and set down in the square in front of it. Here, the local snake-hunters (who have searched for snakes throughout the woodlands all around the village) put large numbers of live snakes on the statue and several villagers carry the statue (and the snakes in procession around the village. Apart from the snakes which are placed on the statue, many other snakes are carried in procession and some of them (nowadays illegally) traded.

2. Data collection: Data on the species used for the Saint Domenico’s rituals were obtained by visiting the religious procession at Cocullo village, each year from 1994 to 2001. All snakes that were found at the ceremony were noted, and the species composition in the ritual was recorded. During the ceremony, every effort was made to avoid multiple counts of the same specimens, but we could not exclude that in a few cases the same individual was counted more than once. The same problem did not occur with free-ranging specimens, because they were individually marked (see below). In addition, as most of the specimens are routinely released after the end of the rituals at the site of capture, it is likely that some of the specimens used in the ritual of a given year were also used again for the rituals in other years.

Field surveys for snakes were conducted between March and September of 1994-2001, in both the woodlands around Cocullo (where local hunters have removed snakes for over 300 years), and in those around Celano (province of L’Aquila – where no such hunter activity is found). On average, one to two field days per week were conducted throughout the spring and summer months at either areas. On each field day, the study areas were surveyed from hrs 0800 to 1600 (Rome standard time). Both sites were situated at about 1000 m a.s.l., and are nearly identical in terms of general habitat (mixed oak forests: Fagus sylvatica, Quercus pubescens, Q. cerris, Fraxinus ornus, Ostrya carpinifolia), exposure (SW facing slopes), and climatic conditions, with 1090-1173 mm of annual rainfall and 11.3 to 12.7°C mean annual temperature between 1886 and 1986 (Ministero dei Lavori Pubblici, 1886-1986). In both areas, we searched for...
snakes along standardized routes in the various micro-habitats frequented by snakes. We captured snakes by hand, but additional free-ranging specimens were found squashed by cars along the main roads. We always recorded the site of capture and the habitat at each capture site. Each snake was measured for snout-vent length (SVL, to the nearest ± 0.5 cm) and tail length, sexed by examining the morphology of the cloacal region, weighed with an electronic balance, and individually marked by ventral scale clipping for future identification.

Snake reproductive seasonality was studied according to standard methods (Capizzi et al. 1996, Capula et al. 1997). Reproductive status of female snakes captured during random transects across the two study areas was assessed by abdominal palpation in the case of living specimens, and by dissection in the case of roadkills or specimens found dead in the field. Clutch size was also established by abdominal palpation (precision ± 1 egg).

In addition, several gravid females were removed from the wild and housed in captivity until egg deposition occurred. The conditions of captivity were identical for specimens of both study areas: each female, of every species, was kept alone in a cage of 100 × 50 × 40 cm. Enclosures were lighted with “Gro-Lux” fluorescent lamps (40 W); humidity level was maintained constant (around 55%); diurnal temperature was 26°C on average, with a spot for thermoregulation in each terrarium, and nocturnal temperature was constant at 22°C. Every terrarium was inspected daily to record precisely the oviposition dates. When eggs were deposited, they were incubated in vermiculite under standard conditions (see Luiselli et al. 1997), and the newborn snakes were then marked by scale clipping, and released at the capture site of their mother. Considering that: (i) the two study areas were nearly identical in terms of habitat and climate, (ii) all snakes were kept captive under identical conditions, and (iii) egg-laying conditions were identical for all specimens and were not more similar to one of the two areas (for example, the ground structure was the same), we do not expect that any major experimental bias will have affected the outcome of the results. Moreover, as all the females were kept individually, there was not any bias caused by the eventual snake density in the terraria, which might have influenced either oviposition dates or nesting behaviour and requirements.

Concerning snakes from Cocullo area, it should be noted that three out of five females of Elaphe quatuorlineata, three out of five females of E. longissima, and one out of five females of Coluber viridiflavus, used for our captive experiments were used in the same year of our captive maintenance for the procession ritual.

All snake specimens, including both offsprings and their mothers, were set free unharmed, immediately after the end of the scientific study.

All statistical tests were two-tailed, with alpha set at 5%. Means are followed by ± 1 SD. Data were processed by STATISTICA (version 4.5 for Windows).

RESULTS

Snakes at Cocullo procession

The numbers of each snake species observed by us between 1994 and 2001 is presented in Fig. 1. These numbers are likely to be the conservative side, because we cannot exclude that some speci-
mens may have “escaped” our attention due to the great confusion and mobs of people moving around the procession. Our data indicates that the species used for “climbing” the statue was mainly *Elaphe quatuorlineata*; other species commonly used by snake-hunters were *E. longissima*, *Coluber viridiflavus*, and more rarely, *Natrix natrix* and *Coronella austriaca*. All these species are well known to occur in the study area (Bruno & Di Cesare 1990, Spinetti 1996, Filippi & Luselli 2000).

The pressure of hunting on the snake natural populations is likely so strong that most of the free-ranging specimens of Cocullo’s woodlands are really captured for the S. Domenico’s rituals: indeed, (1) the three species most frequently used in the procession (i.e. *E. quatuorlineata*, *E. longissima*, and *C. viridiflavus*; see Fig. 1) represented approximately 72% of the total snake sightings in the field (i.e. woodlands situated within 10 km radius from Cocullo) between 8th June and 8th July (total *n* = 428 snakes) (Fig. 2A), but only 4% of the total snake sightings (*n* = 223) in the same areas between the second week of March (onset of the activity period after the hibernation) and the second week of May (Fig. 2A), when they are actively hunted for the purposes of the procession (differences between the two samples: *P* < 0.00001 at χ² test with df = 1);

(2) the number of *Vipera aspis* (a venomous species which is not used in the procession) observed in the field was nearly stable in the same two periods (Fig. 2A), thus indicating that there is no effect of snake-hunters on this species;

(3) the number of *Natrix natrix* (a non-venomous species which is rarely used in the procession) was much higher in the early spring phase than in
the summer phase (Fig. 2A), this depending on the fact that these snakes change behaviour with season, and in spring large numbers are often visible as they go through their mating period, while only a few weeks later they are no longer seen and estimates on their populations would be much changed (Luiselli 1996).

On the other hand, in the control area without snake hunting (see Fig. 2B), all species of snakes were observed much more frequently in March-May than in June-July, as expected from previous studies on the annual activity patterns of Mediterranean snakes (Bruno & Maugeri 1990, Filippi 1995, Capula et al. 1997).

Comparing the two areas

Reproductive seasonality of non-target species

With regard to Vipera aspis, gravid females (n = 31 in Cocullo, n = 23 in Celano) were mostly observed between the beginning of July and the beginning of September in both study areas, with no obvious inter-site differences (P > 0.3 at \( \chi^2 \) test with df = 1; Fig. 3 top). Five specimens from Cocullo and five from Celano were also kept in captivity until parturition; they were nearly identical in birth dates (from 8 to 14 September at Cocullo; from 7 to 12 September at Celano). The parturition dates (calculated by counting the number of days elapsed for giving birth to young after the first parturition event) were not significantly different between Celano and Cocullo (P > 0.45, at Kruskal-Wallis ANOVA).

Reproductive seasonality and clutch size of target species

2.2.1. Elaphe quatuorlineata

Gravid E. quatuorlineata were found in June and early July at Celano (n = 9), but in late July and August at Cocullo (n = 16) (Fig. 3 middle). The monthly frequency of occurrence of gravid females were statistically significant between study areas (P < 0.01 at \( \chi^2 \) test with df = 1). Egg-laying of captive females from Celano occurred on 21 July (two females), 25 July, 28 July, and 29 July. Egg-laying of captive females from Cocullo occurred on 22 August (two females), 23 August, and 26 August (two females). The egg-laying dates were significantly later at Cocullo than at Celano (mean number of days after the first oviposition event = 33.8 ± 0.05 days (Cocullo) versus 4.75 ± 3.59 days (Celano), one-way ANOVA: \( F_{1,23} = 236.3, P = 0.00001 \)).

Mean length of gravid females was similar in both study areas (Cocullo: \( x = 131.6 \pm 10.4 \text{ cm}, n = 15 \); Celano: \( x = 136.7 \pm 12.3 \text{ cm}, n = 10 \); one-way ANOVA: \( F_{1,33} = 1.236, P = 0.277 \)), as was the clutch size (Cocullo: \( x = 10.3 \pm 2.1 \text{ eggs}, n = 15 \); Celano: \( x = 10.7 \pm 2.7 \text{ eggs}, n = 10 \); one-way ANOVA: \( F_{1,23} = 0.143, P = 0.708 \)).

2.2.2. Elaphe longissima

The trend observed in E. quatuorlineata was also found in E. longissima: gravid females were found mainly in June at Celano (n = 12), but in late July at Cocullo (n = 14) (Fig. 3 middle). These inter-site differences were statistically significant (P < 0.01 at \( \chi^2 \) test with df = 1, calculates on the monthly differences in the frequency of gravid females at the two sites). In addition, egg-laying of captive females from Celano occurred on 24 June, 29 June, 30 June, 4 July, and 6 July, and that of captive females from Cocullo occurred on 26 July, 2 August (two females) and 3 August (two females). The egg-laying dates were significantly later at Cocullo than at Celano (mean number of days after the first oviposition event = 39.0 ± 3.39 (Cocullo) versus 8.75 ± 3.86 (Celano), one-way ANOVA: \( F_{1,7} = 156.85, P = 0.0001 \)).

As observed in E. quatuorlineata, in E. longissima the mean length of gravid females was similar in both study areas (Cocullo: \( x = 103.5 \pm 4.9 \text{ cm}, n = 12 \); Celano: \( x = 102.2 \pm 6.4 \text{ cm}, n = 9 \); one-way ANOVA: \( F_{1,19} = 0.268, P = 0.610 \)), as was the clutch size (Cocullo: \( x = 10.0 \pm 2.0 \text{ eggs}, n = 12 \); Celano: \( x = 9.8 \pm 1.7 \text{ eggs}, n = 9 \); one-way ANOVA: \( F_{1,19} = 0.069, P = 0.795 \)).

2.2.3. Coluber viridiflavus

The third target species, C. viridiflavus, exhibited a less obvious pattern. Gravid females were found mainly in June at Celano (n = 17), and in both June and July at Cocullo (n = 21) (Fig. 3 bottom). However, the inter-site differences in terms of monthly frequency of occurrence of gravid females were statistically significant (P < 0.05 at \( \chi^2 \) test with df = 1). Egg-laying of captive females from Celano occurred on 16 June, 18 June, 23 June, 27 June, and 29 June; egg-laying of captive females from Cocullo occurred on 14 June, 23 June, 7 July, 8 July, and 18 July. The egg-laying dates were significantly later at Cocullo than at Celano (mean number of days after the first oviposition event = 22.5 ± 10.28 (Cocullo) versus 8.6 ± 5.59 (Celano), one-way ANOVA: \( F_{1,7} = 6.79, P = 0.035 \)).

The mean length of gravid females was similar in both study areas (Cocullo: \( x = 96.2 \pm 10.2 \text{ cm}, n = 20 \); Celano: \( x = 94.8 \pm 10.8 \text{ cm}, n = 15 \); one-way ANOVA: \( F_{1,33} = 0.154, P = 0.697 \)), as was the clutch size (Cocullo: \( x = 7.6 \pm 2.2 \text{ eggs}, n = 20 \); Celano: \( x = 8.1 \pm 2.3 \text{ eggs}, n = 915 \); one-way ANOVA: \( F_{1,33} = 0.564, P = 0.458 \)).
DISCUSSION

Our study revealed that the hunting activity at the Saint Domenico’s procession rituals was directed specifically towards two *Elaphe* species, and, to a lesser degree, one *Coluber* species. We provided circumstantial evidence that the populations of two main species targeted for the rituals (*E. quatuorlineata* and *E. longissima*) are massively reduced just a few weeks before the onset of their mating season. We based our conclusions on the clear difference in the frequency of encountering these species in the period of, and in the period after, the procession rituals and correlated human activities. We exclude that this pattern may be merely explained by differences in snake behaviour between the two periods (see Bonnet & Naulleau 1996) for the following reason: *E. quatuorlineata* is much more easy to find in the field in the early spring months than in June-July (Filippi 1995), the reverse being true for the case of our Cocullo surveys. The same is true also for the other two species, which are generally peaking their above-ground activity in April-May, and not in June-July (Filippi 1995, Bonnet *et al.* 1999). Thus, we believe that it most likely depended on the effects of the massive removal of free-ranging specimens for the procession rituals.

Thus, the above considerations led us to conclude that (1) hunting for the procession rituals may temporarily remove a consistent portion of the free-ranging adult populations of *E. quatuorlineata, E. longissima* and possibly *C. viridiflavus*, and (2) that this removal has occurred every year for well over 300 years, thus affecting many generations of snakes. In addition, these removals occur immediately at the onset of snake spring activity (which in the study area takes place by mid March), which is before the onset of the mating season (late April, in conspecifics from neighbouring areas where no snake hunting occurs; Filippi & Luiselli 2000). In practice, the human hunting activities are likely to have impeded most adult snakes to have both mat-

Fig. 3. – Top, Monthly occurrence in the field of gravid females of *Vipera aspis* at Cocullo (total $n = 31$) and at Celano ($n = 23$); middle, Monthly occurrence in the field of gravid females of *Elaphe quatuorlineata* at Cocullo (total $n = 16$) and at Celano ($n = 9$); middle, Monthly occurrence in the field of gravid females of *E. longissima* at Cocullo (total $n = 14$) and at Celano ($n = 12$); bottom, Monthly occurrence in the field of gravid females of *Coluber viridiflavus* at Cocullo (total $n = 21$) and at Celano ($n = 17$). Note that the monthly occurrence of gravid females was very different between the two areas (for statistical details, see the text) in the latter three species, whereas it was similar different between the two areas in the case of *Vipera aspis* (for statistical details, see the text).
ing and vitellogenesis at their usual spring-time ev-
ery year for generations, and would have potentially
produced a shift (delaying) of the reproductive
events as a consequence.
Snakes at Cocullo experienced a remarkable de-
lay in the reproductive seasonality of two of the tar-
get species used at Cocullo, i.e. E. quattuorlineata,
E. longissima, and to a lesser extent also C.
viridiflavus, whereas no such effect was observed in
the species which is not used for the procession, i.e.
V. aspis. In contrast, no such pattern was observed
in any target species on mean body size of gravid
females and their clutch size. This delay in repro-
ductive seasonality can be attributed to a shift in
the short term due to disturbance in the current sea-
on or the last season (i.e. effect of stress if, for in-
stance, the snakes are kept in cool conditions with-
out the ability to thermoregulate while being held
before, during, and after the procession, the females
may be unlikely to progress as quickly through
vitellogenesis, and males through spermatogenesis),
which can become an adaptive trait after the contin-
ued removal of reproductive snakes for much over
300 years just before the onset of the mating season.
These strong effects (delaying of oviposition period
of 22-39 days on average) may have important conse-
quences for other aspects of the reproductive biol-
ogy of these animals (e.g., length of the reproduc-
tive cycle, reproductive frequency, etc). Hormonal
samples (corticosterone, estradiol, testosterone, and
progesterone) for comparisons among the sites
would be extremely interesting in this context, and
these hormonal studies will be a further step of our
research.

The alternative hypothesis, of a local variation
due merely to microclimatic differences between the
study areas, can be rejected because it is well known
that the reproductive seasonality of snakes is rela-
tively constant in Italy also when very different cli-
matic conditions are available (e.g., conspecific
populations of snakes in the cold Alpine mountains
and in the dry Mediterranean lowlands may show a
shift in the reproductive timing (for instance, in mat-
ing season and oviposition time) shorter than 3 weeks
(Capula & Luiselli 1997).

Although the hypothesis that snake reproductive
cycles have “evolved” differences in response to
over 300 years of annual human activity is cer-
tainly interesting and noteworthy, there are two is-
ues here which may be against the above hypothe-
sis. The first is that, the evidence for such a shift is
relatively indirect, with not enough data on snake
cycles presented to make firm judgements (we
were able to study only a relatively small sample
size for each species). In this respect, the compari-
sion to a live-bearing snake (Vipera aspis) to
egg-laying snakes (the three species targeted for
the procession) may not be a valid comparison.
Furthermore, the oviparous and viviparous species
differ tremendously in body size, with the ovipa-
rous species being much larger. The second related
issue is that the observed differences (assuming
here they are because of human activity) could just
as easily be explained as a result of proximate ef-
ects, i.e. the disturbance in one year (or the previ-
ous year) delays reproduction because snakes are
disturbed, cannot thermoregulate, feed etc. This ap-
pers to be a reasonable hypothesis, but also if this
is true, it implies a remarkable reproductive plastic-
ty in Mediterranean snakes in response to hu-
man activity, and there is no doubt that document-
ing that human activity affects snake reproduction
is important. If an evolutionary shift is involved in
this process, it is however to be demonstrated via
the mechanism of the shift, i.e., how would animals
that have been disturbed and shifted their reproduc-
tive pattern been favoured with the production of
more offspring over the last 100-200 generations?
Of course, we do not have any firm data about it,
but we may present some working hypotheses. We
suggest that potential advantages for “delayed”
newborns may be that (i) they may have increased
survivorship due to later entry into hibernation (i.e.
they may have consumed less energy reserves) and
(ii) better avoidance of predation. In any case, the
patterns observed may be explained by stress rather
than any evolutionary response, as rates of gene
flow are possibly high enough to prevent local ad-
aptation.

In conclusion, our study provides indication of
the fact that the effects of long-term manipulation
on snakes by humans (for traditional religion-linked
cultural reasons) may strongly alter the reproduc-
tive phenology of free-living snakes in Mediterra-
nean areas. It is likely that similar effects may also
occur in tropical areas (e.g. in some regions of Asia
and Africa), where snakes are totem animals tradi-
tionally used for pagan rituals and other traditional
purposes.

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