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Reproduction ecology of the recently invasive snake *Hemorrhois hippocrepis* on the island of Ibiza

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Abstract

Knowing the causes of biological invasion success can be relevant to combat future invasive processes. The recent invasion of the horseshoe whip snake *Hemorrhois hippocrepis* on the island of Ibiza provides the opportunity to compare natural history traits between invasive and source populations, and to unravel what makes this snake a successful invader that is threatening the only endemic vertebrate of the island, *Podarcis pityusensis*. This study compares the basic reproductive traits of mainland native and invasive populations of the snake. Our results revealed that invasive populations were characterized by female maturity at a smaller size, extended reproductive period, and much lower reproduction frequency compared to the native population. In contrast, some major reproductive traits—the abdominal fat body cycle, clutch size, hatchling body size, and hatchling body condition, did not differ between the two populations. Some of these results must reflect the environmental differences in the recently invaded island with respect to the source area, and overall plasticity of reproductive traits. Plasticity is evolutionarily interesting, and may aid the successful growth of this species in their invasiveness of Mediterranean islands like Ibiza. The most significant finding is that this expression of phenotypic plasticity occurred rapidly in this invasive population, within a period of 14 years maximum. Our results on the reproduction ecology of the invasive population were not conclusive regarding the factors determining the invasiveness of the snake and pointed to alternative causes.

Key words: Ibiza, invasion success, islands, reproduction frequency, snakes

Invasive species affect ecosystems around the planet, mostly threatening island species (Jones et al. 2016), which are particularly vulnerable due to their inherent characteristics. Island species have evolved in relatively isolated conditions, and are adapted to fit the typically innocuous insularity of their environment; newcomers to islands, however, are usually more efficient than native species (Cohen 2002; Simberloff et al. 2013). Therefore, knowing the possible causes of

success regarding biological invasions would give us accurate predictions for invasive processes as well as efficient control and eradication mechanisms for non-indigenous species (Jeschke and Strayer 2006; Hayes and Barry 2008; Rödder et al. 2009; Mahoney et al. 2015).

Diverse factors have been tested in order to determine a causal effect with invasion success, like the reproductive traits of invader species (Cohen 2002): early sexual maturity has been reported as a

facilitator of establishment for the invader turtle *Trachemys scripta* in Southern Europe (Pérez-Santigosa et al. 2008). Evidence for the expansion of the reproduction period or disappearance of reproduction seasonality has been described in the invasive population of the snake *Boiga irregularis* in Guam (Savidge et al. 2007). A greater portion of the population reproducing every year, which implies an increase in reproductive frequency, also happens in the case of *T. scripta* (Pérez-Santigosa et al. 2008). Fecundity rise is also an observed feature in invaders that contributes to their success (Mahoney et al. 2015), as demonstrated with the case of *Lithobates catesbeianus* (Kaefer et al. 2007) along with other aquatic invasive animals in North America (McMahon 2002). Lastly, the neonate size of the invasive snake *B. irregularis* is larger in the invasive population in Guam than in the native population (Rodda and Savidge 2007).

The aforementioned cited shifts on reproductive traits can improve the fitness of organisms, and some of them are expected to be displayed by invasive populations when compared to the source populations of the same species. However, it is not the general rule, and none of the invasive species presented all the shifts at the same time (Parker et al. 2013). For instance, sexual maturity is delayed in the populations of the invasive treefrog *Osteopilus septentrionalis* in the South-eastern United States (McGarrity and Johnson 2009), and females of *B. irregularis* do not appear to increase reproduction frequency in the invasive population of Guam (Rodda and Savidge 2007). With respect to productivity, the *Coccinella septempunctata* beetle lays larger clutches with smaller eggs in the invasive populations of North America (Kajita and Evans 2010), and the invasive *Lampropeltis californiae* in the Canary Islands showed low levels of juvenile recruitment (Fisher et al. 2019).

Therefore, there is an accelerating need to study to what extent reproductive traits can aid invasive population success; understanding the mechanisms of a particular invasion can help managers take actions against it. The recent invasion of the island of Ibiza (Balearic Islands) by the Horseshoe whip snake *Hemorrhois hippocrepis* from the southern Iberian Peninsula yields a good opportunity to study and determine whether reproduction ecology is behind the quick expansion of the invasive population (Fisher et al. 2019). Snakes as invaders frequently cause drastic impacts on the environment (Willson 2017). Moreover, they exhibit such a broad diversity in their intraspecific reproductive strategies (Shine 2003), that this group is considered a model taxa for studies of reproductive ecology (Shine and Bonnet 2000). In parallel, the study of invasive populations offers the opportunity to understand basic processes in natural history (like reproduction), thanks to the velocity of the changes exhibited by invasive organisms (Sakai et al. 2001).

The Balearic archipelago, in the Mediterranean Sea, has been isolated from the mainland for 5.33 million years. Later, it suffered severe climatic oscillations that influenced species range and extinctions (Bover et al. 2008). In Ibiza, the westernmost of the Balearic Islands, a sudden mass extinction occurred during the Pleistocene that is yet to be explained (Alcover and McMinn 1993). Consequently, the only non-flying vertebrates found in the fossil records right after this event are two reptiles: an undescribed dwarf viper (Alcover JA, personal communication) and the Ibiza wall lizard *Podarcis pityusensis*, the latter being the only extant endemic reptile to Ibiza Island.

During the end of the 3rd millennium BCE, humans colonized the islands and the introduction of alien species began; human-mediated extinctions started taking place (Pinya and Carretero 2011), among them, the case of the dwarf viper. By the beginning of the third millennium CE, there were consistent importations of big

and old olive trees from the south of the Iberian Peninsula, which inadvertently caused a surge of stowaway snakes, in a process of multiple introductions at a rather high propagule pressure during several years (Silva-Rocha et al. 2018): *Malpolon monspessulanus*—the Montpellier snake, *Zamenis scalaris*—the ladder snake, and *H. hippocrepis* (Álvarez et al. 2010). *Malpolon monspessulanus* first disappeared from the island of Ibiza a few years after the introduction, *Z. scalaris* maintains a small and stable population, and *H. hippocrepis* is thriving well and spreading quickly throughout the lowlands of the island (Silva-Rocha et al. 2018), occupying an empty functional niche.

Hemorrhois hippocrepis is a slender-bodied, medium to large-sized colubrid, native to north-western Africa and southern Iberian Peninsula, also present in some Mediterranean islands, where it was probably introduced (Pleguezuelos and Feriche 2014). It is a rupicolous snake that usually prowls human dwellings and masonry stone walls, which are used for shelter and foraging (Pleguezuelos and Feriche 2014), man-made and rather simplified habitats that may be favorable for the invasive population (Sakai et al. 2001). The importance of controlling the fast spread of *H. hippocrepis* on Ibiza island lies in the major impact it poses for the only Ibizan endemic vertebrate, *P. pityusensis*, the most consumed prey of the invasive snake on the island (57% in frequency; Hinckley et al. 2017). Indeed, reptiles are not only among the most introduced vertebrate species in Mediterranean islands, but also among the most affected by the introduction of other reptile species (Kraus 2009). However, when studying the feeding ecology of the invasive Ibizan *H. hippocrepis*, we found puzzling results that hardly account for the success of the Ibiza invasive population; despite a rather low percentage of individuals with gut content while maintaining a high body condition (Hinckley et al. 2017). Hence, we searched for other aspects of the snake's natural history, such as its reproductive ecology, that could help us in our understanding of its invasion success as well as in the management of these populations.

The reproduction ecology of *H. hippocrepis* is well known in its native area, including the region where the invaders come from (Pleguezuelos and Feriche 1999). These circumstances offer an excellent opportunity to investigate the degree of potential plasticity of the reproductive traits (Ajtić et al. 2013), and whether the reproductive ecology of the snake is an alternate explanation of its invasion success on the island of Ibiza. In invasive reptiles reproduction is a natural history aspect scarcely ever addressed (Kraus 2015). In addition, just a decade after the first reference to the invasion, some expressions of phenotypic plasticity were observed, like an increase of the maximum body size and the level of fat reserves (Montes et al. 2015; Hinckley et al. 2017). In snakes colonizing islands, body size shifts at a fast pace with respect to the source population (Aubret 2015), and populations with easy access to prey exhibit fast growth and high fecundity (Bronikowski and Arnold 1999). Thus, it is likely that the reproductive ecology of *H. hippocrepis* is already being affected to complement the new phenotypes on the island of Ibiza.

The main goal of this study was to assess whether any trait of the reproductive ecology of the invasive population of *H. hippocrepis* in Ibiza has changed with respect to the source population in the southern Iberian Peninsula. For both sexes, we compared the body size at sexual maturity, the timing of the reproductive cycle, the monthly variation of fat bodies in relation to the reproductive cycle, reproductive frequency and clutch size in females, and newborn size, that is, traits whose variation can also modify fitness in invasive populations (Parker et al. 2013). Our hypotheses are the following: 1) in females, maturity will be reached at the same body size in the invasive

and in the source populations, given that in snakes the mother needs a minimum abdominal space to allocate the eggs (Seigel and Ford 1987); 2) we expect an extension of its reproductive season, favored by warmer thermal envelopes, compared to the mainland population (Shine 2003; Mathies 2016). This is due to the fact that the invasive snake is spreading exclusively on the warm lowlands of the island, whose thermal conditions are different with respect to the southern Iberian Peninsula, and that the reproductive traits of ectothermal organisms may change due to variation in the thermal environment (Huey 1982); 3) we expect that mature snakes on the island do not skip opportunities to reproduce and do so on a yearly basis (Seigel and Ford 1987), due to the excellent body condition of most mature females (Hinckley et al. 2017) and being that reproduction frequency in temperate snakes relies upon this parameter (Bonnet et al. 2001); 4) since we are observing gigantism in the insular population (Hinckley et al. 2017) and fecundity is related to the mother's size (Shine 1992), particularly in *H. hippocrepis* (Pleguezuelos and Feriche 1999; Pleguezuelos and Fahd 2004), we expect larger clutch sizes for the invasive than for the mainland population (topic reviewed in Parker and Plummer 1987); 5) in an alternative but not exclusive way, invasive females, because of their excellent body condition, will produce larger hatchlings and/or in better body condition (Ford 2011; Tuttle and Gregory 2014). These changes refer to the acquisition of fast reproductive traits (Tuttle and Gregory 2014; Allen et al. 2017) and would provide evidence on the success of the invasion process (Sax et al. 2007), a topic that has been scarcely addressed previously in snakes between source and introduced populations (Fisher et al. 2019; but see Reed 2005; Rodda and Savidge 2007).

Materials and Methods

The island of Ibiza is within the Balearic archipelago, in the Western Mediterranean Sea. It has an area of 572 km², and is rather flat, with a maximum altitude of 486 m asl. The mean annual temperature is 17.9°C and the average yearly rainfall 413 mm (Ibiza Airport weather station, 38° 52′ 22″ N, 1° 22′ 23″ E; www.aemet.es). The landscape is shaped by pine forests and cultivated land, with alternating scrubland. Thousands of houses are scattered throughout the island, with stone walls frequently delimiting gardens and crops, both landscape traits favorable for *H. hippocrepis*.

Individuals for this study come from eradication campaigns of *H. hippocrepis* on the island (2014−2017 period), fostered by the Regional Administration, together with road kills and specimens collected by the locals. Specimens were euthanized in the field, right after the capture, with a pentobarbital injection in the heart, and preserved in ethanol in the collection of the Granada University (DBAG). In total, 545 specimens from Ibiza (224 females, 261 males, and 60 immature of undetermined sex) were examined. Comparisons with the characteristics of the source population were made by using raw data from Pleguezuelos and Feriche (1999), updated with other specimens from the DGAB collection, from the same area of the Southern Iberian Peninsula, and processed by the same protocol here explained (477 specimens: 167 females, 182 males, and 128 immature). Before fixation and preservation, snout-vent length (SVL; ±1 mm) and body mass (±0.1 g) were measured. Gonads were examined by making a ventral incision and the following variables were measured after fixation: sex (male, female, or undetermined). In females, we recorded the number of ovarian follicles or oviductal eggs on each side, the biggest diameter of each follicle or oviductal egg (±0.1 mm) and the presence of oviducts with emptied but distinct, incubation chambers (indicative of postpartum

condition; Almeida-Santos et al. 2014); in males, sagittal, transverse, and longitudinal axis of the right testis (±0.1 mm), anterior in the body and larger than the left one. Measurements were only taken from well-preserved specimens and organs, which resulted in some variability in the sample size of the traits. Testicular volume (TV) was obtained using the formula for a flattened ellipsoid (Mayhew 1963). In females, size at maturity was graphically estimated by plotting the length of the largest vitellogenic follicle/oviductal egg against maternal body size (SVL; Figure 1A), and by the presence of oviductal eggs or folded oviducts; in males maturity was graphically deduced by plotting Relative testicular volume (RTV; residuals of the correlation between log TV on SVL) to the male body size (Seigel and Ford 1987; Figure 1B), and by the presence of convoluted ductus deferens. The reproductive cycle was deduced by plotting the length of the largest follicle/oviductal egg, and the RTV against the days of the year (Julian calendar), for sexually mature females and males, respectively (Figure 2). These three deductions were made based on the point at which follicle diameter or RTV suddenly increases, which indicates the beginning of the reproduction activity (sexual maturity or reproductive period). We also recorded when the oviducts were glandular and folded or the ductus deferens thickened. In both sexes, we recorded fat body reserves by scoring abdominal fat body level in five visual categories according to Pleguezuelos and Feriche (1999): zero, no traces of fat; one, small traces of fat among intestine loops; two, fat bodies covering less than half of the intestinal surface; three, fat bodies covering more than half of the intestinal surface; and four, a continuous fat layer on the ventral zone of the abdominal cavity. Body condition index (BCI) is a good estimator of body reserves in snakes (Santos and Llorente 2004), and in general, individuals with better BCI usually increase future survival (Giesel 1976). We estimated the BCI (residuals of log body mass [g] on log body length [SVL in mm]; Whittier and Crews 1990) in hatchlings (individuals not damaged by traffic casualty) from the source and the invasive populations. We considered hatchlings only those individuals within 15 days before and after the average hatching date (10 September), maintaining a fresh vitellogenic scar and without gut content.

Sexual dimorphism in body size (SVL) was assessed by using a Mann–Whitney U test, because of differences between groups in the variances. Student *t*-test was used to compare SVL between the group of mature females within each population that reproduced and the group that did not, as well as between the group of mature males that accomplished spermatogenesis and the group that did not. The clutch size was compared between groups by ANCOVA (maternal SVL as a covariate). Homogeneity of fat bodies among months was tested by the Kruskal–Wallis test. The percentage of reproductive females (gravid and with oviductal scars indicating recent oviposition) in the appropriate period was taken as a measure of the reproduction frequency and was compared between study areas and to biennial theoretical reproduction by a 2×2 contingency table. Mean values are followed by ±1 SD, with alpha set at 0.05. Statistical analyses were carried out by the program STATISTICA version 8.

Results

Size at maturity

The mean SVL of the Ibizan *H. hippocrepis* was 723.4 mm (±216.5 mm, range = 239–1499 mm, $n = 485$), females being on average shorter than males (661.4 ± 177.1 mm, range = 239–1305, $n = 224$ versus 776.6 ± 232.7 mm, range = 283–1499, $n = 261$; U = 21004.0, $P < 0.0001$; all individuals considered). The young
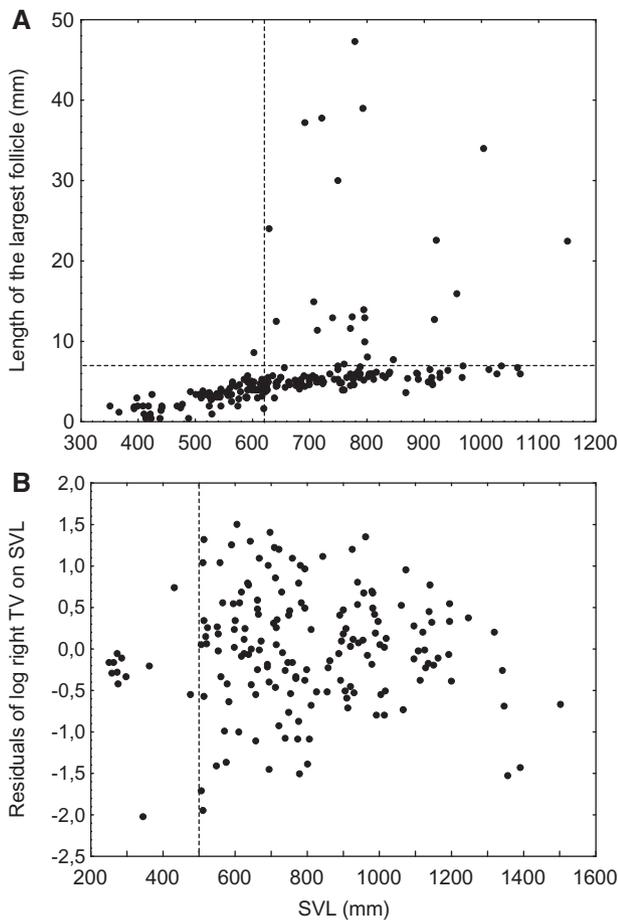
Figure 1. Body size at sexual maturity for females and males of invasive *H. hippocrepis* on the island of Ibiza. (A) shows the length of the largest follicle/oviductal egg plotted against body size (SVL; *n* = 204). Dashed horizontal line tentatively separates between primary and secondary vitellogenic follicles or oviductal eggs; dashed vertical line tentatively separates between immature (on the left) and adult females. (B) shows residual scores of the right testis volume (log-transformed) on SVL plotted against body size (SVL; *n* = 171). The vertical dashed line tentatively separates immature (on the left) from mature males. Each point represents one individual.

females of any month and all adult females from late June to late April (out of the vitellogenesis period) exhibited follicles <8 mm maximum diameter (primary vitellogenic follicles). A female with oviductal eggs was 628 mm SVL, and several females just above this size showed secondary vitellogenic follicles or oviductal eggs (Figure 1A). Thus, we consider that maturity is reached at approximately 628 mm SVL in females. Female sexual maturity was reached at 48% of the maximum SVL on Ibiza, whilst at 63% in the source population (the smallest mature Iberian female was 665 mm SVL).

Males from Ibiza strongly increased RTV at 500 mm SVL (Figure 1B), at which we estimated sexual maturity is achieved. Even though a male with 431 mm SVL (DBAG HEMO-670) had relatively big and turgid testis, we considered this specimen as an exception. Sexual maturity was reached at 33% of maximum SVL and on the south of the Iberian Peninsula it was reached at the same SVL (500 mm), but at 39% of the maximum body size (SVL).
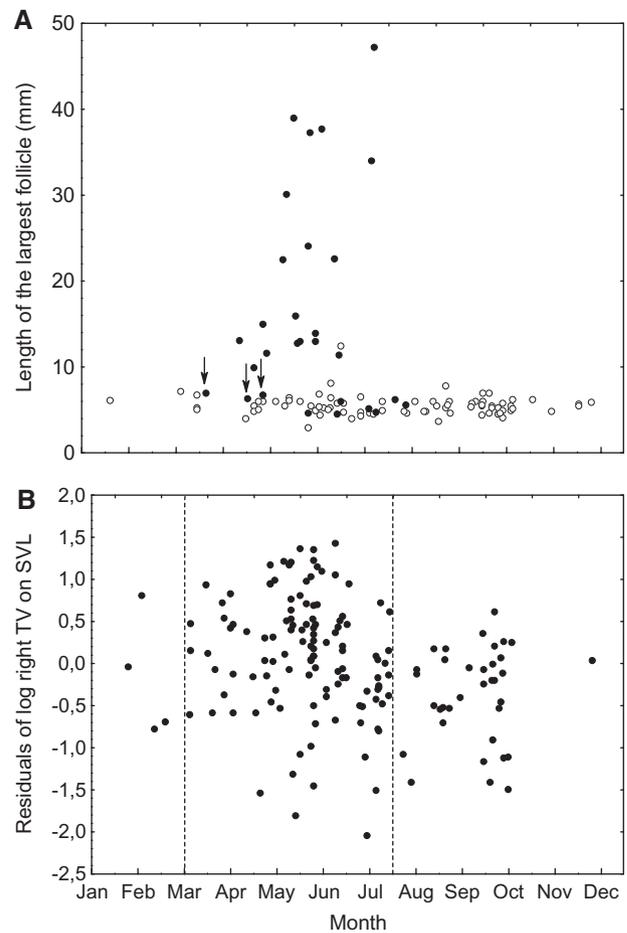
Figure 2. Reproductive cycles of invasive *H. hippocrepis* on the island of Ibiza. (A) shows the length of the largest follicle or oviductal egg in females plotted against the day of the year (only potentially adult individuals, SVL > 628 mm; *n* = 110); empty circles represent non-reproductive females whereas full circles show reproductive females. Three specimens from spring, with no developed follicles, were considered as reproductive females because of maximum fat-body levels and the presence of folded oviducts (indicated by arrows). Full circle cases within the cloud of empty circles correspond to individuals with oviductal scars and/or folded oviducts. (B) shows residual scores of the right testis volume (log-transformed) on SVL plotted against the day of the year (only potentially adult individuals, SVL > 500 mm; *n* = 157). Vertical dashed lines are intended to mark the beginning and end of spermatogenesis. Each point represents one individual.

Reproductive cycles

Females with enlarged follicles (≥8 mm) and expanded and/or folded gonoducts were found throughout the year, with very large follicles (≥14 mm) or oviductal eggs from late April to late July, and with fully developed oviductal eggs (≥38 mm) and oviductal marks of recent oviposition from late May to late July (Figure 2A); thus, we deduced oviposition occurred during June and July.

Testicular recrudescence of mature males (SVL > 500 mm) started in mid-March, with the highest peak of RTV in June, and declined through July. Some individuals showed a small testicular recrudescence between the end of September and October, suggesting a secondary spermatogenesis period (Figure 2B). From that plot, we also calculate that every year, 59.3% (70 out of 118 individuals in the plot) of males from Ibiza island with body size large enough to be adults did accomplish spermatogenesis; there were no significant differences in body length between those which accomplished

spermatogenesis and those which did not ($t = 1.36$, $P = 0.18$). The frequency of mature males in spermatogenesis was not significantly different in the south of the Iberian Peninsula either (70%, $n = 46$; 2×2 table, $\chi^2 = 1.48$, $P = 0.22$).

Female reproductive frequency

Among the 58 adult females from the period of mid-April to mid-August, 29 showed enlarged follicles (>10 mm), oviductal eggs, or clear signs of recent oviposition, meaning that 50% of adult females reproduce every year. Some females with SVL slightly larger than 628 mm but with very small follicles and low-fat body levels were considered still immature and not included in this calculation. SVL of these reproductive females (812.3 ± 122.7 mm, $n = 29$) did not differ from that of the subsample of non-reproductive females in the same period (763.2 ± 89.8 mm, $n = 29$; $t = 1.74$, $P = 0.09$), indicating that this result was not affected for the different size of the specimens in the two subsamples. This frequency of reproduction is below that observed for the south of the Iberian Peninsula (82.6%, $n = 23$; in a comparison between regions of the frequency of reproductive females; 2×2 table, $\chi^2 = 5.97$, $P = 0.01$), and fits to a biennial reproduction frequency (2×2 table, $\chi^2 = 0.00$, $P = 1$).

Abdominal fat bodies

Sexually mature females showed a mean fat body level of 2.77 ± 1.14 ($N = 116$), and sexually mature males 3.24 ± 0.95 ($n = 231$). Mature but non-reproductive females reached maximum fat-body levels throughout the spring, explained by the fact that they do not invest their fat reserves on vitellogenesis, whereas reproductive ones attained elevated fat-body levels at the beginning of the spring, decreased during June and were minimum in July and August, showing that vitellogenesis does have a cost on fat reserves (Figure 3A). In an inter-monthly comparison, fat-body levels were homogeneous in mature but non-reproductive females (Kruskal–Wallis test, $H_{8,87} = 8.60$, $P = 0.34$), different in reproductive females (Kruskal–Wallis test, $H_{4,29} = 18.04$, $P = 0.0012$), and heterogeneous in males (Kruskal–Wallis test, $H_{8,231} = 26.79$, $P = 0.008$), with maximum level during the period May–July (Figure 3B).

Clutch size

The maximum clutch size for the Ibiza population was 13 (Figure 4), larger than in the source population (11), and the mean clutch size was 6.9 ± 2.9 ($n = 9$), but did not differ from the same parameter in the source population (6.5 ± 2.4, $n = 13$). These results hold in absolute ($t = 0.309$, $P = 0.75$) and relative terms (ANCOVA, with clutch size as dependent variable, maternal SVL as covariate, and region as grouping factor, $F = 0.41$, $P = 0.53$; homoskedasticity of the variances, $P < 0.401$; slope of the regression lines, $P = 0.853$; Figure 4).

Hatching period and offspring size

On the island of Ibiza hatchlings were found in the field from the end of August, with mean SVL of 282.9 ± 27.8 mm (range 250–325 mm; $n = 13$) and mean body mass of 6.9 ± 2.4 g (range = 4.2–10.8 g; $n = 13$). There was no difference in SVL neither in body mass from hatchlings from the source population (277.9 ± 24.3 mm, $n = 11$, $t = 0.46$, $P = 0.64$; and 5.7 ± 1.5 g, $n = 10$, $t = 1.37$, $P = 0.18$, respectively for SVL and body mass). There was no significant difference in BCI between Ibizan and
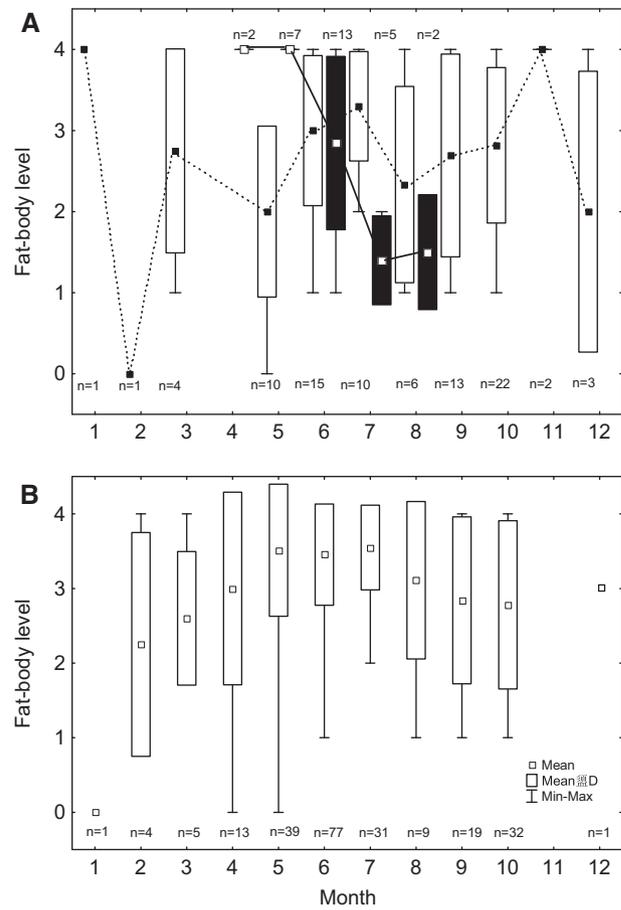
Figure 3. Abdominal fat-body level of sexually mature female (A) and sexually mature male (B) *H. hippocrepis* on the island of Ibiza. For the period April-August we differentiate between reproductive and non-reproductive females, black box-plots represent reproductive females and empty box-plots show non-reproductive females. Fat-body level is scored in five categories, from zero to four (see "Material and Methods" section for more details). Females $n = 116$, males $n = 231$. In both sexes, successive winter months pooled because of a small sample size.
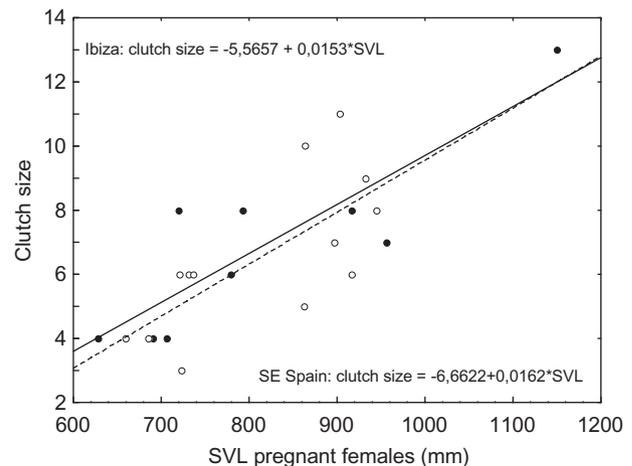
Figure 4. Linear regression between clutch size and maternal SVL of *H. hippocrepis* for the invasive population on the island of Ibiza (solid circles and continuous line, $r = 0.866$; $P = 0.002$) compared to the source and native population from the south of the Iberian peninsula (empty circles and dashed line, $r = 0.708$; $P = 0.006$).

Iberian hatchlings (0.051 ± 0.11, $n = 13$ versus -0.067 ± 0.20, $n = 10$; $t = 1.82$, $P = 0.08$).

Discussion

The invasive population in Ibiza shows some differences in reproductive traits with respect to the source population: maturity is attained at smaller body size in females, the reproductive season is longer, and fat body levels are higher in both sexes (see Pleguezuelos and Feriche (1999) for data of the source population). However, female reproductive frequency is lower, and there are no differences in body size at maturity in males, absolute or relative clutch size, hatchling body size, or hatchling body condition. Whilst the results for the first set of reproductive traits could be supporting the invasion success, some of the latter, vital for population fitness, suggest slow reproductive traits that do not help to explain invasion success.

Size at maturity

Regarding the female body size at which sexual maturity is acquired, our hypothesis is not met. Reproduction represents a risk for female survival regardless of their offspring productivity (Aldridge and Duvall 2002); hence, natural selection forces females to attain a minimum body length to reproduce, which implies a minimum clutch size that overcomes the risks of their first reproductive period (Seigel and Ford 1987). For this reason, we expected females from both populations to become sexually active at the same body length. However, we found smaller reproductive females in Ibiza than in the source population. This suggests a shorter time from hatchling to the age at which reproduction occurs, although we have no data on the growing rate of the individuals. Ford and Seigel (1994) found in the oviparous *Elaphe guttata* that neither age nor size of first reproduction was fixed; Cardozo and Chiaraviglio (2011) found in *Boa constrictor* that females from healthy environments mature at smaller body sizes than those from poor environments; the population of the Lake Erie Water Snake *Nerodia sipedon insularum* accelerated its sexual maturity and raised its offspring production after starting to feed almost exclusively on a newly arrived and abundant, invasive prey, proving that an improvement in diet can actually enhance the reproductive characteristics of a population only in a one or two-generation lapse (King et al. 2006). Something similar may have happened in Ibiza (at least in body size, as we do not have data on age), since the invasive population of *H. hippocrepis* has shifted its diet toward naïve and abundant prey, especially the endemic *P. pityusensis*; the excellent body condition showed by snakes of this population (Hinckley et al. 2017; body condition data in this study) supports this interpretation. In males, the lack of differences in body size at maturity would be just a consequence of less important trade-offs between sexual maturity and other natural history traits, compared to females (Cardozo and Chiaraviglio 2011).

Reproductive cycles

As we hypothesized, the reproductive period expanded in both sexes in the invasive population compared to the source one, representing a fast manifestation of phenotypic plasticity in one of the reproductive traits. Vitellogenesis advanced 1 month and oviductal eggs got delayed by one week compared to populations on southern Iberian Peninsula (Pleguezuelos and Feriche 1999), meaning that the invasive population has expanded the laying period. In temperate reptiles, reproduction seasonality is forced by the thermal requirements of embryogenesis (Vitt and Vangilder 1983), besides, *H. hippocrepis* is a very thermophilic snake. The species shows quite high thermal needs in order to perform its peculiar vernal spermatogenesis, a typical cycle on the warm southern belt of the Mediterranean Basin (Saint Girons 1982), where the species originated (Carranza et al. 2006). The invasive population's use of a wider reproductive window may be explained by Ibiza's favorable climate and habitat. This may allow the population to be less strict regarding their original reproduction boundaries. The same has been observed in the round goby *Neogobius melanostomus* in the Upper Detroit River (MacInnis and Corkum 2000), in the introduced population of *Anolis sagrei* in Taiwan (Norval et al. 2012), and in the well-known invasive *B. irregularis* in Guam (Rodda and Savidge 2007), whose males switch between synchronized seasonal reproduction and asynchronous continuous reproduction (Mathies et al. 2010); this generally occurs in snakes of tropical environments (Mathies 2016). Future eradication campaigns on *H. hippocrepis* in the island of Ibiza can take advantage of the precise knowledge of its reproductive seasonality in the invaded area.

Reproductive frequency

Given the optimal environmental conditions (Seigel and Ford 1987) and since most adult females showed high-fat body level at the start of the breeding season on Ibiza (Figure 3A), we hypothesized that all adult female individuals of the invasive population would reproduce every year. However, reproductive frequency is significantly lower than on the source population in the Iberian Peninsula (Pleguezuelos and Feriche 1999; this study), to the point of being biennial. The male gametal production on the island population, as measured by the frequency of mature males in spermatogenesis, is statistically similar, but with a high portion of males not accomplishing spermatogenesis on Ibiza (40.7%, reproductive cycles "Results" section). Nonetheless, the absence of spermatogenesis is not determinant to dismiss sexual activity, as the sperm might be stored in the ductus and males would fecundate females by sperm produced in the previous season or year (Saint Girons 1982). In addition, mature males might not accomplish spermatogenesis in sequential years because females usually store sperm from from previous matings in their oviducts (Almeida-Santos et al. 2004), as recorded for this species in captivity (Cattaneo 2015). These possibilities can only be tested out by histological examination.

Regarding the rather small amount of reproductive females found in Ibiza, female snakes carrying eggs tend to decrease their locomotory capacity and perform a secretive behavior (Shine 1980; Gregory et al. 1999) that makes them less vulnerable to being captured, producing a bias toward non-pregnant females in field surveys (Fitch 1987); Bonnet and Naulleau (1996) found the opposite in a viper, with a higher catchability in pregnant females. However, these biases, if they occur in *H. hippocrepis*, must happen on both populations, the source and the invasive one, and therefore, it does not explain the results here obtained. Low reproduction frequency has been described for the invasion-front population of cane toads *Rhinella marina* in Australia, suggesting a trade-off where the costs of reproduction are too elevated to achieve an effective dispersal (Hudson et al. 2015). Indeed, scarcity of pregnant females seems to be a common and not well-understood characteristic of invasive organisms, particularly snakes (Savidge et al. 2007), as it happens with *B. irregularis* on Guam (Rodda and Savidge 2007), although in the reptiles there is a clear advantage of frequent clutches for the success of introduced species (Allen et al. 2017).

Fat bodies and clutch size

We knew that fat reserves on the invasive population were much higher than on the source population of *H. hippocrepis* (Hinckley et al. 2017), a circumstance opposite to that observed for invasive *B. irregularis* in Guam (Rodda and Savidge 2007). In this study, we expected to find fat reserves vary, according to the reproduction phases, as usual in temperate snakes (Seigel and Ford 1987). In our study this variation was only evident when differentiating non-reproductive females from reproductive females of the invasive population, the former group showing rather homogeneous and high abdominal fat body levels, indicating an absence of investment on vitellogenesis; furthermore, this fact proves that the choice of not reproducing is not linked to the lack of feeding resources, opposite to what happens for other Eurasian snake species (Pleguezuelos et al. 2007). The group of reproductive females does show a variation that perfectly matches the vitellogenic processes: maximum levels at the beginning of spring and, as follicles are developing during May and June, fat reserves decrease until July and August, when eggs have been laid. The high level of abdominal fat bodies at the beginning of spring suggests a capital strategy (*sensu* Drent and Daan 2002) to fuel vitellogenesis and oogenesis in the invasive females; however, the presence of prey in the gut content during pregnancy (Hinckley et al. 2017) also suggests an income strategy, in which snakes produced better body condition and higher survivorship of females after reproduction (Feriche et al. 2016). For invasive males, there is indeed a variation on fat reserves, but spermatogenesis does not have an impact on their lipid stores (see Mathies et al. 2010 for similar results in the introduced *B. irregularis*); conversely, the fat body levels increase as the spermatogenesis does, and do not seem to be affected by the males actively searching for females in late spring (Pleguezuelos and Feriche 2014). In brief, the results from the study of fat body levels do not support that energetic constraints are behind the low reproduction frequency in both sexes of the invasive population. The increased weight in the invasive snake population of *L. californiae* on the Canary Islands, has been also suggested to be tied to their predation success on naïve prey (Fisher et al. 2019), the same that could be occurring for *H. hippocrepis* on the island of Ibiza.

Despite the good body condition of mature females from the invasive population, the results do not meet our hypothesis on the clutch size; the important accumulation of fat reserves in the invasive population seems to not be diverted into energy for reproduction, and the clutch size failed to differentiate from the same trait on the source population. Although a very large invasive female had the largest clutch recorded for the species in the wild, the female size to clutch size relationship was maintained in the comparison between populations, suggesting that this trait is somewhat genetically fixed for this rather slender bodied snake. In Florida, the invasive potential of *Python bivittatus* is determined by its large size and high reproductive potential (Reed et al. 2012; see Allen et al. 2017 for an interspecific analysis of this topic), but the invasive population of *B. irregularis* on Guam showed smaller values of clutch size than in the native populations, even though invasive snakes were larger (Savidge et al. 2007). Apparently, the invasive success of *H. hippocrepis* on the island of Ibiza does not rely on a larger clutch size for females with very fine body condition, and this rather low reproduction rate suggests also a low population turnover.

Hatchlings

We hypothesized that invasive females would produce bigger hatchlings. Data show no significant differences between invasive hatchlings and native ones in length, weight or body condition, therefore, the hypothesis is not met. In agreement with our findings, in other snake's intraspecific comparisons, egg/hatchling size tend to be less variable than clutch size, as occurred in the Chinese *Naja atra* (Ji and Wang 2005), and in *Thamnophis marcianus*, for which differences in food availability for reproductive females affected both clutch size and clutch mass, whilst offspring size remained unaffected (Ford and Seigel 1989); however, in the congeneric *T. elegans*, females of different populations, under different environmental conditions, produced neonates of different body size (Bronikowski and Arnold 1999), and hatchling mass of *Tropidonophis mairii* is influenced by maternal phenotype (Mayer et al. 2016). Once more, this reproductive trait does not explain the success of the invasive population of *H. hippocrepis*.

We conclude that the reproductive ecology scarcely explains the invasion success that *H. hippocrepis* is showing in Ibiza (similarly to the case of the famous and invasive *B. irregularis* in Guam; Rodda and Savidge 2007). But this study on the reproductive ecology of the invasive population discovered a fast expression of phenotypic plasticity, something that occurs under new environmental conditions such as insularity (Ajtić et al. 2013), and it uncovered too that the degree of plasticity differs among the various life-history traits (Seigel and Ford 2001). This expression of phenotypic plasticity arising from environmental factors (thermal envelope and naïve prey) is interesting because of the rapidity of the process (Sakai et al. 2001). The changes observed in this study, together with the increase in body size (Hinckley et al. 2017), occurred within a period that lasted a maximum of 14 years or 3–4 generations (unpublished data of the authors), whilst reproduction traits of the introduced *A. sagrei* in Hawaii did not change after 20 years (Goldberg et al. 2002). In looking for the reasons for the success of this invasive snake through the analysis of intrinsic factors of the population, like its feeding and reproduction ecology (Hinckley et al. 2017; this study), we obtained rather inconclusive results. However, the reasons for the success could be extrinsic, like the almost absolute lack of threats faced (predators and competitors) in the new and insular environment of Ibiza (Silva-Rocha et al. 2018), favoring high survivorship for this recently invasive snake.

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