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MILITARY ACTIVITES AND BIODIVERSITY CONSERVATION: A CASE STUDY IN AMPHIBIANS

Travail de Maîtrise universitaire ès Sciences en comportement, évolution et conservation
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par

Jenny PANZIERA

Directeur : Prof. Alexandre Roulin
Superviseur : Dr. Amélie Dreiss
Expert : Anonymous
Department of Ecology and Evolution

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Abstract

Military activities and biodiversity conservation are seen as antagonists. However, armies are actively participating in environmental preservation and military bases are home to a rich biodiversity, including threatened species such as pioneer amphibians. The reproduction of the European tree frog *Hyla arborea*, the Natterjack toad *Epidalea calamita* and the Yellow-bellied toad *Bombina variegata* was monitored by counting the number of adults and tadpoles in twenty-five ponds in the training field of the military base of Bière, in order to test the impact of the passage of military vehicles on reproductive success, as well as the effect of pond characteristics on the presence of adults and tadpoles. The study reveals a negative correlation between the number of passages and the presence of tadpoles of *H. arborea*. Tadpole and adult presence of this species is linked to the number of drought events while the vegetation plays a role in the presence of tadpole and adults of *B. variegata.* Avoiding to cross the ponds during the presence of tadpoles should help reducing the mortality induced by military activities. For conservation purposes, cooperation with armies is necessary to preserve biodiversity on their bases and can thus enhance global environmental protection.

Résumé

Activités militaires et conservation de la biodiversité sont généralement perçues comme antagonistes. Pourtant, les armées participent activement à la protection de l'environnement et les bases militaires abritent une biodiversité particulièrement riche, y compris des espèces menacées comme les amphibiens pionniers. La reproduction de la rainette verte *Hyla arborea*, du crapaud calamite *Epidalea calamita* et du sonneur à ventre jaune *Bombina variegata* a été suivie en comptant le nombre d'adultes et de têtards dans vingt-cinq gouilles du terrain d'entrainement de la place d'armes de Bière afin de tester l'impact du passages des véhicules militaires sur le succès de reproduction ainsi que les effets des caractéristiques des gouilles sur la présence d'adultes et de têtards. L'étude a révélé une corrélation négative entre le nombre de passages et la présence de têtards d'*H. arborea*. La présence de têtards et adultes de cette espèce est liée au nombre d'évènements de sècheresse alors que la végétation joue un rôle dans la présence de têtards et d'adultes de *B. variegata*. Eviter de traverser les gouilles lorsque les têtards sont présents pourrait aider à réduire la mortalité induite par les activités militaires. À des fins de conservation, coopérer avec l'armée est nécessaire afin de préserver la biodiversité sur les bases militaires et peut ainsi améliorer globalement la protection de l'environnement.

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Introduction

The negative effects of army activities on the environment are well-known, such as land degradation, soil contamination, massive carbon dioxide production, whereas efforts to minimize their impact and to participate to nature conservation is rarely acknowledged (Jorgenson *et al.*, 2010; Madden and McQuinn, 2014). Yet, the armies sometimes take an active part in preserving the natural resources. In several countries, the army is involved in environmental projects. For example, the U.S. Army Corps of Engineers cooperates with The Nature Conservancy to restore and protect river habitats (Hickey and Warner, 2005). The Israel Defence Forces cooperates with the Society for the Protection of Nature and Nature and Parks Authority since 2014 in about 50 projects, from restoring habitats to preserving rare species through minimizing their impact on biodiversity (NDF, 2016). Switzerland is no exception. According to the Federal Constitution, the Swiss Army has to preserve the environment account while working on the defence of the country and participates in several projects on the protection of the nature (Rey-Mermet, 2014; Aebi, 2015; Vtg.admin.ch, 2018.a and 2018.b). Many Swiss military fields are connected to natural reserves and some can even act as refugees for several rare species (Zangger and Hosbach, 2000). The Nature, Landscape and Army program is referencing natural values and determine measures to conciliate military activities and biodiversity preservation (Zangger and Hosbach, 2000, Vbs.admin.ch, 2018). However, little scientific studies are made on military fields in Switzerland.

As in other countries, the biodiversity in Switzerland is decreasing dramatically, especially because of the disappearance of wet habitats (Secretariat de la Convention sur la diversité biologique, 2014; OFEV 2014 and 2017). Among the victims, the amphibians are particularly threatened because of their dependence on water bodies (Schmidt and Zumbach, 2005; Pellet, 2013). Among the twenty species present in Switzerland, fourteen are on the Red List, including nine that are “endangered” (Schmidt and Zumbach 2005). Pioneer species are the most threatened because their primary habitats disappear at a high rate and are thus relying only on secondary habitats, created by human activities, such as in quarries and military grounds. These habitats are characterized by temporary ponds, which are drying during autumn and winter and have water in spring and summer. This particularity prevents predators such as fish to establish, which is particularly interesting for organisms that can stand with this harsh condition (Ryser *et al.*, 2002; Meyer *et al.*, 2009).

The military base of Bière, used by artillery and infantry, is also a biodiversity refugee for birds and other organisms (Gerber *et al.*, 1998, Bernardi *et al.*, 2002; Perrenoud, 2009). The training field contains also forests, bushes and permanent water bodies. The passage of the tanks in the ground creates temporary ponds, favourable for the reproduction of amphibians. This site hosts seven species of amphibians, among which six are on the red list. The site is of national importance for three pioneer species: the European tree frog *Hyla arborea*, the Natterjack toad *Epidalea calamita* and the Midwife toad *Alytes obstetricans* and shelter the fourth pioneer amphibian, the Yellow-bellied toad *Bombina variegata*, which is also endangered according to the Red List (Schmidt and Zumbach, 2005; Perrenoud, 2009).

Pioneer species, especially *E. calamita* and *B. variegata,* rely on the passage of vehicles on the field, especially heavy machines as tanks, to create new ponds and maintain dynamic conditions necessary for habitat heterogeneity (Pellet *et al.*, 2002; Ryser *et al.,* 2002; Warren *et al.,* 2007; Warren and Büttner, 2008). A ceasing of military activities would be dramatic in this environment, as it would lead to the eutrophication of the ponds and their disappearance. However, too many passages of military vehicles in ponds during the reproductive season may increase mortality, especially of tadpoles and clutches which can be crushed.

In the context of biodiversity conservation, it is primordial to identify threats and to take measures to preserve important environment and their inhabitants, like military fields. We focused on the reproductive season of tree amphibian species: the European tree frog *Hyla arborea*, the Natterjack toad *Epidalea calamita* and the Yellow-bellied toad *Bombina variegata* (figure 1). Our aim is to analyse the impact of military activities on survival by looking at the impact of the passage of the tanks on pioneer amphibian reproductive success. We tested also the effect of pond characteristics on the presence of adults and tadpoles. Based on literature, vegetation and depth of the ponds are suspected to be determinant for the presence of adults while drought and passage of vehicles can increase tadpole mortality. Adults and tadpoles were counted in twenty-five ponds on the military training field of Bière once a week between April and September 2017. To our knowledge, this study is the first carried out on military field to examine the impact of military vehicles on amphibian reproductive success. We hope that it will facilitate and encourage future cooperation between scientists and the army for conservation purpose. This study also provides recommendations for amphibian conservation measures in the military training field of Bière that are compatible with military activities.

Figure 1 From left to right: *Hyla arborea, Epidalea calamita* and *Bombina variegata*

Methods

Ponds' selection and follow up

The study was conducted from March to September 2017 in the training field of the military base of Bière (VD) (Appendix II).

A mapping of all the ponds of the field was done in March and surface area and depth were measured. A maximum of twenty-five ponds were selected (Appendix III.a and III.b) to follow the reproductive season of three pioneer amphibian species: the European tree frog, *Hyla arborea*, the Natterjack toad, *Epidalea calamita*, and the Yellow-bellied toad, *Bombina variegata*. The ponds were selected according to their size (measured with a meter stick), smallest and biggest ponds were excluded. For material limitations, we could not monitor more ponds. As sizes were varying during the season, they were measured again at the end of the field work. The depth of ponds ranges from 19 to 45 centimetres (Appendix I). Presence or absence of vegetation at the edges of the ponds was recorded for each pond. Eighteen of the twenty-five ponds had vegetation, generally at the edges as shown in the figure 2, and seven had no vegetation.

Figure 2 Example of a pond with vegetation at the edges

Ponds were monitored for amphibian reproduction once a week according to the military use of the field during 22 weeks from the 10th of April to the 8th of September. Only during two weeks (weeks 2 and 18, i.e. the weeks of the 17th of April and the 7th of August), no visit could be performed. The following data were collected during the monitoring visits, for each pond:

- from April to June (weeks 1-12), the adults were counted by night by direct seeing and hearing. No visit was performed during the week of the 17th of April.

- from May to September (weeks 6-22), clutches, tadpoles and juveniles were counted during the day. An amphibian net was used to find and catch tadpoles for identification. No visit was performed during the week of the 7th of August.

Passage of vehicles

Initially, camera traps were installed in front of each pond to record the number of passages of the tanks but unfortunately, the cameras have been stolen in the beginning of the season and the data collected was lost. An alternative method was to spread chalk spray around ponds each week from June to August to determine whether vehicles crossed the ponds during the week. An interrupted line meant that at least one vehicle has crossed the pond, as shown in figure 3. The number of passages corresponds to the number of weeks for which passage was observed. Unfortunately, this method did not give a precise count of passages nor the vehicle type. In addition, the rain sometimes erased the marks in between two visits. For these different reasons, the number of passages is underestimated compared to the real number of crossing. However, it still gives information on which ponds are more frequently crossed during the season.

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Figure 3 - Example of chalk spray line interrupted by the passage of vehicle

Data analysis

Choice of variables: Number of adults and presence of tadpoles

According to Pellet and Schmidt (2005), three visits per sites are enough to detect *H. arborea* and *B. variegata* while six are necessary for *E. calamita*. As each pond has been visited eleven times by night for the adults, the detectability is likely to be accurate enough. Furthermore, all the ponds where we found tadpoles were previously occupied by adults at the beginning of the season. This suggests a good detectability of the adults. However, not all the ponds colonized by adults contained tadpoles afterward. The presence of at least one tadpole is then used to test for the reproductive success. Number of adults and presence of tadpoles were analysed according to the number of passages, the presence/absence of vegetation, the number of droughts and the depth of the pond.

Study species

H. arborea, *E. calamita* and *B. variegata* share a similar ecology: the three of them are pioneer species, with a late reproduction in temporary or new ponds (Meyer *et al.*, 2009). They breed later in the season, compared to early amphibian species, such as the common toad *Bufo bufo*. Their breeding season occurs approximately from the end of April until August, depending on the meteorological conditions. However, they showed slightly different distributions across time and ponds and have different behaviours. *H. arborea* and *E. calamita* are leaving the ponds after the eggs are deposited while some adults of *B. variegata* are staying until the end of the reproductive season. *H arborea* lay eggs on vegetation while *E. calamita* seems to avoid it, laying the eggs on the bottom of mineralized ponds. *B. variegata* can lay eggs on vegetation or on the bottom of the ponds but the female split the clutch in different ponds in order to prevent all its eggs to die if the pond is drying out too early. Drying is indeed one of the most important risks for amphibians because eggs and tadpoles cannot survive out of water. However, amphibians have evolved different strategies to cope with this problem. *B. variegata* can survive several dry seasons without reproducing probably because it lives sufficiently long to skip one reproductive event, while tadpoles of *E. calamita* are able to dig small holes to retain water around their body during periods of drought. The temporary ponds are generally shallow and the few centimetres of water are warming very fast, allowing a fast development of eggs and tadpoles of the three species to prevent mortality due to drought. Sexual maturity is generally reached at two years of age in the three species (Müller and Schellenbaum, 1997; Meyer *et al.*, 2009).

Statistical analysis

R logiciel (version 3.4.2) was used to build simple generalized linear models (GLM) for all species together and separately (R core Team 2017). Quasipoisson regression is used for the number of adults and a logistic regression (binomial) for the presence of tadpoles. Probabilities are calculated with variance analysis (ANOVA) and a Chi2 test. The figures were made using packages “ggplot2” (version 2.2.1) and “reshape2” (version 1.4.3) (Wickham, 2007 and 2009). Correlations between the factors are tested with Wilcox_test of the package “coin” (version 1.2.2) (Hothorn *et al.,* 2009) and cor.test using Spearman method (method = spearman) of the standard R library. The ponds are probably not entirely independent because of the geospatiality but the method used is thought to be conservative enough to still do independent statistical tests.

Results

Reproductive period

The adults of the three amphibian species were present by night from the week of the 10th of April until the week of the 19th of June (Figure 4). None of the three species was recorded during the week of the 26th of June.

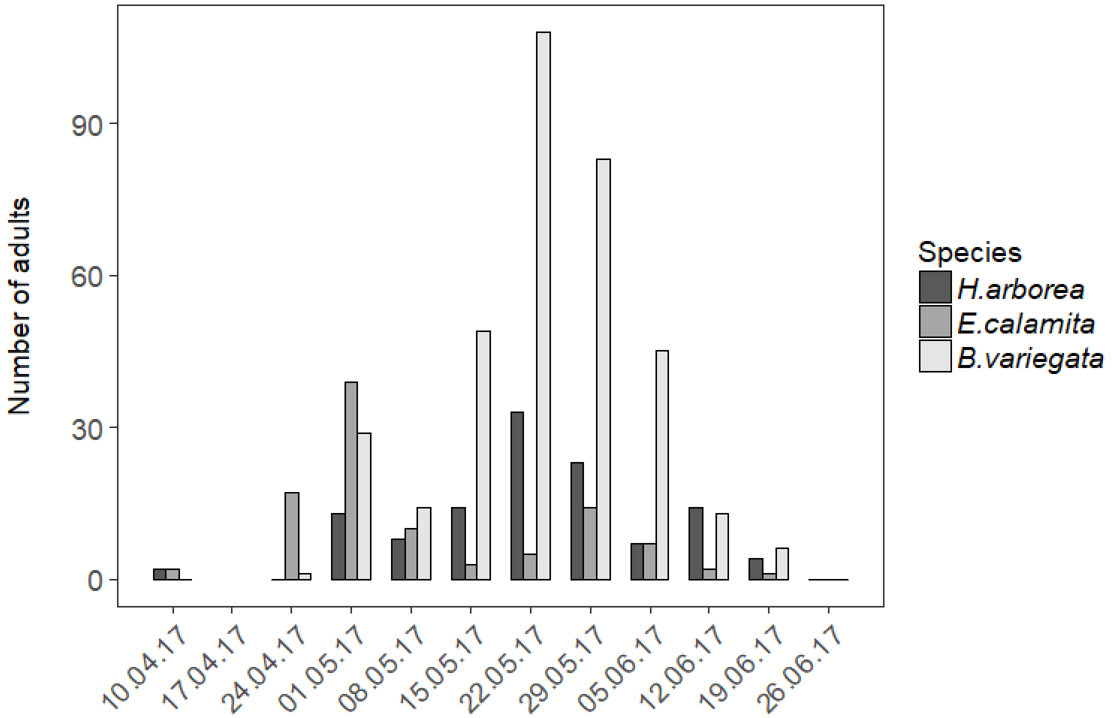

Figure 4 – Number of adults of each species in the 25 ponds sampled in the military base of Bière. The adults of the three species were counted by night by hearing and direct observation from the week of the 10th of April until the week of the 26th of June. The dates are representing weeks and not the precise date. No visit was performed during the week of the 17th of April.

As shown in Table 1, *E. calamita* was the less abundant species, representing only 17 % of the total number of adults counted, while *B. variegata* is the most abundant (63 %). *H. arborea* is also less present (20 %).

Table 1 - Total number of adults counted during the night of monitoring in the military base of Bière.

Species	*H. arborea*	*E. calamita*	*B. variegata*	Total
Number of adults	118	104	372	**594**
Proportion [%]	20	17	63	**100**

Pond occupancy and reproductive success

Of the 25 ponds, 24 were occupied by the adults and 18 by the tadpoles. Adults of *B. variegata* occupied most ponds (22) than *E. calamita* and *H. arborea* (17 and 20 ponds respectively). Tadpoles of *H. arborea* occupied less ponds (8) than *E. calamita* and *B. variegata* (13 and 17 ponds respectively) (table 2). The reproductive success was calculated in the following way:

$$\text{reproductive success} = \frac{\text{nbr ponds occupied by tadpoles}}{\text{nbr ponds occupied by adults}}$$

Globally, the reproductive success is 75 % but looking at the species level reveals that *H. arborea* has a reproductive success of only 40 % while *E. calamita* and *B. variegata* has respectively 76 and 77 % (table 2).

Table 2 – Number of ponds occupied by tadpoles and adults of each species during the reproductive season. The reproductive success is calculated by dividing the number of ponds occupied by tadpoles by the number of ponds occupied by adults.

Species	*H. arborea*	*E. calamita*	*B. variegata*	**all**
Adults	20	17	22	**24**
Tadpoles	8	13	17	**18**
Reproductive success	40%	76%	77%	**75 %**

Number of adults

Presence of vegetation and number of droughts were positively correlated with the total number of adults. The number of adults of *H. arborea* was positively correlated with the number of droughts while the number of adults of *B. variegata* was positively correlated with the presence of vegetation. No correlation was found for the number of adults of *E. calamita* (table 3).

Table 3 – Relationship between the number of adults of the three amphibian species and the number of passages of military vehicles, the presence of vegetation in the ponds, the number of droughts and the depth of the ponds (cm). Results of the generalized liner models (GLM). Chi-square test with quasipoisson distribution

	all				*H. arborea*			
	estimate	**standard error**	**t-value**	**p-value**	**estimate**	**standard error**	**t-value**	**p-value**
passages	-0.1785	0.0899	-1.9857	0.0591	-0.1850	0.1798	-1.0290	0.3142
vegetation	1.2787	0.4006	3.1918	**0.0041**	1.7741	0.9570	1.8537	0.0766
droughts	0.1111	0.0464	2.3912	**0.0254**	0.1943	0.0697	2.7873	**0.0105**
depth	-0.0196	0.0163	-1.2029	0.2413	-0.0220	0.0303	-0.7268	0.4747

	E. calamita				*B. variegata*			
	estimate	**standard error**	**t-value**	**p-value**	**estimate**	**standard error**	**t-value**	**p-value**
passages	-0.1946	0.1477	-1.3175	0.2006	-0.1719	0.1089	-1.5790	0.1280
vegetation	0.0513	0.5429	0.0945	0.9256	1.8262	0.6195	2.9478	**0.0072**
droughts	-0.0303	0.1098	-0.2757	0.7852	0.1109	0.0607	1.8285	0.0805
depth	-0.0255	0.0268	-0.9515	0.3512	-0.0173	0.0207	-0.8345	0.4126

No significant correlation was found between the number of adults and the number of passages for none of the species but there was a negative tendency (figure 5).

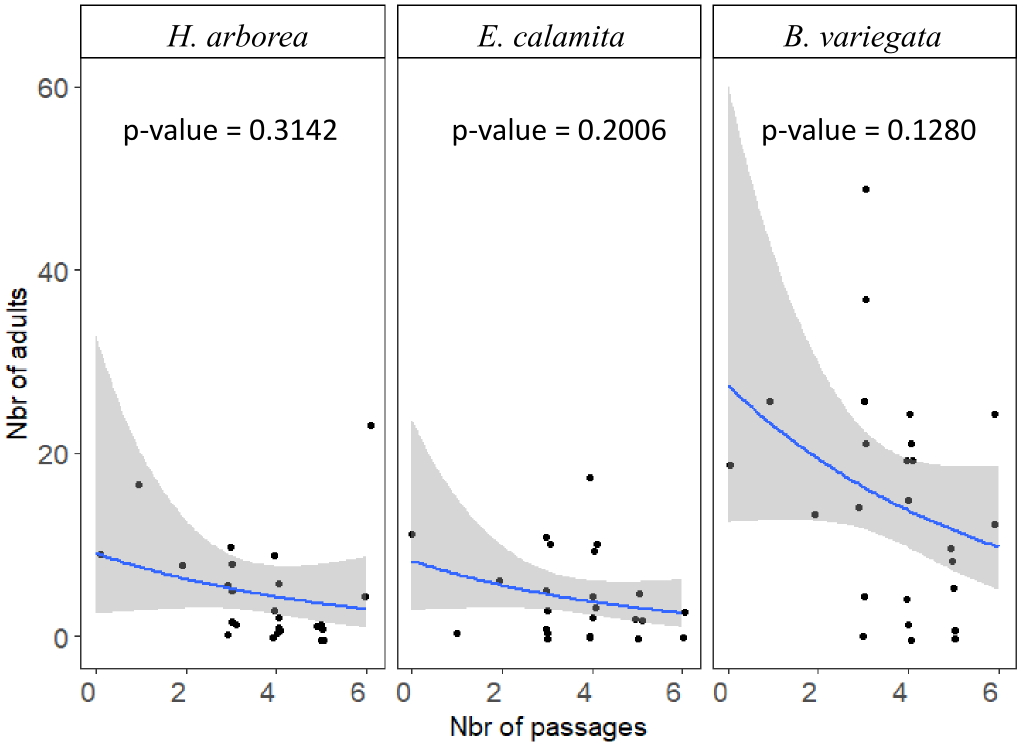

Figure 5 - Number of adults according to the number of passages of the three amphibian species. Chi-square test with a quasipoisson distribution (p-values in table 3)

More adults of *H. arborea* are present in vegetized ponds but it is not significant. The number of adults of *E. calamita* is not significantly different in ponds with and without vegetation. More adults of *B. variegata* are significantly present in vegetized ponds (figure 6).

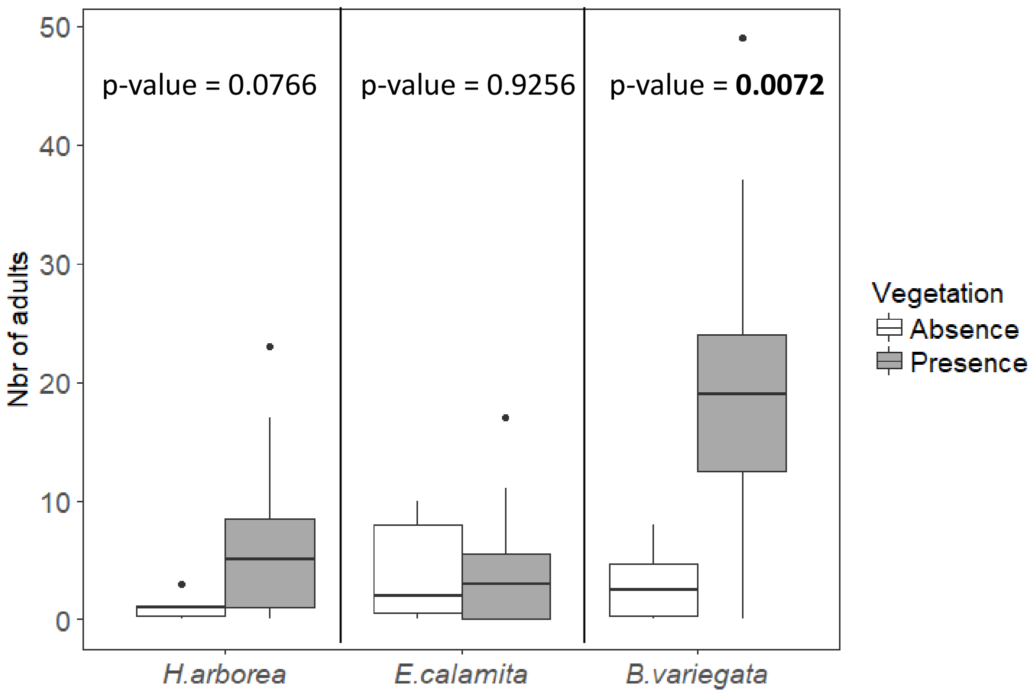

Figure 6 - Number of adults according to presence of vegetation. Chi-square test with a quasipoisson distribution (p-values in table 3)

More adults of *H. arborea* were present in ponds that faced more droughts events. No significant correlation was found for *E. calamita* and *B. variegata* (figure 7).

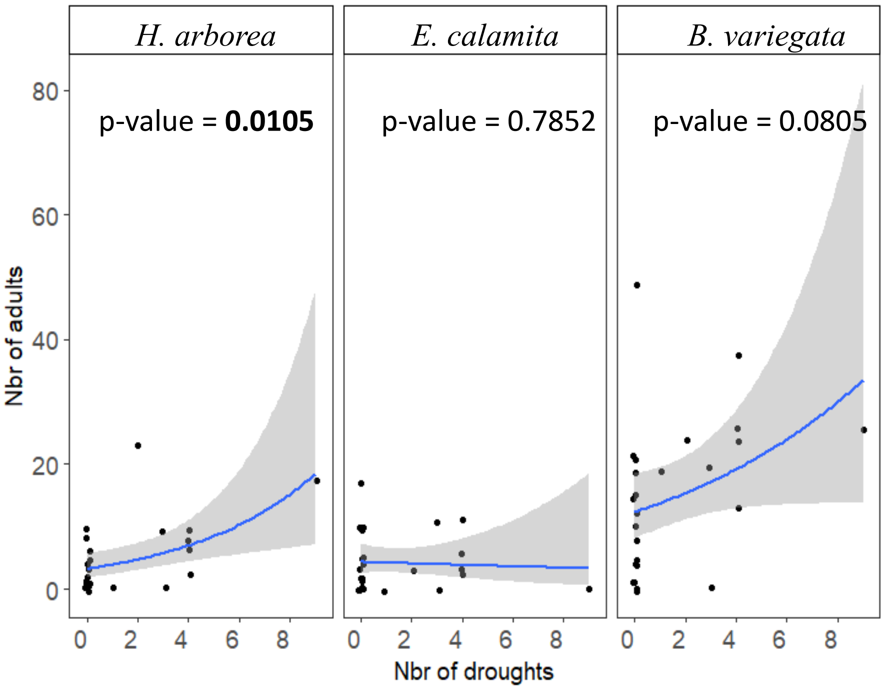

Figure 7 - Relationship between the number of adults according to the number of droughts. Chi-square test with a quasipoisson distribution (p-values in table 3)

Presence of tadpoles

Presence of vegetation is positively correlated with the presence of the tadpoles of the three species together. The presence of tadpoles of *H. arborea* is negatively correlated with the number of passages and positively correlated with the number of droughts. No significant correlation was found for the presence of tadpoles of *E. calamita*. The presence of tadpoles of *B. variegata* is positively correlated with the presence of vegetation (table 4).

Table 4 – Relationship between the presence of tadpoles of the three amphibian species and the number of passages of military vehicles, the presence of vegetation in the ponds, the number of droughts and the depth of the ponds (cm). Results of the generalized liner models (GLM). Chi-square test with binomial distribution

	all				*H. arborea*			
	estimate	**standard error**	**t-value**	**p-value**	**estimate**	**standard error**	**t-value**	**p-value**
passages	-0.6770	0.4415	-1.5335	0.1252	-2.5270	1.1369	-2.2227	**0.0262**
vegetation	2.3671	1.0704	2.2114	**0.0270**	18.2476	2662.8561	0.0069	0.9945
droughts	0.1738	0.2506	0.6936	0.4879	0.6055	0.2807	2.1573	**0.0310**
Depth	-0.0096	0.0524	-0.1826	0.8551	-0.0531	0.0537	-0.9891	0.3226

	E. calamita				*B. variegata*			
	estimate	**standard error**	**t-value**	**p-value**	**estimate**	**standard error**	**t-value**	**p-value**
passages	-0.8342	0.4351	-1.9174	0.0552	-0.6507	0.4197	-1.5505	0.1210
vegetation	1.0116	0.9828	1.0293	0.3033	3.2834	1.2632	2.5992	**0.0093**
droughts	0.4872	0.2871	1.6968	0.0897	0.2346	0.2573	0.9118	0.3619
Depth	0.0277	0.0478	0.5789	0.5627	-0.0054	0.0505	-0.1071	0.9147

Presence of tadpoles tends to be negatively correlated with the number of passages but it is significant only for *B. variegata* (figure 8).

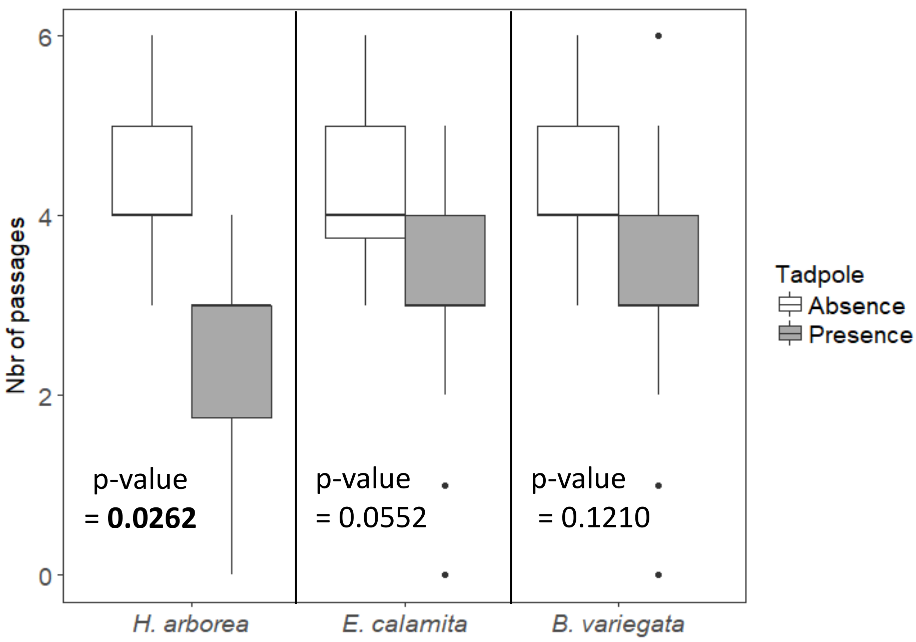

Figure 8 – Relationship between the presence of tadpoles according to the number of passages. Chi-square test with a binomial distribution (p-values in table 4)

Discussion

Our analyses showed that some characteristics of the ponds are related to adult and tadpole presence. The number of adults and the presence of tadpoles of *B. variegata* were positively correlated with the presence of vegetation, while tadpoles of *H. arborea* were observed only in vegetalized ponds. The number of drought is positively correlated with the presence of tadpoles and the number of adults of *H. arborea*. The number of passage of vehicles in ponds does not seem to affect the number of adults of the three species while it was negatively correlated with the presence of tadpoles of *H. arborea*. However, the real number of passages was not successfully measured as planned, so information about the impact on survival is still needed.

Reproduction of the three amphibian species in Bière Camp

As expected according to literature, adults of *H. arborea* and *E. calamita* were observed a bit earlier in the season than *B. variegata*. The latter was also observed during the day across all the breeding season, unlike the two others which are leaving the ponds after reproduction to hide during the day (Barandun and Reyer, 1997; Meyer *et al.*, 2009). *E. calamita* was less abundant than the two other species, as shown in figure 4 (17 % of the total number of adults, table 1) and showed the highest abundance at the beginning of May while the two other species were mostly present at the end of May. *B. variegata* was the most abundant (63 % of the total number of adults, table 1). According to Pellet and Schmidt (2005), *E. calamita* requests six visits on a same site to be detected while three visits are enough to detect the two other species. As ponds have been visited by night during eleven weeks, it was enough to detect the presence of each species but the number of adults can be underestimated for *E. calamita* compared to the two other species due to its lower detectability. Furthermore, the three pioneer species have different behaviour: *B. variegata* tends to stay in the ponds while males of *H. arborea* stay in the bushes to call the females at the beginning of the season and can thus be underestimated by direct observation (Meyer *et al.*, 2009). Moreover, weather and time of the day are known to have an impact on the detection of adults (Paquet, 1994 *in* Morard *et al.*, 2003). However, military time-table limited the possibility to go on the field and the visits were made according to the authorizations provided instead of choosing the days with the best conditions, which might thus result in an underestimation of the number of adults.

The presence of the three species together in most of the ponds suggests that there is none or little competition between the three species. Even though the data collected in this study does not allow to test the competition properly, it is congruent with previous studies showing that density of *H. arborea* and *E. calamita* are unrelated (Warren and Büttner, 2008).

H. arborea showed a lower reproductive success than the two other species (table 2). Most of the time during the monitoring, the water in the ponds had a high turbidity, thus preventing clear observations of tadpoles, especially *B. variegata* and *H. arborea* for which tadpoles are often solitary in the middle of the pond, where it is deeper and darker. They are thus harder to see than tadpoles of *E. calamita* which are more gregarious and stay preferentially at the edges where it is shallower and clearer. Moreover, this latter species lays big clutches which results in a higher abundance of tadpoles while the two other species lay eggs in small patches, even in different ponds for *B. variegata* (Meyer *et al.*, 2009). Principally due to muddy water, the abundance was then difficult to assess, especially when different species were present in the same pond, explaining why the presence of tadpoles was used rather than their abundance. However, the ponds have been visited at least seventeen times each and catching tadpoles with an amphibian net helped minimizing the risk of missing the presence of tadpoles. The reproductive success is then more likely to be due to other factors than just missing the presence of tadpoles. In addition, amphibian males are attracting females with their calls in order to mate, but they do not necessarily distinguish between willing females, females that have already laid eggs, other males and individuals of other species (Meyer *et al.*, 2009). Thus, amplexus, which is the mating position of frogs and toads, does not necessarily result in eggs deposit. Moreover, clutches are not necessarily developing successfully for different reasons, such as predation or water quality (Tester, 1990 *in* Morard and Zuberbühler, 2006, Nöllert and Nöllert, 1992 *in* Morard *et al.*, 2003). Clutches would have been useful to know if the low reproductive success was related to few egg deposits, high egg mortality or low success in embryonic development. However, clutches were not previously detected in all ponds in which tadpoles were found, suggesting that clutches have a low detectability. This is partly due to the high turbidity of the water in the ponds. Considering that clutches laid generally on the bottom of the ponds as well as on vegetation just below water, they are likely to be hidden in the muddy water. Moreover, embryonic development lasts between one to two weeks for the three species and if the temperature is high enough, it can be done in a couple of days (Meyer *et al.*, 2009). It is thus likely to miss clutches considering approximately one visit a week.

Potential impact of passage of vehicles on amphibian reproduction

Our results showed no significant correlation between the number of passages of vehicles in the ponds and the number of adults of the three amphibian species (table 3 and figure 5). This makes sense considering that the vehicles are crossing the ponds during the day while the adults are present for breeding at night. However, this result has to be taken carefully as the passage of vehicles can have indirect impact such as preventing the establishment of vegetation for example, which may affect the attractiveness of the ponds for the adults. Moreover, the data about the passage was collected from June to September while the adults were counted from April to June. Military activities might have been different at the beginning of the season according to the schedule of the army but information about it was difficult to obtain afterwards.

The number of passage is negatively correlated with the presence of tadpoles of *H. arborea* (p-value = 0.0262, table 4 and figure 8) and the result for *E. calamita* is close to the significant threshold (p-value = 0.0552, table 4 and figure 8). Considering the small sample size (n = 8 ponds for *H. arborea*, n = 13 for *E. calamita*, n = 17 for *B. variegata*, table 2) and the underestimation of the real number of passages, this result has to be evaluated with caution. Furthermore, a lot of passages can result in refilling of the pond. This can induce mortality for both eggs and tadpoles which can be buried under the soil and suffocate. In addition, survival of organisms on the field is hard to test in a global manner because a lot of environmental and human factors can induce mortality and affect distribution and density. In the training field of Bière, the surroundings of the ponds are slightly different across the field. Some parts have more forest and more ponds, while retention ponds are present in other parts. This can affect species distribution across the field and induce a bias in the ponds occupancy.

Characteristics of the ponds and amphibian reproduction

Adults of *B. variegata* were more numerous in ponds with more vegetation (p-value = 0.0072, table 3 and figure 6). No such trends were observed for *H. arborea* (p-value = 0.0766, table 3 and figure 6) and *B. variegata* (p-value = 0.9256, table 3 and figure 6). Yet, previous studies suggested that minimal vegetation on ponds was more attractive for *B. variegata* while *E. calamita* showed negative correlation with vegetation (Warren and Büttner, 2008). Furthermore, female of *B. variegata* is known to lay eggs in small patches in several different ponds, either on vegetation or directly in the water without attaching it to a substrate (Meyer *et al.*, 2009). As vegetation generally indicates that the pond is old, it is then likely that the amphibians will prefer ponds without vegetation. In our study, the vegetation was present only at the edges for most of the ponds, which means a low vegetation cover and still pioneer conditions. This particular case can result in the female preference of laying eggs in vegetation because it can provide shelter for the eggs and tadpoles. Regarding *H. arborea*, no correlation between adult density and vegetation was found, however the p-value is close to the significance threshold (p-value = 0.0766, table 3 and figure 6). A higher sample size could show a significant link, which will make sense, considering that the female of this species lays eggs on vegetation (Meyer *et al.*, 2009). This is also suggested by the fact that tadpoles of *H. arborea* were found only in vegetalized pond. Even though the result shows no significant correlation (p-value = 0.9945, table 4), extremely high standard error and estimate are returned by the test (estimate = 18, standard error = 2662, table 4). Vegetation is positively correlated with the presence of tadpoles of *B. variegata* (p-value =0.0093, table 4). This can either be related to a preference of the female to lay eggs in vegetation as explained previously (see chapter Number of adults in Discussion) or a higher survival in ponds with vegetation. As the number of adults of this species also showed a correlation with the presence of vegetation, it suggests that the preference of females is likely. Normally, the passage of vehicles in the pond, especially heavy ones, is preventing the establishment of the vegetation and thus keeping the pioneer conditions necessary for amphibian reproduction (Ryser *et al.*, 2002). A correlation between vegetation and passage is then expected. In this study, vegetation is not colonizing the whole pond but just kept at the edges because the vehicles are preferentially crossing in the middle rather than on the sides. This is probably why no correlation is found between the two variables (p-value = 0.1677, Z = 1.3796).

The number of drought is positively correlated with the number of adults of *H. arborea* (p-value = 0.0105, table 3) but not of the two other species. Drought is an important characteristic of temporary ponds (Ryser *et al.*, 2002). Although they are supposedly dry in autumn and winter, early drought can still happen. Of the twenty-five ponds, nine have dried out several times before the end of the reproductive season and were all located in the same area (Appendix III.b). This may be related to different properties of the ground in the different areas such as compaction of the soil and presence of settling pond which may affect the moisture of the ground in the area, thus the probability of drought. It suggests also that this area may contain more suitable habitats for this species while the two other may be more spread across the field. Presence of tadpoles of *H. arborea* is also positively correlated with the number of drought (p-value = 0.0310, table 4), which is surprising considering that drought is deadly for tadpoles, as observed by the presence of dried tadpoles found in dried ponds during the monitoring of the ponds. However, the sample size is very small for this species as tadpoles were found in only 8 ponds (only 40 % of the ponds) and this may affect the result. Furthermore, the presence of tadpoles and the presence of vegetation are two binary variables. Quantitative data would be more accurate to test for the correlation between vegetation and tadpoles.

A significant correlation was found between drought and depth (p-value = 0.03077, Z = 2.1601). Ponds subject to drought are more likely to be the shallow one but some deep ponds also dried. However, the size of the ponds was variable across the season. Depth at the beginning of the season and at the end of the season was different. Despite the correlation found between initial and final depth (p-value = 0.01, rho = 0.49), some ponds were deep at the beginning of the season and dried at the end of the season. The depth tends to be increased by the passage of the vehicles and ponds were sometimes refilled by the military when too deep. This might create a bias when testing the link between the depth and the number of passages, which does not seem to be correlated (p-value = 0.8507, rho = -0.0397). However, this could also be linked to the underestimated number of passages. No significant correlation was found between depth and the number of adults for none of the species (table 3), yet Warren and Büttler (2008) showed a strong positive correlation for *E. calamita*. This difference might be due to the wide range of depth tested in their study (from 9 cm to 75 cm, mean 28 cm) compared to our range (from 19 cm to 45 cm, mean 32 cm). No correlation between the depth and the presence of tadpoles were found neither for none of the species (table 4).

Limitations and perspectives

Military time-table was problematic in this study because it limited the visits on the site, resulting in a probable underestimation of the number of adults. It is also linked to underestimation of the number of passages due to the rain. The limited authorization for visiting the field made also difficult to detect juveniles, as they are leaving ponds after metamorphosis (Meyer *et al.*, 2009). Furthermore, migrating juveniles could also be threatened by the passage of the vehicles on the field as they can be crushed. Due to the limitations of this study, there is still need for information about a more precise effect of the passage on survival, on tadpoles as well as on juveniles. It is likely that too many passages will have a negative impact and a threshold below which the impact is not too significant would be useful to adjust conservation measures allowing military activities while minimizing the risk for amphibian survival. A close collaboration with the army by integrating them actively in the study could allow us to monitor the number of passage in each pond. It would be thus possible to compare the reproductive success between ponds with different rates of crossing.

In addition, military weapon rejects, such as bullets and gun powder residuals, were found in the field and may have a potential effect on survival. As amphibians are known to be sensitive to physico-chemical characteristics, it would be interesting to evaluate soil and water quality and the potential pollution caused by this material in order to minimize its impact on amphibian reproduction (Nöllert and Nöllert, 1992 *in* Morard *et al.*, 2003). A better knowledge of the factors influencing the success of reproduction in military field will be precious to enhance conservation measures.

Despite the field limitations, it was still possible to make recommendations for pioneer amphibian conservation in the military training field of Bière in the following chapter (Recommendations). Nevertheless, it has to be kept in mind that military grounds are firstly a training fields rather than a natural reserve. Moreover, keeping the use of the field is essential for pioneer species because it maintains dynamic environments necessary for this type of amphibians. The rich biodiversity and the refugee capacity of military fields are precious. These biotopes rely directly on military activity and it is thus essential to have the support and cooperation of military services to preserve these conditions, which are ideal also for other organisms. The cohabitation of army and biodiversity can exist by taking measures to preserve the environment while keeping the military activities and minimizing their impact.

Recommendations

Considering the negative correlation found between *H. arborea* tadpoles and the number of passages, crossing the ponds during the presence of tadpoles should be avoided. It is however difficult to stop military activities for several months in the whole area, but focusing on particular ponds would be a more realistic compromise. To do so, it requires to identify ponds with the best chances of reproductive success. For *E. calamita* and *B. variegata,* the reproductive success was high (76 % and 77 % respectively). Focusing on the factors related to the number of adults is then a good start to find the best ponds. For *B. variegata*, vegetalized ponds should be prioritized. Considering that none of the factors tested in this study were found to be correlated with the number of adults of *E. calamita*, it would be useful to look for other factors that may influence it. Regarding the low reproductive success of *H. arborea*, looking for clutches and tadpoles at the beginning of each reproductive season will be necessary to determine the ponds to avoid in priority, but it would be helpful to study further the reproductive success of this particular species in the military field of Bière. As drought is very deadly for tadpoles and clutches, the zones subject to it are of less importance to preserve amphibians since the survival is already highly compromised. Efforts can be put in ponds and zones with lower risk to dry during the reproductive season. Appendix III.a and III.b show the location of the twenty-five ponds monitored, with the presence of the adults of the three different species and the indication of the ponds that dried out during the reproductive season. All the ponds subject to drought are located on the same zone (in red on appendix III.b).

In addition, only few ponds were monitored and species distribution can vary across years, due to weather and field dynamic linked to military activities. Other ponds showed presence of amphibians and may be important too. Moreover, pioneer species are likely to colonize new ponds and avoid the oldest one. Important ponds are then likely to change across years. It is thus difficult to predict which have to be avoided from one season to another. It is then important to look for the best ponds at each beginning of season.

Moreover, as the impact of vehicles on migrating juveniles is not known for now, passage should be avoided during the massive migration of the juveniles.

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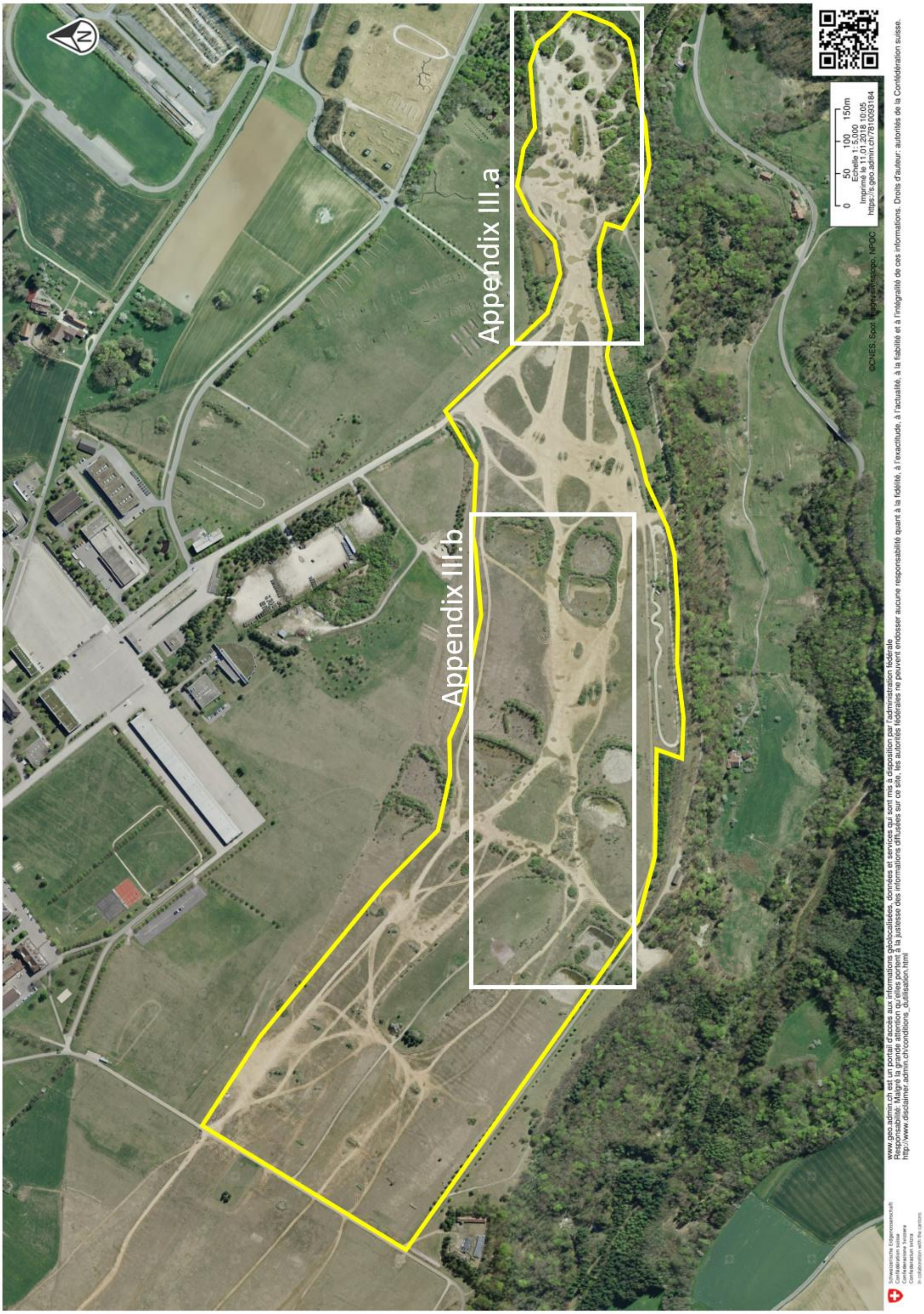
Appendices

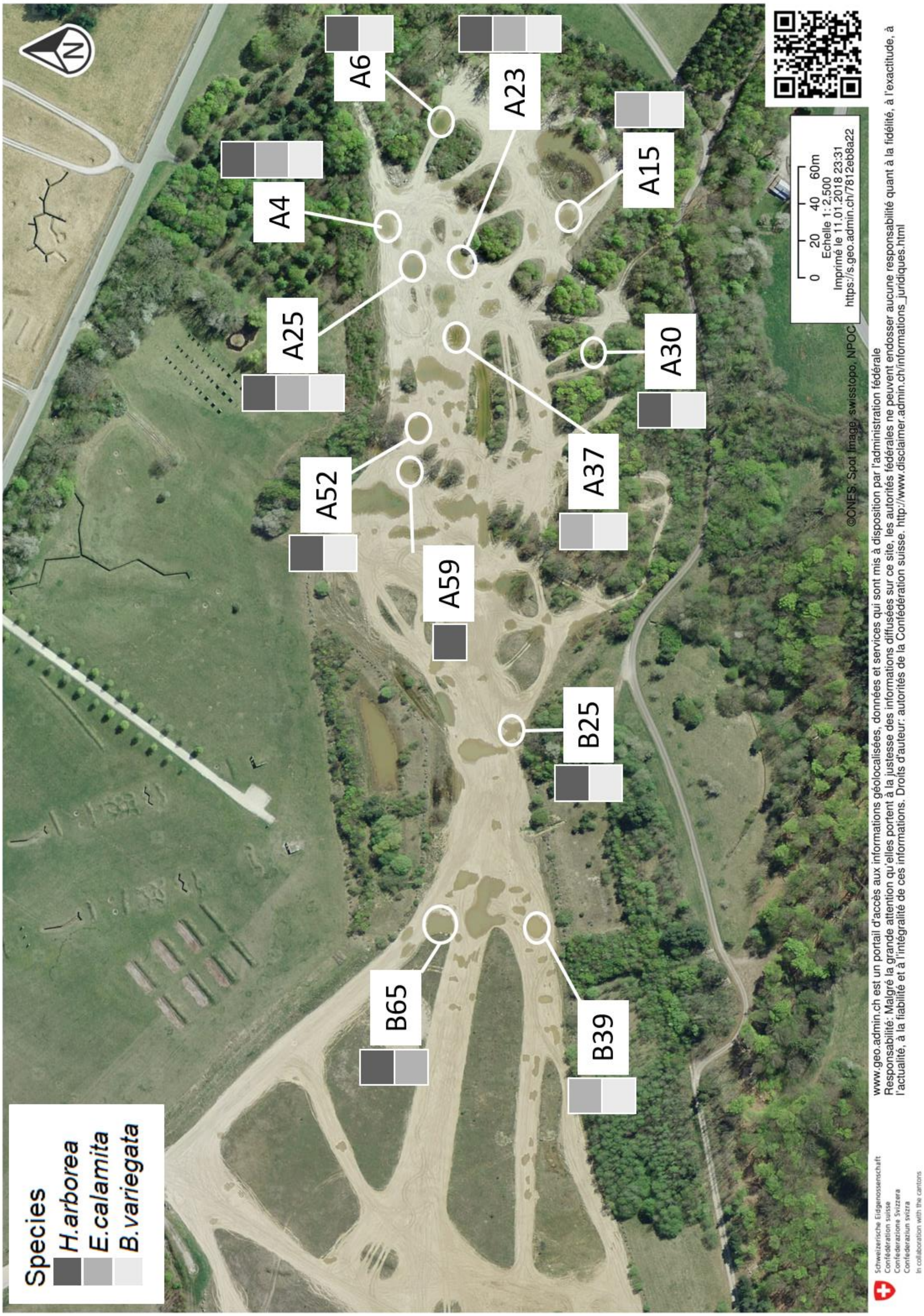
Appendix I – Table of the data collected on the military base of Bière from April to September 2017

Ponds ID	*H. arborea*		*E. calamita*		*B. variegata*		number of passage	number of drought	presence of vegetation	initial depth [cm]	final depth [cm]
	number of adults	presence of tadpoles	number of adults	presence of tadpoles	number of adults	presence of tadpoles					
A15	0	0	9	0	15	0	4	0	1	25	23
A23	1	0	2	1	8	1	5	0	0	25	32
A25	3	0	10	1	1	0	4	0	0	30	36
A30	1	0	0	0	21	1	3	0	1	34	34
A37	0	0	10	0	4	0	3	0	0	45	40
A4	6	1	17	0	19	1	4	0	1	20	21
A52	1	0	0	0	10	1	5	0	1	25	29
A59	1	0	0	0	0	0	4	0	0	37	27
A6	10	1	0	0	14	1	3	0	1	30	26
B25	4	0	0	0	12	1	6	0	1	32	22
B39	0	0	5	1	1	1	5	0	1	45	33
B65	8	1	5	1	0	1	3	0	1	42	39
D1	0	0	0	0	0	0	5	3	0	20	3.5
D32	23	0	3	0	24	0	6	2	1	25	18
D6	2	0	3	1	4	1	4	0	1	43	27
E17	1	0	2	0	5	0	5	0	0	39	30
E20	0	0	0	0	19	0	4	1	1	32	27
E24	1	0	4	1	21	1	4	0	1	37	27
E38	6	1	3	1	26	1	3	4	1	22	7
E40	5	0	1	1	49	1	3	0	1	35	16
E7	2	1	11	1	37	1	3	4	1	33	0
E8	9	0	2	1	24	1	4	4	1	24	0
F3	17	1	0	1	26	1	1	9	1	45	0
F7	8	1	6	1	13	1	2	4	1	19	5
F8	9	1	11	1	19	1	0	3	1	20	12

Appendix II – Map of the training field (in yellow) of the military base of Bière. In white, the two area where the twenty-five ponds monitored are localized (Appendix III.a = 12 ponds, Appendix III.b = 13 ponds)



Appendix III. a - Map of the 12 ponds in zone not subject to drought with the presence of adults of the three amphibian species



Appendix III.b - Map of the 13 pond in zone subject to drought with the presence of adults the three amphibian species. Nine ponds (in red) dried out several times during the reproductive season