

X-ray imaging of smooth snakes *Coronella austriaca* to investigate possible overwintering by gravid females

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INTRODUCTION

The smooth snake is a small non-venomous species with a western palearctic distribution that reaches its north-western limit in England and Norway. In England, its distribution is almost entirely restricted to warm microhabitats within lowland heath, where females are known to have a biennial or triennial breeding cycle and may mate in springtime or the autumn (see for example Limburn et al., 2013). Nevertheless, many aspects of reproductive behaviour remain imperfectly understood as the species is secretive, engaging in mosaic-basking among heather stands, referred to as ‘cryptic heliothermy’ (Gent, 1988).

The possibility that females might enter hibernation in England whilst gravid has been suggested (Spellerberg & Phelps, 1977). There are no records confirming this, however, in April 2011 a smooth snake, which was possibly gravid, was observed basking close to an artificial refuge. Features suggesting this female was gravid were – a clear demarcation of the tail from the body, increased girth of the posterior two thirds of the body (rather than a clear ‘prey bulge’), and a ‘falling away’ of the body from the backbone which is associated with depletion of fat reserves during gestation. The female was weighed and measured and the ratio of mass to body length (excluding tail) was found to be significantly higher than that of several other female snakes sampled at the same time for comparison. The female also showed high site fidelity throughout the spring and summer, never being found more than one metre from the refuge; this highly sedentary behaviour is typical of gravid smooth snakes. Together these signs suggested that a pregnancy had been carried over the winter from the preceding year, but since the female was not taken into captivity this could not be confirmed (Atkins, 2011).

In both England (Limburn et al., 2013) and Norway (Johansen et al., 2022) autumnal matings have been observed but in neither country have gravid females been observed in the springtime. In a total of seven observed matings in Norway, two were autumnal and five were vernal. It has been assumed that sperm from such autumnal matings is stored until the following spring when the females ovulate following which fertilisation takes place and the resulting embryos are gestated over the summer. If gravid females do enter hibernation then it could possibly be the

result either of the autumn mating behaviour, provided that ovulation coincided with mating, or as a consequence of a poor summer that prevented the completion of embryonic development.

To investigate whether apparently gravid female smooth snakes in early spring are genuinely carrying embryos, a non-invasive method of examination was required. The use of ultrasound is problematic because of the small size of smooth snakes (adults of both sexes average between 45–55cm in England although interestingly they are often much larger in Norway, up to 90cm) making resolution of tiny embryo snakes almost impossible. Likewise, palpation (which involves gentle manipulation of a gravid snake to detect embryos), is extremely difficult with small species and as well as not being definitive it is also potentially harmful to the snake and its embryos. The aim of the current study was to test a dental X-ray machine as a means of obtaining clear images of smooth snake embryos within gravid females.

MATERIALS & METHODS

The equipment selected for use was the standard digital dental intra-oral X-ray set with small phosphor plate sensors for capturing the X-ray images. A preliminary feasibility study of the X-ray technique was undertaken in the late summer of 2023 on snakes believed to be gravid. This was successful so that a small-scale study was made of snakes suspected of being gravid by virtue of their size and shape in April 2024 and 2025.

X-ray and handling procedure

In August 2023 a gravid smooth snake was collected from an Amphibian and Reptile Conservation (ARC) Trust reserve. This was about 15 minutes by car from a local dental practice with which prior arrangements had been made to make the X-ray facilities available for use out of hours and at short notice. Much interest was shown by the staff although it was fortunate that the snake’s arrival occurred five minutes after a particularly ‘snake phobic’ patient had left the premises.

The snake was introduced into a 2 cm diameter tube made from thin flexible plastic with the furthest end being covered to encourage it to move further inside. Visibility through the clear plastic tube was useful during the positioning of the snake to exactly align the region of the

body where any snake embryos were likely to be found with the direction of the X-ray beam and the sensor plate below. By simply rotating the tube, the sides of the snake's body could easily be positioned at right angles to the X-ray beam so that the spinal column would not obscure embryos in the resulting image. The X-ray tube head was pointed vertically downwards, touching the tube containing the snake, which was itself lying horizontally on top of an extra-long sensor plate (size 3+). Snakes were handled by the first author and the technical equipment by the second author and dental practice staff according to relevant experience and legislation.

As smooth snakes are small and their bony structures are very thin and not overly radiopaque, and as the skeleton of the embryonic snakes was assumed to be even finer and less ossified, it was decided that the first experimental radiographic exposure would be performed at the lowest possible X-ray dosage setting. The parameters for this were 0.03 sec at 60 kV and 7 mA, which would be used for the radiography of a child's lower primary incisor tooth. Fortuitously, these settings produced a readable image, the contrast of which was later increased digitally. A successful result was obtained at the second exposure. Approximately 2 hours after capture, the snake was released in the exact spot from where it was taken.

In 2024 and 2025, a single potentially gravid female smooth snake was X-rayed following the same procedure.

Animal welfare considerations

The prime concern during this procedure was the welfare of the animals; to minimise radiation dose and exposure to manual handling and stress, to avoid harm to the adult and to any developing embryos, and to keep the duration of captivity to an absolute minimum. The study was undertaken under licence through the ARC Trust and specifically its 'Snakes in the Heather' project, having established that no Home Office licence was required for the use of radiographic imaging on possibly gravid smooth snakes.

The radiography was performed according to current safety protocols [IR(ME)R 2024] and in keeping with the principle of making exposure to X-rays as low as reasonably practicable. Digital radiography requires less X-ray exposure to produce a readable image than with comparable analogue techniques. After exposure to X-rays the phosphor plates were scanned immediately and the resulting images were produced almost instantaneously when compared to traditional X-ray film which needs time to be developed chemically. A key benefit of this is that the handling time of the snake was very much reduced.

RESULTS & DISCUSSION

The results of the test X-ray on a gravid female in August 2023 clearly demonstrated that relatively large, near-term embryos can be detected (Fig. 1). As far as known, this is the first time that dental X-ray imaging has been used to examine the internal anatomy of smooth snakes either in England or elsewhere. The technique could be applied to other small snake species where ultrasound and palpation

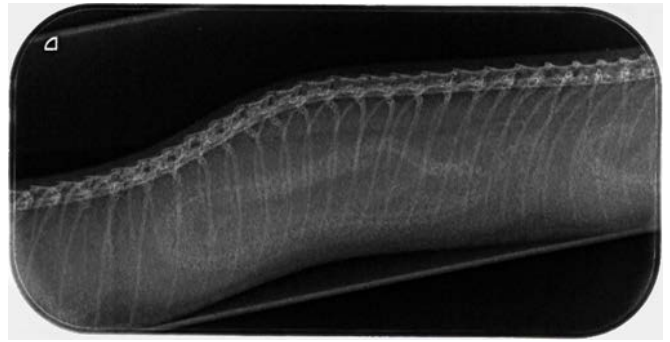


Figure 1. X-ray image of a female smooth snake in August 2023 which shows the complete looped body of the well-developed embryo in the body cavity along with part of a further embryo to its right

cannot provide the required detail. Having established proof of concept the next step was to apply the technique to females with apparent signs of being gravid in the spring.

Unfortunately, the weather in both 2024 and 2025 was not favourable for the collection of potentially gravid smooth snakes. In particular, a very brief spring rapidly became an early and hot summer, meaning that few snakes could be found during the critical springtime 'window of opportunity' for surveying. This 'window' is between emergence from winter torpor to mid-May, after which time females may be gravid as a result of particularly early spring mating rather having overwintered in that condition. However, in both years a single female snake was found that showed at least some of the signs of being gravid and each was subjected to the X-ray procedure.

In April 2024, aside from the snake's own ribs and backbone, it was not possible to discern any distinct structures within the X-rayed snake (Fig. 2). The image obtained of the snake in April 2025 was of more interest. The fragmented remains of a backbone are clearly visible within the body cavity of the snake (Fig. 3). As the sizes of the bones are almost as large as the bones of the snake, this indicates the presence of a prey item. It is not possible to identify the prey species, although the length of the vertebral fragments suggests a snake or slow-worm *Anguis fragilis* rather than a small mammal or legged lizard. Although the possibility that the remains are from a meal consumed the previous autumn cannot be ruled out entirely, this is considered unlikely given the integrity of the vertebrae and the avoidance by snakes

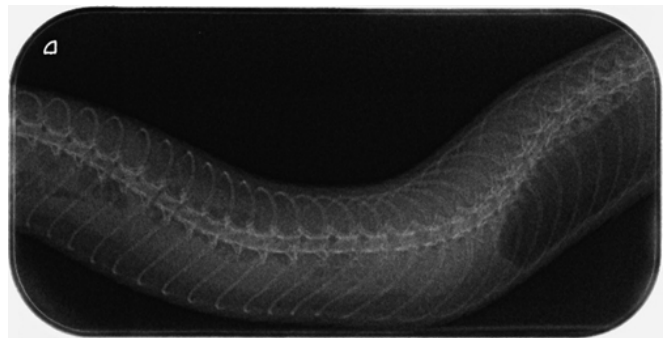


Figure 2. X-ray image of an apparently gravid female smooth snake in April 2024 in which no embryos could be detected

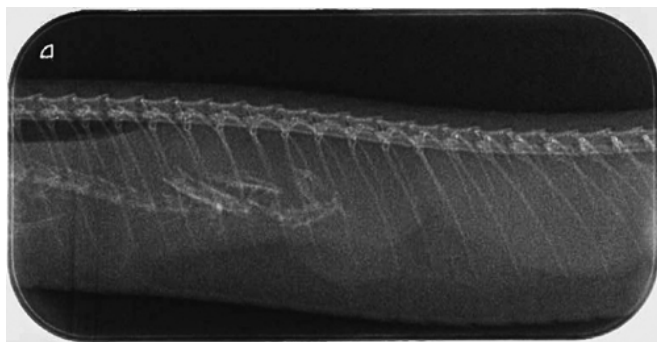


Figure 3. X-ray image of an apparently gravid female smooth snake in April 2025 in which no embryos could be detected but instead the skeletal remains of prey are evident

of feeding late in autumn because of the risk of incomplete digestion during the winter and consequent poisoning of the snake by bacterial decay in the gut. The snake was collected on 23 April 2025, a relatively early date to have already fed, which suggests that smooth snakes will feed at the earliest chance following winter torpor. This is not surprising given the largely sedentary nature of the species and hence the need to be opportunistic with regard to taking prey (as well as to breeding).

In neither case was there any evidence of snake embryos being present, suggesting that those smooth snakes appearing unduly 'large' in spring are likely not gravid. This tentative conclusion is based on only two specimens so that the question of overwintering when gravid remains open. The probability that this does happen occasionally is increased by the fact that overwintering when gravid has been reported in England's other species of ovoviviparous snake, the adder or northern viper *Vipera berus*. A female was found dead under bracken in springtime and subsequent dissection revealed ten fully developed young which must have been the result of a pregnancy initiated the previous year (Hand, 2013).

Although not conclusive in excluding gravid overwintering in smooth snakes, we have shown that dental X-ray imaging provides good resolution of the internal anatomy of the smooth snake, is straightforward to use, and as far as we can tell is safe for the animal. Further work involving the dental X-ray imaging of a larger number of suspected gravid females in spring would be needed before any definite conclusion could be drawn about whether gravid overwintering does indeed occur in England. If so, this characteristic would be seen as a limitation to the range and population size of smooth snakes compared with Norway, where the mean temperature in southern Norway in the warmest month of the year is 1.5 °C warmer than in England (Pernetta, 2009), making the completion of embryonic development there more reliable. Smooth snakes in Norway give birth in August, similar to the species elsewhere in continental Europe and, critically, somewhat earlier than English smooth snakes (Johansen et al., 2022). Failure to give birth in the preceding summer would presumably impact on a female's ability to restore herself to breeding condition during the following year, resulting in a triennial rather than a biennial cycle with consequent negative implications for population growth if this were a common phenomenon. In England, an increase

in the mean summer temperature as a consequence of climate change may well produce a situation similar to that in southern Norway, allowing the species to occupy a greater range of habitats and for more frequent reproduction.

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