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Ecology of the viperine snake (*Natrix maura*) as invasive snake in Mallorca: A first approach

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1 INTRODUCTION

The introduction of invasive species can be a difficult issue to work with, it is known that herpetological fauna shows a great ecological plasticity and invasive potential. One of the most recent and troublesome introduction was *Python bivittatus* in Florida (EEUU) declining local mammal populations (Dorcas *et al.*, 2012). Moreover, island introductions can be fateful such as *Boiga irregularis* was in Guam (Savidge, 1987; Fritts, 1988), where this species was responsible for eliminating ten bird species and abating lizards populations. Other reptiles as the lizard species *Anolis carolinensis* was introduced in Bonin Islands (Japan) causing severe impact in entomological fauna, specially in pollinator species (Hasegawa *et al.*, 1988).

Knowing the special vulnerability of insular ecosystems, in Balearic islands (Spain) endangered species could be leaded to extinction through pressure of invasive predator species as the anthropic historical introduced viperine snake (*Natrix maura*) (Guicking *et al.*, 2006), (See Figure 1).



Figure 1: Specimen of *N. maura* captured in Tramuntana mountain range, Mallorca. Picture of E. Pantoja.

N. maura has a remarkable historical relevance as a predator in the Balearic Islands being responsible for the extinction of *A. muletensis* and improbably of *Discoglossus sp.* species in Menorca. Fossil record shows that *Discoglossus sp.* disappeared before *N. maura* arrival (Bover *et al.*, 2008).

Although this species has a remarkable significance as invasive snake species, nowadays there are four more invasive species in Mallorca: *Rhinechis scalaris*, *Malpolon monspessulanus*, *Hemorrhois hippocrepis* and *Macroprotodon mauritanicus* (Álvarez *et al.*, 2010). While *M. mauritanicus* and *N. maura* were historically introduced, the others were recently introduced in Mallorca (Pinya & Carretero, 2011).

Habits of *N. maura* are mainly aquatic, establishing their populations around reservoirs (See Figure 2), inland waters and karstic canyons (See Figure 3) in Mallorca. Consequently this species feeds on fish, adults amphibians and tadpoles as well as their clutches (Meijide & Salas, 1989; Valverde, 1967; Martínez-Solano, 2000).



Figure 2: An example of *N. maura* reservoir habitat in Tramuntana sampling location, anthropic construction. Picture of E. Pantoja.

In Mallorca, *N. maura* was introduced in Roman times from south east France (Guicking *et al.*, 2006). Settling down as the main predator of the mallorcan midwife toad (*Alytes muletensis*), the viperine snake feeds on adults but mostly on tadpoles (Alcover *et al.*, 1984; Roman & Mayol, 1997). It is assumed that *N. maura* occurrence could affect negatively on *A. muletensis* tadpole populations (Schley & Griffiths, 1998). Predation of tadpoles and adults in canyons of Mallorca was previously observed (Moore *et al.*, 2004).

Presence of *N. maura* could have caused population extinctions of *A. muletensis* in Mallorca. Actually only remaining isolated populations of midwife toad in Tramuntana mountain range where *N. maura* has a relative or a total difficulty to access in some *A. muletensis* populations (Guicking *et al.*, 2006). Due to the conservation status as critically endangered (CR) in Spain and vulnerable (VU) of *A. muletensis* species (Viada *et al.*, 2006), a national level- modification of the Catalogue of Endangered Species (BOE, 2003) allows to remove *N. maura* individuals in *A. muletensis* presence localities.

Predators of *N. maura* are mainly wetland birds and birds of prey (Valverde, 1967; Amat & Herrera, 1977; Blanco *et al.*, 1987). Due to the mountain isolation of the main populations of *N. maura*, bird predation could be a difficult issue in karstic canyons (See Figure 3) and other remote locations. Absence of natural predators was related previously with high density population in this species (Hailey & Davies, 1986). Being an insular ecosystem, another habitats that must be relevant are the humid areas near the coast as the Albufera of Mallorca, where this species presence has been observed (Santos *et al.*, 2002). Populations of *N. maura* were cited near brackish waters in other locations in Spain (Cabot & Olea, 1978).



Figure 3: Karstic canyon habitat of *N. maura* in Mallorca. Typical noticeable isolation and difficult terrain with pools where this species can be observed. Pictures of E. Pantoja.

In spite of this complicated mountain habitat and management procedures, *N. maura* has never disappeared from all the *A. muletensis* localities. Therefore, is essential to provide a first approach to *N. maura* predator impact in midwife toad populations.

In order to sort out the lack of information about its ecology in Mallorca, as a first step, is necessary to characterize the population of *N. maura* in Tramuntana mountain range in order to assess the effectivity of the realized management. Is also essential to characterize the geographical invasive potential related with the current and potential distribution in Mallorca studying habitat suitability of *N. maura* from ecological variables.

2 METHODS

2.1 Historical presence impact

STUDY AREA AND DATA COLLECTION During 1991 to 2015, *N. maura* individuals were removed from different localities of Tramuntana mountain range canyons by the Balearic Islands Government (Criado & Mejías, 1991). Collected data of *A. muletensis* tadpole population of each locality with *N. maura* presence was provided by the majorcan midwife toad recovering plans of the Balearic Islands Government (Criado & Mejías, 1991; of Species Protection, 2007). The annual census of *A. muletensis* was realized visiting each known population of this species and counting visually observed tadpoles from the pool (Oliver *et al.*, 2014).

DATA ANALYSES Locality correlation analysis was performed in six localities which represent the 89,2% of the entire *N. maura* historical removing record. Occurrence of *N. maura* was considered merely representative ($N < 3$) in other localities. Correlation graphics for each locality as well as the historical evolution impact were executed using R project software (Gentleman *et al.*, 1997).

Historical evolution impact was determined between individuals that were yearly removed of *N. maura* and the number of *A. muletensis* tadpoles registered of each next year. Thus, direct correlation was provided because effects of the predator removal were evident in the next year counting of the prey population. Locality correlation was performed following the same procedure but using captured individuals of each locality.

Spearman’s rank correlation, a non parametric correlation rank, was applied to measure data correlation of each locality by the Spearman correlation test of the “pspearman” package of R project software (Gentleman *et al.*, 1997).

2.2 Distribution analysis

DATA COLLECTION Presence data of *N. maura* was extracted from BioAtlas 2.0 (CAIB, 2016). Reference of geographic data of BioAtlas comes from the Spatial Data Infrastructure of the Balearic Islands (IDEIB). Current distribution map in Mallorca of *N. maura* and *A. muletensis* was generated in UTM 5x5 cells of BioAtlas (CAIB, 2016). The maximum resolution coordinate form to work with was limited due to endangered species status of *A. muletensis*.

POTENTIAL DISTRIBUTION MODELING Potential distribution modelization of *N. maura* was performed by Maxent algorithm model (Phillips *et al.*, 2004, 2006). Maxent performs a maximum entropy modelization of species potential distribution determined by given ecological parameters. As ecological parameters, 19 bioclimatic variables from WordClim database (Hijmans *et al.*, 2004) were used to perform the maximum entropy modelization (See Table 1).

The whole bioclimatic variables were downloaded in 30” geographic degree resolution. Additionally, species presence coordinates were translated to latitude and longitude due to software requirements. Modelization maps with Maxent generate cumulative probabilities of presence from 0 to 1 on each pixel considering given ecological variables (Phillips *et al.*, 2004, 2006).

To estimate dependability of the potential distribution model, Area Under Curve (AUC) was established as standard. Comparison between predicted values of habitat suitability versus presence was performed producing a Receiver Operating Characteristic (ROC) plots (Fielding & Bell, 1997) and consequent AUC value. AUC value was a measure to analyze effectiveness of model performance.

Table 1: Climatic variables used for MAXENT modelization.

Var n°	Description
Var 1	Annual Mean Temperature
Var 2	Mean Diurnal Range [Mean of monthly (max temp - min temp)]
Var 3	Isothermality [(var2 / var7) * 100]
Var 4	Mean Diurnal Range [Mean of monthly (max temp - min temp)]
Var 5	Maximum Temperature of Warmest Month
Var 6	Minimum Temperature of Coldest Month
Var 7	Temperature Annual Range (var5 - var6)
Var 8	Mean Temperature of Wettest Quarter
Var 9	Mean Temperature of Driest Quarter
Var 10	Mean Temperature of Warmest Quarter
Var 11	Mean Temperature of Coldest Quarter
Var 12	Annual Precipitation
Var 13	Precipitation of Wettest Month
Var 14	Precipitation of Driest Month
Var 15	Precipitation Seasonality (standard deviation / mean)
Var 16	Precipitation of Wettest Quarter
Var 17	Precipitation of Driest Quarter
Var 18	Precipitation of Warmest Quarter
Var 19	Precipitation of Coldest Quarter

Models of potential distribution were accomplished by using MAXENT 3.3 (Phillips *et al.*, 2006). A total of 15 replica models were used to maximize data reliability.

2.3 Demographic characterization

STUDY AREA AND DATA COLLECTION Populations was analysed from dead and preserved *N. maura* individuals retired within the scope of the recovery plan of *A. muletensis*. Specimens were preserved in 96° ethanol. On the other hand, alive snakes were captured during the day by hand after searching them along the water reservoirs and surrounded areas of Tramuntana mountain range. Hooks and telescopic nets were needed to capture them (See Figure 4).



Figure 4: Field sampling of *N. maura* in Tramuntana reservoir location, sometimes hooks and telescopic tools can be useful to viperine snake sampling in harsh habitats. Picture of X. Truyols.

All *N. maura* body length was measured against steel tape rule and expressed as snoutvent length (SVL, cm) (Hailey & Davies, 1987c). Individuals with amputated tail were also registered.

SEX DETERMINATION *N. maura* individuals sex was determined observing genitalia by popping technique (McDiarmid *et al.*, 2012) and dissection for removed individuals.

SKELETOCHRONOLOGY Age was determined through skeletochronology techniques by counting growth rings of ectopterygoid bone located in the skull (See Figure 5) of preserved individuals. Right ectopterygoid bone was removed from 17 *N. maura* specimens boiling the snake heads in water for 2-4 hours (?). Muscle and adjacent tissue were removed manually from the bone and air dried. Finally, each bone was cleared in glycerol from 2 to 12 hours depending on the size. Cleaned and dried right ectopterygoid bones were observed and photographed with polarized light microscope (Leica DMLP Polarizing Microscope) at 4x amplification (See Figure 6). Growth rings were counted following (Peabody, 1961) methodology.

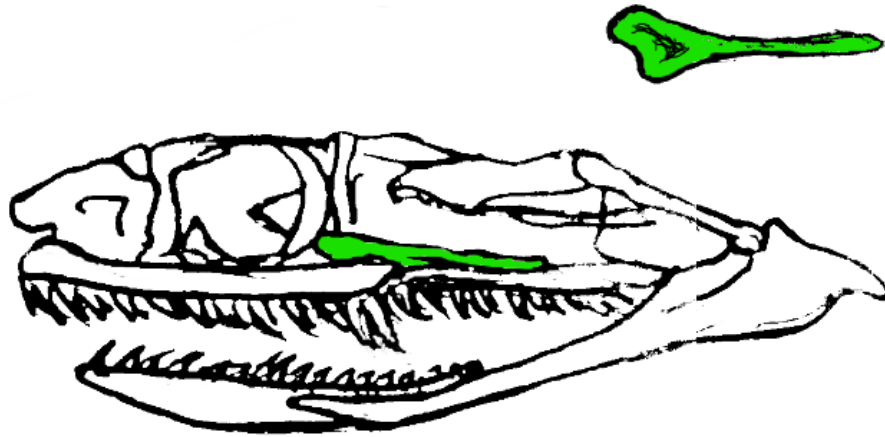


Figure 5: Ectopterygoid bone (green) located in *N. maura* skull, being part of the snake's jaw. Illustration of E. Pantoja.

Age was determined only in dead individuals extrapolating number of growth rings directly to age in years (Hailey & Davies, 1987c). Reproductive individuals were registered from collected individuals by locality following established SVL length criteria being above 22 cm in males and 35 cm in females (Feriche & Pleguezuelos, 1999), and following also age criteria, reaching sexual maturity at 2-3 years in males and females 4-5 years (Duguay & Saint Girons, 1966; Feriche & Pleguezuelos, 1999; Hailey & Davies, 1987a,b,c; Santos & Llorente, 2001). Data set as well as plots and calculations were performed using R project software.



Figure 6: Example of ectopterygoid growth rings of an eight years old *N. maura* (4x amplification). Each growth ring marked with arrows corresponds to a year of the snake's life. Picture of E. Pantoja.

3 RESULTS

3.1 Historical presence impact

Comparisons between historical records of yearly presence of *N. maura* and *A. muletensis* tadpoles (See Figure 7) suggested three clearly differentiated phases:

A first phase, where density of *N. maura* reached its highest in 1992, exceeding 15 individuals and consequently the lowest record of *A. muletensis* tadpoles in 1994 with less than 10000 individuals. A big amount of snakes were removed in this phase.

A second phase, where density of *N. maura* fluctuated never exceeding 10 individuals a year since 1995. In this phase *A. muletensis* tadpole population presented a progressive growth until year 2002.

Finally a third phase that started in 2003, where *A. muletensis* tadpole populations began to grow drastically reaching maximum record in 2009 that matched with one of the lowest record of *N. maura*. In this phase snakes slightly surpassed 5 individuals, while tadpoles never fell from 20000 individuals.

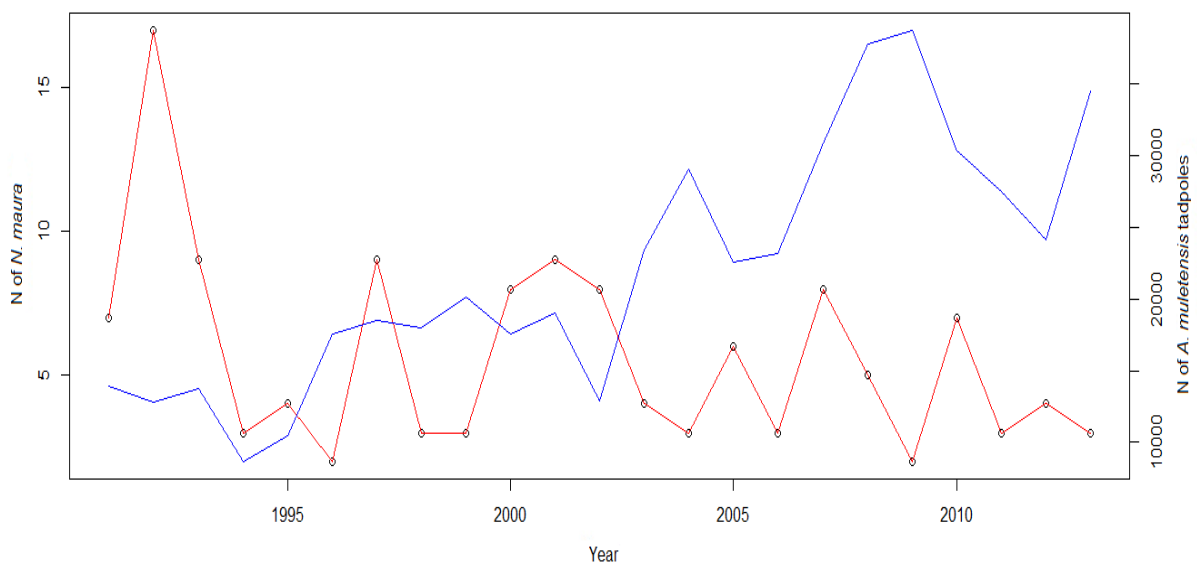


Figure 7: Historical presence evolution of *N. maura* (Red line) and *A. muletensis* tadpoles (Blue line) in Mallorca.

In regard to the comparison between the 6 localities (See Figure 8 and Table 2), a trend of negative correlation between *N. maura* and *A. muletensis* tadpoles was observed in all locations sampled except for locality 4, which showed a positive correlation ($Rho = 0.1500$). Only 2 of 6 localities were statistically significant, locality 1 and 3 (p -value < 0.05).

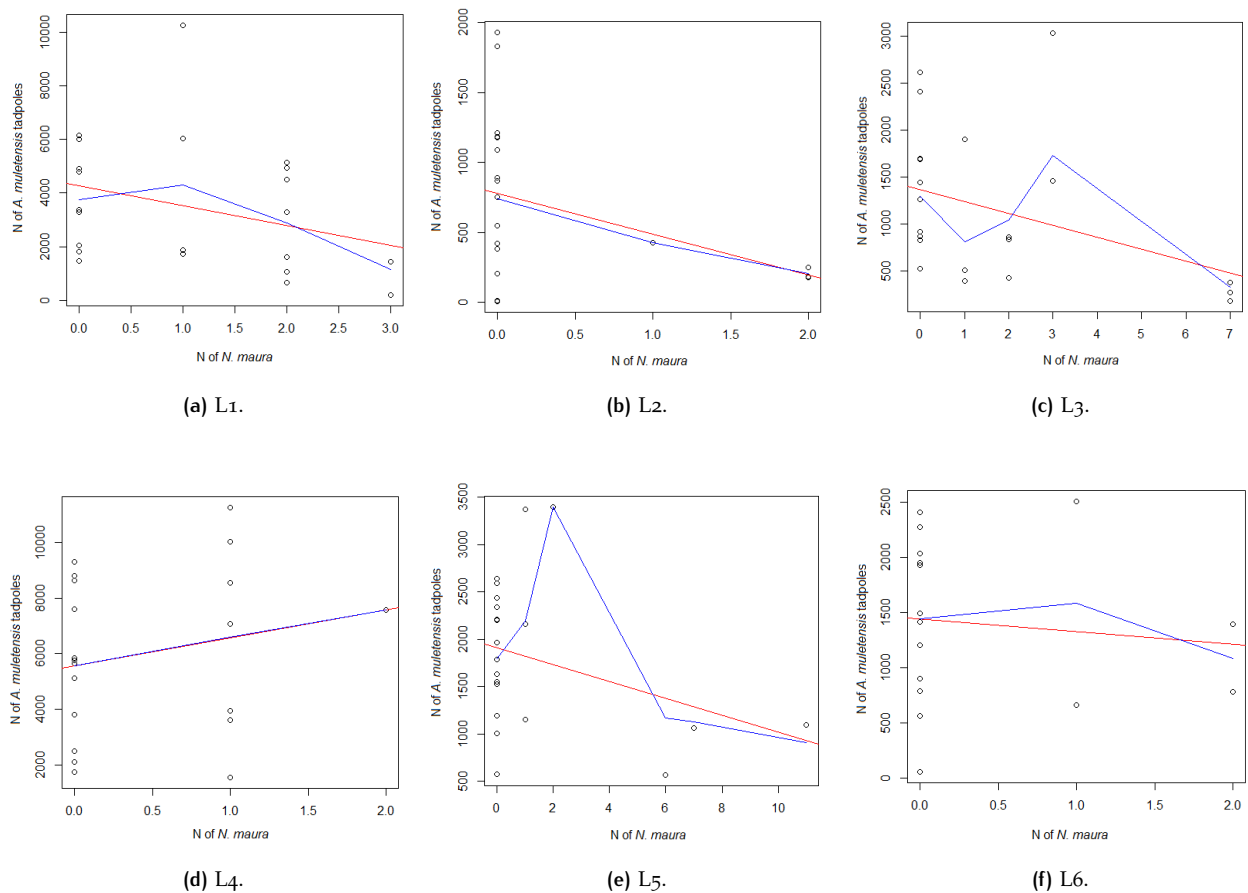


Figure 8: Correlation graphics between number of historical captures of *N. maura* and *A. muletensis* tadpoles for six different locations. Data regression line was represented in red and lowess line in blue.

Table 2: Spearman correlation test

Locality	Spearman rho	P-value
L1	-0.4515	0.0305
L2	-0.3605	0.1185
L3	-0.4649	0.0292
L4	0.1500	0.5278
L5	-0.1429	0.5257
L6	-0.1200	0.6578

3.2 Distribution analysis

N. maura was cited a total of 56 times, 41 cells of 5x5 UTM according to BioAtlas database. Approximately 40% of the citations pertains to the Tramuntana mountain range. On the one hand, 15% of the citations were from the area of Albufera of Mallorca. The remaining 55% citations were from south west, north east and south east coast of Mallorca. Only two citations belong to inlands. (See Figure 9, left)

A. muletensis was cited in 14 cells of 5x5 UTM (See figure 9, right), 12 of which correspond to the *N. maura* area. This means that 85.7% of *A. muletensis* presence area agree with *N. maura* presence.

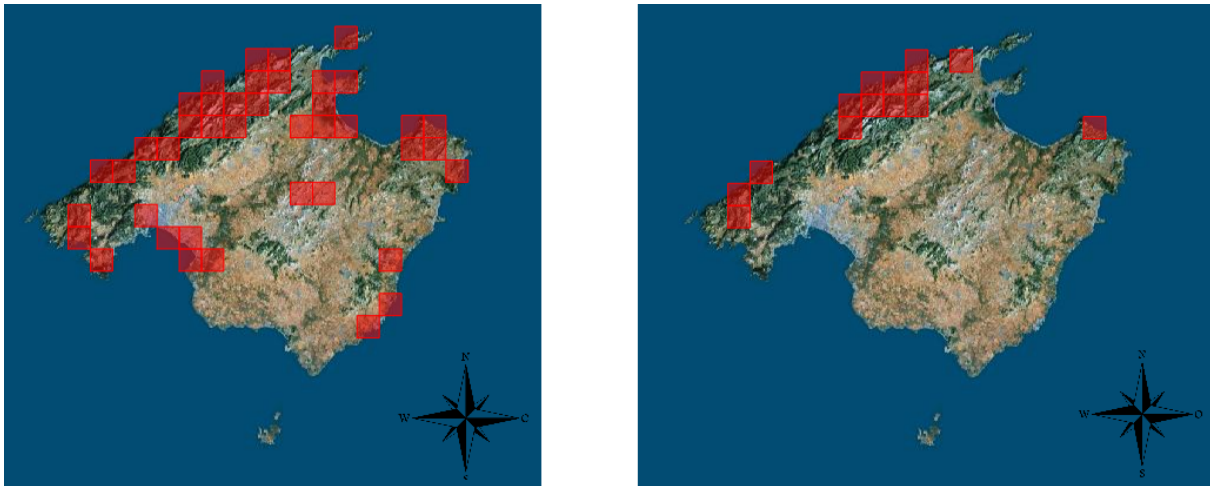


Figure 9: Current distribution map of *N. maura* (Left) and *A. muletensis* (Right) in Mallorca from BioAtlas data. Red cells represent 5x5 UTM coordinates of species presence.

The potential distribution model of *N. maura* manifested a good altogether performance (AUC=0.831, See figure 11). High suitability areas of the potential distribution map (See Figure 10) corresponded principally to Tramuntana mountain range, especially concentrated in north zone. A significant core was also observed in Albufera zone. Wide center and south inland regions of Mallorca showed up almost entirely unsuitable for *N. maura*.

Some coastal areas like coast of Palma, and Artà showed a certain noticeable level of suitability (> 50%). A high suitability (>90%) isolated locations were observed in south east of Mallorca, between Felanitx and Santanyi.

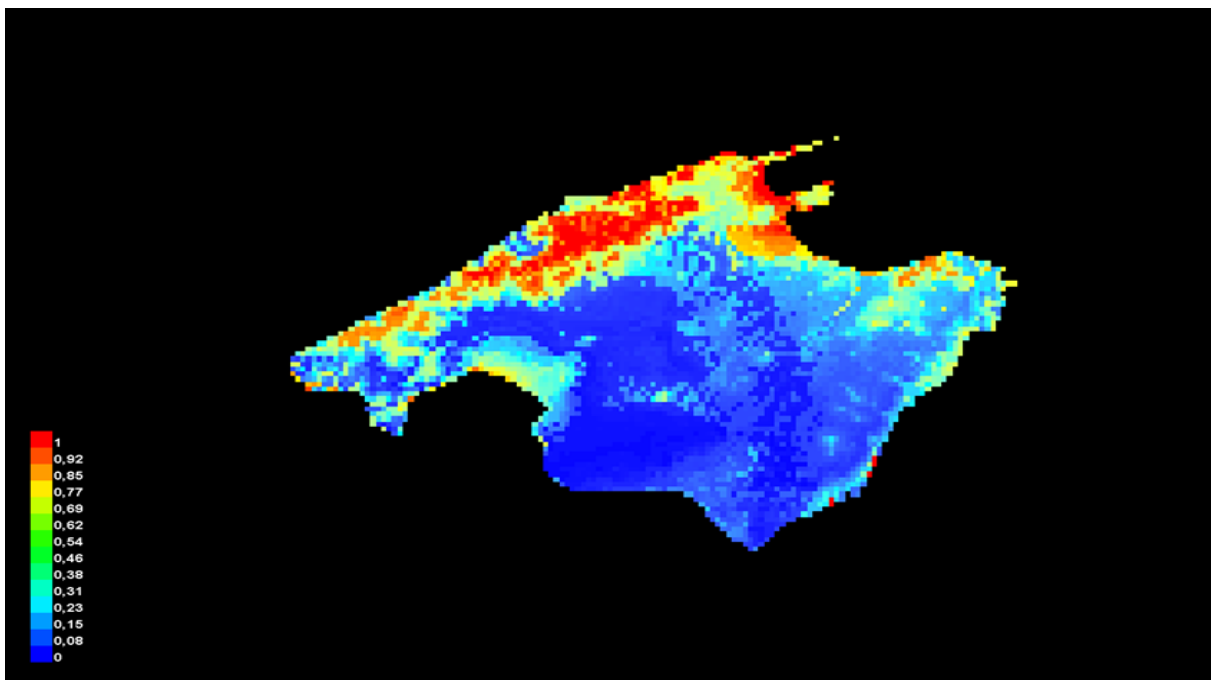


Figure 10: Potential distribution map of *N. maura* in Mallorca based on bioclimatic variable modelization with *Maxent*. Presence suitability was represented from red to blue gradient, being red the most likely (1) and blue the most unlikely (0) presence areas.

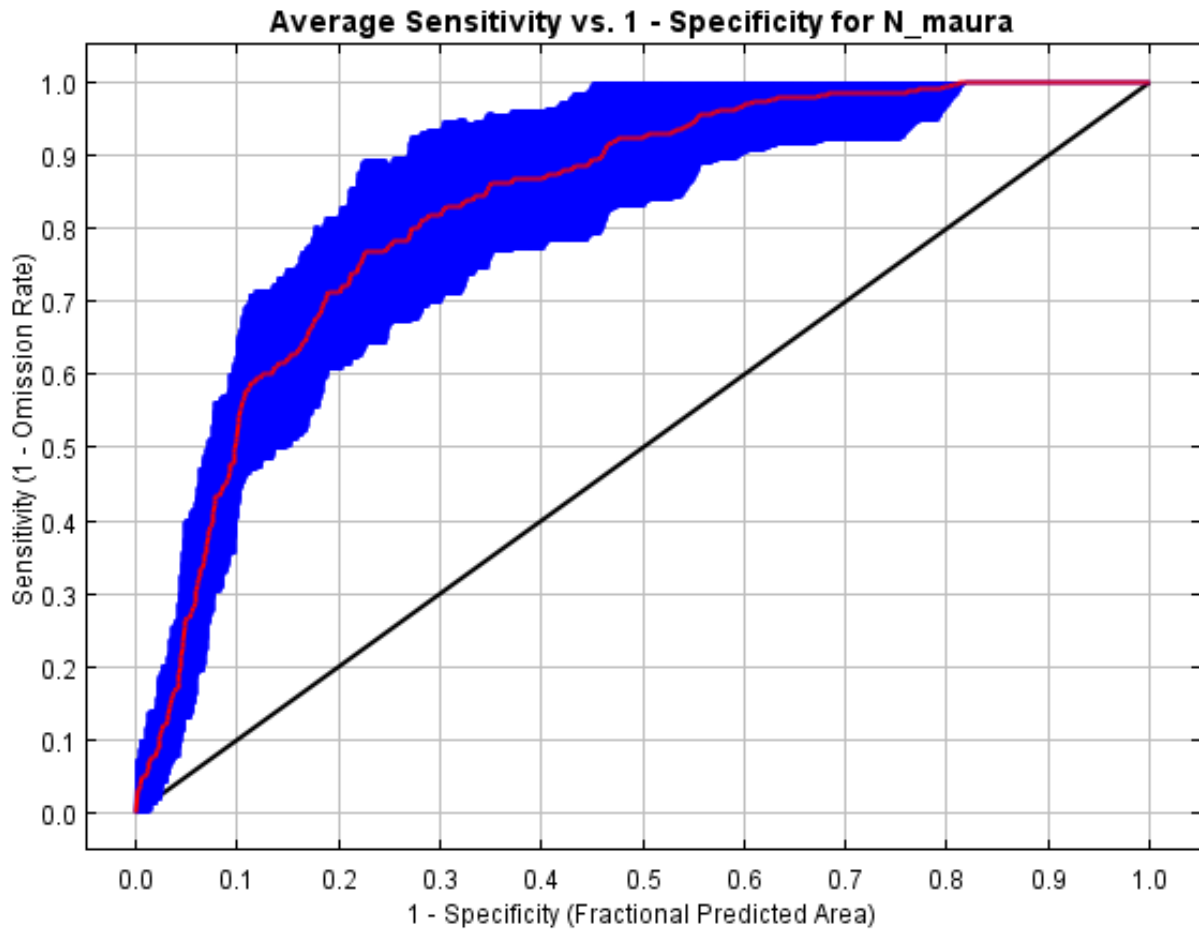


Figure 11: Receiver Operating Characteristic (ROC) plot of *N. maura* potential distribution modelization with MAXENT. Area Under ROC Curve (AUC) values represented in red line. Mean standard deviation area in blue.

In relation to the variable importance used in this model, the environmental variable with highest gain when used in isolation was Var 18 (Precipitation of Warmest Quarter), which therefore appears to have the most useful information by itself. The environmental variable that decreases the gain the most when it was omitted was Var 15 (Precipitation Seasonality), which therefore appears to have the most information that wasn't present in the other variables.

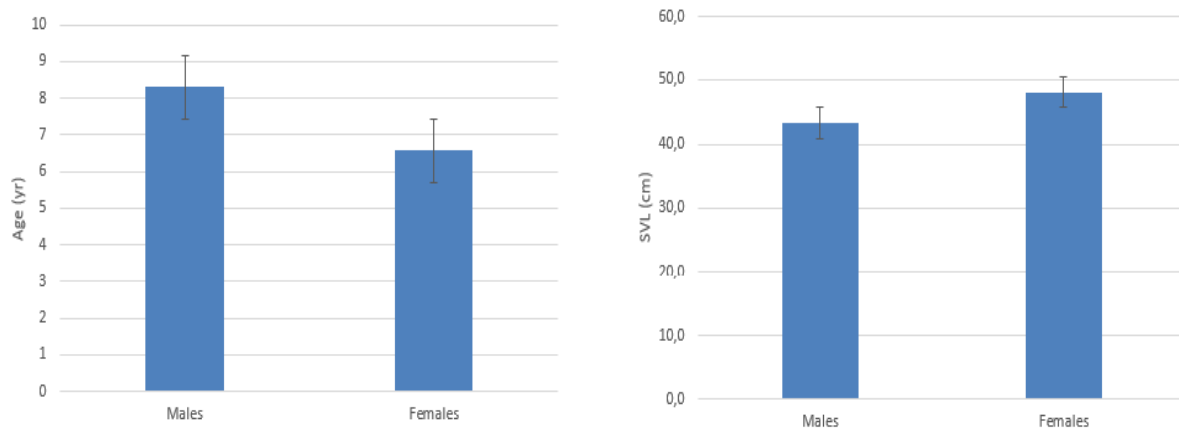
3.3 Demographic characterization

Ages of 17 sampled snakes (10 males and 7 females) were estimated using skeletochronology method. Females had a mean longevity of 6.57 ± 1.25 years (data range was 1 to 11, $n = 7$), which tendency was lower than the longevity of males (8.30 ± 1.12 years, data range was 2 to 12 years, $n = 10$) (See Figure 12, A). Maximum longevity of males was 12 years, while females was 11 years.

A total of 22 snakes were sampled (10 males and 12 females) to measure snout-vent length (SVL) body length. Body length tendency was slightly larger in females than in males (See Figure 12, B). Males have a mean SVL of 43.25 ± 4.32 cm (data range was 19 to 63 cm, $n = 10$), while females have a mean SVL of 53.7 ± 4.99 (data range was 20.5 to 74 cm, $n = 12$). Maximum size in males was 63 cm whilst in females was 74 cm. (See Table 3). In addition, 18% of this 22 snakes showed tail partially amputated.

Table 3: Data set of population Body length (SVL,cm) and Age (Years)

	Body length (SVL,cm)		Age(Year)	
	Males	Females	Males	Females
N	10	12	10	7
Min	19.0	20.5	2	1
Max	63.0	74.0	12	11
Mean	43.25	53.7	8.30	6.57
SE	4.32	4.99	1.12	1.25

**Figure 12:** Mean longevity of *N. maura* in years (A) and mean body size (SVL, cm) (B) comparisons between sexes.

A total of 19 reproductive individuals of *N. maura* (9 males and 10 females) were sampled. L7 locality showed the highest amount of reproductive males (N = 4), while L5 and L8 showed no reproductive males, (See Table 4). Reproductive females were higher in L8 and L1, with 4 and 3 females respectively. No reproductive individuals were found in L5.

Table 4: Reproductive individuals by locality

Locality	N reproductive	
	Males	Females
L1	2	3
L4	2	1
L5	0	0
L6	1	1
L7	4	1
L8	0	4
Total	9	10

4 DISCUSSION

4.1 Impact, habitat selection and distribution

On the one hand, the three phases of historical evolution confirmed the annual general scarcity of *A. mueltensis* tadpoles population and the inflection point where *A. mueltensis* is gradually recovering due to *N. maura* management. Despite all of these management years, the number of annually retired *N. maura* continues to fluctuate even without exceeding remarkable levels. However, the management plan only includes the removal of *N. maura* individuals in areas with *A. mueltensis* presence. Possibly the most important breeding areas could be close water reservoirs and other related anthropic water constructions. There, *N. maura* could generally benefit on high density of prey and produce small stable populations quickly (Rugiero *et al.*, 2000). A good example of this was the anthropic sampled locality (Figure 2), a big reservoir where a high density of *Pelophylax perezi* and consequently a remarkable density of *N. maura* was observed (Pers. obs.). Moreover, this locality was situated near an important sampled canyon locality (L3).

Canyons of mediterranean areas typically show temporary streams. The presence of canyon pools could be an essential factor of *N. maura* presence, since its presence was linked to the aquatic environment and also prey availability. In times of activity, *N. maura* could be forced to expand its vital domain for food seeking and also for breeding reasons, specially in males (Santos & Llorente, 1997). This vital domain expansion could be an explanation for its presence in many *A. mueltensis* locations since karstic canyon pools could act as a trap due to its physical isolated situation. Active *N. maura* individuals that are seeking water environments for food or breeding could be the best subjects to fall into the "trap".

Since *N. maura* was established as the main predator of *A. mueltensis* could be expected the general rarefaction of *A. mueltensis* tadpoles which was assumed previously (Schley & Griffiths, 1998). A negative correlation response that could be expected was observed in most sampled localities. It means that the higher *N. maura* presence, the lower presence of *A. mueltensis* tadpoles. Positive correlation was observed only in locality 4 (L4). It was a big torrent with a very large number of breeding points so the effect of *N. maura* on *A. mueltensis* in a breeding pool could be possibly mitigated by the effect of other breeding pools that were not affected because *N. maura* was not present.

As for the current distribution in Tramuntana mountain range, despite being an unsuitable habitat, is not uncommon to find *N. maura* (Pleguezuelos & Feriche, 2003; Bons *et al.*, 1996). The altitude wasn't a big problem because the presence of *N. maura* is common between 0 and 1200 m but are especially abundant records in the altitude of 500- 600 m (Pleguezuelos & Villafranca, 1997). The highest point of Mallorca in Tramuntana mountain range is Puig Major mountain with 1445 m of altitude, having reached other populations of this species in Sierra Nevada (Spain) 2.050 m of altitude (Pleguezuelos & Feriche, 2003).

Regarding the modeling variables, the variable that contributed more to our model was the precipitation of warmest quarter (Var 18) and the seasonal precipitation (Var 15). It means that dependence on these variables was higher. These variables values were understood into the importance of a possible degree of drought in summer, and the variation of the possible number of pools that year (usually temporary) being our most determining environmental factor because it has been observed that snakes were found in water mainly during the warmest months (Hailey & Davies, 1987c; Jaén-Peña & Pérez-Mellado, 1989). It was directly related to prey availability and consequently also with the variation of distribution of *N. maura* in their active period location where this species could seek for water areas actively due also to its vital domain expansion, this strengthen our previous hypothesis of canyon trapping.

Maximum entropy modelization was successfully used previously in invasive reptiles and amphibians such as *Boiga irregularis* (Rödder & Lötters, 2010) or *Lithobates catesbeianus* (Ficetola *et al.*, 2007) as well as Iberian herps (Sillero *et al.*, 2012; Muthoni, 2010). Our model also showed a good performance result (AUC=0.831), moreover high suitability presence regions usually coincide with *N. maura* actual presence although few high suitability points scattered in south east coast of Mallorca that could be anthropic explained. It should be pointed out that the maximum entropy model with Maxent software only takes environmental variables account and that was not the only factor that could determine the presence of species, future studies could take into account other variables such as habitat fragmentation, availability of prey, water bodies and anthropic factors to improve modelization.

4.2 Demography

The results of SVL body length agrees with the previous values known for the species, being maximum length quite similar to the values of other populations of this species in the Iberian Peninsula (Jaén, 1988). Although this values weren't statistically significant ($p\text{-value} > 0.05$) tendency of the average length between males and females of *N. maura* suggested to be smaller in males than females, being a general expected difference in this species.

Results of the sex ratio in these *N. maura* were slightly biased to females, this feature was previously observed in other *N. maura* populations (Duguy & Saint Girons, 1966; Escarré & Vericad, 1981).

The usefulness of skeletochronology methods in age determination of snakes was tested successfully on several occasions such as *Boiga irregularis* (Collins, 1992), *Tamnophis sp.* (Waye & Gregory, 1998), as well as *N. maura* (Hailey & Davies, 1987c; Filippakopoulou *et al.*, 2014). Thus, number of growth rings showed that our population was relatively long-lived with maximum ages of 11-12 years, most of individuals focused in the age range of sexual maturity being reproductive the vast majority of locality sampled *N. maura* (87%). Despite of the large number of *N. maura* reproductive individuals, it was previously observed that generally most cases of reproduction weren't carried out, possibly due to the lack of optimal habitats according to strong summer drought of typical Mediterranean habitats (Hailey & Davies, 1987b; Santos & Llorente, 2001). Summer drought was also proved to be one of the most important variable affecting our model distribution of *N. maura*. Number of growth rings in the ectopterygoid bone previously indicated a maximum ages of 20 years for females and 13 years for males (Hailey & Davies, 1987a) and consequently a higher longevity in females than males but our results suggested the opposite, although it was not significant, may be due to a reduced sample size.

4.3 Predation risk

The frequency of amputated tails in *N. maura* is generally higher than other Iberian snakes because activity patterns of *N. maura* expose them to a wide array of predators (Pleguezuelos *et al.*, 2007, 2010). The results suggest in this case that the frequency of snakes with tail partially or completely amputated (18%) can be considered quite high having a direct correlation with the species predation risk, being this feature a good indicator (Santos *et al.*, 2011) proposing a remarkable amount of potential predators of *N. maura* on this insular ecosystem despite of being an invasive species.

Potential predators in Mallorca could be small Mustelidae as *Martes martes* and *Mustela nivalis* being part of their diet specimens of the same genera (Amores, 1980; Ruiz-Olmo, 1987). A fairly wide range of wetland birds including Ardeidae (Amat & Herrera, 1977) and several bird of prey such as *Milvus milvus* (Blanco *et al.*, 1987) could be considered potential predators of *N. maura*.

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