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Are fibrocement slabs a useful tool for monitoring reptiles? The case of *Hierophis viridiflavus* in its south-western limit of distribution

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The established methodology in detection and long-term reptile monitoring programmes has so far focused on visual encounter surveys (VES), a method that can yield very high capture rates for snakes, but, even when carried out in favourable habitats, can also be very limited. Therefore, one passive sampling method was tested: fibrocement slabs. A total of 81 slabs were placed in nine sampling stations in the southern part of Irún (Basque Autonomous Community, Spain). During the two years of sampling (2020–2021) a total of 79 reptile records were obtained, of which 33 were records of the European whip snake corresponding to 28 individuals. There was at least one reptile record in each transect and 75% of the reptile species present in the study area were detected. However, we did not identify any trend over time that indicated an increase in the number of individuals captured during the second year. All in, the data obtained have provided positive results; therefore, it could prove to be a very useful methodology for reptile studies, in particular for ophidians.

Keywords: European whip snake, conservation, methodology, artificial refuge, capture, detection, Basque Autonomous Community, Spain

INTRODUCTION

Visual encounter surveys (VES) are a recurring methodology in long-term reptile monitoring programs (Zuiderwijk et al., 1999; Graeter et al., 2013; Andreu et al., 2022), being used in the monitoring programs with the longest tradition in Europe, such as the one led by the RAVON Foundation in the Netherlands 'Monitoring Network of Reptile, Amphibian & Fish Conservation', or the one developed by the Amphibian and Reptiles Conservation (ARC) in collaboration with volunteers in the United Kingdom, 'The National Amphibian and Reptile Recording Scheme' (NARRS). The Spanish Herpetological Association, following these guidelines, set up the Spanish Amphibian and Reptile Monitoring Programme (SARE Programme in Spanish) in 2006, defining the same methodology for the sampling of terrestrial reptiles, based on pre-set itineraries within the most representative habitats of the study area. VES can yield very high capture rates for snakes (e.g. Brown et al., 2013), however, even when carried out in favourable habitats, can also be very limited (e.g. Zuiderwijk et al., 1999; Santos, 2014; Gosá, 2019). The size and/or density of the focused population, detections and counts may depend on a wide range of other variables, as detection rates may be influenced by the organism itself (such as sex effects, size effects or other sources of individual heterogeneity in activity and microhabitat use (Tyrrell et al., 2009; Christy et al., 2010), or by spatiotemporally varying environmental conditions (Vogt & Hine, 1982; Williams & Berkson, 2004; Lardner et al., 2015).

The difficulty in taking records for a rigorous analysis of the conservation status of snakes extends to the struggle that specialists encounter in arguing the inclusion of a species in a threat list (at local scale, Romero-Iraola et al., 2020; or broad scale, Böhm et al., 2013). Faced with this complicated situation and given the good results shown by passive sampling elements (water cisterns, García-Cardenete et al., 2014; cover boards, Halliday & Blouin-Demers, 2015; pitfall traps, Bateman et al., 2009), the application of another passive sampling method was tested: the placement of fibrocement slabs. They have already been used satisfactorily in the monitoring of reptiles in other European countries (e.g. *Hierophis viridiflavus*, Bonnet et al., 2021; *Malpolon monspessulanus* and *Zamenis scalaris*, Ballouard et al., 2016; *Testudo hermanni*, Ballouard et al., 2013). The slabs corrugations ease shelter and provide environments conducive to the thermoregulation of ectothermic animals such as reptiles, and snakes in particular (Lelièvre et al., 2010a), encouraging them to take refuge beneath them and facilitating their detection, as well as the capture of individuals. Given the potential negative consequences caused by trapping animals, cover objects, such as fibrocement slabs, are preferred to funnel and pitfall traps (Grant et al., 1992). Besides,

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fibrocement slabs have alredy been tested succesfully for *Hierophis viridiflavus* (Bonnet et al., 2021; Romero-Iraola et al., 2022), and cover boards have appeared to be underused by snakes (e.g. Eye et al., 2018).

The European whip snake *Hierophis viridiflavus* (Lacépède, 1789) is a medium-sized diurnal colubrid snake (snout-vent length 1089 mm on males and 1023 mm on females, and tail-length 381 mm on males and 354 mm on females; Schätti & Vanni, 1986), with a south-central European para-Mediterranean distribution, occurring across a variety of open environments characterised by temperate climatic conditions (Lelièvre et al., 2010a; Santos et al., 2015; Vanni & Zuffi, 2011; Sillero et al., 2014); whose optimum preferred temperature stands around 30 °C (Lelièvre et al., 2010b).

Hierophis viridiflavus is catalogued as Low Concern (LC) by the IUCN (Vogrin et al., 2009), however, it is indexed within the regional catalogue of endangered species (see Romero-Iraola et al., 2020), as it is the snake species with the lowest occupancy in the Basque Autonomous Community (northern Spain) (Bea, 1985). This population located in the Basque Autonomous Community is the south-western population. Populations along the range edge frequently display high levels of local adaptation and contain distinctive genotypes that may be required for the persistence of the species under future climatic conditions (Hampe & Petit, 2005). Studies focusing on *H. viridiflavus* have a reduced number of occurrence records, which are not representative of the species range, as they did not include this edge population (Rato et al., 2009; Mezzasalma et al., 2015; 2018; Senczuk et al., 2021), making *H. viridiflavus* a compelling target species.

In this study, we set up a network of fibrocement slabs, as we aimed to: i) test them for reptile studies in the local reptile community focusing in *H. viridiflavus*´ south-westernmost population and ii) assess if catchability increases in time using this method.

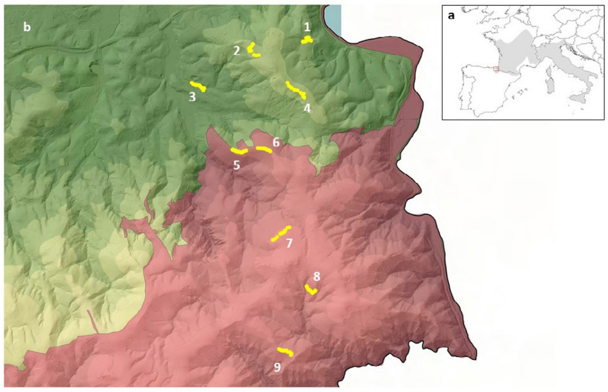

Figure 1. **a)** Global distribution of the European whip snake according to the IUCN (Vogrin et al., 2009) (in grey) and the study area (square in red). **b)** The nine sampling stations spread over the southern part of Irún (Spain) (1–4), five of them (5–9) within the Aiako Harriak Natural Park (in red).

Figure 2. Corrugated fibrocement slabs of 0.99 m² in an ecotonal area within the Aiako Harriak Natural Park

MATERIALS & METHODS

Study area

The study area was located in Irún (Basque Autonomous Community, Spain), distributed in peripheral districts of the same municipality and on land belonging to the Special Area of Conservation (SAC) of Aiako Harriak (ES2120016), on land belonging to the same municipality (Fig. 1). According to the Köppen classification, this Natura 2000 Network protected area, like the municipality of Irún, has an oceanic climate cfb (mid-Atlantic), with annual rainfall of 2800 mm and an average temperature of 12 °C (Meteorological Station of Jaizubia [Agencia Vasca de Meteorología, 2023]).

Regarding vegetation, coniferous plantations are the most abundant, with the most dominant being those of insignis pine *Pinus radiata*, Norway spruce *Pinus nigra* and Japanese larch *Larix kaempferi*. In addition, there are also plantations of deciduous trees such as American red oak *Quercus rubra* and Japanese chestnut *Castanea crenata*. Although in smaller quantities, native flora can also be found, such as common oak *Quercus robur*, beech *Fagus sylvatica* or honey oak *Quercus pyrenaica* forests. Furthermore, in areas with abundant disturbances such as those caused by human activity or fire, there are abundant populations of gorse *Ulex* spp., heather *Erica* spp. and ferns (Arbelaitz et al., 2002). Concerning the reptile fauna of the study area, seven snake species; southern smooth snake *Coronella girondica*, smooth snake *Coronella austriaca*, red-eyed grass snake *Natrix astreptophora*, viperine snake *Natrix maura*, Seoane`s viper *Vipera seoanei*, Aesculapian ratsnake *Zamenis longissimuss* and European whip snake *H. viridiflavus,* and five lacertids can be found; Catalonian wall lizard *Podarcis liolepis*, Common wall lizard *Podarcis muralis*, viviparous lizard *Zootoca vivipara*, slow worm *Anguis fragilis* and Western Green lizard *Lacerta bilineata* (SIARE, 2022).

Transects with fibrocement slabs (hereafter referred to as slabs) (Fig. 2) were established at nine sampling stations spread throughout the study area; four in the southern part of the municipality of Irún and five within the Aiako Harriak Natural Park (Fig. 1; supplementary material). These stations were selected in areas where the species had already been detected in the past, and included other areas with a high potential for its presence even if it had not been recorded so far. A limiting factor in defining the areas was land ownership, and only publicly owned lands were selected to facilitate sampling.

Field procedure

Fieldwork was carried out between April and September 2020 and 2021, although earlier information collected by rangers in the study area (2015–2019) was also taken into account (presented in the discussion and used to select sites). Slabs were checked every 3–4 days, i.e. twice a week during the first four months of sampling (April, May, June and July), the period of highest ophidian activity, and once a week in the following two months (August and September), for a total of 98 days of fieldwork. The time between samplings was not constant, because the most favourable days were chosen, avoiding the hottest days and discarding rainy days. Likewise, the order in which the slabs were examined was alternated so that the same area was not always the first or the last to be examined. At the same time, the sampling times were modified according to the duration of sunlight, starting between 10:00–11:00 h in spring and 09:00–10:00 h in summer, or according to the temperature of the chosen day. The transect sampling could last for a maximum of 300 minutes, as each series of nine transects was carried out in one day of fieldwork.

At each sampling station, the transects were made on foot, with an average length of 220 ± 55 metres (n = 9; range: 170–326 m), establishing an approximate separation between the slabs of 25 metres, with each transect consisting of nine slabs, so that the total number of installed slabs was 81. These had an undulating shape, which facilitates access and shelter for the animals, with an approximate surface area of 0.99 m^2 (1.1 x 0.9 m), and were arranged in preferred environments for the target species (*H. viridiflavus*): ecotonal areas between grasslands, shrublands and woodlands (Capula et al., 1997); all slabs were placed in the open and slightly covered by shrubs.

The protocol established to record the catch data was as follows:

1. Temperature measurement with a digital laser thermometer, both above and below the slabs.
2. Obtaining biometric records of the snakes captured (total length, snout-vent length, tail length and weight), counting the number of ventral scales (data collected for future studies) and noting the age class (juvenile, < 300 mm; subadult, 300–1000 mm; and adult, > 1000 mm). Before release, the individuals were marked using the cutting system on the lower edge of the ventral scales (see Brown & Parker, 1976), and any identifying marks (wounds or malformations) of the individual were photographed. Moults were treated as observations.
3. Similar data were collected for the other snake species captured, and the potential prey of the European whip snake (micromammals and lizards) were also censused; any incidents that may have occurred on the slabs, such as marks, displacements, breakages or deterioration, were also noted.

Placement of the slabs began during January and February 2020, 2–3 months before the proposed start of the species' activity period (Santos et al., 2015) and the start of the planned surveys (Naulleau, 2002). Due to the limitations imposed by the state of alarm resulting from the Covid-19 pandemic, only 45 of the slabs were placed in winter (January–February); the remaining slabs were placed during the month of April, in early spring, 16 days before the start of the fieldwork.

Statistical analyses

To assess if catchabily increases in time, we only used *H. viridiflavus* records, due to slabs that were placed in areas to target *H. viridiflavus*, and the detection of other species (particularly the specialist species) could be biased by the habitat where slabs were placed. For the analyses we discarded recaptures to avoid pseudo-replicates. Since the data did not show normal distribution, non-parametric Mann-Whitney tests were used (as previously seen in Ballouard et al., 2016). All the statistical tests were performed using RStudio (R Core Team, 2022).

RESULTS

Reptile diversity

A total of 79 reptile records of nine different species were obtained during the sampling years (2020–2021), beneath 27 different slabs (33.3%) and with at least one record on each transect. Seventy-five percent of the reptile species present in the Aiako Harriak Natural Park and neighbouring areas were counted. Fifty-five records were of snakes (European whip snake *H. viridiflavus*, n = 33; Red-eyed grass snake *N. astreptophora*, n = 13; Smooth snake *C. austriaca*, n = 5; Aesculapian ratsnake *Z. longissimus*, n = 3; and Seoane´s viper *V. seoanei*, n = 1), while 24 belonged to lacertids (Slow worm *A. fragilis*, n = 9; Western green lizard *L. bilineata*, n = 3; Common wall lizard *P. muralis*, n = 6; and Viviparous lizard *Z. vivipara*, n = 6) (Fig. 3).

***Hierophis viridiflavus* success**

The 33 records of *H. viridiflavus* obtained in 2020–2021 corresponded to 28 individuals, including one juvenile, one subadult and one adult specimen identified by its moult. Of all records, five were recaptures (15% of the total). Broken down by year; in 2020, 12 records were obtained from ten individuals (one of them a moult), two of them recaptures (16%); in 2021, 21 records were obtained from 18 individuals, three of them recaptures (14%) (Table 1).

In 43% of the sampling days in 2020 at least one specimen was caught, while in 2021 catches increased to 84%. Despite this, it cannot be affirmed that there has been an increase in the number of individuals caught

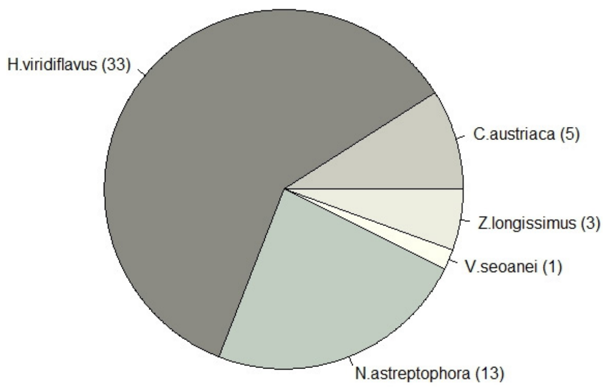

Figure 3. Number of snake records in fibrocement slabs during the years of sampling (2020–2021)

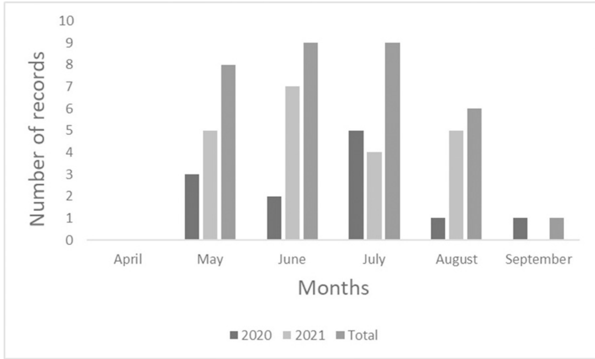

Figure 4. Records per year (2020 and 2021) and monthly totals of *Hierophis viridiflavus* in the set of transects carried out

Table 1. Number of *Hierophis viridiflavus* recorded on the total number of fibrocement slabs installed, in the two sampling years

		Age		
		Adults	Sub-adults	Juveniles
2020	Captures	11	1	0
	Recaptures	1	1	0
2021	Captures	20	0	1
	Recaptures	3	0	0
Total	**Captures**	**31**	**1**	**1**
	Recaptures	**4**	**1**	**0**

Table 2. Days elapsed from the start of the sampling and the placement of the slabs before the first capture by each snake species

Species	Days
Hierophis viridiflavus	14
Natrix astreptophora	51
Coronella austriaca	63
Vipera seoanei	93
Zamenis longissimus	101

during the second year (Mann-Whitney U-test; U = 12; Z = - 0.81354; p-value = 0.4459). Ninety-four percent of the captures corresponded to adults, with the capture of subadults and/or juveniles being residual. The first capture of *H. viridiflavus* was made 14 days after the slabs were placed, while for the rest of the species identified it was necessary to wait more than 50 days after their placement (Table 2).

In 2020, the month with the highest number of captures was July, with 71% of sampling days with at least one specimen captured. In 2021, the most successful months were May and August with 100% of records, i.e. a minimum of one individual was caught on each sampling day. Even so, in 2021 June had a success rate of 80% and July 71% (Fig. 4).

External factors: Temperature and micromammals

The temperature recorded on the slabs depended on the amount of radiation received during the day, and thus varied greatly from day to day, with maximum temperatures of 56.3 °C above the slab and minimum temperatures of 9.1 °C, while under the slab the maximum temperature was 46.3 °C and the minimum was 8.6 °C.

Ninety percent of the slabs showed signs of micromammal activity (genera *Apodemus*, *Myodes* and *Sorex*), detectable through the identification of traces (mostly food remains) and the presence of nests. Furthermore, in 34.5% of the slabs, adult individuals were detected at the time of lifting them.

DISCUSSION & CONCLUSION

Nine of the possible 12 reptile species in the study area were found. Only the most specialist reptile species were not detected (Sanz-Azkue & Alkorta, 2017; Fernández-Arrieta et al., 2019; Bea, 1985), such as *N. maura* (linked to rivers and wetlands) and *P. liolepis* (characteristic of areas with high insolation, especially rocky outcrops), as well as *C. girondica* (an ophidian with similar requirements to the previous lizard, on which it preys). Given that slabs were placed in areas to target *H. viridiflavus*, it was predictable not to detect specialist species. However, these data indicate the use of the slabs by different groups of reptiles (e.g. Bonnet, 2013), giving the method a generalist character. Therefore, it may be useful for herpetological inventories, because explanatory results were obtained for the composition of the reptile community in the area, whereas previous studies required between four and seven years to achieve similar results (Sanz-Azkue & Alkorta, 2017; Fernández-Arrieta et al., 2019). The use of slabs would help in the task of locating terrestrial herpetofauna, such as snakes and lizards, which are cryptic and are often difficult to detect (Gibbons, 1988), reducing the time and effort required to do so, as well as providing the option of capturing the individual to collect data. However, our data have shown that they are more efficient at capturing ophidians and would, therefore, be more useful in studies focused on these species (e.g. Romero-Iraola et al., 2022).

Records of *H. viridiflavus* obtained until the beginning of the study have been scarce, with a total of eight individuals identified between 2015 and 2019 (Romero et al., 2020; X. Rubio, pers. obs.). This is because there was not an adequate methodology for their study, given that VES show very scarce results for snakes in the region (e.g. Gosá, 2019). Furthermore, despite that active searches of natural habitat can yield high snake capture rates (e.g. Rodda & Fritts, 1992), in the study area there is a lack of natural shelters like logs or rocks, so species find shelter in shrubs and burrows where active search is very difficult to carry out. By placing the slabs both in areas where *H. viridiflavus* had previously been detected and in areas with unknown presence to date, but presenting high potential for it, it was possible to verify that it was a relatively common species in the area, due to the number of records obtained. Using this method, the species was detected in nine new 1 x 1 grids, going from seven to 16 (see Romero-Iraola et al., 2022). The 28 individuals detected in the two years of sampling compared to eight during the last five years before the study, would indicate that the slabs can function as a complementary methodology to the visual transects when carrying out snake studies.

The early captures of the first records of *H. viridiflavus* individuals, without the need for a period of 'naturalisation' of the slabs to the environment, contrasts with the results obtained by Naulleau (2002), who suggests a period of 2–3 months for the slabs to homogenise with the environment. This suggests that in the censused population, the first records may be more influenced by the densities of the species (Bonnet et al., 2013), although the time elapsed since the slabs were placed may also be a determining factor, as the data obtained for the rest of the snake species might indicate. Nevertheless, lack of shelter may be a determining factor for species when using the slabs (e.g. *Testudo hermanni*, Ballouard et al., 2013).

As observed by Ballouard et al. (2016) over three years in other snakes such as the Montpellier snake *Malpolon monspessulanus* and the Ladder snake *Zamenis scalaris*, this two-year study also failed to confirm an increase in the number of individuals captured during the second year, i.e. it was not possible to confirm that the effectiveness of the slabs increased over time (Naulleau, 2002). Besides providing protection from predators, fibrocement slabs can facilitate thermoregulation (Lelièvre et al., 2010a). Therefore, the use of the slabs may be influenced by the temperature of the environment and the physiological needs of the snake rather than by the increasing passage of time (Bonnet, 2013). The months in which there have been the greatest number of captures are times that correspond to peaks of activity of *H. viridiflavus* (Santos et al., 2015); even so, individuals were captured up to mid-September 2020.

The high and low temperatures that can be reached by the slabs led to the need to choose a specific day and time to carry out the fieldwork, at times and hours when the temperature recorded by the slabs is within the optimum levels for *H. viridiflavus*, which stands around 30 °C (Lelièvre et al., 2010b). It should also be taken into account that the species changes its activity habits depending on the time of year. In spring and late autumn, daily activity has a unimodal pattern (peaking at midday), whereas in late spring and summer, it has a bimodal pattern (peaking in the early morning and late afternoon) (Capula et al., 1997). It is therefore recommended that rainy and very hot days, as well as the central hours of the day in summer, be ruled out.

The presence of micromammals under the slabs could be an indicator that snakes also use them to hunt potential preys. Such would be the case of *H. viridiflavus*, of whose diet micromammals form part (Vanni & Lanza, 1977).

Given the need for a new methodology to monitor reptile populations (Cox & Temple, 2009), this study of fibrocement slabs to census populations of *H. viridiflavus* in the mid-Atlantic environment, and other accompanying reptile species, has provided positive results. This methodology should be extended in future campaigns in order to carry out a more precise analysis of their effectiveness, which could be very useful for recording the abundance of ophidians and the necessary decision-making for their management. In addition, there were very few incidents related to the slabs. Ruptures/displacements were only recorded in areas with high livestock activity. The results presented in this study have led Navarre (northern Spain) to initiate in 2021 a similar monitoring programme of transects using the same methodology of fibrocement slabs, combining them with VES, in order to carry out snake censuses throughout its territory. All the same, considering the wide range of artificial refuges that could be used, other materials might be as effective as fibrocement slabs. Microhabitat preferences vary between reptile species and are influenced by environmental elements like temperature and moisture. As a result, the ideal environmental parameters for a given species may be met by a specific kind or size of artificial refuge (Engelstoft & Ovaska, 2000; Hecnar & Hecnar, 2011).

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