CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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DEPARTMENT OF ECOLOGY

LONG TERM MONITORING OF AMPHIBIAN POPULATIONS: METHODS AND IMPORTANCE

BACHELOR THESIS

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BACHELOR THESIS ASSIGNMENT

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Applied Ecology

Thesis title

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Objectives of thesis

Amphibian declines during last decades have led to an increasing of emphasis on monitoring of amphibian population abundances. However, great natural fluctuations in amphibian populations are typical. Deciding whether a population is declining requires an understanding of population fluctuations, and therefore, long-term monitoring conducted at a sufficient spatial scale. Because this kind of monitoring are expensive and time-consuming, there is a lack of such studies.

The main goal of this bachelor thesis is to perform literary review on following topics: (i) summary of main threats to amphibian diversity (1–2 pp); (ii) population dynamics in amphibian populations – separating human impacts from natural fluctuations, magnitude of population fluctuations, and relation between p. fluctuations and the risk of local extinction from demographic or environmental stochasticity (3–5 pp); (iii) monitoring of population trends in amphibians – the importance and examples of long-term studies, simple meta-analysis of such studies + examples, evaluation, recommendations (10–15 pp). Furthermore, the student will participate in field work, collection and data processing, as well as in preparing a manuscript on monitoring Rana dalmatina population abundance in Hornojiřetínská spoil heap. The following main questions will be solved: (1) What is the rate of fluctuation of R. dalmatina recruitment and pond occupancy? (2) Is there any trend in the population abundance?

Methodology

1) Literature review – work with databases and other possible sources of literature, mainly scientific papers, (Web of Knowledge, ScienceDirect, Google Scholar etc.).

2) Field work – (i) participation in data collection (counting of Rana dalmatina clutches) in the field (during at least one reproduction season); (ii) simple data processing – detection of trends using linear regression, analysis of frequencies of population abundances using chi-square tests and log-linear models.

3) Comparison of relevant studies according their length of monitoring, spatial scale (local × population × regional), purpose of monitoring, model species, using methods, results etc.

The proposed extent of the thesis

ca 30 pages

Keywords

Amphibian conservation, amphibian declines, population variation, population dynamics, the agile frog, spoil banks, occupancy, monitoring.

Recommended information sources

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Collins J. P. & Storfer (2003). Global amphibian declines: sorting the hypotheses. Diversity and Distribution 9: 89–98.

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Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, a H., & Kuzmin, S. L. (2000). Quantitative evidence for global amphibian population declines. Nature, 404(6779), 752–755.

Marsh D. M. & Trenham P. C. (2001). Metapopulation dynamics and amphibian conservation. Conservation Biology 15: 40–49.

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Semlitsch R. D. (2003). Amphibian Conservation. Smithsonian Books, Washington and London.

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Declaration

I hereby declare that this bachelor thesis was written by me and me alone, merely using the cited sources. I agree with the loan of my work and its publication.

In Prague, April 12, 2016 _______________________________

Liz Mabel Vargas Cáceres

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ABSTRACT

In the last decade, there has been particular concern about the apparent widespread decline of many amphibian populations. Aside from anthropogenic stressors, there are large, natural fluctuations in amphibian populations that need to be established through long-term monitoring programs with sufficient spatial scale to preclude the misinterpretation of preliminary data cause by variations in natural factors. The true utility of multiple, long-term studies is an understanding of how geographic and species-specific differences affect population fluctuations. Despite the obvious importance of time series analyses in this process, they are still rare in amphibian ecology. The main purposes of this bachelor's thesis are the literary review of the main causes of amphibian population declines and dynamics of the amphibians; the meta-analysis of long-term publication reports of amphibian population trends available at this point in time; as well as the active involvement in field work, data-collection, and the preparation of a manuscript that monitored the abundance of the agile frog (*Rana dalmatina*) for 11 years.

Key words: amphibian conservation, amphibian declines, population variation, population dynamics, the agile frog, spoil banks, occupancy, monitoring.

ABSTRAKT

V posledním desetiletí došlo k znepokojení nad zjevným poklesem mnoha populací obojživelníků. Kromě antropogenních stresorů je však potřeba rovněž brát v potaz přirozené kolísání populací obojživelníků, které je třeba zajistit prostřednictvím dlouhodobých programů pro jejich sledování s dostatečnou prostorovou stupnicí, aby se zabránilo chybné interpretaci údajů, na které mají vliv proměnlivé přírodní faktory. Významem studií dlouhodobého sledování je pochopení toho, jak geografické a druhově specifické rozdíly ovlivňují výkyvy populací obojživelníků. I přes zřejmý význam časově dlouhodobých analýz je jejich využití v případech sledování obojživelníků poměrně řídké. Literární rešerše hlavních příčin klesajících populací obojživelníků, populační dynamika obojživelníků, meta-analýza dostupných dlouhodobých pozorování populačních trendů obojživelníků společně s aktivním zapojením v terénních prací, shromažďování dat a příprava rukopisu k 11 let trvajícímu monitorování početnosti skokana štíhlého (*Rana dalmatina*) jsou hlavními cíli této bakalářské práce.

Klíčová slova: ochrana obojživelníků, úbytek obojživelníků, kolísání velikostí populací, populační dynamika, skokan štíhlý, výsypky, obsazenost, monitoring.

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1. INTRODUCTION

Amphibian populations have been suspected to be in a worldwide decline that goes beyond the overall biodiversity crisis (Wake 1991; Blaustein et al. 1994; Houlahan et al. 2000; Stuart et al. 2004). Human activities such as habitat alteration, the introduction of non-native species, diseases, and environmental pollution may negatively influence the abundance, diversity and population structure of amphibian communities (Houlahan et al. 2000; Collins & Storfer 2003; Blaustein et al. 2011).As news of the declines began to surface around the world, scientists wondered if they were simply anecdotal accounts that reflected natural population fluctuations (Pechmann & Wilbur 1994), therefore, the extent to which observed population declines of some species are still uncertain and a topic of debate (Crump et al. 1992; Pechmann et al. 1991).

Amphibian populations suffer natural year-to-year fluctuations (Pechmann & Wilbur 1994), and tends to undergo several years of declines followed by drastic and short increases (Alford & Richards 1999). Amphibian declines in the short term has to be analysed carefully, since this declines could be due to habitat degradation or may be part of a natural cycle of fluctuations in the population due to biotic and abiotic causes (Pechmann & Wilbur 1994). Moreover, the magnitude of population fluctuations may indicate the risk of local extinction from demographic or environmental stochasticity (Leigh 1981). Pond-breeding amphibians with double life-histories, and especially the anurans with high rates of recruitment, present high variability in population fluctuation and high rates of local extinctions (Green 2003).

Despite the growing amount of research investigating ultimate causes of amphibian declines (Houlahan et al. 2000), a major difficulty in assessing the reported declines of amphibians has been the virtual absence of long-term baseline data on population abundances. Without the understanding of magnitude, trends and causes of population variation, and the knowledge of the degree of natural population fluctuations it is impossible to distinguish between normal and abnormal declines (Pechmann & Wilbur 1994; Marsh 2001; Green 2003).

At the present, many of the monitoring studies of amphibians are shorter than 5 years (Gardner 2001); nevertheless, at least 10 years of data collection are needed in order to detect biologically meaningful trends of amphibian populations and to examine the importance decline drivers that affect population dynamics and community structure (Meyer et al. 1998; Alford & Richards 1999).

Besides a sufficient period of population monitoring, research is also needed at the landscape level (Storfer 2003). Due the fact that this kind of monitoring is labour-intensive and represents an expensive proposition (Houlahan et al. 2000), temporal scales integrated within spatial scales are lacking (Cody 1996).

1.1.Aim and objectives of the study

The purpose of this bachelor thesis is to make out the literature review on the main causes of amphibian declines, population dynamics, and monitoring of amphibian populations. In addition to the literary review, the main purposes are:

a) Provide an overview and meta-analysis of the current state of the available long-term monitoring of amphibian populations publication reports.
b) Participate in the data-collection process, selectively examine existing amphibian long-term monitoring reports and research data in other to state supporting interpretations for the results, and subsequently prepare a manuscript of a case of study on population abundances of the agile frog (*Rana dalmatina*) in Hornojiřetínská spoil heap (Appendix IV).

2. LITERATURE REVIEW

2.1. Main threats to amphibian diversity

Treat status of amphibians

There are approximately 7461 species of amphibians in the world (Amphibiaweb 2015), including frogs and toads (Anura or Salientia), newts and salamanders (Urodela or Claudata), and caecilians (Gymnophiona or Apoda) (Duellman & Trueb 1994; Wells 2007). This class of vertebrates leads a double life history: most species lay clutches of soft eggs without an external covering in water where they will develop until the larval stage, and as adults they spend their lives on land where they retain their mood permeable skin, an important factor for water and gas exchange (Beebee 1996; Stebbins & Cohen 1997). Amphibians are also ectothermic, which means they cannot produce their own body heat and rely on heat from the environment to carry out metabolic processes (Duellman & Trueb 1994; Wells 2007). The soft skin and delicate nature of many amphibians have made them very sensitive to changes in their environments (Beebee 1996; Blaustein & Kiesecker 2002).

In recent years, many amphibians have suffered significant worldwide declines, faster than those of birds or mammals (Houlahan et al. 2000; Semlitsch 2003; Stuart et al. 2004). The International Union for Conservation of Nature (IUCN) reports that 41% of amphibian species are at imminent risk of extinction (IUCN 2012). In the Czech Republic the situation is also not good, all of the 21 Czech amphibian species are included in The Red List of Threatened Species of the Czech Republic (Zavadil & Moravec 2003), and 19 of them are listed as protected species under the current Czech legislation (Vojar 2007).

Factors contributing to drive the decline of amphibian populations are complex and may be caused by a synergistic interaction of many extinction drivers, and vary from one region to another, even for different populations of the same species (Blaustein et al. 2011). Human activities such as habitat eradication (in particular aquatic breeding sites) and land use change, as a result of urban development and agricultural reform, are the most significant factors of amphibian declines worldwide (Houlahan et al. 2000).

Summary of the main threats

Many studies have shown that **changes in land-cover** and habitat structure near breeding ponds may have significant implications for amphibian abundance. As examples, the populations of some amphibians in north-western France have been affected by land conversion from grassland to arable and urbanization; consequently, with pond disappearance over three decades a large number of amphibian breeding sites have also been lost (Curado et al. 2011). Monitoring performed by Eskew et al. (2012) along the Broad and Pacolet Rivers in South Carolina indicated that alterations in flow regime associated with damming may have a negative effect on anurans species since such changes can reduce the riparian areas that serve as the breeding habitat for them. Furthermore, it is estimated that clearcutting in U.S. national forests in western South Carolina has led to the loss of approximately 14 million salamanders per year (Petranka et al. 1994). Land conversion may affect amphibians directly through the loss of breeding and terrestrial habitats, and also indirectly through the increased application of pesticides and fertilizers that affect natural habitats situated within agricultural lands (Beebee & Griffiths 2005). Owing to the fact that amphibians rely on waterbodies to reproduce, high concentrations of these chemicals may affect local populations and community structure (Hamer et al. 2004).

Another factor potentially contributing to declines associated with anthropogenic action is **road mortality** (i.e., amphibians being killed by traffic), especially in urban areas. In the last two decades traffic intensity has increased worldwide; as such, it is likely that the mortality of amphibians caused by traffic has increased proportionately (Fahrig et al. 1995; Hels & Buchwald 2001). Many aquatic breeding amphibians have a high vulnerability to death when moving across roads while migrating from their hibernation site to the breeding pond or vice versa (Hels & Buchwald 2001).

Concerning the possible causes of the decline of some species, it is important to mention the **introduction of species** that lead to predation upon adults, larvae and/or eggs, and competition between one or more life stages mainly for the consumption of resources such as food (Collins & Storfer 2003); as well as avoidance behaviour; when exotic predatory fish and crayfish are present, native amphibians reduce activity, use different habitats and increase the use of refuges (Collins & Crump 2009). On the other hand, risk of genetic pollution is increased with the introduction of non-native species.

It has been demonstrated that the introduced marsh frog (*Rana ridibunda*) replaces the native edible frog (*Rana esculenta*) and the pool frog (*Rana lessonae*) in many areas of Western Europe due to genetic pollution, with an expulsion of the *lessonae* genome. The special concern is that, with the spread of *Rana ridibunda* in natural populations, the chance of sterilization of the *lessonae-esculenta* complex is raised (Vorburger & Reyer 2003).

Among the various factors proposed as causes for amphibian decline, **global climate changes** are receiving the attention of scientists. Although the role of climate changes on driving these declines has not yet been systematically addressed (Pounds 2001). Global increases in ultraviolet-B (UV-B) radiation associated with stratospheric ozone depletion are potentially contributing to the decline of numerous amphibian species around the world. The use of chlorofluorocarbons (CFCs) and other chemicals by humans is causing a continual depletion of the ozone layer, while local weather and water conditions may influence the amount of ultraviolet-B radiation that reaches the surface (Blaustein & Kiesecker 2002). Continuous exposure of UV-B radiation along with other environmental stressors may reduce survival and induce a range of deformation and other lethal effects in embryonic and larval amphibians (Blaustein et al. 2003). The increased temperatures and humidity not only affect breeding times in some amphibian species (Beebee 2002), but can also alter amphibian habitats, influence food availability, predation and competitive interactions (Blaustein et al. 2010), and expedite the spread of newly emerging diseases (Carey & Alexander 2003). One of the most widespread **diseases is chytridiomycosis**, caused by *Batrachochytrium dendrobatidis* fungus, which infects the skin of frogs and the mouthparts of tadpoles (Vitt & Caldwell 2013). For example, significant changes in weather conditions have been observed in the same area and over a period of time, while chytrid-related mass mortalities have occurred in Spain (Bosch et al. 2007).

While much effort has been directed at documenting declines and identifying causes, it remains the case that very little is known about the ultimate ecological consequences of these losses (Houlahan et al. 2000; Stuart et al. 2004). It is important to provide detailed knowledge of the significance of several causes – knowledge which can be only attained through critical analysis, detailed monitoring and experiments (Pechmann & Wilbur 1994). In this manner, amphibian conservation measures that ensure success can be more effectively guided (Henle, 2005).

2.2. Dynamics in amphibian populations

Amphibians are demonstrably in general decline and exhibit a great range of dispersal abilities, demographic characteristics, and population sizes (Green 2003). Population sizes of amphibians can fluctuate over several orders of magnitude, to an extent widely variable between species, over multi-year time-scales and separating such fluctuations from long-term declines is a widely acknowledged problem (Pechmann et al. 1991).

The degree to which population size varies with time is tied to several key questions in ecology, for example, population size variability is intrinsically related to the role of density-dependent versus density-independent processes and whether populations possess stable equilibria (Pellet et al. 2006; Raithel et al. 2011). Therefore, it is extremely important to possess a strong foundation of amphibian population dynamics in order to make wise conservation management decisions in the future. (Marsh 2001; Green 2003).

2.2.1. Separating human impact from natural fluctuations

Amphibian populations are known to have normal year-to-year fluctuations owing to changes in the population sizes caused by immigration, emigration, birth and death in response to biotic and abiotic factors (Pechmann et al. 1991; Green 1997; Marsh 2001; Collins & Crump 2009). Unfortunately, given the paucity of such data, their significance, consistency and frequency are difficult to determine (Blaustein 1994). The major concern is that the represented decline may give rise to misleading conclusions about whether amphibian populations are declining beyond the expected natural oscillations (Pechmann et al. 1991; Pechmann & Wilbur 1994); an erroneous conclusion may involve lost money and credibility and consequently could affect future conservation efforts (Blaustein 1994).

Due to the intensification of anthropogenic impacts on the environment, long term monitoring is crucial for any attempt to distinguish those impacts from natural population dynamics and better understanding of the magnitude of the crisis of declining amphibian populations (Pechmann et al. 1991; Blaustein 1994; Blaustein et al. 1994; Stebbins & Cohen 1997; Houlahan et al. 2000; Blaustein et al. 2011).

There are several cases related to the problem of the decline in amphibian species which have received a wealth of attention; for example, the gastric-brooding frog

(*Rheobatrachus silus*) first discovered in 1973, from the rain forest in south-eastern Australia. The uniqueness of this species is that it was the only known animal able to incubate its eggs in its stomach and release fully formed young frogs by vomiting, something attained only by an extensive modification of the gastric function during the brooding period (Fanning et al. 1982). Since 1979, not one of these frogs has ever been found again (Tyler 1991). The golden toad (*Bufo periglenes*) that was endemic to the Monteverde cloud forest reserve of Costa Rica; this species was known to have lived underground all year, except in spring when they emerged during the breeding season. During observations made between 1970 and 1987, more than 1500 individuals were observed in 1987, but during 1988-1990 only 11 toads were found (Crump et al. 1992). According to Crump et al. (1992), this decrease was probably caused by warmer temperatures and the lack of precipitation during the dry season, which led to poor breeding conditions. Golden toads may have declined or may be underground waiting for convenient climatic conditions. Both the golden toad and the gastric brooding frog were discovered recently. Consequently, little is known about their population dynamics; as such, there is no evidence that the declines are abnormal (Blaustein 1994). Given the absence of long-term studies for these species, it is impossible either to confirm or deny the possibility that the drastic decrease in the number of individuals of both species could be linked to random cycles (Blaustein 1994).

In order to ascertain whether observed trends of amphibian populations are declining, it is necessary to determine how much change over what time period falls outside those expected from random fluctuations (Pechmann 2003). Therefore, the use of techniques based on their statistical power and appropriate statistical methods is key to the detection of population trends (Storfer 2003). One way to test those trends is through the use of statistical analyses called null models. Null models attempt to predict the expected pattern in the absence of the mechanism being tested, and allow for the randomization of tests of ecological data (Gotelli 2001). In other words, certain elements of the data remain constant, and others can vary stochastically to create new assemblage patterns (Gotelli & Graves. 1996). Pechmann (2003) analysed different choices of null models and concluded that each one may lead to different conclusions. For example, the correlation between population size and time averages zero is generally tested by the statistical techniques of parametric or non-parametric correlation or regression. A statistically significant trend is one where the observed association between population size and year is highly unlikely to occur by chance, and this is often thought to indicate changes that exceed the natural variation, possibly due to human influences.

Alford & Richards (1999) considered 85 time series of amphibian populations between the period 1951-1997, using the null model 1:1 of declines to increases and following regression analysis concluded that most amphibian populations should decrease more often than they increase against time than would be expected under their null hypotheses of normal population fluctuations, with 67% of relationships correlating negatively. As the authors themselves admit, these results may reflect a general trend of decline, also may reflect that the 1:1 declines-increases model not perfectly reflects the behaviour of the population for all amphibians and would be preferable to use a wide variety of more appropriate null hypothesis for each species.

Houlahan et al. (2000) made by far the most extensive study for 936 populations of 157 species from 6 continents, between 2 and 31 years duration. Although their results identify marked temporal variation in the speed of the declines, and spatial variation as to its extent, a definitive negative relationship is clearly evident. In the light of that fact, criticism has recently been raised as to the validity of the statistical averaging methods used by Houlahan and colleagues.

According to Alford et al. 2001 it is not possible to draw overall conclusions without knowing the population's biology of each species. Pechmann argued that the ratio 1:1 of declines to increases may not be the most appropriate null model for many amphibian populations and tests based on it should be used only for populations where there is sufficient evidence supporting its validity. A model where mean regional and global trends equal zero is a potentially useful null model, a mean trend of zero can be informative for a representative set of protected high-elevation populations for which there is certain expectation that null hypothesis might be true, but can be limited when is applied to all populations at a global scale, due to the magnitude by which human activities were altered habitats of amphibians, rejection of the null hypothesis is expected (Pechmann 2003).

Null models were used by Pounds et al. (1997) to explain the disappearances in the seemingly pristine area of Monteverde, Costa Rica. The null models were based on data from long-term studies on other amphibians from South Carolina and Cleveland County. Was estimating the probability that any one species would disappear, calculating the probability that a given number of species would disappear at the same time, and comparing the observed number of disappearances to those predicted by null models. The results led them to conclude that the observed disappearances at Monteverde (40% of the anuran fauna) are highly improbable to have occurred if populations were just fluctuating normally.

It was noted that if the null models were based on the patterns of population fluctuations of amphibians reported by Pechmann et al. (1991) in South Carolina the chance of many species disappearing form Monteverde under normal circumstances would have been less than one in a one hundred billion. Pechmann (2003) questioned these results, because South Carolina and Cleveland County studies should not be considered as representative of all the other places and other droughts.

The principal problem when population dynamics are analyzed are more biological than statistical. Expanding knowledge about the natural behavior of amphibians and metapopulation dynamics, and consequently establishing an appropriate null model, would be helpful in identifying which populations have been affected in a major or minor degree and focus preservations efforts where they are most needed (Pechmann 2003).

2.2.2. The magnitude of amphibian population fluctuations.

The revelation of the magnitude of natural variability in amphibian numbers gives a clear message that some amphibian species must deal constantly with natural declines. Pechmann et al. (1991) have shown that natural population fluctuations can easily be misinterpreted as declines, particularly if fluctuations are largely relative to the length of the data set. On the other hand, Pechmann & Wilbur (1994) suggested that populations are likely to be in decline most of the time followed by short increases after high recruitment.

However, aside trends in abundance, researchers recently asserted also the importance of understanding the levels of variation to which amphibian populations fluctuate around any trends (Marsh 2001; Green 2003; Whiteman & Wissinger 2005), that knowledge is essential to calculating both the statistical power of a monitoring program and the level of extinction risk from stochastic events, additionally provide information about the processes that drive population fluctuations and serves as base-line for monitoring program designs (Marsh 2001).

Population size variability in general is affected by a variety of different factors including extrinsic factors such as resource availability and intrinsic factors such as individual variation in survival and fecundity (Pellet et al. 2006); is also known to influence the probability of extinction for a population (Marsh 2001). Many years of data are needed before statistical analyses has sufficient power to detect changes in amphibian populations trends (Storfer 2003). Meta-analysis of long-term amphibian population's data demonstrated that even with five years of collected data, it may be difficult to estimate the statistical power of amphibian monitoring programs (Marsh 2001).

Considering the sizes of many amphibian populations, demographic stochasticity (the variable breeding success of species) may be highly important (Pechmann et al. 1991). Among demographic stochasticity, environmental stochasticity and catastrophes, Lande (1993) concluded that demographic stochasticity exerted the least influence, as the other two were independent of populations sizes. Hence, the spatial aspects of amphibian population dynamics also must be taken into account (Gardner 2001).

Marsh (2001) reviewed previous work to address how variation in demographic characteristics and habitat requirements may reflect on the comparative risk of decline in amphibians, and to comprehend the relative degrees of variance among changes in amphibian population sizes. Due to his results, pond-breeding species seemed to fluctuate more than terrestrial-breeding species. What is consistent with Green's (2003) observations; species that live in stable terrestrial environments tend to have stable populations form year to year due to greater demographic and environmental stochasticity, whereas species that live or breed in unstable or fluctuating environments tend to have populations that fluctuate to a much greater degree.

Therefore, estimates of population variability can also be used to calculate risk of extinction from environmental or demographic stochasticity (Leigh 1981). On the other hand, according to Marsh (2001) deficient estimates of population variability may lead to deficient estimates of extinction risk since these calculations are often made from demographic rates, rather than total population sizes, an increase in the variability of population size will usually correspond with an increase in the variability of demographic rates. In this respect, more long-term data and changes in the general statistical approach may be useful to solve these problems .

It was also demonstrated that amphibians exhibit large differences in population size variability between taxonomic families, between males and females, life stages, with juveniles fluctuating more than adults, and among latitudes (Marsh 2001; Green 2003; Pellet et al. 2006; Raithel et al. 2011). This variation leads to issues that biologists need to consider when designing long-term monitoring programs; more sites will have to be monitored to obtain an estimate of long-term population trends for species that exhibit large annual fluctuations among years and fluctuating populations are more vulnerable to local extinction events, and thus, are susceptible to extirpation unless recruits from adjacent ponds can help a local population to recover (Green 2003).

Because of the relationship between population size variability and extinction risk, identifying differences between populations of the same species may help to elucidate underlying causal mechanisms of variability, either natural or anthropogenic derived, which in turn can be used to fine tune conservation strategies (Marsh 2001).

2.3. Monitoring of population trends in amphibians

Amphibian populations are declining worldwide due to the direct and indirect anthropogenic extinction drivers (Wake 1991; Blaustein et al. 1994; Houlahan et al. 2000; Stuart et al. 2004; Chapter 2.1.). On the other hand, an increasing number of studies have shown that many populations and species have disappeared from even seemingly pristine habitats (Pechmann & Wilbur 1994). However, regardless of the nature of the drivers of these changes and whether they are anthropogenic or natural, worldwide there is clearly a need for conservation plans based on coordinated systems of research and monitoring that will determine and characterize significant population trends and identify the causes of declines (Blaustein et al. 1994; Meyer et al.1998; Stuart 2012).

Studies of declining amphibians should examine the effects of multiple factors that potentially affect reproductive success and survival in different habitats. Only when the factors regulating populations of threatened species are known, would be possible to understand why they decline. Hence, to ensure the success of conservation efforts, that knowledge is crucial (Beebee 1996, Nystrom, 2007). Therefore, monitoring amphibian populations and the spatial use of the habitats provide information about the factors (natural and anthropogenic) causing fluctuations and their potential role as source populations at landscape level (Hartel & Moga 2007).

Among many reasonable strategies, long-term monitoring has been recommended as the best tool to provide detailed temporal and spatial information to evaluate and build conservation plans to confront present and future challenges (Pechmann et al. 1991; Blaustein et al. 1994; Pechmann & Wilbur 1994; Storfer 2003). The strongest support for a decline is a long-term data set that has registered population levels for particular species in particular locations (Blaustein et al. 1994; Storfer 2003). However, the accumulation of numerous accounts from shorter-term studies of a variety of amphibian species in diverse habitats and geographic regions lends credence to pleas for concern about declines (Houlahan et al. 2000). Unfortunately, many amphibian populations and species that are thought to be declining have not been monitored over long periods of time, becoming short-term changes in population size difficult to assess critically (Alford & Richards 1999).

2.3.1. Long-term monitoring: importance and examples

Despite the increasing number of reports suggesting that amphibians are undergoing population decline, range reduction, and even extinction (Wake 1991; Houlahan et al. 2000; Stuart et al. 2004); as reported by Pechmann et al. (1991), supporting long-term census data remains unavailable. Only a few of the studies used in these analyses spanned more than 10 consecutive years, or involved two or more interacting species (Meyer et al. 1998; Alford & Richards 1999; Houlahan et al. 2000).

Census studies must last a minimum of 10 successive years to detect biologically meaningful trends of amphibian populations, the analysis of such time-series would provide crucial information to better identify actual population trends and may reveal some of the biotic and abiotic factors that govern the dynamics of amphibian populations, and ultimately determine population distribution and abundance (Alford & Richards, 1999; Blaustein et al., 1994; Lindenmayer & Likens, 2010).

The nature of amphibian population fluctuations has been the subject of considerable debate among ecologists (Blaustein, 1994; Green, 2003; Pechmann & Wilbur, 1994). Alford & Richards (1999) proposed that there is a tendency in amphibian population size for occasional high population recruitment followed by several years of gradual decline, in which reproductive success is low.

Amphibians naturally exhibit extreme population fluctuations, with offspring production frequently varying by several orders of magnitude from one year to the next (Pechmann et al. 1991; Pechmann & Wilbur 1994). Meyer et al.'s (1998) results of a 28 years study conducted in a pristine area in Switzerland, were consistent with this statement. Despite an overall stability, two of the three studied populations showed negative population trends most of the time that were followed by significant short-time increases during the study period. Therefore, short term amphibian declines might be part of a natural cycle of population fluctuations due to abiotic and biotic causes (Pechmann et al. 1991; Daszak et al. 2005), consequently may give a wrong impression about population trends (Meyer et al. 1998). Concluding that amphibians are suffering when they are simply fluctuating within the normal range will waste resources and credibility, which can seriously compromise future conservation attempts (Blaustein, 1994).

In light of that fact, to understand the real extent of the global amphibian decline crisis, biologists need to distinguish between natural population fluctuations and abnormal declines that may be directly or indirectly due to human actions (Pechmann et al. 1991; see Chapter 2.2.).

Long data series serve to show the range of natural fluctuations that the population size of a particular species can exhibit and they can provide testable hypotheses concerning the population's fluctuation history, including possible population declines (Meyer et al. 1998; Marsh 2001; Loman & Andersson 2007). Long-term field studies are also valuable to ecologists and conservation efforts for several other reasons. They are important for identifying factors, such as climate variables such as temperature, rainfall and droughts, that affect population dynamics and community structure (Beebee 2002; McMenamin et al. 2008; Blaustein et al. 2010).

There are a number of key papers that have highlighted the importance of phenological records in demonstrating past changes in abundances, distribution and the individualistic responses of species to climate change (Terhivuo 1988; Reading 1998; Scott et al. 2008; Griffiths et al. 2010). Using anecdotal data, Terhivuo (1988) analyzed changes in amphibian populations over 150 years in Finland to determine the relationship between the temperature and the timing of spawning and suggested that the carbon dioxide concentration in the atmosphere, that increased due to human activity, led to a rise in the mean annual temperature and simultaneously to changes in the common frog (*Rana temporaria*) timing of spawning.

The England-wide 12 years study carried out by Scott et al. (2008) lead to similar conclusions. The results suggested that the reproductive cycles of the common frog (*Rana temporaria*) are becoming earlier since the late 1970s and that this long-term phenomenon. On the other hand, Reading's (1998) study of the common toad (*Bufo bufo*) in a single pond located in south Dorset, UK showed that main arrival to breeding sites was highly correlated with mean daily temperatures over the 40 days intermediately preceding main arrival.

In North America a survey of four amphibian species (1980–1998) showed a close relationship between temperature before breeding and the timing of first breeding for four species, while only the western toad (*Bufo boreas*) displayed a tendency to breed earlier (at one site) and Fowler's toad (*Bufo fowleri*) to breed later (Blaustein et al., 2001). Gibbs & Breisch (2001) reported that, between 1900 and 1999 at Ithaca, four frog species were calling earlier and two were unchanged.

There is also evidence from Japan supporting earlier spawning in amphibians. Kusano & Inoue (2008) looked at a number of datasets ranging from 12 year to 31 year periods. This study found that the first date of spawning was advancing in correlation with the mean monthly temperature from the month just before the breeding season for three amphibian species. This is one of the first amphibian phenology studies from temperate East Asia and shows that shifts in the timing of amphibian breeding may be a global issue.

Additionally, rainfall is an environmental factor that has been shown to affect breeding population sizes of amphibian species through its effect on breeding activity and past recruitment (Semlitsch et al. 1996). The relationship between breeding by the gopher frog (*Rana capito*) and rainfall was investigated over 13 years in Alabama, USA. While no significant fluctuations were found and since these kind of studies are scarce, its importance lies in serving as a baseline for other future research programs (Jensen et al. 2003).

New evidence has recently emerged suggesting that global climate change may also play a role in disease outbreaks and subsequent population declines (Pounds et al. 2006). After years of gathering, Bosch et al. (2007) evidence recently postulated after over 20 years of monitoring that the declines and extinctions of amphibians in the Peñalara Natural Park, Spain were possibly caused by climatic changes that favoured optimal growth of chytrid fungus.

Clearly, proactive strategies include systematic long-term monitoring are needed to understand how climate change affects amphibians, and why the impacts are greater today than they were historically, most importantly can be used as an early warning system for adaptive management (Pellet et al. 2006; Griffiths et al. 2010). Long-running surveys are also valuable to evaluate changes in ecosystem structure and habitat providing base-line data for environmental management (Hossack et al. 2015; Loman & Andersson 2007). Understanding the habitat-amphibian relationship is crucial, since this vertebrate group is highly sensitive to man-induced habitat changes and has undergone alarming declines in recent years (Nyström et al. 2007).

Agricultural landscape modification has been included with urbanisation as a major driver of biodiversity loss and constitutes landscape change that affects water bodies and the amphibians that utilise them (Curado et al. 2011). A three decade study of 12 amphibian species by Curado et al. (2011) documented extensive land use change and a marked reduction in the number of ponds in France. Findings, result of intensive monitoring, led to the conclusion that man-made ponds play a crucial role for amphibian persistence in farmland for the reason that wetlands provide crucial ecosystem services for maintaining regional biodiversity and metapopulation connectivity (Marsh & Trenham 2001; Curado et al. 2011).

Because the major reason for the amphibian decline seems to be habitat loss, habitat restoration should therefore be an important counter measure (Beebee 1997). Along the lines of habitat loss, habitat restoration projects require pre and post development studies to determine success, and specifically long-term monitoring is required to evaluate sustainability of the population (Petranka et al. 2007).

A replicated, before-and-after study by Beebee (1997) in 1977–1996 of ponds on chalkland in England, UK found that pond restoration and creation resulted in an increased occupancy by amphibians but not species richness per pond. On the other hand, Pickett's et al. (2013) long-term surveys in Brickpit, Australia, showed no net loss for a habitat restoration program. Petranka et al. (2007) conducted a 13-year study to examine the persistence of amphibian populations at a geographically isolated restoration site in western North Carolina and additionally the possibility to document the frequency of outbreaks and local epidemiology of the disease over a decade, as well as other environmental stressors.

Successful implementation of habitat offset enables infrastructure projects to contribute to conservation efforts through mitigation programs, whilst long term monitoring programs to evaluate success can provide a much needed insight into the population dynamics of threatened species and communities (Pickett et al. 2013; Petranka et al. 2007). The research on habitat loss and degradation should, at best, be closely linked to practical conservation management both by improving and using the information gained by management efforts and long-term monitoring. That gap of knowledge can be highlighted by the scarcity of successful examples of habitat restoration for threatened amphibians, even when restoration has been specifically targeted to such species (Nyström et al. 2007).

Long-term monitoring data also provide information for local, regional and global comparisons; this type of study ascertains the spatial scale at which planning should occur (Petranka et al. 2004). The problems associated with the short timescale of most studies are exacerbated when spatial scale is considered (Wheeler et al. 2003). Although some local populations can dramatically decline (or even go extinct), regional densities can remain relatively stable because other populations in the same area may experience little change or growth during the same time period. Therefore, monitoring a single population over a short period of time may lead to overestimates of population instability (Pechmann & Wilbur 1994; Alford & Richards 1999).

On the other hand, year-to-year monitoring are also useful for the evaluation of changes due to ecological processes such as disturbance or environmental stochasticity and increase the possibility of discovering the results of those slow processes (Whiteman & Wissinger 2005). An 11 year study of Fowler's toads (*Bufo fowleri*) in Ontario Canada conducted by Green (1999) shows the synergy of deterministic and stochastic factors influencing population size trends. Severe storms in 1985 and 1986 swept through the area and thus with the community of tadpoles. This study gave the author the opportunity to evaluate the gradual recovery and evolution of the population.

Long-term monitoring data also provides empirical data for testing ecological theory and models (Storfer 2003). Marsh (2001) suggested, based on recent meta-analysis of 29 amphibian time series, it may be challenging to estimate the statistical power of amphibian monitoring programs even if five years of data has been collected. Besides the reasons exemplified above, multiyear data sets also generate new and important questions about population, community and ecosystem dynamics (Raithel et al. 2011); and serves as guiding evidence-based environmental legislation (Lindenmayer & Likens 2010).

Despite the obvious importance of time series analyses in this process, they are still scarce in amphibian ecology (Semlitsch et al. 1996; Meyer et al. 1998), and consequently that pronounced lack of long-term data still makes it impossible to determine the status of most amphibian populations with their complex life cycles (Blaustein et al. 1994; Houlahan et al. 2000; Whitfield et al. 2007).

3. METHODS

3.1. Meta-analysis of available long-term studies

Sample of studies

Were retrieved long-term monitoring of amphibian populations published reports available by the present time by means of various procedures:

Firstly, reports were searched in computerized databases, including Science Direct, Web of Knowledge, Springer Link, and JSTOR using the key words *long-term monitoring, amphibians, trends, population variation, and population dynamics.* These databases provided the most comprehensive coverage of the journal reports in this field.

Secondly, after identifying the core body of reports, were examined the references of these reports and other relevant reviews to retrieve additional reports that were not included initially.

Thirdly, if the full-text versions of publications were not available in the databases named above, these publications were requested directly from the authors through Research Gate, a social network that allows scientists to upload their research, collaborate and learn from each other's work (Available at: https://researchgate.net).

Finally, reports were searched in other Internet-based databases such as Google Scholar, and College of Letters and Science maintained by the University of California, Berkeley (Available at: https://ib.berkeley.edu).

Selection Criteria

The following criteria was used to select studies for inclusion in the meta-analysis:

a) Based on the knowledge that especially for amphibian monitoring programs a minimum of 10 years of data collection are reasonable in order to begin to understand population status and to measure the extent of variation, were included only long-term monitoring published reports with 10 or more years of duration, and with any usable qualitative and quantitative information on amphibian population trends.

b) Only three publications with less than 10 years on average were included, since these were the only available reports that analyzed trends of amphibian populations on a global scale.
c) Studies in which researchers analysed amphibian population trends using provided historical data were also included.

Database information processing

For descriptive purposes, the publications were distributed by spatial scale in which the long-term surveys took place (i.e., global, regional, and local scale).

Each publication was organized by (a) the year and (b) the authors of each report; as well as (c) description of the study (i.e., number of ponds visited during each study period, findings and goals resulted from performed long-term monitoring surveys); (d) the target species (i.e., monitored species such as *Rana, Salamandra, Bufo,* etc.); (e) the amphibian order (i.e., Anura, Caudata, Gymnophiona); (f) the study duration, and (g) the country in which the study was conducted (Appendices I, II, III).

Graphics were used to obtain a quick overall view of the relevant information, as well as detailed information for evaluation and comparison purposes. To determine in which geographic areas these studies were carried out the most; the reports were summarized according to the country in which they were conducted by continent (Fig. 1); to ascertain which amphibian species were taxonomically targeted the most these were counted by order of species. When the data of a report was the result of the monitoring of both, frogs and salamanders, this publication was summarized independently to get a more general view (Fig. 2); to evaluate the spatial scale in which studies were carried out the most, the studies were summarized on a global, regional and local scale (Fig. 3).

Statistical analysis

Pearson's Chi-squared tests (χ^2) were used to compare the number of studies among continents, the orders of species, and spatial scales. For these purposes, the statistical analysis were performed using the computing environment R (R Core Team 2012).

3.2. Case study: variation of the agile frog (*R. dalmatina*) population fluctuation

Study site

The study area is located in the North Bohemian Brown Coal Basin (50°35'N, 13°35'E). Covering an area of approximately 7 km^2, the Hornojířetínská spoil bank is one of the largest in the Czech Republic. Due to the fact that half of the spoil bank has been technically modified, there is a marked reduction in the number of ponds in this area. On the other hand, the other half is an area unspoilt by major human activity where more than 300 waterbodies have been identified (Doležalová et al. 2012; Appendix IV).

The developed vegetation in this pristine area that include scattered shrubs, trees, and light forest (Prach, 1987; Prach et al., 1999), as well as the considerable number of possible breeding sites and a suitable terrestrial environment, provides valuable habitat for amphibian species, including a large agile frog's (*Rana dalmatina*) population (Doležalová et al. 2012; Appendix IV).

Study species

The agile frog (*Rana dalmatina*) is a widely distributed species common in the southern and central part of Europe (Arnold & Ovenden 2004). *R. dalmatina* male species are regarded as a breeding territorial and monogamous, for that reason, each female lays one well-separated and distinguishable egg clutch per breeding season, therefore, population size can be easily estimated using egg mass counts (Lesbarreres & Lode 2002; Hartel et al. 2009).

Data collection

Each of 134 ponds were visited every consecutively year from 2005 to 2015 during breeding season, during this period, egg mass counts were conducted to estimate the abundance of the agile frog (*Rana dalmatina*) in the Hornojířetínská spoil bank.

To achieve the requirements of this bachelor thesis, I have participated in the field work and data collection during one breeding season in 2015.

Sample of studies

For this study were also retrieved long-term monitoring of amphibian populations published reports available by the present time by means of the following criteria:

a) Were retrieved only long-term monitoring published reports with 8 or more years of duration, and with any usable qualitative and quantitative information on amphibian population trends.
b) Only long-term monitoring published reports of frogs species (i.e., *Rana*) populations were included in the research.
c) Studies in which researchers analysed amphibian population trends using provided historical data were also included.

This selection criteria led to a database of 25 long-term monitoring publication reports that subsequently served as background for the analysis of the 11-years monitoring of the agile frog (*Rana dalmatina*).

Statistical analysis

Pearson's Chi-squared tests (χ^2) were used to compare whether clutch numbers in 134 permanently monitored ponds differ over years and to compare pond occupancy of the agile frog (*R. dalmatina*). Simple linear regression was used to ascertain the existence of linear trends in both clutch numbers and pond occupancy (dependent variables) during the study period (independent variable). Pairwise Spearman rank correlation coefficients and Pearson's product-moment correlation were calculated for both clutch numbers and pond occupancy. For these purposes, the statistical analysis were performed using the computing environment R (R Core Team 2012).

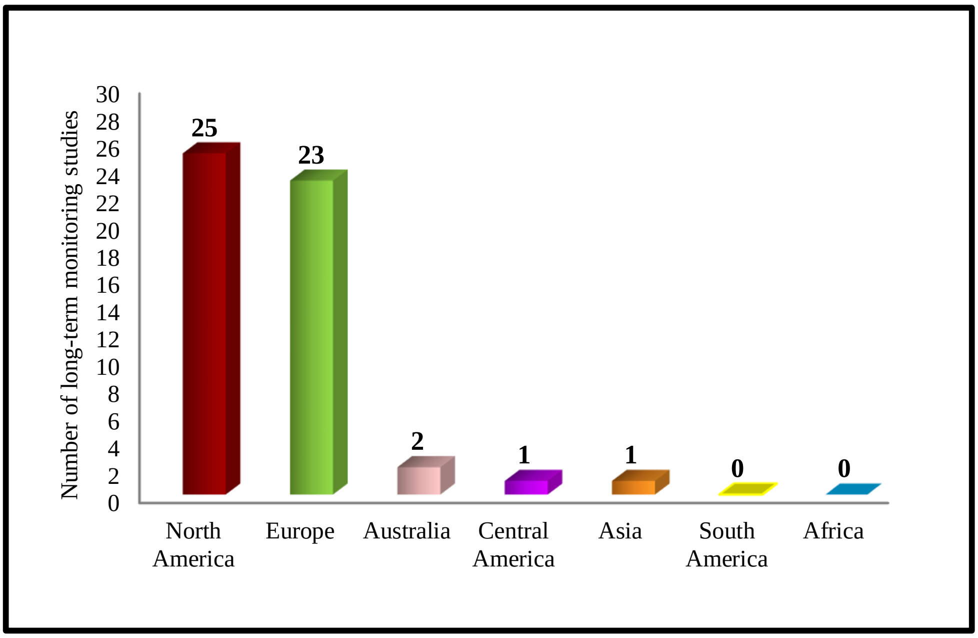
4. RESULTS

4.1. Meta-analysis of available long-term studies

Comparisons among continents

A total of 55 long-term monitoring publications reports listed in Appendices I, II, III, were included in the study. Significant statistical difference was found in the number of studies conducted in each continent (χ^2 = 115.04, df = 7, $p < 10^{-6}$). Long-term monitoring studies are fairly well developed in North America and Europe, but absent in Australia, Central America, South America, Asia and Africa (Fig. 1).

Fig. 1 Number of long-term studies of amphibian populations by continent.



Comparisons among amphibian groups

Similarly, significant statistical difference was found among targeted taxonomical orders (χ^2 = 46.1, df = 2, $p < 10^{-6}$). Attention has been focused principally on anurans (Fig. 2) in a lesser degree in salamanders, and there was not found any long-term monitoring reports about caecilians.

Comparisons among amphibian groups

Significant difference was also found in the spatial scale in which the studies were conducted (χ^2 = 37.7, df = 2, $p < 10^{-6}$). Long-term monitoring studies were carried out more at the local scale, and only a few long-term studies of amphibians have focused on entire communities at larger scales (Fig. 3). According to the results, regional monitoring studies, as well as worldwide research are lacking.

Fig. 2 Number of orders of species targeted in each long-term report.

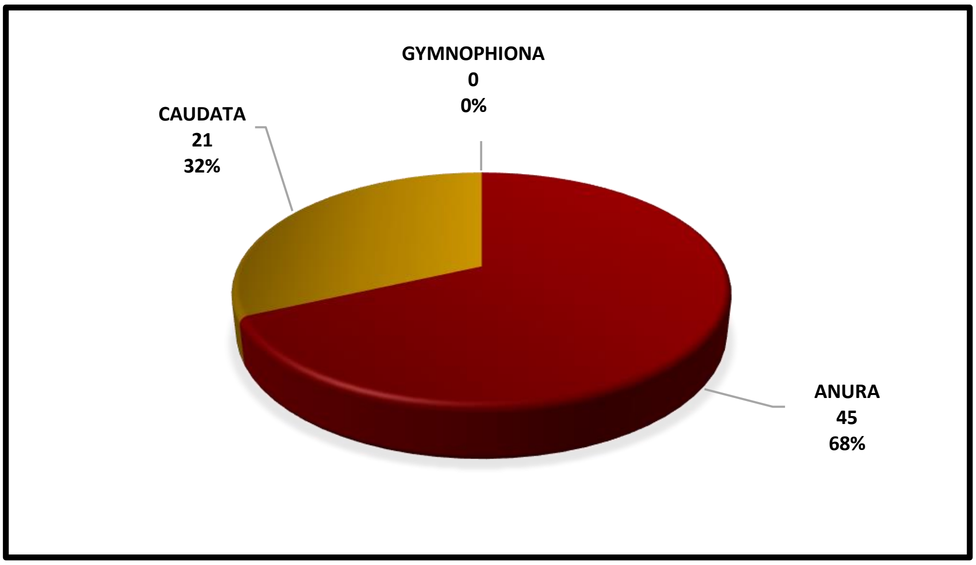
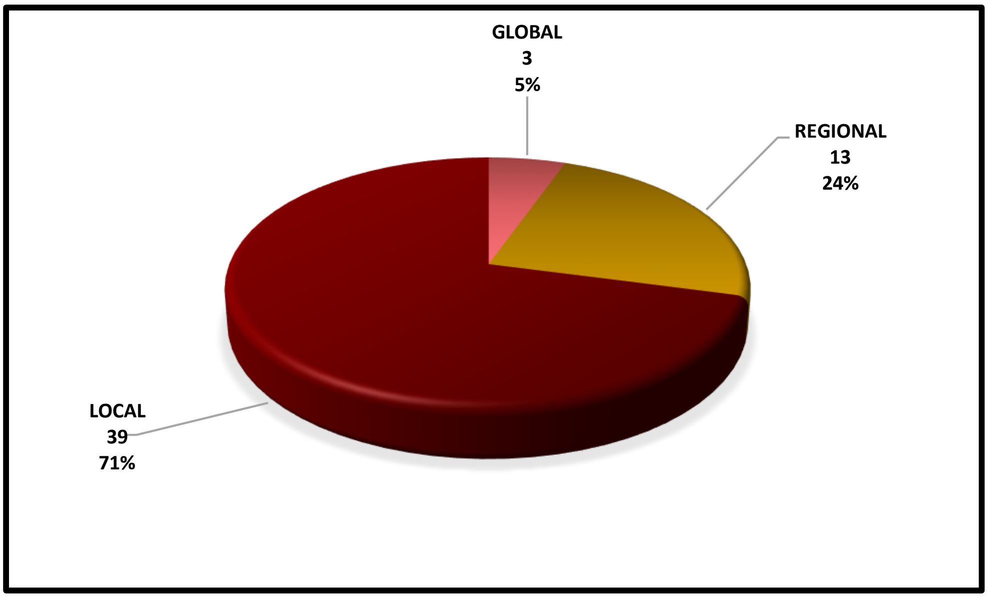


Fig. 3 Number of studies at the local, regional and global scale.

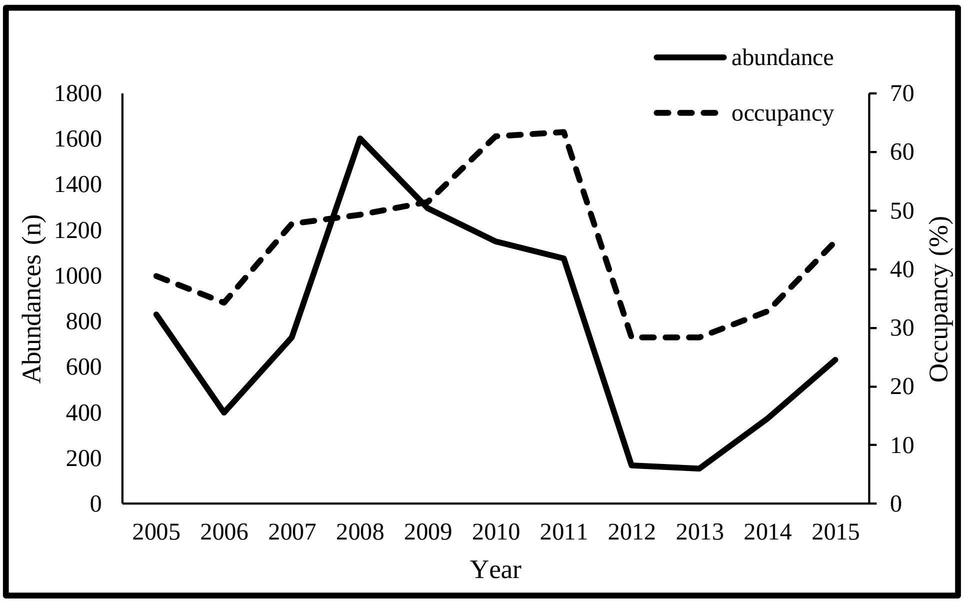


4.2. Case study: variation of the agile frog (*R. dalmatina*) population fluctuation

It was found a total of 8410 clutches during the 11 years monitoring of the agile frog (*R. dalmatina*). The number of clutches annually counted showed considerable statistical differences among years (χ^2= 2965, 69, df = 10, $p < 10^{-6}$), and the total number of clutches showed a variation of more than tenfold (10.34×) during the study period (mean ±SD: 765 ± 417 clutches, min. = 155, max. = 1,603). On the other hand, no linear trend was observed (F = 1.47, df = 1, p = 0.26; Fig. 4; Appendix IV).

In the same way, the number of ponds with the presence of the agile frog (*R. dalmatina*) clutches showed an important variation during the study period (χ^2= 47, 68, df = 10, $p < 10^{-6}$), without any linear trend (F = 0.22, df = 1, p = 0.65; Fig. 4; Appendix IV). The total clutch numbers correlated positively with the numbers of colonized ponds (r = 0.80, 95% CI for r = 0.39–0.95, t = 4.06, df = 9, p = 0.003).

Fig. 4 Rana dalmatina clutch numbers (Abundance) and the proportions of ponds occupied by the species (Occupancy) within 134 permanently monitored ponds on Hornojiřetínská spoil bank during 11-year study period (2005–2015)



5. DISCUSSION

5.1. Meta-analysis of available long-term studies

Widely reported declines in amphibian species in many regions of the world have necessitated field studies conducted in order to assess the importance of environmental factors in determining habitat use and population fluctuations of amphibians (Beebee 1997). Despite the growing amount of research investigating ultimate causes of amphibian declines, a noticeable lack of long-term data still makes it impossible to determine the status of most populations (Houlahan et al. 2000). Therefore, it is crucial to implement monitoring programs that are sufficiently robust to detect trends that account for natural fluctuations in populations (Pechmann & Wilbur 1994; Meyer et al. 1998; Storfer 2003).

Comparisons among continents

A total of 55 long-term monitoring reports with more than 10 years duration were summarized and analysed in this bachelor thesis. The results indicate that long-term surveys for amphibian populations are currently very advanced in North America and Europe (Beebee 1996), but absent in Central America, South America, Asia and Africa (Stuart et al. 2004).

While the summarized studies from Australia are underrepresented, it is known that different amphibian survey and monitoring programs are underway in Australia where several databases on species and population time series are available or in development (Wilkinson). Although there is evidence that amphibian populations are globally declining, contributions to the Houlahan et al.'s (2000) extensive review of studies of 936 amphibian populations worldwide, were mainly from scientist based in the United States, Europe, and Australia.

Amphibian population data from the tropical regions of Africa and Asia are limited and there are no reported die-off or enigmatic population declines form either area (Weldon et al. 2004). Thus, the threat status and population trends of amphibian populations on these continents remain unknown (Stuart et al. 2004). There is also a paucity of long-term well-documented studies in Latin America (but see Whitfield et al. 2007), limitations are mainly due to fact that densities of many species, especially those that inhabit primary forests, are low or at least the species are observed very infrequently (Pearman et al. 1995). This makes reliable estimation of population a very time-consuming and expensive matter (Houlahan et al. 2000).

Comparisons among amphibian groups

On the other hand, most of the available data on fluctuations of amphibian populations pertain to frogs (Fig. 2), probably due to their greater diversity and worldwide existence, anurans are seasonally active that arrive over restricted periods of time at breeding sites where they congregate in greater densities than at any other time of the year, which give the researchers the possibility to record their arrival and abundance, and detect their breeding activity (Blaustein et al. 1994; Pechmann & Wilbur 1994). Salamanders have been observed to a lesser extent (e.g. because of their secretive nature and nocturnal lifestyle, are one of the least studied groups of animals (Vitt & Caldwell 2013). Even though caecilians are potentially as threatened as frogs and salamanders; their largely tropical distribution, subterranean habitats, and taxonomic instability that lead to difficulties in identification make it complicated to obtain data concerning their status (Gower & Wilkinson 2005).

Comparisons among spatial scales

It has been emphasized that conservation efforts need research at the landscape level that focus on the ecological value of nature preserves (Storfer 2003). However, judging by the results, few long-term studies of amphibians have focused on entire communities on large scales (Fig. 3). Most of the studies have been focused on the local scale while studies assessing trends at regional and global scale are scarce, and therefore, temporal scales integrated within spatial scales are lacking (Cody 1996).

It is difficult to estimate amphibian declines from a global perspective from individual research studies. The most recent reports that have attempted to draw wide conclusions are Alford &Richards (1999), Houlahan et al. (2000) and Stuart et al. (2004). The first, collected data over the period 1951-1997 and found amphibian populations decreased more than their model predicted. However, there was no evidence that the agents of decline were becoming more prevalent over time. Houlahan et al. (2000) used data from 936 populations to assess variations in amphibian population trends on a global scale. Unfortunately, their results indicate relatively rapid declines from the late 1950s to the late 1960s, but a reduced rate of decline was observed to the present.

There has been a debate between the two groups of scientists, Alford et al. (2001) argued that by emphasising the global mean, Houlahan and his colleagues have masked spatial and temporal variation in amphibian population trends. On the other hand, according to Houlahan et al. (2001) this is a problem emphasised by many herpetologists and can only be minimised if a long time series of monitoring data is provided. Finally, Stuart et al. (2004) found that 43 percent of the amphibian species are experiencing some form of population decrease, 32.5 percent of the species are globally threatened, and that 122 species are possibly extinct; most of those losses are recent, having occurred since 1980.

Even though, these extensive reviews of amphibian populations status worldwide averaged less than 10 years in length, they were included in this study because were the only large scale studies that suggested that amphibian declines were a global phenomenon and demonstrated that the declines had been occurring at a global scale over the last decades. These studies are important because they highlighted the urgent need to identify formally those amphibian species that were declining and needed conservation attention.

5.2. Case study: variation of the agile frog (*R. dalmatina*) population fluctuation

The studied agile frog (*Rana dalmatina*) population fluctuated more than 10-fold over the 11-year study period, while other Central European pond-breeding ranid frogs (*R. temporaria*, *R. dalmatina*, and *R. arvalis*) fluctuated in a lesser degree over the same time study (see Appendix IV). The ranges of fluctuation determined in these studies did not increase with monitoring duration, which disagree with Pechmann & Wilbur's 1994 assumptions.

While there have been studies on amphibian population trends (e.g., Marsh 2001; Green 2003), and that there is an increasing suggestion that amphibian declines may be nothing more than natural fluctuations (Alford & Richards 1999); there is still a paucity of long-term studies of amphibian populations describing the magnitude of its fluctuations (Pechmann & Wilbur 1994).

Over the years of study the population size decrease more than increase (Fig. 4), this trend agreed with Alford & Richards (1999) statement that proposed that there is a tendency in amphibian population size for occasional high population recruitment followed by several years of gradual decline, in which reproductive success is low**.**

Although, the number of ponds with the presence of agile frog clutches strongly correlated with the total abundance, these variables did not follow the same trend over years. While during some years has been a decrease in the overall numbers, the percentage of occupied sites increased. These facts point to the complicated structure of the agile frog (*R. dalmatina*) population in a post-mining habitat.

5.3. Recommendations

Long-term monitoring of amphibian populations is crucial and must be supported by the establishment of standard methods and techniques. In the same way, it is important that the academic community, land managers, and conservation organizations realize that exhaustive field programs focusing on the distribution, abundance, status, and fluctuations of amphibian populations and species are needed especially in the countries where little is known about amphibian population's status.

More long-term monitoring programs on large scales are needed because regional persistence is probably governed at the spatial scale much larger than that of a single site. Without these long-term monitoring reports the understanding of how ecological communities function is limited, as well as the detection of the losses of amphibian populations that have resulted from the anthropogenic changes. When long-term and widespread monitoring becomes a standard part of environmental assessment programs, declines are likely to become less ambiguous and the causes less enigmatic.

6. CONCLUSIONS

In recent years, there has been a drastic decline in the number of amphibians around the world, and most of the species are currently listed as threaten (IUCN, 2012) therefore, finding solutions to counter amphibian declines and extinctions is one of the greatest conservation challenges of the century. Nonetheless, any attempt to achieve an understanding of the potential factors affecting amphibian populations is important to ameliorate their impact on amphibian populations and their habitats.

Quantifying the direct and indirect effects of habitat destruction or alteration, environmental pollution, diseases, and global climate change is complicated, and will be more challenging to change in the short term. In the same way, the fact that ecological communities constantly experience temporal turnover, and that consequently some species will not only fluctuate markedly but also become apparently either locally or globally extinct, makes it difficult to use short-term studies as a base-line for deciding if a population is increasing or decreasing in the long term.

Long-term monitoring of amphibian populations are needed to achieve the understanding of how the causes affecting amphibian populations operate independently and synergistically. However, a variety of studies are now collectively giving a much better overview of the numbers and how amphibian population and species are changing.

The literary review of the main causes of amphibian population declines and dynamics of the amphibians *per se*; the meta-analysis of the available long-term publication reports of amphibian population trends, as well as the active involvement in field work, data-collection and the preparation of a manuscript of 11-year monitoring of the abundances of the agile frog (*Rana dalmatina*) are the main purposes of this bachelor's thesis.

Meta-analysis of long-term monitoring reports showed that long-term data sets on species and population time series are currently available or in development. Unfortunately, amphibian databases with population time series have far less information for the tropics, Africa, and Asia compared with Europe and North America, which are well studied regions herpetologically (Appendices I, II, III; Fig.1).

In addition, salamanders and caecilians are both poorly represented in the current available long-term monitoring reports, probably due the fact that in some cases, the nature and habitats of the species themselves, make it harder to monitor trends in their population size (Appendices I, II, III; Fig. 2). In the same manner, there is a paucity of long-term monitoring at larger scales, most of the studies have been focused in the local scale, while regional and global scale remain scarce (Appendices I, II, III; Fig. 3).

The results of 11-year monitoring of populations in 134 ponds in Hornojiřetínská spoil bank in the Most Basin in the Czech Republic of the agile frog (*Rana dalmatina*) clutches, showed that the overall number of *R. dalmatina* clutches varied more than 10-fold within the study period, despite the fact that decreases in the number of clutches surpass increases, the data did not show any trend. On the other hand, the number of clutches and occupied ponds were positively correlated.

With this 11-year study, a reference database was created that will be useful in tracking further changes in the dynamics of these populations. The results led to conclude that amphibian population sizes may vary naturally during certain period of time without any effect on long-term population trends.

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APPENDICES

Appendix I. Studies documenting trends of amphibian populations at the global scale.

Appendix II. Long-term studies (> 10 years consecutively) documenting trends of amphibian populations at the regional scale.

Appendix III. Long-term studies (> 10 years consecutively) documenting trends of amphibian populations at the local scale.

Appendix IV. Submitted manuscript of 11-years monitoring of the agile frog (*Rana dalmatina*) for the journal Current Zoology.