



UNIVERSITÀ
DEGLI STUDI
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ENDANGERED SPECIES CONSERVATION IN PERI-URBAN HABITATS

*Study of Yellow-bellied Toads populations
(Bombina variegata) in Geneva (Switzerland) -
Ecological, ethological and genetic
approaches*

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To my homozygote

Once upon a time... at 3 o'clock in the morning... after 6 hours of field work...

*Petit crapaud, tout au fond de ta gouille !
Petit crapaud, nous aimons bien ta bouille-bouille-bouille,
Petit crapaud, deviendra tout orange,
Si beaucoup d'insectes tu manges !*

*Piccolo rospo, in fondo al tuo pozzo!
Piccolo rospo, amiamo il tuo visino-sino-sino,
Piccolo rospo, di venterai color arancio,
Se mangi un tanto gross' insetto!*



ABSTRACT

Our planet is strongly suffering from human activities and their consequences. Obviously, the animals and plants have to cope with the variable environmental conditions that result from these anthropic activities. The aquatic species are even more vulnerable as, often, their habitats is affected by climate. As global warming causes the temperature to rise, the increase of evaporation and consequent dryness of ponds can highly affect the survival and fitness of their inhabitants. The amphibians are the mostly threatened vertebrate group, and the Yellow-bellied toad (*Bombina variegata*) is one of the most endangered amphibian species in Switzerland.

In an effort to improve the knowledge on *Bombina variegata* in Geneva to develop more efficient and robust conservation measures, we monitored 27 sites (21 Genevan and 6 French) during 1 to 5 years using capture-recapture method. In these sites, we monitored 2713 ponds of three different categories: artificial ponds (n=1302), semi-natural ponds (n=1147) and natural ponds (n=264). The first category concerns plastic buckets that were installed in the field. The second category concerns artificial ponds that were dug in the field and made waterproof thanks to clay, or other ancient man-made ponds. The last category concerns natural depressions that were mapped in each site. The sites were monitored at least once a month during the activity period of the toads, for at least one year, but the prospectations were much more regular in some sites (TEP: up to 21 sessions during a year, 5 years of monitoring).

This census allowed the global observation (sum of capture events) of 2655 toads from 2012 to 2016 in the whole Geneva area. These represent 1587 adults (975 males, 612 females), 737 subadults (444 males, 293 females) and 331 juveniles (52 males, 70 females and 209 not sexed). Among these observations, we were able to recognize 1107 different individuals thanks to the colouration of their belly. We were also able to count 4770 tadpoles and 7819 eggs. The global sex-ratio on the whole study area is 53:47. The observed population size per site range from 2 to 433 individuals. Out of the 27 sites, 6 never allowed the observation of *Bombina variegata*. Among the 21 sites occupied by the toads, 3 did not allow the observation of breeding indices.

Thanks to the analyses of the environmental data, we found that the presence of *Bombina variegata* is especially influenced by water availability. We also showed that the toads preferentially choose ponds filled between 50% and 75% of water, probably in the aim of balancing the risks due to predators that may feed on larvae and eggs, and the risk of desiccation. Our results indicate that adults *Bombina variegata* are less tolerant to the presence of Marsh frogs (*Pelophylax ridibundus*) than Italian crested newt (*Triturus cristatus*), and that there is no clear effect of the vegetation on the toads. The eggs and tadpoles seem to be the most demanding stage, which means that adults might consider the conditions of the ponds before spawning to increase the survival probability of their larvae. We also suggest some bias of observation due to the turbidity of water and the presence of algae or other vegetation in the ponds.

Since water is so important for toads, we considered the impact of the hydroperiod on the movement and breeding of *Bombina variegata*. We classified the ponds into two categories: predictable ponds (buckets) and unpredictable ponds (semi-natural and natural ponds). It appeared that the detection of toads and breeding indices is higher in buckets. In both categories, the toads stay for one season without breeding, which is consistent with the idea that the animals inspect the conditions of the ponds before starting to breed. Moreover, the toads are more likely to stop breeding in and leave unpredictable ponds. Finally, the probability that an unoccupied pond welcomes breeding is higher in predictable ponds, as well as the probability that an occupied pond without breeding welcomes breeding the following year.

We also investigated the resistance of the canton of Geneva to the toads' movement using the ArcGIS tool Linkage Mapper. It appeared that the sectors of Jussy (East) and Versoix (North) are very isolated from all the other Swiss sites. The Western part of the canton seems better connected, but the Rhône acts as an impassable barrier. A more detailed analysis of the sector of Jussy showed that a potential corridor passes through the area in a North-South axis and especially through the site CAR. The protection of this site is important to keep the whole area connected.

Finally, to assess the genetic diversity of *Bombina variegata* in Geneva, we collected DNA samples using the non-invasive buccal swab method. We analysed 12 microsatellite loci developed either for our target species or for its sister species *Bombina bombina*. It appeared that the genetic diversity is low, as it was demonstrated in other studies in Switzerland, though the expected heterozygosity is within the range of other studies in Europe. Moreover, the genetic differentiation is moderate. However, even though the values are similar to those in other studies, it is preoccupying that we have such a structure in such a small area. In fact, landscape fragmentation in Geneva is high and it could then likely lead to the isolation of populations. Using Structure and GeneLand softwares, we found that the animals of Geneva come from four original clusters, but that there is not a lot of difference in the attribution of the populations to the clusters. It means that the genetic material of the individuals in each population is not very different. The contrast between this result and the moderated differentiation previously found can be due to missing values in our dataset that reduced the power of the analyses, but can also have a biological explanation. Indeed, amphibians have a generation length long enough to potentially prevent the impact of landscape fragmentation to be noticed on the short term.

To place all these considerations in a conservation framework, we can suggest four important guidelines to insure the protection and survival of *Bombina variegata* in Geneva. First, water is important for this species, so we need to protect and restore humid habitats, especially the ones that includes many temporary ponds. We need to pay particular attention to the key-sites, meaning the sites that are still presenting connections with surrounding areas where the toads are present. Then, we need further

field work to detect breeding grounds that were missed and whose absence may have caused the underestimation of reproduction rate. Further prospections on French grounds could allow us to better understand the potential of dispersion around sites that are located on the border. Moreover, we need to improve the ability of dispersal within and between sites. For this, more ponds must be dug to restore or create connections between colonised areas. Amphibian passageways may also be a good way to prevent the barrier effect of important roads. Finally, the degree of genetic differentiation must be regularly monitored to notice any important increase.

ABSTRACT

Il nostro pianeta soffre enormemente delle attività antropiche e delle loro conseguenze e, chiaramente, gli animali devono confrontarsi con le condizioni ambientali variabili che ne conseguono. Le specie acquatiche sono particolarmente vulnerabili in quanto, spesso, il loro habitat è fortemente influenzato dal clima del pianeta. Dato che il riscaldamento climatico implica un innalzamento delle temperature, la maggior evaporazione ed il conseguente aumento del prosciugamento di stagni e specchi d'acqua può influenzare in maniera grave la sopravvivenza e il successo riproduttivo delle specie presenti in questi ambienti. Tra i vertebrati, gli anfibi sono i più minacciati, e tra gli anfibi più minacciati in Svizzera vi è il rospo ululone dal ventre giallo (*Bombina variegata*).

Per meglio conoscere *Bombina variegata* a Ginevra e ottenere informazioni utili ai fini conservazionistici, la specie è stata studiata, mediante un approccio di cattura-ricattura, in 27 siti (21 ginevrini e 5 francesi). In questi siti abbiamo controllato 2713 tra stagni e pozze d'acqua, suddivisi in tre diverse categorie: artificiali (n = 1302), semi-naturali (n = 1147) e naturali (n = 264). La prima categoria è rappresentata da secchi di plastica appositamente sistemati sul terreno. La seconda categoria si riferisce a siti d'acqua artificiali, scavati nel terreno e impermeabilizzati con argilla durante questo studio, oppure ad altri punti costruiti dall'uomo e già presenti in loco. L'ultima categoria è rappresentata da depressioni naturali che sono state cartografate in ognuno dei siti. I siti sono stati ispezionati almeno una volta al mese durante il periodo di attività dei rospi, per almeno un anno, ma in certi siti le prospezioni sono state più regolari e prolungate (TEP: fino a 21 sessioni in un anno e per 5 anni).

Questo censimento ha permesso l'osservazione globale (somma degli episodi di cattura) di 2655 rospi dal 2012 al 2016 su tutto il bacino ginevrino. Si tratta di 1587 adulti (975 maschi, 612 femmine), 737 sub-adulti (444 maschi, 293 femmine) e 331 giovanili (52 maschi, 70 femmine e 209 con sesso non determinato). Complessivamente, grazie alla colorazione del ventre, è stato possibile riconoscere 1107 diversi individui tra le 2655 osservazioni. Sono stati inoltre osservati 4770 girini e 7819 uova. Il rapporto tra i sessi, calcolato complessivamente per tutta la zona studiata, è di 53:47. Le popolazioni osservate variano di numero da 2 a 433 individui per sito. Sui 27 siti studiati, 6 non hanno permesso di osservare *Bombina variegata*. La riproduzione non è stata osservata in 3 dei 21 siti in cui sono stati trovati rospi.

Grazie all'analisi dei dati ambientali è stato possibile rilevare come la presenza di *Bombina variegata* sia influenzata soprattutto dalla disponibilità di acqua. È stato anche dimostrato che i rospi preferiscono stagni e pozze d'acqua con un riempimento d'acqua che va dal 50% al 75%, probabilmente allo scopo di controbilanciare i rischi legati ai predatori che possono nutrirsi di larve e di uova e il rischio di prosciugamento. I risultati ottenuti indicano che gli adulti di *Bombina variegata* tollerano meno la presenza di rane verdi maggiori (*Pelophylax ridibundus*) che quella di tritoni crestati italiani (*Triturus*

carnifex), e che non c'è un effetto marcato della vegetazione sull'abbondanza di rospi. Uova e girini sono le classi di età più esigenti, il che implica che la scelta da parte degli adulti, prima di deporre le uova, di un punto d'acqua con condizioni adeguate, è fondamentale per aumentare la probabilità di sopravvivenza delle uova stesse. Lo studio effettuato ha inoltre fornito alcune indicazioni sul fatto che alcuni fattori, come la torbidità dell'acqua e la presenza di alghe o di altra vegetazione nei punti d'acqua, potrebbero avere influito sulle osservazioni.

Data l'importanza dell'acqua per i rospi, abbiamo preso in considerazione l'impatto dell'idro-periodo sul movimento e sulla riproduzione di *Bombina variegata*. I punti d'acqua sono stati classificati in due categorie: punti d'acqua prevedibili (vasche) o imprevedibili (aree semi-naturali e naturali). È chiaro che il reperimento di rospi e gli indici di riproduzione sono più elevati nelle vasche. In entrambe le categorie i rospi rimangono per una stagione senza riprodursi, il che è consistente con il concetto che gli animali valutano le condizioni dei punti d'acqua prima di cominciare a riprodursi. Per di più, i rospi possono interrompere la riproduzione e abbandonare i punti d'acqua imprevedibili. Finalmente, la probabilità che un punto d'acqua libero diventi un sito di riproduzione è più alta nelle aree prevedibili, come pure la probabilità che un punto d'acqua occupato ma senza riproduzione diventi sito di riproduzione l'anno successivo.

Utilizzando l'ArcGIS Linkage Mapper abbiamo pure studiato la resistenza del cantone di Ginevra verso il movimento dei rospi. Si è evidenziato che i settori di Jussy (Est) e di Versoix (Nord) sono molto isolati rispetto agli altri siti svizzeri. La parte Ovest del cantone sembra meglio collegata, ma il Rodano svolge un ruolo di barriera invalicabile. Un'analisi più dettagliata del settore di Jussy ha dimostrato che un corridoio potenziale passa attraverso la zona in un asse Nord-Sud e soprattutto attraverso il sito CAR. La protezione di questo sito è importante per far sì che la zona intera resti collegata.

Infine, per studiare la diversità genetica di *Bombina variegata* a Ginevra abbiamo raccolto campioni di DNA col metodo non invasivo dello striscio buccale. Abbiamo analizzato 12 microsatelliti sviluppati sia per la nostra specie in studio sia per la sua specie sorella, *Bombina bombina* (ululone dal ventre rosso). Si è evidenziato che la diversità genetica è bassa, come dimostrato in altri studi in Svizzera, nonostante che l'eterozigosi attesa rimanga nell'ambito degli altri studi europei. Inoltre la differenziazione genetica è modesta. Ciò nonostante, è preoccupante che vi sia una tale struttura in una zona così ristretta. Ciò suggerisce che la frammentazione del territorio di Ginevra sia alta e suscettibile di causare l'isolamento delle popolazioni di rospi. Attraverso il programma Structure e GenLand abbiamo trovato che gli animali di Ginevra discendono da quattro gruppi d'origine, ma che non esiste una grande differenza nell'attribuire le popolazioni ai raggruppamenti. Ciò vuol dire che il materiale genetico degli individui in ogni popolazione non è molto differente. Il contrasto tra questo risultato e la differenziazione moderata trovata in precedenza può essere dovuto a dei valori mancanti nella nostra serie di dati che riducono la potenza dell'analisi, ma può avere ugualmente una spiegazione biologica. In effetti gli anfibi hanno tempi di

generazione abbastanza lunghi perché l'impatto della frammentazione del paesaggio sia evidente in tempi brevi.

Per porre tutte queste considerazioni nel quadro della conservazione delle specie, possiamo suggerire quattro importanti linee di condotta per assicurare la protezione e la sopravvivenza di *Bombina variegata* a Ginevra. In primo luogo, per questa specie l'acqua è importante, per cui dobbiamo proteggere e ripristinare le zone umide, in particolare quelle che includono punti d'acqua temporanei. Dobbiamo prestare un'attenzione particolare ai siti-chiave, cioè ai siti che presentano ancora collegamenti con le zone circostanti dove sono presenti i rospi. Poi, abbiamo bisogno di un lavoro supplementare sul terreno per individuare siti di riproduzione che non sono stati rilevati portando a una sottostima del tasso di riproduzione. Ulteriori prospezioni nelle zone francesi possono permetterci di comprendere meglio il potenziale di dispersione intorno ai siti localizzati sulla frontiera. Dobbiamo inoltre migliorare la capacità di dispersione all'interno e tra i siti. A questo fine punti d'acqua supplementari devono essere creati per ripristinare le connessioni tra le zone colonizzate. Degli ecodotti per batraci possono anche essere un buon metodo per prevenire l'effetto-barriera rappresentato da strade importanti. Infine, stime del grado di differenziazione genetica devono essere effettuate regolarmente per evidenziare un aumento importante.

Notre planète souffre énormément des activités anthropiques et de leurs conséquences. Bien évidemment, les animaux et les plantes doivent faire face aux conditions environnementales variables qui en résultent. Les espèces aquatiques sont d'autant plus vulnérables que leur habitat est souvent lié au climat de la planète. Sachant que le réchauffement climatique implique une hausse des températures, l'augmentation de l'évaporation et la sécheresse des points d'eau qui en découlent peut gravement affecter la survie et le succès reproductif de leurs habitants. Les amphibiens représentent le groupe de vertébrés le plus menacé, et le Crapaud sonneur à ventre jaune (*Bombina variegata*) est l'une des espèces d'amphibiens les plus menacées de Suisse.

Afin d'améliorer les connaissances sur *Bombina variegata* à Genève pour développer des mesures de conservation plus efficaces et plus robustes, nous avons suivi 27 sites (21 genevois et 6 français) pendant 1 à 5 ans grâce à la méthode de capture-recapture. Dans ces sites, nous avons contrôlé 2713 points d'eau classés en trois différentes catégories : points d'eau artificiels (n=1302), points d'eau semi-naturels (n=1147) et points d'eau naturels (n=264). La première catégorie concerne des bacs en plastique qui ont été placés sur le terrain. La seconde catégorie concerne des points d'eau artificiels qui ont été creusés sur le terrain et rendus imperméables grâce à de l'argile ou d'autres points d'eau construits par l'homme et déjà en place. La dernière catégorie concerne des dépressions naturelles qui ont été cartographiées dans chaque site. Les sites ont été suivis au moins une fois par mois durant la période d'activité des crapauds, pendant au moins une année, mais les prospections ont été bien plus régulières dans certains sites (TEP : jusqu'à 21 sessions en une année, 5 ans de suivi).

Ce recensement a permis l'observation globale (somme des événements de capture) de 2655 crapauds de 2012 à 2016 sur tout le bassin genevois. Ils représentent 1587 adultes (975 mâles, 612 femelles), 737 subadultes (444 mâles, 293 femelles) et 331 juvéniles (52 mâles, 70 femelles et 209 individus non sexés). Parmi ces observations, nous avons pu reconnaître 1107 différents individus grâce à leur coloration ventrale. Nous avons également compté 4770 têtards et 7819 œufs. Le sex-ratio général de toute la zone d'étude est 53:47. La taille de population observée par site varie de 2 à 433 individus. Sur les 27 sites étudiés, 6 n'ont pas permis l'observation de *Bombina variegata*. Parmi les 21 sites accueillant des crapauds, 3 n'ont pas permis l'observation d'indices de reproduction.

Grâce à l'analyse de données environnementales, nous avons trouvé que la présence de *Bombina variegata* est surtout influencée par la disponibilité en eau. Nous avons aussi démontré que les crapauds choisissent de préférence des points d'eau remplis d'eau entre 50% et 75%, sans doute dans le but de contrebalancer les risques liés aux prédateurs qui peuvent se nourrir de larves et d'œufs et le risque de dessiccation. Nos résultats indiquent que les adultes *Bombina variegata* tolèrent moins

la présence de Grenouilles rieuses (*Pelophylax ridibundus*) que de Tritons crêtés italiens (*Triturus carnifex*), et qu'il n'y a pas d'effet marqué de la végétation sur les crapauds. Les œufs et les têtards semblent être les classes d'âge la plus exigeantes, ce qui signifie qu'il est possible que les adultes considèrent les conditions des points d'eau avant de pondre afin d'améliorer la probabilité de survie de leurs larves. Nous suggérons aussi certains biais d'observation liés à la turbidité de l'eau et à la présence d'algues ou d'autre végétation dans les points d'eau.

Etant donné que l'eau est si importante pour les crapauds, nous avons considéré l'impact de l'hydropériode sur le mouvement et la reproduction de *Bombina variegata*. Nous avons classé les points d'eau en deux catégories : point d'eau prévisible (bacs) et imprévisible (points d'eau semi-naturels et naturels). Il est apparu que la détection de crapauds et d'indices de reproduction est plus haute dans les bacs. Dans les deux catégories, les crapauds restent une saison sans se reproduire, ce qui est cohérent avec l'idée que les animaux inspectent les conditions des points d'eau avant de commencer la reproduction. De plus, les crapauds sont plus susceptibles d'arrêter la reproduction et de quitter des points d'eau imprévisibles. Finalement, la probabilité qu'un point d'eau inoccupé accueille de la reproduction est plus haute dans les points d'eau prévisibles, tout comme celle qu'un point d'eau occupé sans reproduction accueille de la reproduction l'année suivante.

Nous avons également étudié la résistance du canton de Genève envers le mouvement des crapauds en utilisant l'outil ArcGIS Linkage Mapper. Il est apparu que les secteurs de Jussy (Est) et de Versoix (Nord) sont très isolés des autres sites suisses. La partie Ouest du canton semble mieux connectée, mais le Rhône agit en tant que barrière infranchissable. Une analyse plus détaillée du secteur de Jussy a montré qu'un potentiel corridor passe à travers la zone dans un axe Nord-Sud et surtout à travers le site CAR. La protection de ce site est importante pour garder la zone entière connectée.

Finalement, pour estimer la diversité génétique de *Bombina variegata* à Genève, nous avons collecté des échantillons d'ADN grâce à la méthode non-invasive du frottis buccal. Nous avons analysé 12 microsatellites développés soit pour notre espèce-cible, soit pour son espèce-sœur *Bombina bombina*. Il est apparu que la diversité génétique est faible, comme il a été démontré dans d'autres études en Suisse, bien que l'hétérozygotie attendue soit dans la gamme d'autres études en Europe. De plus, la différenciation génétique est modérée. Cependant, bien que les valeurs soient similaires à celles d'autres études, il est préoccupant que nous ayons une telle structure dans une si petite zone. En réalité, la fragmentation du territoire à Genève est haute et elle serait susceptible de mener à l'isolation des populations. Grâce au programme Structure et GeneLand, nous avons trouvé que les animaux de Genève proviennent de quatre groupes d'origine, mais qu'il n'y a pas de grande différence dans l'attribution des populations aux regroupements. Cela peut signifier que le matériel génétique des individus de chaque population n'est pas très différent. Le contraste entre ce résultat et la différenciation modérée trouvée précédemment peut être dû à des valeurs

manquantes dans notre jeu de données qui réduisent la puissance de l'analyse, mais peut également avoir une explication biologique. En effet, les amphibiens ont un temps de génération suffisamment long pour que l'impact de la fragmentation du paysage se remarque à court terme.

Pour placer toutes ces considérations dans le cadre de la conservation des espèces, nous pouvons suggérer quatre importantes lignes de conduite pour assurer la protection et la survie de *Bombina variegata* à Genève. Tout d'abord, l'eau est importante pour cette espèce, donc nous devons protéger et restaurer les zones humides, en particulier celles incluant des points d'eau temporaires. Nous devons porter une attention toute particulière aux sites-clefs, c'est-à-dire aux sites qui présentent encore des connexions avec les zones environnantes où les crapauds sont présents. Ensuite, nous avons besoin de travail de terrain supplémentaire pour détecter des sites de reproduction qui ont été manqués et qui ont impliqué une sous-estimation du taux de reproduction. Davantage de prospections dans les zones françaises peuvent nous permettre de mieux comprendre le potentiel de dispersion autour des sites localisés sur la frontière. De plus, nous devons améliorer la capacité de dispersion à l'intérieur et entre les sites. Pour cela, des points d'eau supplémentaires doivent être créés pour restaurer ou créer les connexions entre les zones colonisées. Des crapauds-ducs peuvent aussi être un bon moyen de prévenir l'effet de barrière que représentent les routes importantes. Finalement, des estimations du degré de différenciation génétique doivent être réalisées régulièrement pour remarquer toute augmentation importante.

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ABBREVIATIONS

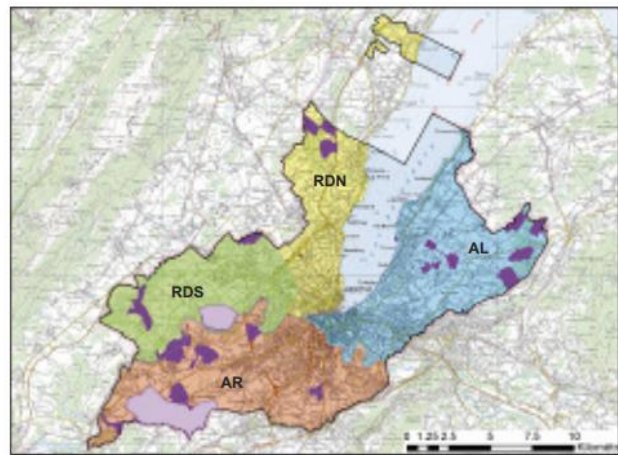
SWISS AMPHIBIAN SPECIES

Species	Abbrev.	Species	Abbrev.
Alpine salamander <i>Salamandra atra</i>	SAAT	Common toad <i>Bufo bufo</i>	BUBU
Fire salamander <i>Salamandra salamandra</i>	SASA	European green toad <i>Bufo viridis</i>	BUVI
Alpine newt <i>Ichthyosaura alpina</i>	ICAL	Natterjack toad <i>Epidalea calamita</i>	EPCA
Palmate newt <i>Lissotriton helveticus</i>	LIHE	European tree frog <i>Hyla arborea</i>	HYAR
Common newt <i>Lissotriton vulgaris</i>	LIVU	Agile frog <i>Rana dalmatina</i>	RADA
Italian crested newt <i>Triturus carnifex</i>	TRCA	Italian agile frog <i>Rana latastei</i>	RALA
Northern crested newt <i>Triturus cristatus</i>	TRCR	Common frog <i>Rana temporaria</i>	RATE
Common midwife toad <i>Alytes obstetricans</i>	ALOB	Green frogs complex : <i>Pelophylax esculentus</i> , <i>P. lessonae</i> and <i>P. ridibundus</i>	PEES PELE PERI
Yellow-bellied toad <i>Bombina variegata</i>	BOVA		

GENEVA SITES AND AREAS

Site	Abbrev.	Area
Arales	ARA	AL
Bardograve	BAR	AR
Bouchets	BOU	AR
Carprière	CAR	AL
Châtillon	CHA	AR
Champ-Grillet	CHG	AR
Colombière	COL	RDN
Corbeille	COR	AL
Coulouvrière	COU	AR
Dolliets	DOL	AL
Epeisse	EPE	AR
Jussy	JUS	AL
Mategnin	MAT	RDS
Peney	PEN	RDS
Plaine	PLA	RDS
Repentance	REP	FR
Rougemont	ROM	AR
Roulave	ROU	RDS
Teppes	TEP	RDS
Touvière	TOU	AR
Vernand	VED	FR
Versoix	VER	RDN
Vernaz	VEZ	FR
Viry	VIR	FR

Area	Abbrev.
Arve-Lac	AL
Arve-Rhône	AR
Rive droite Nord	RDN
Rive droite Sud	RDS
France	FR



Map showing Geneva divided in four areas referred above.

I. INTRODUCTION

A. Global background

i. Global warming and consequences

As it has already strongly been demonstrated, global warming is completely changing our Earth's climate (Houghton, 1999; Stocker et al., 2013). It is not the first time that the temperature of the planet changes, as geologists determined that multiple glacial ages happened during the Pleistocene, alternating with warmer interglacial intervals (Press et al., 2003). Some were longer, and some were shorter, more regular and less extreme. The causes of these changes are not fully understood, but a consensus says that several factors are implied. Among others, the Earth's orbit around the sun and the angle of Earth's rotation axis are modified through astronomical cycles, called Milankovitch cycles, which lead to changes in the amount of heat the planet receives from the Sun (Hays et al., 1976; Clark et al., 2009). Another factor may be the motion of tectonic plates that leads to the modification and shifting of the planet's crust that will have consequences on the ocean currents (Lüthi et al., 2008). However, the actual warming is not only due to natural factors. Studies revealed that it is explained by the accumulation of Carbon dioxide that increases Greenhouse effect (Krupa & Kickert, 1989). Its production is strongly linked to human activities such as fossil fuels burning and destruction of forests. This Greenhouse effect is usually natural and is explained by the fact that our atmosphere absorbs much of the thermal radiation emitted by the Earth thus warming the air and the surface of the planet, making thus life possible. However, as part of the CO₂ is sequestered and cannot escape out of the atmosphere, and as the emitted amount of this gas increases, the Greenhouse effect is strongly intensified by anthropic activities and contributes to the abnormal rise of the temperatures (Solomon et al., 2007). This is a continuously growing problem as the more the human activities and demography increase, the greater the amount of gas is produced. It has been shown that land and sea surface temperatures have risen over the last hundred years (+1°C: Stocker et al., 2013, +0.6°C: Root et al., 2003). Even if all the emissions stopped now, the temperature would not drop significantly for at least a thousand of years (Solomon et al., 2008). The increasing temperatures have several effects that can be observed as indicators of climate change. For example, the frozen areas of the Earth are melting, causing the elevation of sea level. During the XXth century, the sea surface globally rose of 1,7 (±0.2) mm per year, but since 1990, it rose of 3,2 (±0,4) mm per year (Church & White, 2011).

The consequences of climate change, both natural and human-induced, are that the occurrence of extreme weather and climatic events, such as extreme precipitation events or warm spells, is increased (Seneviratne, 2012). However, life on Earth depends on specific conditions that are currently being modified. Animal and plant species have to respond to be able to survive in spite of these changes in their environment. They did

so throughout their evolutionary history, but they now have to accelerate because of the pace of today changes. One way to survive is to shift one's distribution with the aim of keeping the same niche as required by physiological constraints. Evidences show that several plant and animal species display such temperature-related distribution shift, making clear that the impact of global warming is already noticeable (Root et al., 2003). However, global warming is not the only stress that hovers over these species. Combined for instance with habitat destruction, which is one of the main actual threats, these stresses could easily lead to changes in populations or in communities composition and structure within ecosystems. These ecological modifications can ultimately lead to both local and global extinctions (McCarty, 2001; Walther et al., 2002).

The threat can concern a wide variety of taxonomic and functional groups, but the ones that are first affected and suffer the most from such local extinctions are the ones whose geographic range is the most restricted or fragmented and whose ecological niche is narrow. These species often depend on small habitat patches and they cannot easily move somewhere else (Thuiller et al., 2005). These are mainly the arctic species (Callaghan et al., 2004) and the mountain-top species (Gottfried et al., 1999) that cannot move to keep suitable conditions for their survival. In the Alps, for example, Brunetti et al. (2009) showed that during the last century, the temperatures increased twice as much in this area as the global world average. Many studies showed the impact of this warming on plants (Leonelli et al., 2011; Jay et al., 2012; Moradi et al., 2012), and a few focused on the impact on animals (for example: marmot: Tafani et al., 2013; yellow-bellied toad: Cornetti et al., 2016). The second most concerned group of species are aquatic ones. Among them, tropical coral reefs, particularly thermo-sensitive (Hoegh-Guldberg, 1999), and amphibians, whose complex life cycle is half-aquatic and half-terrestrial (Wilbur, 1980), are the most negatively affected (Parmesan, 2006). Moreover, this latter group also suffers from the UV-B radiation that becomes stronger due to ozone depletion and increases vulnerability to infectious diseases in larval stage (Kiesecker et al., 2001).

ii. Urbanisation and consequences

Unfortunately, global warming is not the only threat that globally or locally endangered species face. As human communities grow both in number and in occupied space, both plant and animal species are affected (McKinney, 2006; Niemelä et al., 2000; Venn et al., 2003). Furthermore, global warming is directly linked to other global environmental changes such as urban development, as the expansion of cities and industries contributes to the increase of Carbon dioxide released into the atmosphere (Didham, 2010). The effects of the growing abundance of human cities are various and can be seen at different time (short and long) and space (local and global) scales (McKinney, 2008; Ray et al., 2002). In their study conducted in China, Sun et al. (2016) recorded the elevation of temperature in the country and quantified the contribution of urbanization and other forces to this warming. The researchers concluded that about one third of the observed warming was due to urban warming influences, the rest being explained by other anthropogenic forces and by natural forces. This percentage is not

the same if we consider the whole planet, but it is clear that the effect of urbanization can increase the warming of local areas and lead to many problems as mentioned above (Cui et al., 2012).

However, urbanization does not always affect biodiversity negatively (Pautasso, 2007). For example, the effects are different whether we look at native or non-native species (Pauchard et al. 2006). When the environment becomes more urbanized, implying the apparition of inhospitable lands such as roads or construction works, native species will likely disappear as they are not able to cope with these sudden changes (Czech et al., 2000). On the other hand, the new city will provide a new diversity of habitat patches that will promote the arrival and survival of non-native species (McKinney, 2006). Moreover, according to the intensity of urbanization and some other variables such as the taxonomic group and the spatial scale, the effects can also vary (McKinney, 2008). Obviously, extreme urbanization, such as the one occurring in central urban core areas, tends to reduce species richness, especially in birds (Marzluff, 2001). However, if urbanization stays moderate and remains under a critical threshold, like in suburban areas, species richness can be increased thanks to the creation of various new small niches. It has been repeatedly demonstrated in plants (McKinney, 2008; Young et al., 1996), but also in a few studies on invertebrates and vertebrates (Niemelä et al., 2011; Pautasso, 2007). The global impact of urban development on biodiversity is thus not always negative, as the reduction of native species is outpaced by the establishment of the new species. However, even if biodiversity in itself is not affected by urbanization because of the diversity in newly settled species, the competition induced by these non-native species can have a dramatic impact on endemic species and even cause local extinctions (Rahel, 2002).

The major consequence of urbanization is that, as cities extend on the countryside, wild habitats are destroyed. This invasion usually happens very quickly, and often without plans to compensate for the negative impacts on local populations, which will not be able to resist and survive the changes in their habitat. For some decades, landscapes worldwide went through changes such as urbanization, expansion of agricultural lands or intensification of renewable energy uses, to the point where their sustainability is at risk (Plieninger et al., 2016). Yet, the land-use changes can diminish and isolate suitable habitats resulting in a fragmented landscape instead of the original large and continuous habitat. The problems of such divided environments are that they generate spatial variation in resource availability and that groups of organisms find themselves isolated from each other's (Ahlers et al., 2016; Hoffmeister et al., 2005). Landscape fragmentation and thus habitat loss result in declines in population sizes and species richness and limits population connectivity. Thus, they are key drivers to species loss (Fischer & Lindenmayer, 2007). In the same way as for global warming, the impacts of habitat fragmentation on several indicators can be observed: community composition, species interactions and ecosystem functioning (Didham, 2010; Collinge, 1996) and it might have dramatic consequences. It was shown for example that over 40% of amphibian species are currently declining (Stuart et al., 2004), particularly because of habitat loss and fragmentation of landscape due to the extension of

urbanisation and agriculture, which has no precedent in any other animal class. It was even demonstrated that the extinction rate of amphibians could currently be more than 200 times higher than during former periods (McCallum, 2007; Roelants et al., 2007). Lehtinen et al. (1999) explained that amphibian diversity is negatively correlated with the proportion of urban land-use at all spatial scales, especially because these animals suffer from the diminution of both aquatic and terrestrial habitats which are crucial for them (Cushman, 2006; Becker et al., 2007). As explained by Didham (2010), the underlying mechanisms that can lead a population close to local extinction can be classified into four categories: environmental stochasticity (randomness of the environmental variables that might lead to population extinctions), demographic stochasticity (randomness of the birth and death rates that might lead to population extinctions), natural catastrophes (unpredictable event that might wipe out small and isolated populations) and genetic diversity loss (increase in the frequency of deleterious alleles that might lead to the diminution of individual fitness and thus to the decline of populations). These four categories can interact and create “extinction vortices” (Gilpin & Soulé, 1986; Mills & Smouse, 1994; Brook et al., 2002) and as the actual situation seems to trigger a sixth massive extinction (Chapin et al., 2000), it is crucial to understand what can be done to change this trend.

iii. Consequences of genetic diversity loss

In biodiversity conservation, genetic diversity is a key indicator because it is directly linked to long-term survival and adaptability of a population to changes such as global warming or habitat fragmentation (Wilson, 1989). Inversely, a heterogeneous environment enhances genetic diversity, because organisms that manage to colonize different ecological niches evolve afterwards each on their own and become more and more genetically distinct (Nevo et al., 1984). Charles Darwin described in 1859 his theory of Natural Selection. The principle is that the organisms are all different, but some of them are more adapted to their environment and so, will more likely survive and produce more offspring that will transmit their genes to the next generations and thus allow the appropriate features to persist and evolve through generations. He demonstrated this using the beak of finches that changed through time according to the food that the birds found in different areas. Actually, the underlying principle is that the first birds were generalists with a diversified genetic material that allowed them to colonize different habitats. It is only then that they progressively showed a beak that was the most suitable for their food. Said in a more global perspective, among the various gene alleles possible, some will increase the fitness in a particular environment and will thus lead to the survival and better breeding success of the individual carrying them.

When a population includes many individuals that present many variants of each gene, the chances that the population is able to perform adequately in different environmental conditions are high (Barret & Schluter, 2007). If we add the mutation and recombination rates to this primary genetic diversity, the total potential allele diversity is large enough for the population to cope with most of unpredicted events (Wagner &

Altenberg, 1996). Another important process linked to the genetic aspect of a population is the genetic drift. It is a random selection of variants that happens at each generation and that makes the frequency of each allele go up and down, eventually reaching fixation (Masel, 2011). The strength of this process increases in small populations (Ellstrand & Elam, 1993). Thus, problems appear when the population is reduced, for example because of landscape fragmentation. In this case, the number of individuals is often too small to allow the population to survive in case of external environmental perturbations such as an intensive drought, an important habitat destruction (quarry in activity), the arrival of a new predator or the emergence of a new disease (McCarthy & Thompson, 2001). Indeed, the population is not extended enough in the territory to insure some individuals to be out of the damaged or endangered area and thus to be protected and sheltered from the problem. Moreover, consequences of a reduced population are that the random sampling of organisms remaining in this group does not show all the gene variants that could exist in a larger group. This implies a less variable gene pool, a reduced genetic diversity, and thus, a smaller ability to adapt (Freeman & Herron, 2004). In addition, the genetic drift strength will grow, increasing the number of variants that will be lost from the population, reducing even more the genetic diversity (Masel, 2011). The consequences are that the surviving individuals can lose the ability to face a change and survive it. Data from several taxa show that populations that have a reduced genetic diversity face a high risk and also show reduced growth and increased extinction rates (Keller & Waller, 2002). Furthermore, if the population is reduced, it is most likely also isolated, affecting migration patterns, interrupting connections with other populations (other gene pools). The gene flow is thus strongly compromised and inbreeding rate increases as local organisms cannot find foreign individuals as mates, and outsiders cannot reach the populated area (Lacy, 2000). Many new problems can consequently emerge (Brook et al., 2002), such as the augmentation of homozygous individuals implying potential expression of recessive deleterious alleles (Charlesworth & Charlesworth, 1987) resulting in a reduced fitness (Mills & Smouse, 1994; Morten & O'Brien, 2010). For all these reasons, the persistence of such a small population is usually strongly jeopardized.

Concerning the genetic diversity loss, one can note that all taxa are not evenly impacted by a reduced population size. As mentioned for urbanization, in plants, for example, the gene flow appears to be rather increased when fragmentation occurs (Young et al., 1996). However, this is unfortunately not the general rule, and more than often, changes in the environment negatively affect the gene flow and lead to genetic erosion. As we saw earlier, amphibians are a group of vertebrates that is particularly vulnerable because of the fragmentation of its habitats and the current and quick diminution of its populations. It was shown that the genetic diversity and divergence in amphibians is better explained by ecological variables than by life-history and demographic variables (Nevo & Beiles, 1991). Moreover, their breeding strategy is by definition very dangerous as, among other reasons, they usually lay their eggs in temporary ponds that can suffer from drought, leading to few juveniles produced.

Another risky characteristic of these animals is their rather poor dispersal ability. Indeed, even though some studies showed that individuals can cover great distances (Schroter et al., 2005; Smith & Green, 2005), most of amphibian species are highly philopatric which directly limits the connectivity between groups and leads to a natural fragmentation of the gene pool. They are thus even more sensitive to habitat destruction as, if their environment becomes fragmented, they will even more struggle to move and the genetic connection between populations will be reduced (Morten et al., 2010; Cushman, 2006).

The work presented here will focus on amphibians and especially on the order of Anurans because this whole taxonomic group is potentially subjected to global warming, urbanization and genetic diversity loss.

iv. *Situation in Europe and Switzerland*

Although the European continent is the second smallest in surface (2% of Earth surface), it has the third rank of human population sizes (11% of world human population is in Europe). This leads to an ecological footprint that is 2.6 times larger than what the continent can provide as resources (Temple & Cox, 2009). As a consequence, it is the most densely occupied continent with Asia, and the most urbanised one. Many studies tried to identify the drivers of landscape changes across Europe, but they often consider only medium-term time scales and local spatial scales, thus the knowledge about the main driving forces that guide these changes and the impact on several isolated landscapes is limited (Plieninger et al., 2016). Anyway, the result of these changes is that the landscape of Europe is highly fragmented and the part that remains wild is really tiny. During the last century, the land-use in Europe strongly intensified as the agricultural production increased. However, the last decades seem to show a global stabilisation across the continent that can be a result of various environmental and agricultural policies (van der Sluis, 2016). Anyway, even though the situation is not currently getting worse, animals are under a great pressure and have been forced to adapt to semi-natural habitats created and maintained by humans. However, Europe is also considered as a worldwide biodiversity hotspot thanks to its geographical context displaying a great diversity of habitats and landscapes (sea, mountains, plains, forests, artic areas, etc.).

Pressures on fauna and flora are various and their impacts are different according to the region and species involved. We can separate them in two different groups: direct effects due to human activities and indirect effects. In the first group, we can cite the expansion of agriculture and urbanisation, and in the second one, the exhaustion of soil with the acidification and desertification, the eutrophication, the introduction of alien species and, obviously, climate change. Humans change their planet according to their needs, but they forget that they need animals and plants for example to pollinate their crops, sequestrate Carbon and purify water (Temple & Cox, 2009).

Concerning amphibians, they are very particular as their life cycles take place in two different environments. Indeed, the adults stay in terrestrial habitats and go to humid areas or water points to spawn (Wilbur, 1980). The eggs develop inside the water into tadpoles that have gills. The tadpoles of Anurans are herbivorous. During the metamorphosis, the tail is resorbed and used as an energetic resource, the hind legs are formed, and as soon as the front legs appear, the lungs are functional and the juveniles must breathe air. The breeding strategies depend on the species. The frogs spawn their eggs into clutches that can form a mattress of eggs if the species is social (Common frog, *Rana temporaria*: Tournier & Tournier, 2012). The toads usually spawn in necklaces that are deposited on the ground between grasses. Some species have more specific spawning behaviour. The Tree frog (*Hyla arborea*: HYAR) and the Yellow-bellied toad (*Bombina variegata*: BOVA) make small clusters that look like raspberry and that are tied to a branch or grass (HYAR: Gunzburger, 2006; BOVA: Rafinska, 1991). Some Anurans, and especially males of these species, show some parental care such as carrying eggs or tadpoles around their hind legs or on their back and taking care of their humidity rate (*Alytes sp.*: Márquez, 1993; *Hyloxalus subpunctatus*: Duellman & Trueb, 1994).

Currently, we estimate between 7000 and 8000 the number of amphibian species living on the whole planet (Frost, 2016), most of them being in South America, as it is the centre of this biodiversity and is constantly updated on the online site of the American Museum of Natural History (*in date of 26.01.2017*: 7590 amphibian species in the world). Out of these species, 90% are Anuran (Alford et al., 2007). In Europe, more than 70 species are present (73: Sillero et al., 2014; 85: Temple & Cox, 2009), two out of the three orders are represented and 75% of the species are endemic to Europe and must thus be particularly protected (TABLE 1.1). Among the European species, 19 are threatened and 13 are near threatened. Among all European animal species, amphibians are the most suffering because of the reduction of wetlands. Their declines are thus the strongest (59% of amphibian species are declining).

TABLE 1.1. DIVERSITY AND ENDEMISM IN AMPHIBIAN ORDERS AND FAMILIES IN EUROPE (Temple & Cox, 2009).

Class	Order	Family	Europe	
			Number of species	Number of endemic species (% endemic)
Amphibia	Anura	Alytidae	9	8 (88.8%)
		Bombinatoridae	3	2 (66.7%)
		Bufoidea	7	3 (42.9%)
		Hylidae	5	2 (20.0%)
		Pelobatidae	3	1 (33.3%)
		Pelodytidae	2	2 (100%)
		Ranidae	21	16 (76.2%)
		Plethodontidae	8	8 (100%)
		Caudata	Proteidae	1
	Salamandridae		26	21 (80.8%)
	Total			85

Among these European species, 19 can be observed in Switzerland. Seventy percent of the indigenous species are present on the Red list of threatened amphibians in Switzerland (Schmidt & Zumbach, 2005). According to the KARCH, the Coordination Centre for the Protection of Amphibians and Reptiles in Switzerland (KARCH website), three species are widely distributed (BUBU, TRAL and RATE: see Abbreviations list p.0). They are the only ones we can find high in the mountains, with the SAAT, which occurs only above 800m. Some of them occur only in certain parts of the country. For example, RALA is only found in Tessin, BUVI is only present between Zurich and Bern and used to be in Tessin and TRCA used to be only in Tessin but was accidentally introduced in Geneva. RADA is present in Tessin, in Geneva and in Schaffhouse. Seven species are especially present on the plateau, meaning the upper half of the country (TRCR, TRHE, PERI, HYAR, ALOB, BOVA, BUCA) in various abundances. The last four species occur on the plateau and in Tessin (TRVU, SASA, PEES and PELE).

In Switzerland, as in many places around the world, the climate has considerably changed over the past century. According to MeteoSuisse (website), the federal office of meteorology and climatology in Switzerland (last update in 2014), during the XIXth century, temperatures in Switzerland were going very low during falls and winter. However, through the XXth century, it was recorded that they increased until 1997 and 1998 when a brutal change drove the winters to be warmer. It is still the case up to now and the cold seasons do not reach incredibly low temperatures anymore. We also observed a rise of temperatures during spring and summer and some years were exceptionally hot (1940's). Since 1990, by average, the temperatures are 1,5°C higher than previously (Beniston et al., 1994) (FIGURE 1.1).

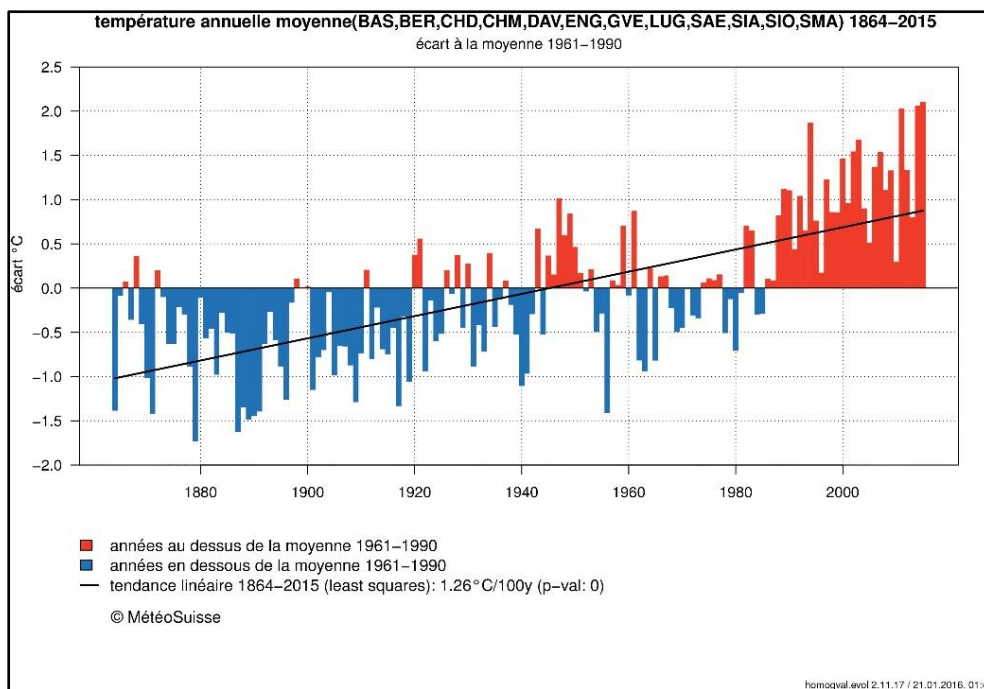


FIGURE 1.1. AVERAGE ANNUAL TEMPERATURES RECORDED IN SWITZERLAND FROM 1864 TO 2015 (MeteoSuisse website). Red: years with temperatures above the mean (1961-1990). Blue: years with temperatures below the mean (1961-1990). Line: linear trend (1854-2015).

About the precipitations, there are great differences between the North and the South of the country because of the Alps. The only trend that can be noticed on the long-term occurs in the North part and during the winter. Indeed, there is a significant increase of rainfalls during spring periods in the last years, and a significant decrease during winter periods. This latter point is also true for the Western and alpine part of Switzerland (FIGURE 1.2).

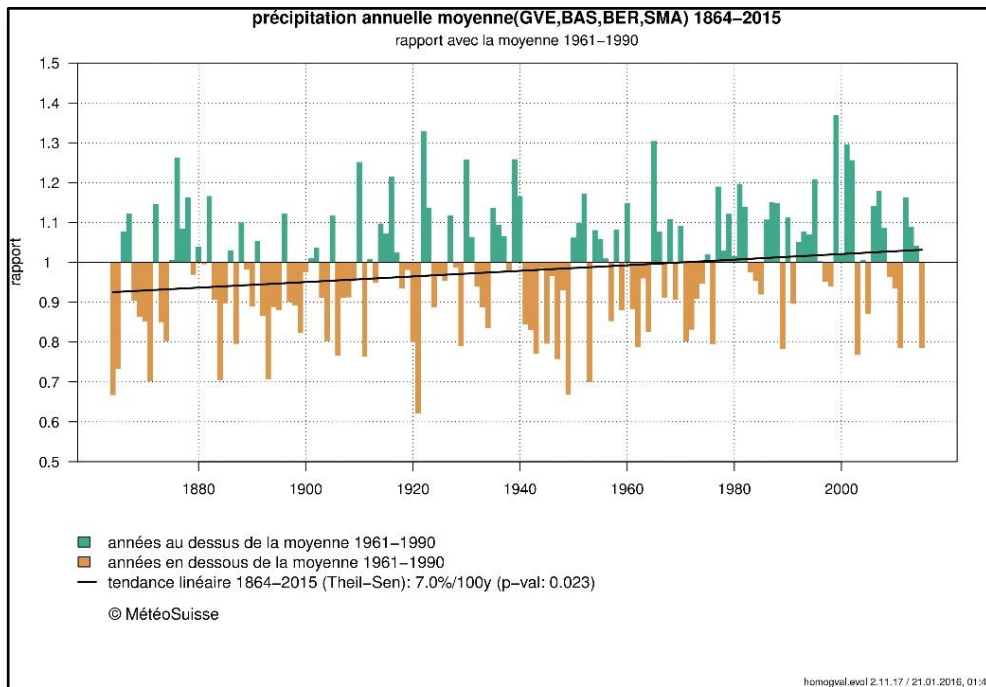


FIGURE 1.2. AVERAGE ANNUAL PRECIPITATIONS RECORDED IN SWITZERLAND FROM 1864 TO 2015 (MeteoSuisse website). Green: years with rainfall above the mean (1961-1990). Gold: years with rainfall below the mean (1961-1990). Line: linear trend (1854-2015).

Researches forecast two scenarios in the future, but all conclude that this warming and drying trends should continue and lead to an increase of several degrees in the whole country during the whole year (2,7°C - 4,8°C before the end of XXIst century) and reduced rainfalls in summer (-18% to -28%). More heat waves should occur that will last longer and less snow but more rain should fall, leading to higher risks of flood especially in the plain region. A third scenario called “stabilisation” predicts that these changes should be less intense, but the researchers find it less likely (MeteoSuisse website).

Switzerland, though being a small country, shows a wide variety of natural habitats (plains, mountains, lakes, warmer local areas, etc.). However, as many other densely populated landscapes, the intensification of land-use and recent anthropogenic landscape modifications affected the distribution of the wild species. Thus, even those that used to be widely distributed a century ago find now their geographical range strongly reduced (Grossenbacher, 1988). In Western Switzerland, the agricultural landscape changed a lot in the last decades. Natural areas such as meadows and pastures suffered from peri-urban development or agricultural intensification and gave

way to new districts extending the cities. The humid areas were drained for crops and new communication networks divided even more the countryside, fragmenting the environment (Pellet et al., 2004).

Five types of natural habitats are considered in Switzerland as biotopes of national importance. They represent 1,8% of the territory, occur in the whole country and are listed and monitored to follow which species can be observed within their range. They are high- and transitional marshes, low-marshes, alluvial areas, breeding sites for amphibians and meadows and pastures. Many of them need an urgent restoration (OFEV website). The protection of amphibians is ensured since 1966 by the Federal Law on Nature Protection (LPN) and the protection of their reproductive sites is official since a reinforcement of this law in 2001 (Ordonnance sur la Protection des Sites de Reproduction de Batraciens d'Importance Nationale: OBat). Indeed, most of the Swiss species are on the red list of threatened species as they are very vulnerable to human activities (Hotz & Broggi, 1982) and the Confederation decided that measures needed to be taken to ensure their persistence. Since then, 897 sites are protected and monitored, and for some rare species, such as the Italian agile frog (*Rana latastei*), they can only be found in these areas.

The analyse of the OBat sites includes data on the location (map, surface types), a short historical and geographical description, other data about the ownership of the ground, comments on the amphibian and other natural values of the place and finally the threats and conservation measures currently running or planned in the future. Each site was defined according to two perimeters. The sector A was the centre of the area and included the breeding areas and natural habitats that are directly connected to the reproduction. The sector B was the peripheral area including terrestrial habitats and migratory routes that guarantee Amphibians survival during the whole year (Thiébaud, 2008).

Several factors were taken into account to determine the amphibian value of the sites. The formula was elaborated in 1994 by Grossenbacher & Dalang. It includes the present indigenous species, their rarity at the national level and the importance of the population in the area. Thanks to this mathematical value, one can compare the different sites. The population size used in the formula ranks from 1 to 4 according to the estimated number of breeding individuals in the area. These four classes vary between species as some are more common than others (TABLE 1.2).

TABLE 1.2. POPULATION CLASSES ACCORDING TO GROSSENBACHER (1988) used to calculate the amphibian value of each OBat site. See Abbreviations list for all names (p.0).

Number of breeding adults	Small (1)	Medium (2)	Big (3)	Very big (4)
Newts	1-3	4-10	11-40	40+
HYAR + ALOB	1-5	6-20	21-60	60+
BOVA, BUCA + PERI	1-5	6-30	31-100	100+
BUBU, RATE + RADA	1-5	6-50	51-200	200+

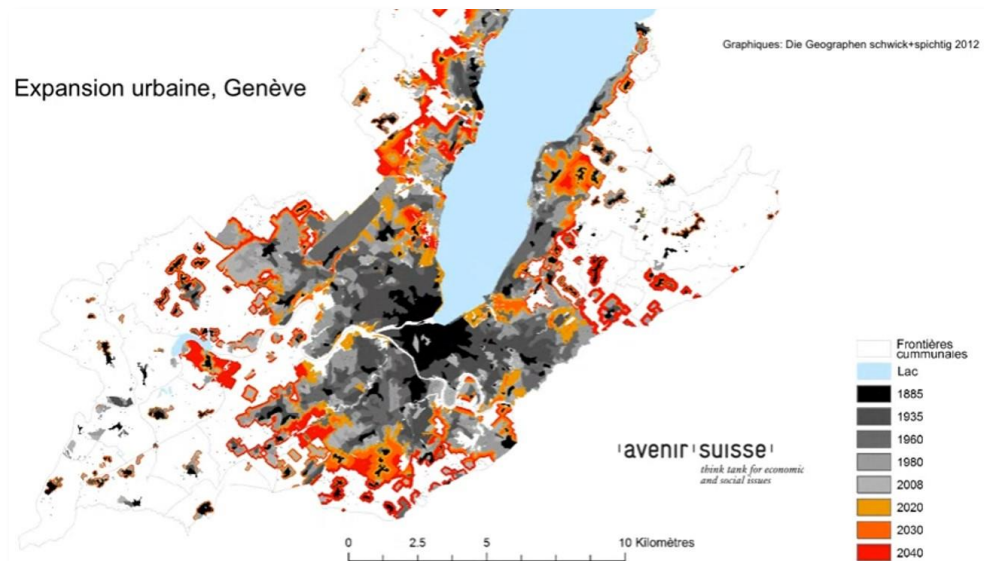
v. Geneva

Geneva is a canton located at the extreme South-West of Switzerland at a mean altitude of 370 meters. It surrounds the end of the Lemman Lake and is itself surrounded by France with which it shares 95% of the borders. It covers almost 280 km². It is well known for its city, but it is also a huge agricultural canton as the rural part represents more than half of its surface. The forests cover only 35 km², meaning about 12% of the territory and are split in three main parts: Chancy, Jussy and Versoix, in addition to small other forested areas. The main variety present in Geneva is the Oak which goes well and is not affected by the European spruce bark beetle (*Ips typographus*). However, the quality of the wood is medium and has thus a weak economic value. Geneva is the only canton where hunting is forbidden, and the regulation of ungulates is only conducted by wildlife officers (OFEV website).

In 2005, a new law about the promotion of Genevan agriculture was adopted in the aim of conserving an outreach agriculture that stays competitive, diversified and respectful of the environment and consumers. A first Swiss and unique label was created to federate the producers, distributors, unions and consumers under the same banner (Geneva website). The Genevan agriculture represents only 1% of the Swiss agriculture, but it corresponds to 11 thousands hectares of agricultural land and about 400 professional exploitations. Geneva has the 3rd rank of wine-making industry in Switzerland, the 3rd rank for utilization of greenhouses and crops tunnels and the 7th rank for oil-seed rape production. It is also the biggest Swiss producer of eggplants and it has the biggest herd of bison (160 heads).

Unfortunately, there is no national park in the Geneva area, but there are five types of sites that are important at national or even wider scale. Indeed, there are many forested reserves and many meadows and dry pastures, three (out of 283 in Switzerland) nationally important alluvial areas and three huge internationally important areas for water and migratory birds such as along the Rhône (which is also an internationally important humid area as described by the Ramsar Convention) and the sides of the lake. The whole alluvial complex of the Rhône is also considered as an Emerald site, because it shelters species that need specific protection measures on the European level (OFEV website). Moreover, the urban part of Geneva increased a lot in the last century, reducing the wild habitats and threatening wild species (DieGeographen website) (MAP 1.1). However, even if the intensity of human activities and occupancy is high, there are a significant number of nationally important sites for amphibians (23) exist in the area. These places cover a good part of the surface occupied by waterways, forests and agricultural land. Particularly, the forest cover is important for the amphibian distribution (Joly et al., 2001; Cayuela et al., 2015a) and they strongly avoid cultivated areas (Pichenot, 2008; Cayuela et al., 2015a). For this study, the whole canton was divided in four areas (AL, AR, RDN, RDS), each including several sampling sites. For ten years now, a big urban conglomeration project is being planned between Geneva, Vaud and France, so there really is an opportunity to work on the improvement of the natural regional heritage (Thiébaud, 2008). The monitoring and the management of the natural

Genevan sites are carried out by the Nature and Landscape Department of Geneva (DGNP, which recently became DGAN). In 1993, Keller & al., among others, observed that existing databases on amphibians in Geneva were old and out of date, so in 2003, the DGNP launched a review of these data, which then started in 2006 with the Bachelor thesis of Vincent Jaggi (2010).



MAP 1.1. URBAN EXPANSION IN GENEVA. The city in the center of the canton strongly expanded in the last century (DieGeographen website). Colours: time intervals.

According to the last review of OBat published in 2008, twelve species of amphibians (out the 19 species present in Switzerland) live in Geneva among which 9 are indigenous. Three species used to be present but disappeared in the last decades due to competition with other species or to habitat loss (TRCR, HYAR and PEES).

Among the indigenous species, 6 are common in stable forested habitats and can use a large variety of waterways. They are the Fire salamander (SASA), the Alpine newt (ICAL), the Palmate newt (LIHE), the Common toad (BUBU), the Agile frog (RADA) and the Common frog (RATE). The 3 other indigenous species are more demanding because they need pioneer habitats, meaning habitats whose grounds were just disturbed or created and in which no vegetation is installed yet (Warren & Büttner, 2008). These habitats are less frequent than others in the nature so these species are more linked to human activities (quarries, paths in forests, etc.). These species are the Yellow-bellied toad (BOVA), the Midwife toad (ALOB) and the Natterjack toad (EPCA). Ecological requirements of these three endangered indigenous toads are different so that conservation measures need to be specific to each species.

Finally, three species are present in Geneva without being indigenous. They were involuntary introduced in the canton and quickly colonised the habitats because they do not have any predator when they arrive, they often are bigger than indigenous species, so they take their place in the ecological niches and act as competitors for the resources (Moyle & Light, 1996; Losos et al., 1993). They thus participated to the disappearance of

the three above-mentioned species. These invasive species are the Italian crested newt (TRCA), the Common newt (LIVU) and the Marsh frog (PERI).

The following work will focus on BOVA, one of the three indigenous species that require specific conditions to survive, and are thus more vulnerable.

B. Studied species

i. Description

The Yellow-bellied toad (*Bombina variegata*: BOVA) is a little amphibian, unique Swiss representative of the Bombinatoridae family, whose length is usually smaller than 5 centimetres. It has heart-shaped eyes, a dark mud-coloured back which allows a perfect camouflage in the environment, and a bright yellow belly (Linnaeus, 1758) (FIGURE 1.3). Such colour is aposematic, which means it indicates its toxicity to potential predators (Cott, 1940). It is spattered with dark blue stains whose organization is unique to each individual. Juveniles show quite early their colouration so, even if the dots and lines get thicker as the individual grows, the pattern remains the same and can be recognized. This fact is an advantage for working with this species as it is relatively easy to differentiate visually one individual from the others and, therefore, thanks to the capture-mark recapture method (Delarze et al., 2000), the estimation of the population is made easier. Moreover, the natural marking of the animals makes the experimental marking useless, which facilitates practical work. In this study, the use of a non-invasive methodology was a deliberate choice so the natural marking was used to replace the toe-clipping method, which anyway is often contested (Funk et al., 2005; May, 2004; McCarthy & Parris, 2004).

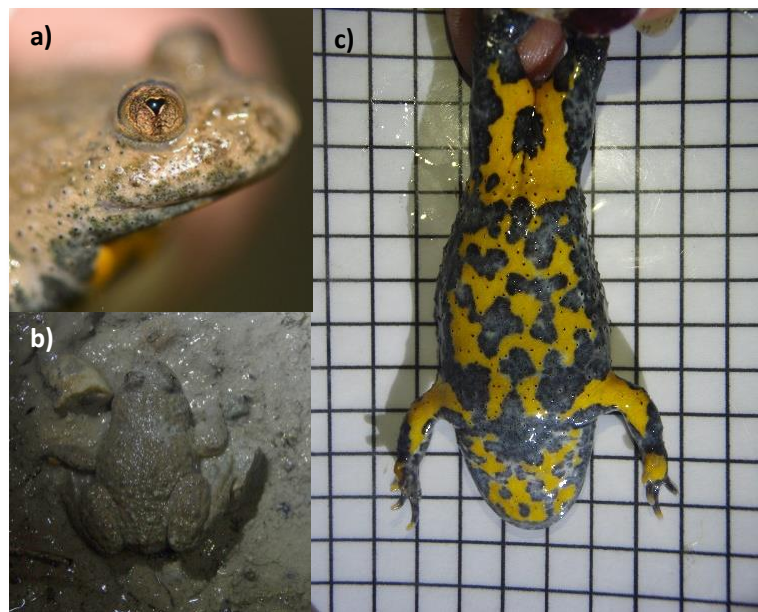


FIGURE 1.3. YELLOW-BELLIED TOAD. a) heart-shaped eye. b) dark back colouration. c) belly colouration (individual TEP013).

ii. Behaviour

As most of the amphibians, the BOVA is mostly active during the night and the hot season. Indeed, during winter, it goes to the forest to hide and reduces its biological rhythm to spend the cold season. It does not hibernate (Puissauve et al., 2015). The distribution of this species extends over much of central and southern Europe, from the altitude of 100 meters to 2'000 meters. It is listed as Least Concern by the IUCN in 2004 due to its wide distribution, but some populations have experienced local extinctions or declines as in Switzerland. Such as most amphibian species, the BOVA is highly philopatric, implying that it does not usually move over great distances. Several studies have been conducted on the movement of amphibians during several periods of their life cycle, especially during breeding seasons. Beshkov & Jameson (1980) assumed that if animals move consistently in some directions, the distance covered will increase in proportion to the time between captures. On the other hand, if they move randomly, within a kind of home range or around a preferred site, the distance covered between captures will be representative of the activity of the animals. He also found in his study that the BOVA travelled differently between two captures according to the period (no difference between the genders). During the spawning period, the recorded distances are 20-60m, during the whole season, they reached more than 600m and between years, the records were of 100-300m, knowing that the time interval between two captures was random. Since the behaviour of the BOVA is highly dependent on the temporary ponds that are formed after heavy rains, it makes sense that a potential trend of movement is more likely to be seen when it seldom rains than when the rain is abundant, as they will search for a suitable habitat in a more active way and on a larger scale if their current territory is dry (Barandun & Reyer, 1998).

The diet of the BOVA does not differ much from other amphibians. The highest diversity of prey taxa is found in adults, and most of the preys are terrestrial (95%) (Sas et al., 2005). A study analysed stomach contents to assess the feeding habits of this species. It appeared that the toads mainly feed on invertebrates, most of them being terrestrial objects (45%: Formicida, Araneida and Coloptera). They can also feed on low mobility preys such as Gasteropoda, since the snails are attracted by humidity when the season is dry and thus approach the water points, or Lepidoptera larvae that might fall from the trees into the water (Groza et al., 2008). Other studies advanced that the BOVA might also feed on aquatic crustaceans (Amphipoda and Cladocera) (Sas et al., 2004; Groza et al., 2006) and it seems that juveniles prefer feeding on Collembola (Sas et al., 2005). The tadpoles, as for them, are herbivorous and macrophagous, feeding on large plants and algae (Günther, 1996).

When a *Bombina* feels threatened, it can show different behaviours. When in the water, the defensive response is usually to swim away and hide somewhere in the pond. On the other hand, when the toad is on land and perceives a threat, it arches strongly its back, lifts its legs and bloats itself in an attempt to look larger. This is called the Unken reflex and is done in the aim of showing its ventral colouration to indicate its toxicity to the predator (aposematic colouration: Kuchta, 2005). It will then stay immobile for a

while. Toxins are especially released from the parotid glands during such reflexes (Marco & Leguia, 2001; Harkewicz, 2004).

iii. Habitat

There are several important habitats for the Yellow-bellied toad, because it changes its location during the year. Indeed, during winters, the animals shelter in the forest where they hide in the litter, in the ground, under rocks or stumps to keep the humidity without risking a frost. When spring arrives and the air temperatures rises, it gets active again and will move toward the breeding area (Puissauve et al., 2015).

The BOVA need very specific conditions to survive. Indeed, during this active period, it is known to live in pioneer habitats, such as sunny ruts filled by the rain in various habitats ranging from agricultural (meadows, marshes, humid forests) to industrial places (quarries, landfill sites, building sites) (Bauer, 1987; Mermoud et al., 2010). In addition to the aquatic habitat, toads also need terrestrial refuges during the breeding season. Indeed, as the breeding activity takes mostly place during the night, the animals go out of the water during the day and need to hide under wood piles, or bushes or in the vegetation surrounding. The survival and breeding success of the BOVA depend on the presence of both types of habitats. The problem with temporarily humid areas is that their small surface makes them very sensible to an intense evaporation. Yet, if the drying of the area happens too fast, the tadpoles which would not have had the time to complete their metamorphosis would be condemned. If a larger permanent pond better retains the water, it also welcomes more predators or competitors, whereas a small pond suffers the risk of desiccation, but its size allows the water to reach higher temperatures more and will be less attractive to other amphibians as resources are limited.

There are many hypothesis on what type of pond toads use for spawning. Barandun & Reyer (1997a) studied the spawning pattern and toads habitat choices habitat in Zurich. They considered eight factors for they analyses: pond duration (how many days each pond contained water), water temperature on sunny days, vegetation cover (percentage of the surface area that was covered by plants), period of the year when spawning was observed, surface area of the pond and presence of newts, invertebrates and other anuran competitors. The research in Zurich lead to the conclusion that the toads preferred spawning in ponds of intermediate duration because they were the best trade-off between water availability and predator and competitor presence. They also concluded that the pond duration was a more important factor than predation or competition for larval survival.

iv. Development and Breeding habits

The *Bombina* is sexually mature after two to four years (Barandun & Reyer, 1997b; Puissauve et al., 2015). The spawning period lasts three months, between April and July (Barandun, 1990). It is only then that sexual dimorphism can be seen. Indeed, males have nuptial pads on the forearms that serve during the amplexus allowing the male to hold the female. They disappear at the end of the season. The call can be heard

during the first half of the reproduction period. It sounds like a weak “hou hou hou” that does not carry far away for human ears. Eggs are laid by the females in small masses on branches or other stalks found in the water (FIGURE 1.4A). They hatch after less than ten days. Then, the metamorphosis occurs after a maximum of 60 days, but is often observed after 38 to 45 days (Barandun & Reyer, 1997b; Puissauve et al., 2015). The tadpoles have a length of 30 to 35mm when they are at an advanced stage. The tail is proportionally short and does not exceed 1.5x the length of the body. The eyes are small and placed rather on the top of the head. A transparent envelope allows to see the violin shape of the body in the youngest tadpoles. The mouth is facing downward (Bühler et al., 2007) (FIGURE 1.4B).

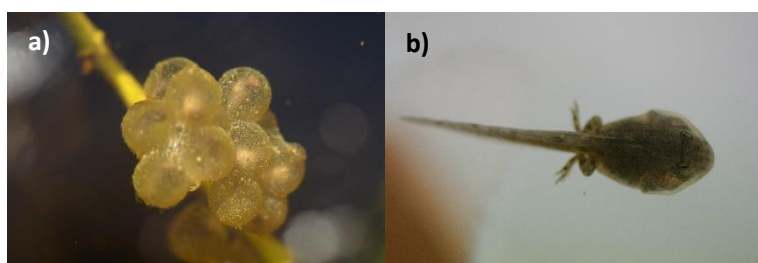


FIGURE 1.4. YELLOW-BELLIED TOAD. a) eggs in clutch. b) tadpole.

As mentioned above, the survival chances of tadpoles are highly dependent to rainfall and evaporation (Barandun & Reyer, 1997b). Globally, it is known that the survival rate is very low. A study in Romania showed that less than 4% of the eggs grow to sexually mature adults (Fuhn, 1970; Beshkov & Jameson, 1980).

As explained globally for other amphibians, tadpoles live in water and breathe thanks to gills, whereas juveniles and adults have lungs and breathe air. These latter also breathe, and drink, through the skin.

v. Threats

As in other parts of the world, habitat loss and landscape fragmentation are the main threats for Swiss amphibians and the disappearance of humid areas concerns important superficies (Cushman, 2006). In this country, we estimate that 90% of these habitats have been reduced in the last 150 years, even 100% in some regions (Imboden, 1975). This is due to the fact that cities expand and take the place of natural areas. Another consequence of urbanization is that the road network is denser than before and might affect animals. Indeed, several consequences follow on from the road network densification. First, as we saw earlier, habitat fragmentation leads to the isolation of populations and thus to their genetic diversity loss. Besides, as many roads are illuminated with street lamps, the light pollution increases and can disturb nocturnal animal behaviours that might change their mating, feeding and breeding strategies (Wise, 2007). Moreover, as the traffic becomes denser, risks for animals increase. For many amphibians, wintering grounds are different from breeding grounds and when they need to travel from one habitat to the other, they might need to cross a road. There is thus a high risk of road kill (Santo et al., 2007; Fahrig et al., 1995). The risk is also

present if the light pollution attracts the amphibians that want to follow their prey (Baker, 1990).

Another major threat that leads to the diminution of populations seems to be the droughts due to climate change (Cayuela et al., 2015b). As in most amphibian species, the highest mortality occurs at the larval stage, because tadpoles cannot survive if the water disappears (Barandun & Reyer, 1997b). It means that tadpoles survival rate is a determinant factor for toads population dynamics (Berven, 1990; Banks, 1993) and that a rapid larval development is an efficient strategy to reduce risks and thus improve survival rate.

Predation is obviously also a great threat, as it can eliminate a whole wave of breeding (Werner, 1986; Banks & Beebee, 1987). The main aquatic known predators are fishes, salamanders, newts, other anuran species and several invertebrate species. When fishes are not present, as in temporary ponds, insects can become very abundant and thus also become the first aquatic predators (Batzer & Wissinger, 1996). Birds are also considered as predators but they will not be taken into account in this study as no event was observed or could be estimated (Barandun & Reyer, 1997b).

Competition with other species or among larvae also tends to reduce the survival and growth rates (Berven, 1990; Beebee & Beebee, 1978).

Adults are expected to avoid short-lived ponds, or cool ponds, or high densities of predators and competitors, which implies a dilemma when they choose their spawning site (Smith et al., 1983). Indeed, the optimal conditions (high temperature, high productivity, few predators) are found in small ponds displaying high desiccation risk. Concerning BOVA, more predators will occur in large ponds, which can be a reason why it evolved to prefer smaller ponds, but small ponds that can quickly dry will lead to a higher concentration of tadpoles in the remaining water or to the total dryness before the metamorphosis is completed (Schmuck et al., 1994). For tadpoles, the best strategy to maximize the survival would be to wait until they reach a large size to metamorphose because it would improve juvenile survival and get them faster to sexual maturity (Riis, 1991). However, some tadpoles choose the strategy not to wait until full size to metamorphose to survive in case the pond dries (Werner, 1986). Since BOVA is specialised in breeding in small and sunny temporary ponds, it evolved a development plasticity to be able to cope with variable environmental conditions in the aim of maximizing its reproductive success (Dittrich et al., 2015), such as in other amphibian species (Philippi & Seger, 1989; Buschmann, 2002).

In some regions, other mortality causes are, that this species is captured to be used as bait by fishermen, as pet (Harkewicz, 2004) or as laboratory tool by scientists (Kawamura et al., 1972; Simmaco et al., 1991). Moreover, the hybridization with the Fire-bellied toad (*Bombina bombina*) might also lead to the loss of pure populations in Transylvania (Vines et al., 2003) or in other contact areas. Diseases are also responsible for amphibian declines. The chytridiomycosis is an infectious disease provoked by an aquatic fungus, *Batrachochytrium dendrobatidis*. It was first isolated in 1997 in a Blue poison dart frog (*Dendrobates azureus*) in an American park (Longcore et al., 1999). It

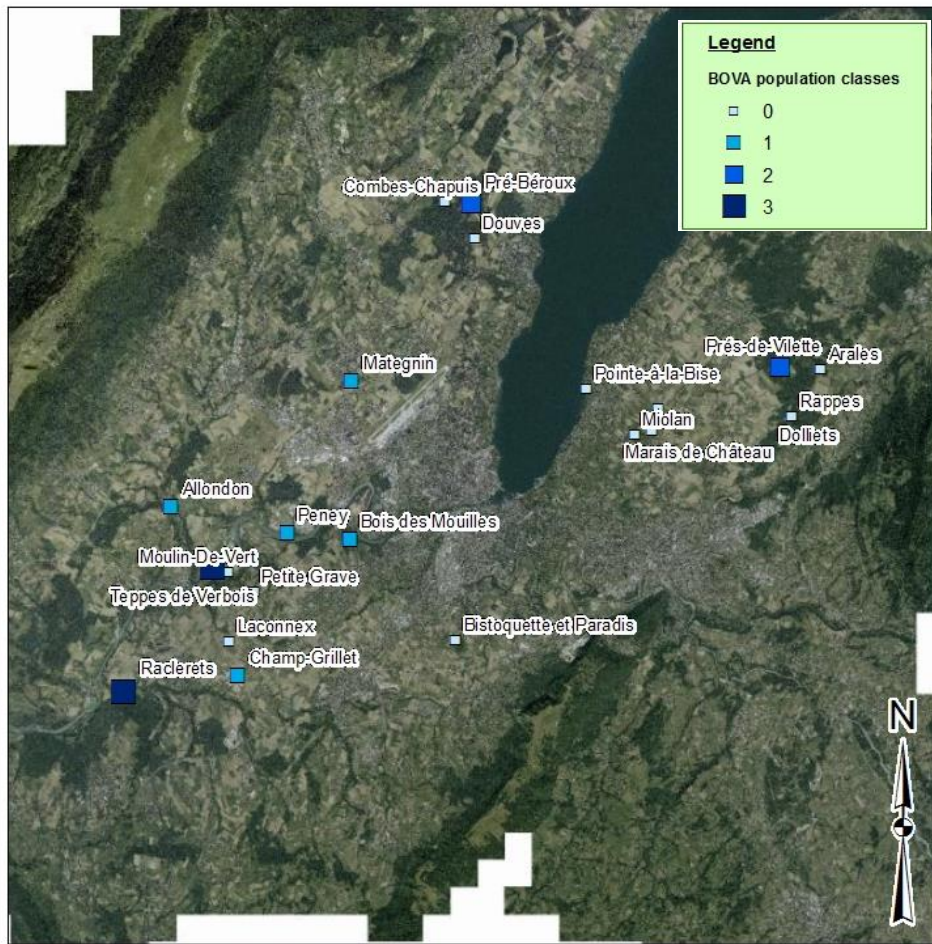
was detected in 387 species over 45 countries and manages to reach new geographical areas because of the expansion of invasive species. It is even more dangerous, because as it reduces the immunity system of contaminated animals, its effects can be combined with other threats such as pollution (Dejean et al., 2010; Fisher et al., 2009). Indeed, the contamination of water can play a role in the decline of some amphibian populations. These animals are very vulnerable to the quality of water as their skin is permeable. It was shown in Central America that high level of nitrogen were recorded in a site where a population suffered a huge decline (Whitfield et al., 2016). Until now, the impact of the infection by this fungus was demonstrated for its sister species *Bombina pachypus* (Stagni et al., 2004). About *Bombina variegata*, some studies showed that it can be contaminated, but that most of individuals are asymptomatic carriers (Sztatecsny & Glaser, 2011; Vacher, 2013).

vi. *Yellow-bellied toads in Geneva*

According to the last census, BOVA is listed as endangered in Switzerland and in Geneva. It was still present in all regions but suffered a huge decline and only 10 out of the 23 OBat sites showed its presence (TABLE 1.3, MAP 1.2).

TABLE 1.3. POPULATION CLASSES OF BOVA in the 10 sites where it occurred in the last review of OBat (Thiébaud, 2008). 1: small population (1-5 individuals). 2: medium population (6-30 individuals). 3: large population (31-100 individuals).

Site	Vilette	Dolliets	Raclerets	Mategnin	Mouilles	Raclerets
BOVA in 2007	2	1	3	1	1	3
Site	Champ-Grillet	Pré-Bérroux	Teppes	Allondon	Peney	Pré-Bérroux
BOVA in 2007	1	2	3	1	1	2

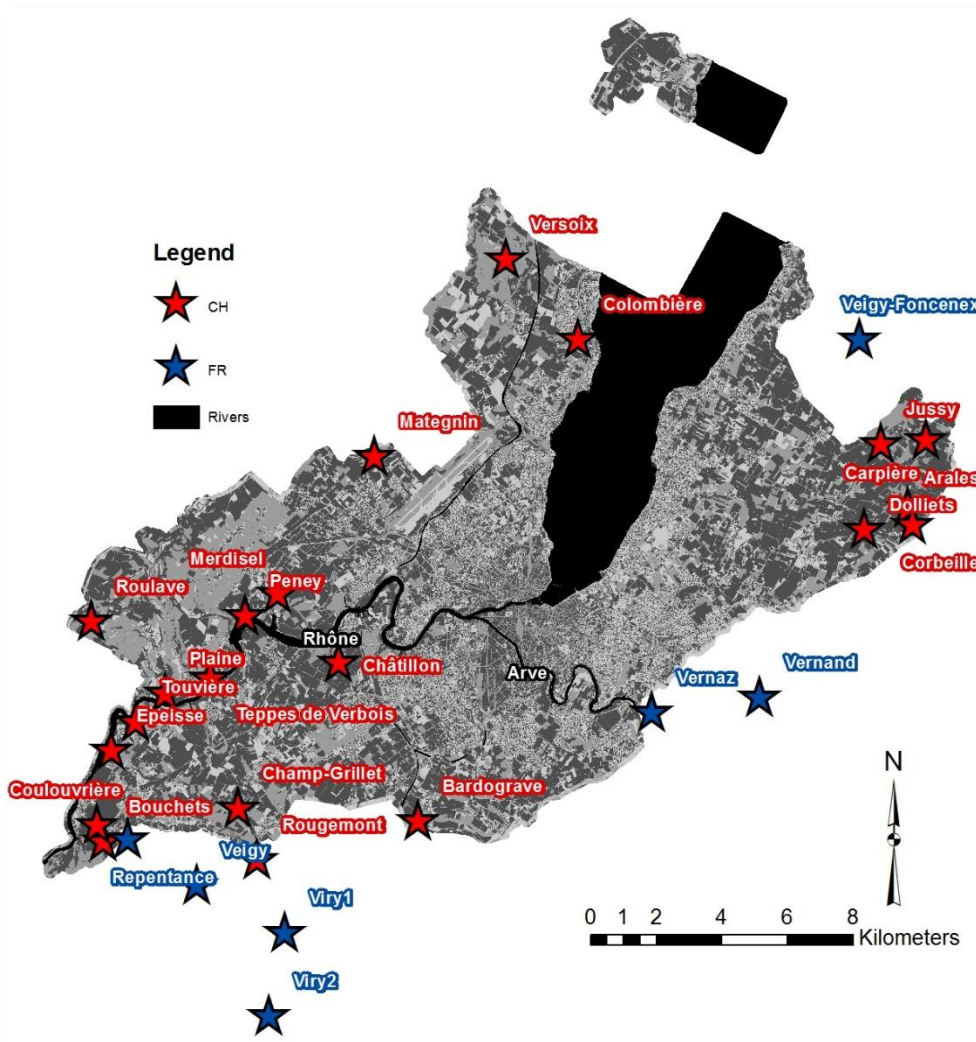


MAP 1.2. OBAT SITES CONSIDERED IN THE LAST REVIEW OF 2008. Only 10 sites allowed the observation of BOVA. 0: absence of BOVA. 1: small population (1-5 individuals). 2: medium population (6-30 individuals). 3: large population (31-100 individuals)

In Geneva, the global estimation of the Yellow-bellied toads populations started then in 2006 (Jaggi, 2010). Its aim was to assess the evolution of this species and the impact of climatic changes on it. More precisely, the first step of the study was to estimate the populations (2006 to 2008) and the second step was a global survey (2009 to 2010). It has been observed that populations are declining in the last 25 years and, in 2009, only 300 to 600 individuals were recorded in the Geneva area (Thiébaud, 2011). Cayuela et al. (2015a) showed that the presence of toads is negatively correlated with the diminution of forests, the development of agriculture urbanisation and road network densification, while it is positively correlated with the number of ruts on dirt paths and tracks made by vehicles. In Geneva, the settlement of the woody areas often destroys the favourable areas for the development of ruts. It is thus very important to maintain a constant observation of the populations to be able to determine the areas that must be protected. In 2011, the small density of BOVA in Geneva led to the elaboration of an Action Plan to improve the conditions of the population.

C. Studied sites

Based on the last review, only some of the OBat sites are considered in the study presented here. During the first year, the monitoring took place in a single site, as the aim was to assess the success of several measures installed in the Teppes de Verbois (TEP). Seven other sites were monitored by volunteers and the data, although incomplete, were used in some of the analyses. During the following years of the study, other sites were added either after some anecdotal observations, or to update the knowledge and estimate the remaining places where the Yellow-bellied toad (*Bombina variegata*: BOVA) occurred. In total, over 5 years of monitoring, 18 Swiss sites were checked for the presence of the species on a regular basis over one up to five years. Seven sites on the French side of the border were also checked at least once (MAP 1.3).



MAP 1.3. SITES MONITORED OVER THE 5 YEARS OF THE STUDY. 10 correspond to OBat Sites. Red: CH: Swiss sites. Blue: FR: French sites. Black: main rivers and lake.

Concerning the OBat sites, ten of them were chosen to be monitored during this study, among which two did not show any BOVA observation in the last review

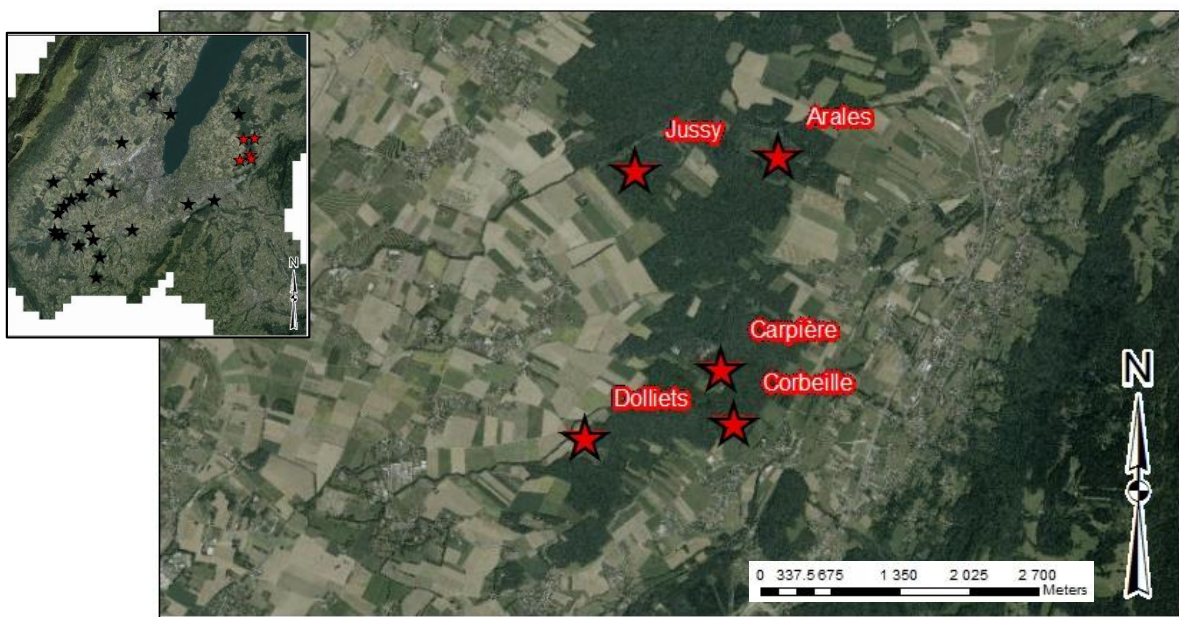
(Arales and Rappes). Some sites were slightly different than the exact location of the OBat, so their names were changed (Rappes became Carpière and Raclerets became Coulouvrière), and some different OBat sites were grouped in one big site with subsites (Versoix grouped Pré-Béroux, Combes-Chapuis and Douves, and Pré-de-Vilette was grouped with other new sites under the name of Jussy). The Allondon and the Bois des Mouilles are the only OBat sites with BOVA's presence in the review of 2008 that were not monitored in the present study because some informal monitoring during 2008 and 2012 did not reveal any toad.

For the OBat, Geneva was divided in four regions, but for our study, we changed this categorisation into five classes. This decision was made as we wanted to group sites that were potentially connectable by migrating toads. This is why the most isolated sites were grouped in the “outsiders” category.

The abbreviations used in this chapter will be developed in the methods part. PA means a Prospection Area, which means that the presence of BOVA needed to be confirmed so we widely explored the area. EA means an Emergency Area, which means that the presence of BOVA was confirmed, but that measures were installed to improve the fitness of the individuals.

i. Jussy Area

The Jussy Area extends on the Bois de Jussy which are some of the largest woods of Geneva. It includes five sites (**MAP 1.4**). The main threats for the toads are the Route de Monniaz and the Route de Juvigny which are intensely used by people travelling from France to Switzerland. The water connectivity is good in this area as it is covered by several small streams.

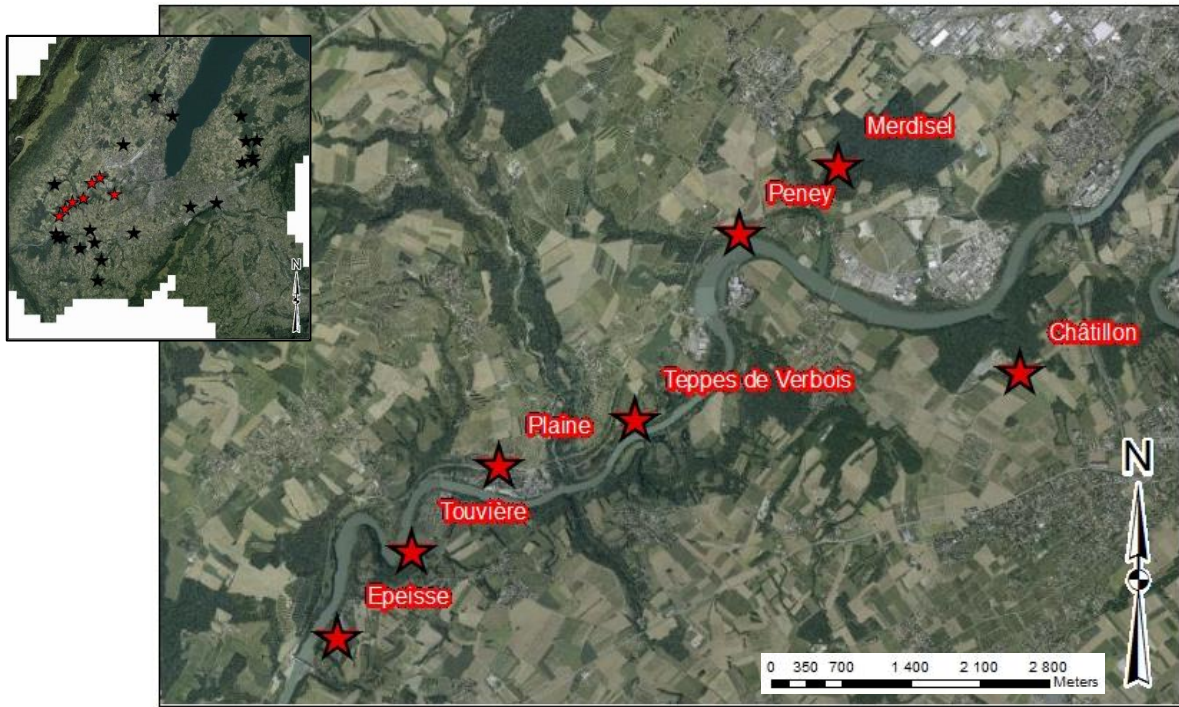


MAP 1.4. JUSSY AREA, EAST PART OF GENEVA. 5 sites are in this area.

- Arales (ARA): It is a surface of 25 ha on which three EA were monitored. The whole site is a forested area even if some sectors were opened in the last decade to favour oaks. It includes among others a meadow in which four lakes exist (two since 1973, and two since 2009). It seems that a part of these lakes is subject to eutrophication because of the surrounding agricultural lands. A ditch borders the area.
- Jussy (JUS): It is a surface of 54 ha on which six PA were monitored. The whole site is a forested area with various tree densities. Indeed, some places are opened (meadows), and some regions are liable to flooding. A forested path passes through the site. Visitors can use a sportive trail that cuts into the woods.
- Dolliets (DOL): It is a surface of about 3 ha that was monitored as a PA. The whole site is a forested area bordered by the Route de Juvigny. This site is of national importance and welcomes many amphibian species. Every winter since 2011, an amphibian barrier is installed to block the animals during their migration and protect them from being killed by vehicles. Wood works were conducted to cut some trees, creating wood piles and new ponds.
- Corbeille (COR): It is a surface of 4 ha where the oak is favoured. It thus includes areas with low tree densities surrounded by forest.
- Carpière (CAR): It is a surface of 16 ha that was monitored as a PA. The whole site is a forested area. It was created in 1950 to serve as water retention during floods and reservoir in case of wildfires. It is situated in the middle of the Jussy area, thus potentially playing the role of connector between the different sites.

ii. Rhône Area

The Rhône is a river that crosses Europe from the Alps to Camargue. In Geneva, it is used to create electricity thanks to the dam of Verbois, as a means of transport and as a relaxing place. Many efforts have been done to help fish, to improve their genetic mixing and their breeding success. The Vallée du Rhône is an important corridor that allows southern species to survive up North. A large diversity of animal species can be observed next to the river, because it is often surrounded by woods, reed beds, ponds, quarries and meadows. It is the case of the Reserve of Moulin-de-Vert that shelters an impressive birdlife as well as many species of reptiles and amphibians. Mammals are not outdone as several species of mustelids inhabit next to the river. Due to its high flow, the Rhône needs to be frequently monitored and the dams must be regularly drained. Thanks to the wooded corridor on the riverbanks of the Rhône, the humid areas on the right side are still well connected to the other Genevan regions. For our study, this area includes seven sites (**MAP 1.5**).



MAP 1.5. RHÔNE AREA, CENTRAL PART OF GENEVA. 7 sites are in this area.

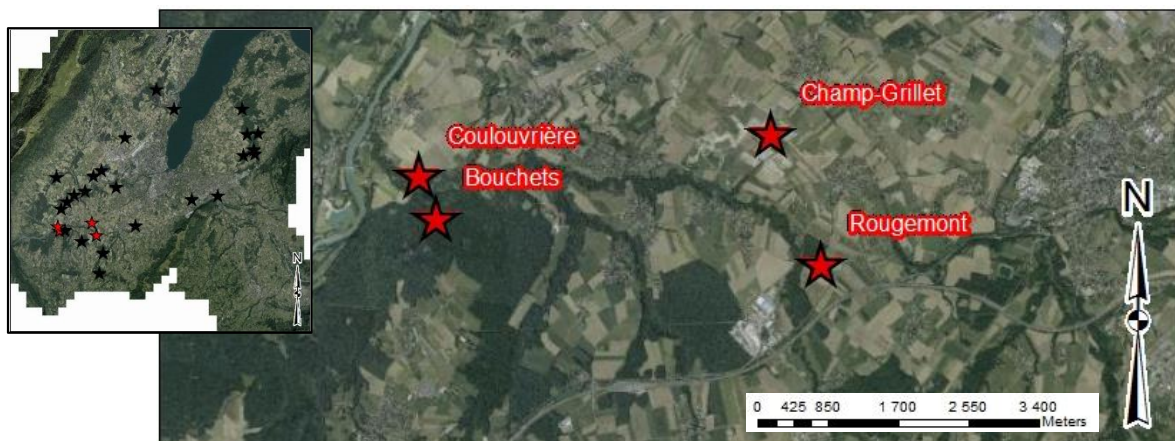
- Châtillon (CHA): It is a surface of 0.5 ha monitored as a PA and situated on the left side of the Rhône that takes place on an ancient meadow of Black locust (*Robinia pseudoacacia*) that is now well maintained as it is located under electric lines. This site stands next to the cantonal landfill site and it used to include all the surroundings (126 ha) before they were abandoned. The area is partly forested and partly open. It is limited to the East with the highway and reaches living area close to a riding school to the North.
- Epeisses (EPE): It is a surface of 17 ha situated on the left side of the Rhône that is used as a military exercise field. The open central place is surrounded by buildings and then by a woody sloping cord including ditches and drains. It is monitored as an EA area.
- Merdisel (MER): It is a very small surface of 0.3 ha situated a bit above the Rhône. It was not a real monitored site, but a potential observation of BOVA during the season 2015 lead us to check this site for the presence of the species. Unfortunately, it was not confirmed.
- Peney (PEN): It is a small surface of 0.3 ha situated on the right side of the Rhône. It is established on a steep slope of clay that suffers a permanent erosion between an agricultural and wine-producing area and the woody cord next to the Rhône. Seepages areas make it constantly humid. Bushes alternate with open areas but the layout of the site always changes due to rock slides. It is monitored as an EA area.
- Plaine (PLA): It is a surface of about 8 ha situated on the right side of the Rhône and located between a wine-making hill and railway line. The chemical products used in the winery can run down the slope and reach the area

where the toads live. A retention tank connected to a canal is the main water recipient of the area. It is monitored as an EA area.

- Touvière (TOU): It is a surface of 2 ha situated on the left side of the Rhône between a reed bed and a pasture. In case of a flood, the whole area can be submerged as the river flows as close as 5 meters away. The site is rather open but is surrounded by forested areas upstream and downstream. It is monitored as a PA area.
- Teppes de Verbois (TEP): It is a surface of 23 ha situated on the right side of the Rhône, opposite to the Reserve du Moulin-de-Vert. It used to be a quarry running for twenty years before its natural value was recognized and is now also classified as a reserve of national and international importance. It is about half agricultural land and half forested areas. The site is divided in five areas: 4 PA and 1 EA. The latter one is the area of Etang de Pêche and is accessible to visitors which can lead to disturbances. This site is limited to the North by the railway line. It includes lakes that shelter fish, which limit the development of amphibian populations. The wild fauna is well developed and it can help creating convenient habitats for the BOVA. Some areas of this site can be flooded.

iii. Laire Area

The Laire is a river that runs over France and Switzerland, crosses the municipality of Viry and flows into the Rhône in the municipality of Chancy. The human pressure is weak, which allowed the presence of a diversified flora. It appears that some areas dry in full summer and that the general flow of the river is weak. The valley of the Laire was the target of a management plan aiming to the land restoration of some parts of the river and, thus, favoured the biodiversity in these habitats. The exploitation of the quarries in this sector is still intensifying and threatens the pioneer amphibian populations. For our study, this area includes four sites (**MAP 1.6**).

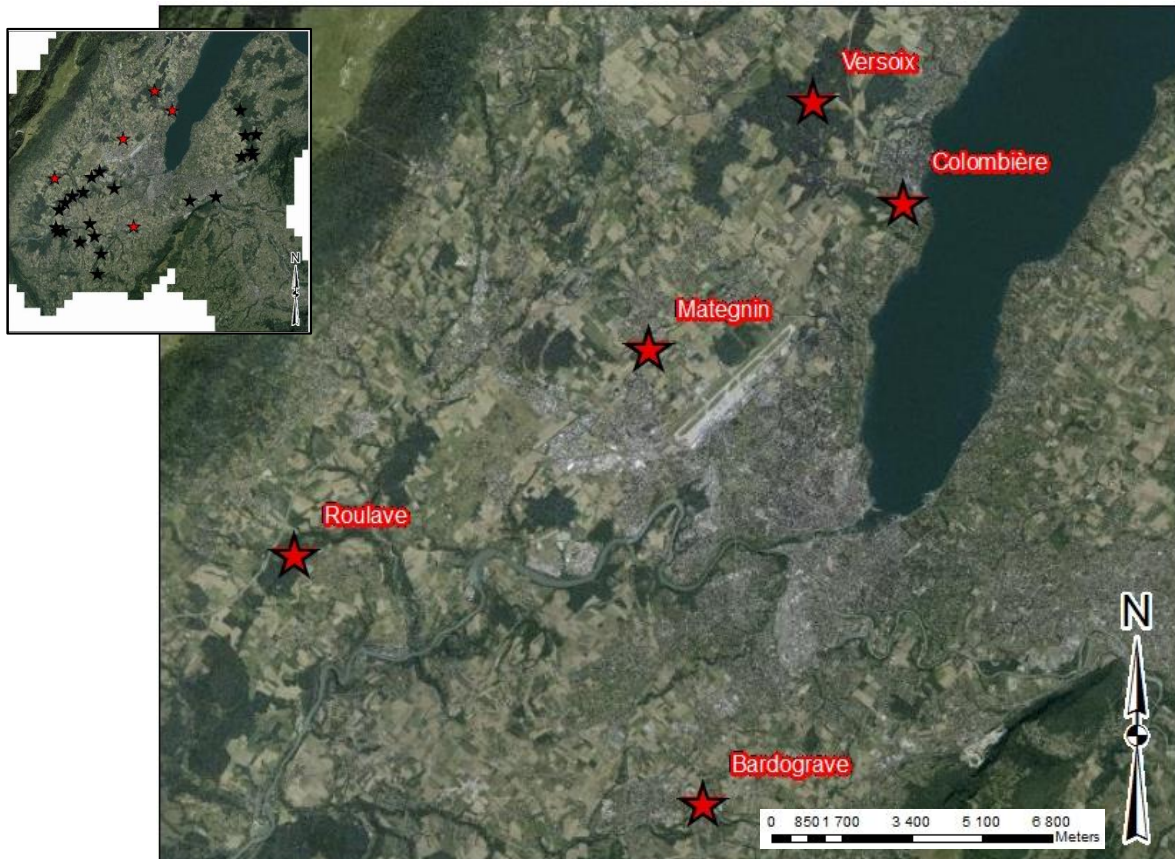


MAP 1.6. LAIRE AREA, SOUTH-WEST PART OF GENEVA. 4 sites are in this area.

- Bouchets (BOU): It is a surface of 30 ha globally monitored as a PA. The whole site is a forested area, composed by both oaks and pines, and especially the “pinède à molinies” which is a rare habitat of national importance that shelters threatened plant species. It also alternates flat and sloppy areas, such as valleys. In the aim of protecting these rare habitats, a sustainable forest management was set after an intensive exploitation. Besides, a part of the area is managed as a forested regeneration area. The monitoring could not take place in the whole area as some protected orchid species were not to be disturb.
- Champ-Grillet (CHG): It is a surface of 14 ha that has been running as a quarry since 1963. For a long period, it was also used as a landfill site for various materials coming from construction sites. Since 2000, only unpolluted excavation materials are accepted. The continuous use of the site created several ponds that led this place to be of national importance for amphibians, among other for the Natterjack toad (*Bufo calamita*), but it was monitored as a PA for the BOVA.
- Coulouvrière (COU): It is a small private area of 0.1 ha that is lightly sloppy. The area is covered by rather open, bushy meadows. A path borders it and the river. It was monitored as an EA.
- Rougemont (ROM): It is a surface of about 8 ha completely covered by forest. It was monitored as a PA. It is located next to the municipality of Soral, on the border with France and is limited by a road to the West and by fields all around. The ground is very flat and includes only few holes that can retain water, which means that the whole area is flooded when it rains but that then the water quickly disappears.

iv. Outsiders

Other places were monitored for the presence of BOVA but they are dispersed through Geneva and cannot be grouped in a specific sector (MAP 1.7).



MAP 1.7. OUTSIDERS. 5 sites are in this group.

- Versoix (VER and COL): VER is a surface of 2.9 ha, completely covered by forests. The Bois de Versoix also are some of the biggest woods of Geneva. They act as a connector between humid area of Geneva and Vaud. It is divided in three areas monitored as PA. The Creuson, a small river, passes through the site, but the monitoring did not take place around it as there are plenty of Marsh frogs. Some part of the site are used as regeneration places for oaks. Works were conducted to cut woods and create wood piles as shelters. The area which is the most favourable for BOVA is the northeast, because it includes forests and sunny meadows as well as artificial measures created for the species. The construction of a gas pipeline lead to the formation of many ruts and a ditch. COL is a small private surface of about 2 ha. The owners accepted to install buckets or other structures to welcome the toads. Hedges separate the different properties.
- Bardograve (BAR): It is a surface of 19.5 ha monitored as a PA and situated on a running quarry, thus the disposition of the elements can change during the season. This place is an important biotope for many plant and animal species. The managers of the quarry have the will to favour biodiversity that is why they regularly create rut or ponds to maintain amphibian populations.

Since 2008, workers are trained to learn how to manage nature in a quarry. North of these activities, stands a private field that was monitored as a PA.

- Mategnin (MAT): It is a surface of 5.3 ha located on a nature reserve classified as of national importance for amphibians. ProNatura, one of the main Swiss associations for the protection of environment, fights since 1928 to protect these important sites and thanks to the extension of marshes, many rare Swiss species found a shelter. Part of the marshes are situated inside a forest and the rest borders agricultural land and surrounds a lake. A green corridor runs from the marshes of Mategnin to the Rhône. Concerning BOVA, the lack of pioneer habitats prevents it to establish. The last observation probably comes from the industrial area when it used to be abandoned and humid, so this site was monitored as a PA.
- Roulave (ROU): It is a surface of 6.5 ha located in the Bois de Roulave. It is important for its floristic diversity, among others for the numerous bryophytes that exist there, and for its role as a shelter for many birds. In 2012, a forested sanctuary was created. Some parts are totally forested and some others are regeneration areas or pasture for horses. This site was monitored as a PA.

v. French areas

We choose some sites to have a comparison between the Swiss and the French populations. Indeed, the management of the land is not the same on both sides of the border and it seems that the French populations are in better condition than the Swiss ones. We collaborated with the municipality of Ambilly and Gaillard as well as with the association Apollon 74 who is in charge of many projects about biodiversity in the Haute-Savoie (France, 74) and more globally in the Rhône-Alpes region.

All the French sites were not monitored as the Swiss ones, but were checked once or few times, this is why information about them is less complete than for the previous sites. They are represented in blue on **MAP 1.2**.

- Vernaz (VEZ): It is a surface of 0.15 ha located in the Bois de Vernaz, on the sides of the Arve which is the main affluent of the Rhône. It is an important place thanks to high floristic, animal and ecological diversities and a pedagogic trail is installed. Visitors can pass through the forest to reach a meadow with picnic areas which can be a great source of disturbance. We mapped many natural and semi-natural measures, but they were filled by a heavy rain and then dried quickly. We took in consideration only one pond where toads were present.
- Repentance (REP): It is a surface of 3 ha, located in the Bois de la Joux. The site is surrounded by forests but the centre part is more open and rolling. We checked the whole area but only few depressions were of interest. We took in consideration only one pond where toads were present.

- Veigy (VEI): It is a surface of 2.5 ha located in a forested area but mainly under the electric lines. The place is thus quite open with the edge of the forests all around. We checked the whole area but only few depressions were of interest and no toad was observed.
- Veigy-Foncenex (VEF): It is a very small site displaying two ruts on a forested path of 100 meters long. This site is shown on our global map, but was not monitored during this study. However, it used to be an important site with about a hundred toads in 2006.
- Vernand (VED): It is a small site of 100 meters long on the side of a major road with much traffic. The municipality decided to create some measures to promote biodiversity and dug three ponds where toads were present.
- Viry (VIR): This site is divided in two places. One is a surface of 0.2 ha in a private place. It consists of two rather large artificial ponds created by the farmer to retain water before using it to irrigate his fields. No toad was observed in this part. The second part is a surface of 1.5 ha located in Bois du Ban. The area is totally forested and rather humid as some small streams runs through it. We mapped the whole area as Apollon 74 installed buckets for the conservation of BOVA. We also recorded some humid suitable places.

D. Aims of the thesis

The aim of this study is to improve the knowledge on an endangered species to be able to better protect it in a highly urbanised area. This work is divided in four main chapters: Ethology, Ecology, Eco-ethology and Genetic. In this way, the study and the conservation of the Yellow-bellied toad can be approached from several perspectives.

The first part is the Ethological part. This term is here used in a broad sense. We grouped in this part the analyses concerning the **Yellow-bellied toad's** (*Bombina variegata*: BOVA) **distribution in Geneva** and its **migratory behaviour**, as well as those linked to **other living organisms**. The first objective was to perform a wide compilation of everything that was done in Geneva on the Yellow-bellied toad. Indeed, as this species is one of the most threatened amphibians in Switzerland, a lot of prior work has been done to update the knowledge of this animal in this region, but they have not been reviewed to summarize the results. Our first step was to compile these data to be able to decide where the field work had to take place. Then we aimed at updating the data on the population's status thanks to field work and capture recapture method. Urbanisation in Geneva is rapidly growing, changing the landscape constantly by works and constructions which degrades the environment and destroys many natural habitats. We thus checked whether the previous censuses of BOVA were still valid, meaning if the previously recorded populations still exist. We also needed to prospect for new suitable sites where the BOVA could have established itself. Moreover, the project of installing artificial ponds started in 2006. These measures, as well as semi-natural habitats that were dug on the field, have been created to improve the survival and reproductive success of BOVA, so a report needed to be done to assess their efficiency and their impact on toad populations. We divided the factors linked to these measures in three clusters. In our first part, we assessed the impact of the presence of other living animals that could act as predators or competitors for BOVA (predators/competitors cluster), such as Marsh frogs, newts and predatory insects. Finally, we investigated how the toads move in their environment.

The second part is the Ecological part. We grouped here the analyses concerning the influence of **abiotic ecological factors** on BOVA (water cluster and vegetation cluster). To assess how our measures affected the fitness of BOVA, we needed to evaluate the impact of the types of ponds and of several types of vegetation on the different stages of BOVA (adults, subadults and juveniles). The assessment of the conditions in which we observed tadpoles and eggs was also a clue to determine the suitable conditions in which BOVA can breed. In this part, we also investigated the importance and impact of **landscape fragmentation** on our populations. We used the software Linkage Mapper, developed to determine corridors in which wildlife can transit within an area. This allowed us to measure how our sampling sites are isolated from each other.

Next, the Eco-ethology part aims to be a link between the first two chapters. We here investigated the **movement patterns of BOVA** linked to the ecological variables,

and especially linked to the water availability. Indeed, since we installed plastic recipients on the field, we experimentally **manipulated the hydroperiod** of the area. We examined thus whether the animals showed specific movement patterns according to the type of habitat in which they are.

In the last part, Genetic, we used microsatellites data to assess **the isolation and the structuration** of our sampling sites. We used two different programs, Structure and GeneLand, to infer the presence of several populations within our study area, and to assign individuals to a certain population. Here, we also made a connection with the Ecological part as we conducted basic analyses of **landscape genetics**.

Finally, our work can find its roots in the field of conservation. We make recommendations to conduct better long-term the **conservation** of this species in Geneva. The Ethological and Ecological analyses allowed us to assess the **preferences** of BOVA for a certain type of habitats and especially to determine what factors play a major role in their habitat selection and migration behaviour, whereas the Genetic analyses allowed us to offer several views on solutions to improve survival and fitness of BOVA in Geneva.

II. METHODOLOGY

A. Methods background

i. How to study amphibians

The major problems to study amphibians are that they are mainly nocturnal and usually hidden in the environment, so the detection of individuals in natural conditions is difficult. Statistical tools exist now to overcome this issue (MacKenzie et al., 2002; Royle & Nichols, 2003) as well as specific sampling methods.

The timing of data collection is very important. First, it is much easier to observe amphibians during their breeding period as they come in numbers toward a water place. Then, their activity reaches a peak during the first part of the night, meaning that the data collection should take place about one hour after sunset (GHRA-LPO Rhône-Alpes, 2015).

Different methods were developed by researchers to conduct their studies. None of them are exhaustive but these approaches can lead to good estimates of the population sizes. Moreover, the efficiency of the method depends on the target-species and on the period during which the sampling is conducted (Petitot et al., 2014).

A first method to monitor the presence of amphibians is to observe the animals in their natural habitat. One procedure is to go on a site where the presence must be confirmed or studied and to check all the water points (natural or human-made) with a torch to assess whether there are individuals at the surface, on the sides or inside the water. This practice is useful to be able to tell whether the species is present or not, or to globally assess the number of individuals present at each field session. The count of eggs can be very interesting as it can give information about the breeding activity and then, if we link the number of eggs to the tadpoles and then juveniles number, we can estimate the survival rate (Berven, 1990; Banks et al., 1993).

If we need to precisely estimate the population parameters (abundance, survival rates, etc.), we need to conduct a Capture-Mark-Recapture methodology (hereafter referred to as CMR). The capture of the animals, especially adults, can be done by hand, with a net, or thanks to creels (GHRA-LPO Rhône-Alpes, 2015; Klop-Toker et al., 2016). Manipulating the animals allows to identify individuals and thus to have an estimation of the population size over a long period. This identification can be done according to several methods. Some species have particular colourations on their body that help the researchers to know how many different individuals are present in an area without the need to mark them. The spots or lines are used to differentiate the individuals without being invasive. It is especially the case with the Yellow-bellied toad (*Bombina variegata*: BOVA) and the Fire Salamander (*Salamandra salamandra*: SASA), but also for several species of newts (Barandun & Reyer, 1997c; Pellet & Pellet, 2003; Mettouris et al., 2016). In some studies, the animals cannot be recognized thanks to their bodies, so one needs

to mark them. This is done by adding some marks (tattoos, bands, tags) or by mutilations (toes, scales). This is very invasive and can induce stress and impact the survival or behaviour of the animals (McCarthy & Parris, 2004; Langkilde & Shine, 2006; Antwis et al., 2014). The toe-clipping method is commonly used for amphibians' studies, especially to mark juveniles, but also to take DNA (Campanella & Smalley, 2006). The principle is that researchers cut one or several toes of the animals according to a code for each season (Williamson & Bull, 1996). Thus, when they catch an individual, they can see either if it was already captured during this season or during a previous one, or if it is a newly captured individual. Some researchers also used this invasive recognition method for naturally marked species (Barandun & Reyer, 1997c; Beshkov & Jameson, 1980; Hartel, 2008) which is useless. To quote May (2004), the toe-clipping is a "casual barbarity" that can be avoided.

Another way to monitor anurans is thanks to their call. This method must take place during the mating period, when the singing males are active. The aim is to record the calling activity of all anuran species present in an area during a given length of time (Villena et al., 2016; Klop-Toker et al., 2016). As each individual sings on a slightly different tone, this method allows researchers to collect information on location, presence and number of individuals (especially males). Moreover, it is possible to link these call surveys with the assessment of population's persistence (Schmidt & Pellet, 2005). This method is especially useful for volunteers-based surveys as this method is easily taught and learned (Genet & Sargent, 2003; Villena et al., 2016).

In addition to the studies conducted in the field, lab work and experiments can also be used to improve the knowledge of the concerned species. Indeed, in wild conditions, the environmental factors are always difficult to assess and cannot be controlled, whereas it is possible to do so in the lab. When we want to study the biology of amphibian species, and as the environmental factors can strongly influence their development (Barandun & Reyer, 1997b), lab work can be useful to compare the development of several species that are placed under the same conditions (Rafinska, 1991). On another hand, when we want to study the impact of certain conditions on the biology of species, lab work allows researchers to control the variables and thus to assess their impact, whereas in the field, there can always be other external unknown factors that contributed to the observed results (Kruuk et al., 1999).

Field experiments are another way to test factors without the need to raise individuals in the lab. The conditions must be normalised at maximum for all the factors that are not tested in the aim of reducing their impact on the study. For example, Dittrich et al. (2015) installed tanks into the field in different habitats and took eggs from each. The aim was to monitor the effect of temperature, so the water chemistry was controlled (pH and conductivity) and the tanks were protected to avoid predators. Only 15 emerging tadpoles were kept in each tank in order to control for the density, the others being released into their original pond.

Obviously, lab work can be useful to raise individuals before releasing them to rebuilt extinct wild populations (Kinne et al., 2006). Indeed, for endangered species, the lab conditions protect individuals from any predators or diseases and thus, the reproductive success and the number of individuals that can be released is increased. This said, an important point needs to be considered here as if the species got extinct into the wild, it might be because the habitat is not suitable anymore. Thus, a study must be conducted before releasing any individuals into the wild to assess the suitability of the environment or recreating adequate habitat. This conservation measure will be explained below.

ii. *How to promote amphibians conservation*

Humans are changing the world. They are becoming more and more abundant and their impact on wildlife increases as well. For a long time, human expansion was done without any consideration for the environment and its inhabitants, and irreversible damages were caused. Luckily, movements are slowly appearing to change this trend and works are conducted to try to rebuild what was destroyed and fix what was damaged. As we saw, amphibians are the most affected group of animals and their decline is mostly due to the loss of their habitats. Many researches and conservation programs were created to prevent the disappearance of other species (Mendelson et al., 2006), especially in hotspots where a great diversity was observed. In 1990, a group was formed within the Species Survival Commission of IUCN “to determine the nature, extent and causes of declines of amphibians throughout the world, and to promote means by which declines can be halted or reversed”. Then, the first Global Amphibian Assessment (GAA) was organised and it led to the Amphibian Conservation Summit (ACS) in September 2005. This group designed the Amphibian Conservation Action Plan (ACAP) to set priorities for conservation and research actions (Gascon et al., 2007).

Four kinds of interventions were determined that, at that time, had to start immediately. First, there was the need to expand our understanding of the causes of declines and extinctions. Second, the documentation about amphibian diversity needed to continue, as well as the one about the changes that occurred. Then, long-term conservation programs needed to be developed and implemented. Finally, responses to emergencies and immediate crises needed to be found. In addition to that, eleven thematic areas grouped several priorities for conservation and research about amphibians. As we saw, one of the main threats to these vertebrates is the destruction of suitable habitats. One of the themes is “freshwater resources and associated terrestrial landscapes”. It thus concerns both the aquatic and terrestrial habitats of amphibians and is built in three points:

- Securing existing areas: The aims are to identify the requirements of the habitats, to improve the education of the public, especially of the young one, and of the policy makers, and provide management guidelines for managers and owners of land sheltering amphibians.
- Preventing future habitat loss: The aims are to quantify anthropogenic effects on amphibians, improve the education of the public to change

individual behaviours and industries impacts, provide management guidelines to minimize future habitat loss and identify methods to restore amphibian habitat and improve population sizes.

- Restoring disturbed or compromised habitats: The aims are to improve the education of the public and policy makers about the value of habitat restoration and healthy amphibian communities and ecosystems and to collaborate to develop effective restoration practices.

Before these international recommendations, the concept of the Green infrastructures (in French: Trames vertes) appeared in Europe in the 1990's and was especially developed after 2000 in France. It comes from an even older topic first created in Germany in the 1920's which aimed at connecting several green spaces with little paths (Renard, 1999). Two kinds of Green infrastructures exist now. On one hand, there are these green spaces that are natural and spontaneous and that were not ecologically planned. They often surround or follow paths or hiking trails. On another side, there is an ecological meshing on different scales that is scientifically planned, created and monitored. It goes with SIG mapping. The concept of Green infrastructure is the sum of all the biological connection areas and connected natural habitats. It is a factor of ecological coherence and cooperation as inhabitants, industrials as well as official politics are concerned and must work together to make it successful (Drobenko, 2004). The principle is to add or maintain hedges or other vegetal structures such as ditches, quarries, wastelands or slopes on the side of the roads, to keep the various habitats connected and allow animals to migrate between them while staying protected and finding resources. It is not focussed especially on amphibians, and the targeted species are often mammals or birds, but all species can benefit from it, as its aim is to reverse landscape fragmentation (Beier & Noss, 1998). The structures included in the Green infrastructures are various. They can be continuous or dispersed, they can be permanent or temporary and they can also be immaterial, such as migratory axes for birds or fishes. Recently, the trend was more towards clearing huge open areas to improve the surface of crops (Clergeau, 2007). Many people were not aware of the importance of these bushy zones for biodiversity and did not realise that the connectivity within the land was capital for the survival of many animals. Now that we know how rich in species are the hedges and other bushy patches within an open area, and how useful these shelters are, for example when a farmer mows his crops and forces the animals to flee, the development of biological corridors is more and more abundant and widely extended.

Within the wide topic of Green infrastructures which are the global principle of connecting areas, we often talk about biological corridors. They precisely are these patches that link several vital habitats for a species, a population, a metapopulation, a group or a community. They include various environment types, as moving animals need to feed, sleep and protect themselves against predators (Chetkiewicz et al., 2006). Among others, the importance of field margins was shown in the Genevan Champagne in Switzerland in a study concerning endangered farmland birds (Meichtry-Stier et al., unpublished). These structures are "uncropped but cultivated areas, either

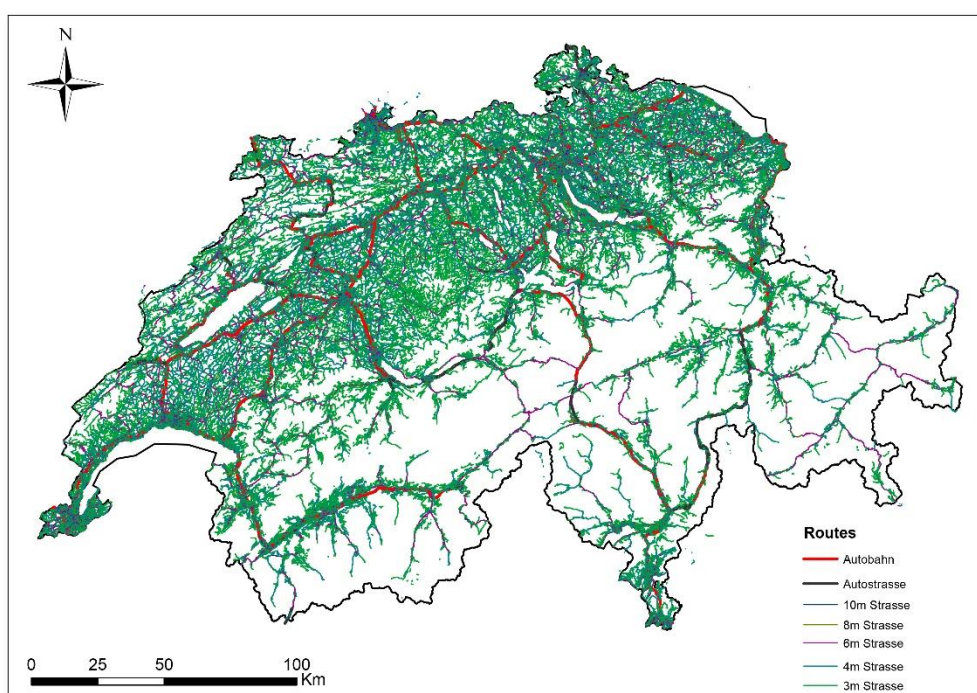
spontaneously vegetated or sown with species-rich seed-mixes and [on which] no or only few fertilizers and pesticides are allowed". 82 field margins were monitored during 11 years and the abundance of 13 farmland bird species of conservation concern was recorded. It appeared that as the proportion of field margins increased from 0,4% to 11%, the number of territories of nine out of the 13 species significantly increased during the whole or part of the study (Meichtry-Stier et al., unpublished).

Landscape fragmentation of landscape is due to many factors, but the road network is one of the most important threat, as it will separate previously continuous habitats and the roads can act as a minor up to a complete barrier (Jones, 2012; Trocmé, 2011). The roads themselves have many negative impacts on wildlife. Indeed, their construction means a sudden huge disturbance for the animals living in the area as well as ecological changes between before and after the construction. Once built, the traffic will lead to the increase of local noise, pollution and vibration that will have cumulative negative effects on wildlife. We talk about a "road-effect zone" (Beckmann & Hilty, 2010). Moreover, next to these indirect effects, the collisions between vehicle and animals are important direct sources of mortality (Glista et al., 2009; Kintsch & Cramer, 2011). It concerns many taxa, amphibians included. These animals are particularly affected when they annually move between their wintering grounds and their breeding habitats (Smit et al., 2006; Trocmé, 2005). The roads can kill a large part of the adults in a population which will affect the population dynamics (Vos & Chardon, 1994; Means, 1999; Carr & Fahrig, 2001; Hels & Buchwald, 2001; Matos et al., 2012). As mentioned earlier, and for many reasons, a population that suffers a reduced size increases its extinction risk (Bennett et al., 1999).

The presence of roads has different impacts according to the species and populations living around them. Some species can be attracted by the grassy areas alongside, but most of them will avoid these disturbing areas and will be negatively impacted (Jones, 2010). In the aim of avoiding or at least reducing this "road barrier effect", the concept of fauna passages emerged. It first appeared in Europe as a consequence of the post-war expansion of the road network that led to the isolation of mammal populations. The hunters started to ask for measures to counter these negative impacts. These passages are now worldwide spread, but Europe is still in advance in the number of installations in comparison to other places in the world thanks to the high standards of compliance for infrastructure developments (Jones, 2010). The fauna passages can be created over or under highways, railways or any major road. Their aim is to provide effective landscape connectivity despite the presence of the road and to reconnect habitats that became isolated since the road construction (Jones, 2012). These structures are rapidly accepted and used by wildlife (Soanes et al., 2013). First, they were not created for a particular species, but for some decades now, the construction of such passages is designed according to targeted species. Many passages are large and covered with vegetation to ensure shelter to rather large animals such as ungulates and other mammals. Amphibians also have their dedicated tunnels that are usually underpasses and must show specific conditions to be efficient, such as having constant and stable moisture and temperature (Jackson, 1996) and have a diameter of

100cm (Vos & Chardon, 1994). Again, Europe lead the way for these particular tunnels (Forman et al., 2003). For most of the animals, it seems that the wider the passages are, the more frequently used they are. However, amphibians do not show such a trend and might even use non-adapted passages or passages devoted to mammals (Veenbaas & Brandjes, 1999; Smit et al., 2006).

Switzerland has one of the densest infrastructure networks of the whole Europe (Oggier et al., 2001) (MAP 2.1). It led to problems for a large variety of species, such as the lynx, the roe deer or many species of amphibians. About ten years ago, a program of defragmentation was launched aiming at identifying bottlenecks where infrastructure intercepts wildlife corridors and to restore the connection where possible (Trocmé, 2005). This program is currently conducted and should still be running for ten more years.



MAP 2.1. ROAD MAP OF SWITZERLAND, including main roads and country roads (Swisstopo, 2016). Red: highways. Green: 3m-wide roads.

Various methods are used to confirm the efficiency of the passages and to see how they are used by the wildlife. Infrared detectors show how many individuals pass on the structure, whereas sandbeds or inkbeds can give information on the species concerned (Keller, 1999; Veenbaas & Brandjes, 1999; Smit et al., 2006; Martinig & Belanger-Smith, 2016). Camera traps can even allow the identification of single individuals (Guzvica et al., 2014). An experimental study tested the preferences of three amphibian species for different types of passages. It appeared that concrete tunnels are less often used than tunnels whose ground is made of soil, but some species do not like tunnels and will avoid them to reach grassy patches (Lesbarrères et al., 2004). Even if such trends can be noticed, it seems that the type of passage is less important than its location. Indeed, for species with low mobility like the amphibians, the passage will be

particularly efficient if it is located near suitable habitats and will help to reconnect habitats at a local scale (Smit et al., 2006; Rodriguez et al., 1996).

In Geneva, several roads cause a high mortality in amphibian populations. Three major axes are monitored. The Route de Loëx is a major road located in the center of Geneva. It connects the two sides of the Rhône. It is monitored since 2007 and permanent fauna passages were built under the road in 2009. The Route de Juvigny is located in the Eastern part of Geneva and is a major axe to connect France and Switzerland. It is monitored since 2009. Finally, the Chemin des Combes, also located in the Eastern part of Geneva, is monitored since 2013 after 94 dead toads were found. The two last ones, as well as the first one before the permanent passage was built, are followed in spring when the temperatures rise and the amphibians wake up to reach their breeding sites. A temporary barrier is placed on the sides of the roads and recipients are installed along it at regular intervals. The aim is to block the way of the animals before they reach the road. As they will try to pass around the obstacle, they will fall in a recipient. Volunteers check the presence of animals every morning and make them safely cross the road to reach the breeding pond (KARCH-GE website). In the next years, permanent measures are planned to be installed. A last point must be noted here. Even if these fauna passageways are important structure to mitigate the impacts of roads, they are also one of the most expensive tools to counter landscape fragmentation (Jones, 2012). Studies must be conducted to assess their importance before starting any work and specific designs must be elaborated to ensure that their characteristics meet the needs of the animals concerned (Martinig & Belanger-Smith, 2016).

iii. How to explore genetic variation in amphibians

As mentioned earlier, the study of amphibians in the wild is not always easy. They hide and disperse which makes it difficult to identify them and to analyze every biological aspects of these discreet animals if the study relies only on sightings. Luckily, the methods to investigate the life and behaviour of such wild animals are improving, and in particular, genetic protocols become very helpful. Since the 1970's, researchers are able to describe different species using protein variants (Lewontin, 1991), but it stayed difficult to go into more details and reach smaller levels than taxonomic groups. It is only later that the PCR (Polymerase Chain Reaction) technology allowed the study of other kinds of organization such as geographically distinct units. Indeed, even if a species has a certain range, it is possible that small parts of its populations experimented historical isolation and thus, became Evolutionary Significant Units that need to be preserved as they show distinct genetic diversity (Moritz, 1994; Ryder, 1986).

First of all, several ways to collect DNA exist for amphibians. The one that is surely the most ancient (Bogert, 1947) but also the most controversial is the toe-clipping method. For some researchers, it has a double advantage to both insure a great amount of DNA (Cornetti et al., 2016) and to help individual marking (Hartel, 2008). The impact seems to be various. Some studies certify that the animals are not affected by this technique (Hartel, 2008), some that there is no sign of impact (Van Gelder & Strijbosch,

1996) and some that it causes great damages (Clarke, 1972; Golay & Durrer, 1994; McCarthy & Parris, 2004). As in many species, blood samples can be collected from adults or post-metamorphic individuals (Baranowski-Smith & Smith, 1983; Wright, 1995), but the amount of blood that can be safely removed must be calculated and it usually requires the anesthesia of the animals (NWHC, 2001), which is also very invasive. The most ethical and inoffensive way of collecting DNA is to take buccal swabs which, even if the amount of DNA is clearly reduced next to the other techniques, was proven to be particularly efficient (LeVin et al., 2011; Broquet et al., 2007; Pidancier et al., 2003).

Recently, the use of high variation molecular genetic markers increased, as the costs of laboratory techniques decreased, and their analysis became more powerful, as the computing technology improved over the last decades. Moreover, they are of great interest in conservation biology. Indeed, they allow researchers to address ecological questions such as measuring local gene flow and local migration. Indeed, it helps identifying the most probable population origin for various individuals, measuring the effective population size, meaning individuals contributing to breeding or detecting potential past demographic bottleneck events (Jehle & Arntzen, 2002). Another advantage is that genetic material can be taken from both recent and old specimen, so we are able to compare them (Wayne & Morin, 2004).

Several markers are used for such conservation studies. Allozymes are common biological enzymes that are coded by different alleles at the same locus and exhibit high levels of functional evolutionary conservation throughout specific phyla and kingdoms (Parker et al., 1998). They can be used to provide evidences of the linkage between geography and genetic in fragmented populations, such as the effects of human-induced landscape fragmentation (Hitchings & Beebee, 1997). A single-nucleotide polymorphism (SNP) is a variation in a single nucleotide that occurs at a specific position in the genome. In the human genome, it is the most frequent type of variation (Wang et al., 1998). They can be used to estimate population structure and identify outliers (Allendorf & Seeb, 2000). Microsatellites loci are also part of these genetic markers. The principle of their utility is that they are very abundant in every genome of every eukaryote organism. They are repetitive units of DNA of usually 1 to 6 basepairs in length. The most commonly used repeats for molecular genetic studies are dinucleotides, trinucleotides and tetranucleotides (Selkoe & Toonen, 2006). The number of repeats is highly variable even within a species or within an extended population, which allows researchers to detect differences in the genomes of even closely related individuals. This high number of alleles is an advantage of the microsatellites compared to SNP that usually show only two alleles (Morin et al., 2004).

To be able to use them, one needs to amplify them. Therefore, we targeted the flanking regions of a microsatellite locus. The sequences of such regions are usually very similar between individuals even between different species. Thanks to primers, short sequences of DNA, that are designed to match these sequences, we can bind these flanking regions and guide the amplification of the targeted microsatellite locus (Selkoe & Toonen, 2006). It will result in fragments of DNA of varied length according to the

initial size of the marker. We can then sequence them and compare the length found for each microsatellite locus for each sample. As all eukaryotes organisms are diploids, each individual will show two alleles for each locus. In comparing these alleles among individuals and population samples, we can estimate the genetic diversity of a group (Freeman & Herron, 2004).

Another new tool was recently designed: the environment DNA. The basic idea under this new technique is that many organisms live in the environment without humans knowing about them, whether it is because of their small size or because of their discretion (Gotelli & Colwell, 2001; MacKenzie et al., 2006). However, it is logical that they let tracks of their passage and it is these tracks that we can use to detect their presence. Rondon et al. (2000), instead of culturing microorganisms under laboratory conditions as it was usually done (Hugenholtz & Pace, 1996; Staley & Konopka, 1985), built libraries of genomic DNA thanks to the isolation and amplification of sequences directly taken from soil. The advantage is that environmental DNA can be detected at very low concentrations (Ficetola et al., 2008). This approach is also often used with water samples to be able to discover new genes and species and to learn more about the species richness of an environment (Venter, 2004). Some studies also applied this technique to identify extinct communities of macro-organisms since short sequences of DNA can persist for long time periods in the environment (Hofreiter et al., 2003; Willerslev et al., 2003; Willerslev et al., 2007). Ficetola et al. (2008) even used this method to detect the presence of current macro species (amphibians) in natural environment, showing that this technique can be widely used for studies focusing on species' presence.

B. Focus on this study

As shown above, methods designed to help and protect amphibians are numerous and they differ depending on the focused species and the studied sites. According to the historical data about the Yellow-bellied toad's (*Bombina variegata*: BOVA) presence in Geneva, the Direction of Nature and Landscape (DGNP) decided to improve the conditions in the wild to make them more suitable for this species. Thus, artificial habitats were created in the different studied sites while considering what was still present in the area. Different degrees of emergency were estimated that would lead to different types of measures to protect this species. In the sites where the presence of BOVA was not confirmed for several years, a prospection area (PA) was determined. In the sites where the presence was recently confirmed, but the current number of individuals unknown, an emergency conservation area (ECA) was determined.

i. Types of measures

The measures installed or determined in each site were of three types (FIGURE 2.1).

- Type I "Artificial measures": Plastic buckets were installed in the field. They are mainly circular with a diameter and depth of 40 cm, but some are rectangular (65x35x29 or 72x42x30). In some sites, other recipients were installed, like flower pots.
- Type II "Semi-natural measures": Water points were dug and were made waterproof with a clay substrate. Their sizes vary but they all present the important characteristics needed by BOVA, meaning slightly sloped sides, a maximum depth of 50cm and a maximum surface of 15m² (Jaggi, 2010; Marchesi & Zanini, 2009) In case of a drought, a small part of the water point was dug deeper to act as a refuge if the water disappears.
- Type III "Natural measures": These were not created. We mapped all the natural holes, depressions and cavities that could retain water and potentially welcome toads. We searched for these places in a perimeter of 100m around the buckets or the semi-natural ponds, or, in places where there was no works done, we mapped all we could find in a given area.

In total, at the beginning of the study, we determined eight classes of water bodies (the abbreviations come from the French words) (FIGURE 2.1):

- Buckets (bacs: B): plastic recipients
- Ponds (étangs: E): artificial water point made waterproof
- Ditches (fossés: F): linear water point, often along a road
- Lakes (lacs: L): artificial big water point (which became also E)
- Natural (naturels: N): natural water point
- Ruts (ornières: O): water point created by the passage of a vehicle
- Muds (souille: S): water point created by wild fauna (which became also N)
- Others (autres: X): other water points, as the old hole of a bucket



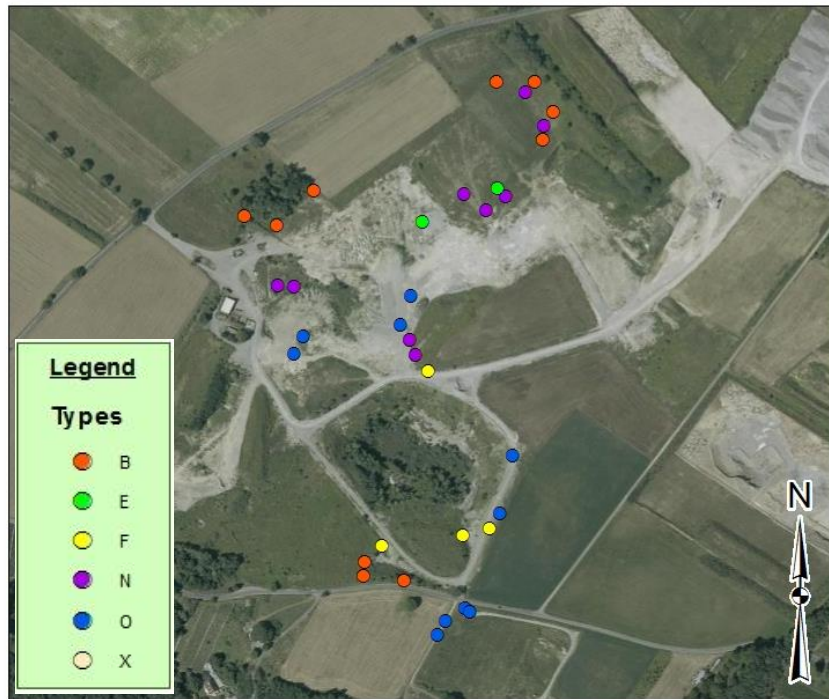
FIGURE 2.1. EXAMPLES OF SOME MEASURE TYPES. a) round bucket. b) rectangular bucket. c) rut. d) lake. e) pond.

ii. Characteristics

All the ponds were measured using a laser distance measuring device DeWalt DW040 for the length and width and using a scaled stick for the depth. All the lengths considered were those measured when the water point was potentially at its maximum water capacity. Most ponds were equipped with a pole and a tag giving its name contact information in case visitors wonder what these measures were for and wanted more information. The alphanumeric code of each pole was two letters and two numbers, though some ponds added later during the study got an extra letter to identify them.

The location of each measure point was decided according to the knowledge of the species, the previous data and the most recent ones. If no data was available, locations considered as Prospection Areas (PA), we either only mapped the natural depressions we could find, or we installed buckets randomly through the area in the aim of maybe reaching the last individuals remaining, and we mapped around them (MAP 2.2). If BOVA's presence was recently confirmed, locations considered as Emergency Areas (EA), buckets were regrouped in islands in the areas where we knew there were individuals to try to supply them with habitats that will keep water during the whole season (MAP 2.3). In some places, works were conducted to dig small ponds to add some semi-natural habitats that would hopefully keep water. In the areas where some populations seemed well established, we also installed the buckets in lines to prospect for their presence further away and to connect different colonised subsites. The

mapping was conducted in as many sites as we could to increase the chances of contacting toads.



MAP 2.2. EXAMPLE OF PA. Champ-Grillet is a place where the presence of BOVA was not confirmed for some years, so the buckets were placed randomly to survey the whole area. Orange: buckets. Other colours: natural and semi-natural ponds.



MAP 2.3. EXAMPLE OF EA. Corbeille is a place where the presence of BOVA was confirmed, so the island disposition allowed us to improve the breeding success of the animals. Orange: buckets.

iii. Population census procedures

At the beginning of each field season (March-April), the buckets were installed thanks to the Association Ok Forêt. The workers were in charge of placing the buckets in the previous holes or digging new holes to place new buckets. In each bucket, they added two bricks (one laying flat on the bottom and one on its side to reach the edge of the bucket) and one small branch. They made sure that there was neither a gap between the edge of the bucket and the top brick nor a huge step thanks to a little earth bridge. The bricks play the role of hiding places for the toads and the branch is useful for the females to attach their clutch (Barandun & Reyer, 1998). Ok Forêt was followed by SITEL SA workers who carried a water tank and filled the buckets once they were installed. The water used was taken from fire hydrants as it is important that the water doesn't carry any smell of potential predators (insects, fishes) (Hartel et al., 2007a).

During the whole study, the number of studied sites varied according to the data collected. In 2012, only one site was monitored to follow both the new built measures and the installed buckets. There were two census sessions per week for five months. In 2013, we monitored 12 sites dispersed in Geneva once a month, except for the main site which was followed twice a month. In 2014, three sites were abandoned because no BOVA were detected during the previous season and the populations were declared locally extinct. We monitored 6 sites once a month. In 2015, we monitored 11 sites once a month. Three sites on the French part of the border were added to allow some comparison with the Swiss populations and were checked once. In 2016, we monitored 7 sites once a month. Each year, we also got some incomplete data that will only be used in part of the analyses. The **APP. B1** shows the exact list of monitored sites per year and the reason why some were added or abandoned. The column AD shows where we got some anecdotal observations which are incomplete (for example data obtained from volunteers with no environmental data, no exact location).

The number of water points in each site varied as some were discovered afterwards and some were abandoned because they were considered as not suitable (full of water during the mapping, but dry during the whole season or full of vegetation that forbid an accurate monitoring). Indeed, drying ponds rarely welcome toads and we recorded only few encounters on dry land (personal observation). Furthermore, we also abandoned and chose not to consider lakes in the analysis, because their exhaustive monitoring and census was impossible. But abandoning their monitoring was deemed acceptable as, according to Duellman & Trueb (1994), this type of habitat is not used by most of the anuran species for spawning as it leads to high larval mortality rates (Licht, 1974). Works to add some semi-natural water points and the mapping of the areas were also conducted at different time of the study. The **APP. B2 AND B3** shows the exact number of type I, II and III measures in each site per year.

C. Data collection

Once all the sites were decided and all the measures installed or mapped, we started the data collection which took place from April to September as suggested by other studies (Barandun & Reyer, 1997a). The sites' names were each given an abbreviation coded in three letters. The toads observed in a site were named after the site with a code putting together the abbreviation of their site and a 3-digit number. For each session, we checked every measure in each site, and for each one, we noted abiotic and biotic factors. As there were several groups of observers, protocols were devised for each point of the data collection (**APP. B4: DATA COLLECTION, B5: PHOTO-IDENTIFICATION, B6: DNA SAMPLING**). The Yellow-bellied toad (*Bombina variegata*: BOVA) being mainly active during the night as most of the amphibians, the field sessions took place after sunset (Barandun & Reyer, 1998). It randomly happened for us to be in the field during the day and we estimated that less than a third of the animals could be observed by day-light (personal observation), knowing that this number obviously depends on the weather and temperature.

Although we did not record the pond duration (number of days that ponds contain water) (Barandun & Reyer, 1997a), we did consider the percentage of available water at each visit. Combined with the dimensions of each pond, we are able to estimate the volume of water available for the toad at each session. The ponds range from 0,4m² to 30'000m² and the volume from 45m³ to 43'200m³. These numbers obviously varied as a consequence of desiccation, this is also why we preferred considering the water availability which then ranged from 0 to 43'200m³. As for the vegetation cover, we did not record the percentage of coverage, but only if there was vegetation on the surface or in the depth forming places that the animals could use as a refuge or hiding place. This factor was also important to determine whether there were underestimated data as the visibility was reduced. Finally, we did not consider the water temperature for individual census because of practical reasons.

Practically, we first collected data on paper, but then, as the weather was often uncertain, we used a voice recorder in the field and then transferred the data on computer. The chytridiomycosis is not present in our study area yet, however, we try to minimize the potential risk of transmission of the fungus. As we caught the toads by hand, we did not need any material that could touch different toads and contaminate them. The only danger was if we stepped in the ponds as the boots could transmit the disease, but we avoided it as much as possible. In case it happened, we made sure that the material dried for at least three hours between two sessions, as the fungus cannot survive if it is exposed to air during such a time period (Johnson et al., 2003).

i. Abiotic factors

- Water quantity: To assess the potential water availability, we estimated the quantity of water in each measure. To reduce the observer's bias, we decided to classify the water availability into 7 ranks. The principle is to compare the actual water capacity of the measure with its potential maximum filling and to estimate the percentage of water present during the data collection. The 7 ranks were: **0%** = empty or dry pond / **1%** = wet pond (1cm of water) / **15%** = only refuge zone with water / **25%** = one-quarter full / **50%** = half full / **75%** = three-quarter full / **100%** = maximum water capacity reached. The term refuge originally concerned the semi-natural ponds, as a deeper zone was dug to retain the water longer in case of a drought, but it was then generalised to all ponds, meaning it retained only 15% of the water. During the last two seasons, an emergency call to the DGNP was made if the water level of an area was dangerously low and the water tank came to refill some ponds.
- Turbidity: To assess the potential visibility and thus the accuracy of the data, we estimated the turbidity of the water. To reduce the observer's bias, we decided of 3 ranks linked to the probability of seeing a toad: **clear (C)** = if a toad dives, we can follow it to the bottom of the pond and see where it stops / **clear-trouble (CT)** = if a toad dives, we can see where it goes, but we are unable to see where it stops / **trouble (T)** = if a toad leaves the surface, we are not able to see where it goes.
- Weather: At the beginning of each field session, we recorded the air temperature, the cloud cover of the sky according to three classes: clear = no cloud at all / covered = as soon as some clouds were present / rainy = when it was raining. We considered only the weather characteristics at the beginning of the session even if the conditions changed during the evening.
- Other data: Next to the environmental variables, we also recorded the date of the session, the time at the beginning of the field work and the time at the end of the field work.

ii. Biotic factors

- Presence of algae: To assess the potential visibility and the probability of missing a hidden animal, we noted whether there were algae at the surface or inside the water. The algae are an important food resource for many marsh insect, so their presence also enhances the presence of these insects (Batzler & Wissinger 1996) and thus it also influences the presence of the toads and especially the survival of the eggs and larvae.
- Presence of branches: To assure that the ponds were adequate to the toads' needs, we added branches to each one of them during the installation period, so that the toads had a support to lay their eggs (Barandun & Reyer, 1997b). During the data collection, we checked if the branches were still present in each pond. If not, we added some more.

- Presence of leaves: To assess the probability of missing a hidden animal, we noted whether there were leaves at the bottom of the ponds. The leaves might also enhance the presence of aquatic insects as they provide food or habitat to them. Globally, the forested floodplains show a high insect productivity as the fallen leaves act as a habitat enrichment (Batzer & Wissinger 1996).
- Presence of growing vegetation: To assess the degree of vegetalisation in the ponds, we noted whether there were plants growing inside the pond. This factor concerned the natural and semi-natural ponds where seeds could grow. It is linked to the water capacity, as a seed growing will lead to the crackling of the bottom and thus the loss of water in the ground. Moreover, a study of Miesler & Gollmann (2000) showed that the importance of vegetation could depend on the stage of BOVA, so it will allow us to check the preferences of each stage.
- Presence of predatory insects and arachnids: To assess the survival probability, we noted visually whether there were predatory arthropods that could feed on eggs or tadpoles and reduce the fitness of the toads. The considered predators were: Notonectidae, Dytiscus, larvae of Odonata and Dolomedes (Barandun & Reyer, 1997b; Engelhardt, 1989; Chovanec, 1992).
- Presence of other species: To assess a potential predatory risk or some competition levels, we noted the presence of any other amphibian or reptile and their stage (eggs, tadpoles, juveniles, subadults, adults).

iii. Bombina's data

- At each BOVA's encounter, the animals were caught by hand, kept in buckets and released after a few minutes in the pond of origin. In the rare sites where the number of individuals was important, we captured the BOVAs and let them in big boxes until they were manipulated. In the wild, the toads tend to foam when they feel threatened, as they release antimicrobial peptides against a potential predator (Lai et al., 2002; Chen et al., 2003). During our manipulations, they can also feel in danger, but they are less likely to be stressed when they have more space (personal observation). We released them after a maximum of half an hour.
- If two individuals were observed mating, the amplexus was not disturbed to identify the animals that were thus noted as "missed" in the data.
- For any individual that completed the metamorphosis and already had some colours on its belly, we took a photography of its underparts (even for juveniles) while lying on a scaled card, snout down, to stretch it in a standardized position.
- For each observation, we noted the location of the capture, the stage of the individual and its sex. The stage was determined according to three ranks of sizes (snout-vent length): adult = > 3.5cm / subadult = 3 - 3.5cm / juvenile = < 3cm (Cayuela et al., 2016a, 2016b). The sex of the individuals was

assessed directly into the field while manipulating the animals (adults and subadults), and double-checked on the pictures once back to the office. As the males tend to lose their nuptial pads at the end of the breeding season (Barandun & Reyer, 1997c), all individuals whose length was bigger than 3cm and that did not show any nuptial pad was assumed to be a female and the data were then checked as soon as further recaptures occurred to verify the sex of the individuals. The juveniles were also confirmed in this way if they were recaptured once sexually mature.

- For the tadpoles, we noted their size according to 3 ranks: **p** = as small as the head of a pin / **T-** = bigger tadpole which hasn't got any leg yet / **T+** = big tadpole with legs.
- The number of eggs was recorded where they were observed. However, during the first year of the study, the ponds were not checked for eggs in details, so their number might have been underestimated.
- For each individual measuring more than 2cm, we took a DNA sample with a cotton-swab. As mentioned by Le Vin et al. (2011) the belly was not used for the swab as it could be contaminated by some other DNA. The aim was to get the toad's own DNA, rather than to test for the presence of parasites (Klop-Toker et al., 2016). We swabbed the inside of the toad's mouth, rubbing delicately to avoid hurting the animal, while obtaining enough DNA (Campanella & Smalley, 2006). Here again, the use of a non-invasive method was deliberate in order not to hurt the animals.

The methodology used to analyse the data is explained in the following chapters.

III. ETHOLOGY

A. Introduction

In this day and age, humankind is growing to such an extent that the occupied land encroaches more and more upon wildlife territories. Animals have thus to cope with disturbed habitats and fragmented landscapes (McKinney, 2006; Niemelä et al., 2000; Venn et al., 2003). Among them, the amphibians are the most endangered group of animals. Indeed, they have a complex life cycle that makes them rely on both aquatic and terrestrial habitats (Wilbur, 1980), and so they are affected on both sides. According to the IUCN (Baillie et al., 2004), the Yellow-bellied toad (*Bombina variegata*: BOVA) is of least concern thanks to its wide distribution, but is declining. Moreover, some local populations are endangered, already disappeared or might become extinct if they do not receive any protection measure. In Belgium and in Luxembourg, this species has always been considered as rare and its disappearance seems to be linked to humans (Parent, 1974). The most likely causes are various: abusive use of insecticides, road metalling in forests that destroyed the breeding areas or modification of river beds. Forty years ago, already, it was noticed that the condition of the species was much better on French ground (Lorraine) than in Belgium and Luxembourg (Parent, 1974). The situation did not improve as a report of Jacob (2006) suggests that it remained only one population of BOVA on Belgian ground and that even if the species is protected since 2001, it is listed as in critical danger of extinction. An atlas of 1984 recorded the species in 56 squares whereas it occupied only 3 squares in 2003 (Jacob, 2006). In Germany, BOVA is also considered as locally endangered. It seems that it is more common in highly disturbed habitats such as military training fields (Warren & Büttner, 2008) where it may be surprisingly common. In Italy, BOVA used to be considered as common (Vandoni, 1914), but seemed to suffer a huge decline in the last decades in most of its distributional range (Di Cerbo & Ferri, 1996; Caldonazzi et al., 2002). In 2004, Barbieri et al. conducted a research in several regions of Italy to assess the distribution and conservation status of BOVA. It appeared that the number of populations in northern Italy seemed stable, but in some regions, for example in Lombardy, the number of observed individuals decreased a lot.

In Switzerland, BOVA is one of the mostly threatened amphibian species. It was studied in several regions such as in the Rhône Plain (Zanini, 2006) or in a military training area near Zürich (Barandun & Reyer, 1997a) and all populations show an important decline. Geneva is an area where urbanization is expanding and takes more and more place in the countryside, destroying favourable habitats. To our knowledge, only few studies focussed on BOVA in this area (Jaggi, 2010) and none was conducted on a long-term basis. The major work that was done there is linked to the Action Plan written in 2011 and aimed at helping this species survive in this region thanks to the improvement of the conditions of the habitat. However, no study was conducted

afterwards to compile the effects of these improvements. To fill this caveat, we conducted a five years monitoring of the Genevan populations using capture recapture methods, in order to know if their conditions improved and to assess the level of extinction's risk they still face.

B. Methodology

Field work was conducted in different part of Geneva's canton. In each site, we mapped and monitored ponds by night. For each pond, we took data on the occurrence of amphibians (Yellow-bellied toad (*Bombina variegata*: BOVA, Marsh frog: *Pelophylax ridibundus*: PERI and Italian crested newt: *Triturus carnifex*: TRCA) and predatory insects (Notonectidae, Dytiscus, larvae of Odonata and Dolomedes). For each BOVA's encounter, we took a picture of the toad's belly as the individual's "fingerprint" and we recorded its stage, its sex and its location. (SEE DETAILED METHODOLOGY IN PART II.C. DATA COLLECTION).

i. Bombina's presence and global considerations

Using ArcGIS 10.3, we mapped the sites where BOVA was observed or not, and where we observed breeding indices, meaning tadpoles and eggs.

We considered the number of toads observed during each field session to see if there was a regularity in the observation rate in each site. We also used the total number of capture events versus the total number of observed (including not captured) events to estimate our capture success rate. We tested whether this success rate was stable or varied between our sampling sites using a Kruskal-Wallis rank sum test followed by a pairwise comparison using Tuckey & Kramer (Nemenyi) test (with Tuckey-DIST approximation for independent samples) on the standard deviation data of each site through the whole study (data for 2 to 5 years). All statistical tests were conducted on R v3.3.2 (www.r-project.org).

ii. Populations' characteristics

The pictures of the toad's belly were compared manually one by one and one file per site was created with the catalogue of all the observed bellies in the area (SEE AN EXAMPLE IN APP. C1). While into the field, we checked a first time whether we knew the manipulated toad and we conducted a double check once back to the office. This method worked perfectly, so it allowed us to confirm that the toe-clipping method was not needed to recognize these naturally marked individuals, even for most of the juveniles (FIGURE 3.1), differently from what was suggested in other studies (Barandun & Reyer, 1997b). We also double-checked the sex and the stage of the individuals. The size of all animals was also recorded to monitor the toads' growth.



FIGURE 3.1. EXAMPLE OF THE RECOGNITION OF THE SAME TOAD, ONCE JUVENILE AND ONCE ADULT. TEP055 was 2.1cm on the 19th of June 2013, and was 4.1cm on the 3rd of August 2016, its belly pattern was clearly recognisable from the beginning.

According to Rovero and Zimmermann (2016), if we take the cumulative number of identified individuals for each session, we can estimate the size of a population supposedly closed or with limited immigration and dispersion. Indeed, at each session, the number of known toads that are recaptured increases and the number of unknown toads newly captured decreases, so we are able to estimate a threshold toward which the real number of toads in a given population tends. We thus considered the observed population size (OPS) as the number of identified toads per site during the whole study period. We also considered the current annual population size (CPS), meaning the number of different individuals that we captured within a year. Indeed, not all the known individuals are observed each year as some can have moved, died or stayed hidden in the environment. We first had a general look at the populations' states by looking at the abundance of individuals at each site and the number of recaptured individuals. We also looked at the sex-ratio of the different populations through the years. To verify whether the sex-ratios differ from a 50:50 distribution within sites and between years, we estimated the probability that a given individual is a male using a binomial test (using `binom.test` in R). Finally, we looked at the relation between CPS and OPS.

The real population size (RPS) was calculated thanks to the recapture probability calculated with the program MARK (White & Burnham, 1999; Cooch & White, 2017). We used the Cormack-Jolly-Seber model (CJS) that works with an input data in which live encounters only were coded in a binomial way. For each capture event, a one means that a certain individual was seen, and a zero means that it was not seen (which does not mean it is dead: emigration is indistinguishable and treated the same as mortality).

We first compared the apparent survival probability (ϕ) and the recapture probability (p) of each site and each year. To do that, we compiled our data to have one data per individual per year. We thus considered 5 encounters occasions (2012 to 2016). 987 individuals in 12 sites were included in this analysis as we considered only populations with more than 10 individuals. We added to the encounter data one column per site so that MARK can attribute each individual to its group. We started by fitting a fully-time and -group dependent model $\{\phi(g*t), p(g*t)\}$, where g is the group (meaning the sampling site), and t the time, and then we removed factors one by one to test all combinations. The models were ranked according to their Akaike Information Criterion (AICc) and weighted using the AICc weight.

We also ran this model with a dataset comprising all individuals of all groups, but considering the age of the animals. Here, the aim was to determine whether there was a difference in survival and recapture for young individuals, so we grouped together subadults and adults. We thus considered two classes of age: juveniles and bigger than juveniles, and we added to the encounter data two columns. We started by fitting a fully-time and -age dependent model $\{\phi(a*t), p(a*t)\}$, where a is the age of individuals, and then we tested all combinations. For these two CJS models, we used model averaging to have real estimates of survival and recapture probabilities. Indeed, this tool weights the results according to the probability of each model to be the best for our data.

Then, to estimate the population size, we used the formula $N=c/p$, where N is the population size, c is the number of identified individuals and p is the recapture probability. As the CJS model allowed us to estimate the recapture probability while considering the population open (potential movements between the seasons), we can now use the formula for closed population to estimate the population size for each year (no movement on or off the study area during a season) (Lukacs, 2017).

iii. Presence according to factors from the “competitors/predators” cluster

The analyses aimed at assessing the effect of different ecological variables and was based on the total number of capture events, rather than the number of identified toads. To assess the impact of the environmental variables on the presence of BOVA, we grouped them into 3 clusters: **competitors/predators** (Marsh frog: PERI, Italian crested newt: TRCA, predatory insects), **vegetation** (algae in surface and in depth, growing vegetation, branches, leaves) and **water** (surface and type of the ponds, water level, turbidity). Here, we will analyse the first cluster to know whether the competitors and predators influence the presence of BOVA. For the predators, we monitored the presence of newts, especially the Italian crested newt (*Triturus carnifex*), as well as four insect groups (Notonectidae, Dytiscus, larvae of Odonata and Dolomedes). Concerning these latter, we grouped them together for the analyses to show the effect of 1 to 4 predators' types (cumulative presence of each type). We first conducted a visual exploration of our data. We then conducted Kruskal-Wallis tests to assess the significance of the differences between the repartition of each stage in each class of

factor, followed by Nemenyi test and we used a Pearson's correlation test to check whether the BOVA's and competitors' or predators' abundance were correlated.

We then ran a Generalized Linear Model (GLM) in R including only one factor or pairwise comparisons of factors. For this, we used the densities of BOVA in ponds presenting each factor. We then investigated the significance of the relationship for each stage of BOVA. We linked the strength of this relationship with the mean densities of BOVA in the extreme classes of the factors (if two classes of factors: low vs high; if more than two classes: lowest and highest). In the case of a cumulative effect of two factors, we analysed the influence of the two combined factors (1st fact low - 2nd fact low / 1st fact low - 2nd fact high / 1st fact high - 2nd fact low / 1st fact high - 2nd fact high).

iv. Migration patterns

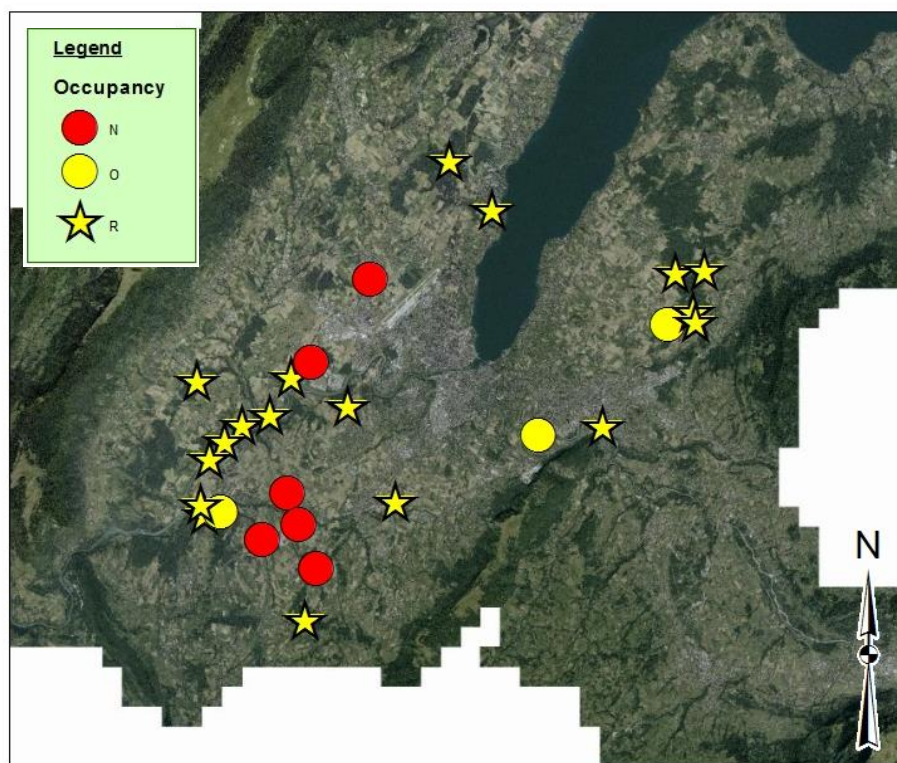
To assess the distance covered by a single individual, we considered only the individuals that were recaptured at least twice in different ponds (n=352 individuals). Using ArcGIS 10.3 and the Analysis tool "Point Distance", we got the distance between each pair of ponds in Geneva. Then, we calculated the distances covered by the toads during a certain time according to two successive captures. We then removed the inter-annual distances in calculating the distances only within a year (n=271 individuals). Finally, we calculated the mean daily travel distance (number of meters reduced to one day) for Geneva and for each site.

C. Results

i. Bombina's presence and global considerations

During the first two years of data collection, the class “sub-adults” was not considered and was grouped with adults. We added this class in the protocols for the following years, but we were also able to correct the first data thanks to the pictures, this is why the subadult class can be considered in the analyses. In the following analysis, adults, subadults and juveniles are grouped on the same graphs as their numbers are similar, whereas tadpoles and eggs numbers are shown on different graphs.

In totality, we monitored 27 sites, 21 being in Switzerland, and 6 being in France, just at the border. We observed Yellow-bellied toads (*Bombina variegata*: BOVA) in 21 sites, since no toad was observed in 4 Swiss sites and 2 French sites (MAP 3.1, red=no toad, yellow=toads). After 5 years of study, 2655 events of capture were realized, representing 1587 adults (975 males, 612 females), 737 subadults (444 males, 293 females) and 331 juveniles (52 males, 70 females, 209 not sexed). The three stages were determined thanks to the size of the captured toads (Cayuela et al., 2016a, 2016b). We observed but not identified because of unsuccessful captures, 619 toads, 324 being juveniles. For all the sites where several capture sessions took place, we obtained a percentage of capture success of 81.1%. We also recorded breeding indices (tadpoles and eggs) in 18 sites out of the 21 occupied (MAP 3.1, star=reproduction). In totality, we observed 4770 tadpoles and 7819 eggs.



MAP 3.1. MAP SHOWING THE OBSERVED PRESENCE OF BOVA over the whole study period. Red: No observation. Yellow: Observations of BOVA. Star: Reproduction of BOVA.

We considered the number of observations realised in each site per session to see if there was a regularity in the observation rate. In most of the sites, no trend was noticeable, some sessions allowed the observation of many individuals, and some of very few. However, we recorded a trend on the long term for 7 sites that showed a global increase in the observation rate (**FIGURE 3.2**).

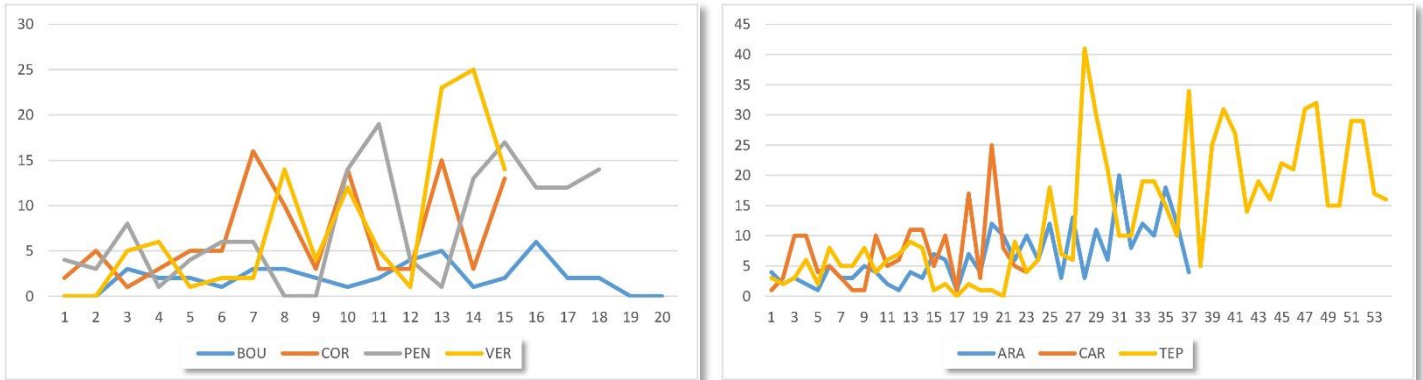


FIGURE 3.2. LONG-TERM TREND IN OBSERVATION RATE. Seven sites showed an increase in the global observation rate over the study period. Numbers on X-axis are field sessions. Colours: sites.

The success rate, meaning the percentage of caught individuals versus the totality of observed animals (including missed ones), ranged from 40% to 100% according to the sites and the years. Considering the standard deviation, it appeared that the global success rate over the whole study period was rather constant between the sites (KW: $\chi^2(16)=16$, $p=0.453$, $\alpha=0.05$ / Nemenyi: comparisons range from 0.79 to 1.00).

ii. Populations' characteristics

Thanks to the pattern on the bellies, the identity of the captured toads is easily recorded (Delarze et al., 2000). During the whole study period, we identified 1107 different toads. We created a histogram to have a visual approach on the observed population sizes (OPS) in each site, meaning the number of different individuals found in an area during the whole study period (**FIGURE 3.3**). On this graph, we can see that the OPS in each of our sites ranges from 2 to 433 individuals. We also show here that the percentage of recaptured individuals ranges from 16% to 100%. The average percentage of recaptured individuals is 50.3% (530 recaptured toads out of the 1054 identified toads - VIR is not included in these numbers as only one session was conducted).

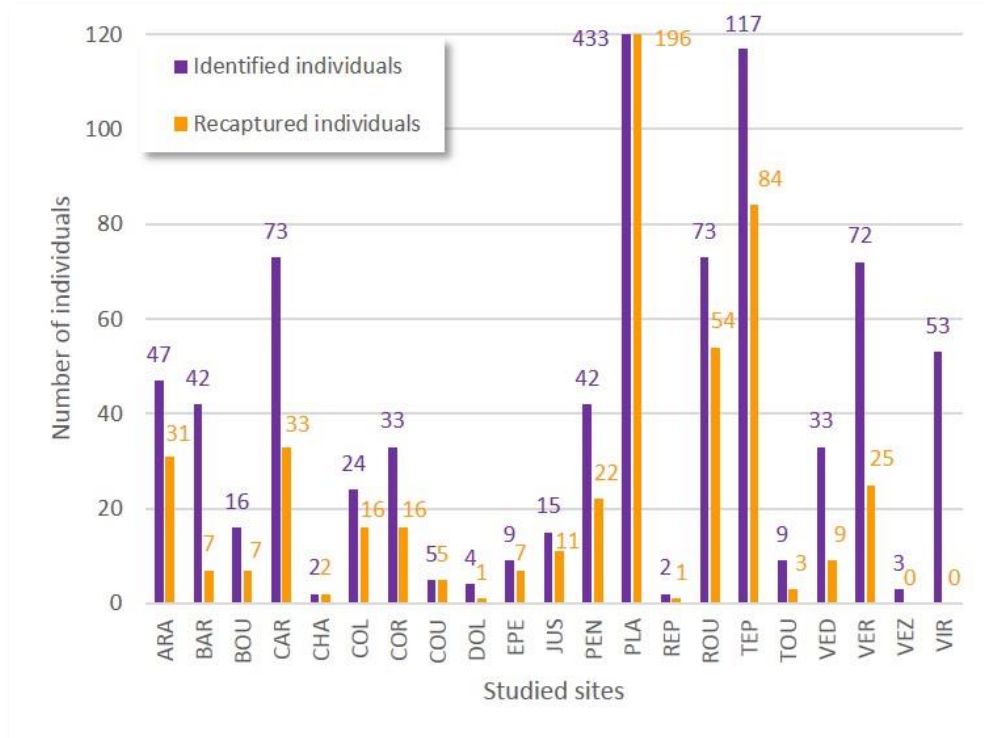


FIGURE 3.3. IDENTIFIED INDIVIDUALS AND RECAPTURED INDIVIDUALS IN EACH SITE. Purple: identified individuals. Orange: Recaptured individuals. The bars of PLA were cut for readability reason, but their numbers are correctly written.

For each site, we checked whether the sex-ratio was balanced or not. For the statistical analyses, we used the total number of identified sexed individuals, meaning we did not consider the number of juveniles that were not sexed. We considered thus 928 individual data (492 males, 436 females) (FIGURE 3.4). It appeared that the number of individuals of each sex on Geneva for the whole study period was not significantly different ($P_{\text{male}}=0.53\pm 0.06$, $p\text{-value}=0.07$, $\alpha=0.05$). However, in 2013, 2014 and 2015, we observed significantly more males than females in the whole Geneva, (2013: $P_{\text{male}}=0.57\pm 0.22$, $p\text{-value}=0.0132$; 2014: $P_{\text{male}}=0.62\pm 0.1$, $p\text{-value}=0.0002$; 2015: $P_{\text{male}}=0.55\pm 0.09$, $p\text{-value}=0.0315$, $\alpha=0.05$) which can be linked with the fact that some sites showed significant differences in sex-ratio for these years (TABLE 3.1, SEE APP. C2 FOR ALL DATA). The only site that presents a significant difference in 2016 is ARA, but it did not affect the global trend of Geneva.

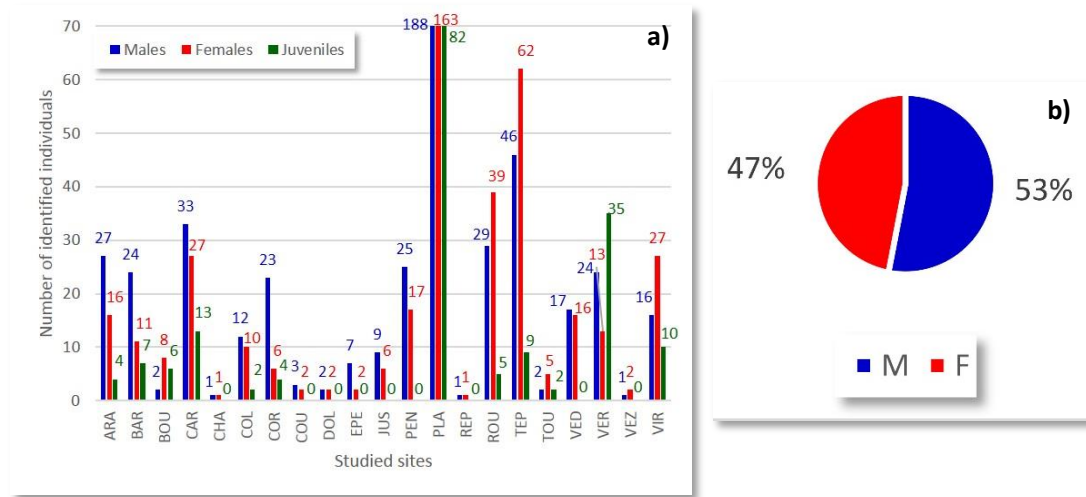


FIGURE 3.4. SEX-RATIO, Blue: males. Red: females. Green: not sexed juveniles. a) Sex-ratio in each site. The bars of PLA were cut for readability reason, but their numbers are correctly written. b) Global sex-ratio in Geneva.

TABLE 3.1. SIGNIFICANT DIFFERENCES IN SEX-RATIO. Some sites are different for a unique year, but some are different for the whole study period. GVA: Geneva. See Abbreviations list for all sites' names (p.0).

Site	Year	p-value	
GVA	2013	0.0132	
	2014	0.0002	
	2015	0.0315	
ARA	2016	0.0414	
BAR	2013	0.0414	
	TOT ₍₂₀₁₃₋₂₀₁₄₎	0.0409	
	COR	2012	0.0078
COR	2013	0.0026	
	2014	0.0075	
	TOT ₍₂₀₁₂₋₂₀₁₃₋₂₀₁₄₎	0.0023	
	PEN	2014	0.0044
	2015	0.0357	
PLA	2015	0.0177	
VER	2014	0.0294	

Obviously, not all the known toads are observed during each year. We considered thus the current population size (CPS) as the number of individuals observed within a year. The CPS are much more reduced and range from 1 to 268. An example of the link between OPS and CPS is shown in **FIGURE 3.5**. TEP is a site that was followed for 5 years (end of 2012: 23 different observed toads, end of 2013: 54 toads, end of 2014: 53 toads, end of 2015: 58 toads, end of 2016: 47 toads) (**TABLE 3.2, APP. C3 FOR ALL DATA**).

TABLE 3.2. CPS AND SEX-RATIOS FOR EACH SITE FOR EACH YEAR. See Abbreviations list for all sites' names (p.0).

Number of identified individuals

M: males. F: females. J: juveniles

ID	2012			2013			2014			2015			2016			Population							
	M	F	J Total	M	F	J Total	M	F	J Total	M	F	J Total	M	F	J Total	M	F	J Total					
	ARA	7	3	1	5	3	0	8	9	5	3	17	15	8	8	31	15	5	1	21	27	16	4
BAR				15	5	4	24	14	6	3	23									24	11	7	42
BOU				2	2	1	5	1	3	1	5	1	4	4	9	1	3	1	5	2	8	6	16
CAR				7	9	5	21	13	7	3	23	22	17	8	47					33	27	13	73
CHA				0	0	0	0	1	0	0	1	1	1	0	2					1	1	0	2
COL	10	8	0	8	5	0	13	7	3	2	12								12	10	2	24	
COR	8	0	0	19	4	4	27	15	3	0	18								23	6	4	33	
COU				3	2	0	5	3	2	0	5								3	2	0	5	
DOL				0	1	0	1	1	1	0	2	1	0	0	1				1	0	0	1	
EPE				6	2	0	8	6	1	0	7								7	2	0	9	
JUS				5	0	1	6	5	3	0	8	5	5	0	10				9	6	0	15	
PEN	8	4	0	11	4	0	15	16	3	0	19	20	8	0	28				25	17	0	42	
PLA	4	9	11	70	63	48	181	3	4	2	9	134	97	33	264				188	163	82	433	
ROU				20	21	1	42	19	18	4	41	15	12	2	29	16	13	1	30	29	39	5	73
TEP	11	12	0	18	24	12	54	21	25	7	53	27	28	3	58	18	27	2	47	46	62	9	117
TOU	0	1	0	1	1	2	4	0	3	0	3	1	2	0	3	0	2	0	2	2	5	2	9
VER				5	1	6	12	22	9	37	68								24	13	35	72	
GVA	48	37	12	195	147	84	426	156	96	62	314	242	182	58	482	52	50	5	107	457	390	169	1016

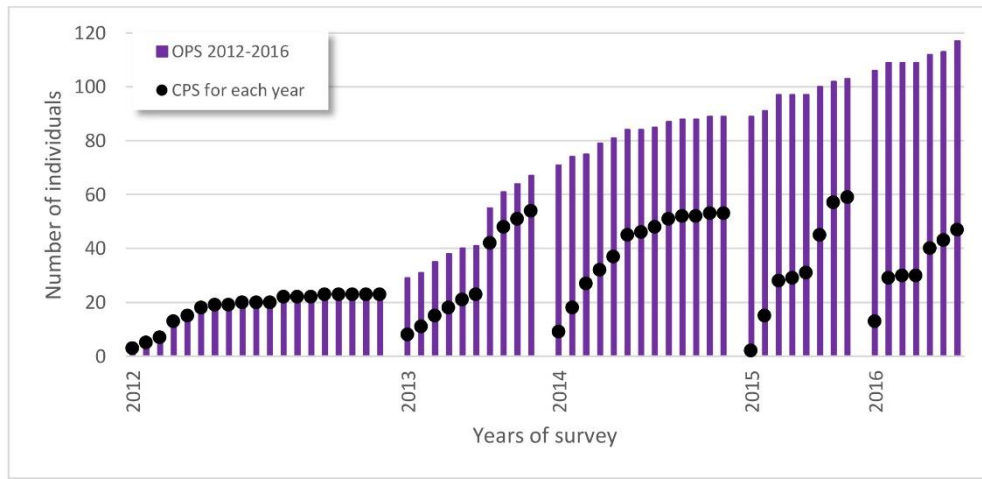


Figure 3.5. CURRENT POPULATION SIZES VS OBSERVED POPULATION SIZES FOR TEP. Each pack of data corresponds to a different year. Purple: OPS. Black: CPS.

Thanks to MARK software (Cooch & White, 2017), we estimated the apparent survival (ϕ) and recapture probabilities (p) and the population size (N) (meaning our RPS).

For a first analysis, we used the CJS model and we tried to estimate survival and recapture probabilities according to the sampling sites (groups) to which belong the individuals. In the models, g means group, t means time and a point means that the parameter is constant. It appeared that the model that best fits our data is a model in which the apparent survival probability depends on the group and the time, whereas the recapture probability depends on the group only. Its AICc weight shows that this models fits our data at 75% (75% of chances that this is the correct model). The second best model is the one in which both the survival and the recapture depend on the group and the time. This second model has 25% of chances to be the best model, but is less parsimonious (55 parameters vs 48 parameters) (TABLE 3.3).

TABLE 3.3. MODEL SELECTION PROCEDURE FOR SURVIVAL AND RECAPTURE PROBABILITIES ACCORDING TO GROUPS (CJS MODEL). All sites with more than 10 individuals were considered here. The model that appears to fit best the data is the $\{\phi(g^*t), p(g)\}$. ϕ : survival. p : recapture. g : group (site). t : time. \cdot : constant. r : rank of the best models. k : number of parameters. AICc: Akaike Information Criterion.

r	Model	k	AICc	AICc Weight
1	$\{\phi(g^*t), p(g)\}$	48	2738.0550	0.74659
2	$\{\phi(g^*t), p(g^*t)\}$	55	2740.2162	0.25338
3	$\{\phi(g), p(g^*t)\}$	51	2758.6520	0.00003
4	$\{\phi(t), p(g^*t)\}$	45	2762.0913	0.00000
5	$\{\phi(\cdot), p(g^*t)\}$	43	2770.3698	0.00000
6	$\{\phi(g^*t), p(\cdot)\}$	39	2776.3765	0.00000
7	$\{\phi(g^*t), p(t)\}$	41	2776.7307	0.00000
8	$\{\phi(t), p(g)\}$	16	2946.6372	0.00000
9	$\{\phi(g), p(t)\}$	16	2954.3794	0.00000
10	$\{\phi(g), p(g)\}$	24	2997.7001	0.00000
11	$\{\phi(\cdot), p(g)\}$	13	3034.4862	0.00000
12	$\{\phi(g), p(\cdot)\}$	13	3044.5199	0.00000
13	$\{\phi(t), p(\cdot)\}$	5	3091.8343	0.00000
14	$\{\phi(t), p(t)\}$	7	3093.6104	0.00000
15	$\{\phi(\cdot), p(t)\}$	5	3095.7207	0.00000
16	$\{\phi(\cdot), p(\cdot)\}$	2	3187.1439	0.00000

We used the tool “model averaging” to get an average value of survival and recapture according to the weight of the best models (TABLE 3.4, AND SEE APP. C4). As we consider five years of captures, the models calculate the survival probabilities during each time interval between two years. The number 1 is the interval between 2012 and 2013, the number 2 between 2013 and 2014, and so on. However, the recapture probabilities are calculated for a given year, and not between the years (FIGURE 3.6). In the CJS, the recapture probability at time 0 is not calculated, because p is the probability of encountering a marked (=known) individual. For sites monitored during five years, as no individuals were known prior to occasion 0, no p_0 (=2012) could be captured. For sites monitored less than five years, the p_0 corresponds to their first year of monitoring.

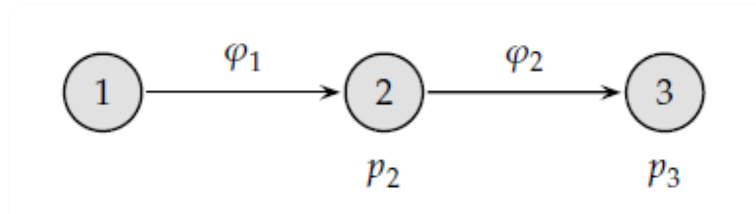


Figure 3.6. BASIC STRUCTURE OF CJS. ϕ : survival probability. p : recapture probability. Numbers 1 to 3: field sessions (Cooch & White, 2017).

In the TABLE 3.4, survival and recapture probabilities were calculated for years even if no data were collected in some sites during some years. Indeed, MARK extrapolates probabilities for missing years according to the data provided for other years.

TABLE 3.4. ESTIMATES FOR SURVIVAL AND RECAPTURE PROBABILITIES FOR EACH GROUP AFTER MODEL AVERAGING. “- -”: values that could not be calculated. Nb: assigned number of the groups. Numbers 1 to 4 - survival: intervals between years of monitoring. Numbers 1 to 4 - recaptures: years, without the season zero.

Group	nb	Survival				Recapture			
		1	2	3	4	1	2	3	4
ARA	1	0.72	0.49	0.65	0.33	0.6	0.55	0.58	0.48
BAR	2	0.58	0.74	0.88	0.50	1.0	1.0	1.0	0.91
BOU	3	0.44	0.5	--	0.21	0.41	0.41	0.33	0.36
CAR	4	0.44	0.59	0.4	0.39	0.86	1.0	0.99	0.89
COL	5	0.44	0.09	0.73	--	0.86	0.99	0.91	0.75
COR	6	0.78	0.54	0.07	0.24	0.84	0.84	0.63	0.63
JUS	7	0.99	0.51	--	0.27	1.0	0.9	0.74	0.76
PEN	8	0.45	0.99	0.74	--	0.61	0.66	0.67	0.49
PLA	9	0.66	0.89	0.99	--	0.5	0.63	0.62	0.44
ROU	10	0.45	0.71	0.78	0.9	0.56	0.62	0.58	0.63
TEP	11	0.49	0.6	0.83	0.62	0.91	0.93	0.93	0.88
VER	12	0.45	0.71	--	0.95	0.81	0.93	0.9	0.9

The mean survival probability varies from 0.38 (BOU) to 0.84 (PLA). The standard deviation between years ranges from 0.1 (CAR) to 0.37 (JUS), and it is 0.24, ± 0.05 for the time intervals. It is thus very variable and depends on the site and the year. Moreover, we see that the survival probability tends to be lower during the last time interval. However, as we did not collect data for many sites during the last year, these lower values may be underestimated by MARK. The mean recapture probability varies from 0.38 (BOU) to 0.98 (BAR). The standard deviation ranges from 0.02 (TEP) to 0.12 (JUS), which shows that the recapture probability was quite constant between the years. The high value of BAR is surprising when we link it to the relief of the area, because many individuals were not captured, so it might be an artefact from MARK.

For the second analysis, we used the CJS model and we tried to estimate survival and recapture probabilities according to the age of the individuals. It appeared that the model that fits best our data is a model in which the apparent survival and the recapture probabilities depend only on time. Its AICc weight shows that this model fits our data at 55%. The second best model is the one in which the survival is constant among age classes and between each encounter occasion (23%) and the third best model suggests that the survival depends on the age class (13%) (TABLE 3.5). The most parsimonious model is the second best one (5 parameters).

TABLE 3.5. MODEL SELECTION PROCEDURE FOR SURVIVAL AND RECAPTURE PROBABILITIES ACCORDING TO AGES (CJS MODEL). All sites with more than 10 individuals were considered here. The model that appears to fit best the data is the $\{\phi(t),p(t)\}$. ϕ : survival. p : recapture. a : age class. t : time. \cdot : constant. r : rank of the best models. k : number of parameters. AICc: Akaike Information Criterion.

r	Model	k	AICc	AICc Weight
1	$\{\phi(t),p(t)\}$	7	3159.2596	0.54714
2	$\{\phi(\cdot),p(t)\}$	5	3161.0258	0.22626
3	$\{\phi(a),p(t)\}$	6	3162.0531	0.13536
4	$\{\phi(t),p(\cdot)\}$	6	3164.7374	0.03537
5	$\{\phi(a^*t),p(t)\}$	11	3165.8109	0.02068
6	$\{\phi(t),p(a^*t)\}$	11	3165.9395	0.01939
7	$\{\phi(\cdot),p(a^*t)\}$	9	3167.7130	0.00799
8	$\{\phi(a),p(a^*t)\}$	10	3169.3981	0.00344
9	$\{\phi(a^*t),p(\cdot)\}$	9	3170.1662	0.00234
10	$\{\phi(a^*t),p(a^*t)\}$	14	3171.6644	0.00111
11	$\{\phi(a^*t),p(a)\}$	10	3171.9976	0.00094
12	$\{\phi(\cdot),p(\cdot)\}$	2	3341.6313	0.00000
13	$\{\phi(a),p(t)\}$	3	3342.7846	0.00000
14	$\{\phi(\cdot),p(a)\}$	3	3342.9531	0.00000
15	$\{\phi(a),p(a)\}$	4	3344.7471	0.00000
16	$\{\phi(\cdot),p(\cdot)\}$	2	3187.1439	0.00000

We used the tool “model averaging” to get an average value of survival and recapture according to the weight of the best models (TABLE 3.6, AND SEE APP. C5 FOR A EXTRACT OF THE MODEL AVERAGING). Again, we consider here four time intervals between the five years of capture. We see that the models that find an influence of the age class on the survival and recapture probabilities have only small weights next to the best models. Indeed, in the next table, the probabilities are similar between both age classes (standard deviation values < 0.01).

TABLE 3.6. ESTIMATES OF SURVIVAL AND RECAPTURE PROBABILITIES FOR EACH AGE CLASS AFTER MODEL AVERAGING. Numbers 1 to 4 - survival: intervals between years of monitoring. Numbers 1 to 4 - recaptures: years, without the season zero.

Age	Survival				Recapture			
	1	2	3	4	1	2	3	4
Adults-subadults	0.64	0.54	0.59	0.44	0.72	0.51	0.64	0.32
Juveniles	0.65	0.55	0.6	0.44	0.72	0.51	0.65	0.32

These results show that the age class does not influence survival and recapture, whereas the group and time seem to have an impact. Using the population respective recapture probability and the number of identified individuals of each age class (TABLE 3.7), we are thus able to calculate their size for each group for each year ((TABLE 3.8). Here, we removed in the table the data for years that were not considered by MARK (first year of capture in each site and years where no monitoring took place).

TABLE 3.7. RAW DATA TO CALCULATE POPULATION SIZE. Numbers 1 to 4: years or recaptures, without the season zero. The recapture probability will be used for both age classes. The empty cells are either years during which the site was not monitored, or the first year of monitoring that corresponds to the p_0 of the site.

Group	Recapture prob				Nb identified juv				Nb identified ad+sub			
	1	2	3	4	1	2	3	4	1	2	3	4
ARA	0.6	0.55	0.58	0.48	0	14	23	1	8	3	8	20
BAR		1.0				3				20		
BOU		0.41	0.33	0.36		1	4	4		4	5	1
CAR		1.0	0.99	0.89		3	8			20	39	
COL	0.86	0.99			0	2			13	10		
COR	0.84	0.84			4	0			23	18		
JUS		0.9	0.74			0	0			8	10	
PEN	0.61	0.66	0.67		0	0	0		15	19	28	
PLA	0.5	0.63	0.62		48	2	33		133	7	231	
ROU		0.62	0.58	0.63		4	2	1		37	27	29
TEP	0.91	0.93	0.93	0.88	12	7	3	2	42	46	55	45
VER		0.93				37				31		

TABLE 3.8. ESTIMATED POPULATION SIZES. Numbers 1 to 4: years or recaptures, without the season zero. The population size was calculated thanks to the formula $N=c/p$.

Group	Juv population size				Ad+Sub population size			
	1	2	3	4	1	2	3	4
ARA	0	25	40	2	13	5	14	42
BAR		3				20		
BOU		2	12	11		10	15	3
CAR		3	8			20	39	
COL	0	2			15	10		
COR	5	0			27	21		
JUS		0	0			9	14	
PEN	0	0	0		25	29	42	
PLA	96	3	53		266	11	373	
ROU		6	3	2		60	47	46
TEP	13	8	3	2	46	49	59	51
VER		40				33		

The annual capture data range from 0 to 48 juveniles and from 1 to 231 adults, whereas the estimates range from 0 to 96 juveniles and from 3 to 373 adults. The populations for which the recapture probabilities are high obviously have estimated sizes that are close to our capture data, whereas our data underestimated the population sizes where the recapture probabilities are low.

iii. Presence according to factors from the “competitors/predators” cluster

On the histogram showing the total number of BOVA observations (FIGURE 3.7), we noticed that it seems that all stages were mostly seen when there is no competitor. This is validated by a Kruskal-Wallis test that showed us that the differences in abundance are significant (all p-values $< 4.75e^{-7}$, $\alpha=0.05$).

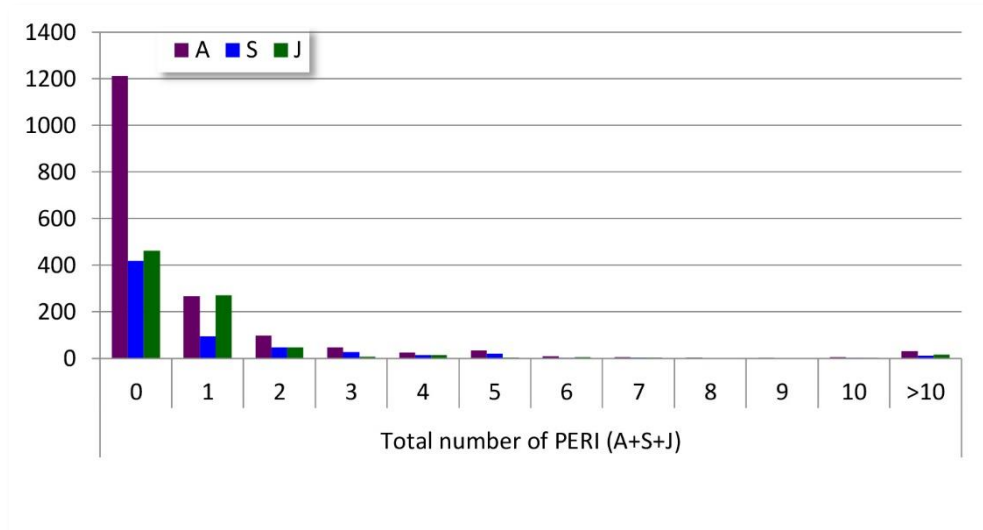


Figure 3.7. DISTRIBUTION OF BOVA’S OBSERVATIONS ACCORDING TO THE PRESENCE OF COMPETITORS. Purple: adults (A). Blue: subadults (S). Green: juveniles (J). PERI (A+S+J): total number of Marsh frogs (adult+subadult+juveniles). Most observations were realised when there was no competitor.

However, we conducted a Pearson’s correlation test to see whether the abundance of the competitors is correlated with the abundance of BOVA and it appeared that nothing is correlated (PERI: $r_{adults}=0.0023$, $p=0.7814$; $r_{sub}=0.0132$, $p=0.1083$; $r_{juv}=0.039$, $p=0.6337$, $\alpha=0.05$).

The global histogram of the abundance of BOVA according to the presence of predators (adult Italian crested newts: TRCA, and predatory insects) showed that there are less BOVA when there are more types of predators (FIGURE 3.8). A Kruskal-Wallis test showed that the differences are highly significant (TRCA: all p-values $< 8.41e^{-7}$; insects: all p-values $< 2.74e^{-5}$) and the Pearson’s correlation test showed that there is a correlation only for insects (TRCA: $r_{adults}=-0.0011$, $p=0.8886$; $r_{sub}=-0.0006$, $p=0.9451$; $r_{juv}=-0.0002$, $p=0.9782$ / insects: $r_{adults}=0.058$, $p=1.38e^{-12}$; $r_{sub}=0.038$, $p=4.19e^{-6}$; $r_{juv}=0.018$, $p=0.03$, $\alpha=0.05$).

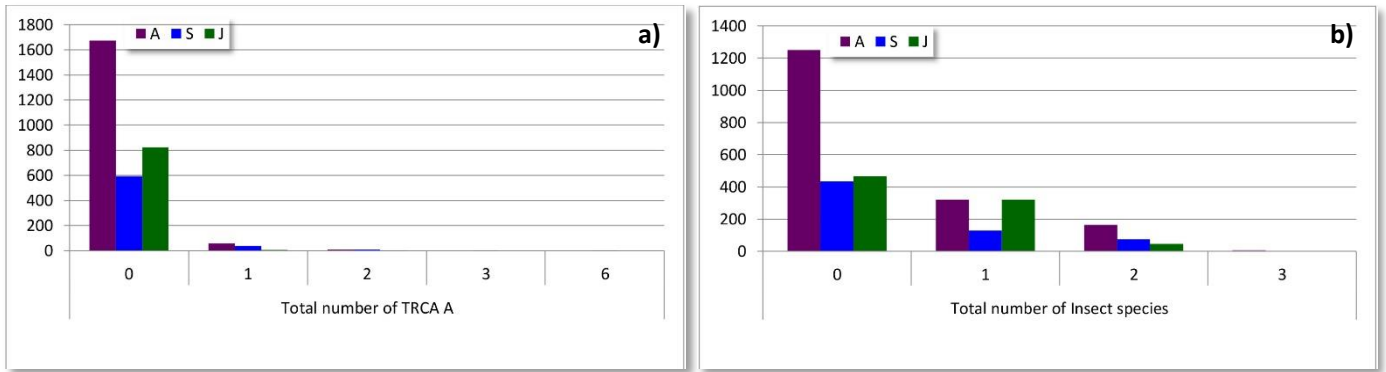


Figure 3.8. DISTRIBUTION OF BOVA'S OBSERVATIONS ACCORDING TO THE PRESENCE OF PREDATORS Purple: adults (A). Blue: subadults (S). Green: juveniles (J). a) adult Italian crested newts. b) predatory insects.

We also investigated whether there was an influence on the choice of the breeding site, so we checked the distribution of the observations of tadpoles and eggs according to the presence of competitors (FIGURE 3.9) and predators (FIGURE 3.10). Here, we also used the sum of capture events. We see that, again, most observations were realised in ponds where there was no competitor or predator.

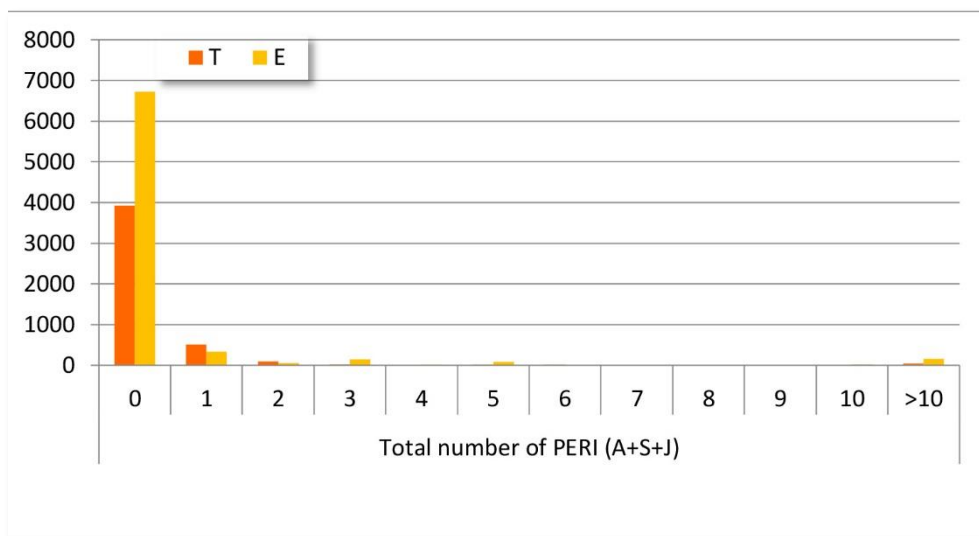


Figure 3.9. DISTRIBUTION OF BREEDING INDICES' OBSERVATIONS ACCORDING TO THE PRESENCE OF COMPETITORS. Orange: tadpoles (T). Yellow: eggs (E). PERI (A+S+J): total number of Marsh frogs (adult+subadult+juveniles). Most observations took place when there was no competitor.

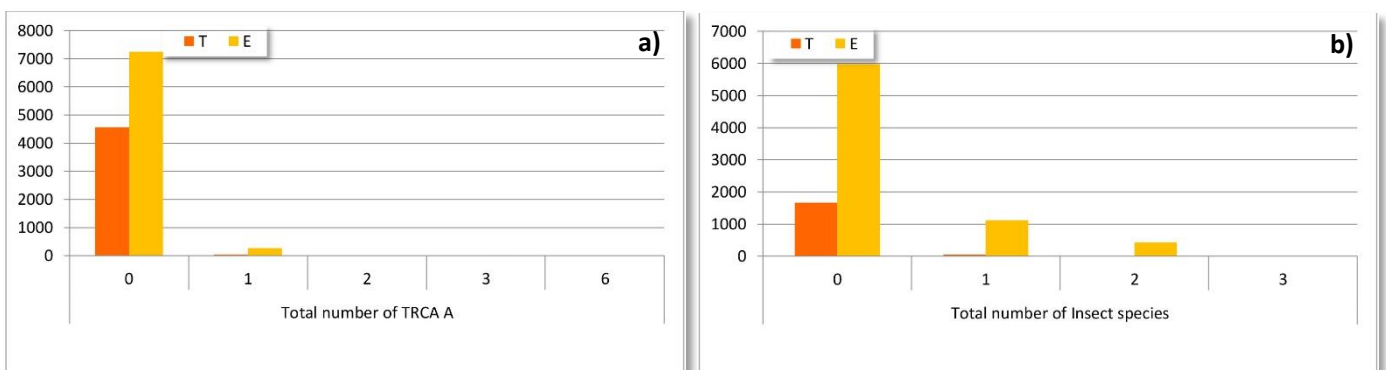


Figure 3.10. DISTRIBUTION OF BREEDING INDICES' OBSERVATIONS ACCORDING TO THE PRESENCE OF PREDATORS. Orange: tadpoles (T). Yellow: eggs (E). a) adult Italian crested newts. b) predatory insects.

After analysing the results of the GLM (FIGURE 3.11), we can notice that, in most cases, the subadults and the juveniles do not show any preferences (SEE APP. C6 FOR ALL VALUES). Overall, most influences concern eggs. Indeed, except for Odonata, the presence of other amphibians at the same time as predatory insects influences negatively their densities. Moreover, the densities of BOVA at all stages are strongly negatively correlated with the presence of PERI (all p-values < 8.49e⁻¹³, except for tadpoles: p=0.73, α=0.05) and it appeared that BOVA's adults and eggs are especially present when there is at maximum one TRCA. The impact of Notonectidae and Dolomedes is negative for tadpoles and eggs and the impact of Dytiscus is negative for eggs. Surprisingly, the Odonata larvae have a positive impact on tadpoles and eggs when they are alone, or a mixed impact if the Odonata are present at the same time as another amphibian. The adults alone do not appear to be influenced by predatory insects. If we take together PERI and TRCA, the impact is strongly negative for eggs, as well as when PERI is present with Notonectidae. The cumulative effect of several insects is mixed, or tends to be negatively correlated with tadpoles and eggs.

	A Influence	S Influence	J Influence	T Influence	E Influence
PERI_PRES	*** -	*** -	*** -		*** -
TRCA_PRES	*** -	*** 0		*** 0	*** -
DYT		* 0			*** -
NOT	*** 0	*** 0		** -	** -
DFY	*** 0	*** 0	*** 0	*** +	*** +
DOL	*** 0	* 0		*** -	*** -
PERI_PRES:TRCA_PRES					*** -
PERI_PRES:DYT	** 0		*** 0	*** 0	
PERI_PRES:NOT	*** 0	*** 0	*** 0	* 0	** -
PERI_PRES:DFY	*** 0	*** 0	*** 0		*** mixed
TRCA_PRES:DYT					*** -
TRCA_PRES:NOT	*** mixed	*** mixed	* 0		
TRCA_PRES:DFY				*** mixed	
DYT:NOT	*** 0	** 0		** -	*** -
DYT:DFY			* 0	*** mixed	*** mixed
NOT:DFY	* 0	* 0	** 0		
NOT:DOL	*** 0				
DFY:DOL				*** mixed	

Figure 3.11. SUMMARY OF GLM RESULTS FOR THE COMPETITORS/PREDATORS CLUSTER. A: adults. S: subadults. J: juveniles. T: tadpoles. E: eggs. Stars: strength of significance. Grey: not significant comparisons. Red: negative values of GLM estimates. Green: positive values of GLM estimates. 0: no influence on distribution. +: positive influence on distribution. -: negative influence on distribution. mixed: no clear influence.

iv. Migration patterns

Considering all the individuals that were captured at least twice, we calculated the distance they covered between two recaptures occasions (FIGURE 3.12).

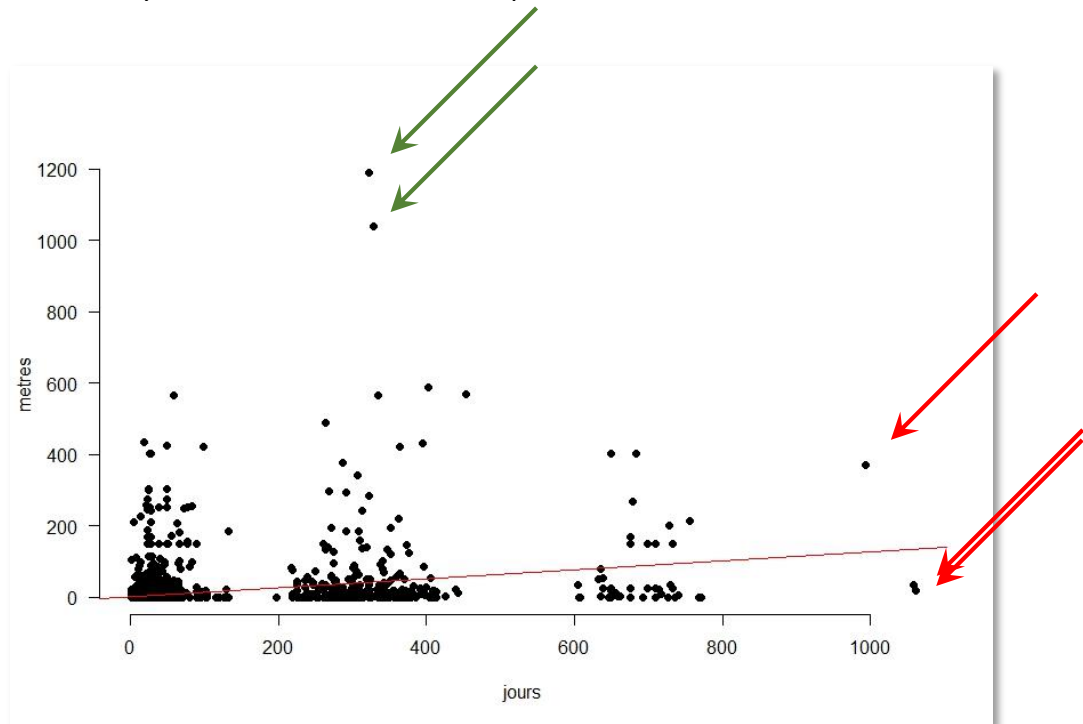
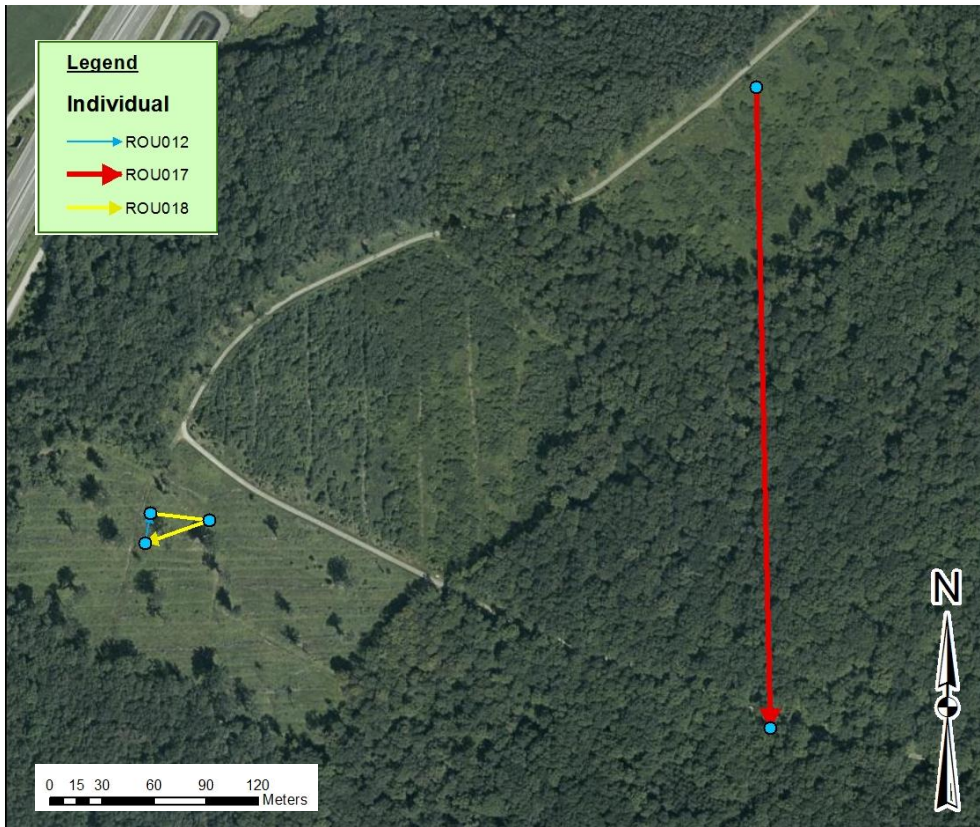


FIGURE 3.12. PLOT OF THE DISTANCES COVERED BY INDIVIDUALS BETWEEN TWO RECAPTURES. Green: individuals that covered greater distances. Red: individuals that were recaptured after 1000 days. We see several clusters: corresponding to inter-annual recaptures.

We see that the data are grouped into 3 main clusters that correspond to three consecutive field seasons. Since each point is a recapture of a certain toad, the first cluster represents the recaptures within a season, the second one represents the recaptures two years apart and the third one represents the recaptures three years apart. We even see that **three individuals** were recaptured after more than 1000 days. ROU012 was observed the 21st of August 2013 and recaptured only on the 17th of July 2016, and he moved over 17 meters. ROU018 was observed the 17th of June 2013 and recaptured only on the 10th of May 2016, and he moved over 34 meters. ROU017 was observed the 21st of August 2013 and recaptured only on the 10th of May 2016, and he moved over 370 meters (crossing through ROU site) (**Map 3.2**). We also see that **two individuals** travelled over greater distances than the other individuals. JUS002 covered 1190 meters in 323 days, passing from JUS site to ARA site, and VER001 covered 1039 meters in 328 days, passing from one subsite of VER site to another.



MAP 3.2. ITINERARIES OF ROU INDIVIDUALS THAT WERE RECAPTURED AFTER THE LONGEST TIME INTERVALS. Blue: ROU012. Red: ROU017. Yellow: ROU018. ROU017 travelled across the whole site.

Then, we removed the distances covered between two seasons to have a better view of the short-term travel distances (FIGURE 3.13). During a single season, the biggest time interval between two recaptures was 132 days (TEP050) and the greatest distance covered was 567 meters (ROU025).

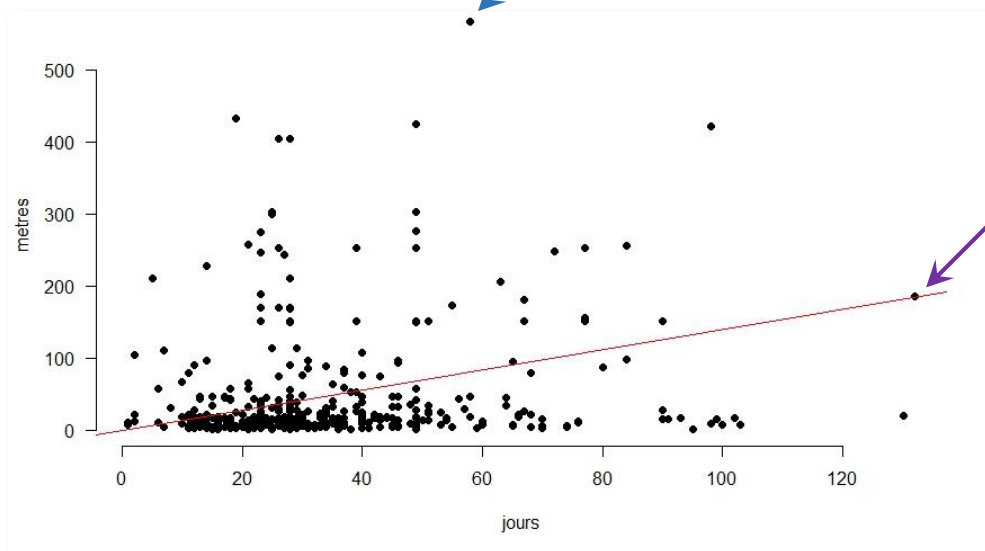


FIGURE 3.13. PLOT OF THE DISTANCES COVERED BY INDIVIDUALS BETWEEN TWO INTRA-ANNUAL RECAPTURES. Blue: biggest time interval. Purple: greatest distance. The distances are reduced to globally less than 120 meters within a year.

These numbers obviously vary a lot as the time interval between two recaptures may be very different. We thus reduced the distance covered between two recaptures to the daily travel distance to compare the individuals and the sites. The daily distances ranged from 0.01 meter (**ROU070**) to 52 meters (**ARA030**) (mean= 2.011; median= 0.68). We plotted with bars the mean daily distance per individual (**FIGURE 3.14**) or per site (**FIGURE 3.15**).

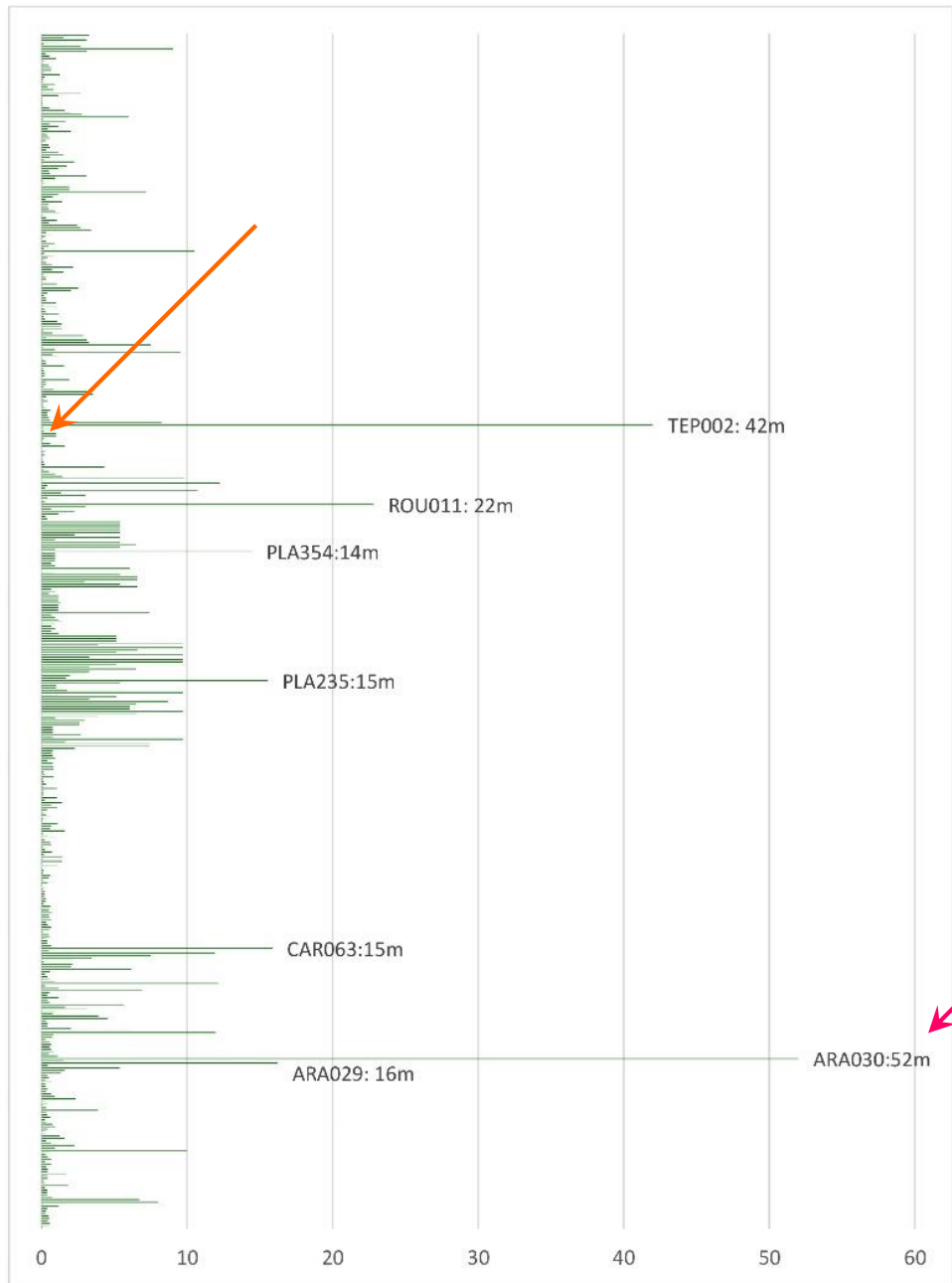


FIGURE 3.14. BAR-PLOT OF THE MEAN DAILY DISTANCE PER INDIVIDUAL. Orange: smallest mean daily travel distance. Pink: greatest mean daily travel distance. Most individuals do not cover more than 10 meters per day.

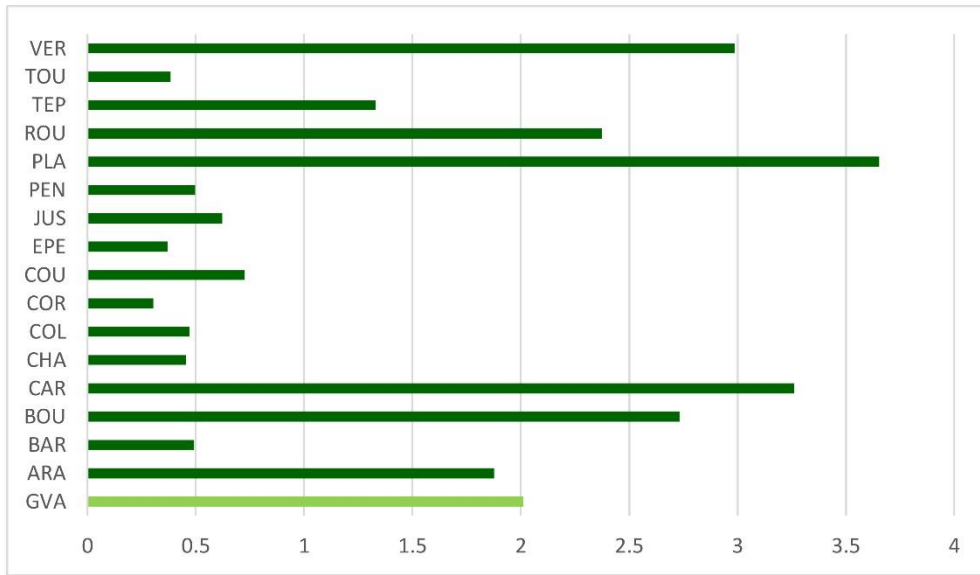


FIGURE 3.15. BAR-PLOT OF THE MEAN DAILY DISTANCE PER SITE. X-axis: mean daily travel distance (m). Y-axis: sites. The mean daily distance is usually less than 2 meters. GVA presents the average over all sites. See Abbreviation list for all sites' names (p.0).

D. Discussion

i. Global considerations

The work conducted for this study allowed to update the knowledge on the Yellow-bellied toad in Geneva. Indeed, only few studies took place in this region and none was conducted on a long-term basis. First, we will present some global considerations about this study.

The study lasted five years. However, not all the 27 sites were monitored during the whole study. Indeed, different actors (associations, research units, volunteers) were in charge of some parts of the prospection and the Department of Nature and Landscape of Geneva decided, according to the funds allocated each year for the *Bombina* project, how many sites could be followed. Thus, the survey effort varied over the whole study area. However, thanks to the calculation of the success rate (number of individuals captured vs number of individuals observed), we managed to catch most toads. One site can be an exception: BAR. This site is located in a running quarry and some of the monitored ponds were hardly accessible due to their big size or the relief of the ground. This can explain why the success rate of BAR is only about 40%. In most other sites, the features of the area allowed the researchers to go close enough to the ponds to be able to catch at least 75% of the observed animals.

Many people involved in the project were surprised that we preferred taking time to recognize the individuals thanks to the pattern of their belly, instead of clipping toes. The decision to use a non-invasive method was made for ethical reasons. Indeed, we believe that hurting the animals goes against the aim of this study which was to conduct a better conservation of the Yellow-bellied toad (*Bombina variegata*: BOVA). Moreover, the method of toe-clipping is more and more controversial (May, 2004; Antwis et al., 2014), and it is totally useless and non-sensed when studying naturally marked animals (Mettouris et al., 2016). It was even shown that the standard error of population sizes estimations was bigger when using toe clipping than where comparing bellies (Cevik et al., 2008). Still with a view to keeping this study ethically correct, we reduced the manipulations of the animals as much as possible. Indeed, BOVA is known for showing stress behaviour, called Unken reflex (Kuchta, 2005) when it feels threatened. We never observed this behaviour while the toads were manipulated by ourselves, but it happened when other less trained people manipulated them. Moreover, we also observed in these cases another stress-induced behaviour: the foaming. It comes out as a skin secretion of the toad which is accompanied by a strong vinegar smell (personal observation). This reaction is linked with the fact that BOVA is a toxic animal (Harkewicz, 2004) and produces toxins thanks to the parotid glands to protect itself.

After five years of monitoring, we were able to confirm the regions where BOVA is present or not. If we have a look at the distribution of the BOVA's observations in Geneva, we can see that the places where no toad was observed are quite grouped (**MAP 3.1**). Indeed, the sites that were monitored in the centre of Geneva, in the Champagne region, did not allow any observation of our target species. To explain that, we considered the expansion of the main rivers within the area. It appeared that the sites where most of the observations were realised are close to one or more running waters (Rhône, Arve, Laire, Aire, Allondon) or in areas where many small streams exist (Jussy area). Thus, these sectors permanently keep a certain rate of humidity. In opposition, the centred area is not connected to any stream. Indeed, the Champagne area is known to be warm and dry (Meichtry-Stier et al., unpublished). The drier conditions can explain why the BOVA could not establish itself as it needs a certain distance to source and density of streams (Pichenot, 2008). However, we observed other amphibian species in this sector, among others the Natterjack toad (*Bufo calamita*: BUCA). It is often said in the literature that the needs of this species are close to those of the Yellow-bellied toad (GHRA-LPO Rhône-Alpes, 2015), so we wondered why we could not find any BOVA. The depressions that are filled with rainfall in the Champagne quickly dry and are often very wide and thus not adequate for BOVA. Moreover, even if these two species' needs are often compared, the BUCA is the extreme European amphibian for breeding in temporary waters. Its development rate is much faster than the one of BOVA, which can explain how this species can survive in the Champagne whereas BOVA did not (Díaz-Paniagua, 1988; Banks & Beebee, 1988).

ii. Status of populations

We explained previously that we considered three types of population sizes: the observed population size (OPS), which is the total number of identified toads observed during the whole study period, the current population size (CPS), which is the total number of identified toads observed within a year, and the real population size per year (RPS) using data from the program MARK. First of all, as shown on **APP. C3**, we can see that the CPS (black dots) of 13 out of the 17 Swiss sites that allowed the observation of BOVA increased along the study. This is already a good note to assess the efficiency of the installed measures for improving the fitness and conditions of BOVA's populations. A more detailed analysis of which factors contributed to this efficiency and really influenced the presence of BOVA will follow in the *Ecology part*. We can also see on **APP. C3** that the OPS (purple bars) tend to increase each year, meaning that each new field season allowed the observation of new unknown toads. These new individuals may be juveniles or adults. New identified juveniles may mean that we discovered a new area where breeding occurs, which was the case in VER in 2014, and in BOU and CAR in 2015, or may mean that breeding started in the area as the conditions improved, which was the case in ARA in 2015. New identified adults may mean that some hidden animals were finally discovered, or may mean that new individuals immigrated in the area. Indeed, thanks to MARK, we considered our populations as open between seasons, and closed during seasons. The estimated real population sizes (N) are different than our OPS (c)

because they depend on the recapture rate (p) (formula: $N=c/p$), which depends on the habitat, on the availability of the ponds and on the humidity of the surrounding. Indeed, if there are many ponds in the area, but the environment is dry, the recapture probability increases because the toads are more likely found in the ponds that we monitor. However, if the environment is humid, the toads can stay out of the ponds and, thus, not be detected during field work (Bartelt et al., 2004; Bartelt et al., 2010; Sullivan et al., 2008). In addition to that, the spatial distribution of the monitored ponds also has an impact on the detection of animals.

If we have a look at the results given by MARK, we see that the RPS of ARA, BOU, PEN and ROU are much higher than our OPS, due to a smaller recapture probability. This can be explained because in these sites, the toads can find good habitats out of the monitored ponds. ARA, BOU and ROU are located in forests, but the monitored ponds are widespread in a big area, so it is likely that we miss individuals when they are travelling through the site. Moreover, we showed that in ROU, some individuals were not seen during several consecutive years, which proves that the animals stay hidden for observers and find other suitable habitats that are not monitored. PEN is a site located in a clay slope. It can be very dry, but as soon as it rains, the whole area becomes suitable for toads as many small holes are suddenly filled with water. That explains why we may miss individuals. On the other hand, we see that the RPS of CAR, COL, COR, JUS, and TEP are quite close to our OPS, due to a very good recapture probability. This can be explained because in these sites, even though the areas can be vast, the monitored ponds are numerous and well distributed in the site. The toads are thus more likely seen in ponds that are monitored, and will thus be detected. Moreover, as these sites are quite dry, the toads will stay in the ponds instead of in the environment.

Three sites give unexpected results: BAR, VER and PLA. MARK gave a recapture probability of 1 to BAR, which is very surprising because only 8 out of 44 individuals were recaptured at least twice. Moreover, we saw above, that the success rate of BAR is only 40%. We assume that as this site was monitored only during two years, MARK could not accurately calculate this recapture rate and it overestimated it. We thus think that the RPS of BAR is well underestimated. The problem of VER is that it is located in forests with widespread monitored ponds, so we expected a low recapture probability as for ARA, BOU and ROU. However, MARK gave a recapture probability of 0.93. We assume that the bias is the same as for BAR: the site was monitored only two years, so the dataset was not large enough for MARK to have an accurate calculation. About PLA, MARK gave a recapture probability of 0.5 to 0.63, whereas the area is small and the availability of other not monitored habitats is not likely. This can be explained because the main pond is a huge retention tank with very difficult access and deep water, which probably hides many animals. Moreover, the canal connects this site with some further areas and, as soon as it rains, it probably allows the toads to leave the area.

Thanks to our capture-recapture data, we are able to compare our observed recapture probabilities with those of MARK (TABLE 3.9). We see here that for most sites, the estimated probability is much higher than the observed probability. It is only for

ARA, BOU and ROU that the observed probability is higher, but the difference is large only for BOU. For the other sites, the differences between estimated and observed probabilities is large for five sites (BAR, CAR, COR, JUS and VER). We tried to investigate whether these biased estimates might be related to the size of the available ponds. Indeed, the recapture probability also depends on the accessibility of the ponds for humans. BOU is a site with only 16 identified individuals and a very small CPS (5 in 2016). Considering all sites with more than 10 identified individuals, it is the only site where the ponds have a surface smaller than 18m². This point, put together with the small population, might explain the bias. About the other differences, BAR and CAR comprise huge ponds (max 240m² and 8000m² respectively) which means that the observers struggle to reach the water and may miss individuals that are not on the edges of the pond. Moreover, if we consider the type of habitat, factor that MARK cannot take into account, we noticed that the biased estimates concern sites that are located in forests, but where the ponds were on the edges of the trees. As mentioned by DeMaynadier et Hunter (1995), the amphibians are more hardly caught in forests than in open areas. Moreover, Rothermel & Semlitsch (2002) said that the forests can be good habitats for food resources, cover, and thus to prevent dehydration, which implies that the animals might disperse more and be thus less observable. As that the concerned sites are places where most ponds are in open areas, we may hypothesize that the toads do not stay much in the ponds and prefer to go into the covered forest to stay in humid habitat. This might be why the observed probability is smaller than the estimated one.

TABLE 3.9. COMPARISON BETWEEN RECAPTURE PROBABILITIES. **Bold:** highest value. Underlined: large difference between both estimates. The observed probabilities were calculated thanks to capture-recapture data, the estimated probabilities were given by MARK. See Abbreviation list for all sites' names (p.0).

Group	Recapture prob observed	Recapture prob estimated
ARA	0.63	0.55
BAR	0.22	<u>0.98</u>
BOU	<u>0.75</u>	0.38
CAR	0.44	<u>0.94</u>
COL	0.74	0.88
COR	0.47	<u>0.74</u>
JUS	0.58	<u>0.85</u>
PEN	0.53	0.61
PLA	0.45	0.55
ROU	0.73	0.6
TEP	0.77	0.91
VER	0.41	<u>0.89</u>

To summarize some points about the recapture probability, we can say that it depends at the same time on the humidity of the environment, the spatial distribution of our monitored ponds and the type of habitat. As all these considerations are not used by MARK, we can expect to have differences between both the observed and estimated recapture probabilities and real population sizes.

The program MARK also allowed us to estimate the survival probabilities. The CJS model that fits the most our data says that the survival depends on the group and changes over time. Indeed, we can see in the estimates that the groups show big differences. The survival probabilities ranges from 0.07 (COR) to 0.99 (JUS, PEN, PLA), with means ranging from 0.38 to 0.85. Here, we need to take into account that some sites were only monitored during a few years, so MARK had to extrapolate the estimates over five years from fewer data than for sites that were monitored during five years. It may lead to biased estimates that are difficult to interpret. With our capture-recapture data, we are unable to estimate the survival rate of our toads, and thus we cannot compare an observed survival probability with the estimates of MARK.

The global sex-ratio in the whole study area appears to be quite balanced (53:47) (FIGURE 3.4B). However, we showed that during some years of data collection, we caught and identified more males than females (different individuals). We also noticed that among all capture events, more recapture events concerned males than females (sum of captures) (binomial= $9.86e^{-24}$, $\alpha=0.05$), meaning that males seem more catchable than females. This is consistent with the results of Cayuela et al. (2014) who showed that the females may go to the breeding sites only one year out of two and stay there for a shorter period. They are thus less likely to be caught. The fact that the sex-ratio is balanced in Geneva is good in a conservation perspective, because it means that all individuals may find a mate. Moreover, it was shown that the sex of amphibians is not determined by the temperature (Hayes, 1998) as it is particularly the case for reptiles (Rhodes & Lang, 1995). Indeed, a study conducted in lab on *Rana sylvatica* lead to the results that the sex-ratio was always close to 50:50 even if the temperature of incubation varied (Witschi, 1929). This can thus show that even if global warming increases the temperatures of the planet, the sex-ratio of amphibians should not be biased. However, further studies should take place to assess whether all males and all females breed, or if a certain percentage of the male or female populations participate to the reproduction (effective population size).

We saw that breeding occurred in 18 out of 21 monitored sites. However, the juveniles are only a minor part of the identified individuals (GVA: 179 juveniles out of 1107 individuals: 16% of the total population). This is low, especially when we consider that 82 juveniles were observed in PLA (46%), 35 were observed in VER (19%) and 13 were observed in CAR (7%), meaning that 72% of all juveniles come from 3 sites only. It suggests that even if breeding occurs, it is not abundant and may not be enough to insure the conservation of the species on a large scale and on the long-term. However, as mentioned above, the number of juveniles participates to the increases seen in OPS, but more field work should take place to discover some new breeding grounds.

iii. Strategies of *Bombina* according to threats

According to the GLM and the distribution of our observations in ponds presenting different factors, we found that the presence of Marsh frogs is negatively correlated with the presence of BOVA, meaning that when there are frogs, there are no BOVA. Indeed, we saw that there was a significant difference between the number of toads present in ponds where there is no frog and when there are frogs. We also saw that this trend is only true for adult BOVA and that there is no difference between any number of frogs, which suggests that the only presence of frogs, and not the number of frogs, dissuade the toads to come in the pond. This is different from the conclusion of Cayuela et al. (2012), but in their studies, they noticed that the overlap of the distribution of both species was very small. In our study, the distribution of PERI has the same extent as the one of BOVA, so it may explain why we found a negative influence.

Moreover, it appeared that there are more BOVA when there is at maximum one Italian crested newt. As this species is considered as a predator for BOVA's eggs and larvae, this is surprising. We may suggest here that the toads choose ponds in which there are newts for their ability to feed on insects, even though they can also be a threat to BOVA. Moreover, in their research Barandun & Reyer (1997a) said that if the toads have the choice, they will try at maximum to avoid areas where there are newts and invertebrate predators. However, if the threat of predators is overpassed by the threat of desiccation, the latter risk seems more important and so, the adults will preferentially spawn in ponds with predators, but with more water. Then, the induced threat of predation may be countered by changes in behaviour and morphology of the tadpoles that will grow more important caudal muscles to be able to swim faster and that will have a reduced activity and will prefer staying under shelters (Hartel et al., 2007b).

Globally, it seems that the frogs are the only factor that influences the presence of BOVA for all stages. All other influences are especially seen for eggs and tadpoles, meaning the spawning adults, which appear to be the most demanding age class. Moreover, the mixed effect of Odonata with PERI can suggest, as for the newts, that the BOVA choose sites where PERI can feed on Odonata and thus remove some danger for its eggs. It was also advanced by Cicort-Lucaciu et al. (2011) that BOVA itself may feed on Odonata larvae, which can confirm our results that show that the Odonata is the only insect species that does not influence negatively the presence of BOVA's adults.

iv. Low mobility

The amphibians are known to have a low mobility (Smit et al., 2006; Rodriguez et al., 1996) and to be highly philopatric (Schroter, 2005; Smith & Green, 2005), which does not help them to keep the connection between populations. Thanks to our capture-recapture data, we were able to know the exact location where any toad was seen for each capture and we thus calculated a mean daily travel distance thanks to the time interval between two captures. We wanted to be able to compare the distances covered by several individuals. In the article of Beshkov (1980), it is said that the toads

can reach 20 to 60 meters during a season, that the average distance between seasons is about 600 meters and that the record between two recaptures is about 300 meters. However, he did not consider the time during which the toads could have travelled. Barandun & Reyer (1998) did not consider the time interval either, but found that the toads usually do not move more than 50 meters away from their original pond. In our data, we found that between two recaptures within the same season, the record was 567 meters (in 58 days), and that between seasons it was 1190 meters (in 323 days). Globally, we found that most toads travelled less than 1 meter per day, which means about 150 meters during our yearly study period, and if we consider that the spawning period lasts three months, the average distance is about 90 meters. We also wanted to show the link between the covered distance and the weather, as Barandun & Reyer (1998) advanced that BOVA may move over biggest distances when the conditions are dry as they look for suitable breeding areas, but we recorded the weather only during our field sessions and not in the interval between them. The link was thus not possible to make.

We also considered the covered distance according to the age class. We found that adults move globally less than subadults and juveniles (intra-annual daily means: adults=1.82m, subadults=2.43m, juveniles=2.63m, inter-annual daily means: adults=1.21m, subadults=1.86m, juveniles=2.34m). However, the differences are less obvious if we consider the medians (intra-annual daily medians: adults=0.58m, subadults=1.13m, juveniles=1.06m; inter-annual daily medians: adults=0.31m, subadults=0.73m, juveniles=0.97m). Overall, juveniles appear to move more than the other classes, which is consistent with the reported higher dispersal ability of juveniles in the literature (Barandun & Reyer, 1998). Indeed, they have to find new breeding grounds, even if they may return to their natal site afterwards. Gollmann & Gollmann (2000) also suggested that juveniles may move over considerable distances during the first years of their lives.

Obviously, the covered distance is also linked to the relief of the area, which means that if there are lots of obstacles around the ponds, the toads have more difficulties to travel far from them. This point will be approached in the *Ecology part* where we analysed the resistance of the environment thanks to the ArcGIS tool Linkage Mapper.

E. Conclusion

Overall, we saw that the size of the populations in Geneva increased during our monitoring, which suggests that the conditions became more favourable for our target species in the last years. Moreover, it appeared that the parts of Geneva that the Yellow-bellied toad (*Bombina variegata*: BOVA) did not colonise are geographically and topographically not adequate for this species, so there is no sense in trying to make it settle there. The breeding is not abundant everywhere, but further field work should take place to improve the data on this aspect. Indeed, we already noticed that some new breeding grounds may be discovered, so more prospections could allow us to detect some unknown important zones. We showed that the presence of other amphibians, and especially the Marsh frog, is avoided by BOVA, so the protection measures need to be created with care to be sure we do not favour the wrong species. The creation of small semi-natural habitats and the installation of buckets with a reduced surface is a good way to promote BOVA only, as this species prefers smaller ponds than its competitors. As other authors did, we found that BOVA does not disperse on great distances. However, we calculated an average daily travel distance to be able to compare the individuals' movements without adding a bias with the time between two recaptures. To our knowledge, our study is the first to date which focussed on BOVA on a long-term basis Geneva area. We showed that some results cannot be summarize over the whole study because of annual trends, which proves that short-term studies cannot reveal the importance of some of the studied factors. To be able to protect in an efficient way an endangered species such as BOVA, one needs to have good knowledge on the variation of the parameters that may influence its presence.

IV. ECOLOGY

A. Introduction

When a species is impacted by human activities affecting its habitat, one way to help it is to stop damaging the area and to try to restore suitable conditions (Gascon et al., 2007). This is not simple, because the creation of new habitats might not benefit the concerned species if they are not adequate and do not respond to the needs of the target (Gascon et al., 2007). In Geneva, the Yellow-bellied toad strongly suffered from the expansion of the anthropogenic activities that reduced the areas with appropriate ecological conditions. A first study conducted in 2006 in the East part of Geneva (Jussy) aimed at positioning artificial recipients in the field and assessing the impact of this action on the conservation of the Yellow-bellied toad (*Bombina variegata*: BOVA) (Jaggi, 2010). Three different types and shapes of recipients were used (rectangular, elongated, circular). The advantage of these plastic buckets is that the loss of water into the ground is avoided. Moreover, as their surface is relatively small (between 1500 and 5000 cm²), the evaporation rate is slow. The results show that this habitat type allowed the observation of 22 different individuals in a 2 months' time-interval in an area where BOVA was not considered abundant and easy to observe. Even if this study can only give a short-term idea of the impact of these buckets, it served as a starting point to set up a long-term study aimed at assessing the efficiency of such artificial measures for the conservation of BOVA.

In our study, we thus decided to monitor the whole area of Geneva to assess the presence of BOVA in historically occupied sites. We installed plastic buckets, mapped existing natural depressions and created new semi-natural ponds. Then, we monitored the presence of BOVA in these habitats to understand their efficiency for the conservation of this threatened species. We conducted preliminary analyses at the end of 2013 to assess the efficiency of these measures and the effect of environmental factors (Tournier & Tournier, 2013). This also allowed us to tune up our protocols for the following years of monitoring. At the end of the study, we conducted the same analyses again, but with the full dataset including data for five consecutive years.

Moreover, we presented in a previous chapter the movements recorded for the toads between two recaptures and a potential travel rate per day (see *Part III: Ethology*). However, if we consider movement, we need to consider how the landscape affects the travel ability of the toads. Indeed, these animals are known to have low mobility (Smit et al., 2006; Rodriguez et al., 1996), and either new disturbances caused by many works ongoing in the city and around, or the landscape itself, or even old human-made structures can prevent the animals from moving from one place to another (Joly et al., 2003). As the dispersal is linked to population dynamics, it is important to understand which factors can affect it to be able to achieve better conservation strategies (Graves

et al., 2014). One way to assess the impacts of habitat fragmentation is to introduce the concept of resistance. The resistance of a surface is a value that is given to a grid cell according to its characteristics and how it can constrain movement or gene flow (Spear et al., 2010). It depends on both the potential obstacles located on a path and on the willingness of an organism to pass through a certain type of habitat. Moreover, it links several factors that can interact with each other to produce different impacts (Spear et al., 2015). It is thus very difficult to assess the resistance of different types of habitats. In addition to that, the effect of a habitat can vary according to the region or according to the animal. Stevens et al. (2006a and 2006b) presented a study on the Natterjack toad (*Bufo calamita*) in which they used artificial arenas including different types of habitats. They checked whether the toads passed more easily through certain land types and also whether they could detect a preference of the animals for some land types. It appeared that these two types of resistance were not always consistent. Another study also showed that the impact of a habitat on mobility strongly depends on the concerned species, but even then, the impact can differ according to the region (Zanini et al., 2009). This means that the resistance cannot be globally defined and must be reconsidered for each case. The setting of the resistance values of a landscape can be done in two ways (Ray et al., 2002). As noted above, one can conduct an experiment to assess the resistance of each landscape type. The results of such an empirical method are likely to be the closest to reality (Spear et al., 2015), and this can explain why their popularity is increasing in the recent years (Richards-Zawacki, 2009; Emaresi et al., 2011; Hagerty et al., 2011). The other possibility is to use an expert opinion. It is clearly the most subjective way of determining the resistance values, but it is more cost-efficient than an empirical method, and the knowledge of an expert can improve the data in a way difficult to achieve with many experiment (Murray et al., 2009).

B. Methodology

i. Environmental factors affecting *Bombina*'s presence

As a first step, started with a visual description of the data,. We first looked at the preferences of the individuals depending on the type of pond, and then on the age class.

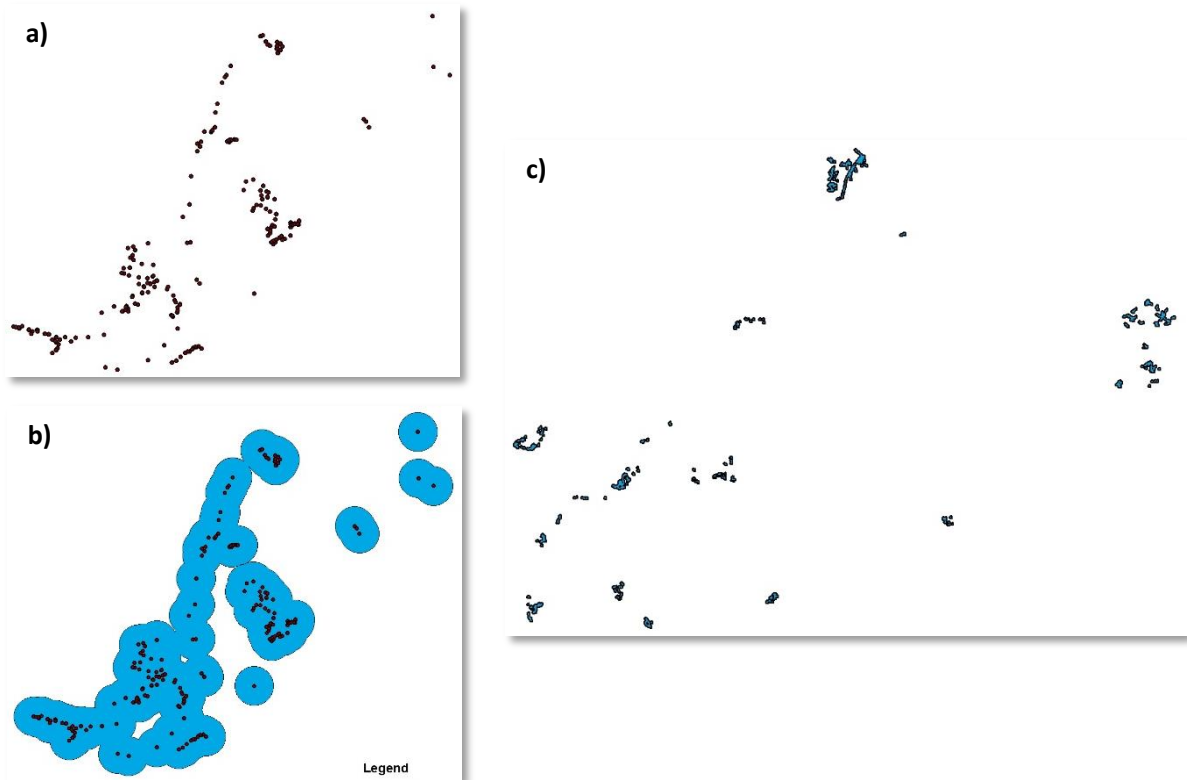
We then conducted several analyses considering the number of individuals of each stage that were seen in the ponds according to various characteristics. We consider here two different clusters of variables: (1) the cluster “water”, including the water level, type of ponds, turbidity and surface; (2) the cluster “vegetation”, including the presence of algae, branches, dead leaves and growing vegetation. We looked at the global distribution of our observations. To prevent a bias in our data, we used densities instead of abundances of toads because the number of ponds presenting a given characteristic vary a lot according to the considered characteristic. We also conducted Kruskal-Wallis tests (with Nemenyi correction for pairwise testing when more than two classes are present), Chi-square test (two classes) or two-sample Kolmogorov-Smirnov test (for distributions) to check for significance in differences between groups or distributions.

Finally, we ran Generalized Linear Models to estimate the influence of the environmental factors of the cluster “water” and “vegetation” on the presence of BOVA.

ii. Permeability of landscape

To investigate how the toads can move in their habitat, we used Linkage Mapper, a GIS tool created in 2010 by McRae & Kavanagh (publication in 2011) that runs on ArcGIS with the Spatial Analyst extension. It works in the same way as some GIS packages that existed previously and where used in studies on landscape resistance (Joly et al., 2003; Ray et al., 2002), but is designed to automate the mapping of wildlife habitat corridors. To run, the tool requires a core area polygon GIS file and a resistance raster GIS file. For the users who do not have an ArcGIS license, a text file is also needed with Euclidian distances between the polygons.

To create our core area polygon file, we used the map of each pond that we created and updated along the study. Thanks to the movement analysis presented in the previous chapter, we know that most of the toads do not cover a distance bigger than 50m. We thus decided to build a buffer of 50m around each pond. We used the option “dissolve type” according to the site’s name, so that all the points belonging to a single sampling site where considered as a unity (**MAP 4.1**).



MAP 4.1. STEPS TO CREATE THE POLYGON FILE. a) Points for each pond. b) Buffer of 50m around each point. c) Polygons for the whole Geneva.

To create our resistance raster file, we took advantage of a habitat map of Geneva that was recently created using 39 variables (slope, geology, distance to water, etc.) (FIGURE 4.1, MAP 4.2A), predictions, cadastral plans and satellite pictures of Geneva and that is continuously updated thanks to management plans (SITG website). In total, more than 257'000 objects are divided into 32 classes and 84 categories.

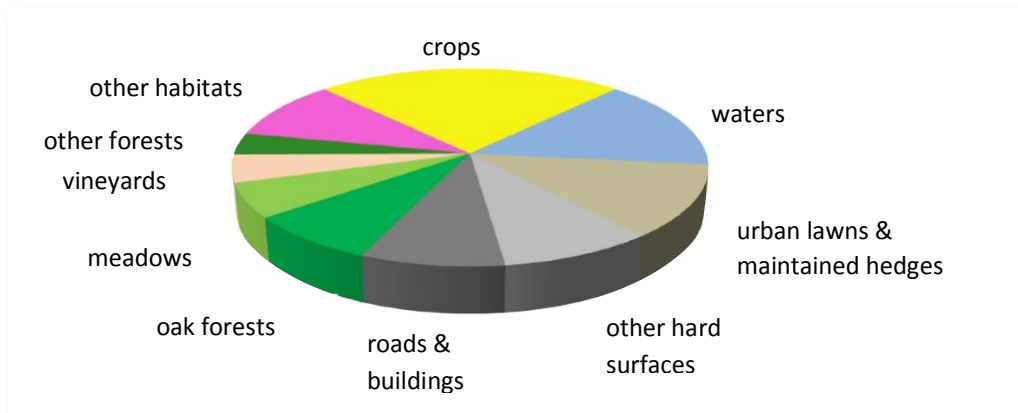


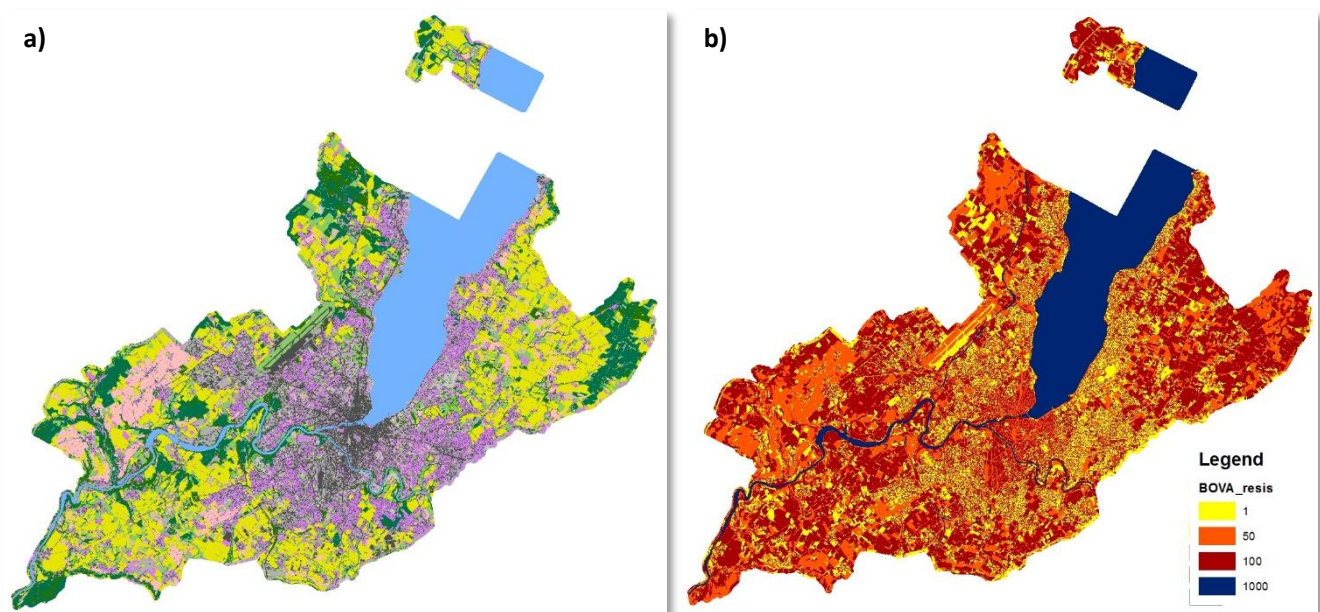
FIGURE 4.1. PIE CHART SHOWING THE REPARTITION OF THE HABITATS IN THE MAP OF GENEVA (SITG website). Colours: different habitat types.

We decided to define 4 classes of resistance. As advised by WHCWG (2010), we assigned 1 to putatively ideal habitats, meaning habitats through which a toad can move easily without a particular effort, and as advised by Beier et al. (2011), we assigned to

the main barriers a value higher than 100. The value 50 was assigned to habitat showing medium resistance. Our values of resistances are thus 1-50-100-1000. The maximum value was decided to be a lot bigger than the other ones to be able to model the effect of impassable barriers, impossible to cross for our target species (lake, flowing rivers, highways). Each habitat and its assigned resistance value are shown in TABLE 4.1.

We noticed that in the habitat map, all the roads were not further subdivided in different categories, but they all correspond to paved roads (routes revêtues, code MN 903) (TABLE 4.1). As we wanted to be able to show the effect of the highway present in our study area, we created a separate polygon shapefile on which we drew the highway that crosses Geneva on a North-South axe. We then used one of the “Analysis tools” of ArcGIS called “Update” to merge this shapefile with the one of the habitats to obtain the highway effect covering all other habitats present in the map where the highway passes. We then ran the tool “Features to Raster” available in ArcGIS to convert our map into a raster, and specified 10 meters for the size of each cell grid (MAP 4.2b).

Finally, we ran Linkage Mapper for all the 5 steps available. The step number 4 is optional but we decided to use it to limit the nearest neighbours to 4 cores.



MAP 4.2. CANTON OF GENEVA. a) Habitat map (colours according to pie chart above). b) Raster resistance map (10m grid) (colours: resistance to BOVA's movement).

TABLE 4.1. LIST OF HABITATS INCLUDED IN THE GENEVA HABITAT MAP (IN FRENCH). Code MN: code for each habitat type. Resist: resistance value according to BOVA's mobility. 1: no resistance. 50: medium resistance. 100: high resistance. 1000: impassable barrier. The 1000 category was manually added as the four concerned items are normally part of other categories.

CODE MN	VEGETATION	Resist	CODE MN	VEGETATION	Resist	CODE MN	VEGETATION	Resist
101	Eaux calmes	1		Mégaphorbiaies		908	Trottoirs	50
210	Prairies humides	1	214	marécageuses	50	909	Ilôts routiers	50
301	Alluvions nues des rivières	1		Végétations annuelles		919	Ilôts verts	50
302	Glariers	1	215	temporairement inondées	50	999	Chemins végétalisés	50
403	Gazons et terrains de sport	1		Enrochements (rivage sans			Végétations des sols argileux	
404	Accotements	1	218	végétation)	50	1002	calcaires	50
406	Pelouses sèches	1	407	Pelouses mi-sèches	50	1003	Massifs entretenus	50
409	Prairies artificielles extensives	1	408	Prairies artificielles intensives	50	1004	Bosquets arborés urbains	50
	Prairies semi-naturelles		502	Ourllets mésotrophes	50	107	Eaux courantes	100
410	extensives	1	510	Haies taillées	50	203	Roselières	100
411	Pâturages extensifs	1		Buissons mésophiles ou			Végétations des rives d'eau	
412	Pâturages intensifs	1	515	thermophiles	50	206	courante	100
	Formations graminéennes		516	Ronciers	50	208	Magnocaricaies	100
413	rudérales	1	517	Régénérations forestières	50	501	Ourllets eutrophes	100
416	Pelouses arborées	1		Saulaies buissonnantes		609	Hétraies mésophiles	100
520	Fourrés xérophiles alluviaux	1	518	alluviales	50	610	Forêts de feuillus mélangés	100
603	Arbres isolés	1		Saulaies buissonnantes		613	Chênaies à charmes à gouet	100
605	Saulaies blanches	1	519	marécageuses	50		Chênaies à charmes à herbe	
701	Terrains piétinés et rudéraux	1	601	Plantations de feuillus	50	614	aux gouteux	100
801	Pépinières	1	602	Plantations de conifères	50	619	Forêts secondaire de robiniers	100
804	Vergers haute tige	1		Forêts inondables des grands		807	Grandes cultures	100
805	Vergers basse tige	1	604	cours d'eau	50	811	Jachères florales	100
903	Routes revêtues	1	606	Aulnaies	50	901	Bâtiments	100
905	Sols nus sans végétation	1		Forêts riveraines des petits		902	Serres	100
910	Places d'aviations	1	607	cours d'eau	50	911	Bassin de STEP	100
1000	Chemins	1	611	Cordons d'espèces ligneuses	50	912	Objets durs divers	100
1006	Alignements d'arbres en	1	612	Chênaies à charmes	50		Hangars/dépôts agricoles	
	bordure de route			Chênaies à charmes à		913	temporaires	100
4444	Surfaces dures	1	615	molnies	50	924	Piscines	100
201	Rivages avec végétation	50	616	Chênaies sèches	50		Parois rocheuses et débris	
211	(prairies à litière mésophile)	50	623	Pinèdes	50	1005	rocheux	100
	Prairies humides à populage			Rudérales à annuelles et		101	Lac Léman	1000
212	mésotrophes)	50	708	pluriannuelles	50	107	Rhône	1000
	Prairies humides à globulaire		802	Pépinières de feuillus	50	107	Arve	1000
213	(prairie à litière sèche)	50	803	Pépinières de conifères	50	903	Autoroute A1	1000
			806	Vignes	50			
				Cultures herbacées,				
			808	marâchères	50			
			809	Jardins potagers individuels	50			
			904	Gravières	50			
			906	Voies ferrées	50			

C. Results

i. Presence according to factors from the “water” cluster

We first considered all the types of ponds in which observations took place to know which stage of the Yellow-bellied toad (*Bombina variegata*: BOVA) was observed in which type (FIGURE 4.2). It seems that all types of ponds allowed the observation of each age class, even if the proportions were not the same. Obviously, the number of tadpoles tends to drive the data here as they are generally much more abundant than the other stages. Tadpoles were less observed in ditches (F), whereas juveniles were observed only few times in buckets (B) and in the “other” class (X). However, as this graph shows percentages, the number of available ponds cannot be seen, so the differences between each type can be misinterpreted.

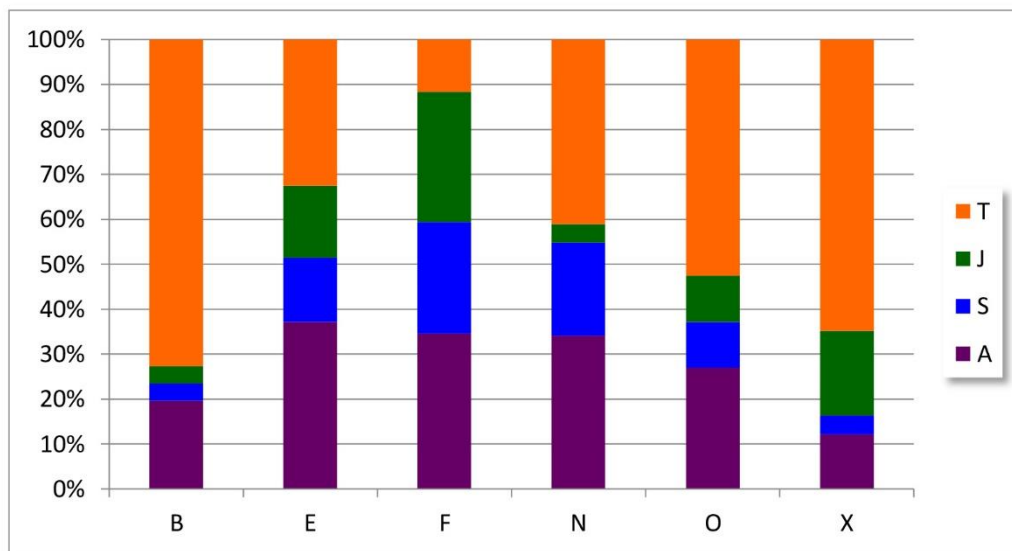


FIGURE 4.2. HISTOGRAM SHOWING THE PROPORTION OF OBSERVED STAGE IN EACH TYPE OF PONDS. A: adults. S: subadults. J: juveniles. T: tadpoles. B: buckets. E: ponds. F: ditches. N: natural. O: ruts. X: others. All stages are present in all types.

We thus investigated the question in the opposite way to know where each stage was mostly observed (FIGURE 4.3). We considered all the observations and their location. It appeared that tadpoles indeed are mostly seen in buckets and in the “other” class. The juveniles are mostly seen in the “other” class and in ditches. The subadults are mostly seen in ditches and buckets. The adults are mostly seen in buckets.

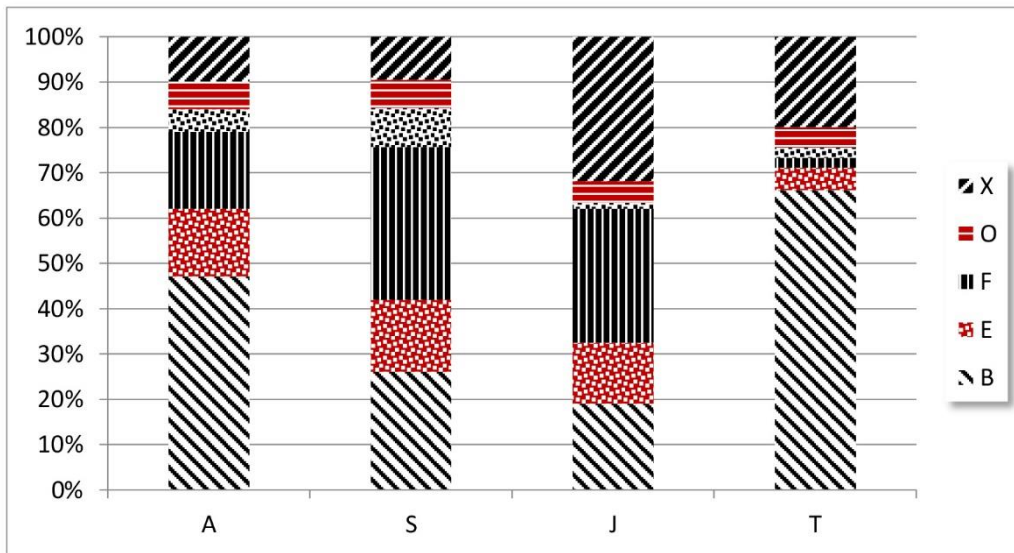


FIGURE 4.3. HISTOGRAM SHOWING THE PROPORTION OF POND TYPES FOR EACH OBSERVED STAGE. A: adults. S: subadults. J: juveniles. T: tadpoles. B: buckets. E: ponds. F: ditches. N: natural. O: ruts. X: others. The buckets are well occupied but are not the preferred type for subadults and juveniles.

Then, we investigated which characteristics of the ponds influence the presence of our target species. We first looked at their ability to retain water (FIGURE 4.4). We considered the percentage of each pond type that presented each water level. We can see that the buckets retain highly significantly more water than any other types (Nemenyi test for independent samples: $\chi^2(5)=5848$, $p=2.2e^{-16}$).

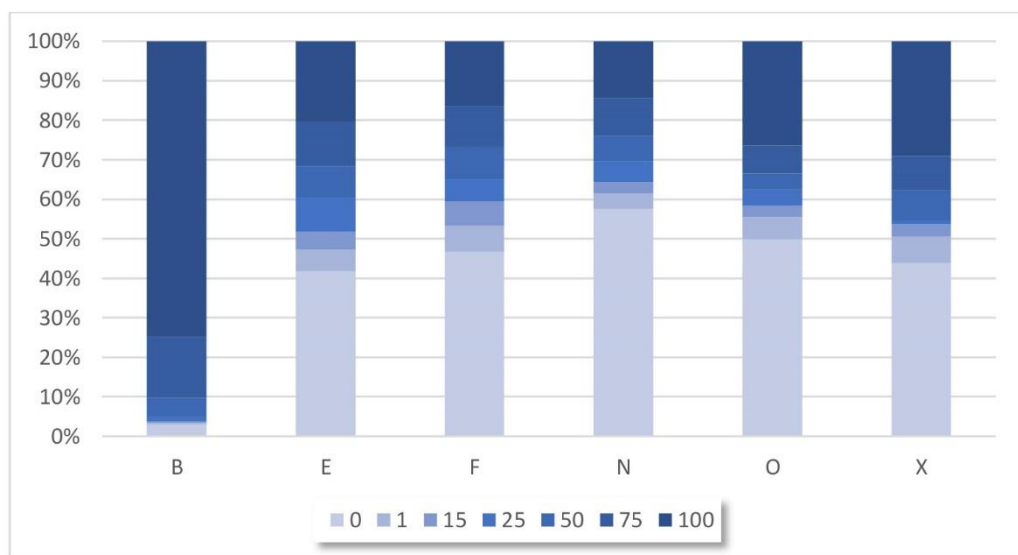


FIGURE 4.4. HISTOGRAM SHOWING THE PERCENTAGE OF WATER PRESENT IN EACH TYPE. B: buckets. E: ponds. F: ditches. N: natural. O: ruts. X: others. Numbers 0 to 100: water level. The buckets clearly retain more water than the other types (90.1% of buckets observations with more than 75% of water).

To know if the quantity of available water influences the presence of the toads, we compiled all the observations for each stage (sum of capture events) according to the level of water in the ponds (FIGURE 4.5). We did not consider tadpoles here as they do not choose their living environment, it is the choice of the breeding adults (next chapter).

Even if it seems on the following graph that there are more BOVA in ponds that are filled with water at their maximum, the differences between water classes are overall not significant (only 2 out of 63 significant pairwise comparisons with Nemenyi test: BOVA adults - 0 vs 75%, $p=2.1e^{-5}$; 0 vs 100%, $p=2.5e^{-8}$) (SEE APP. D1 FOR ALL P-VALUES AND GRAPHS OF DISTRIBUTIONS).

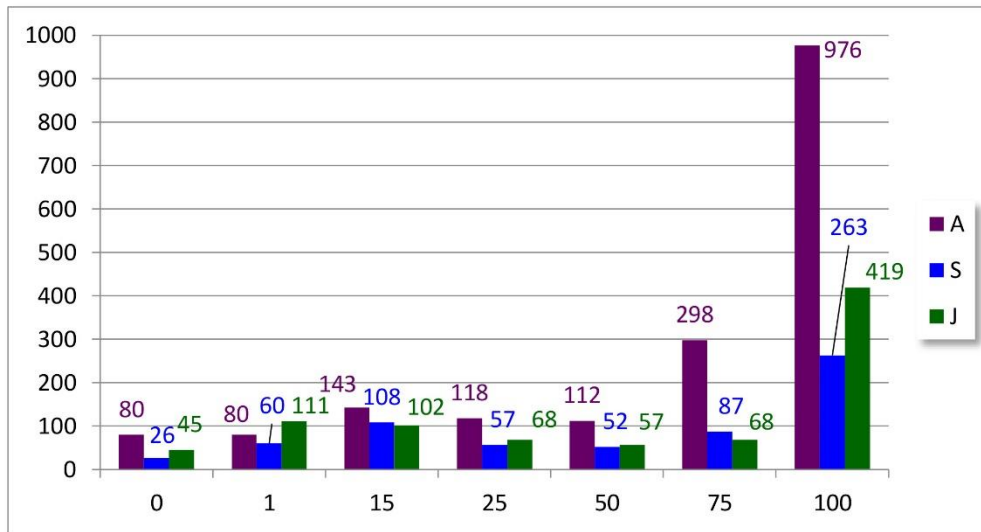


FIGURE 4.5. HISTOGRAM SHOWING THE NUMBER OF TOADS OBSERVED IN PONDS ACCORDING TO THE WATER AVAILABILITY. Purple: adults (A). Blue: subadults (S). Green: juveniles (J). Numbers 0 to 100: water level. It seems that all stages are more present in ponds filled with water. X-axis: levels of water availability. Y-axis: number of toads.

In addition to that, to prevent any bias due to the abundance of ponds presenting a certain characteristic, we used densities of BOVA (number of individuals - adults/subadults/juveniles - per surface - m^2) to conduct a two-sample Kolmogorov-Smirnov test to test whether the distribution of each stage of BOVA was different in each water class and the direction of the differences. For this analysis, for pertinence reasons, we removed the pairs that presented less than 5 data points because the Kolmogorov-Smirnov test compares distributions, and no real trend could be seen with less than 5 individuals. It appeared that for each stage, the distribution is maximal from 50% of water. If we consider the strength of the differences, it appeared that the differences are all highly significant from 75% of water for adults and subadults, and only for 100% of water for juveniles (SEE APP. D2 FOR GRAPHS OF DISTRIBUTIONS).

We further investigated the effect of water availability by using Kruskal-Wallis tests to see if there was a significant difference between the numbers of toads observed in ponds filled with 15% to 100% of water. Here, we removed the PLA site from the analyses because it presents particular conditions and as it is the biggest site, it could have biased the global trend. As there were no significant differences (all p -values > 0.99), we tested two groups of data, one including the observations in 0 and 1% of water, and one including the observations in more than 15%. In this case, the difference is very significant (adults: $p=3.9e^{-11}$, subadults: $p=0.02$, juveniles: $p=0.25$, **BOVA_{tot}: $p=2.4e^{-14}$** , $\alpha=0.5$).

We conducted very similar analyses to test the influence of the surface on the presence of toads. The global graph shows that the abundance of BOVA is reduced when the surface is large (FIGURE 4.6). The Spearman test indicated that there is no correlation between the number of toads and the surface (adults: $r_s = -0.082$; subadults: $r_s = -0.0009$; juveniles: $r_s = -0.006$).

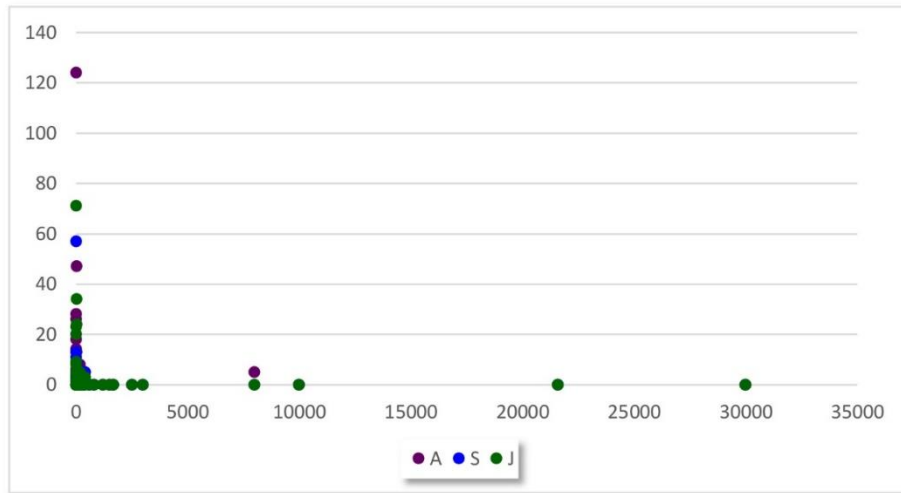


FIGURE 4.6. PLOT OF THE ABUNDANCE OF BOVA ACCORDING TO THE SURFACE OF THE PONDS. Purple: adults (A). Blue: subadults (S). Green: juveniles (J). X-axis: surface of the ponds (m²). Y-axis: number of toads. Most observations took place in ponds whose surface is smaller than 24 m².

Concerning the turbidity, we looked at the global distribution of the observations of BOVA between the three classes (sum of capture events). The category 0 corresponds to water that is clear, the category 1 corresponds to water that is between clear and completely trouble and the category 2 corresponds to water that is completely trouble (see *Methodology 2Ci* for complete explanation) (FIGURE 4.7). A Nemenyi test showed us that globally the category “clear” (0) is significantly different from the others ($\chi^2(2)=131.63$, $p=2.2e^{-16}$).

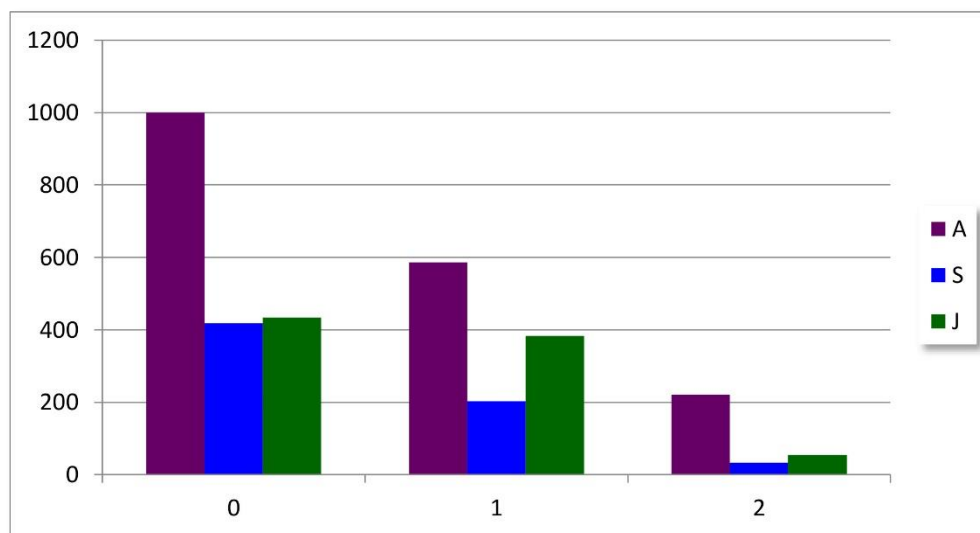


FIGURE 4.7. HISTOGRAM SHOWING THE NUMBER OF TOADS OBSERVED IN PONDS ACCORDING TO THE TURBIDITY. Purple: adults (A). Blue: subadults (S). Green: juveniles (J). 0: clear water. 1: clear to trouble water. 2: trouble water. Y-axis: number of toads. Most of the observations occurred in clear water.

In addition to that, we ran a GLM to investigate how the factors of the “water” cluster influence the presence of toads when they are considered all together (FIGURE 4.8). Adult BOVA’s densities are positively influenced by the water level and by the combination of water level and the type of ponds E (“ponds”). The densities of subadult BOVA are positively influenced by the combination of water level and the type E, as well as by ponds N (“natural”). The highest density in type E is when there is 75% of water, and the highest density in type N is when there is 50% of water. The juvenile BOVA’s densities are positively influenced by the combination of water level and the types of ponds E and O (“ponds” and “ruts”) (SEE APP. D3 FOR ALL VALUES).

	A Influence	S Influence	J Influence	T Influence	E Influence
A.SURFACE	*** 0	*** 0	*** 0		
A.DEPTH			*** 0	* 0	*** +
V	*** 0	*** 0	** 0	*** -	*** -
B	*** +	*** 0	** 0	*** +	*** +
L		*** 0		* +	*** -
A.SURFACE:A.DEPTH	*** 0	* 0	*** 0	** +	*** +
A.SURFACE:V	*** -	*** 0		*** -	
A.SURFACE:B	*** +	*** 0	*** 0	*** mixed	*** -
A.SURFACE:L			*** 0	*** mixed	*** -
A.DEPTH:V	** -	*** 0	*** 0		*** -
A.DEPTH:B	*** +	** 0	*** 0	*** +	*** +
A.DEPTH:L	*** 0	* 0	* 0		*** -
V:B	*** +	*** 0	*** 0	*** +	*** mixed
V:L	*** +	*** 0	*** 0	*** +	** mixed
B:L		*** 0		*** -	*** mixed

Figure 4.8. SUMMARY OF GLM RESULTS FOR THE WATER CLUSTER. A: adults. S: subadults. J: juveniles. T: tadpoles. E: eggs. Stars: strength of significance. Grey: not significant comparisons. Red: negative values of GLM estimates. Green: positive values of GLM estimates. 0: no influence on distribution. +: positive influence on distribution. -: negative influence on distribution. mixed: no clear influence.

ii. Presence according to factors from the “vegetation” cluster

For the following analyses, we considered the factors included in the “vegetation” cluster, meaning the presence of algae on the surface or in the depth of the pond, the presence of growing vegetation, branches and dead leaves. We first had a look at the global distribution of the observations (sum of capture events) (FIGURE 4.9).

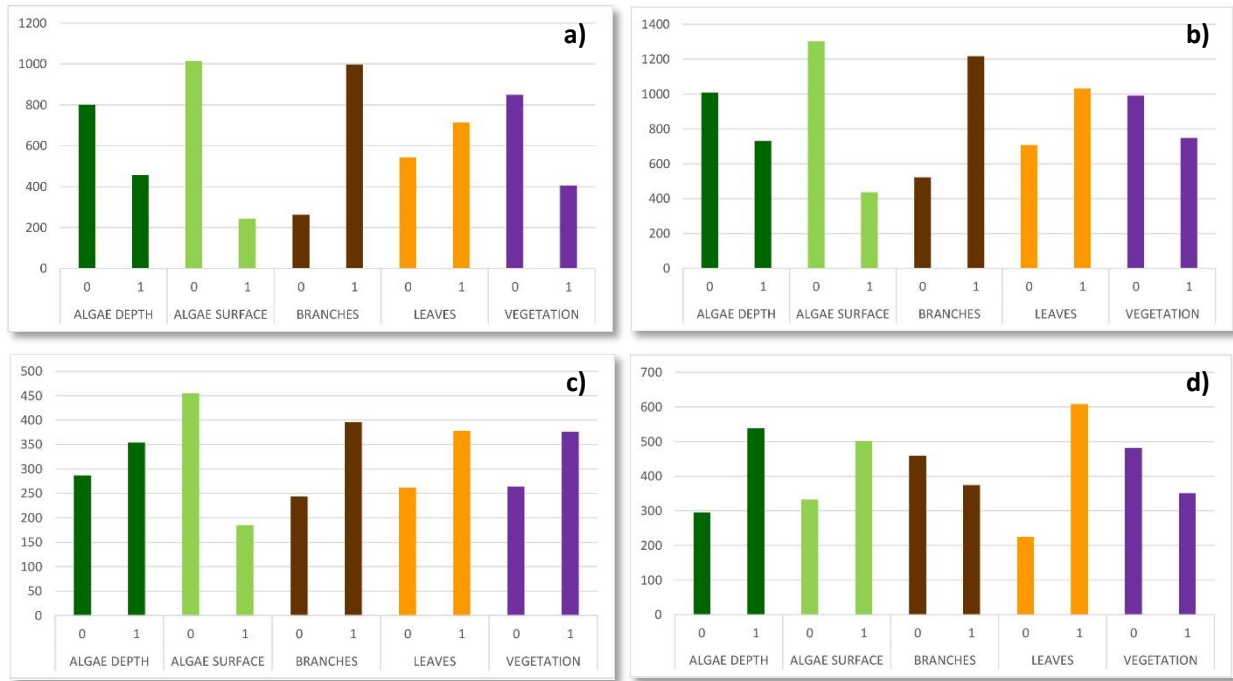


FIGURE 4.9. HISTOGRAMS SHOWING THE NUMBER OF TOADS OBSERVED IN PONDS ACCORDING TO THE VEGETATION CLUSTER FACTORS. a) BOVA_{tot}. b) Adults. c) Subadults. d) Juveniles. Dark green: Algae in depth. Light green: Algae on surface. Brown: Branches. Orange: Dead leaves. Purple: Growing vegetation. 0: factor is absent. 1: factor is present. Y-axis: number of toads.

In this analysis, we show the results in different graphs because the trend is not the same for all the age classes. If we look globally at the previous histograms, we can see that the algae in depth are apparently avoided by adult toads, but not by the other stages. The general trend goes in the same direction as the adults. The surface algae are avoided by all except by juveniles. The branches are linked with a higher abundance of adults and subadults, but a lower abundance of juveniles. The dead leaves seem to be preferred by all stages. Finally, the growing vegetation is avoided by all stages except the subadults. We then conducted Chi square tests to check for significance between the two classes of each factor (0: absent, 1: present). They revealed that all comparisons are significant (TABLE 4.2), even though the standard deviation is big. To check the influence of our biggest site (PLA), we removed it from the analyses, but the standard deviation and the trends did not change much. We also checked the influence of our smallest sites, but the results did not change if we remove them.

TABLE 4.2. P-VALUES OF CHI-SQUARE TESTS FOR EACH STAGE VERSUS EACH FACTOR. All comparisons are significant, to different levels ($\alpha=0.1$, $\alpha=0.05$, $\alpha=0.01$, $\alpha=0.005$).

	Algae depth	Algae surface	Branches	Leaves	Vegetation
BOVA	$<2.2e^{-16}$	$1.8e^{-5}$	$<2.2e^{-16}$	$1.17e^{-10}$	0.03087
Adults	$<2.2e^{-16}$	$2.5e^{-6}$	$<2.2e^{-16}$	$1.22e^{-6}$	7.694e⁻⁴
Subadults	$1.4e^{-11}$	$5.84e^{-4}$	$3.36e^{-7}$	0.0033	$1.34e^{-5}$
Juveniles	$3.67e^{-6}$	$1.49e^{-5}$	0.02695	0.06635	$6.73e^{-4}$

To obtain a more reliable interpretation of the influence of these factors, we ran a GLM for the vegetation cluster (FIGURE 4.10). According to the model, the juveniles and the subadults presence is not significantly influenced by any factor. The influence on adults appears to be positive as soon as there are branches or leaves in the combination (SEE APP. D3 FOR ALL VALUES).

	A	Influence	S	Influence	J	Influence	T	Influence	E	Influence
WATER	***	+	***	0	***	0	***	+		
T	***	0	***	0	***	0	***	-		
SURFACE:TYPEE			*	0	*	0	*	0		
SURFACE:TYPEF	***	0	***	0	***	0	***	0		
SURFACE:T	***	0	***	0	***	0	***	mixed	***	mixed
TYPEB:WATER	***	0	*	0	*	0	*	+		
TYPEE:WATER	***	+	**	+	**	mixed	**	0		
TYPEF:WATER	***	0	***	0	***	0	***	0		
TYPEN:WATER	***	0	**	mixed	**	0	**	+		
TYPEO:WATER	***	0	***	0	***	mixed	***	mixed		
TYPEB:T	**	0	*	0	*	0	*	-		
TYPEE:T	**	0	***	0	***	0	***	mixed		
TYPEF:T	**	0	***	0	***	0	***	0		
TYPEO:T	*	0	***	0	***	0	***	0		
WATER:T	***	0	***	0	***	0	***	-	***	-

Figure 4.10. SUMMARY OF GLM RESULTS FOR THE VEGETATION CLUSTER. A: adults. S: subadults. J: juveniles. T: tadpoles. E: eggs. Stars: strength of significance. Grey: not significant comparisons. Red: negative values of GLM estimates. Green: positive values of GLM estimates. 0: no influence on distribution. +: positive influence on distribution. -: negative influence on distribution. mixed: no clear influence.

iii. Presence of breeding indices according to the factors

Up to now, we talked about the preferences of the toads. However, tadpoles and eggs do not show any preferences as they cannot decide where they go. They rather allow us to see the preferences of adults BOVA to spawn.

Concerning the quantity of available water, breeding seems to occur more in ponds filled with water (FIGURE 4.11). However, a Nemenyi test showed that the difference between 100% of water filling and the other water levels are not significant (all p-values >0.31, except tadpoles: 100vs75: p=0.013). The high bars of the following histogram in 100% are pulled by outsiders (SEE APP. D1 FOR ALL P-VALUES AND GRAPHS OF DISTRIBUTIONS). A two-sample Kolmogorov-Smirnov test using densities of BOVA showed us that the distribution of breeding indices in 100% of water filling is significantly different from their distribution in the other water levels (SEE APP. D2 FOR GRAPHS OF DISTRIBUTIONS).

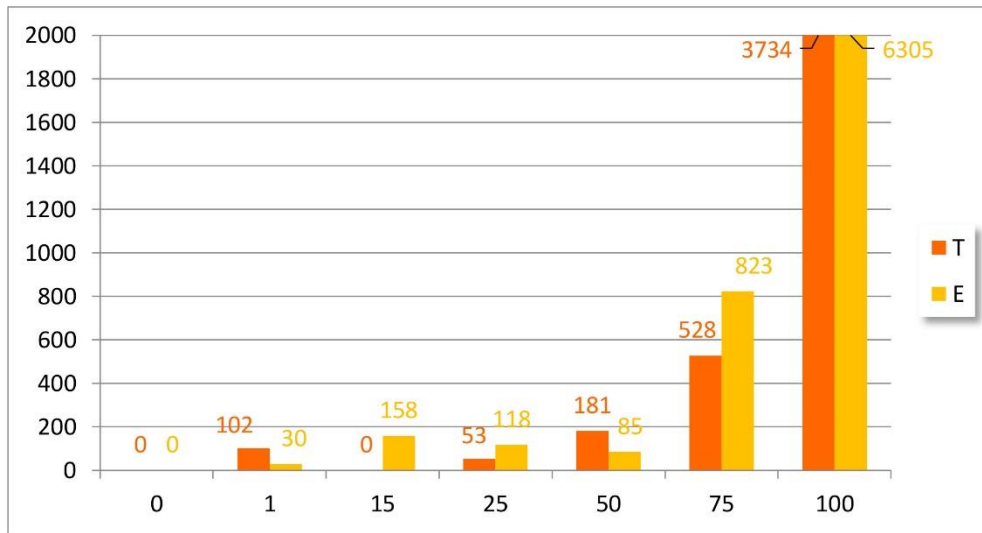


FIGURE 4.11. HISTOGRAM SHOWING THE NUMBER OF BREEDING INDICES OBSERVED IN PONDS ACCORDING TO THE WATER AVAILABILITY. Orange: tadpoles (T). Yellow: eggs (E). Numbers 0 to 100: water level. Y-axis: number of toads. It seems that breeding occurs especially when ponds are full of water.

The surface shows the same trend as for the other stages of BOVA: almost all observations of breeding indices occurred in small ponds (max surface: 400m²) (FIGURE 4.12).

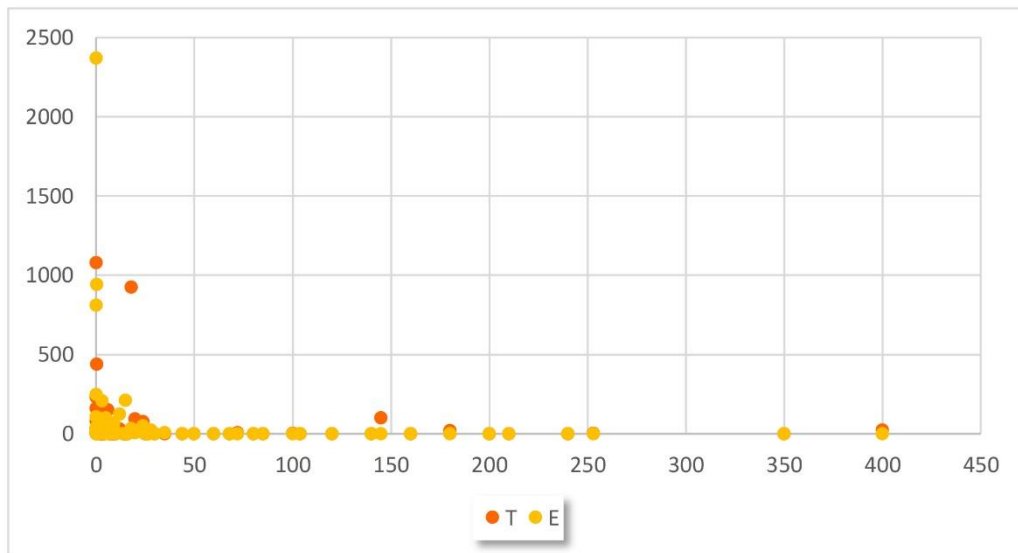


FIGURE 4.12. PLOT OF THE ABUNDANCE OF BREEDING INDICES ACCORDING TO THE SURFACE OF THE PONDS. Orange: tadpoles (T). Yellow: eggs (E). X-axis: surface of the ponds (m²). Y-axis: number of toads. All observations took place in ponds smaller than 400m².

On the following graph, we can see that more tadpoles and especially more eggs were observed in clear waters (FIGURE 4.13). A Kruskal-Wallis test showed that there are globally no significant differences in the abundance of tadpoles in different turbidity classes ($\chi^2(2)=3.74$, $p=0.15$). However, when we refined the analyses in removing the data where tadpoles were absent, the differences appear significant ($\chi^2(2)=10.92$, $p=0.004$). Then, a Nemenyi test shows that this significance is true for the comparison

between the number of tadpoles in turbidity classes 2 and 0 ($p=0.01$). The results for eggs show the opposite situation. The Kruskal-Wallis test on the whole dataset showed a significant difference for eggs ($\chi^2(2)=23.9$, $p=6.37 \cdot 10^{-6}$). However, the Nemenyi test did not show any significant pairwise comparison. We thus also refined the analyses in removing the data where eggs were absent. The results then appeared not significant ($\chi^2(2)=4.01$, $p=0.135$).

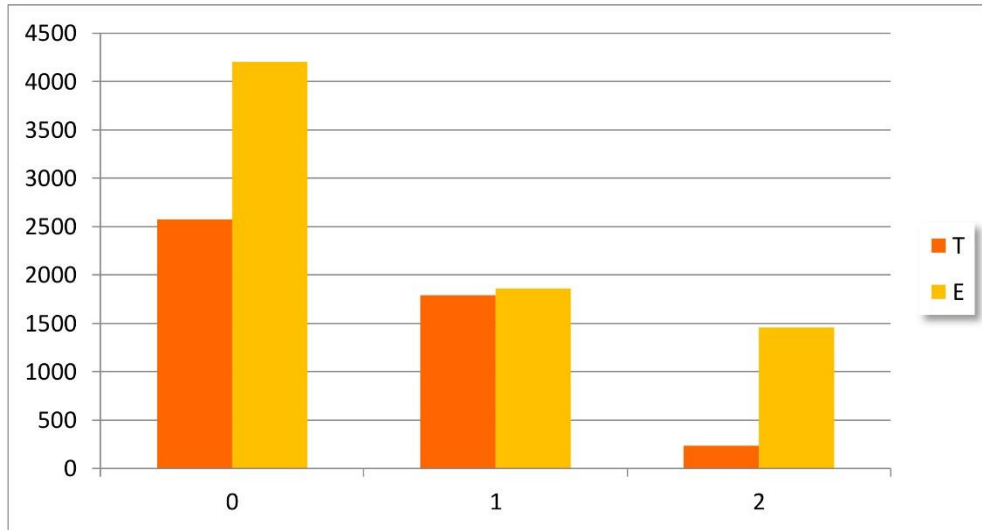


FIGURE 4.13. HISTOGRAM SHOWING THE NUMBER OF BREEDING INDICES OBSERVED IN PONDS ACCORDING TO THE TURBIDITY. Orange: tadpoles (T). Yellow: eggs (E). 0: clear water. 1: clear to trouble water. 2: trouble water. Y-axis: number of toads. It seems that most of the observations, especially eggs, occurred in clear water.

According to the GLM (FIGURE 4.8), the preferences of tadpoles and eggs appear again to be stronger than the ones of the other classes. The water level in buckets (B) and in natural ponds (N) is strongly positively correlated with the density of tadpoles. However, this class of BOVA, as well as eggs, are negatively influenced by turbidity, especially in buckets (B), for any water level. The surface does not seem to influence the presence of BOVA (SEE APP. D3 FOR ALL VALUES).

According to the cluster “vegetation” (FIGURE 4.14), the only apparent difference between the abundance of tadpoles and eggs is for ponds with leaves. However, Chi square tests showed that only three comparisons are significant. The trend is opposite to the one of the other stages (TABLE 4.3).

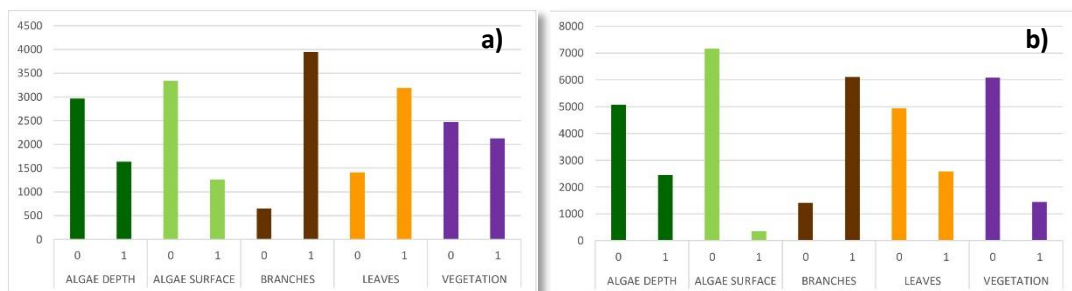


FIGURE 4.14. HISTOGRAMS SHOWING THE NUMBER OF BREEDING INDICES OBSERVED IN PONDS ACCORDING TO THE VEGETATION CLUSTER FACTORS. a) Tadpoles. b) Eggs. Dark green: Algae in depth. Light green: Algae on surface. Brown: Branches. Orange: Dead leaves. Purple: Growing vegetation. 0: factor is absent. 1: factor is present. Y-axis: number of toads.

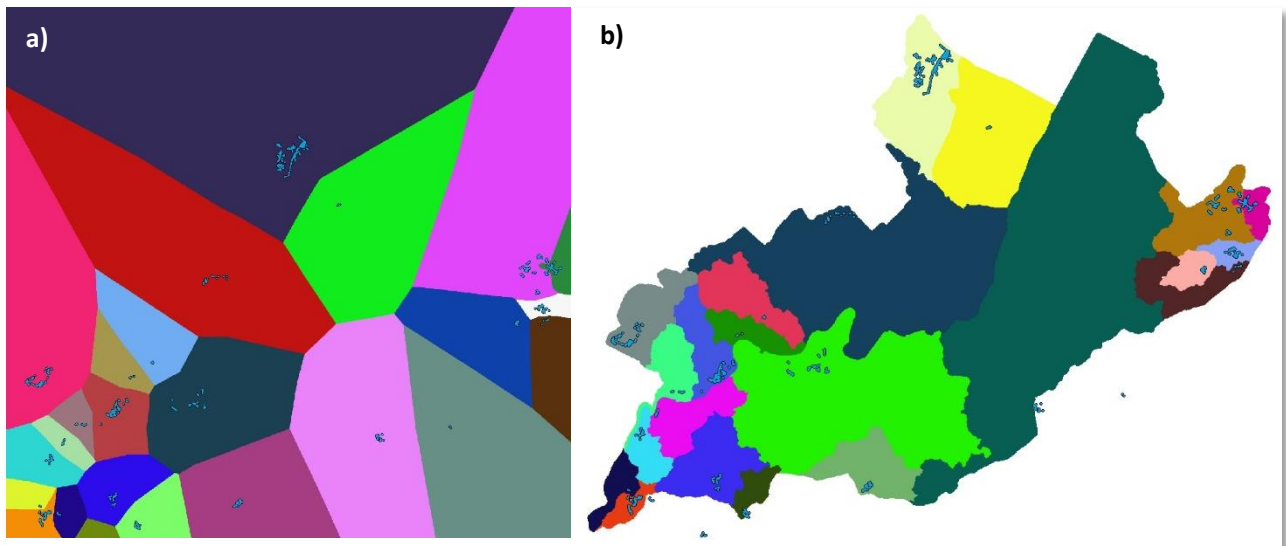
TABLE 4.3. P-VALUES OF CHI-SQUARE TESTS FOR TADPOLES AND EGGS VERSUS EACH FACTOR. Only four comparisons are significant ($\alpha=0.005$, $\alpha=0.01$ or $\alpha=0.05$).

	Algae depth	Algae surface	Branches	Leaves	Vegetation
Tadpoles	6.13e⁻⁶	0.2062	0.001657	0.04801	0.2521
Eggs	0.1508	0.7309	0.009709	0.6429	0.3469

The GLM for the cluster “vegetation” (FIGURE 4.10) reveals that the eggs are positively influenced by algae in depth, whereas the vegetation influences negatively the densities of tadpoles and eggs. The leaves influence positively tadpoles, but negatively the eggs. However, the presence of branches influences significantly positively adults, tadpoles and eggs. It seems that any combination of vegetation, branches and leaves tends to have a positive impact on both classes, whereas both algae combined with another factor tend to have a negative impact, except when they are both together, or in combination with branches (SEE APP. D3 FOR ALL VALUES).

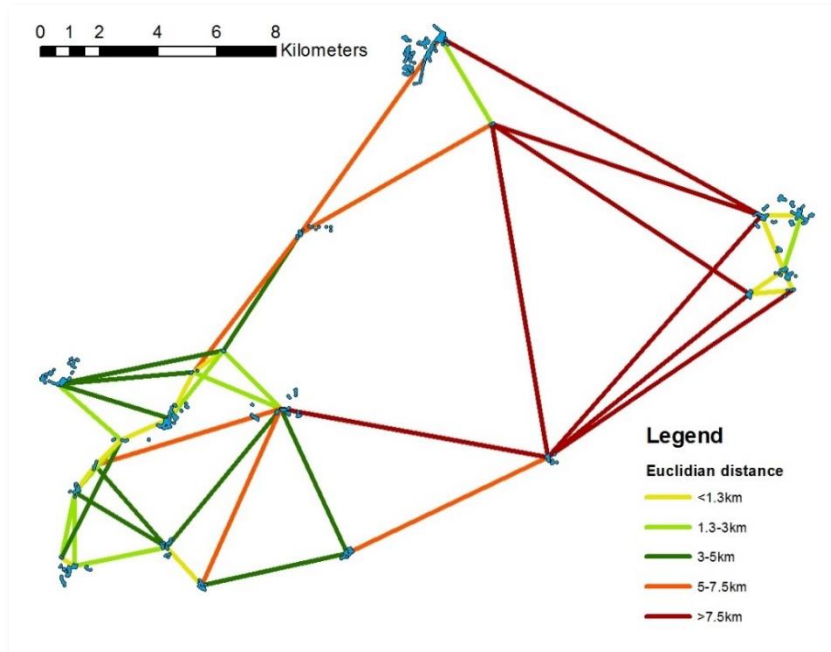
iv. Linkage Mapper

The first step of Linkage Mapper is to identifying adjacent neighbouring core areas (MAP 4.3). This is done according to Euclidian distances and according to Cost-weighted distances extracted from our resistance raster. It means that each grid cell is allocated to the nearest core polygon according to one or the other distance.



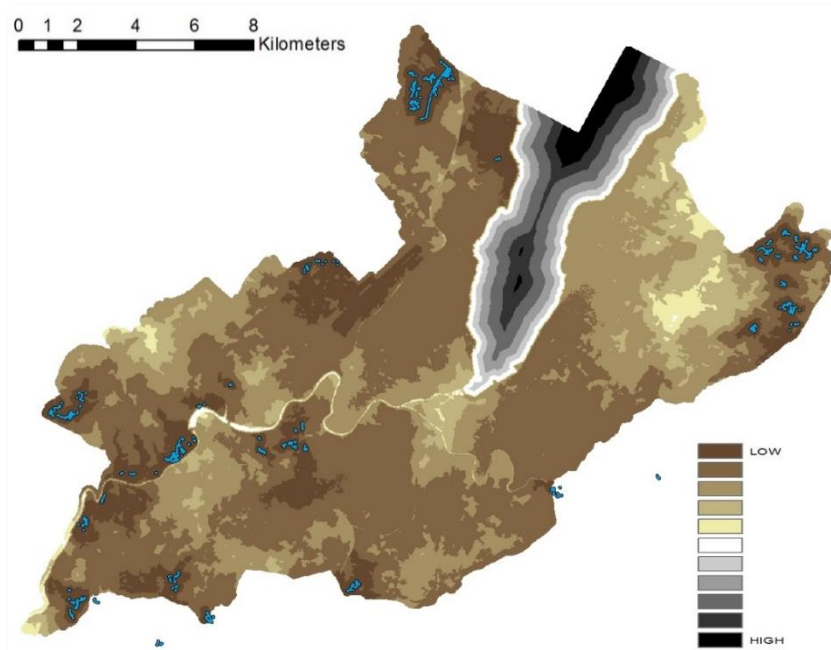
MAP 4.3. ADJACENT CORE AREAS. a) According to Euclidian distances. b) According to Cost-weighted distances. Colours are random.

The second step is to construct a network of core areas using the adjacency estimated in the first step and the distance data. All the core area polygons are thus connected by lines and each pair is a potential candidate for the corridor mapping that will occur later (MAP 4.4).

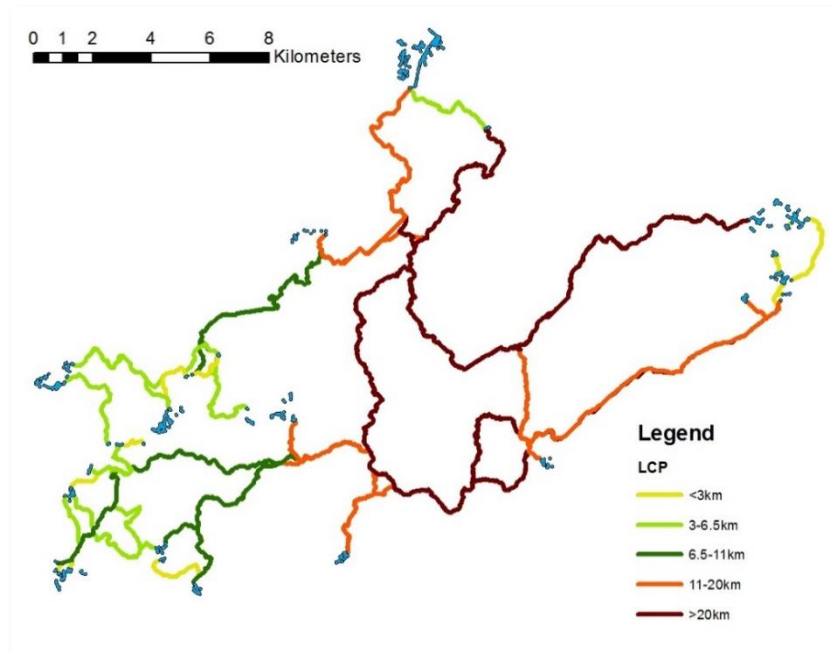


MAP 4.4. NETWORK OF CORE AREAS ACCORDING TO EUCLIDIAN DISTANCES. Colours: distances. The connections between the sites do not depend on the landscape features.

The third step calculates the cost-weighted distances from each core area and extracts from them the least-cost paths (MAP 4.5, MAP 4.6), meaning the route along which the least resistance is accumulated (McRae & Kavanagh, 2011), between each pair of cores. For this analysis, we asked the tool to drop any corridor that intersect another core area, which means that if a path passes through an intermediate core area, it was not considered.



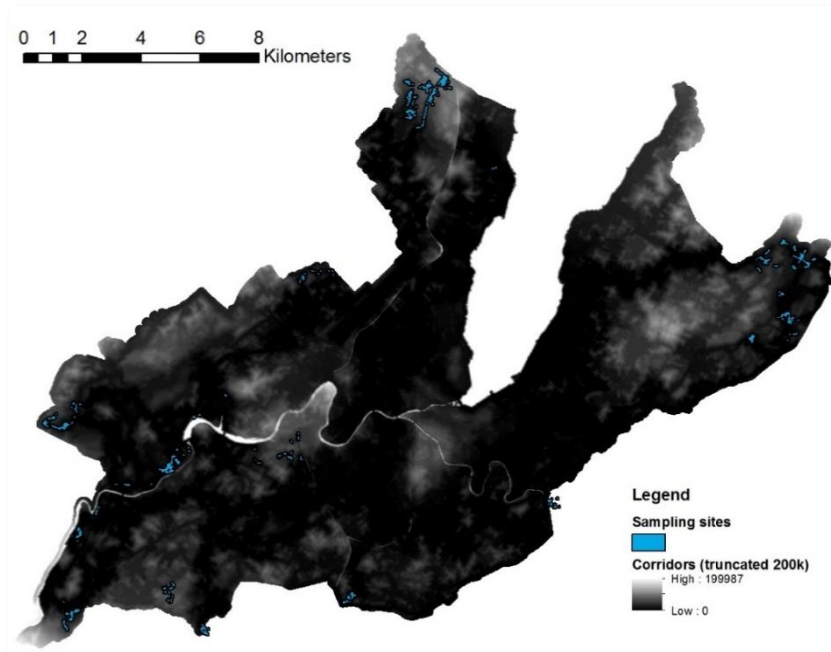
MAP 4.5. COST-WEIGHTED DISTANCE TO NEAREST CORE AREA. Colours: resistance of the ground. The crossing of some areas is more costly.



MAP 4.6. LEAST-COST PATHS BETWEEN ADJACENT CORE AREAS. Colours: distances. The shape of the lake that cannot be crossed appears in the LCP.

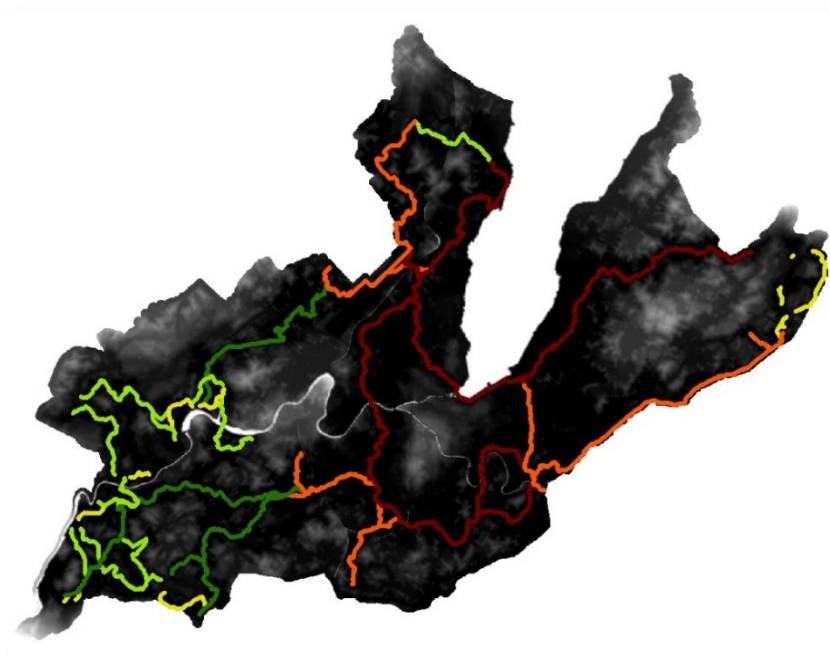
As we were able to notice on this map, some sampling sites were quite isolated from the others. We then decided to use the optional Step 4 to connect each core to its 4 nearest neighbours, and then to connect disjoint clusters to form constellations. We believed that this should have allowed us to remove some of the paths determined in Step 3. However, it appeared that there was no difference between the two maps, which means the tool already determined that there was no more than four neighbours to any core area.

Finally, we ran the Step 5 which calculates the least-cost corridors from the cost-weighted distance rasters and show them into a single map (**MAP 4.7**). The darker zones mean the costs to cross them are lower than for white zones.



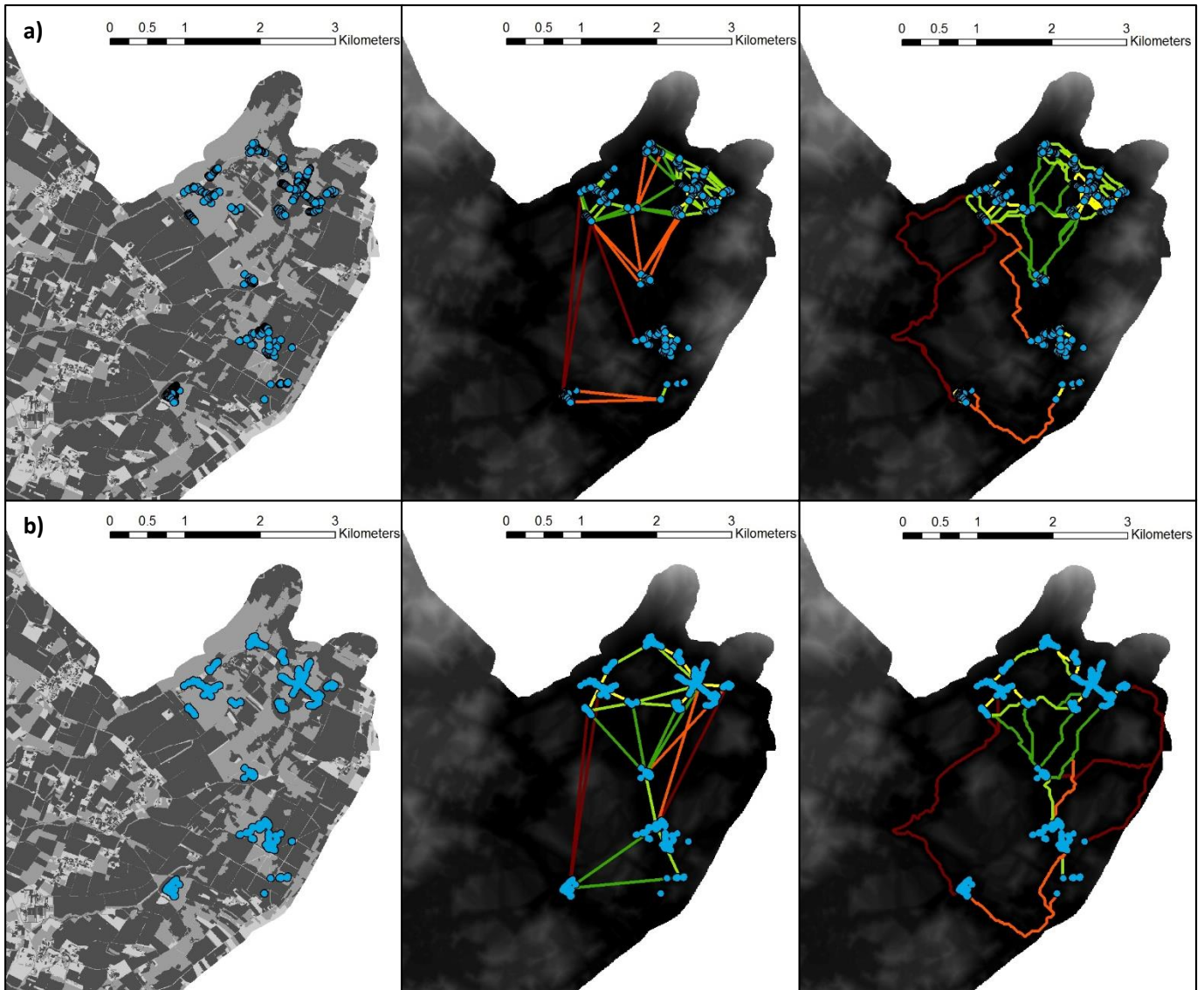
MAP 4.7. NORMALIZED AND MOSAICKED LEAST COST CORRIDORS. Blue: sampling sites. White: high resistance. Black: low resistance.

The corridors are thus directly linked to the least-cost paths previously calculated (**MAP 4.8**).



MAP 4.8. MAP COMBINING CORRIDORS AND LEAST-COST PATHS. White: high resistance. Black: low resistance. Colours: distances of the LCP. The sites are globally divided in three isolated areas: Jussy (East), Versoix (North) and the Western part of the canton.

With the aim of assessing the movement in a specific area, we decided to do the same analysis concentrated in a smaller area (Jussy area). For this, we used the same buffer of 50m, but we did not dissolve the buffers thanks to the tool. We compared the results of Linkage mapper with a dataset where all the buffered ponds were individually considered, and when they were groups. For the second part, we manually dissolved the buffers that were overlapping using the tool “union” to create small monitored areas. We also created Euclidian distances and least-cost paths between core areas and a corridor map (MAP 4.9).



MAP 4.9. MAP OF JUSSY PONDS. Blue: sampling sites. White: high resistance. Black: low resistance. Colours: distances. a) Ponds considered individually - Euclidian distances - LCP. b) Ponds unified according to overlap - Euclidian distances - LCP.

D. Discussion

i. The importance of water

The first consideration that motivated this study was that the amphibians need water. However, as a consequence of global warming, heat waves are happening more often and can have a dramatic impact on pioneer species that live in temporary habitats. We thus decided to improve the ability of the habitat to retain water by digging new ponds with waterproof compacted bed, and by installing plastic buckets that will not leak water into the ground. And it seems in our results that this water factor has a really big influence on the presence of the Yellow-bellied toad (*Bombina variegata*: BOVA) as shown by the GLM (water on all stages: $p < 8e^{-7}$). It is thus very important for the protection of this species and of all amphibian species that their habitats stay humid.

We also see, thanks to the GLM, that there is a positive influence of the combined factors ponds (E) and water level, but that the types of ponds do not have any influence when we consider them alone. If we adopt a conservation point of view, the type of ponds should actually be considered as the corresponding conditions they represent and whether these conditions fit the needs of our target species. In other words, an adult BOVA may choose to stay in a bucket not because it is a bucket, but thanks to the adequate conditions of such a habitat. This is exactly what say our GLM results. Indeed, all significant influences are those related to water alone, or water combined with another factor. This importance of water is well documented in the literature (Hartel et al., 2007b; Scheele et al., 2014; Cayuela et al., 2011; Barandun & Reyer, 1997b). The temperature of water is also well studied (Barandun & Reyer, 1997a, 1997b; Dittrich et al., 2015). Indeed, a warmer water can increase the development rate of eggs and tadpoles and thus improve the survival in case of a drought (Smith, 1983; Prema, 1981). We did not monitor the temperature for practical reasons, but we can make a parallel with these studies as we noticed that the juveniles were more observed in ruts. This can be explained by the fact that the quantity of water is less important there, so it will heat more quickly, and favour a quick growth of the tadpoles that will thus metamorphose in mass.

Concerning the tadpoles and eggs, they obviously do not choose themselves the conditions in which they prefer to grow, but are representative of the breeding adults' choices. The only influences that appear to be significant concern the tadpoles. It seems that their presence is correlated with the buckets. As we saw that this type of measures keeps more water than the others (**FIGURE 4.4**), we can make the hypothesis that the toads manage to have clues about the ability of buckets to retain water, and thus, they preferentially choose this type of habitat to spawn in the aim of increasing the survival probability of their larvae. However, we obviously cannot rule out that the toads also spawned in other pond types, but that the eggs were predated or lost due to desiccation, leading to a quasi absence of tadpoles.

As a last remark about water, we showed that the biggest abundance of individuals was in ponds filled between 50% and 75% of water, and not in full ponds. We can interpret that as a solution to manage the compromise between enough water to survive, but no predators. Indeed, most predators of BOVA settle in big ponds filled with water. BOVA could spawn in ponds that present only a small quantity of water in order to avoid predation, but then the risk of losing a wave of eggs and tadpoles would be too high. Choosing half-full ponds is thus a good solution for this dilemma (Smith, 1983; Barandun & Reyer, 1997a). Once again, there is also the possibility that the predation is higher in bigger ponds, hence less tadpoles are observed.

ii. *The importance of a support*

When investigating the influence of the factors included in the “vegetation” cluster, the GLM showed that the juveniles and subadults are absolutely not influenced by any on them. the impact of vegetation is only seen on adults, tadpoles and eggs, which suggests that the vegetation is especially influential on breeding.

If we consider the effect of the different factors alone, there is only the branches that show a positive influence on the three stages. This is consistent with the knowledge that BOVA needs a support to tie its eggs (Barandun & Reyer, 1997b; Kinne, 2006). Some researchers advanced that it may lay its eggs on the bottom of a pond (Cayuela, personal communication), but we have never observed that in our study area. According to our results, the branches have such a strong effect that they pull the influence as soon as they are included in an interaction with another factors.

Concerning the other factors, it is difficult to notice any trend in the influence. For example, dead leaves have a positive influence on tadpoles, but a negative one on eggs, whereas the combined algae do not influence the adults, but strongly positively influence the tadpoles and eggs. However, according to our observations and to the literature, we can suggest that the tadpoles and eggs need a shelter to hide from predators (Semlitsch, 2002; Kinne, 2006; Harkewicz, 2004). The shelter can consist of a layer of algae or leaves. It was shown with Green frogs (*Lithobates clamitans*) that the survival of tadpoles was correlated with the density of vegetation as it protects the larvae from invertebrate predators (Tarr & Babbitt, 2002), so the same explanation is likely to be true for BOVA as well. We saw on the field that when we arrive next to a pond with tadpoles, they are first close to the surface of the pond (often a bucket in our study), but quickly go under cover. Moreover, the positive effect of algae on breeding indices is consistent with some studies that say that tadpoles feed on algae, the quality of whose may increase the rapidity of their development (Kupferberg, 1997; Ranvestel et al., 2004).

iii. *Bias of observation*

For these analyses, we noticed several aspects that can act as bias in our observations. First of all, we tried to conduct exhaustive prospections on the field since we monitored each time in the same way the same locations. However, as mentioned earlier, ponds presenting big surfaces could not be correctly checked for the presence

of BOVA as we could often only reach part of the edges of these ponds. It is one reason why we abandoned some ponds after the first year, because our observations could not be reliable in such places. Moreover, we decided to monitor the factor “turbidity” as it is often said in the literature that BOVA likes muddy waters (Ghiurca & Gherghel, 2007; Pupina & Pupins, 2008). However, we quickly noticed that dim waters could hide animals, which would bias the data. This aspect can be seen in the results of the GLM. Indeed, there is no significant influence of the turbidity on the presence of adults, subadults and juveniles, whereas it appeared that there is a strongly negative influence on tadpoles and eggs. In practice, this noticing seems quite logic, as in dim waters, animals that breath on the surface will be easier to see. Since the tadpoles and eggs are on the bottom of the ponds, they were not seen when the turbidity was high. We can make the hypothesis that the breeding does not occur in trouble waters as there is less Oxygen available in such places, and the survival of larvae is thus compromised, but because of a bad visibility, we cannot be sure there was not plenty of unseen tadpoles and eggs in these trouble waters. The bias due to bad visibility is also true for algae in surface or in depth that can make us underestimate hidden animals.

Another bias must be considered when we investigate the effect of the presence of branches. Indeed, as this study aimed at improving the breeding success of our populations, we wrote in the protocol a step saying that if there was no branch in the pond, one had to be added. We noticed that it was useful as all observed eggs were attached to this support. Although this addition tends to improve the habitat, the branches were then present almost everywhere, which made it difficult for us to assess the impact of their absence.

In some of the analyses, we removed the data from the PLA site. The reason was that the characteristics of this site were very particular, did not reflect the global conditions in the whole canton and, as it is the biggest sites of our study (433 individuals out of 1107 identified individuals, 777 capture events out of 2655), it drove the global trend of the whole dataset. PLA consists of a big retention tank of 18m² located downhill of vineyards and a canal of 1.2 meter-height and 500 meters-long that we recorded as a ditch (F) that can have at maximum 20 centimetres of water. Both these structures are built with concrete, so the conditions are not supposed to be adequate for BOVA.

When we considered PLA in the analyses, the “ditch” type (FIGURE 4.15), as well the 15% water level exploded in the apparent preferences of BOVA, whereas these conditions were not at all the preferred ones for the other sites.



FIGURE 4.15. EFFECT OF PLA ON THE PREFERRED POND TYPE. Purple: data without PLA. Green: data with PLA. A: adults. S: subadults. J: juveniles. B: buckets. E: ponds. F: ditches. N: natural. O: ruts. X: others. When we consider PLA, the importance of ditches and the “other” class explode.

Moreover, PLA allowed us to see an incredible quantity of juveniles in comparison of the other sites (FIGURE 4.16). If we had considered it in the analyses, it would have strongly influence the breeding estimations.

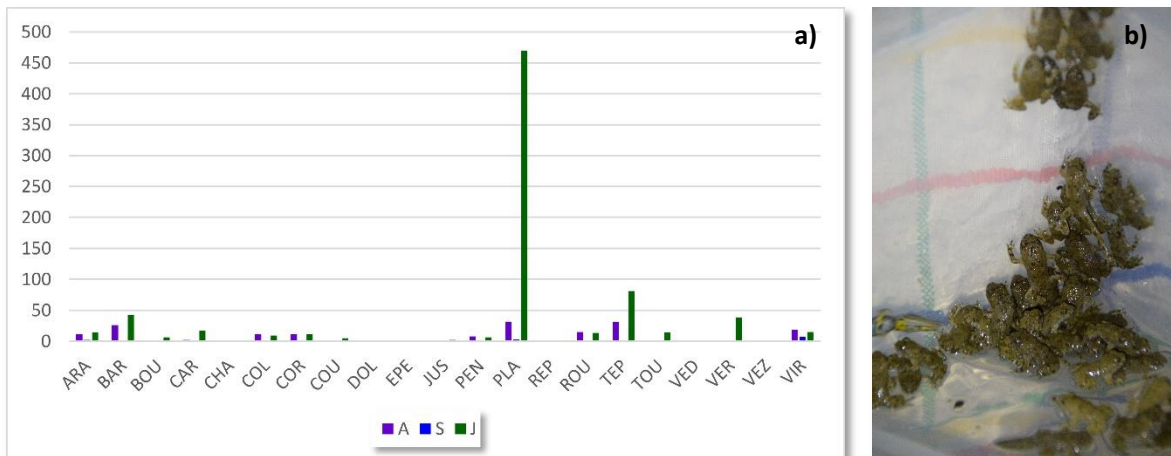


FIGURE 4.16. EFFECT OF PLA ON THE NUMBER OF JUVENILES. Purple: adults (A). Blue: subadults (S). Green: juveniles (J). a) Number of small juveniles observed in each site. Y-axis: number of toads. b) Session of July 2015 in PLA. See Abbreviation List for all sites’ names (p.0).

iv. Corridors through Geneva

On the final map that shows the end of the analyses of Linkage Mapper (MAP 4.8), we quickly see that three areas are isolated: Jussy in the extreme East, Versoix in the extreme North and the Western part that makes a pack on itself. A first global observation is that the BOVA does not have a homogenous distribution over the whole canton, which implies that the sites located at the far ends of Geneva are very unlikely to be connected. Moreover, considering the landscape features, we see that Jussy is surrounded with crops to which we attributed the resistance of 50 or 100 according to

the difficulty for a toad to cross a cultivated area (Ray et al., 2002). Versoix is located in the middle of a dense forest and is bordered by the highway. The program managed to find a way to connect both sites within this area (VER and COL), but we do not think it is very likely that animals physically manage to pass from one to the other. Indeed, there is no fauna passageways under or above the highway, so they should use human roads if they want to travel. The Western part of Geneva seems well connected on the result map. However, Linkage Mapper found least-cost paths that crossed over the rivers (Arve and Rhône) as some bridges where mapped on the reference habitat map. We also find that it is not likely that these bridges are used by the toads, especially because one of them is followed by a railway (PLA), and the other one is supposed to connect a site where no BOVA was observed (MER). We can see that ROU, which is the site on the extreme North-West of Geneva, has two paths toward the Rhône. If we link that to the habitat map, we see that between the two paths, there is the river Allondon which also appears as a barrier. The area South of the Rhône seems more connected, but we previously explained that it is a very dry area (Champagne). Linkage Mapper shows that this area is not very resistant to the movement of the toads, but the biological explanation would say it is not very likely that they will travel through it. Overall, it is difficult to see real corridors through which the animals can move to connect the different part of the canton, at the scale of a toad.

In the configuration of the tool, we attributed the resistance value of 50 to the roads. First, we did this because the different kinds of roads were not distinct on the habitat map. We manually added the highway effect, but we could not distinguish small dirt roads from principal roads. Moreover, we wanted to show that toads could move on dry flat surface, but that it is not very likely that they will cross over concrete, so we attributed a medium value. We also wanted to see whether the tool could find that important roads are bigger barriers than small roads without us giving the data to calculate the risk effect on survival on such roads. In our results, we see that busy roads, for example Juvigny road in Jussy could not be passed by any least-cost path. In parallel, we attributed to any flowing stream the value of 100, because the literature says that BOVA is more likely to be found in standing waters or temporary streams (Canessa et al., 2013; Vines et al., 2003). However, we could not differentiate permanent rapid flows from temporary slow flows on the habitat map. We only changed the values of the main rivers to make them impossible to cross (resistance of 1000). It is thus possible in some areas that we underestimated the mortality risk on roads and overestimated the barrier effect of some rivers.

Concerning the area of Jussy, as we explained above, it appears isolated from the other areas. Moreover, even within the area which is about 4km², the core areas are not all easily connected. However, in the calculation of corridors on the whole Geneva, we grouped the ponds according to the sites. For the Jussy area, the grouping was decided for convenience reasons linked to field work, but if we look more closely at the distribution of the ponds, the sites are interlocked in each other and, thus, do not really

reflect the potential connection between the ponds. This is why we ran Linkage Mapper a second time, including only the ponds of Jussy area. We compared the two approaches. First, we considered each pond as a site and we analysed all sites separately. Then, we grouped together the ponds according to their location and their overlapping (MAP 4.9). We can quickly see that these approaches give different results, both for the Euclidian distances and for the least-cost paths. When we consider all ponds independently (MAP 4.9A), there are enough close and easily connectable ponds for the tool to not consider connections that could go further. However, when we group the ponds (MAP 4.9B), the tool has less choices for connections in the direct surroundings, so it has to look for further paths. Biologically speaking, we showed that BOVA usually does not cover great distances (see *Ethology part*), and that it is more likely moving from pond to pond to travel across an area. Thus, if we have a look at the North-East part of Jussy, we see a bunch of ponds that are placed in a cross shape. If all ponds are considered independently, Linkage Mapper finds that the extremities of the cross may be connected by cost-effective paths. However, if we consider that the toads will move within the area from pond to pond, the second version is more plausible.

Moreover, DOL is at the South-West end of Jussy and is driven along the Juvigny road which is one of the main access from France to Switzerland. It is used by many border dwellers who cause an intense traffic. We see here that Linkage Mapper shows that this site cannot connect easily with the northern part and that the only possible path is toward East and then North to connect the next site, which is COR. The effect of Juvigny road was thus considered in the calculation of the LCP.

Overall, we can see that one main corridor appears on the map on a North-South axe, and that CAR is in the middle of it and could act as a transition area. Linkage Mapper does not consider the sizes of the populations. If we add this consideration to our reflexion, CAR is the most occupied site of Jussy with 73 individuals. We can thus imagine that, as it is quite centred in the corridor designed by the tool, the individuals could connect both North and South sites from there. This site must thus be well protected to insure the viability of the Jussy population.

Concerning the isolation of Jussy area, we have to add another consideration about the design of the resistances. As explained above, we attributed the value 100 to the rivers as BOVA is not supposed to live in flowing waters. As Jussy presents a great density of small rivers, this setting provided to Linkage Mapper the basis to create higher resistance zones within Jussy. However, many of these streams are small tributaries of the Seymaz that do not flow very strongly. It is thus possible that we overestimated the isolation of Jussy because of potentially great resistances wrongly attributed to small temporary streams. There would be here the opposite effect of the Champagne where the resistance is probably underestimated. Anyway, there is no Swiss population of BOVA that is located near Jussy, so it is not very likely that connections with another part of Geneva would appear if we manage to attribute a small resistance to these streams. To explore further potential connection between Jussy and another area, we would need the map of the French ground to be able to detect cross-border corridors. It is also the case all around Geneva between sites at the border and French areas.

Linkage Mapper is continuously updated, and the authors warned the users that there might be problems according to the area, the type of map on which the raster is based and the shape of the core area polygons. As our own polygons were created using the Buffer tool around each pond, they do not have easy shapes. That can explain some paths that seem to be badly placed. Moreover, our habitat map does not cover any French area, so we must be careful when considering the connections between our sites when they are located next to the border. Indeed, there might be least-cost paths that connect our sites with French sites, but they were not considered in our analyses. Further analyses including French ground could improve these results and increase the knowledge on potential least-cost paths and connections between populations.

E. Conclusion

Among all factors monitored during this study and linked to the presence of the Yellow-bellied toad (*Bombina variegata*: BOVA), it clearly appeared that the water level is the most important, and the only one that influences all stages. We previously showed that Marsh frogs were avoided by BOVA, so it is probably in the aim of avoiding these competitors that the toads preferentially choose ponds half-filled with water. Even if the presence of branches or other supports for the eggs seems important, the factors included in the “vegetation” cluster do not seem to affect the presence of the toads. In a wider scale, we used the tool Linkage Mapper to see if low-cost corridors existed in Geneva for the toads to move between sites. It appeared that the only strongly connected area is located on both sides of the Rhône, knowing that the tool found passages on human bridges that are not likely used by toads in the wild. It would thus mean that there are a North part and a South part that are well connected within each other but not between. The Jussy area is quite isolated, but connections exist within the area between the groups of ponds. As road works are planned in 2018 on Juvigny road, it will be interesting to add new wildlife passageways to the habitat map to see whether the connections between DOL and the Northern part of Jussy increase.

V. ECO-ETHOLOGY

The chapter presented here consists of the manuscript of a paper written in collaboration with Aurélien Besnard, Virginia Tournier and Hugo Cayuela. To conduct these analyses, the data were modified to be able to compare them and enter them into the modeling program, this is why the numbers may differ from the other analyses presented in the other parts of this work. After a final revision by the co-authors, it will be submitted first to *Oecologia*, or, as a second choice, to *Freshwater Biology*.

Even if many parameters can be studied to know if they influence the presence of toads, many authors suggested that the main factor for the Yellow-bellied toad (*Bombina variegata*: BOVA) is the water retention of the environment and the duration of the ponds (Barandun & Reyer, 1997b; Cayuela et al., 2011; Hartel et al., 2007b). The same conclusion was reached in the previous chapter. Now, we will investigate the effect of hydroperiod on the movement of toads.

A. Introduction

The ecology of disturbances received a considerable attention during the last four decades (Levin & Paine, 1974; White & Pickett, 1985; Battisti & Fanelli, 2016). Disturbances consist in sudden changes in environmental conditions (e.g. fire, flood and drought) caused by natural and anthropogenic factors whose effects can be temporary or long-lasting. Their regime can be quantified in terms of spatial distribution, frequency, periodicity and intensity (White & Pickett, 1985; Rykiel, 1985). These disturbances can have consequences at different levels of biological organization, from ecosystems, through communities and populations, to individuals (Pickett et al., 1989; White & Pickett, 1985; Battisti et al., 2016). During the last century, human activities broadly altered disturbance regimes (Turner, 2010), resulting in an overall increase of the intensity and frequency of disturbances (flood: Syvitski et al., 2009; drought: Easterling et al., 2000; fire: Johnstone et al., 2016). Understanding how such changes impact ecological mechanisms at individual and population levels is a critical challenge for ecologists and conservationists.

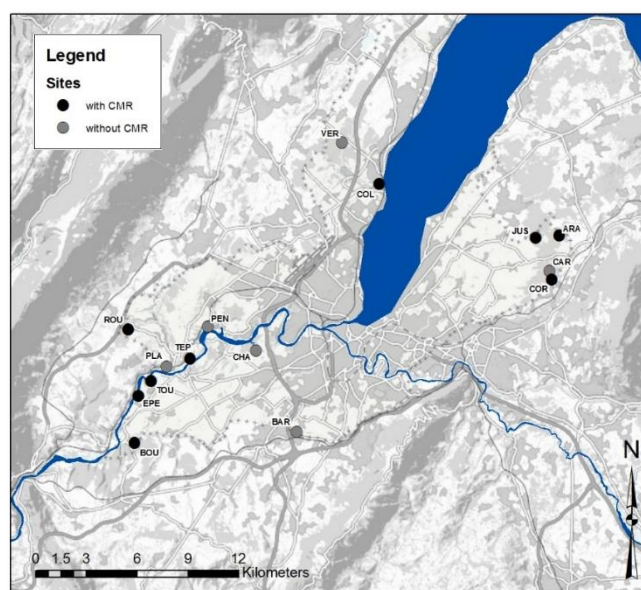
At the individual level, dispersal strategies play a critical role in organisms' response to disturbances. Dispersal is usually thought as a three-stage process with a distinction between emigration, transfer into the landscape matrix and immigration (Clobert et al., 2009). A recurrent finding of evolutionary models of dispersal is that dispersal depends on the balance between costs and benefits of this behaviour at each stages of the process (Bonte et al., 2012; Travis et al., 2012). The cost-benefit balance is influenced by the internal state of individuals (*phenotype-dependent dispersal*) and environmental factors (*condition-dependent dispersal*). Theoretical studies have demonstrated that condition-dependent dispersal is likely to evolve as a response to spatio-temporal variation of the environment (McPeck & Holt, 1992; Travis & Dytham,

1999), including disturbances (Reigada et al., 2015). When directly triggered by a disturbance, dispersal is said *pulsed* (*sensu*, Reigada et al., 2015). This form of condition-dependent dispersal implies that individuals adjust their emigration and immigration decisions according to the impact of the disturbance on their own and offspring survival (Bates et al., 2006; Altermatt & Ebert, 2008, 2010). Emigration and immigration are thus “informed” decisions (Clobert et al., 2009), namely that they are based on abiotic (Bowler & Benton, 2005; Matthysen, 2012) or social information (Boulinier et al., 2008; Jacob et al., 2015) allowing an evaluation of the risks triggered by the disturbance.

At the population level, disturbances can have a wide influence on the demographic processes underlying the dynamics of spatially structured populations including metapopulations (Levins, 1969), patchy populations (Harrison, 1991) as well as source-sink and pseudo-sink systems (Pulliam, 1988; Dias, 1996). These populations are classically composed of subpopulations occupying distinct breeding patches that are linked by dispersing individuals (Thomas & Kunin, 1999; Revilla & Wiegand, 2008). In these systems, dispersal is of a critical importance for the long term persistence of the population as it influences the chances of rescue effect (Hanski, 1998) and the colonization of newly available patches (Johst et al., 2002). By affecting the availability and the quality of patches, disturbances increase subpopulation extinction rates, resulting in higher turn-over rates at the population level (Kallimanis et al., 2005; Elkin & Possingham, 2008; Reigada et al., 2015). Yet, theoretical models demonstrated that increased dispersal results in higher (re)colonization rates that reduce the risk of population extinction in disturbed environments (Hastings, 2003; Elkin & Possingham, 2008; Reigada et al., 2015).

Surprisingly, few empirical studies have examined the effects of disturbances on individual dispersal decisions (e.g. Bates et al., 2006; Altermatt & Ebert, 2010). Moreover, no empirical work has yet investigated how changes in disturbance regimes may simultaneously affect dispersal decisions and patch occupancy dynamics. To fill this gap, pond-breeding amphibians are excellent biological models. To avoid the risk of larval mortality caused by the predation of fishes, many amphibians reproduce in temporary ponds (i.e. breeding patches) whose hydroperiod (the time during which the pond is filled of water) broadly fluctuates over space and time (Hecnar & McCloskey, 1997; Werner et al., 2007a, 2007b). In these organisms, the breeding success strongly depends on pond desiccation and intraspecific competition at larval stage (Wilbur, 1976; Van Buskirk & Smith, 1991; Hartel et al., 2011; Green et al., 2013; Barandun & Reyer, 1997a). When the risk of pond drying out is spatially heterogeneous and thus differs between breeding ponds, it is expected that adults adjust their dispersal decision to minimize offspring mortality before metamorphosis. These individual decisions should then affect pond occupancy dynamics. Especially, correlative studies showed that the turn-over rates of reproduction increases in ponds with short and variable hydroperiod (Baber et al., 2004; Cayuela et al., 2012).

In this paper, we examined how contrasted perturbation regimes affect dispersal decisions and pond occupancy dynamics in an endangered amphibian, the yellow-bellied toad (*Bombina variegata*). In 15 populations located in the Geneva region (MAP 5.1), we experimentally manipulated pond hydroperiod by installing plastic containers whose hydroperiod was expected to be more predictable (i.e. longer and less variable) than in the ponds where the reproduction usually takes place (i.e. ruts, ditches and residual puddles). First, we verified that the probability of pond water filling was higher in plastic containers (called thereafter *predictable ponds*) than in other ponds (thereafter *unpredictable ponds*). After establishing this pre-requisite, we examined how the predictability of pond hydroperiod affects emigration/immigration processes in breeders at both intra-annual and inter-annual levels. For that purpose, we collected capture-recapture data during a 5-year period (2012-2016) in 9 populations. Using capture-recapture multievent models, we tested the hypothesis that emigration rates were lower in predictable ponds than in unpredictable ones. By contrast, we hypothesized that immigration rates were higher in predictable ponds. Then, we analyzed how pond hydroperiod predictability affects inter-annual changes (disappearance and colonization) in the occurrence of adults and reproduction in ponds. For that purpose, we collected presence/absence data in 334 ponds spread in the 15 populations during the 5-year study period. Using multiple season occupancy models including state uncertainty, we tested the hypothesis that the probability of reproduction disappearance was higher in unpredictable ponds. As well, we predicted that the probability of disappearance of adults without effective reproduction was higher in unpredictable ponds. Concerning colonization, we expected that the probability of pond colonization with an effective reproduction was higher in predictable ponds. By contrast, we hypothesized that pond colonization without reproduction was higher in unpredictable ponds.



MAP 5.1. MAP OF THE STUDY AREA. Grey: Presence-absence data. Black: Capture-recapture data. Fifteen populations of BOVA were considered in the region of Geneva (Switzerland) during a 5-year period (2012-2016).

B. Methodology

i. Biological model

Bombina variegata breeds in small water bodies as ruts and residual puddles whose hydroperiod usually varies between few days and several months (Barandun & Reyer, 1997a, 1998), depending on rainfall events and the ability of the soil to hold rainwater. The risk of pond drying out is the main mortality factor at egg and larval stages (Barandun & Reyer, 1997b). Pond hydroperiod unpredictability results in frequent breeding failures and a high inter-annual variance of the breeding success (Barandun et al., 1997; Cayuela et al., 2016b). A relatively high adult survival and degree of iteroparity allows individuals to skip reproduction opportunities (Cayuela et al., 2016a) especially when drought conditions are experienced during the breeding season (Cayuela et al., 2014). To avoid the risk of a complete breeding failure, females may spread their eggs in multiple clutches among different water bodies (Buschmann, 2002).

ii. Study area and data collection

The study took place in the region of Geneva (Switzerland) where *B. variegata* populations have suffered a drastic decline during the last three decades due to habitat loss and fragmentation resulting from an increasingly growing urbanization. In our study, we focused on the 15 main populations located the Geneva region (TABLE 5.1, MAP 5.1), which were surveyed during a 5-year period (2012-2016). These populations strongly vary in terms of size (TABLE 5.1), from 4 to 92 identified adults in populations TOU and TEP respectively. Each of these population occupy a set of different ponds ranging from 5 (population CHA) to 73 (TEP). In 13 populations, we manipulated pond hydroperiod by installing plastic containers. The number of buckets installed in each population varied according to the prospected area. Other ponds were also mapped in a buffer area of 100m around the buckets or in areas where the toads were known to be present.

To examine how pond hydroperiod predictability affects dispersal between ponds, capture-recapture data were collected in 9 populations out the 15 considered in this study (TABLE 5.1, MAP 5.1). These 9 populations were surveyed during 2 to 5 years and 5 capture sessions were considered each year. At each capture sessions, all the available ponds that could potentially be occupied were exhaustively checked. The type of pond, i.e. plastic containers or other ponds, occupied by each individual at each capture session was recorded. We assumed that toads became sexually mature at 3 years old, with a mean body length (snout–vent length) of 35 mm in males and 36 mm in females (Cayuela et al., 2016a); smaller individuals were excluded from the analysis. We identified gender on the basis of strong forearms and the presence of nuptial pads in males. We identified each individual by the specific pattern of black and yellow mottles on its belly, recorded by photographs.

To examine how pond hydroperiod influences pond occupancy dynamics, we collected presence-absence data in 334 ponds (168 plastic containers and 166 other ponds) spread in the 15 populations (TABLE 5.1, MAP 5.1).

TABLE 5.1. DESCRIPTION OF SURVEY DESIGN. In the fifteen populations, we collected presence data during a period ranging from 2 to 5 years. In the nine first populations (ARA to TOU), capture-recapture data were also collected.

See Abbreviation List for all sites' names (p.0).

ID	Population	Number of containers	Number of pools	Number of years of survey	Number of capture sessions	Number of adults identified	Number of captures
ARA	ARALEs	25	16	5	23	33	151
BOU	BOUCHETS	4	11	4	20	10	25
COL	COLOMBIERE	5	2	3	12	22	65
COR	CORBEILLE	23	0	3	14	25	67
EPE	EPEISSE	6	3	2	7	9	23
JUS	JUSSY	9	2	3	15	11	26
ROU	ROULAVE	11	15	4	20	53	170
TEP	TEPPES	39	34	5	25	92	408
TOU	TOUVIERE	8	4	5	19	4	11
BARD	BARDOGRAVE	0	29	2	10	-	-
CAR	CARPIERE	0	26	3	15	-	-
CHA	CHATILLON	4	1	4	17	-	-
PEN	PENEY	16	1	4	17	-	-
PLA	PLAINE	15	4	4	17	-	-
VER	VERSOIX	3	18	2	10	-	-

iii. Assessing pond hydroperiod predictability

We aimed at demonstrating that the plastic containers have a longer and more predictable hydroperiod than the ponds usually used by the species in the study area. For that purpose, we examined whether the probability of pond water filling differed according to pond type. We used field records carried out in 334 ponds spread in the 15 studied populations (TABLE 5.1) during the 5-year study period. At each capture session, the absence or presence of water in the pond was recorded, resulting in 3770 observations (1972 in plastic containers and 1798 in other ponds). We then used a Generalized Linear Mixed Model (GLMM) implemented in the R package lme4 (Bates et al., 2014). Pond identity was included in the model as a random effect while the pond type, a discrete variable with two modalities (plastic containers vs other ponds), was included as fixed effect. The model fit was assessed graphically by plotting the residual distribution.

iv. A multievent capture-recapture model to estimate condition-dependent dispersal

Our goal was then to demonstrate that reducing pond hydroperiod predictability affects movement behaviour of breeders. For that purpose, we used capture-recapture multievent models recently developed by Cayuela et al. (2017a) that allow to estimate movement between sites located in similar or different habitat types. As usual in multievent capture-recapture models, a distinction is made between events and states

(Pradel, 2005). An event is the field observation coded in the individual capture history that is related to the latent state of the individual. These observations can come with a certain degree of uncertainty regarding the latent state and multievent models aim at modeling this uncertainty in observation process using hidden Markov chains.

The CR multievent model proposed by Cayuela et al. (2017a) is based on states that include the movement status, the previous and current capture statuses and the current occupied habitat. This information is embedded in composite states as following. Individuals that changed of site between $t-1$ and t are coded M for 'moved' while individuals that remained in the same site are coded S for 'stayed'. They can also occupy different categories of site. In our study case, individuals can occupy ponds with a highly predictable hydroperiod (H, plastic containers) or with a lowly predictable hydroperiod (L, other ponds). These codes are prefixed by the previous capture status and suffixed by the current capture status (+ for 'captured', o for 'not captured'). This leads to the consideration of 13 states, including the dead state (TABLE 5.2). In the model, we considered six possible observations made on the field (i.e. event). For individuals captured at t and $t-1$, a code of 1 or 4 was attributed if they did not change of pond and occupy a pond of category H or L respectively; 2 or 5 were attributed if they changed of ponds and occupy a pond of category H or L respectively. For individuals that were not captured at $t-1$ and are captured at t in a pond of category H or L, we attributed a code of 3 or 6 respectively, and individuals not captured at t are given a code of 0.

TABLE 5.2. CR MULTIEVENT MODEL TO ESTIMATE EMIGRATION/IMMIGRATION RATE. Thirteen states and six events are considered in the model. S: stayed. M: move. H: plastic container. L: other ponds. + captures. o: not captured. D: dead state.

State	State description
+SH+	Captured at $t-1$ and t in the same pond with a highly predictable hydroperiod
+MH+	Captured at t in a pond with a highly predictable hydroperiod different from the pond where captured at $t-1$
+SL+	Captured at $t-1$ and t in the same pond with a lowly predictable hydroperiod
+ML+	Captured at t in a pond with a lowly predictable hydroperiod different from the pond where captured at $t-1$
oSH+	Captured at t in the same pond with a highly predictable hydroperiod occupied at $t-1$ when not captured
oMH+	Captured at t in a pond with a highly predictable hydroperiod different from the pond occupied at $t-1$ when not captured
oSL+	Captured at t in the same pond with a lowly predictable hydroperiod occupied at $t-1$ when not captured
oML+	Captured at t in a pond with a lowly predictable hydroperiod different from the pond occupied at $t-1$ when not captured
SHo	Not captured at t and in the same pond with a highly predictable hydroperiod as at $t-1$
MHo	Not captured at t and in a pond with a highly predictable hydroperiod different from the pond occupied at $t-1$
SLo	Not captured at t and in the same pond with a lowly predictable hydroperiod as at $t-1$
MLo	Not captured at t and in a pond with a lowly predictable hydroperiod different from the pond occupied at $t-1$
D	Dead

The model parameterization presented in Cayuela et al. (2017) allows to estimate all the possible movements between sites including between-habitat (L to H and H to L) and within-habitat movements (L to L and H to H). Because we were only interested in quantifying emigration and immigration rates in the two pond types in the present study, we thus modified the original model parameterization. At their first capture, individuals can be in two states: oSH+ and oSL+. We then considered four state-state transition steps at which a piece of information embedded in the composite state is updated: (1) survival, (2) emigration, (3) immigration and (4) recapture. When updated, this piece of information appears in bold in the transition matrices following the convention established by Souchay et al. (2014).

(1) Survival is first modeled and individuals may survive with a probability φ or may die with a probability $1-\varphi$, resulting in a transition matrix with 13 states of departure and 7 intermediate states of arrival (TABLE 5.2, FIGURE 5.1). (2) Emigration is updated and individuals that survived may leave the pond they occupied at $t-1$ with a probability ε or may remain in the same pond with a probability $1-\varepsilon$. This leads to a transition matrix with 7 states of departure and 13 states of arrival (TABLE 5.2, FIGURE 5.1). Emigration can be made dependent on the type of pond by forcing different ε values for the rows 1, 2 and 3 (type L) and for the rows 4, 5 and 6 (type H) of the corresponding transition matrix. (3)

Immigration is then modeled and individuals that left the pond they occupied at $t-1$ may reach a pond of type L with a probability α and may move to a pond of type H with a probability $1-\alpha$, which leads to a transition matrix with 13 states of departure and arrival (TABLE 5.2, FIGURE 5.1). (4) Recapture is updated and individuals may be recaptured with a probability p or may be missed with a probability $1-p$, resulting in a transition matrix with 13 states of departure and arrival (TABLE 5.2, FIGURE 5.1). The last component of the model links events to states; each state corresponds to only one possible event (FIGURE 5.1).

This parameterization was implemented in program E-SURGE (Choquet et al., 2009). We first checked parameter identifiability using E-SURGE diagnostic. Competing models were ranked through a model-selection procedure using Akaike information criteria adjusted for a small sample size (AICc) and AICc weights. Our hypotheses concerning recapture and state–state transition probabilities were tested from the general model [$\varphi(\text{POP})$, $\varepsilon(\text{HYDR})$, $\alpha(\text{HYDR})$, $p(\text{POP} + Y)$] that includes three effects: the pond hydroperiod predictability (HYDR) coded as states in the model (L and H); a population effect (POP) introduced as a discrete covariate with 9 modalities (corresponding to the nine populations surveyed by capture-recapture); and yearly-specific variation (Y). From this general model, we first examined whether recapture p varied among populations (POP) and years (Y). Then, retaining the best combination of effects on recapture, we investigated whether survival φ differed among populations (POP). Next, keeping the best combination of effects on recapture and survival, we tested whether emigration ε depended on pond hydroperiod predictability (HYDR). Lastly, we examined how hydroperiod predictability affects immigration by comparing the estimates and the overlap of 95% of immigration parameters; individuals that emigrated can indeed arrive either in a pond of type L or a pond of type H. For recapture, the effects POP and Y were introduced in an additive way. Moreover, we did not considered sex-specific variation in emigration/immigration processes as males and females usually respond similarly to pond-specific factors that affect offspring fitness in this species (Cayuela et al., 2016c, 2017b).

<p>Initial probabilities</p> <table style="margin-left: auto; margin-right: auto;"> <tr> <td style="padding: 0 10px;">N</td> <td style="padding: 0 10px;">A</td> <td style="padding: 0 10px;">R</td> </tr> <tr> <td style="padding: 0 10px;">$(1 - \psi)$</td> <td style="padding: 0 10px;">ψ</td> <td style="padding: 0 10px;">ψ</td> </tr> </table>	N	A	R	$(1 - \psi)$	ψ	ψ	<p>State-state transitions</p> <table style="margin-left: auto; margin-right: auto;"> <tr> <td style="padding: 0 10px;">N</td> <td style="padding: 0 10px;">A</td> <td style="padding: 0 10px;">R</td> <td style="padding: 0 10px;">D</td> </tr> <tr> <td style="padding: 0 10px;">N</td> <td style="padding: 0 10px;">$\begin{pmatrix} 1 - \rho & \rho & \rho & 0 \end{pmatrix}$</td> <td style="padding: 0 10px;">A</td> <td style="padding: 0 10px;">$\begin{pmatrix} \rho & 1 - \rho & \rho & 0 \end{pmatrix}$</td> </tr> <tr> <td style="padding: 0 10px;">R</td> <td style="padding: 0 10px;">$\begin{pmatrix} \rho & \rho & 1 - \rho & 0 \end{pmatrix}$</td> <td style="padding: 0 10px;">D</td> <td style="padding: 0 10px;">$\begin{pmatrix} 0 & 0 & 0 & 1 \end{pmatrix}$</td> </tr> </table>	N	A	R	D	N	$\begin{pmatrix} 1 - \rho & \rho & \rho & 0 \end{pmatrix}$	A	$\begin{pmatrix} \rho & 1 - \rho & \rho & 0 \end{pmatrix}$	R	$\begin{pmatrix} \rho & \rho & 1 - \rho & 0 \end{pmatrix}$	D	$\begin{pmatrix} 0 & 0 & 0 & 1 \end{pmatrix}$									
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FIGURE 5.1. CR MULTIEVENT MODEL TO ESTIMATE EMIGRATION/IMMIGRATION RATE. N: unoccupied. A: occupied by adult without effective reproduction. R: occupied for reproduction. D: dead state. Thirteen states and six events are considered in this model (see Table 5.2).

v. A multistate occupancy model to analyse pond occupancy dynamics

To examine how hydroperiod predictability affects pond occupancy dynamics, we used site occupancy models. We were interested in quantifying changes in occurrence (extinction and colonization) for both adult without effective reproduction and breeding. For that purpose, we used multiple season occupancy models with state uncertainty proposed McKenzie et al. (2009), which allow to deal with imperfect detection of the true state of the occupied locations (breeding vs not breeding sites). For the need of our study, we reformulated Kendall's multistate model as a Hidden Markov model following the method proposed by Gimenez et al. (2014). Three states were considered in the model: unoccupied (N), occupied by adult without effective reproduction (A), occupied for reproduction (R). Three observations were considered: undetected adult without any breeding indices (coded 0), i.e. eggs and/or tadpoles; adult detected without breeding indices (coded 1); breeding indices detected (coded 2). The model is built around three pieces of information (FIGURE 5.2): the vector of initial probabilities, the matrix of state-state transition and the two matrices of observation probabilities.

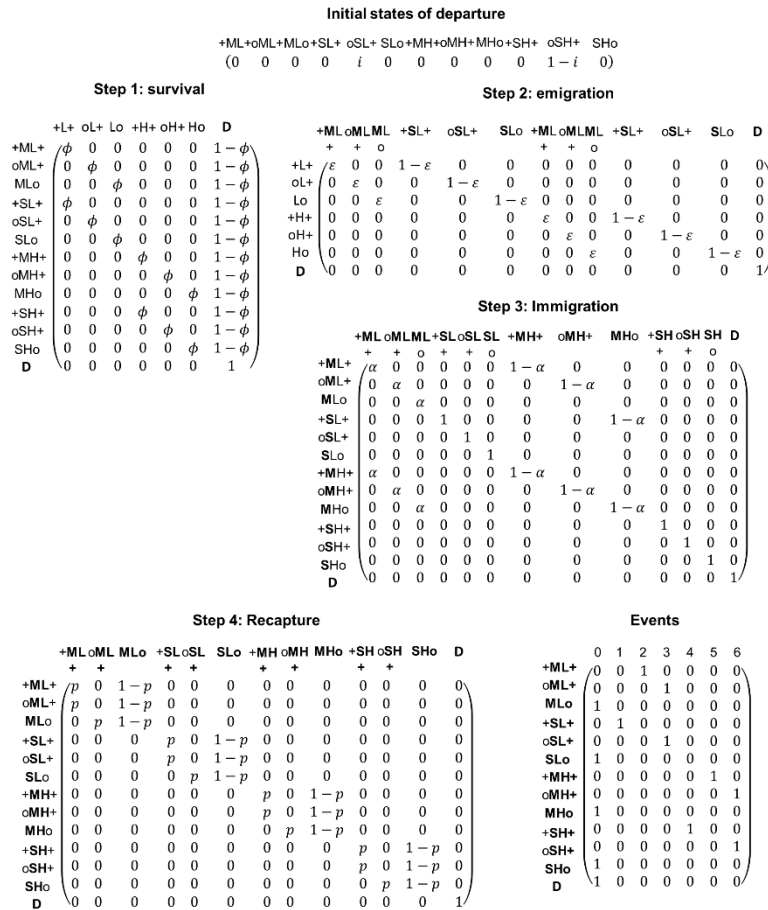


FIGURE 5.2. MULTIPLE SEASON OCCUPANCY MODEL WITH STATE UNCERTAINTY: VECTOR OF INITIAL PROBABILITIES, STATE-SATE TRANSITION AND FIELD OBSERVATIONS. N: unoccupied. A: occupied by adult without effective reproduction. R: occupied for reproduction. D: dead state. The two matrices of events link the latent states of site with field observations. Three observations were considered: undetected adult and breeding indices (coded 0), i.e. eggs and/or tadpoles; adult detected without breeding indices (coded 1); breeding indices detected (coded 2).

At the first sampling occasion, the ponds can be unoccupied, occupied by adult without effective reproduction (A) or occupied for reproduction (R) (FIGURE 5.2). Then, occupancy changes are modeled and the pond may change of state between $t-1$ and t with a probability ρ or may remain in the same state with a probability $1-\rho$. This leads to a transition matrix with four states of departure N, A, R and D (the dead state, that is always forced at 1) and four states of arrival N, A, R and D (FIGURE 5.2). Six potential changes occupancy are considered: ponds may be colonized by adults without (line 1, column 2) or with effective reproduction (line 1, column 2); a pond occupied by adults without (line 2, column 1) or with effective reproduction (line 3, column 1) may be abandoned; a pond occupied by adults without effective may become a breeding site (line 2, column 3); conversely, a pond used for breeding may become a site occupied by adults without effective reproduction. The observation process conditional on underlying occupancy states is then modeled. It is split in two modeling steps (FIGURE 5.2) to highlight the successive processes of detection and breeding state ascertainment. In the first matrix, we introduce a set of intermediate observation: undetected, detection of adults without breeding, and detection of adult with effective breeding. The second matrix specifies the probabilities of reproduction conditional on the intermediate observations.

This parameterization was implemented in program E-SURGE. Competing models were ranked through a model-selection procedure using AICc and AICc weights. We tested our hypotheses concerning initial probabilities, inter-annual changes in occupancy and detection probabilities from the general model, $[\psi(\text{HYDR}), \rho(\text{HYDR}), p(\text{HYDR})]$, where HYDR is a discrete covariate with two modalities: ponds with highly predictable hydroperiod (plastic containers) vs ponds with a lowly predictable hydroperiod (other ponds). All the possible combinations of effect were tested, leading to the consideration of 8 competitive models. As in classic multiple season site occupancy models, the occupancy state was assumed to be fixed at the intra-annual level while changes were allowed to occur at the inter-annual level. For that purpose, all the parameters ρ were forced at 0 at intra-annual level.

C. Results

i. Assessing pond hydroperiod predictability

Our GLMM indicated that the probability of water filling differed between plastic containers and other ponds (TABLE 5.3). The probability that a pond was filled at a given sampling occasion was 0.94 (95% CI 0.93-0.96) in plastic containers while it was 0.63 (95% CI 0.49-0.75) in other ponds. Hence, we considered that plastic containers have a predictable hydroperiod (and thus called “predictable ponds”) while other ponds displayed an unpredictable hydroperiod (“unpredictable ponds”).

TABLE 5.3. MANIPULATION OF POND HYDROPERIOD IN BOVA POPULATIONS. The GLMM included the pond as a random effect and the type of ponds (plastic containers vs other ponds) as a fixed effect. Model outputs are provided: parameter estimates and their 95% CI, z-values and p-values.

Fixed effects	Estimate	95% CI	z	P
Intercept (plastic containers)	2.81 (0.13)	2.56; 3.07	21.64	<0.0001
Other ponds	-2.28 (0.16)	-2.59; -1.98	-14.50	<0.0001

ii. Influence of hydroperiod predictability on dispersal decisions

The mean survival rate (provided by the model in which survival was held constant among populations) was 0.64 (95% CI 0.53-0.76). The mean emigration rates were 0.51 (95% CI 0.44-0.61) and 0.56 (95% CI 0.59-0.65) at intra-annual and inter-annual levels respectively; the mean immigration rates were 0.26 (95% CI 0.20-0.34) and 0.30 (95% CI 0.25-0.37) at intra-annual and inter-annual levels respectively.

The best-supported model, [$\varphi(\text{POP})$, $\varepsilon(\text{HYDR})$, $\alpha(\cdot)$, $p(\text{POP} + Y)$] (SEE THE MODEL SELECTION PROCEDURE IN TABLE 5.4), indicated that recapture probability varied among years and populations (FIGURE 5.3). Recapture was the highest in ARA where it ranged from 0.35 to 0.55 and was the lowest in EPE where it varied from 0.13 ± 0.04 to 0.25 ± 0.07 (FIGURE 5.3c). Survival also varied between populations, ranging from 0.36 in JUS and 0.80 in ROU (FIGURE 5.3A). More importantly, our results showed that emigration and immigration depended on hydroperiod predictability (FIGURE 5.3B-D). Inter-annual emigration was drastically lower in predictable ponds (0.17 ± 0.10) than in unpredictable ones (0.64 ± 0.07). This pattern was slighter at the intra-annual level at which the probability of leaving a pond was 0.41 ± 0.03 and 0.54 ± 0.04 in predictable and unpredictable ponds respectively. By contrast, the immigration probability was drastically higher in unpredictable ponds. At the inter-annual level, it was 0.80 ± 0.06 and 0.19 ± 0.06 in predictable and unpredictable ponds respectively. Similarly, the intra-annual immigration probability was 0.77 ± 0.03 in unpredictable ponds and 0.23 ± 0.03 in predictable ones.

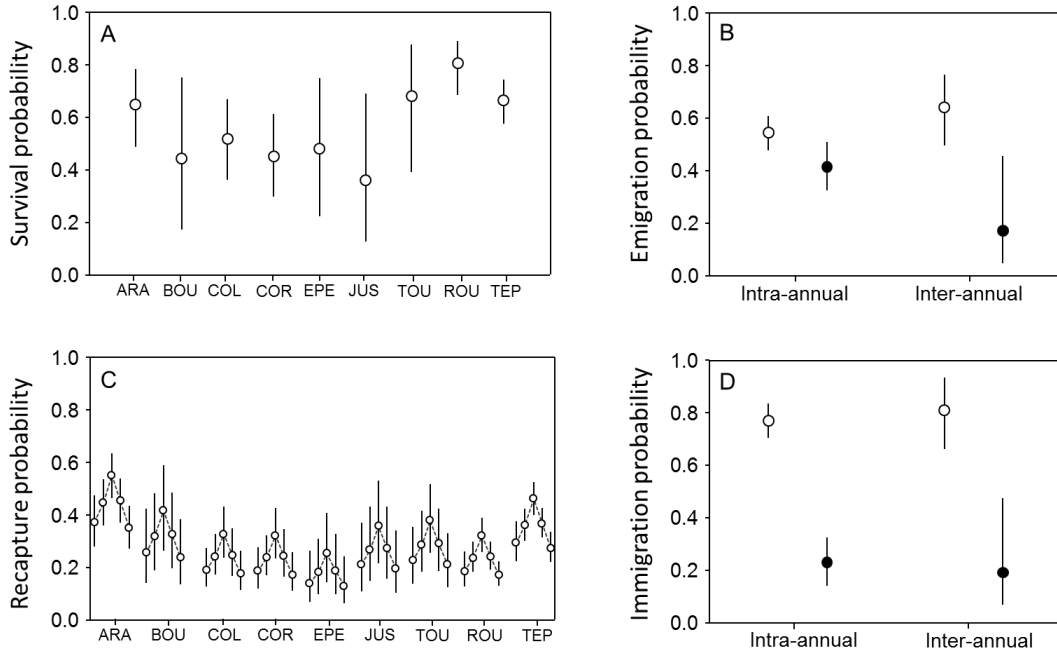


FIGURE 5.3. INFLUENCE OF POND HYDROPERIOD PREDICTABILITY ON EMIGRATION AND IMMIGRATION IN BOVA. Full circle: high predictability ponds. Empty circle: low predictability pond. A) Survival differs between populations. B) Emigration rates are lower in ponds with high hydroperiod predictability than in ponds with low hydroperiod predictability. C) Recapture probability differs among years and populations. D) Immigration rates are lower in ponds with high hydroperiod predictability than in ponds with low hydroperiod predictability.

TABLE 5.4. INFLUENCE OF POND HYDROPERIOD PREDICTABILITY ON EMIGRATION (ϵ) AND IMMIGRATION (α) IN BOVA: MODEL SELECTION PROCEDURE. ϕ : survival. p : recapture. AICc: Akaike Information criterion adjusted for small sample size. k : number of parameters. r : model rank. POP: the parameter varies among populations. HYDR: the parameter differs according to pond hydroperiod predictability. Y : the parameter varies among years. \cdot : the parameter is constant.

r	Model	k	Deviance	AICc
1	$\phi(\text{POP}), \epsilon(\text{HYDR}), \alpha(\cdot), p(\text{POP} + Y)$	29	4039.91	4100.00
2	$\phi(\cdot), \epsilon(\text{HYDR}), \alpha(\cdot), p(\text{POP} + Y)$	21	4060.66	4103.76
3	$\phi(\text{POP}), \epsilon(\cdot), \alpha(\cdot), p(\text{POP} + Y)$	27	4054.82	4110.64
4	$\phi(\cdot), \epsilon(\cdot), \alpha(\cdot), p(\text{POP} + Y)$	19	4075.58	4114.48
5	$\phi(\text{POP}), \epsilon(\text{HYDR}), \alpha(\cdot), p(\text{POP})$	25	4067.69	4119.25
6	$\phi(\text{POP}), \epsilon(\text{HYDR}), \alpha(\cdot), p(Y)$	21	4080.30	4123.40
7	$\phi(\cdot), \epsilon(\text{HYDR}), \alpha(\cdot), p(Y)$	17	4089.47	4124.20
8	$\phi(\text{POP}), \epsilon(\cdot), \alpha(\cdot), p(\text{POP})$	23	4082.61	4129.93
9	$\phi(\cdot), \epsilon(\text{HYDR}), \alpha(\cdot), p(\text{POP})$	13	4105.86	4132.29
10	$\phi(\text{POP}), \epsilon(\cdot), \alpha(\cdot), p(Y)$	19	4095.22	4134.12
11	$\phi(\cdot), \epsilon(\cdot), \alpha(\cdot), p(\text{POP})$	15	4104.39	4134.96
12	$\phi(\text{POP}), \epsilon(\text{HYDR}), \alpha(\cdot), p(\cdot)$	17	4101.96	4136.68
13	$\phi(\cdot), \epsilon(\cdot), \alpha(\cdot), p(Y)$	11	4120.77	4143.08
14	$\phi(\cdot), \epsilon(\text{HYDR}), \alpha(\cdot), p(\cdot)$	9	4126.18	4144.39
15	$\phi(\text{POP}), \epsilon(\cdot), \alpha(\cdot), p(\cdot)$	15	4116.87	4147.44
16	$\phi(\cdot), \epsilon(\cdot), \alpha(\cdot), p(\cdot)$	7	4141.10	4155.23

iii. *Influence of hydroperiod predictability on pond occupancy dynamics*

The mean rate of disappearance (extracted from the model in which parameters describing pond occupancy changes were held constant among ponds) was 0.53 (95% CI 0.43-0.62) for adult with an effective reproduction and around 0.45 (95% CI 0.37-0.53) for reproduction. The mean rate of colonization was 0.24 (95% CI 0.19-0.29) and 0.17 (95% CI 0.13-0.21) for adult and reproduction respectively.

The best-supported model, [$\psi(\cdot)$, $\rho(\text{HYDR})$, $p(\text{HYDR})$] (SEE THE MODEL SELECTION PROCEDURE IN TABLE 5.5), indicated that detection probabilities varied according to pond hydroperiod predictability (FIGURE 5.4A). The probability of detecting adults was only slightly higher in predictable ponds (0.24 ± 0.03) than in unpredictable ones (0.18 ± 0.02). Similarly, the probability of detecting breeding indices (eggs or larvae) was higher in predictable ponds (0.54 ± 0.02) than in unpredictable ones (0.36 ± 0.04). Initial occupancy did not differ according to hydroperiod predictability and was drastically higher for adults without effective reproduction (0.13 ± 0.02) than for reproduction (0.02 ± 0.01) (FIGURE 5.4B). More importantly, our results show that extinction probabilities are always lower in predictable ponds than in unpredictable ones (FIGURE 5.4C-D). The probability that a pond occupied by adults only at year $y - 1$ become unoccupied at year y was 0.48 ± 0.06 and 0.60 ± 0.06 in predictable and unpredictable ponds respectively. In addition, the probability that a pond occupied by adults with an effective reproduction at year $y - 1$ become unoccupied at year y was 0.37 ± 0.10 and 0.73 ± 0.13 in predictable and unpredictable ponds respectively. Our analyses also revealed that colonization probabilities differed according to pond hydroperiod (FIGURE 5.4E-F). The probability that a pond unoccupied at year $y - 1$ become colonized by adults without an effective reproduction at year y was higher in unpredictable ponds (0.31 ± 0.09) than in predictable ones (0.21 ± 0.06). By contrast, the probability that a pond become colonized by adults with an effective reproduction was higher in predictable (0.21 ± 0.02) than in unpredictable ponds (0.09 ± 0.02). Moreover, our results show that the probability that a pond occupied by adults without effective reproduction at year $y - 1$ becomes a breeding site at year y is higher in predictable ponds (0.22 ± 0.05) than in unpredictable ones (0.09 ± 0.04) (FIGURE 5.4G). Yet, the probability that a breeding site at $y - 1$ remained occupied by adult without effective reproduction at year y was not affected by pond hydroperiod predictability (FIGURE 5.4H).

TABLE 5.5. INFLUENCE OF POND HYDROPERIOD PREDICTABILITY ON DYNAMICS OF REPRODUCTION OCCURRENCE. ψ : initial occupancy. ρ : yearly changes in site occupancy. p : detection probability. r : model rank. k : number of parameters. AICc: Akaike Information Criterion. HYDR: the parameter differs according to pond hydroperiod predictability.

r	Model	k	Deviance	AICc
1	$\psi(\cdot), \rho(\text{HYDR}), p(\text{HYDR})$	19	6261.65	6300.40
2	$\psi(\text{HYDR}), \rho(\text{HYDR}), p(\text{HYDR})$	21	6259.81	6302.73
3	$\psi(\cdot), \rho(\text{HYDR}), p(\cdot)$	17	6288.04	6322.65
4	$\psi(\cdot), \rho(\cdot), p(\text{HYDR})$	13	6305.91	6332.27
5	$\psi(\text{HYDR}), \rho(\cdot), p(\text{HYDR})$	15	6303.61	6334.09
6	$\psi(\text{HYDR}), \rho(\text{HYDR}), p(\cdot)$	19	6314.29	6353.05
7	$\psi(\text{HYDR}), \rho(\cdot), p(\cdot)$	13	6353.47	6379.83
8	$\psi(\cdot), \rho(\cdot), p(\cdot)$	11	6384.77	6407.03

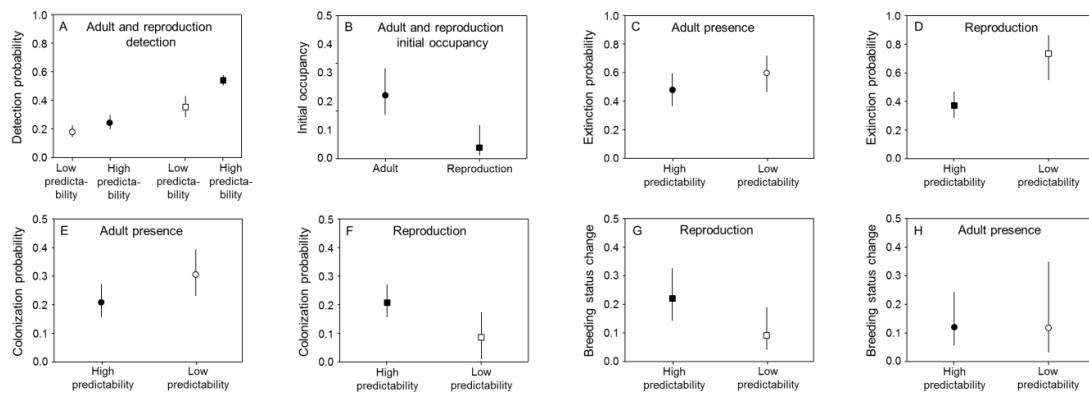


FIGURE 5.4. INFLUENCE OF POND HYDROPERIOD PREDICTABILITY ON DYNAMICS OF REPRODUCTION OCCURRENCE. (A) The probability of detecting adult (circles) and reproduction indices (egg, larval; square) depends on pond hydroperiod predictability (full = high, empty = low). (B) The probability of initial pond occupancy is higher without effective reproduction (circle) than with reproduction (square). (C) The probability that a pond occupied by adults only at year $y - 1$ becomes unoccupied at year y is lower in ponds with high hydroperiod predictability (full circle) than in ponds with low hydroperiod predictability (empty circle). (D) The probability that a pond occupied for the reproduction at year $y - 1$ becomes unoccupied at year y is lower in ponds with high hydroperiod predictability (full circle) than in ponds with low hydroperiod predictability (empty circles). (E) The probability that an unoccupied pond at year $y - 1$ is colonized at year y by adults without an effective reproduction is lower in ponds with high hydroperiod predictability (full circle) than in ponds with low hydroperiod predictability (empty circle). (F) The probability that an unoccupied pond at year $y - 1$ is colonized at year y by adults with an effective reproduction is higher in ponds with high hydroperiod predictability (full circle) than in ponds with low hydroperiod predictability (empty circle). (G) The probability that a site occupied by adults without an effective reproduction at year $y - 1$ become a reproduction site at year y is higher in ponds with high hydroperiod predictability (full circle) than in ponds with low hydroperiod predictability (empty circle). (H) The probability that a breeding site at year $y - 1$ becomes occupied by adults without an effective reproductive at year y is similar in ponds with high (full circle) and low (empty circle) hydroperiod.

D. Discussion

Our results showed that pond hydroperiod predictability affected breeders' emigration and immigration. The emigration probability was lower in predictable ponds while the immigration probability was higher in unpredictable ones. Furthermore, the reproduction occurrence dynamics strongly depended on hydroperiod predictability. Reproduction disappearance was less frequent in predictable ponds than in unpredictable ones. In addition, pond colonization with an effective breeding more frequently occurred in predictable ponds.

i. Influence of hydroperiod predictability results in condition-dependent dispersal

Our results highlighted a high emigration in the studied populations. Indeed, mean emigration rates were around 0.50 at both intra-annual and inter-annual levels. In addition, apparent survival appeared relatively low (with a mean of 0.64) in these populations compared to the one estimated in a previous study (between 0.72 and 0.85 in 5 populations in France; Cayuela et al., 2016b). This probably indicates a substantial permanent emigration out of the study area as apparent survival is always a mixture of mortality and this kind of movement in capture-recapture studies. These outcomes thus reflect a relatively high dispersal that is likely linked to the geographic proximity of ponds and their hydric instability.

Our analyses also showed that an increase in the degree of pond hydroperiod predictability results in lower emigration rates, in particular at the inter-annual level. This result indicates a condition-dependent dispersal. Adults adjust their emigration decisions according to the risk of pond desiccation, which is the main mortality factor at egg and larval stages (Barandun & Reyer, 1997b, Barandun et al., 1997). This outcome is congruent with theoretical models predicting that this form of dispersal is likely to evolve to reduce the impact of disturbances on individual fitness (Reigada et al., 2015). It is also in agreement with two previous studies that have experimentally demonstrated that *B. variegata* adults select their breeding sites according to environmental factors (e.g. intraspecific competition) influencing larval growth and survival (Cayuela et al., 2016c, 2017b). Taken together, these results show that breeders tend to improve the environmental conditions prevailing during offspring growth by avoiding ponds where the risks of larval mortality are high. Such a pond choice and condition-dependent emigration could be widespread in amphibians reproducing in stochastic aquatic habitats.

Contrary to our expectations, our analyses revealed that immigration probabilities were drastically higher in unpredictable ponds than in predictable ones. This indicates that dispersing individuals frequently reach unpredictable ponds where reproduction does not frequently occur. These ponds could thus be used for other activities between breeding peaks or at the end of the reproduction period. Indeed, a recent study showed that adults frequently occupy ponds where no reproduction occurs

for resting and foraging activities (Cayuela et al., 2017a), especially when the breeding ponds hold high density of growing tadpoles.

ii. *Influence of hydroperiod predictability on pond occupancy dynamics*

Our study revealed relatively high rates of inter-annual occupancy changes in the studied populations. The mean rate of disappearance was around 0.53 for adults with an effective reproduction and around 0.45 for the reproduction. The mean rate of colonization was around 0.25 and 0.17 for adults and reproduction respectively. These results are thus congruent with previous studies showing that amphibians as *B. variegata* that occupy small, fishless and unpredictable ponds display relatively high turn-over rates (Baber et al., 2004; Cayuela et al., 2012).

Our analyses showed that colonization more frequently results in effective breeding in predictable ponds than in unpredictable ones. In addition, when a pond is already occupied by adults, the chance that it becomes a breeding site the following year is higher in predictable ponds. Moreover, inter-annual pond disappearance is lower in predictable ponds. Overall, our study shows that a high level of hydroperiod predictability reduces the inter-annual changes in reproduction occurrence, which is congruent with theoretical models predicting that turn-over rates are lower in spatially structured populations experiencing low disturbance frequencies (Kallimanis et al., 2005; Elkin & Possingham, 2008; Reigada et al., 2015). Our results are also in agreement with previous studies in amphibians showing that a reduced and more variable hydroperiod results in an increase in extinction rates and a decrease in colonization rates (Baber et al., 2004; Cayuela et al., 2012). In these amphibian species, breeding in temporary aquatic habitats allows reducing the mortality risks due to fish predation before offspring metamorphosis but also results in frequent breeding failures due to pond desiccation (Hartel et al., 2011; Green et al., 2013). In our study case, as pond drying out is the main cause of mortality before metamorphosis (Barandun & Reyer, 1997b; Barandun et al., 1997), higher reproduction disappearance rates in unpredictable ponds could be due to a higher mortality at egg and larval stages. Indeed, we cannot rule out the possibility that breeding can occur in unpredictable ponds and remains undetected due to desiccation events occurring between two sampling sessions. Yet, this pattern is also likely to result from a pond choice and a condition-dependent dispersal at adult stage. As showed in our capture-recapture analyses, emigration is higher in unpredictable ponds, which probably results from a risk-avoidance tactic to reduce mortality risk at larval stage.

E. Conclusion

Our analyses revealed that hydroperiod predictability affects processes at different biological organization levels, from individual dispersal decision to patch occupancy dynamics. At the individual level, we highlighted that breeding success uncertainty regulated by changes in hydroperiod predictability results in condition-dependent dispersal. These informed dispersal decisions are then likely involved in patch occupancy dynamics. At the population level, a decrease in reproduction turn-over rates is associated with increased pond hydroperiod predictability. In a context where human activities durably alter disturbance regimes, our study emphasizes the central role of individual dispersal strategies in population responses to disturbances. They show that condition-dependent dispersal allows individuals to respond to modifications in perturbation regimes. However, landscape fragmentation could limit their response capacities and might therefore increase the detrimental effects of man-driven changes in perturbation intensity and frequency on long term population viability. Further studies should be undertaken to analyze these possible synergic effects and their consequences on the extinction risks of wild populations.

A. Introduction

The study of living organisms, following technological and theoretical progresses, changed a lot on recent years. We previously saw that we can study animals through direct observations of their behaviour and of the environment surrounding them. That can give us information about their biology, their habits, their feeding habits, their sexual behaviour, and so on. However, in this way, we can only observe what we see directly, but we cannot recreate events that happened without any witness, or that did not leave any track. Unfortunately, such events occurred frequently through time and scientists worked to find a way to get the missing information. One solution lies in the genetics, and especially in the population genetics whose aim is to investigate the processes that caused changes in allele and genotype frequencies in populations of living organisms (Freeman & Herron, 2004). Schwartz (2005) suggested that the studies using genetics to address evolutionary questions can be grouped into two classes. The first one tends to rebuild the previous relationships between organisms (molecular systematics), and the second one focusses on the apparition of morphological novelties (evolution & development). In the former category, the aim is to find in DNA sequences some similarities and some differences that allow the researchers to group animals in clusters according to their evolutionary history (McKelvey, 1982). Many techniques were developed to conduct such comparisons and with each progress, scientists are eager to apply it to humans to understand how *Homo sapiens sapiens* DNA evolved from the first humanoids and to reconstruct human population history (Cann, 2001). However, humans are not the only species of interest on our planet, and molecular genetics can also be used to improve the knowledge and thus the conservation of many other species. This is precisely the framework of a subfield of population genetics called Conservation genetics whose aim is to estimate genetic diversity and genetic differentiation between groups to understand the threats that may linger upon them (Narum, 2006). Indeed, the observation of a reduced genetic diversity is a clue to know that the concerned populations probably suffered from reproductive isolation or genetic drift (Narum, 2006).

In our study, this aspect is very important because Geneva, as globally Switzerland, is an area where human activities keep on growing. This strongly increases landscape fragmentation, destroys valuable grounds and reduces landscape diversity (Feranec et al., 2016). The wild species have to cope with the destruction of their habitat and often find their populations isolated from each other. This is the case of the Yellow-bellied toad (*Bombina variegata*: BOVA) whose habitat was dramatically reduced during the last decades and whose population's connectivity became strongly compromised (Jaggi, 2010). To assess the genetic diversity and the potential risk of extinction that the

Genevan populations face, we took DNA samples using buccal swabs (Campanella & Smalley, 2006; LeVin et al., 2011; Broquet et al., 2007) and we analysed 22 microsatellites markers. We conducted classic analyses to estimate gene diversity and differentiation in our samples and we then ran several programs to investigate population structures. We finally conducted some Mantel tests to do some basic analyses of landscape genetics. These are only preliminary results and, in the future, more detailed analyses will be conducted.

B. Methodology

i. Lab work for extracting and amplifying DNA

As mentioned earlier, genomic DNA of 462 individuals was obtained thanks to buccal swabs conducted directly on the field. The swabs were then dried for 24 hours and stored in 95% ethanol.

The DNA was extracted from samples in the lab of the University of Lyon 1 using the Chélex method and the proteinase K (EU0090 EUROMEDEX 24 rue des Tuileries BP684 67460 SOUFFELWEYERSHEIM) following Casquet et al. (2012). Chelex®100 is an “ion-exchange resin composed of styrene divinylbenzene copolymers with paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions” (instruction sheet of Chelex) (Casquet et al., 2012). The ions will remove magnesium from the samples to inactivate nucleases and DNA destroying enzymes, the aim being to protect the DNA molecules from being degraded. Proteinase K is a broad spectrum peptidase, commonly used in nucleic acid preparations, because it rapidly destroy nucleases that might otherwise degrade the DNA or RNA during purification (Lewis et al., 2001). The digestion time was of 16 hours at 56°C. It was followed by 15 minutes at 90°C to denature the proteinase K. After the extraction, classic PCR procedures, conducted using the Type-it™ Microsatellite PCR Kit (QIAGEN), were performed to amplify a total of 22 microsatellites markers. The best amplification conditions were determined thanks to preliminary tests for 4 different multiplex PCR mixes. The characteristics of each locus and each PCR are presented in TABLE 6.1 and TABLE 6.2. Out of the 22 markers, 3 were taken from Nürnbergger et al. (2003), developed for *Bombina variegata*, 5 were taken from Hauswaldt et al., (2007), developed for *Bombina bombina* and 10 were taken from the PhD thesis of Hugo Cayuela (2016). For this latter research, 119 markers were tested and their amplification was visualized on agarose gel to check polymorphism. Since the Yellow-bellied toad (*Bombina variegata*: BOVA) usually does not show much polymorphism and the number of different alleles is poor, only 10 markers did not appear monomorphic (FIGURE 6.1).

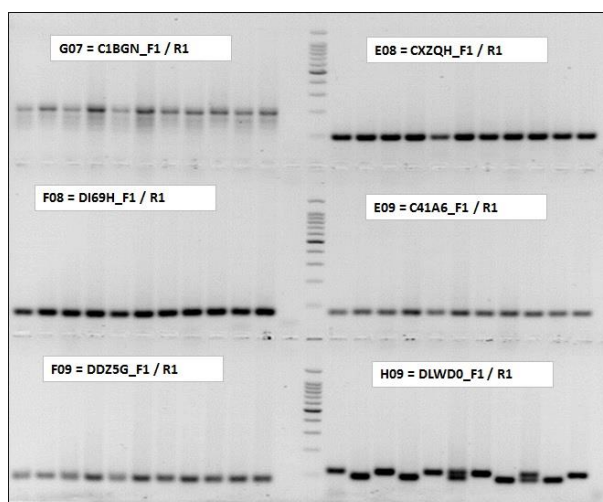


FIGURE 6.1. EXAMPLE OF AGAROSE GEL TO TEST FOR POLYMORPHISM OF MARKERS. The only marker that appeared polymorphic for BOVA in this set is H09 DLWDO (bottom right). C1BGN did not appear polymorphic on this gel, but was then tested with fluorescent tail and was added to the list of markers.

For these 18 microsatellite loci (mix1 to mix3), the Forward primer of each primer pair was labelled at the 5' end with a fluorescent tag (yellow, red, green or blue) (Griffiths et al., 1996). The mix 4 (4 loci) was newly developed for the present study and the primers used to amplify these 4 microsatellite markers were created with the fluorescent labelling method of universal primers described by Blacket et al. (2012). This approach allows any researcher to reduce the costs linked to the amplification and analysis of genetic markers. Indeed, instead of the two usual primers, it uses three different primers: a Forward sequence coupled with a tail whose sequence is universal (four tails exist: A, B, C and D), a Reverse sequence and a third primer that is complementary to the tail and which is coupled to a fluorescent tag (different for each type of tail). Concerning the costs, the two usual primers are very cheap, and it is only the third primer that is expensive. However, once the four different tailed primers are ordered, one only needs to order the F (with universal tail) and R sequences without any expensive fluorescent tag. Moreover, if the colour of the primer must be changed, one just need to buy a new F sequence with another tail, which again is very cheap.

After each amplification, four randomly chosen samples, as well as a control sample and a reference sample were checked using electrophoresis on 3% agarose gels with TAE (hand-made solution). The mixes were then sent to the National Genotyping Platform Gentyane for fragment analysis using GS600 LIZ size standard (Applied Biosystems). The microsatellite alleles data were read on GeneMarker v.1.95 (SoftGenetics).

TABLE 6.1. CONDITIONS USED TO CONDUCT PCR FOR EACH MIX OF MICROSATELLITES. Within the Mix 1, one microsatellite had to be separated during PCR for a better amplification.

Markers	TAQ	Conditions
CUSGH_F1/R1	QIAGEN	52°C x 36 cycles
Mix 1	QIAGEN	52°C x 36 cycles
Mix 2a	QIAGEN	48°C x 38 cycles
Mix 2b	QIAGEN	58°C x 31 cycles
Mix 3	QIAGEN	59°C x 37 cycles
Mix 4a	QIAGEN	57°C x 38 cycles
Mix 4b	QIAGEN	57°C x 38 cycles

TABLE 6.2. CHARACTERISTICS OF THE MICROSATELLITES. The Mix 4 was used for the first time in the present study and the primers were elaborated with a fluorescent labelling of universal primers (Blacket et al., 2012)

Mix	Locus	Repeat	Sequence	Origin	Fluorescence
MIX 1	CUSGH	Dinucl	AC	Cayuela	Green (HEX)
	DM3QM	Tetranucl	TATG	Cayuela	Red (ATTO565)
	DLWD0	Dinucl	AC	Cayuela	Blue (6FAM)
	DORC3	Tetranucl	CTAT	Cayuela	Yellow (ATTO550)
	Cons941	Dinucl	CA interrupted	Cayuela	Green (HEX)
	Cons470	Trinucl	TTA	Cayuela	Blue (6FAM)
MIX 2	Cons266	Dinucl	CA multisites	Cayuela	Red (ATTO565)
	4EMX7J	Tetranucl	CTAT	Cayuela	Yellow (ATTO550)
	Bv11.7	Dinucl	GT	Nürnberg	Blue (6FAM)
	Bv11.2	Dinucl	CA	Nürnberg	Green (HEX)
	Bobom8A	Tetranucl	AGAT; GATA	Hauswaldt	Blue (6FAM)
MIX 3	CWL3Z	Tetranucl	CTAT	Cayuela	Green (HEX)
	Bobom9H	Tetranucl	AGAT; CATA	Hauswaldt	Red (ATTO565)
	Bobom12F	Tetranucl	GATA	Hauswaldt	Blue (6FAM)
	Bobom10F	Tetranucl	GATA	Hauswaldt	Green (HEX)
	Bobom5F	Tetranucl	GACA; GATA	Hauswaldt	Yellow (ATTO550)
	4EGYJ3	Tetranucl	TAGA	Cayuela	Red (ATTO565)
MIX 4	Bv24.12	Dinucl	CA; TA	Nürnberg	Blue (6FAM)
	DN2N8	Tetranucl	TATC	new	Tail A (6FAM)
	DI69H	Dinucl	TG	new	Tail B (HEX)
	4EQ4WY	Tetranucl	ATCT multisites	new	Tail B (HEX)
	C1BGN	Tetranucl	TCTA	new	Tail D (ATTO565)

ii. Microsatellite analysis

Using MicroChecker v2.2.0.3 (Van Oosterhout et al., 2004), the microsatellite markers were tested for the presence of null alleles in each population. Then, FSTAT software v2.9.3.2 (Goudet, 1995) was used to test for genotypic disequilibrium for each pair of loci, gene diversity (H_e), allelic richness (A_r) and inbreeding coefficient (F_{IS}). We manually calculated H_o . We also investigated the degree of differentiation between populations (F_{ST}) and we tested the significance of pairwise F_{ST} values between populations using FSTAT software. We also conducted an AMOVA on Arlequin v3.5.2.2 (Excoffier et al., 2005) to assess the percentage of molecular variation among populations or individuals and its significance.

Then, Structure software v2.3.4 (Pritchard et al., 2000) was used to determine the most plausible number of groups (K) that are genetically different within our study. This analysis does not require any a priori about the geographical origin of the samples which are clustered based on their genotypes. We determined the correct number of clusters according to the comparison of likelihood at different K values that were then confronted to the Delta K values (Evanno et al., 2005). The results of Structure were organized with the online tool Structure Harvester. It also allowed us to estimate the genetic composition of each individual according to the admixture coefficients. To have a comparison, we also ran GeneLand v1.0.7 (Guillot et al., 2005) which is an add-on to R Studio (Ihaka & Gentleman, 1996), to estimate the number of population groups and delimit their spatial boundaries (Vörös & Arntzen, 2010). This tool is more robust for populations that do not present a clear structure. The setting used for the two programs are shown in TABLE 6.3 and TABLE 6.4.

TABLE 6.3. CHARACTERISTICS OF THE MODEL RAN BY STRUCTURE. The data for 12 microsatellites markers were used to determine the most plausible number of groups (K) and the assignment of individuals to K clusters.

STRUCTURE	
Length of burnin period	50'000
Number of MCMC Reps after burning	100'000
K tested	1 to 23
Ancestry Model info	No admixture
Frequency Model info	Independent
LOCPRIOR	off
Number of iterations	10

TABLE 6.4. CHARACTERISTICS OF THE MODEL RAN BY GENELAND. The data for 12 microsatellites markers were used to determine the most plausible number of groups (K) and the assignment of individuals to K clusters.

GENELAND	
Length of burnin period	200
Number of simulations	100'000
Number of thinning	100
K tested	1 to 20
Ancestry Model info	No admixture
Number of iterations	10

Then, we conducted several tests to see if some landscape features had an impact on the genetic diversity of our sites. We used a one-way ANOVA test, Mantel tests (Mantel, 1967) (using the function mantel.rtest from the ade4 package - Thioulouse et al., 1997, with 9'999 replicates) and Spearman correlation to test the effects of the rivers and of the city of Geneva. We also performed a Mantel test to test the effect of distance on genetic differentiation, according to the method of Vacher & Ursenbacher (2014) to have a more accurate evaluation of the level of isolation by distance. For that, we corrected the F_{ST} values ($F_{ST}/[1-F_{ST}]$) and the geographical distance ($\ln[\text{eucl_distance}]$).

C. Results

As we already mentioned, the following analyses were done on a dataset that included many missing values due to poor amplification. We must thus emphasize that these are preliminary results that will be confirmed with further laboratory work.

i. Pre-analyses

After microsatellite checking on GeneMarker, two markers (Mix2: Cons266; Mix4: DI69H) were abandoned due to poor amplification. It also appeared that some individuals were badly amplified. In total, 462 individuals were genotyped. We removed 20 individuals from Romania and 4 Swiss (but not from the region of Geneva) that we genotyped to check for the efficiency of the markers for outsiders but that will not be included in this work. Out of the 438 individuals, 64 individuals were amplified for less than a quarter of loci, so we removed them for the analyses of genetic diversity. These 374 samples coming from 20 populations were used in the analyses about the structure of the population. To have consistent results, we also removed population samples whose individuals did not have any microsatellite data for a locus. We thus investigated the genetic diversity with 305 individuals coming from 12 populations.

We used MicroChecker to test for null alleles. It appeared that out of the 20 considered microsatellites, 5 presented null alleles in 3 to 5 sites, meaning there was a possibility that the concerned populations were not in Hardy-Weinberg equilibrium with these loci. We thus removed them from further analyses (meaning markers Bobom5F, C1BGN, Bobom10F, DN2N8 and 4EQ4WY). Moreover, PLA appeared to have many null alleles, so we will test some analyses on this site alone to see if these results are due to interne structuration.

We used then FSTAT to analyse the remaining 15 markers. It revealed that some pairs showed a significant genotypic disequilibrium. After several trials, we removed further 3 loci and ended up with a final panel of 12 loci.

ii. Global considerations

It appeared that all loci are polymorphic. The number of alleles per locus ranges from 3 (Bv11.7 and Bv24.12) to 11 (CUSGH) for the 305 analysed individuals of 12 populations. The mean value of allelic richness is 1.39, but it might be biased by our small sample sizes.

The gene diversity per population is very variable. Mean H_e values range from 0.34 (COR) to 0.50 (JUS), with a mean of 0.41, whereas H_o values range from 0.13 to 0.47, with a mean of 0.20. We checked whether H_e was linked with the number of samples per populations. We divided the sites into two groups (less than 10 individuals, more than 10 individuals). For the small populations, it appeared that H_e has a mean of 0.44 (0.39-0.50), whereas for the big populations, H_e has a mean of 0.40 (0.34-0.49). The amplitude and mean of H_e per population thus does not depend on the population size.

Beside, a one-way ANOVA test showed that the differences between the molecular variances of big and small groups is not significant ($p=0.709$). All groups show a H_o smaller than H_e , except TOU.

F_{IS} values ranges from -0.214 to 0.268, with a mean of 0.076 and it appeared significant only for one site (PLA). The significant F_{IS} of PLA indicates that this group presents a certain structure, and as the value is positive (0.227), it means that the individuals in this population are more related than one would expect under random mating.

With regard to population differentiation, F_{ST} values ranged from 0 (corrected values, because, according to Nei (1973), F_{ST} values cannot be negative) to 0.5 (pairwise comparison JUS-REP), with a mean of 0.12, and 35 out of 66 comparisons are significant when permuting individuals among populations ($p<0.00$). However, no global trend can be seen for one site in particular. AMOVA revealed that the variation among populations (11.5%) is highly significant ($p<0.0001$), though there is an important percentage of molecular variation within individuals (77.6%) and among individuals within populations (10.9%). The TABLE 6.5 summarizes the genetic diversity of the populations.

TABLE 6.5. GENETIC DIVERSITY OF POPULATIONS. n: number of samples. H_o : observed heterozygosity. H_e : expected heterozygosity. F_{IS} : inbreeding coefficient. The data were averaged from 12 microsatellites (underlined: $H_o>H_e$). See Abbreviation list for all sites' names (p.0).

N° pop	Population	n	H_o	H_e	F_{IS}
1	ARA	16	0.19	0.39	0.197
2	BAR	23	0.15	0.35	0.058
3	BOU	5	0.13	0.43	0.268
4	CAR	33	0.17	0.37	0.059
5	COR	17	0.18	0.34	-0.011
6	JUS	3	0.14	0.50	0
7	PEN	12	0.19	0.49	0.205
8	PLA	79	0.22	0.41	0.227
9	ROU	43	0.19	0.40	0.037
10	TEP	50	0.18	0.46	0.068
11	TOU	3	<u>0.47</u>	0.39	-0.214
12	VER	21	0.17	0.36	0.014

iii. Population structure

In the aim of better understanding the genetic organisation of the individuals in the Geneva area, we also conducted analyses using Structure Software v2.3.4. We used here the dataset including 374 samples from 20 populations. Tough the missing values likely bias our results, several populations are missing data for the same loci. Thus, if differences are find between them, they do not reflect only the missing values. The values of the likelihood suggest that the most relevant partition of the data is with 4 inferred genetic clusters (FIGURE 6.2).

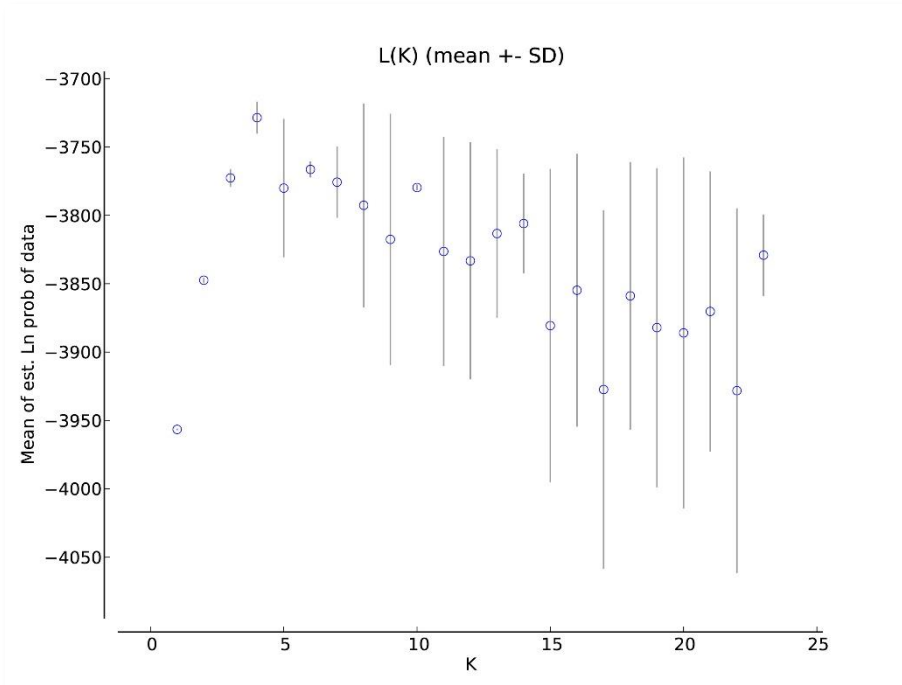


FIGURE 6.2. LIKELIHOOD VALUES GIVEN BY STRUCTURE FOR OUR 12-LOCI MICROSATELLITE DATA. X-axis: number of potential clusters. Y-axis: Ln. The values give that the most relevant partition of the data is with $K=4$.

Thanks to Structure, we can obtain the assignment of each individual to a given cluster. In the **FIGURE 6.3**, we can see the partition corresponding to the best run estimating $K=4$. The populations that appear the most separated in the analysis is the population 18 (VED). The population 13 (PLA) appears also different.

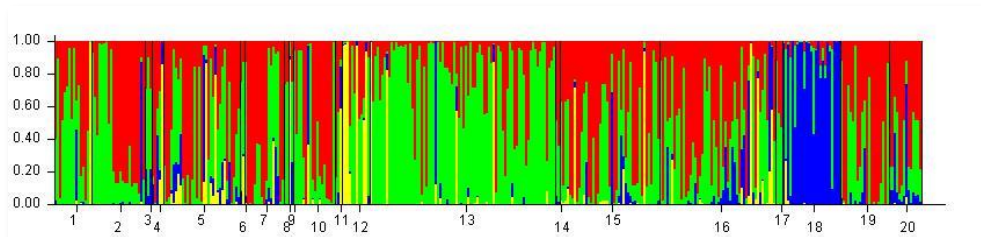


FIGURE 6.3. STRUCTURE RESULTS GIVING THE ASSIGNMENT OF EACH INDIVIDUAL ACCORDING TO $K=4$. The highest probability gives four inferred populations (each colour is a cluster). X-axis: populations (each row is an individual) (1: ARA, 2: BAR, 3: BIE, 4: BOU, 5: CAR, 6: CHA, 7: COR, 8: COU, 9: DOL, 10: GEN, 11: JUS, 12: PEN, 13: PLA, 14: REP, 15: ROU, 16: TEP, 17: TOU, 18: VED, 19: VER, 20: VIR).

In the aim of better visualising the repartition of each cluster within Geneva, we first reorganised the previous graph according to the geographical sector (**FIGURE 6.4**). It seems that the Jussy sector is quite homogenous, showing mainly red and green cluster, with some yellow individuals, but is different from VED (blue) even though their locations are close. The S-Rhône sector is also homogenous with mainly two clusters. The N-Rhône sector seems separated in two. Indeed, PLA (mainly red) and PEN (yellow) appear different from the other sites (green and red).

As mentioned above, some sites are missing values for the same bunch of loci (BAR:2, CAR:5, TEP:16, VED:18 and VIR:20), but as we still see differences between them, it means that the remaining loci showing values are different. However, this must be confirmed with further analyses of a full dataset.

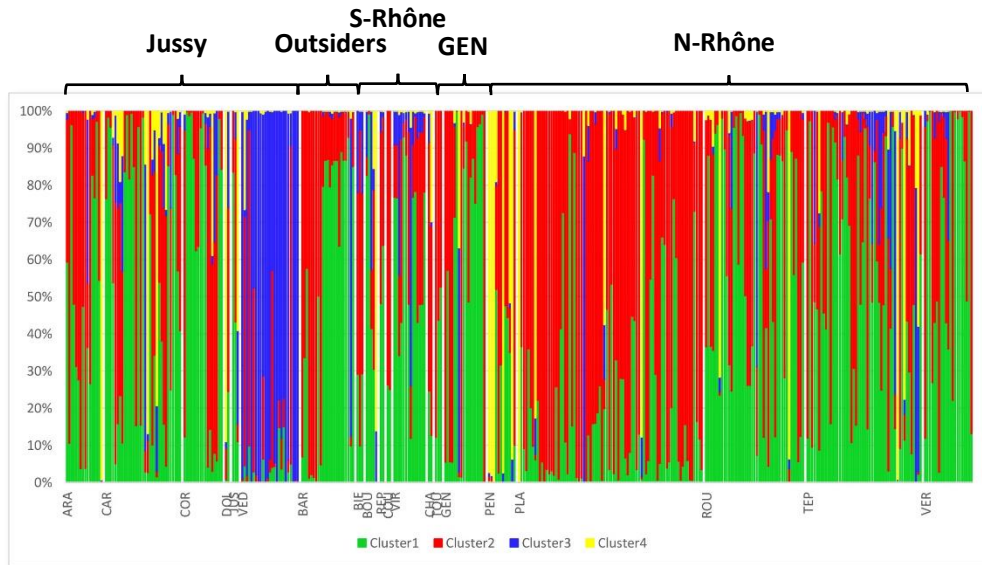
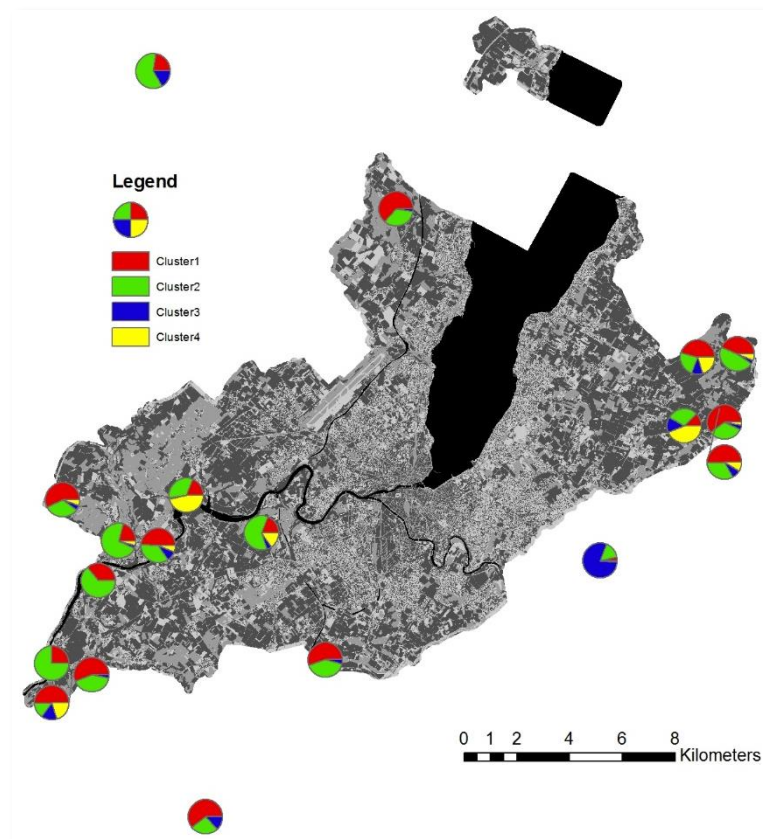


FIGURE 6.4. STRUCTURE RESULTS REORGANISED ACCORDING TO THE SECTOR. Colours: different clusters. Each row is an individual. S-Rhône: South of Rhône. N-Rhône: North of Rhône. PLA appears different from the other sites in the sector North Rhône. See Abbreviation list for all sites' names (p.0).

Then, we pooled together all the individuals belonging to a single site, and we presented the average assignment on a map using ArcGIS (**MAP 6.1**). A global consideration of the whole canton reveals that the Swiss populations show similar attributions to the inferred clusters, especially those to cluster 1 (red) and cluster 2 (green). The attribution to cluster 4 (yellow) especially appears in few sites of the Jussy area (East), in BOU (Laire area - West), and surprisingly, in two sites in the center of the study area: PEN (above the Rhône) and CHA (below the Rhône). Finally, the cluster 3 (blue) is mostly shown in a French site close to Jussy area (VED).

From here, we removed GEN from the analysis. Indeed, it concerns samples for which we did not have any location. We expected to be able to infer its origins thanks to the results, but as many sites appeared similar to it, we were not able to do so.



MAP 6.1. AVERAGES ASSIGNMENTS PLACED ON A MAP OF GENEVA ACCORDING TO $K=4$. Colours: different clusters. The pie chart that is on top of the legend corresponds to BIE which is a site located outside of Geneva, in the Vaud canton. We used it to compare our data with an outsider, but its location is wrong on this map.

We also used the tool GeneLand to have a comparison with the results of Structure. It revealed that there is 80% of probability that our dataset is divided into 4 populations (**FIGURE 6.5**). The results of the best run were put together to create another map of the mean attributions of each site. We can compare the results of the two tools on **MAP 6.2**.

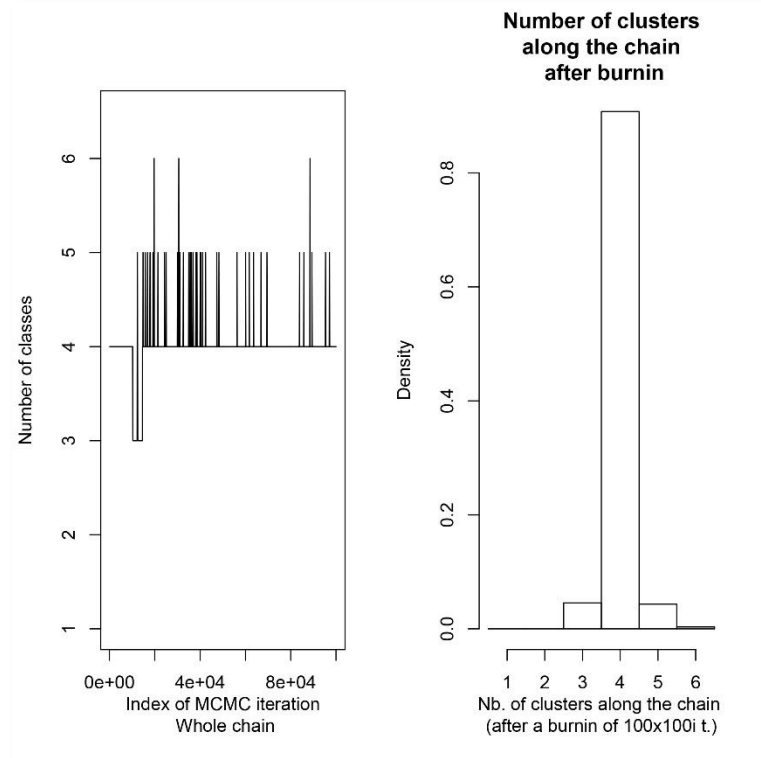
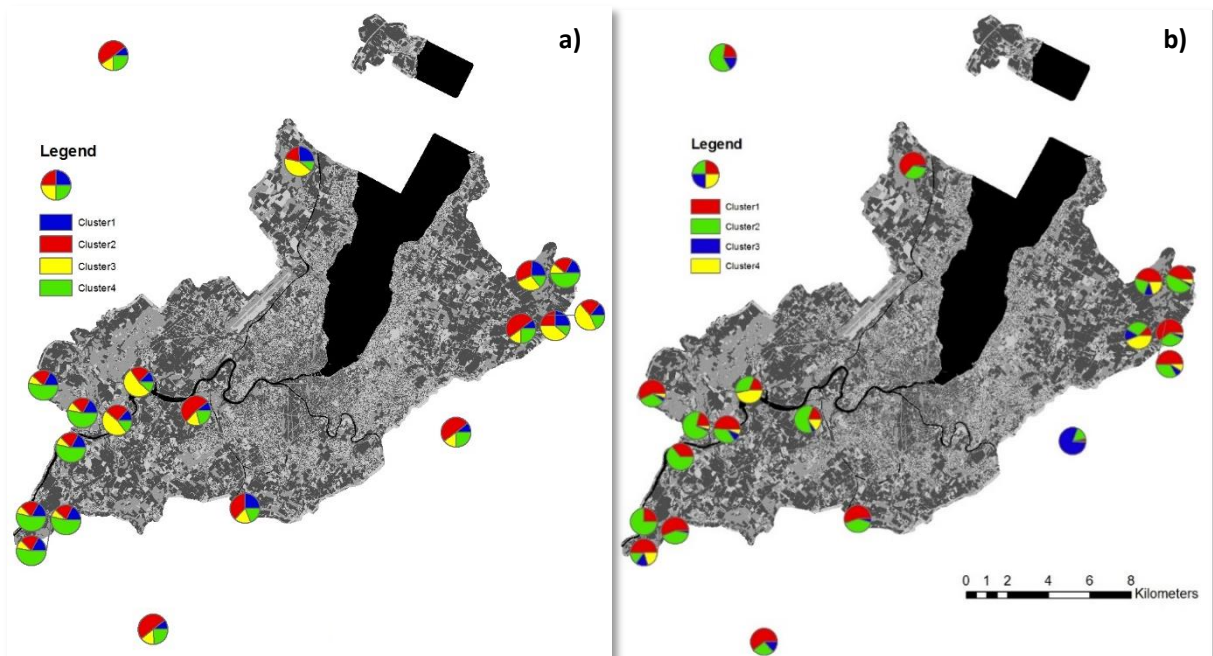


FIGURE 6.5. RESULTS OF GENEland SHOWING THE MOST LIKELY PARTITION OF OUR DATA. The most likely partition is $K=4$.

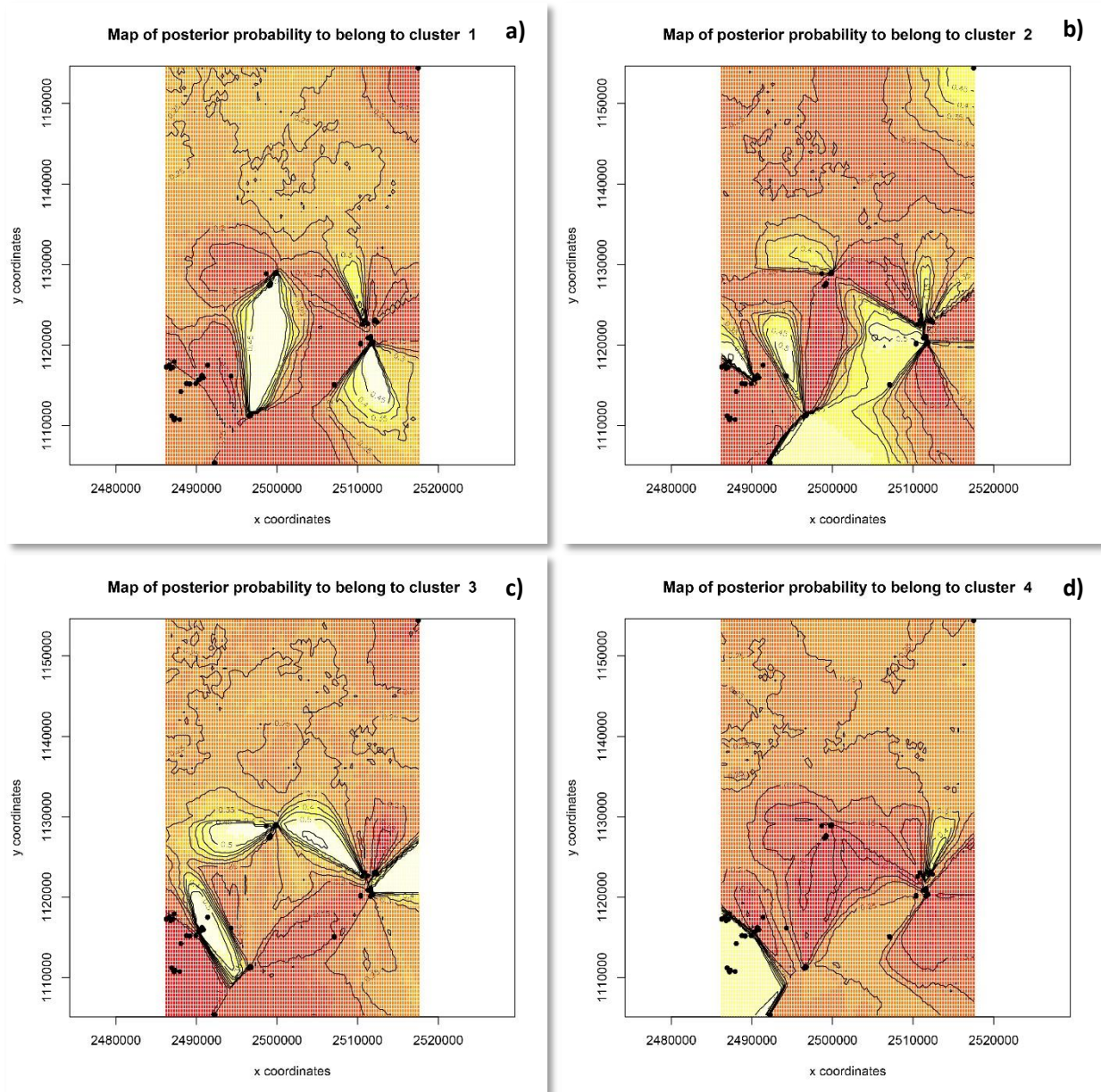


MAP 6.2. COMPARISON OF $K=4$ RESULTS FROM TWO PROGRAMS. Colours: different clusters. a) GeneLand $K=4$ b) Structure $K=4$. The pie chart that is on top of the legend corresponds to BIE which is a site located outside of Geneva, in the Vaud canton. We used it to compare our data with an outsider, but its location is wrong on this map.

The results of both programs give $K=4$ as the best partition, but we see that the attribution of each site to a cluster is slightly different. GeneLand gives a similar trend

to the sites located to the West, whereas Structure did not give them a different attribution from the other Swiss sites. Moreover, the French site that appeared different (blue) in Structure does not show an especially different trend in GeneLand, which suggests a bias due to missing values.

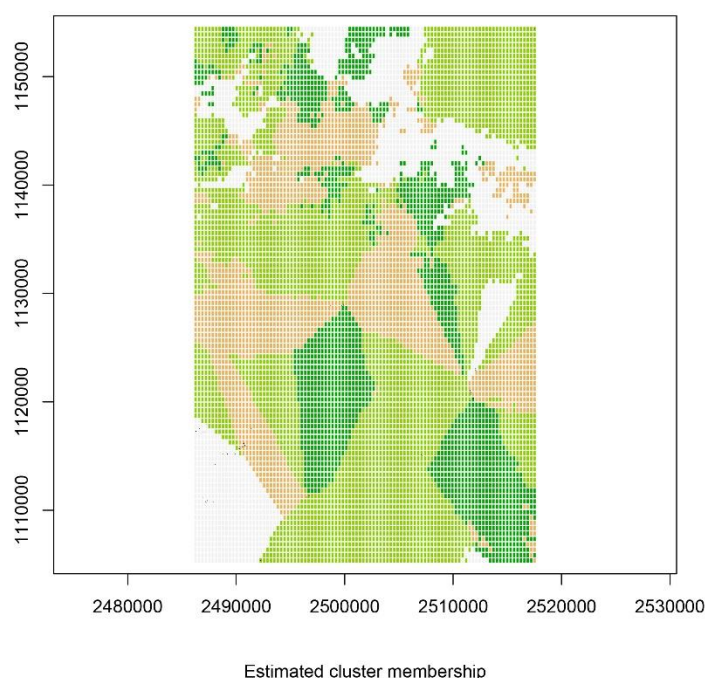
GeneLand also gives us maps of probabilities for each individual to belong to the inferred cluster 1, 2, 3 or 4 (MAP 6.3). The white areas are grouping the individuals with greater probabilities of belonging to the same genetic unit. Here, the software used individual data to make the groupings, and not a mean for each site as we used in MAP 6.2A.



MAP 6.3. MAPS OF PROBABILITIES TO BELONG TO ONE CLUSTER. White: greater probability to belong to a cluster. Red: lower probability to belong to a cluster. a) Cluster 1. b) Cluster 2. c) Cluster 3. d) Cluster 4. The site on top right of the maps is the outsider BIE.

If we compare the **MAP 6.2A** and **MAP 6.3**, we see that the groupings of the Western sites (green on **MAP 6.2A**) correspond to the cluster 4 (**MAP 6.3D**). We also see that the cluster 3 (**MAP 6.3C**) makes a grouping with individuals coming from the sites that appear more yellow on **MAP 6.2A**. However, the results of Structure (**MAP 6.2B**) do not show the same trends. Since GeneLand is usually more robust for populations that are less clearly divided, its results might be more reliable. We will check the correct trend once we will have the full dataset.

Finally, we obtained a map of assignment of each pixel to a cluster according to its geographical location (**MAP 6.4**).



MAP 6.4. MAPS OF ASSIGNMENT OF EACH PIXEL. Colours: different clusters. X-axis and Y-axis: Swiss coordinates.

iv. Landscape genetics

The next analyses were performed to see if the genetic diversity was related to some landscape features. We first investigated the impact of the main rivers (Rhône and Arve). We started by conducting a one-way ANOVA tests to see if there was an important variation of genetic diversity between our sites according to some groupings. We grouped our sites according to the sector in which they are located, meaning East of the Arve river (Jussy area), between the Rhône and Arve rivers (Laire area and South part of Rhône area) and North of the Rhône river (North part of Rhône area). The outsiders sites were added to the group according to their localisation (**MAP 1.2**). The ANOVA showed that the variance is not significantly different between our three groups ($p=0.671$). The variance was not significant neither if we consider only the sites North or South of the Rhône ($p=0.46$) nor East and West of the Arve ($p=0.92$). Then, the Mantel tests (using Euclidian distances) showed that the Arve has an impact on the genetic variance of the populations, but not the Rhône ($r_{\text{Rhône}}=0.137$, $p=0.164$; $r_{\text{Arve}}=0.377$, $p=0.026$). The

Spearman correlation did not show any correlation between the distance to town ($\rho = -0.032$, $p = 0.92$), but a last Mantel test (using corrected F_{ST} and distances) showed that the distance between sites is correlated with the genetic isolation ($r = 0.208$, $p = 0.028$, $\alpha = 0.05$) (FIGURE 6.6).

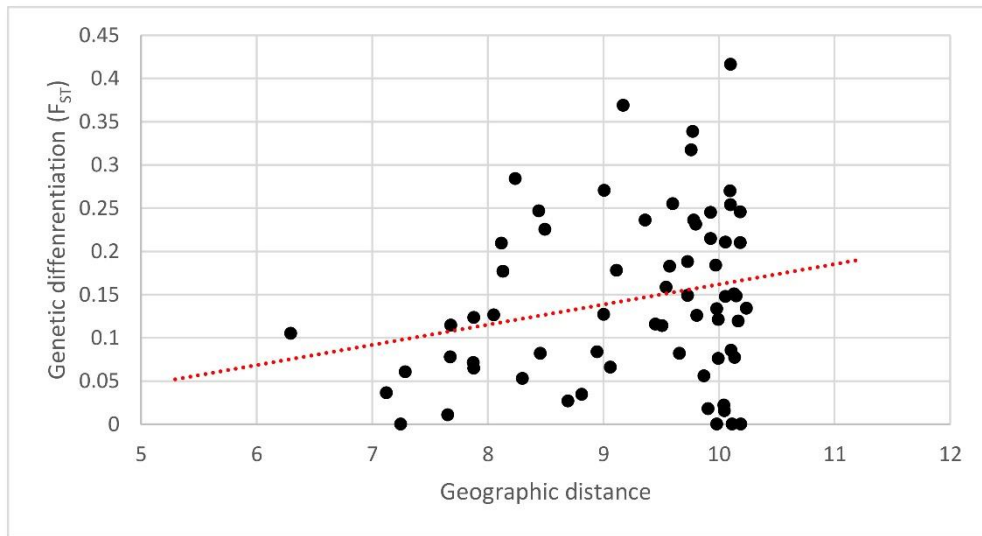


FIGURE 6.6. COMPARISON BETWEEN GENETIC DIFFERENTIATION AND GENETIC DISTANCE. Dot: couple of populations. Red line: linear regression. X-axis: geographic distance. Y-axis: genetic differentiation. A Mantel test revealed a correlation between the two values.

D. Discussion

One of the aims of this study was to determine the genetic status of Geneva's populations to be able to assess the risk of potential genetic isolation. Since the level of urbanisation in Geneva strongly increased in the last century (www.geographen.ch) (MAP 1.1), we expected that the remaining populations of the Yellow-bellied toad (*Bombina variegata*: BOVA) are not connected anymore and that they are going towards isolation, which would lead to a loss of genetic diversity (Lacy, 2000). Such a loss would endanger the populations in this area and, since this species is already considered as one of the mostly threatened amphibians in Switzerland (Thiébaud, 2008), its conservation must be a priority. To have a minimal impact on these fragile animals, we decided to use a non-invasive method of DNA sampling: the buccal swabs. Even if the quantity of DNA is reduced, it is a method that was recognized as efficient for amphibians (LeVin et al., 2011), so we gave priority to animal welfare instead of insuring high quantities of DNA.

About our microsatellite data, we saw that some sample plates were not well amplified or genotyped, which results in missing data for a given locus for a bunch of individuals. Sometimes, a whole population did not get any data for a locus, which means some analyses were not successful at all or badly worked. We thus had to reduce our dataset for some analyses to reduce the bias due to missing data. We will interpret our results as they are, but we intend on applying for additional funding to analyse again the samples that did not work and to add some more samples that were collected in 2016, but could not be included in our dataset.

i. Genetic diversity of the Genevan population

Globally, the mean value of expected heterozygosity in Geneva is medium ($H_e=0.41$) and that a general deficit of heterozygotes occurs ($H_o=0.2$). This is preoccupying because, even if with microsatellites it is difficult to exclude the effect of artefacts such as null alleles or genotyping errors, it may suggest non-random mating and inbreeding which may be dangerous for wild populations as it could result in genetic drift, fixation of deleterious alleles and thus decline of the populations (Didham, 2010; Li & Horvitz, 1953). Beside, the significant genetic differentiation detected ($F_{ST}=0.12$) indicates the presence of genetically isolated populations, which is consistent with the high fragmentation of the Genevan landscape. However, for amphibians, a certain level of differentiation is expected due to their low mobility, their high philopatry and the distances between the sites (Vacher & Ursenbacher, 2014), and, given that our results can be biased by the quality of our data, the conclusions about isolation must be taken with caution.

Some useful indications about the status of the Geneva BOVA populations can be obtained by the comparison with other studies in Europe conducted with

microsatellites on the same species (TABLE 6.6). We compiled data from four studies conducted in five different locations in Switzerland, France and Italy; some of the analysed microsatellites loci are common between studies. We indicated the width of the study areas (studies in grey: 140 to 150 km, studies in white: 30 to 45km) to keep in mind that the genetic values can vary according to the size of the considered area.

TABLE 6.6. COMPARISONS OF GENETIC DIVERSITIES BETWEEN THREE STUDIES USING MICROSATELLITES. CH: Switzerland, FR: France, IT: Italy. n: number of samples. N_{pop} : number of populations. $N_{microsat}$: number of markers. $N_{alleles}$: number of alleles. H_o : observed heterozygosity. H_e : expected heterozygosity. A_r : allelic richness. F_{IS} : inbreeding coefficient. F_{ST} : genetic differentiation. The * value is a mean for both Valais and Vaud. Sites in grey are wider than sites in white (~150km vs ~40km).

Study	Location	n	N_{pop}	$N_{microsat}$	$N_{alleles}$	H_o	H_e	A_r	F_{IS}	F_{ST}
Tournier, 2017	Geneva (CH)	305	12	12	3-11	0.20	0.41	1.39	0.08	0.12
Vacher & Ursenbacher, 2013	Lot (FR)	46	2	6	1-6	0.31	0.33	3.53	-0.03	0.02
Vacher & Ursenbacher, 2014	Alsace (FR)	290	10	6	5-11	0.57	0.51	4.79	-0.11	0.13
Cornetti et al., 2016	Trentino (IT)	200	9	11	2-11	0.49	0.47	3.14	--	0.05-0.32
Ursenbacher et al., unpublished	Valais (CH)	107	4	6	1-6	0.22*	0.17	1.36	-0.01	0.29
Ursenbacher et al., unpublished	Vaud (CH)	89	5	6	1-6	0.22*	0.23	1.45	-0.04	0.1

First of all, the genetic diversity of Swiss populations seems to be lower than the one of French and Italian populations. We did not use here the criterion of private allelic richness (Foulley & Ollivier, 2006) to compare the populations of each study separately because it was not used by all authors, but it could be interesting to evaluate the richness of each population if more data become available. It appeared that the value of allelic richness found in our study is consistent with the one of Ursenbacher et al. (unpublished), even if our value is based on a small sample size. To explain the differences between the Swiss values and the other ones, we compared the location of the studied sites with the distribution of BOVA. Indeed, a reduced allelic richness can be due to highest distances from the initial centre of distribution of a species (Hewitt, 2000). However, according to the distribution map of the IUCN (IUCN website), we found that all the sites were on the edges of BOVA's range, which did not explain the differences in genetic diversity. We thus investigated the degree of urbanisation of the areas, because, according to Araújo (2003) and Burgess et al. (2007), allelic richness may be reduced in case of high human density. We found that the Swiss locations had a higher density of habitants per kilometre square than the other ones (Table 6.7), which can explain the lower genetic diversity in these sites.

TABLE 6.7. COMPARISONS OF ALLELIC RICHNESS ACCORDING TO HUMAN DENSITY. CH: Switzerland, FR: France, IT: Italy. A_r : allelic richness. Grey: low allelic richness. The genetic diversity diminishes when human density increases.

Location	Human density (hab/km ²)	A_r
Lot (FR)	33	3.53
Ardèche (FR)	56	4.79
Trentino (IT)	86	3.14
Valais (CH)	278	1.36
Vaud (CH)	450	1.45
Geneva (CH)	2281	1.39

Secondly, the mean expected heterozygosity value found in Geneva seems to be in the range of the other studies, but is higher than the mean of the other Swiss study, whereas, our mean observed heterozygosity value is consistent with this study. However, the biggest difference in our dataset is that the H_o is much lower than H_e . Indeed, the F_{IS} value is close to zero in all studies, which suggests that the populations are homogenous and that there is no inbreeding (Ursenbacher et al., unpublished). Our study is the only one that presents a positive F_{IS} value, which may point to the bad quality of this preliminary dataset and will require further investigations to know if our population is panmictic or not. And, anyway, our F_{IS} value is not significant.

Finally, all the values of F_{ST} appear to be in the same range and indicate a certain degree of structuration. The highest value for Switzerland is in the Valais region, which suggests that populations in this area are the most isolated each to another. The F_{ST} in the Geneva region is about 60% lower than the mean of Vaud and Valais together ($F_{ST-GV}=0.12$; $F_{ST-VD/VS}=0.2$), but if we compare the width of the study sites, we see that Geneva presents distances between the populations that are about 30% smaller than in Vaud and Valais. In general, the existence of significant genetic differentiation over short distances might be linked to the high level of habitat fragmentation. It indicates the need to continue and further strengthen the government efforts aimed at protecting the environment and reducing the impact of human activities on wild habitats (Bachmann et al., 2010).

ii. Genetic structure of the Genevan population

The analysis of the structure of populations did not reveal clear differences between the sites. The potential isolation that was shown by the least-cost paths by AMOVA and F_{ST} analysis is not clearly supported by the analysis in clusters. Indeed, all Swiss sites seem quite similar on Structure results, and the different attributions given by GeneLand results do not support the LCP. On Structure results, the most different site appears to be VED, which is located in France, close to Jussy area. Though the proportion of the blue cluster is much lower, it is also represented in most sites located on the border of the canton or in France. We monitored another site near VED, with which it would be interesting to compare the genetic data of the individuals. Unfortunately, VEZ allowed the observation of only three individuals and the swabs

could not be analysed. Further genetic work on this area, linked with resistance maps on French ground could allow us to see if this area is particularly isolated. On GeneLand results, it is rather the Western sites that appear different, so field work on this side of Geneva could also improve the knowledge about potential movements across the border.

An interpretation is here difficult because of the quality of our dataset, but, we can offer some assumptions. The reason why the moderate genetic differentiation does not appear in the results of cluster population structure may be that changes in the environment due to human activities are recent. The situation in Geneva appears complex, and if the fragmentation of landscape implies a reduction in the connection network, it either only started to limit the genetic exchanges between populations (Safner et al., 2011) and we are likely to see much more clusters on the long-term, or the missing values are hiding clusters already existing. Anyway, the repercussions of the interrupted gene flow are less detected for amphibians that do not have a short generation length (Safner et al., 2011; Miaud et al., 1999; Barandun & Reyer, 1997b).

iii. *Effect of landscape features on genetic diversity*

In Geneva, the most important landscape features that could have a historical effect on the genetic diversity of the toads' populations are the two main rivers: the Rhône and the Arve. However, the results show that only the Arve has an impact on the populations located on each of its sides.

To understand why one of the rivers has an impact and not the other one, we considered the spatial disposition of the sites according to the rivers. We noticed that the sites around the Rhône are much closer to the water and thus to each other than the sites around the Arve. Indeed, when testing the effect of the Arve, we compared the Jussy sector's sites and the Laire sector's sites including the outsiders and French sites. These sectors are distant of about 30km (BAR is half way from each other). So, even if the Arve seems to have an impact, we can believe that this effect is mainly due to the great distance between the sites, and not really due to a river effect. We suggest thus that there is no effect of the rivers on the genetic differentiation between our sites, but an effect of the distance only. This is confirmed by the other Mantel test that showed a significant correlation between the distance and the F_{ST} .

E. Conclusion

Our results indicate that the genetic diversity in Geneva is low, but consistent with other studies in Switzerland. However, it is much lower than the ones found in other studies in Europe. This can be linked to the degree of urbanisation as the concerned Swiss regions show a higher human density than the regions in France and Italy. Such an urbanisation implies landscape fragmentation and limits the gene flow between local populations. In Geneva, the differentiation is moderated, but as the geographic distances between the sites are small, we would expect it even lower. We also collected preliminary indications suggesting a potential risk of inbreeding, not detected in studies conducted in other areas. On the other hand, the results of Structure did not show big differences between the sites. As the software also uses the microsatellite data, it means it did not find differences big enough to establish differences in the attribution of populations to the clusters. Since GeneLand found some differences, it suggests that these results can be biased by our dataset as it includes many missing values that make the analyses unreliable. However, we can also find a biological explanation. Indeed, it is possible that, in the past, the populations were connected, insuring gene flow. The actual observed genetic differentiation would be thus due to recent increase of human activities that lead to the disconnection of the populations. The consequences of such a situation can be observed through the moderate differentiation, but we would need many generations to have a more obvious noticing.

VII. GLOBAL DISCUSSION

In an effort to promote Yellow-bellied toad's conservation, we first had to elucidate what might affect or influence its presence. We thus monitored three types of ponds, acting as conservation measures, in varied habitats where the Yellow-bellied toad (*Bombina variegata*: BOVA) was known to be historically present and we collected data about nine different environmental factors. For each encounter with our target species, we took a picture of the toad's belly to be able to estimate population size and investigate animal's dispersal (Delarze et al., 2000). This non-invasive method of capture-recapture was acceptable as BOVA is a naturally marked species. This study took place in Geneva, a fast-growing canton where demography considerably increased in the past ten years with a rapid expansion of the city and an intensification of agriculture (DieGeographen website; Ray et al., 2002). We thus conducted genetic analyses to assess the genetic variability amongst Genevan toads and estimate the risk of isolation due to landscape fragmentation. To do so, we collected buccal swabs to stay in line with our non-invasive study (May, 2004).

i. *The efficiency of the measures is linked to the importance of water*

To stop BOVA's population's decline, we improved the conditions of the habitat, and especially, we manipulated the hydroperiod of the ponds. Indeed, we showed, as many other studies (Barandun & Reyer, 1997b; Cayuela et al., 2011), that among many factors, water availability is the most important for amphibians. Of course, it is not natural to find artificial plastic buckets in the wild, and our aim was not to create many small terrariums on the field, but they have the advantage to conserve water as the loss into the ground is inexistent and the evaporation is strongly limited thanks to their small surface. They allow thus a potential clutch to reach the final metamorphosis after which the animals can breathe air and survive even in case of a drought (Bühler et al., 2007). During the study, we observed particularly hot seasons that lead to the drought of almost all natural and semi-natural measures. Even though they did not dry totally, the buckets also showed a reduction of their water quantity. Some especially strong heat waves forced us to add some emergency additional buckets to counter the dryness of a whole area. These sudden new humid habitats were colonized very quickly. This highlights the dangers of global warming on pioneer species, because temporary ponds in which they preferentially breed are likely to last less than the breeding period and to endanger the survival of the larvae. This shows how the measures placed in the canton of Geneva were beneficial for the toads.

We considered three types of measures, two of them leading to the modification of the habitat. Indeed, the mapping of natural depressions did not affect the conditions in which the toads lived before this study, whereas the buckets and the semi-natural ponds improved the water availability. We need to consider here that the 27 studied sites were not modified in the same way, as the number of ponds of each type varied

(APP. B3). The impact on the animals, since the modifications, was thus not the same everywhere. In the sites where ponds were added at the beginning of our study, we noticed an increase in the current population size (CPS) in COR, ROU, TEP and VER (SEE CPS GRAPHS IN APP. C3). There, the number of observed toads increased during the first year or between the two first seasons, suggesting that our conservation measures had a beneficial effect on the habitat quality and thus on the toad populations. We also added some more ponds during the study in sites where there was no observed improvement or where a potential intra-connection could be enhanced. This is the case in ARA, CAR, ROU and TEP. In the first two ones, we saw an increase in the population after the improvement, and in last two, we did not see any increase in the CPS, but we noticed in the field that some new areas allowed the observation of toads. That means that the additional habitats responded to the needs of the animals that quickly colonized them.

Another consideration about the new ponds is that we installed or created them in a spatial disposition that goes along with the fact that amphibians have a low mobility (Smit et al., 2006; Schroter, 2005). Indeed, in areas where the presence of BOVA was recently confirmed, we used the emergency configuration in “island” in the aim of providing to the toads many new habitats in which they could breed within their range of movement. As we showed that the mean daily travel distance for an adult toad is about one meter, the probabilities for them to quickly find a suitable habitat in case their current one dries out, was higher after the installation of our measures. Moreover, we found that the emigration rate is lower in ponds where the hydroperiod is predictable. In the wild, if a pond dries out, the toads have to leave it and find a new one, which has an energetic cost. Thanks to the buckets that do not dry, in addition to smaller risks of breeding failure, the necessity of moving is reduced. Barandun & Reyer (1998) suggested that BOVA moves randomly through its habitat in search of suitable habitat for breeding. Many habitats allowed them to assess the quality of the habitat before spawning and to choose where to breed to avoid the costs linked to a potential loss of clutches in case of a drought. Despite that, we often observed toads in drying habitats, but without any reproduction. As Cayuela et al. (2017a) explained, these other habitats are also used for other activities such as foraging or resting.

ii. *Human-made features compromise more the gene flow than natural landscape features*

The analysis of Geneva’s ground showed that the canton does not present a homogenous resistance to the movement of the toads. Indeed, Linkage Mapper showed that the exact centre of Geneva is highly resistant (at the tip of the lake, between the two main rivers), which is obvious because of the many buildings that prevent the toads from moving (MAP 4.7). It also appeared that the surroundings of the Arve river have a low resistance, whereas the surroundings of the Rhône have a very high resistance. However, as only one population is known around the Arve (VEZ), with three identified individuals, we cannot assess the travelling distance in this area and the potential connection with other sites.

Moreover, it was quite surprisingly to see in the genetic results, that it is especially the distance that influences the genetic differentiation and not the Rhône even though it is an impassable barrier. This was confirmed by both Structure and GeneLand that did not show differences in the attribution of the sites according to their location on each side of the Rhône. We mentioned that Linkage Mapper found paths through the river using the bridges, but that we thought not likely that the toads use them. We maybe have to reconsider our conclusions here, knowing that the toads apparently find ways to connect both sides of the rivers to insure the gene flow.

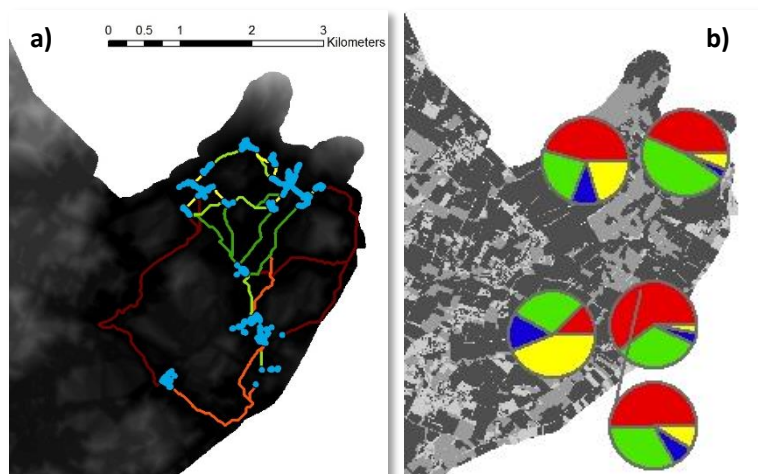
This said, the term “distance” must be considered carefully because the width of the study area is only about 30km. Normally, small distances mean smaller genetic differentiation, and high distances mean higher differentiation. As explained before, the mean F_{ST} value of Geneva is moderated (0.12). However, this value is similar to the one of populations that are much more distant (Ursenbacher et al., unpublished). It means that the resistance of the landscape prevents the toads from moving, even over a much smaller range, which assumes that the degree of fragmentation is higher than expected. It would be interesting to assess the effect of other landscape features on the genetic differentiation. As the habitat map did not allow us to isolate a given type of roads, more data are needed to do this comparison.

The least-cost paths showed that the areas of Jussy and Versoix do not have many connections with other parts of Geneva. This is due to the fact that they are surrounded by crops that cannot be crossed by toads (Ray et al., 2002), but it is also linked to the absence of known populations around them. However, the genetic data did not show any significant differences between these sectors, so it is not likely that they are used to be historically isolated in a genetic point of view. However, as the urbanisation and human-made features density increase in Geneva, the isolation could be more important in a few years.

Inside the Jussy area, Linkage Mapper showed that many connections exist, especially in the central part. Indeed, DOL (South-West) appeared more isolated than all the other sites (**MAP 7.1A**). This point was confirmed by the genetic data, since Structure showed that the main cluster to which DOL is attributed is different from the others (**MAP 7.1B**). GeneLand also showed that the main cluster of DOL is different from the other sites of Jussy. This isolation is probably linked to the presence of a highly frequented road (route de Juvigny) along the site of DOL. As we already mentioned, road works will take place in 2018 in this sector and wildlife passageways will be created under Juvigny. It will be very interesting to assess the genetic diversity of DOL compared to the one of the other Jussy sites in a few years.

Moreover, we found that CAR seems to be the central connection point in Jussy area to link the North sites (ARA and JUS) to the South sites (COR and DOL). We can thus assume that the movements of the individuals within the area are still possible and thus, that the gene flow is not compromised. From a conservation point of view, CAR, and

also the most South JUS ponds, are key-sites that need to be protected to guarantee the survival of the population.



MAP 7.1. COMPARISON BETWEEN LINKAGE MAPPER AND STRUCTURE RESULTS. a) LCP map. b) Population structure. DOL appears isolated in both maps.

To finish the investigation of the link between genetic and landscape, we need to consider the sites located at the border of Geneva. Indeed, as shown on the **MAP 6.2B**, the main trend of Geneva's populations is to be attributed to two main clusters (red and green). The trend changes for sites close to the South and South-East border of the canton. Indeed, most of them were also partly attributed to the blue cluster, appearing thus genetically closer to the French South-East site (VED). Jussy, in particular, seems to have experienced connections with this site, even though their blue attribution is not important. GeneLand also show some trends for the Western border versus the Eastern border (**MAP 6.2A**). The two programs do not show exactly the same results, though, and VED seems less isolated on GeneLand map than on Structure map. Here again, we need data on the French ground to assess the potential LCP that could connect these areas and a better quality of the dataset. Moreover, more field work should take place around VED to maybe discover remnant populations of toads. This would improve the knowledge on the possible gene flow in this sector.

iii. Bias of the study

This study was conducted as a response to the Action Plan concerning the danger that BOVA are facing in Geneva. It was required by the Department of Nature and Landscape who asked us to coordinate the work of several actors. We set up protocols to normalize the data collection and reduce at maximum the observatory bias. However, some of the actors were not scientists or were volunteers and so, even with the protocols, the data were not always collected in a complete way. Among others, we missed surface data and characteristics of ponds, so depending on the analyses, we could not take into account all the data collected in all the sites. When it was possible, we also corrected the data for wrong age class or sex of toad, if pictures were available, infinite number of Marsh frogs and missing weather data.

When considering the OPS, it allowed us to have an idea of the amount of toads present in an area. However, as previously explained, all the present and alive toads are not observed during each year of survey. According to Rovero & Zimmermann (2016), if we repeat a survey several times, the cumulative number of identified individuals should reach a threshold that can help the researchers to estimate the real population size. As we can see in TEP on [FIGURE 3.5](#), this threshold is obvious in 2012 and 2014, but not in the other years. It is also the case for the other sites, the threshold is not always clear. An explanation to that is either that the number of field sessions within a year was not large enough to capture all the present toads, or that the immigration and emigration rates are too high to be able to capture all the toads before they leave or before newcomers arrive.

At the beginning of the season 2014, we experienced vandalism on one of our sites (TEP). Indeed, during the previous winter, all the buckets were removed to be cleaned, as every year, except the ten rectangular buckets that stayed into the field that year. Before beginning the data collection period, we planned on cleaning them directly on the field, but they had disappeared. The problem was that as some eggs were already observed in other parts of this site, it means that we might have lost the first spawning wave in these buckets that are among the most successful ones. Luckily, as soon as we placed them back, the toads colonised them rapidly and we soon observed clutches inside. This episode might have had several consequences among which the perception of an unsuccessful habitat for the toads that spawned the first wave. According to Barandun & Reyer (1998), toads show site fidelity within and between years if the reproduction is successful, but they can disperse if the habitat becomes unpredictable. Our conclusions about consequences of hydroperiod predictability are consistent with their study. We noticed that before the vandalism, we had the same toad (TEP013) in one of the buckets that were removed (TE10) ten times out of twelve recaptures between June 2012 and July 2013 (83%). We never saw this toad again. We cannot rule out the possibility that it died, but as this individual was not the only one that disappeared after such an event, it may be linked.

As explained in different parts of this thesis, the site of PLA was sometimes removed from some analyses, especially when we were investigating a global trend on Geneva. Indeed, PLA is a site that presents particular conditions with a human-made retention tank and a long deep concrete canal. Surprisingly, it is the biggest site in Geneva with 433 identified individuals and half of the observations of juveniles (46%). The weight of the observations conducted there was thus very important, and when we noticed that it countered the other trends, we removed it to see the global average.

Finally, concerning the genetic part, we first had problems with lost samples before they were analysed. Then we had trouble with the amplification of some loci in some populations. Indeed, it appeared that entire plates were badly genotyped, and as individuals from the same populations were grouped on a plate, we had missing values

for some loci in a whole population. It considerably reduced the number of data that could be used in the genetic analyses, and the pertinence of the results including many missing data. As explained, we intend on running again the genetic analyses to improve the quality of the data and add some more individuals, as the field work continued up to fall 2016.

iv. *Guidelines for the conservation of an endangered amphibian species*

Bombina variegata is an endangered amphibian species in some areas, which needs to have access to water. In a conservation perspective, we need to protect the ponds that are already in place and to restore the ones that do not retain water anymore (Schmidt et al., 2015). Lots of semi-natural ponds became dry during the study, because of the heat. The problem with such dryness events is that they cause the bottom of the pond to crack, allowing seeds to go settle and, as soon as it rains, the seeds start to grow. Their roots make the soil crack even more, rendering the pond useless for the toads. When creating new habitats, the bottom of ponds must be properly made waterproof with clay or any other geotextile to insure good water retention. Here, we need to note that the diminution of the water level is not always bad. Indeed, it limits the development of pests, such as predatory insects. However, the water must not disappear completely to prevent any cracks from appearing. A new type of ponds was recently tested in Geneva: the ponds with outlet, or Lippuner ponds, called after a Swiss amphibian specialists, Mario Lippuner. The particularity of this pond is to be drainable thanks to an evacuation pipe. This new pond was especially designed to promote the conservation of the Natterjack toad (*Bufo calamita*), but BOVA is also promoted by this kind of ponds (Lippuner, 2013a; Lippuner, 2013b). Thus, more of them should be created in Geneva to help the local populations. Indeed, this solution is a good trade-off to remove the pests without risking the damage of the ponds due to the germination of seeds (for a complete explanation of the construction of this kind of pond, see KARCH website).

We explained that new areas were found during the study. Some of them were very important and the number of individuals found in these new places had a considerable impact on the population size of the whole site. In other places, no breeding indices were observed, but new juveniles gradually arrived in the prospected area. More field work must thus be conducted to find these non-monitored sites and to have a better idea of the breeding rate and the dispersion network. Moreover, some sites in Geneva are close to the border with France. As the genetic results showed, these populations seem to have a different genetic diversity that might be due to French migrant toads. A collaboration with French research units should take place to improve the knowledge on toads' dispersion across the border and to inspect the resistance of the landscape on the other side of the border to see if such a dispersion is possible.

The landscape fragmentation in Geneva is quite high and prevents the toads from moving out of the area they are currently occupying. A way to help them colonizing

new sites would be to create more ponds, to expand the favourable territory, and to create more connections between sites. If there are obstacles on the way, larger works must be conducted, such as the creation of wildlife passageways, to limit the mortality risks linked to road traffic. Forests are used by the amphibians to feed and rest (Rothermel & Semlitsch, 2002), but the undergrowth of forests is often too dense for the toads to pass freely through. A way to counter that can be to create conservation measures along forest paths. Indeed, paths are more accessible for these small amphibians, and as they are still covered by the canopy, the animals can travel without risking any desiccation.

Finally, we suggest to keep on collecting DNA samples to have a long-term monitoring of the genetic diversity. For now, the gene flow seems well widespread, but the positive impacts of recently implemented conservation measures as much as the negative effects of recent new infrastructure buildings are still hidden by the generation length of the toads. Moreover, as some sites are in the center of areas through which toads can move to disperse and breed, they must be conserved as a priority, because they represent a crucial link between separated areas. If this connection disappears, the toads will not be able to move as far as they used to do, and the genetic diversity may decrease, endangering the smaller populations. With a continuous survey, we would be able to notice if the genetic differentiation becomes critical or if, thanks to conservation management, it is reduced. In this case, the future of *Bombina variegata* would be insured in the Geneva area and the populations would then participate to the increase of the effectives in Switzerland, maybe leading to a change of its local endangered status.

VIII. CONCLUSION

The Yellow-bellied toad (*Bombina variegata*) is one of the most threatened amphibian species in Switzerland. Like most aquatic species, it suffers from habitat destruction, which is linked to urbanisation and landscape fragmentation, and the rapidity of their decline is alarming and much faster than planned. Geneva is an interesting canton, because the expansion of its city was important during the last decades, pushing aside natural habitats and endangering wildlife. We thus chose it to conduct a study on natural populations of *Bombina variegata* to determine the real risks the toads are exposed to and to fight against these threats to minimize their impact on the future of this animals. Thanks to the installation of artificial conservation measures, the populations globally increased over the last five years. The main factor that need to be considered is the water availability. Toads need to have access to filled ponds during the whole breeding season, even in case of unexpected heat waves. If a pond dries out, the animals need to be able to find another one close by. The problem with amphibians is that they have a low mobility and thus a low dispersal rate, which implies that they cannot connect two areas if the distance between them is great. The habitat map of Geneva is an important improvement for the study of wildlife conservation because it allows the researchers to analyse the spatial distribution of the obstacles that might prevent the migration of the animals. In Geneva, two sectors show good connections between sites, but further field work should take place to find new populations in the surroundings. This concerns especially the sites located close to the border, so more prospection should also take place on the French ground. These potentially new sites could improve the knowledge of the gene flows and show us that there is enough movements to prevent a loss of genetic variability. For now, the Genevan populations do not seem to be affected by this problem, as the genetic differentiation is still moderate. However, it is of main concern to maintain corridors, and even create new connections thanks to wildlife passages through the canton to be sure that the situation does not become critical for *Bombina variegata*. Populations should then be surveyed in a genetic way as much as in an ethological way in the next years to monitor the long-term impact of the conservation measures that started during this study. The increase of Geneva populations' effectives is already cheerful and if this trends keeps on going up, the management procedure proposed in this work could be transposed to other areas to improve the health of other locally threatened populations.

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LIST OF MONITORED SITES PER YEAR

Site	2012	2013	2014	2015	2016	AD
ARA	X	X	X	X	X	X (2010)
BAR		X	X	*		
BOU		X	X	X	X	
CAR		X	X	X	*	
CHA		X	X	X	X	
CHG		X	**			
COL	X	X	X	***		
COR	X	X	X	***		
COU		X	X	***		
DOL		X	X	X	X	
EPE		X	X	***		
JUS		X	X	X	****	
MAT		X	**			
PEN	X	X	X	X	****	
PLA	X	X		X	****	X (2014)
REP						X (2015)
ROM		X	**			
ROU		X	X	X	X	
TEP	X	X	X	X	X	
TOU	X	X	X	X	X	
VED (FR)						X (2015)
VER	X	X	X	****		
VEZ (FR)		X	X	****		
VIR (FR)						X (2015)

AD: Anecdotal data. *: viable population, extended area, difficult recapture. **: extinct population. ***: end of COGEFé project. ****: viable population and/or insufficient funding.

TYPES OF MEASURES IN EACH SITE

PA: Prospection Area. ECA: Emergency Conservation Area.

Type I: Artificial measures. Type II: Semi-natural measures. Type III: Natural measures

Site	PA/ ECA	Type I	Type II	Type III
ARA	ECA	X	X	X
BAR	ECA/PA	X	X	X
BOU	PA	X	X	X
CAR	ECA		X	X
CHA	PA	X	X	X
CHG	PA	X		X
COL	ECA	X		
COR	ECA	X		X
COU	ECA			
DOL	PA	X	X	X
EPE	ECA			X
JUS	PA	X		X
MAT	PA			X
PEN	ECA	X		
PLA	ECA	X		X
REP	PE			X
ROM	PA			X
ROU	ECA	X		X
TEP	ECA	X	X	X
TOU	PAS	X	X	X
VED	ECA		X	
VER	ECA	X		X
VEZ	PA			X
VIR	ECA	X		X

NUMBERS OF MEASURES IN EACH SITE

Type I: Artificial measures. Type II: Semi-natural measures. Type III: Natural measures

Site	2012	2013	2014	2015	2016
ARA	I: NA II: NA III: NA	I: 43 II: 7 III: 4	I: 44 II: 9 III: 6	I: 29 II: 49 III: 2	I: 19 II: 29 III: 1
BAR		I: 10 II: 24 III: 3	I: 10 II: 46 III: 1		
BOU		I: 35 II: 23 III: 1	I: 36 II: 24 III: 2	I: 35 II: 28 III: 2	I: 36 II: 28 III: 2
CAR		I: - II: 44 III: 4	I: - II: 44 III: 4	I: - II: 32 III: 1	
CHA		I: 27 II: 50 III: 16	I: 28 II: 25 III: 10	I: 14 II: 3 III: -	I: 14 II: 4 III: -
CHG		I: 10 II: 16 III: 9			
COL	I: NA II: NA III: NA	I: 10 II: - III: -	I: 11 II: - III: -		
COR	I: NA II: NA III: NA	I: 24 II: - III: -	I: 24 II: 24 III: -		
COU		I: 11 II: - III: -	I: 11 II: - III: -		
DOL		I: - II: 41 III: -	I: - II: 48 III: -	I: 12 II: 36 III: -	I: 12 II: 36 III: -
EPE		I: 13 II: 22 III: -	I: 13 II: 22 III: -		
JUS		I: 47 II: 12 III: 19	I: 50 II: 10 III: 11	I: 41 II: 3 III: 2	

Site	2012	2013	2014	2015	2016
MAT		I: - II: 14 III: 3			
PEN	I: NA II: NA III: NA	I: 10 II: - III: -	I: 10 II: - III: -	I: 15 II: 2 III: -	
PLA	I: NA II: NA III: -	I: 10 II: 2 III: -	I: 8 II: 2 III: -	I: 8 II: 2 III: -	
REP				I: - II: 14 III: 3	
ROM		I: - II: 6 III: 27			
ROU		I: 31 II: 13 III: 19	I: 30 II: 7 III: 4	I: 25 II: 14 III: 2	I: 35 II: 15 III: 3
TEP	I: 40 II: 71 III: 1	I: 60 II: 72 III: 7	I: 71 II: 50 III: -	I: 86 II: 41 III: 1	I: 88 II: 44 III: 1
TOU	I: 10 II: - III: -	I: 10 II: - III: -	I: 10 II: 4 III: -	I: 1 II: 11 III: -	I: 5 II: 12 III: -
VED (FR)				I: - II: 3 III: -	
VEI				I: - II: 5 III: 3	
VER		I: 59 II: 23 III: 40	I: 59 II: 28 III: 43		
VEZ (FR)		I: - II: 13 III: 8			
VIR (FR)				I: 2 II: 11 III: -	

PROTOCOL FOR DATA COLLECTION (IN FRENCH)

Cette marche à suivre reprend les principales étapes du protocole A, mais présente également les données supplémentaires à ajouter afin de collecter de manière exhaustive toutes les données pouvant être utilisées pour étudier le Crapaud sonneur.

- **Matériel**

Le matériel nécessaire pour une sortie sur le terrain est le suivant:

- 1) un gilet réfléchissant (jaune, vert, DGNP, etc...)
- 2) des bottes en caoutchouc
- 3) une lampe de poche et une lampe frontale (et des piles de rechange)
- 4) une époussette
- 5) un exemplaire du fichier excel de collecte de données (*Annexe: Saisie_Suivi_Complet*) et un crayon
- 6) un appareil photo (et une batterie de rechange si besoin)
- 7) un papier gradué plastifié
- 8) des écouillons en suffisance et un marqueur indélébile
- 9) une série des photos des individus du site déjà capturés (demande à exposer à Emilie Tournier qui fournira les documents actualisés au fur et à mesure des captures)

- **Collecte de données**

La collecte de données se déroule **uniquement de nuit ou à la tombée du jour**. Elle comporte plusieurs étapes, comme suit, selon l'annexe *Saisie_Suivi_Complet*:

- 1) **Noter le nom du ou des observateur(s)**
- 2) **Noter la date et l'heure de début**
- 3) **Noter la météo:**
 - ciel clair
 - ciel nuageux
 - pluie
 - vent
 - ou toute combinaison des exemples précédents
- 4) **Noter la température**

(si vous ne disposez pas de thermomètre digital (voiture, téléphone ou autre), vous pouvez remplir cette donnée avant de partir sur le terrain ou en rentrant, en consultant sur internet: http://www.meteosuisse.admin.ch/web/fr/meteo/temps_actuel.html)
- 5) **Vérifier les points d'eau**

Si vous avez un site où les bacs et les points d'eau naturels sont nombreux, il est important d'inverser le sens des vérifications à chaque sortie en faisant attention à ne pas mélanger le nom des différents points d'eau.

En passant d'un point d'eau à un autre, il est important de vérifier le sol alentour afin de trouver les individus hors des gouilles et de ne pas les écraser.

A chaque point d'eau, il faut prendre les données suivantes:

- *quantité d'eau dans le point d'eau:*
 - 100 = plein à 100% de la capacité
 - 75 = plein à 75%
 - 50 = plein à 50%
 - 25 = plein à 25%
 - 15 = plein à 15%
 - 1 = quelques centimètres d'eau
 - 0 = vide
- *qualité de l'eau dans le point d'eau:*
 - 0 = eau claire, fond du point d'eau visible
 - 1 = eau relativement claire, fond non visible
 - 2 = eau très trouble, seuls les premiers centimètres du point d'eau sont visibles
- *présence de débris végétaux:*
 - 1 = oui
 - 0 = non
- *présence d'algues:*
 - 1 = oui
 - 0 = non
- *présence de prédateurs:*
 - 1 ou 0 pour larves de libellules
 - 1 ou 0 pour notonectes
 - 1 ou 0 pour dytiques
- *présence d'amphibiens:*
 - hors du point d'eau, dans un rayon de 100m
 - dans ou en bordure proche du point d'eau (0-5m)

Pour toute rencontre, il faut remplir le tableau en donnant les indications sur:

→ **l'espèce observée** (BOVA: *Bombina variegata*, RADA: *Rana dalmatina*, RATE: *Rana temporaria*, RARI: *Rana ridibunda*, BUBU: *Bufo bufo*, BUCA: *Bufo calamita*, SASA: *Salamandra salamandra*, TRAL: *Triturus alpestris*, TRHE: *Triturus helveticus*, TRCX: *Triturus carnifex*)

→ **le nombre d'individus de chaque stade**

A: adulte

S : subadulte

J: juvénile dès disparition de la queue

T+: têtard de gros diamètre dès l'apparition des pattes avant

T-: têtard de gros diamètre jusqu'à apparition des pattes arrières

P: têtard dès la sortie de l'œuf jusqu'à un petit diamètre (quelques millimètres) de type "tête d'épingle")

O: œufs

- *présence de Crapaud sonneur:* Si un individu sonneur est observé, il faut impérativement:

- attraper l'individu sans le blesser
- le saisir par les pattes arrières et le poser sur le dos sur le papier gradué
- prendre une photo nette de son ventre en suivant rigoureusement les instructions (voir [Annexe: Protocole_Photos](#))
- comparer les marques sur son ventre aux photos des individus déjà échantillonnés
- s'il n'y apparaît pas: prendre un échantillon buccal d'ADN en suivant rigoureusement les indications (voir [Annexe: Protocole_ADN](#))
- noter sur l'écouvillon: la date, l'heure et le code du point d'eau
- noter sur l'écouvillon: le nom de l'individu (si l'individu était inconnu jusque là, le nommer avec le numéro suivant le dernier sur la liste des individus déjà connus) et son sexe

- relâcher l'individu au même endroit que sa capture (si plusieurs crapauds sont au même endroit, il faut prévoir un récipient pour les garder de côté le temps de les avoir tous capturés)
- inscrire dans le tableau (voir *Annexe: Donnees_Sonneurs*) le code du plan d'eau (tous les noms se trouvent sur des piquets plantés à côté de chaque plan d'eau), le numéro de la photo ainsi que le nom du crapaud et son sexe et cocher si la prise d'ADN a été effectuée

6) Noter l'heure de fin

- **Transfert de fichiers**

De retour du terrain, il faut préparer les données à transmettre.

- 1) Mettre les échantillons ADN à sécher en ouvrant légèrement le bouchon pendant 30 minutes
- 2) Mettre les échantillons ADN dans le congélateur après avoir bien refermé les bouchons
- 3) Retranscrire informatiquement les résultats de la feuille de collecte de données et de la feuille de données Sonneurs
- 4) Envoyer les documents et les photos prises lors de la sortie (non redimensionnées) par mail à etournier@naries.ch
- 5) Préciser dans le mail combien d'échantillon ADN ont été pris, afin que Emilie Tournier puisse vous contacter pour les récupérer.

En cas de questions sur la méthodologie ou sur le suivi, veuillez contacter Emilie Tournier:

etournier@naries.ch

076/396.97.40

PROTOCOL FOR PHOTO-IDENTIFICATION (IN FRENCH)

Cette marche à suivre est obligatoire, afin de normaliser toutes les photographies des ventres des Crapauds sonneurs de manière à faciliter la reconnaissance d'individus déjà échantillonnés.

- **Matériel**

Le matériel nécessaire pour prendre un bon cliché est le suivant:

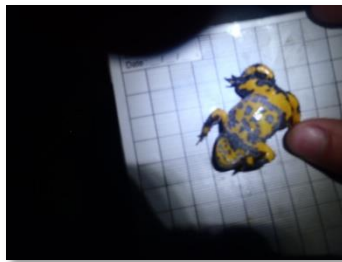
- 1) un papier gradué plastifié
- 2) un appareil photo avec un mode macro (si le mode macro n'est pas disponible, faire d'autant plus attention à la netteté)
- 3) une lampe de poche ou une lampe frontale

- **Position du Crapaud sonneur**

- 1) Afin de pouvoir examiner au mieux les taches ventrales, il faut impérativement que la photographie soit nette et proche (la disposition "portrait" convient mieux).



trop loin: **NON!**



flou: **NON!**



proche et net: **OK!**

- 2) Afin d'assurer une position optimale du Crapaud sonneur, il faut le maintenir par les pattes arrières, sans cacher les premières taches de son bas ventre.



bas du ventre caché: **NON!**



bas du ventre caché: **NON!**

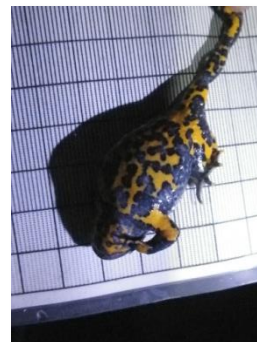


bas du ventre visible: **OK!**

- 3) Afin de pouvoir distinguer au mieux les taches ventrales, il faut que le Crapaud sonneur soit parfaitement plat et sur le dos.



crapaud tordu: **NON!**
EMILIE TOURNIER



crapaud tordu et dans l'ombre: **NON!**



crapaud plat et allongé: **OK!**
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4) Afin de pouvoir distinguer au mieux les taches ventrales, il faut faire attention au reflet du flash sur le ventre du Crapaud sonneur.



crapaud flou, loin et reflet au milieu du ventre: NON!



crapaud surexposé: NON!



pas de reflets, taches visibles: OK!

5) Afin de pouvoir reconnaître au mieux les individus, il faut que les taches de l'entrejambe soient bien visibles.



cuisse invisibles: NON!



une cuisse invisible: NON!



taches sur les cuisses bien visibles: OK!

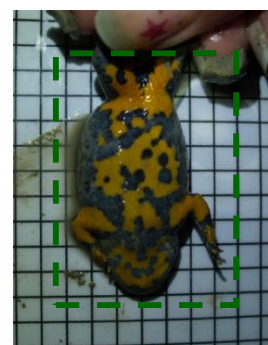
6) Afin de pouvoir rogner les images et faire des cartes de visites normalisées, il faut placer le Crapaud sonneur au milieu du papier gradué et centrer la photo. En outre, pour pouvoir le mesurer, le crapaud doit être dans l'axe des quadrillages.



crapaud en diagonale, impossible à mesurer: NON!



crapaud recroquevillé et impossible à recadrer: NON!



rognage de photo possible: OK!

D'une manière générale, il est toujours plus sûr de faire deux clichés du même individu. En outre, grâce à l'ère numérique, un rapide contrôle de la photographie permet immédiatement de savoir si le cliché est bon ou pas. Il ne faut donc pas hésiter à en refaire un si des doutes subsistent quant à sa qualité!

PROTOCOL FOR DNA SAMPLING (IN FRENCH)

Cette marche à suivre est obligatoire, afin d'assurer la validité des échantillons d'ADN, en restant une méthode non intrusive.

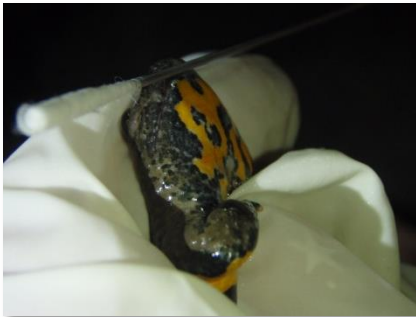
- **Matériel**

Le matériel nécessaire pour prendre un échantillon d'ADN buccal est le suivant:

- 1) des gants en plastique à usage unique
- 2) des écouvillons stériles de 2mm de diamètre
- 3) un marqueur indélébile

- **Marche à suivre**

- 1) Afin de ne pas contaminer l'échantillonnage, il faut impérativement mettre des gants en plastique avant toute manipulation de crapaud, ainsi que les changer entre chaque crapaud.
- 2) Afin de pouvoir échantillonner l'ADN d'un Crapaud sonneur, il faut lui prendre la tête entre le pouce et l'index en faisant passer les pattes avant sous le pouce et en maintenant **doucement** la tête grâce à l'index.
- 3) Lorsque le Crapaud sonneur est bien immobilisé, il faut ouvrir le tube de l'écouvillon (en étant seul, il faut prévoir cette étape avant de manipuler le crapaud).
- 4) Il faut alors faire ouvrir la bouche du crapaud. La meilleure méthode est de partir de la partie métallique en la faisant rouler légèrement d'avant en arrière et la faisant glisser contre son museau. Cette étape peut demander une certaine dose de patience... Dès que la bouche s'ouvre, il faut **rapidement et délicatement** insérer la pointe de l'écouvillon dans la bouche du Crapaud sonneur, sans ne toucher rien d'autre que ledit crapaud et ladite bouche. Lorsque l'écouvillon est dans la bouche du Crapaud sonneur, il faut frotter l'intérieur de la bouche doucement en tournant l'écouvillon à plusieurs reprises, puis retirer l'écouvillon sans brutaliser le Crapaud qui, souvent, mord l'écouvillon.



Immobilisation du Crapaud sonneur



Prise d'ADN buccal

- 5) Finalement, il faut remettre l'écouvillon dans son tube, en faisant attention à ce que la partie en coton ne touche rien, bien le refermer et inscrire le nom du Crapaud sonneur, la date, l'heure et le site de capture.

En cas de grande affluence de Crapauds sonneurs en une sortie, et même dans tous les cas, il peut être utile de prendre une photo du ventre du crapaud, puis de l'étiquette de l'écouvillon renommée avant de passer à un autre individu, de manière à pouvoir s'assurer de la concordance des photos des individus et de leur échantillon et à éviter toute confusion.

EXAMPLE OF CATALOGUE OF PHOTO-ID

Code for each toad

Date of first capture

Pond of first capture



Appendix C2 (see p. 55)

BINOMIAL TEST: SEX-RATIO'S DIFFERENCES AMONG ALL SITES

Captured events

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	total	1471	975	9.861849e-24	0.60139	0.5816705	0.6208664	TRUE

GVA

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2010	2	2	1.00000000	0.5000000	0.06758599	0.9324140	FALSE
2	2012	48	37	0.277999289	0.5647059	0.45277350	0.6719978	FALSE
3	2013	196	149	0.013151284	0.5681159	0.51400167	0.6210509	TRUE
4	2014	159	98	0.000170729	0.6186770	0.55625489	0.6783279	TRUE
5	2015	274	225	0.031549136	0.5490982	0.50425668	0.5933547	TRUE
6	2016	52	50	0.921191049	0.5098039	0.40887503	0.6101488	FALSE
7	total	492	436	0.070944883	0.5301724	0.49747017	0.5626826	FALSE

ARA

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2010	2	2	1.00000000	0.5000000	0.06758599	0.9324140	FALSE
2	2012	7	3	0.34375000	0.7000000	0.34754715	0.9332605	FALSE
3	2013	5	3	0.72656250	0.6250000	0.24486322	0.9147666	FALSE
4	2014	9	5	0.42395020	0.6428571	0.35138011	0.8724016	FALSE
5	2015	15	8	0.21003962	0.6521739	0.42734396	0.8362364	FALSE
6	2016	15	5	0.04138947	0.7500000	0.50895413	0.9134285	TRUE
7	total	27	16	0.12628947	0.6279070	0.46725095	0.7702483	FALSE

BAR

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	15	5	0.04138947	0.7500000	0.5089541	0.9134285	TRUE
2	2014	14	6	0.11531830	0.7000000	0.4572108	0.8810684	FALSE
3	total	24	11	0.04095959	0.6857143	0.5071200	0.8314828	TRUE

BOU

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	2	2	1.0000000	0.50	0.067585986	0.9324140	FALSE
2	2014	1	3	0.6250000	0.25	0.006309463	0.8058796	FALSE
3	2015	1	4	0.3750000	0.20	0.005050763	0.7164179	FALSE
4	2016	1	3	0.6250000	0.25	0.006309463	0.8058796	FALSE
5	total	2	8	0.109375	0.20	0.025210726	0.5560955	FALSE

CAR

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	7	9	0.8036194	0.4375000	0.1975341	0.7012231	FALSE
2	2014	13	7	0.2631760	0.6500000	0.4078115	0.8460908	FALSE
3	2015	22	17	0.5223974	0.5641026	0.3962080	0.7218924	FALSE
4	total	33	27	0.5189580	0.5500000	0.4161191	0.6787785	FALSE

CHA

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2014	1	0	1	1.0	0.02500000	1.0000000	FALSE
2	2015	1	1	1	0.5	0.01257912	0.9874209	FALSE
3	2016	1	0	1	1.0	0.02500000	1.0000000	FALSE
4	total	1	1	1	0.5	0.01257912	0.9874209	FALSE

COL

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2012	10	8	0.8145294	0.5555556	0.3075717	0.7846985	FALSE
2	2013	8	5	0.5810547	0.6153846	0.3157776	0.8614207	FALSE
3	2014	7	3	0.3437500	0.7000000	0.3475471	0.9332605	FALSE
4	total	12	10	0.8318119	0.5454545	0.3221048	0.7561381	FALSE

COR

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2012	8	0	0.007812500	1.0000000	0.6305834	1.0000000	TRUE
2	2013	19	4	0.002599478	0.8260870	0.6121881	0.9504924	TRUE
3	2014	15	3	0.007537842	0.8333333	0.5858225	0.9642149	TRUE
4	total	23	6	0.002315700	0.7931034	0.6027531	0.9200582	TRUE

COU

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	3	2	1	0.6	0.1466328	0.947255	FALSE
2	2014	3	2	1	0.6	0.1466328	0.947255	FALSE
3	total	3	2	1	0.6	0.1466328	0.947255	FALSE

DOL

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	0	1	1	0.0	0.0000000	0.9750000	FALSE
2	2014	1	1	1	0.5	0.01257912	0.9874209	FALSE
3	2015	1	0	1	1.0	0.02500000	1.0000000	FALSE
4	2016	1	0	1	1.0	0.02500000	1.0000000	FALSE
5	total	2	2	1	0.5	0.06758599	0.9324140	FALSE

EPE

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	6	2	0.2890625	0.7500000	0.3491442	0.9681460	FALSE
2	2014	6	1	0.1250000	0.8571429	0.4212768	0.9963897	FALSE
3	total	7	2	0.1796875	0.7777778	0.3999064	0.9718550	FALSE

JUS

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	5	0	0.0625000	1.000	0.4781762	1.0000000	FALSE
2	2014	5	3	0.7265625	0.625	0.2448632	0.9147666	FALSE
3	2015	5	5	1.0000000	0.500	0.1870860	0.8129140	FALSE
4	total	9	6	0.6072388	0.600	0.3228698	0.8366357	FALSE

PEN

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2012	8	4	0.387695312	0.6666667	0.3488755	0.9007539	FALSE
2	2013	11	4	0.118469238	0.7333333	0.4489968	0.9221285	FALSE
3	2014	16	3	0.004425049	0.8421053	0.6042154	0.9661738	TRUE
4	2015	20	8	0.035698138	0.7142857	0.5133317	0.8677635	TRUE
5	total	25	17	0.279956239	0.5952381	0.4328209	0.7437091	FALSE

PLA

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2012	4	9	0.2668457	0.3076923	0.09092039	0.6142617	FALSE
2	2013	70	63	0.6030464	0.5263158	0.43794757	0.6134864	FALSE
3	2014	3	4	1.0000000	0.4285714	0.09898828	0.8159484	FALSE
4	2015	134	97	0.0176635	0.5800866	0.51358988	0.6445008	TRUE
5	total	188	163	0.2001166	0.5356125	0.48190116	0.5887182	FALSE

REP

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2015	1	1	1	0.5	0.01257912	0.9874209	FALSE
2	total	1	1	1	0.5	0.01257912	0.9874209	FALSE

ROU

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	20	21	1.0000000	0.4878049	0.3287790	0.6486576	FALSE
2	2014	19	18	1.0000000	0.5135135	0.3439968	0.6807859	FALSE
3	2015	15	12	0.7011080	0.5555556	0.3532642	0.7452012	FALSE
4	2016	16	13	0.7110711	0.5517241	0.3569387	0.7355447	FALSE
5	total	29	39	0.2749904	0.4264706	0.3072261	0.5523282	FALSE

TEP

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2012	11	12	1.0000000	0.4782609	0.2681962	0.6941220	FALSE
2	2013	18	24	0.4407991	0.4285714	0.2772067	0.5903887	FALSE
3	2014	21	25	0.6587381	0.4565217	0.3089794	0.6098663	FALSE
4	2015	27	28	1.0000000	0.4909091	0.3535362	0.6292947	FALSE
5	2016	18	27	0.2326932	0.4000000	0.2569759	0.5566865	FALSE
6	total	46	62	0.1485763	0.4259259	0.3312690	0.5247496	FALSE

TOU

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2012	0	1	1.0000000	0.0000000	0.000000000	0.9750000	FALSE
2	2013	1	1	1.0000000	0.5000000	0.012579117	0.9874209	FALSE
3	2014	0	3	0.2500000	0.0000000	0.000000000	0.7075982	FALSE
4	2015	1	2	1.0000000	0.3333333	0.008403759	0.9057007	FALSE
5	2016	0	2	0.5000000	0.0000000	0.000000000	0.8418861	FALSE
6	total	2	5	0.453125	0.2857143	0.036692566	0.7095791	FALSE

VED

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2014	3	2	1	0.6000000	0.1466328	0.9472550	FALSE
2	2015	15	15	1	0.5000000	0.3129703	0.6870297	FALSE
3	total	17	16	1	0.5151515	0.3354445	0.6920366	FALSE

VER

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	5	1	0.2187500	0.8333333	0.3587654	0.9957893	FALSE
2	2014	22	9	0.02944937	0.7096774	0.5196393	0.8577715	TRUE
3	total	24	13	0.09887175	0.6486486	0.4746113	0.7979002	FALSE

VEZ

	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2013	1	2	1	0.3333333	0.008403759	0.9057007	FALSE
2	total	1	2	1	0.3333333	0.008403759	0.9057007	FALSE

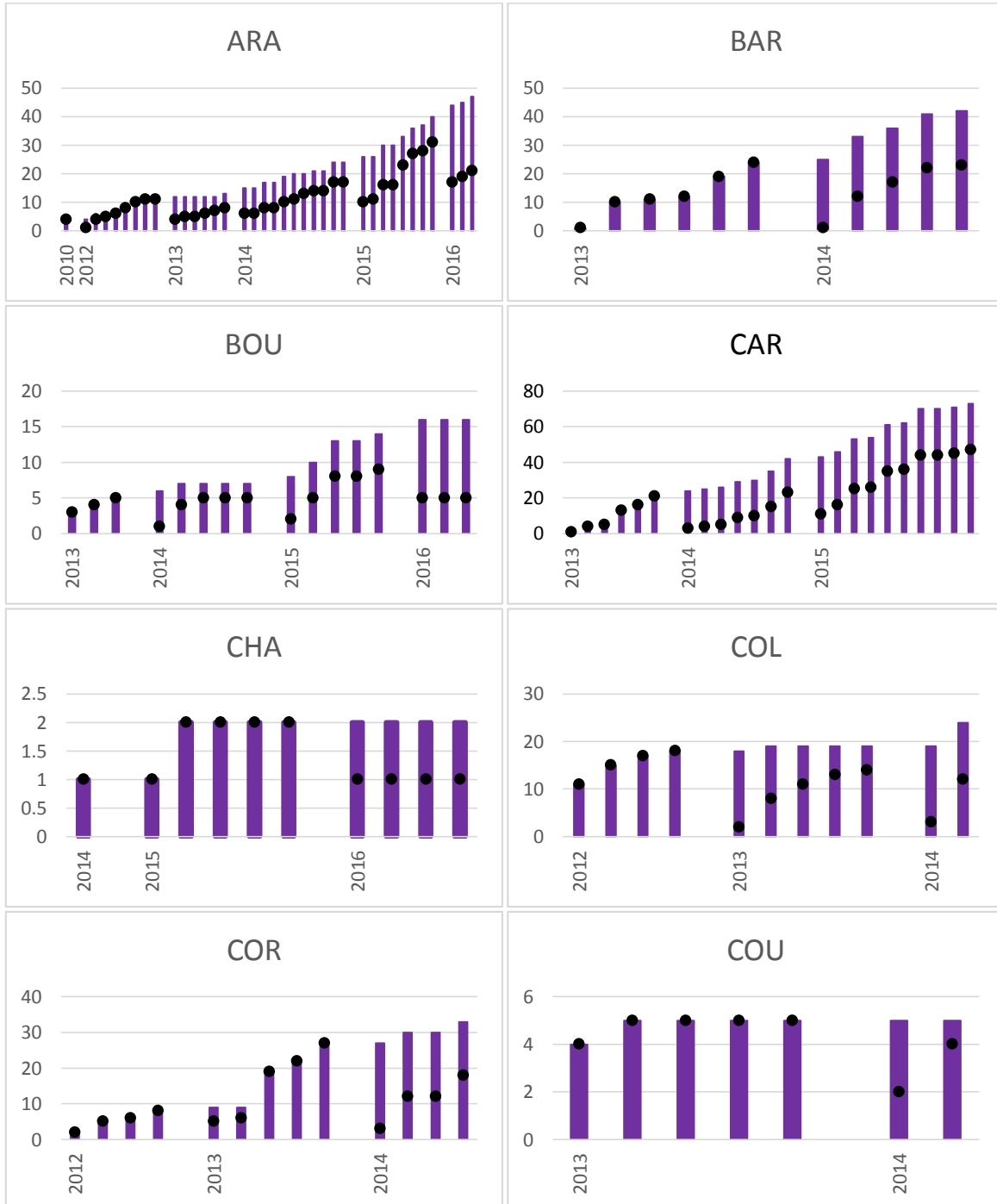
VIR

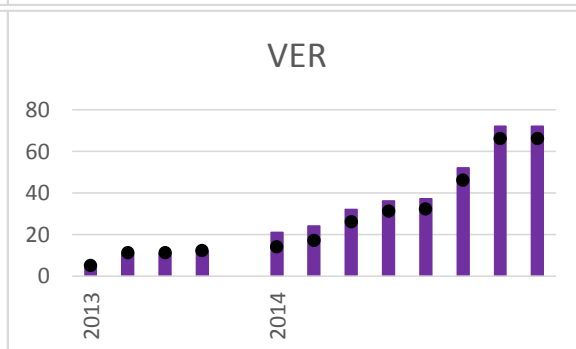
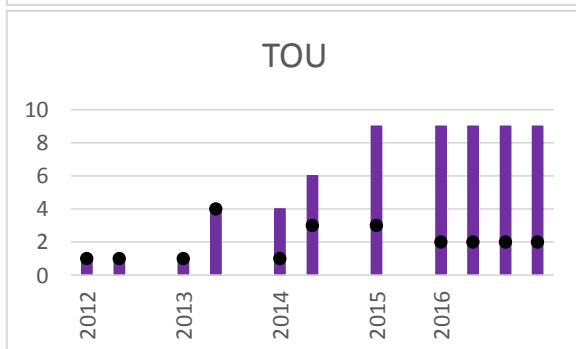
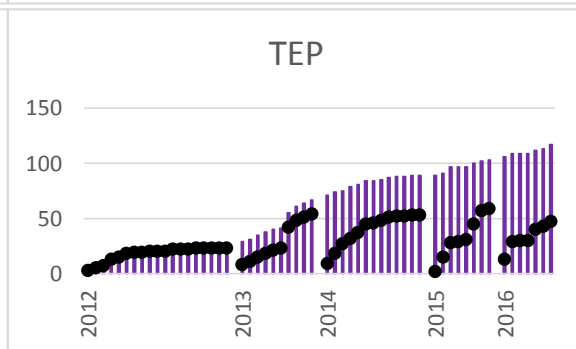
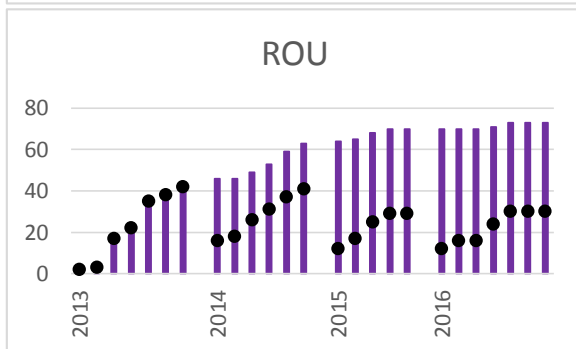
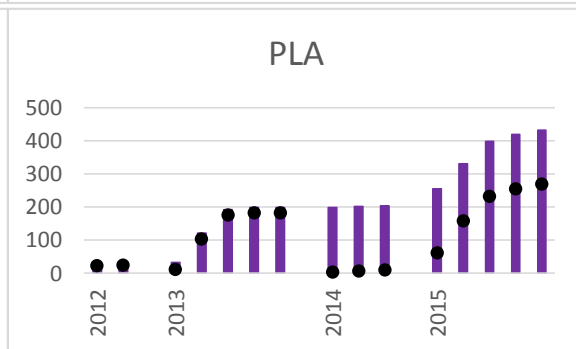
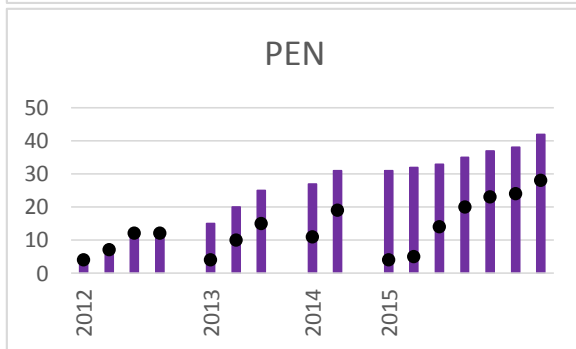
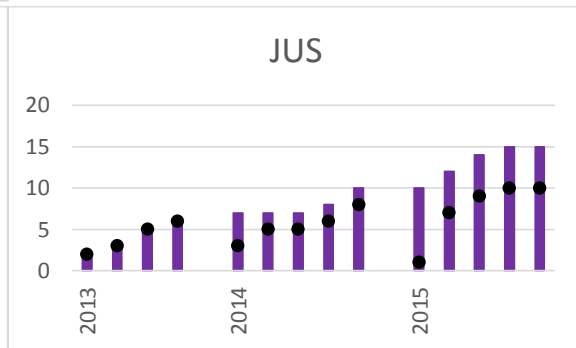
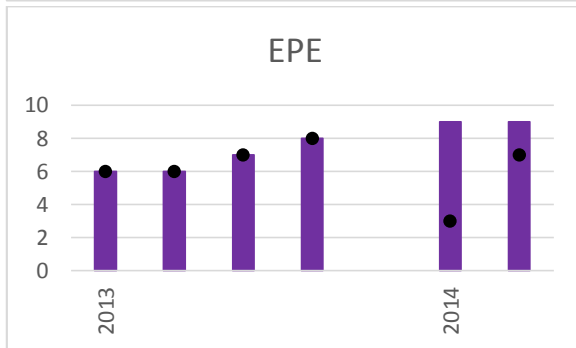
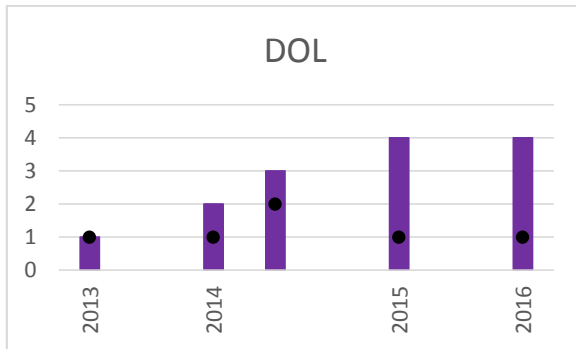
	year	males	females	pval	probaMale	confProbInf	confProbSup	isDifferent
1	2015	16	27	0.1262895	0.372093	0.2297517	0.5327491	FALSE
2	total	16	27	0.1262895	0.372093	0.2297517	0.5327491	FALSE

CPS AND OPS GRAPHS FOR EACH SITE

OPS 2012-2016

CPS for each year





EXTRACT OF MODEL AVERAGING - CJS MODEL "GROUP"

Model	Apparent Survival Parameter (Phi) Group 1	Parameter 1	Weight	Estimate	Standard Error
{Phi(g*t),p(g)}			0.74659	0.7424637	0.0745151
{Phi(g*t),p(g*t)}			0.25338	0.6644734	0.0769042
{Phi(g),p(g*t)}			0.00003	0.5710346	0.0288158
{Phi(t),p(g*t)}			0.00000	0.6774128	0.0510518
Weighted Average				0.7226975	0.0751192
Unconditional SE					0.0824336
95% CI for wgt. Ave. Est. (logit trans.) is 0.5378446 to 0.8537218					
Percent of Variation Attributable to Model Variation is 16.96%					

Model	Apparent Survival Parameter (Phi) Group 1	Parameter 2	Weight	Estimate	Standard Error
{Phi(g*t),p(g)}			0.74659	0.4858620	0.0345810
{Phi(g*t),p(g*t)}			0.25338	0.5250425	0.0428197
{Phi(g),p(g*t)}			0.00003	0.5710346	0.0288158
{Phi(t),p(g*t)}			0.00000	0.5724483	0.0325011
Weighted Average				0.4957923	0.0366684
Unconditional SE					0.0405955
95% CI for wgt. Ave. Est. (logit trans.) is 0.4169948 to 0.5747994					
Percent of Variation Attributable to Model Variation is 18.41%					

Model	Apparent Survival Parameter (Phi) Group 1	Parameter 3	Weight	Estimate	Standard Error
{Phi(g*t),p(g)}			0.74659	0.6708585	0.0488970
{Phi(g*t),p(g*t)}			0.25338	0.5947254	0.0708347
{Phi(g),p(g*t)}			0.00003	0.5710346	0.0288158
{Phi(t),p(g*t)}			0.00000	0.7908175	0.0470970
Weighted Average				0.6515655	0.0544552
Unconditional SE					0.0644454
95% CI for wgt. Ave. Est. (logit trans.) is 0.5173809 to 0.7653619					
Percent of Variation Attributable to Model Variation is 28.60%					

Model	Apparent Survival Parameter (Phi) Group 1	Parameter 4	Weight	Estimate	Standard Error
{Phi(g*t),p(g)}			0.74659	0.2301053	0.0265661
{Phi(g*t),p(g*t)}			0.25338	0.6318215	120.8822700
{Phi(g),p(g*t)}			0.00003	0.5710346	0.0288158
{Phi(t),p(g*t)}			0.00000	0.7644528	0.0000000
Invalid parameter estimate with zero SE?					
Weighted Average				0.3319051	30.6495856
Unconditional SE					60.8491979
95% CI for wgt. Ave. Est. (logit trans.) is 0.0000000 to 1.0000000					
Percent of Variation Attributable to Model Variation is 74.63%					

EXTRACT OF MODEL AVERAGING - CJS MODEL "AGE"

Apparent Survival Parameter (Phi) Adults-subadults Parameter 1

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.6858911	0.0556166
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5697535	0.0246299
{Phi(t),p(.)}	0.03537	0.7575376	0.0534691
{Phi(a*t),p(t)}	0.02068	0.6645311	0.0701933
{Phi(t),p(a*t)}	0.01939	0.6865914	0.0557157
...
Weighted Average		0.6477475	0.0436157
Unconditional SE			0.0728324

95% CI for wgt. Ave. Est. (logit trans.) is 0.4958795 to 0.7746554
Percent of Variation Attributable to Model Variation is 64.14%

Apparent Survival Parameter (Phi) Adults-subadults Parameter 2

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.5307458	0.0314025
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5697535	0.0246299
{Phi(t),p(.)}	0.03537	0.4935739	0.0255597
{Phi(a*t),p(t)}	0.02068	0.5188517	0.0385890
{Phi(t),p(a*t)}	0.01939	0.5309678	0.0313917
...
Weighted Average		0.5465067	0.0280888
Unconditional SE			0.0381267

95% CI for wgt. Ave. Est. (logit trans.) is 0.4712926 to 0.6196560
Percent of Variation Attributable to Model Variation is 45.72%

Apparent Survival Parameter (Phi) Adults-subadults Parameter 3

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.6071586	0.0538634
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5697535	0.0246299
{Phi(t),p(.)}	0.03537	0.6878478	0.0368590
{Phi(a*t),p(t)}	0.02068	0.5887354	0.0579337
{Phi(t),p(a*t)}	0.01939	0.6078845	0.0537546
...
Weighted Average		0.5987998	0.0416849
Unconditional SE			0.0498384

95% CI for wgt. Ave. Est. (logit trans.) is 0.4984644 to 0.6914861
Percent of Variation Attributable to Model Variation is 30.04%

Apparent Survival Parameter (Phi) Adults-subadults Parameter 4

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.3613876	55.7735990
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5697535	0.0246299
{Phi(t),p(.)}	0.03537	0.2208192	0.0192863
{Phi(a*t),p(t)}	0.02068	0.7041806	0.0000000
...
Weighted Average		0.4409595	30.5252789
Unconditional SE			41.2553770

95% CI for wgt. Ave. Est. (logit trans.) is 0.0000000 to 1.0000000
Percent of Variation Attributable to Model Variation is 45.25%

Model	Apparent Survival Parameter (Phi) weight	Juveniles Parameter Estimate	11 Standard Error
{Phi(t),p(t)}	0.54714	0.6858911	0.0556166
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5977088	0.0266003
{Phi(t),p(.)}	0.03537	0.7575376	0.0534691
{Phi(a*t),p(t)}	0.02068	0.7089353	0.0732171
{Phi(t),p(a*t)}	0.01939	0.6865914	0.0557157
...
Weighted Average		0.6527192	0.0439680
Unconditional SE			0.0701063
95% CI for wgt. Ave. Est. (logit trans.) is 0.5062066 to 0.7750761			
Percent of Variation Attributable to Model Variation is 60.67%			

Model	Apparent Survival Parameter (Phi) weight	Juveniles Parameter Estimate	12 Standard Error
{Phi(t),p(t)}	0.54714	0.5307458	0.0314025
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5977088	0.0266003
{Phi(t),p(.)}	0.03537	0.4935739	0.0255597
{Phi(a*t),p(t)}	0.02068	0.5448108	0.0428343
{Phi(t),p(a*t)}	0.01939	0.5309678	0.0313917
...
Weighted Average		0.5509988	0.0284695
Unconditional SE			0.0414771
95% CI for wgt. Ave. Est. (logit trans.) is 0.4690665 to 0.6302540			
Percent of Variation Attributable to Model Variation is 52.89%			

Model	Apparent Survival Parameter (Phi) weight	Juveniles Parameter Estimate	13 Standard Error
{Phi(t),p(t)}	0.54714	0.6071586	0.0538634
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5977088	0.0266003
{Phi(t),p(.)}	0.03537	0.6878478	0.0368590
{Phi(a*t),p(t)}	0.02068	0.6331030	0.0659397
{Phi(t),p(a*t)}	0.01939	0.6078845	0.0537546
...
Weighted Average		0.6037760	0.0421657
Unconditional SE			0.0493298
95% CI for wgt. Ave. Est. (logit trans.) is 0.5042668 to 0.6953770			
Percent of Variation Attributable to Model variation is 26.94%			

Model	Apparent Survival Parameter (Phi) weight	Juveniles Parameter Estimate	14 Standard Error
{Phi(t),p(t)}	0.54714	0.3613876	55.7735990
{Phi(.),p(t)}	0.22624	0.5822238	0.0213734
{Phi(g),p(t)}	0.13536	0.5977088	0.0266003
{Phi(t),p(.)}	0.03537	0.2208192	0.0192863
{Phi(a*t),p(t)}	0.02068	0.6567476	0.0000000
...
Weighted Average		0.4437716	30.5867152
Unconditional SE			41.2962810
95% CI for wgt. Ave. Est. (logit trans.) is 0.0000000 to 1.0000000			
Percent of Variation Attributable to Model Variation is 45.14%			

Recapture Parameter (p) Adults-subadults Parameter 21

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.7046793	0.0607224
{Phi(.) ,p(t)}	0.22624	0.7638185	0.0483888
{Phi(g),p(t)}	0.13536	0.7638423	0.0483843
{Phi(t),p(.)}	0.03537	0.5665296	0.0317424
{Phi(a*t),p(t)}	0.02068	0.7053354	0.0606505
{Phi(t),p(a*t)}	0.01939	0.6918206	0.0789818
...
Weighted Average		0.7211498	0.0556006
Unconditional SE			0.0701132

95% CI for wgt. Ave. Est. (logit trans.) is 0.5663063 to 0.8366551
Percent of Variation Attributable to Model Variation is 37.11%

Recapture Parameter (p) Adults-subadults Parameter 22

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.5203028	0.0349653
{Phi(.) ,p(t)}	0.22624	0.4911157	0.0304592
{Phi(g),p(t)}	0.13536	0.4913664	0.0304607
{Phi(t),p(.)}	0.03537	0.5665296	0.0317424
{Phi(a*t),p(t)}	0.02068	0.5208782	0.0349644
{Phi(t),p(a*t)}	0.01939	0.5046544	0.0433568
...
Weighted Average		0.5108215	0.0334331
Unconditional SE			0.0381449

95% CI for wgt. Ave. Est. (logit trans.) is 0.4363709 to 0.5847950
Percent of Variation Attributable to Model Variation is 23.18%

Recapture Parameter (p) Adults-subadults Parameter 23

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.6507437	0.0562528
{Phi(.) ,p(t)}	0.22624	0.6554772	0.0428222
{Phi(g),p(t)}	0.13536	0.6562148	0.0428199
{Phi(t),p(.)}	0.03537	0.5665296	0.0317424
{Phi(a*t),p(t)}	0.02068	0.6506329	0.0562636
{Phi(t),p(a*t)}	0.01939	0.6230456	0.0612769
...
Weighted Average		0.6485876	0.0505116
Unconditional SE			0.0538447

95% CI for wgt. Ave. Est. (logit trans.) is 0.5373807 to 0.7457119
Percent of Variation Attributable to Model variation is 12.00%

Recapture Parameter (p) Adults-subadults Parameter 24

Model	weight	Estimate	Standard Error
{Phi(t),p(t)}	0.54714	0.3709177	57.2443900
{Phi(.) ,p(t)}	0.22624	0.2309476	0.0228841
{Phi(g),p(t)}	0.13536	0.2311173	0.0228859
{Phi(t),p(.)}	0.03537	0.5665296	0.0317424
{Phi(a*t),p(t)}	0.02068	0.1961305	0.0000000
...
Weighted Average		0.3276583	31.3306352
Unconditional SE			42.3432327

95% CI for wgt. Ave. Est. (logit trans.) is 0.0000000 to 1.0000000
Percent of Variation Attributable to Model Variation is 45.25%

Model	Recapture Parameter (p)	Juveniles Weight	Parameter 31 Estimate	Standard Error
{Phi(t),p(t)}		0.54714	0.7046793	0.0607224
{Phi(.),p(t)}		0.22624	0.7638185	0.0483888
{Phi(g),p(t)}		0.13536	0.7638423	0.0483843
{Phi(t),p(.)}		0.03537	0.6035494	0.0342260
{Phi(a*t),p(t)}		0.02068	0.7053354	0.0606505
{Phi(t),p(a*t)}		0.01939	0.7174496	0.0775004
...
Weighted Average			0.7231111	0.0556599
Unconditional SE				0.0674227
95% CI for wgt. Ave. Est. (logit trans.) is 0.5744266 to 0.8347894				
Percent of Variation Attributable to Model Variation is 31.85%				

Model	Recapture Parameter (p)	Juveniles Weight	Parameter 32 Estimate	Standard Error
{Phi(t),p(t)}		0.54714	0.5203028	0.0349653
{Phi(.),p(t)}		0.22624	0.4911157	0.0304592
{Phi(g),p(t)}		0.13536	0.4913664	0.0304607
{Phi(t),p(.)}		0.03537	0.6035494	0.0342260
{Phi(a*t),p(t)}		0.02068	0.5208782	0.0349644
{Phi(t),p(a*t)}		0.01939	0.5394783	0.0473204
...
Weighted Average			0.5132096	0.0336584
Unconditional SE				0.0408407
95% CI for wgt. Ave. Est. (logit trans.) is 0.4335052 to 0.5922479				
Percent of Variation Attributable to Model Variation is 32.08%				

Model	Recapture Parameter (p)	Juveniles Weight	Parameter 33 Estimate	Standard Error
{Phi(t),p(t)}		0.54714	0.6507437	0.0562528
{Phi(.),p(t)}		0.22624	0.6554772	0.0428222
{Phi(g),p(t)}		0.13536	0.6562148	0.0428199
{Phi(t),p(.)}		0.03537	0.6035494	0.0342260
{Phi(a*t),p(t)}		0.02068	0.6506329	0.0562636
{Phi(t),p(a*t)}		0.01939	0.6862783	0.0671824
...
Weighted Average			0.6517857	0.0508085
Unconditional SE				0.0527241
95% CI for wgt. Ave. Est. (logit trans.) is 0.5427903 to 0.7469129				
Percent of Variation Attributable to Model variation is 7.13%				

Model	Recapture Parameter (p)	Juveniles Weight	Parameter 34 Estimate	Standard Error
{Phi(t),p(t)}		0.54714	0.3709177	57.2443900
{Phi(.),p(t)}		0.22624	0.2309476	0.0228841
{Phi(g),p(t)}		0.13536	0.2311173	0.0228859
{Phi(t),p(.)}		0.03537	0.6035494	0.0342260
{Phi(a*t),p(t)}		0.02068	0.1961305	0.0000000
...
Weighted Average			0.3284784	31.3919166
Unconditional SE				42.3830999
95% CI for wgt. Ave. Est. (logit trans.) is 0.0000000 to 1.0000000				
Percent of Variation Attributable to Model Variation is 45.14%				

P-VALUES FOR GLM ANALYSES - CLUSTER "COMPETITORS/PREDATORS"

signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Competitors and Predators Cluster

Adults

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.0679	-0.4099	-0.4099	-0.4099	22.0965

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-2.47667	0.03195	-77.505	< 2e-16	***
RARI_PRES	0.95375	0.06189	15.411	< 2e-16	***
TRCA_PRES	1.17003	0.11723	9.980	< 2e-16	***
DYT	-0.12761	0.13956	-0.914	0.360526	
NOT	1.14674	0.12632	9.078	< 2e-16	***
LIB	2.06655	0.10331	20.004	< 2e-16	***
DOL	1.40798	0.25269	5.572	2.52e-08	***
RARI_PRES:DYT	-0.54909	0.17364	-3.162	0.001566	**
RARI_PRES:NOT	-1.15703	0.14888	-7.772	7.74e-15	***
RARI_PRES:LIB	-1.85666	0.21354	-8.695	< 2e-16	***
TRCA_PRES:NOT	-2.07173	0.48814	-4.244	2.19e-05	***
TRCA_PRES:DOL	-1.85085	1.07794	-1.717	0.085975	.
DYT:NOT	0.58725	0.17373	3.380	0.000724	***
DYT:DOL	-14.35948	180.62147	-0.080	0.936635	
NOT:LIB	-0.50694	0.23017	-2.203	0.027629	*
NOT:DOL	2.24723	0.43009	5.225	1.74e-07	***
LIB:DOL	-14.93526	304.16504	-0.049	0.960838	

 Null deviance: 11948 on 14878 degrees of freedom
 Residual deviance: 11191 on 14862 degrees of freedom
 AIC: 13304
 Number of Fisher Scoring iterations: 13

Subadults

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.8390	-0.2032	-0.2032	-0.2032	17.3989

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-3.87981	0.06359	-61.017	< 2e-16	***
RARI_PRES	1.79338	0.09355	19.170	< 2e-16	***
TRCA_PRES	1.68572	0.15087	11.173	< 2e-16	***
DYT	-0.66105	0.27563	-2.398	0.016469	*
NOT	1.96548	0.17685	11.114	< 2e-16	***
LIB	2.71934	0.15778	17.235	< 2e-16	***
DOL	1.01526	0.45088	2.252	0.024338	*
RARI_PRES:DYT	-0.48614	0.28757	-1.690	0.090932	.
RARI_PRES:NOT	-2.50510	0.22707	-11.032	< 2e-16	***
RARI_PRES:LIB	-2.41931	0.28352	-8.533	< 2e-16	***
TRCA_PRES:NOT	-1.65645	0.45669	-3.627	0.000287	***
DYT:NOT	0.94220	0.29324	3.213	0.001313	**
DYT:DOL	-12.58222	251.92668	-0.050	0.960167	

```

NOT:LIB      -0.59260    0.28923   -2.049  0.040471 *
LIB:DOL      -1.47579    1.10979   -1.330  0.183585

```

```
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```

```

Null deviance: 5892.5 on 14878 degrees of freedom
Residual deviance: 5168.7 on 14864 degrees of freedom
AIC: 5911.5
Number of Fisher Scoring iterations: 13

```

Juveniles

Deviance Residuals:

```

      Min      1Q  Median      3Q      Max
-0.959 -0.263 -0.263 -0.263  48.681

```

Coefficients:

```

              Estimate Std. Error z value Pr(>|z|)
(Intercept)  -3.36691    0.05052  -66.648 < 2e-16 ***
RARI_PRES     0.76545    0.10701   7.153  8.49e-13 ***
TRCA_PRES     0.38308    0.30519   1.255  0.20941
DYT           0.37546    0.22270   1.686  0.09181 .
NOT           0.11451    0.28707   0.399  0.68996
LIB           2.59108    0.14028  18.471 < 2e-16 ***
DOL          -13.68431  294.20684  -0.047  0.96290
RARI_PRES:DYT -1.73998    0.27836  -6.251  4.08e-10 ***
RARI_PRES:NOT  1.69391    0.30700   5.518  3.44e-08 ***
RARI_PRES:LIB -2.47502    0.46060  -5.373  7.72e-08 ***
TRCA_PRES:DYT -11.95177  207.64706  -0.058  0.95410
TRCA_PRES:NOT -2.16805    1.04747  -2.070  0.03847 *
TRCA_PRES:LIB -11.81578  204.84146  -0.058  0.95400
DYT:LIB       -1.33124    0.55700  -2.390  0.01685 *
NOT:LIB       -2.01237    0.67631  -2.975  0.00293 **

```

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```

```

Null deviance: 9964.9 on 14878 degrees of freedom
Residual deviance: 9110.2 on 14864 degrees of freedom
AIC: 9659.1
Number of Fisher Scoring iterations: 16

```

Tadpoles

Deviance Residuals:

```

      Min      1Q  Median      3Q      Max
-3.183 -0.801 -0.801 -0.801 104.773

```

Coefficients:

```

              Estimate Std. Error z value Pr(>|z|)
(Intercept)  -1.13669    0.01666  -68.230 < 2e-16 ***
RARI_PRES     0.01723    0.04910   0.351  0.725691
TRCA_PRES    -1.16056    0.23000  -5.046  4.52e-07 ***
DYT          -0.05977    0.09562  -0.625  0.531952
NOT          -0.44499    0.14701  -3.027  0.002470 **
LIB           0.98812    0.09622  10.270 < 2e-16 ***
DOL           2.75937    0.08280  33.324 < 2e-16 ***
RARI_PRES:DYT -1.50963    0.19899  -7.586  3.29e-14 ***
RARI_PRES:NOT  0.37506    0.16449   2.280  0.022601 *
RARI_PRES:LIB -0.34037    0.18734  -1.817  0.069247 .
RARI_PRES:DOL -13.80181  100.77011  -0.137  0.891060
TRCA_PRES:NOT -12.85352  264.82479  -0.049  0.961289
TRCA_PRES:LIB  2.85718    0.34294   8.331 < 2e-16 ***
TRCA_PRES:DOL -13.76957  332.66947  -0.041  0.966984
DYT:NOT       0.57290    0.19615   2.921  0.003492 **
DYT:LIB      -1.73823    0.46994  -3.699  0.000217 ***

```

```

DYT:DOL      -14.19714  118.79431  -0.120  0.904871
NOT:LIB      -14.11368  140.44432  -0.100  0.919953
LIB:DOL      -1.35804   0.39836  -3.409  0.000652 ***

```

```
---
```

```

Null deviance: 47204 on 14878 degrees of freedom
Residual deviance: 46204 on 14860 degrees of freedom
AIC: 47338
Number of Fisher Scoring iterations: 14

```

Eggs

Deviance Residuals:

```

  Min      1Q  Median      3Q      Max
-4.764 -0.966 -0.966 -0.966  46.331

```

Coefficients:

```

              Estimate Std. Error z value Pr(>|z|)
(Intercept)   -0.76156    0.01379  -55.229 < 2e-16 ***
RARI_PRES     -0.37657    0.04803   -7.840 4.52e-15 ***
TRCA_PRES      1.35147    0.06436   20.999 < 2e-16 ***
DYT            0.74146    0.05450   13.604 < 2e-16 ***
NOT           -0.35314    0.10980   -3.216 0.001298 **
LIB            1.89346    0.05207   36.363 < 2e-16 ***
DOL            1.40159    0.13554   10.341 < 2e-16 ***
RARI_PRES:TRCA_PRES -0.88107    0.24233   -3.636 0.000277 ***
RARI_PRES:DYT  -0.18745    0.10101   -1.856 0.063498 .
RARI_PRES:NOT   0.37173    0.12572    2.957 0.003108 **
RARI_PRES:LIB  -3.71356    0.32068  -11.580 < 2e-16 ***
RARI_PRES:DOL -15.69236   512.93796  -0.031 0.975594
TRCA_PRES:DYT  -2.11821    0.41591   -5.093 3.52e-07 ***
TRCA_PRES:NOT -16.13326   596.34988  -0.027 0.978417
TRCA_PRES:LIB -13.85334   129.20433  -0.107 0.914614
TRCA_PRES:DOL -15.36500   343.83829  -0.045 0.964357
DYT:NOT       -0.70904    0.12298   -5.765 8.15e-09 ***
DYT:LIB       0.55563    0.09564    5.810 6.25e-09 ***
DYT:DOL      -17.35868   631.98802  -0.027 0.978087
NOT:LIB       0.32739    0.18278    1.791 0.073272 .
LIB:DOL      -12.20296    76.30456  -0.160 0.872941

```

```
---
```

```

Null deviance: 71784 on 14910 degrees of freedom
Residual deviance: 69056 on 14890 degrees of freedom
(1422 observations deleted due to missingness)
AIC: 70054
Number of Fisher Scoring iterations: 16

```

Appendix D1 (see p. 86 and p. 90)

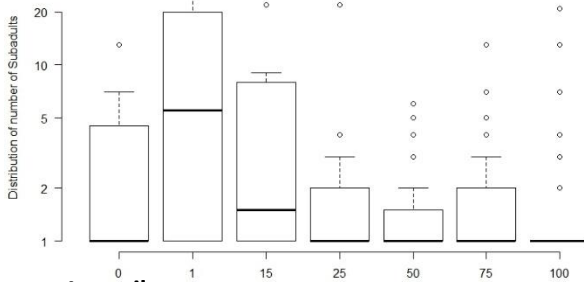
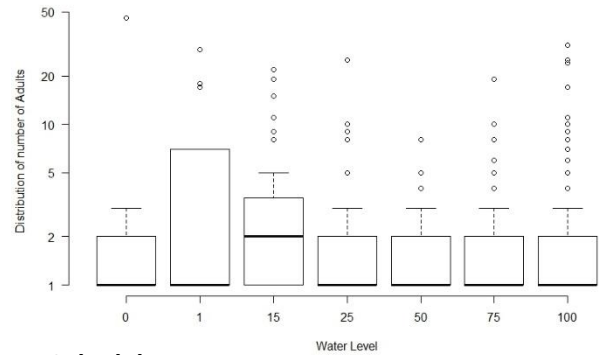
TUKEY AND KRAMER (NEMENYI) TEST: P-VALUES FOR COMPARISONS OF BOVA'S ABUNDANCE ACCORDING TO

WATER LEVEL

Adults

	0	1	15	25	50	75
1	0.9740	-	-	-	-	-
15	0.2467	0.9732	-	-	-	-
25	1.0000	0.9178	0.0596	-	-	-
50	1.0000	0.9474	0.0615	1.0000	-	-
75	1.0000	0.9378	0.0221	0.9999	1.0000	-
100	0.9999	0.8433	0.0029	1.0000	0.9995	0.9923

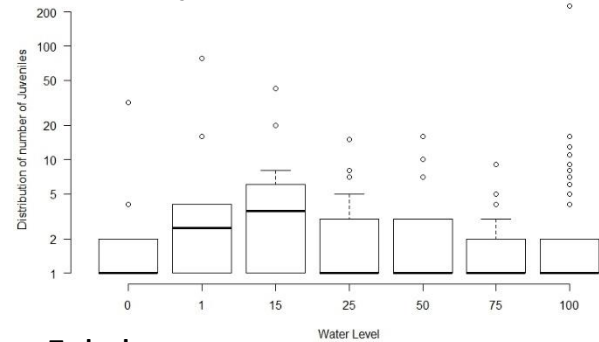
P value adjustment method: none



Subadults

	0	1	15	25	50	75
1	0.995	-	-	-	-	-
15	0.983	1.00	-	-	-	-
25	1.000	0.96	0.78	-	-	-
50	0.975	0.65	0.19	0.94	-	-
75	0.999	0.87	0.47	1.00	0.99	-
100	0.924	0.47	0.04	0.68	1.00	0.77

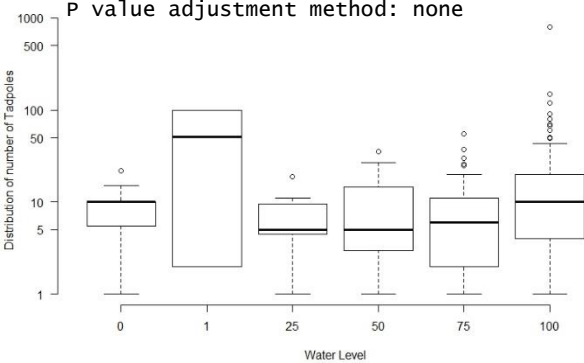
P value adjustment method: none



Juveniles

	0	1	15	25	50	75
1	0.95	-	-	-	-	-
15	0.67	1.00	-	-	-	-
25	1.00	0.80	0.28	-	-	-
50	1.00	0.90	0.45	1.00	-	-
75	1.00	0.71	0.16	1.00	1.00	-
100	1.00	0.65	0.11	1.00	1.00	1.00

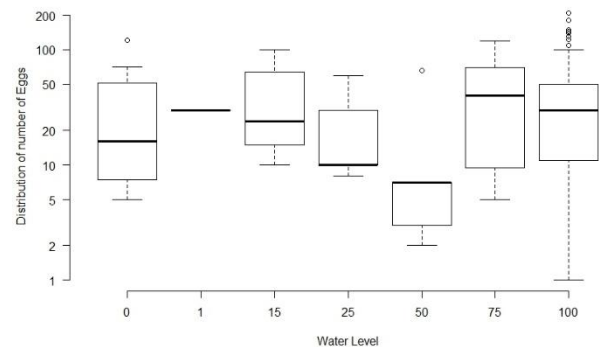
P value adjustment method: none



Tadpoles

	0	1	25	50	75
1	0.997	-	-	-	-
25	0.993	0.973	-	-	-
50	0.966	0.961	1.000	-	-
75	0.885	0.948	1.000	1.000	-
100	0.940	1.000	0.826	0.356	0.013

P value adjustment method: none



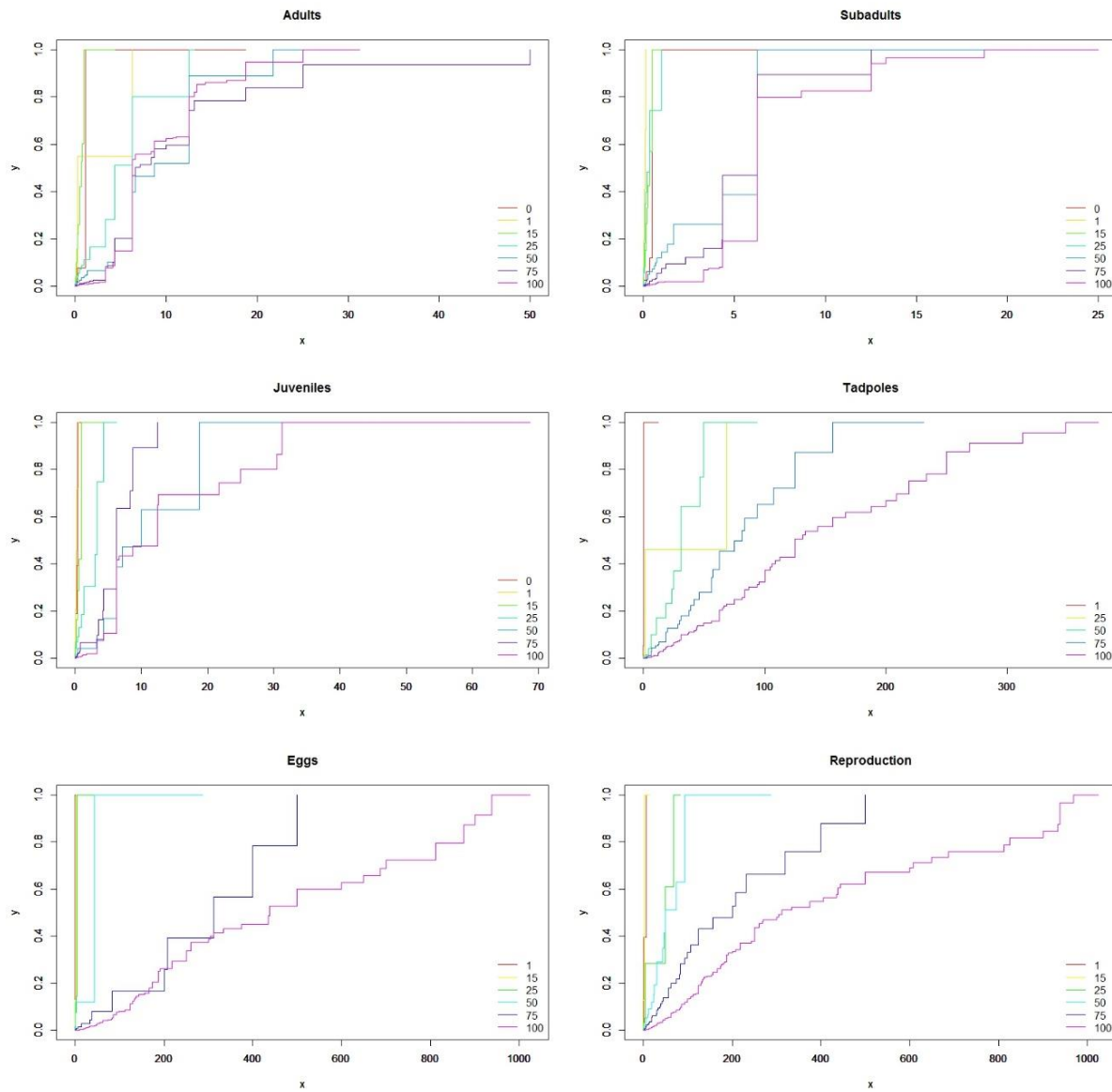
Eggs

	0	1	15	25	50	75
1	1.00	-	-	-	-	-
15	1.00	1.00	-	-	-	-
25	1.00	1.00	1.00	-	-	-
50	0.85	0.97	0.79	0.95	-	-
75	0.93	1.00	1.00	0.97	0.31	-
100	0.98	1.00	1.00	1.00	0.38	0.99

P value adjustment method: none

KOLMOGOROV-SMIRNOV TEST: CUMULATIVE DENSITY FUNCTION (CDF) OF TOADS ACCORDING TO WATER

LEVEL



Appendix D3 (see p. 88, p. 90, p. 92 and p. 93)

P-VALUES FOR GLM ANALYSES - CLUSTER "WATER" AND CLUSTER "VEGETATION"

signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Water Cluster

Adults

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.5136	-0.4529	-0.4328	-0.2283	13.7894

Coefficients: (3 not defined because of singularities)

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-1.369e+01	2.013e+02	-0.068	0.94578	
SURFACE	1.320e-03	9.776e-04	1.351	0.17681	
TYPEB	1.117e+01	2.013e+02	0.055	0.95576	
TYPEE	9.686e+00	2.013e+02	0.048	0.96162	
TYPEF	1.174e+01	2.013e+02	0.058	0.95351	
TYPEN	1.005e+01	2.013e+02	0.050	0.96017	
TYPEO	1.091e+01	2.013e+02	0.054	0.95676	
TYPEX	9.185e+00	2.013e+02	0.046	0.96360	
EAU	5.465e-02	6.307e-03	8.665	< 2e-16	***
T	1.185e+00	1.633e-01	7.257	5.97e-13	***
SURFACE:TYPEB	-1.634e-03	1.725e-02	-0.095	0.92456	
SURFACE:TYPEE	-1.239e-03	9.779e-04	-1.267	0.20501	
SURFACE:TYPEF	5.976e-03	1.040e-03	5.749	8.98e-09	***
SURFACE:TYPEN	-4.178e-03	2.250e-03	-1.857	0.06335	.
SURFACE:TYPEO	-2.047e-03	2.381e-03	-0.860	0.38992	
SURFACE:TYPEX	NA	NA	NA	NA	
SURFACE:T	-4.336e-03	8.659e-04	-5.007	5.52e-07	***
TYPEB:EAU	-5.311e-02	6.556e-03	-8.100	5.48e-16	***
TYPEE:EAU	-3.571e-02	6.498e-03	-5.496	3.90e-08	***
TYPEF:EAU	-5.700e-02	6.565e-03	-8.683	< 2e-16	***
TYPEN:EAU	-3.237e-02	6.940e-03	-4.664	3.10e-06	***
TYPEO:EAU	-3.676e-02	6.754e-03	-5.442	5.26e-08	***
TYPEX:EAU	NA	NA	NA	NA	
TYPEB:T	-3.381e-01	1.142e-01	-2.959	0.00308	**
TYPEE:T	-4.147e-01	1.344e-01	-3.086	0.00203	**
TYPEF:T	-6.285e-01	2.061e-01	-3.050	0.00229	**
TYPEN:T	-3.407e-02	1.804e-01	-0.189	0.85024	
TYPEO:T	-3.383e-01	1.630e-01	-2.076	0.03791	*
TYPEX:T	NA	NA	NA	NA	
EAU:T	-7.555e-03	1.382e-03	-5.467	4.58e-08	***

Null deviance: 10445.2 on 14107 degrees of freedom
 Residual deviance: 8744.9 on 14081 degrees of freedom
 (162 observations deleted due to missingness)

AIC: 10540

Number of Fisher Scoring iterations: 10

Subadults

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.7265	-0.2435	-0.2285	-0.1582	15.3305

Coefficients: (3 not defined because of singularities)

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-15.929012	547.147852	-0.029	0.976775	
SURFACE	0.002250	0.001465	1.535	0.124730	
TYPEB	9.456924	547.148260	0.017	0.986210	
TYPEE	11.281983	547.147898	0.021	0.983549	
TYPEF	13.810301	547.147862	0.025	0.979863	
TYPEN	11.548306	547.147930	0.021	0.983161	
TYPEO	12.534396	547.147923	0.023	0.981723	
TYPEX	9.396275	547.148575	0.017	0.986298	
EAU	0.063707	0.012908	4.935	8.00e-07	***
T	1.950619	0.271841	7.176	7.20e-13	***
SURFACE:TYPEB	-0.016667	0.078967	-0.211	0.832835	
SURFACE:TYPEE	-0.021805	0.009192	-2.372	0.017685	*
SURFACE:TYPEF	0.005863	0.001523	3.850	0.000118	***
SURFACE:TYPEN	-0.002240	0.001544	-1.451	0.146901	
SURFACE:TYPEO	-0.006276	0.005876	-1.068	0.285472	
SURFACE:TYPEX	NA	NA	NA	NA	
SURFACE:T	-0.006581	0.001706	-3.858	0.000114	***
TYPEB:EAU	-0.035423	0.014475	-2.447	0.014396	*
TYPEE:EAU	-0.042213	0.013094	-3.224	0.001265	**
TYPEF:EAU	-0.071145	0.013104	-5.429	5.66e-08	***
TYPEN:EAU	-0.038986	0.013359	-2.918	0.003519	**
TYPEO:EAU	-0.046824	0.013341	-3.510	0.000448	***
TYPEX:EAU	NA	NA	NA	NA	
TYPEB:T	-0.498965	0.200562	-2.488	0.012852	*
TYPEE:T	-0.905425	0.223247	-4.056	5.00e-05	***
TYPEF:T	-1.670933	0.380779	-4.388	1.14e-05	***
TYPEN:T	-0.245618	0.243583	-1.008	0.313284	
TYPEO:T	-0.987133	0.282829	-3.490	0.000483	***
TYPEX:T	NA	NA	NA	NA	
EAU:T	-0.013594	0.002335	-5.822	5.83e-09	***

Null deviance: 5622.1 on 14107 degrees of freedom
 Residual deviance: 4421.7 on 14081 degrees of freedom
 (162 observations deleted due to missingness)
 AIC: 5146.2

Number of Fisher Scoring iterations: 12

Juveniles

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.7265	-0.2435	-0.2285	-0.1582	15.3305

Coefficients: (3 not defined because of singularities)

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-15.929012	547.147852	-0.029	0.976775
SURFACE	0.002250	0.001465	1.535	0.124730
TYPEB	9.456924	547.148260	0.017	0.986210
TYPEE	11.281983	547.147898	0.021	0.983549
TYPEF	13.810301	547.147862	0.025	0.979863
TYPEN	11.548306	547.147930	0.021	0.983161
TYPEO	12.534396	547.147923	0.023	0.981723
TYPEX	9.396275	547.148575	0.017	0.986298

EAU	0.063707	0.012908	4.935	8.00e-07	***
T	1.950619	0.271841	7.176	7.20e-13	***
SURFACE:TYPEB	-0.016667	0.078967	-0.211	0.832835	
SURFACE:TYPEE	-0.021805	0.009192	-2.372	0.017685	*
SURFACE:TYPEF	0.005863	0.001523	3.850	0.000118	***
SURFACE:TYPEN	-0.002240	0.001544	-1.451	0.146901	
SURFACE:TYPEO	-0.006276	0.005876	-1.068	0.285472	
SURFACE:TYPEX	NA	NA	NA	NA	
SURFACE:T	-0.006581	0.001706	-3.858	0.000114	***
TYPEB:EAU	-0.035423	0.014475	-2.447	0.014396	*
TYPEE:EAU	-0.042213	0.013094	-3.224	0.001265	**
TYPEF:EAU	-0.071145	0.013104	-5.429	5.66e-08	***
TYPEN:EAU	-0.038986	0.013359	-2.918	0.003519	**
TYPEO:EAU	-0.046824	0.013341	-3.510	0.000448	***
TYPEX:EAU	NA	NA	NA	NA	
TYPEB:T	-0.498965	0.200562	-2.488	0.012852	*
TYPEE:T	-0.905425	0.223247	-4.056	5.00e-05	***
TYPEF:T	-1.670933	0.380779	-4.388	1.14e-05	***
TYPEN:T	-0.245618	0.243583	-1.008	0.313284	
TYPEO:T	-0.987133	0.282829	-3.490	0.000483	***
TYPEX:T	NA	NA	NA	NA	
EAU:T	-0.013594	0.002335	-5.822	5.83e-09	***

Null deviance: 5622.1 on 14107 degrees of freedom
Residual deviance: 4421.7 on 14081 degrees of freedom
(162 observations deleted due to missingness)
AIC: 5146.2
Number of Fisher scoring iterations: 12

Tadpoles

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.7265	-0.2435	-0.2285	-0.1582	15.3305

Coefficients: (3 not defined because of singularities)

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-15.929012	547.147852	-0.029	0.976775	
SURFACE	0.002250	0.001465	1.535	0.124730	
TYPEB	9.456924	547.148260	0.017	0.986210	
TYPEE	11.281983	547.147898	0.021	0.983549	
TYPEF	13.810301	547.147862	0.025	0.979863	
TYPEN	11.548306	547.147930	0.021	0.983161	
TYPEO	12.534396	547.147923	0.023	0.981723	
TYPEX	9.396275	547.148575	0.017	0.986298	
EAU	0.063707	0.012908	4.935	8.00e-07	***
T	1.950619	0.271841	7.176	7.20e-13	***
SURFACE:TYPEB	-0.016667	0.078967	-0.211	0.832835	
SURFACE:TYPEE	-0.021805	0.009192	-2.372	0.017685	*
SURFACE:TYPEF	0.005863	0.001523	3.850	0.000118	***
SURFACE:TYPEN	-0.002240	0.001544	-1.451	0.146901	
SURFACE:TYPEO	-0.006276	0.005876	-1.068	0.285472	
SURFACE:TYPEX	NA	NA	NA	NA	
SURFACE:T	-0.006581	0.001706	-3.858	0.000114	***
TYPEB:EAU	-0.035423	0.014475	-2.447	0.014396	*
TYPEE:EAU	-0.042213	0.013094	-3.224	0.001265	**
TYPEF:EAU	-0.071145	0.013104	-5.429	5.66e-08	***
TYPEN:EAU	-0.038986	0.013359	-2.918	0.003519	**
TYPEO:EAU	-0.046824	0.013341	-3.510	0.000448	***
TYPEX:EAU	NA	NA	NA	NA	
TYPEB:T	-0.498965	0.200562	-2.488	0.012852	*

TYPEE:T	-0.905425	0.223247	-4.056	5.00e-05	***
TYPEF:T	-1.670933	0.380779	-4.388	1.14e-05	***
TYPEN:T	-0.245618	0.243583	-1.008	0.313284	
TYPEO:T	-0.987133	0.282829	-3.490	0.000483	***
TYPEX:T	NA	NA	NA	NA	
EAU:T	-0.013594	0.002335	-5.822	5.83e-09	***

Null deviance: 5622.1 on 14107 degrees of freedom
Residual deviance: 4421.7 on 14081 degrees of freedom
(162 observations deleted due to missingness)
AIC: 5146.2
Number of Fisher Scoring iterations: 12

Eggs

Deviance Residuals:
Min 1Q Median 3Q Max
-2.869 -1.327 -0.654 -0.174 46.465

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-2.137e+01	3.599e+03	-0.006	0.995262	
SURFACE	-3.040e-03	1.226e+03	0.000	0.999998	
TYPEB	1.878e+01	3.599e+03	0.005	0.995836	
TYPEE	1.820e+01	3.599e+03	0.005	0.995964	
TYPEF	1.881e+01	3.599e+03	0.005	0.995830	
TYPEN	-2.449e-02	3.730e+03	0.000	0.999995	
TYPEO	-4.109e+01	3.680e+03	-0.011	0.991093	
TYPEX	1.968e+01	3.599e+03	0.005	0.995637	
EAU	4.371e-03	9.585e+01	0.000	0.999964	
T	1.128e+00	5.549e+03	0.000	0.999838	
SURFACE:TYPEB	-4.739e+00	1.226e+03	-0.004	0.996917	
SURFACE:TYPEE	-1.110e-01	1.226e+03	0.000	0.999928	
SURFACE:TYPEF	-3.664e-02	1.226e+03	0.000	0.999976	
SURFACE:TYPEN	3.433e-03	1.226e+03	0.000	0.999998	
SURFACE:TYPEO	-1.952e-02	1.226e+03	0.000	0.999987	
SURFACE:TYPEX	1.224e-03	1.226e+03	0.000	0.999999	
SURFACE:T	-1.937e-02	5.338e-03	-3.629	0.000284	***
TYPEB:EAU	2.783e-02	9.585e+01	0.000	0.999768	
TYPEE:EAU	2.675e-02	9.585e+01	0.000	0.999777	
TYPEF:EAU	3.544e-02	9.585e+01	0.000	0.999705	
TYPEN:EAU	-1.918e-03	9.799e+01	0.000	0.999984	
TYPEO:EAU	6.307e-01	9.616e+01	0.007	0.994767	
TYPEX:EAU	1.422e-02	9.585e+01	0.000	0.999882	
TYPEB:T	9.719e-01	5.549e+03	0.000	0.999860	
TYPEE:T	7.824e-01	5.549e+03	0.000	0.999888	
TYPEF:T	3.405e-01	5.549e+03	0.000	0.999951	
TYPEN:T	7.262e-01	5.665e+03	0.000	0.999898	
TYPEO:T	1.661e-01	5.549e+03	0.000	0.999976	
TYPEX:T	6.031e-01	5.549e+03	0.000	0.999913	
EAU:T	-1.800e-02	8.976e-04	-20.051	< 2e-16	***

Null deviance: 71611 on 14740 degrees of freedom
Residual deviance: 61514 on 14711 degrees of freedom
(1592 observations deleted due to missingness)
AIC: 62531
Number of Fisher Scoring iterations: 19

Vegetation Cluster**Adults**

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.6035	-0.4945	-0.4514	-0.3290	22.9933

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-2.91682	0.05791	-50.367	< 2e-16	***
A.SUP	2.36193	0.21388	11.043	< 2e-16	***
A.PROF	-0.34107	0.20260	-1.683	0.092288	.
V	2.51619	0.12243	20.552	< 2e-16	***
B	0.81542	0.08745	9.325	< 2e-16	***
F	0.19412	0.16185	1.199	0.230366	
A.SUP:A.PROF	-0.80804	0.18415	-4.388	1.14e-05	***
A.SUP:V	-0.74072	0.16172	-4.580	4.64e-06	***
A.SUP:B	-1.90090	0.18359	-10.354	< 2e-16	***
A.PROF:V	0.40077	0.14075	2.847	0.004408	**
A.PROF:B	0.63613	0.18956	3.356	0.000791	***
A.PROF:F	0.60101	0.11948	5.030	4.91e-07	***
V:B	-1.42165	0.14791	-9.612	< 2e-16	***
V:F	-1.22162	0.12898	-9.471	< 2e-16	***
B:F	-0.24960	0.14633	-1.706	0.088057	.

Null deviance: 11906 on 14704 degrees of freedom
 Residual deviance: 10915 on 14690 degrees of freedom
 (174 observations deleted due to missingness)

AIC: 13024

Number of Fisher Scoring iterations: 7

Subadults

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.4998	-0.2656	-0.2324	-0.1607	16.1236

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-4.3502	0.1153	-37.728	< 2e-16	***
A.SUP	2.8025	0.3504	7.997	1.27e-15	***
A.PROF	-0.1656	0.2960	-0.559	0.57584	
V	2.9338	0.2011	14.587	< 2e-16	***
B	0.7387	0.1658	4.456	8.34e-06	***
F	1.1299	0.2316	4.879	1.06e-06	***
A.SUP:A.PROF	-0.8021	0.3414	-2.349	0.01880	*
A.SUP:V	-1.4609	0.2530	-5.774	7.72e-09	***
A.SUP:B	-2.0944	0.2335	-8.969	< 2e-16	***
A.SUP:F	-0.3793	0.2315	-1.639	0.10130	
A.PROF:V	1.1600	0.2091	5.548	2.89e-08	***
A.PROF:B	0.7381	0.2417	3.053	0.00226	**
A.PROF:F	0.4751	0.2008	2.367	0.01795	*
V:B	-0.9972	0.2172	-4.592	4.40e-06	***
V:F	-1.7108	0.1850	-9.248	< 2e-16	***
B:F	-1.1919	0.1993	-5.981	2.21e-09	***

Null deviance: 5877.2 on 14704 degrees of freedom
 Residual deviance: 4912.6 on 14689 degrees of freedom
 (174 observations deleted due to missingness)

AIC: 5657.5

Number of Fisher Scoring iterations: 8

Juveniles

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.730	-0.276	-0.194	-0.194	33.796

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-3.97720	0.09957	-39.946	< 2e-16	***
A.SUP	1.53406	0.34510	4.445	8.78e-06	***
A.PROF	2.80779	0.24338	11.537	< 2e-16	***
V	0.85693	0.26131	3.279	0.001041	**
B	0.47684	0.16150	2.953	0.003151	**
F	-0.47338	0.35092	-1.349	0.177345	.
A.SUP:A.PROF	-2.20730	0.27657	-7.981	1.45e-15	***
A.SUP:B	-2.16913	0.28687	-7.561	3.99e-14	***
A.SUP:F	3.53534	0.30836	11.465	< 2e-16	***
A.PROF:V	-1.46978	0.22995	-6.392	1.64e-10	***
A.PROF:B	-2.14626	0.24382	-8.803	< 2e-16	***
A.PROF:F	0.72051	0.28458	2.532	0.011346	*
V:B	1.22721	0.20257	6.058	1.38e-09	***
V:F	-0.81212	0.22847	-3.555	0.000379	***
B:F	-0.56900	0.31654	-1.798	0.072245	.

Null deviance: 9944.5 on 14704 degrees of freedom
 Residual deviance: 7312.0 on 14690 degrees of freedom
 (174 observations deleted due to missingness)

AIC: 7860.9

Number of Fisher Scoring iterations: 9

Tadpoles

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.582	-0.910	-0.822	-0.432	97.656

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-2.37063	0.04560	-51.993	< 2e-16	***
A.SUP	-0.07884	0.33105	-0.238	0.811761	.
A.PROF	0.62513	0.26609	2.349	0.018807	*
V	-3.06529	0.24304	-12.612	< 2e-16	***
B	1.83788	0.05751	31.958	< 2e-16	***
F	0.35691	0.17817	2.003	0.045151	*
A.SUP:A.PROF	0.73924	0.22960	3.220	0.001284	**
A.SUP:V	2.12434	0.20462	10.382	< 2e-16	***
A.SUP:B	-3.38143	0.28037	-12.061	< 2e-16	***
A.SUP:F	1.73341	0.21996	7.880	3.26e-15	***
A.PROF:V	-0.31262	0.15990	-1.955	0.050576	.
A.PROF:B	-1.00537	0.26062	-3.858	0.000114	***
A.PROF:F	0.19839	0.11320	1.752	0.079697	.
V:B	2.48001	0.19577	12.668	< 2e-16	***
V:F	0.60771	0.15310	3.969	7.21e-05	***
B:F	-0.72902	0.18105	-4.027	5.66e-05	***

Null deviance: 47092 on 14704 degrees of freedom
 Residual deviance: 44265 on 14689 degrees of freedom
 (174 observations deleted due to missingness)

AIC: 45393

Number of Fisher Scoring iterations: 10

Eggs

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.380	-0.758	-0.697	-0.642	39.544

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)	
(Intercept)	-1.41382	0.02825	-50.050	< 2e-16	***
A_SUP	-0.45642	0.34356	-1.329	0.18401	
A_PROF	-1.43829	0.20217	-7.114	1.12e-12	***
V	0.55775	0.13803	4.041	5.33e-05	***
B	2.04986	0.03432	59.720	< 2e-16	***
F	-0.70924	0.15831	-4.480	7.46e-06	***
A_SUP:A_PROF	1.32700	0.29086	4.562	5.06e-06	***
A_SUP:V	0.26204	0.15197	1.724	0.08466	.
A_SUP:B	-1.87527	0.22417	-8.365	< 2e-16	***
A_SUP:F	-2.55587	0.15725	-16.254	< 2e-16	***
A_PROF:V	0.68322	0.07221	9.461	< 2e-16	***
A_PROF:B	1.28369	0.19881	6.457	1.07e-10	***
A_PROF:F	1.97806	0.06219	31.808	< 2e-16	***
V:B	-0.68115	0.14046	-4.849	1.24e-06	***
V:F	-0.20643	0.07407	-2.787	0.00532	**
B:F	-1.17502	0.15801	-7.436	1.04e-13	***

Null deviance: 71607 on 14736 degrees of freedom
 Residual deviance: 63228 on 14721 degrees of freedom
 (1596 observations deleted due to missingness)

AIC: 64216

Number of Fisher Scoring iterations: 11