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**IMPACTS OF CLIMATE
CHANGE ON AMPHIBIANS:
PAST DECLINES,
PREDICTED TRENDS, AND
FUTURE PERSPECTIVES**

Tutor: Prof. Marco A. Bologna



DOCTORAL SCHOOL IN BIOLOGY
SECTION: Biodiversity and Ecosystem Analysis
XXIII CYCLE, A.A. 2010/2011



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**Impacts of climate change on amphibians:
past declines, predicted trends, and future
perspectives**

**Impatto dei cambiamenti climatici sugli anfibi:
declino recente, trend previsto e prospettive future**

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A.A. 2010/2011

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“Environments are forever changing [...] with relative rapidity they circulate about over the surface of the earth, and the species occupying them are thrust or pushed about, herded as it were, hither and thither.”

Joseph Grinnell, 1924, Ecology 2:113-224

“Because of the rapid rate of warming, many species will be unable to adjust. Historically, organisms have responded to climate change by shifting their distributions. In today's world, physical changes wrought by humans constrain such response”.

Amphibian Conservation Action Plan, IUCN 2007

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SUPPLEMENTARY MATERIALS

Appendix A: Materials and Methods of Chapter 2 - Global warming and biodiversity: Evidence of climate-linked amphibian declines in Italy

Appendix B: Materials and Methods of Chapter 3 - Human-provoked amphibian decline in central Italy and the efficacy of protected areas

Appendix C: Materials and Methods of Chapter 4 - Will climate change reduce the efficacy of protected areas for amphibian conservation in Italy?

Other papers arising from the PhD researches:

Appendix D: Within-*taxon* niche structure: Niche conservatism, divergence and predicted effects of climate change.

ABSTRACT

Mounting evidences indicate that global climate is changing, that biological responses to warming are under way, and that current conservation strategies will need to be revised for being effective in the face of future climate change. Because temperature and moisture affect multiple aspects of amphibian biology, this class of vertebrates is especially sensitive to the alteration of global climate. Amphibians are particularly interesting because they decline more rapidly than either birds or mammals and are already threatened by a plethora of interacting human-caused factors including pathogens, exotic species, pollution, habitat destruction, and ultraviolet radiation. In this context, the overall goal of my Ph.D. thesis was to give a contribution for clarifying the effects of climate change on amphibian diversity. I mainly focused on Italian fauna to address the following objectives: (i) studying the contributions of climate change to amphibian past declines; (ii) predicting the potential range shifts due to future changes in Italian climate, and (iii) evaluating whether the most important areas for amphibian long term conservation are included in the Italian reserve network. Additionally, this thesis aimed at (iv) examining the implications of within-*taxon* variation in niche occupancy for predictions of the effects of climate change. This thesis is structured along a number of independent studies that, taken together, provide a broad picture of current and potential effects of climate change on amphibian diversity.

To assess the effects of past climatic variation on amphibians in Italy I conducted a multi-factorial study based on the spatial patterns of recent declines, testing contemporarily several potential culprits. Focusing firstly on the national extent, I utilized amphibian data with 10 Km resolution, and I demonstrated that complex influences of several factors are associated with amphibian population declines and vary across species and areas. At the same time, the study revealed that, while various factors have contributed to declines, climate change has been a major cause of population disappearances for multiple amphibian species in past decades. Furthermore, moving to a smaller extent and increasing the resolution of the study a general agreement was evidenced both in terms of factors interactions and of climate change importance. In fact, all species identified as influenced by climate variations at the regional level were also associated with at least one climatic variable at the

national level. The climatic shifts were important even for those species with the lowest rates of decline, suggesting that these species may be quite vulnerable to ongoing climatic changes in the region.

IPCC projections forecast changes in the global climate during the 21st century even larger than those observed during the 20th century. Biological responses will likely be more pronounced because climate is projected to change faster than in the recent past. Considering climatic projections for mid 21st century, I forecasted amphibian range shifts in Italy through an ensemble niche modelling exercise, utilizing two carbon emission scenarios (A1FI and B1) and two dispersal assumptions. Under all the future settings, range modifications are predicted for every amphibian species. Predictions under different emission scenarios and dispersal assumptions agree in indicating that the species most sensitive to climate change are *Pelobates fuscus*, *Salamandra atra*, and *Triturus carnifex*. As reduction of distributional area is consistently a good predictor of extinction risk, my forecasts suggest increasing danger for these species by the middle of the current century.

As species range adjustments take place, protected areas may lose their importance for the protection of some habitats and populations in the future. Based on the predicted distributions under future alternative climatic and dispersal scenarios I analyzed the efficacy of the Italian reserve network for protecting multiple amphibian species, considering both nationally designated areas and Natura 2000 sites. Gap and irreplaceability analyses demonstrated that the Italian reserve network incompletely represents current amphibian diversity and its geographic pattern. This inadequacy will get even worse on the long-term, as climate change will decrease the amount of suitable range falling into reserves for many species, regardless of the assumptions about dispersal. By analyzing the spatial pattern of conservation value, I identified some currently unprotected areas that have high irreplaceability scores for species conservation and that maintain their importance under all the considered future scenarios.

If predictive modeling of species distribution is to be useful for estimating extinction risk under climate change, we need to ensure that we use the best methods available. Species are made up of local populations that may be locally adapted to different portions of their range. Geographic variation in environmental tolerances suggests

that regional populations should be modeled to capture these environmental dependencies. The analyses presented in this thesis explore the implications of within-*taxon* variation in niche occupancy for predictions of the effects of climate change. The subclade models capture the climate-distribution relationship for groups of populations with similar evolutionary histories and shared selective regimes. In comparison to models that ignore sub-*taxon* structure, models that incorporate this structure generally predict larger areas of suitable conditions, consistently perform better, and can reveal divergent potential effects of climatic variation on sub-*taxa*. This richness of additional detail in the potential response of *taxa* and sub-*taxa* to climate change can be relevant for conservation purposes.

In sum, the results of this thesis reveal that shifting climatic conditions are already affecting amphibian communities. This represents important evidence of global warming's impacts on amphibians in a region where declines have nearly always been ascribed to habitat loss. Future threats can derive from the predicted changes in the global climate during the 21st century. The existing protected area network provides a precious service in shielding habitat from destructive use and hence in reducing biodiversity loss. However, this research clearly demonstrates that the network is still far from complete. The most valuable areas for amphibian conservation highlighted in this thesis can direct reserve designation policy to efficiently protect species both in current and future conditions. Such measures are essential in preventing a further loss of amphibian diversity. Finally, this thesis suggests that the incorporation of phylogeographic information into modeling exercises can contribute considerably to an improved theoretical understanding of responses to climate forcing, and to the forecasting of future changes. With regard to conservation, this can help preserving biodiversity both at specific and genetic levels in a rapidly changing world.

RIASSUNTO

Il riscaldamento globale sta producendo importanti conseguenze sulla biologia di numerose specie animali e vegetali e sulla complessa rete di interazioni che intercorrono fra loro. In tale contesto emerge la necessità di riesaminare le attuali strategie di conservazione per poterne assicurare una efficacia a lungo termine. Poiché gli anfibi dipendono dall'ambiente acquatico per la riproduzione e per il mantenimento dell'omeostasi interna, l'aumento della temperatura ambientale e la generale diminuzione delle precipitazioni annuali possono avere un'influenza profonda sulla biologia delle popolazioni, rendendo questa classe di vertebrati straordinariamente sensibile alla variazione del clima. Gli anfibi rivestono un grande interesse conservazionistico poiché a livello globale stanno subendo un declino più veloce di altri vertebrati, come uccelli e mammiferi. Inoltre, essi sono soggetti a numerose altre minacce tra loro interagenti, come l'insorgenza di patologie infettive, la competizione con specie invasive, la distruzione dell'habitat, l'inquinamento e l'aumento delle radiazioni ultraviolette. In questo ambito, il principale obiettivo della mia tesi di dottorato è quello di fornire nuovi strumenti per valutare gli effetti dei cambiamenti climatici sulla diversità di anfibi. Prendendo in considerazione la fauna italiana, ho perseguito i seguenti obiettivi specifici: (i) determinare se la variazione del clima osservata negli scorsi decenni abbia contribuito al declino delle popolazioni di anfibi; (ii) stimare il potenziale spostamento degli areali specifici in risposta ai cambiamenti climatici previsti per la metà del 21° secolo; (iii) valutare se le aree più importanti per la conservazione a lungo termine della batracofauna siano già incluse nel *network* nazionale di aree protette. Un ulteriore obiettivo è stato quello di (iv) verificare se l'inclusione di informazioni sulle relazioni evolutive dei *taxa* possa consentire di migliorare l'efficienza e l'affidabilità dei modelli elaborati per la predizione degli impatti dei cambiamenti climatici.

Innanzitutto è stato condotto uno studio basato sul pattern spaziale del recente declino delle popolazioni di anfibi in Italia, testando allo stesso tempo numerose cause potenziali. L'analisi condotta a livello nazionale si è basata su dati ad una risoluzione di 10 km. I risultati ottenuti hanno identificato i fattori associati al declino delle diverse specie. Tra questi fattori, il cambiamento climatico è emerso come uno degli elementi più importanti. Inoltre, prendendo in

considerazione una regione più ristretta (Lazio, Italia centrale), ma utilizzando un set di dati a più alta risoluzione, sono stati ottenuti risultati paragonabili. Tutte le specie influenzate dal cambiamento climatico a livello regionale, erano associate ad almeno una variabile climatica anche a livello nazionale. La variazione dei parametri climatici è risultata significativa anche per quelle specie caratterizzate dai più bassi tassi di declino locale: ciò lascia ipotizzare che i futuri, più accentuati, cambiamenti climatici possano determinare una accelerazione del declino anche di queste specie.

Secondo vari modelli climatici elaborati dall'IPCC, l'alterazione del clima nel 21° secolo sarà maggiore e più veloce di quella registrata nel 20° secolo. Le risposte biologiche a tali cambiamenti saranno di conseguenza più pronunciate. Nel presente studio sono stati elaborati dei modelli di idoneità ambientale per stimare i potenziali cambiamenti nella distribuzione degli anfibi in Italia sulla base di due scenari climatici attesi per la metà del 21° secolo. Per ciascuna specie sono stati utilizzati differenti algoritmi di calcolo per stimare la risposta più probabile e sono state considerate due alternative relative alla possibilità di dispersione. Le predizioni ottenute in questa fase, relative ai possibili scenari alternativi considerati, hanno mostrato che la distribuzione degli anfibi in Italia subirà modificazioni più o meno drastiche. I modelli matematici concordano nell'indicare che le specie più vulnerabili al cambiamento climatico saranno: *Pelobates fuscus*, *Salamandra atra*, e *Triturus carnifex*. Poiché la riduzione dell'areale è considerato un buon indicatore del rischio di estinzione, le elaborazioni ottenute indicano una crescente minaccia per la salvaguardia di queste specie già a partire dalla metà del 21° secolo.

Il potenziale cambiamento nella distribuzione delle specie, potrebbe modificare nel futuro l'importanza delle aree protette. In base ai modelli, è stata condotta un'analisi dell'efficacia delle aree protette per la conservazione a lungo termine delle popolazioni di anfibi, considerando sia le aree designate al livello nazionale, sia quelle appartenenti al *network* europeo Natura 2000. I risultati ottenuti dalle analisi *gap* e di *irreplaceability*, hanno mostrato che il *network* Italiano di aree protette non garantisce una completa protezione della diversità di anfibi nelle condizioni attuali. Questa carenza si aggraverà verosimilmente nel lungo termine, indipendentemente dalla possibilità di dispersione. Sono state inoltre identificate le aree

indispensabili per la efficace conservazione della batracofauna italiana, le quali non sono attualmente soggette a regimi di tutela ambientale. Tali aree mantengono un'elevata importanza in tutti gli scenari futuri considerati.

L'uso di modelli matematici predittivi per stimare la vulnerabilità delle specie ai cambiamenti climatici, richiede l'utilizzo delle migliori tecniche disponibili. Poiché le specie sono costituite da diverse popolazioni che potrebbero presentare degli adattamenti locali, ciò suggerisce la necessità di elaborare dei modelli di distribuzione potenziale separatamente per ciascuna linea evolutiva. Sulla base di differenti set di dati, sono state analizzate le differenze tra modelli elaborati tenendo conto o meno dell'appartenenza a differenti linee filetiche, in termini di predizioni relative alle condizioni presenti e future. In confronto ai modelli formulati senza tener conto del contributo differenziale di sub-*taxa*, quelli che invece considerano tale contributo generalmente predicono aree più ampie di condizioni idonee, hanno una migliore performance e possono rivelare risposte differenti dei sub-*taxa* al cambiamento climatico. Tali progressi nella comprensione della risposta potenziale dei *taxa* e dei sub-*taxa* alla variazione del clima possono essere molto importanti per la programmazione di azioni di tutela a lungo termine.

In sintesi, i risultati ottenuti dalla presente tesi mostrano che l'alterazione delle condizioni climatiche ha già provocato il declino di numerose specie di anfibi in Italia, in un'area in cui il declino degli anfibi era sempre stato attribuito solo all'alterazione degli habitat. Gli effetti negativi della variazione del clima si manifesteranno ancor più nel futuro. L'attuale sistema di aree protette è fondamentale per garantire la salvaguardia della biodiversità, nonostante ciò non tutte le aree importanti per la tutela degli anfibi sono comprese nel *network* italiano. La creazione di nuove zone di tutela nelle aree evidenziate come altamente insostituibili, risulta particolarmente importante per la protezione a lungo termine della batracofauna in Italia. Infine, un notevole progresso nella nostra comprensione degli effetti potenziali del cambiamento climatico sulla distribuzione delle specie, può derivare dall'inclusione di informazioni sulla sub-struttura filogenetica dei *taxa*. Tale innovazione può aiutare a preservare la diversità genetica e la conservazione di specifiche linee evolutive in un ambiente soggetto a sempre più rapidi mutamenti.

Preface

This thesis encompasses a common introduction, four independent researches, and a section of general conclusions. In the common introduction I clarify the overall design of the thesis and trace out a *fil rouge* that links the following chapters. Chapter 2, 3, 4, and Appendix D are based on papers published or in press in peer-reviewed journals. In particular:

Chapter 2. This chapter bases on the work published in Biological Conservation: D'Amen M., Bombi P., 2009. *Global warming and biodiversity: Evidence of climate-linked amphibian declines in Italy. Biological Conservation* 142, 3060-3067.

Chapter 3. This chapter bases on the work accepted for publication by Wildlife Research: D'Amen M., Pietrangeli B., Bologna M.A., *In press. Human provoked amphibian decline in central Italy and the efficacy of protected areas. Wildlife Research.*

Chapter 4. This chapter bases on the work accepted for publication by Biological Conservation: D'Amen M., Bombi P., Pearman P.B., Schmatz D.R., Zimmermann N.E., Bologna M.A., 2010. *Will climate change reduce the efficacy of protected areas for amphibian conservation in Italy? Biological Conservation* doi:10.1016/j.biocon.2010.11.004

Appendix D. This section bases on the work accepted for publication by Ecography: Pearman P.B., D'Amen M., Graham C., Thuiller W., Zimmermann N.E., 2010. *Within-taxon niche structure: Niche conservatism, divergence and predicted effects of climate change. Ecography* doi: 10.1111/j.1600-0587.2010.06443.x

In the final section I utilize the out comings of these papers for drawing conclusions on the general problem of climate change effects on amphibian diversity.

For brevity, the Material and Methods sections of the Chapter 2, 3 and 4, are reported as Appendices in the Supplementary Materials.

General Introduction

1.1 Biological consequences of climate change.

Warming of the climate system is unequivocal, as is now evident from observations of increases in global average air and ocean temperatures, widespread melting of snow and ice, and rising global average sea level (IPCC, 2007). The increase over the 20th century of mean surface temperature (by about 0.6°C) is greater than the change for any other century in the past one thousand years. Simulation studies individuated anthropogenic activities as proximate cause of such alterations (e.g. Santer *et al.*, 2003). Climate change can include shifts in patterns of precipitation across the landscape or shifts in the severity and frequency of drought and flood cycles, increases in the range of minimum and maximum daily air temperature, reduction in cloud cover (especially at high elevations), and altered timing of seasonal temperature changes (IPCC, 2007). Continued emissions at or above current rates would cause further warming and induce many changes in the global climate system during the 21st century that would very likely be larger than those already observed (IPCC, 2007).

Climate change has already been observed in Europe. Temperatures have on average increased by 0.9°C from 1901 to 2005, together with changes in precipitation regimes, wind patterns and an increase in extreme events (Alcamo *et al.*, 2007). In particular, all over Italy a number of climate studies described a positive trend for mean temperature, a reduction of number of wet days, and an increase in precipitation intensity during the last century (e.g. Brunetti *et al.* 2004, 2006; Toreti and Desiato, 2008). Moreover, projections for the 21st century indicate that the European mean annual temperature is likely to increase by 2.2°C to 5.3°C according to IPCC scenarios (Nakićenović and Swart, 2000), and annual precipitation is very likely to decrease in the Mediterranean region mostly in the summer (Christensen and Christensen 2007; Christensen *et al.*, 2007).

The changes in global climate come at a cost to natural systems. There is growing evidence that climate change will become one of

the major drivers of species extinctions in the 21st Century (Foden *et al.*, 2008). When the modification of the environment due to the alteration of climatic conditions, exceeds the species' niche tolerance, species may be forced to respond in different ways. A rising number of peer-reviewed papers documents such phenologic, morphologic, behavioral or genetic alterations (e.g. Bradshaw and McNeilly, 1991; Huges, 2000; Parmesan, 2006; 2007). Moreover, changes in the places where species are found can be detected, due to the dispersion towards newly suitable conditions (e.g. Parmesan *et al.*, 1999; Huges, 2000; Walther *et al.*, 2002; Parmesan, 2006). For some species this means moving to cooler climates, often up-hill or southward. Such changes will also be affected in unpredictable ways by the anthropogenic alteration of land, other interacting species, and the availability of suitable habitats. When neither adaptation nor shifting range is possible, extinction is a likely scenario (Rosenzweig *et al.*, 2007). Besides, a direct impact of changing climate on one species could lead to an indirect impact on another: the alteration of inter-specific relationships would affect the community stability and composition (e.g. Huges, 2000; Parmesan, 2006, 2007).

As well as affecting species and ecosystems directly, climate change will also influence other major threats to biodiversity (e.g. soil sealing, alien species, and bushfires). Changes in these other threats may have a greater impact than climatic shifts alone (Brown *et al.*, 2008). These pressures may interact amongst themselves and interact with climate change, to produce further indirect effects (Mitchell *et al.*, 2007). Protecting biodiversity from these combined outcomes will be challenging. Monitoring schemes are needed to evaluate the rate and direction these changes are taking. Dispersal corridors and new conservation planning tools for selecting, managing, and monitoring protected areas should be further developed (Hannah *et al.*, 2002; Araújo *et al.*, 2004; Heller and Zavaleta, 2009). In addition, in many situations there will be trade-offs between aspects of biodiversity and important economic and social values (Dunlop and Brown, 2008).

1.2 Global amphibian decline

As a part of a general biodiversity crisis, amphibians are facing an imminent extinction emergency (Wake and Vredenburg, 2008). Since 1970, scientists have observed precipitous population declines

(e.g. Pounds and Crump 1994; Young *et al.*, 2001; Ron *et al.*, 2003), and an increasing incidence of developmental malformations (e.g. Blaustein and Johnson, 2003; D'Amen *et al.*, 2008). This phenomenon has been observed worldwide and major extinctions are documented, especially of tropical mountain species, in Central and South America (e.g. Pounds *et al.*, 1994; Young *et al.*, 2001; Ron *et al.*, 2003). In spite of the skepticism due to the natural wide fluctuations that characterize many amphibian populations (Pechmann *et al.*, 1991), the declines were far more widespread and severe than would be expected under normal conditions of demographic variation (Pounds *et al.*, 1997; Green, 2003). The latest assessment of the Status of the World's Vertebrates reports that 41% of amphibian species are threatened (Baillie *et al.*, 2010). Amphibians have the highest proportion of threatened species among vertebrates, but also the highest proportion of Data Deficient and the lowest proportion of Least Concern species (Baillie *et al.*, 2010).

There appears to be no single cause for amphibian population declines. A number of culprits have been recognized, including habitat loss and alteration, diseases, invasive species, global climate change, chemical contaminants, and overexploitation (e.g. Alford and Richards, 1999; Pimm and Raven, 2000; Collins and Stofer, 2003; Stuart *et al.*, 2004). Each is plausible, all are likely, but none is mutually exclusive and synergistic effects rush the declines. Among these stressors, habitat loss and fragmentation are the most widespread factors driving amphibians to extinction (Gardner *et al.*, 2007). The construction of roads, housing developments and commercial strips, and the agricultural practices can destroy wetlands and alter ecological processes. All these anthropogenic practices fragment the landscape, reduce wetlands, and isolate them from one another. In addition, they produce pollution that degrades the water where amphibians live and breed. All these factors may breakdown critical metapopulation processes and jeopardize structure and persistence of populations (Beebe, 1997; Semlitsch, 2000).

Several experimental studies illustrate that ultraviolet radiation (UV-B) damage all stages of amphibian' life cycle (Blaustein and Bancroft, 2007). In fact, UV-B (280–315 nm) is the most significant biologically harmful radiation at the terrestrial surface (Blaustein *et al.*, 1995). The increase of UV-B is primarily due to stratospheric

ozone depletion (Kerr and McElroy, 1993; Middleton *et al.*, 2001) and it is considered to be one important factor involved in the population declines of some amphibian species (e.g. Blaustein and Kiesecker, 2002). The effects of this radiation on amphibians can be direct (e.g. Blaustein *et al.*, 1995) or can be produced by interactions with other stressors, as climate shifts, or emerging infectious diseases (EIDs) (e.g. Kiesecker *et al.*, 2001). Such effects include reduced growth, developmental abnormalities, and behavioral changes (e.g. Blaustein *et al.*, 1995; Blaustein and Kiesecker, 2002; Bancroft *et al.*, 2008).

Recently, great attention has been focussed on the role of the emerging infectious disease, chytridiomycosis (Berger *et al.*, 1998), in causing amphibian declines. This infection is produced by the fungal pathogen *Batrachochytrium dendrobatidis*, which belongs to a phylum of non-hyphal ‘zoosporic’ fungi (the Chytridiomycota). *B. dendrobatidis* is the first and only known chytrid species pathogenic to vertebrates. The origin of this pathogen is southern Africa, and the international trade in *Xenopus laevis*, that began in the mid-1930s, was the means of its propagation (Weldon *et al.*, 2004). Out of his primary range it was observed causing amphibian mortality in Neotropics in 1974 and in Australia in 1978 (Carey *et al.*, 1999). In Europe, this disease was not observed until 1997, when it first appeared in Spain (Bosch *et al.*, 2001). Although it is increasingly clear that various climate anomalies can alter the dynamics of host–pathogen systems (Pounds *et al.*, 2007), still uncertain remains whether the chytrid-related amphibian declines are mediated through extreme climatic events (climate-linked epidemic hypothesis) (Bosh *et al.*, 2007; Simoncelli *et al.*, 2005; Di Rosa *et al.*, 2007; Kriger, 2009). Unfortunately the relationships between global climate change and disease emergence are complex and difficult to understand, thus further study are needed to explain this possible association (Lips *et al.*, 2008).

1.3 Climate change impacts on amphibians

Amphibians are suffering widespread extinctions even in seemingly undisturbed environments (Stuart *et al.*, 2004). Pathogens have been frequently implicated in these cases, but mounting evidences individuated global warming as a further proximate cause (e.g. Pounds *et al.*, 1999, 2006; Parmesan, 2006; Kriger and Hero, 2007;

Crawford *et al.*, 2010). Temperature changes have been repeatedly correlated with population declines (Heyer *et al.*, 1988), suggesting that environmental changes associated with climate change are likely to affect amphibians. Moreover, severe drought conditions, unusually heavy frosts, and excessive rainfalls in cooler months, were all proposed as causes of decline and extinction for different amphibian populations, both in America and in Australia (Corn and Fogleman, 1984; Heyer *et al.*, 1988; Weygoldt, 1989; Osborne, 1989; Ingram, 1990). Nevertheless, these studies were based mainly on anecdotal evidence, although together they suggest a causal relation (Carey and Alexander, 2003).

Only more recently, there have been attempts to demonstrate the role of climate warming in determining past species decline or population extinctions more directly (e.g. Pounds and Crump, 1994; Kiesecker *et al.*, 2001; Pounds *et al.*, 2006; McMenamin *et al.*, 2008). It has been shown that amphibian declines in Yellowstone National Park were associated to wetland desiccation due to climatic change (McMenamin *et al.*, 2008). Moreover, the report of major declines in the primary forest La selva, Costa Rica, for populations of all terrestrial amphibians and for all lizards as well, offers as ultimate explanation the global warming (Whitfield *et al.*, 2007). The potential mechanisms underlying local extinctions have become clearer recently, with the demonstration of a connection involving increase in mean temperatures, body condition decline, and decrease in fecundity (Reading, 2007). In some cases, the interaction between disease epidemics and climate change, has been demonstrated (Pounds *et al.*, 2006; Cunningham *et al.*, 2006). Anyway, the presence of *B. dendrobatidis* in amphibian communities does not always implicate chytrids as a cause of declines. For example, a long-term study in South Carolina, USA, found that amphibian declines were more likely caused by an increasing aridity at the breeding site than by chytrid (Daszak *et al.*, 2005). Because of the complexity involved in climate change effects on amphibian survival, future research should increase our understanding of threats mechanisms, and advance our capacity to manage and conserve populations.

Regardless of the role that climate changes may have played in past and current amphibian declines, future shifts in climate will certainly pose challenges for the survival of populations, especially when

occurring together with other already well-established stressors, particularly habitat destruction. Predictive studies showed that the distribution of many species, including amphibians, in Europe may be affected by climate change, and that the effects are likely to differ between species (Bakkens *et al.*, 2002; Thuiller *et al.*, 2005; Araújo *et al.*, 2006; Levinsky *et al.*, 2007; Girardello *et al.*, 2009; Carvalho *et al.*, 2010). The ability of species to exploit new suitable habitat will depend on the dispersal of individuals or propagules (Huges, 2000). Species unable to adapt or move will face local or global extinction and this is more likely to happen to species with narrow climatic and habitat requirements and limited dispersal abilities, such as amphibians (Carvalho *et al.*, 2010). In addition to specie-specific ability to disperse, physical changes wrought by humans would likely constrain amphibian shifts and greatly reduce the pool of replacement populations (e.g. Thomas *et al.*, 2004; Araújo *et al.*, 2006).

1.4 Conservation strategies in a changing world

Extinctions of amphibians in pristine protected habitats and projections of further declines in the decades to come, raise concerns about the effectiveness of existing conservation strategies (IUCN, 2006). Current conservation commitments rarely take into account the potential impacts of climate change (Hossell *et al.*, 2003). For species that have ranges limited by climate, studies that model their responses to projected future climatic conditions can inform policy makers about likely shifts in the suitability of regions and help identify those species which are likely to be vulnerable in the future. In particular, one of the key protection strategies adopted worldwide, the “*in-situ*” conservation, needs to be re-examined. National parks and nature reserves are fundamental tools for preserving viable populations in natural ecosystems but they have fixed boundaries (Williams *et al.*, 2005). If global climate change alters the geographic distribution of habitats and wildlife, the ability of parks to retain and protect species in the future is highly uncertain (e.g. Hannah *et al.*, 2002; Araújo *et al.*, 2004; Rodriguez *et al.*, 2004; Heller and Zavaleta, 2009).

A typical approach for the selection of new reserves seeks to maximize the amount of biodiversity that can be represented in networks of conservation areas (Margules and Pressey, 2000;

Hannah *et al.*, 2002). Using species distribution data only for a particular time, reserve selection mostly has not taken into account the possibility of species range dynamic, especially under changing climatic conditions (Araújo *et al.*, 2004). The ultimate goal of conservation planning should be the design of systems that enable biodiversity to persist in the face of natural and human-induced changes (Araújo *et al.*, 2004; Williams *et al.*, 2005; Maiorano *et al.*, 2008). To this end the integration of the potential impacts of climate change with selection of protected areas has recently gained attention (Heller and Zavaleta, 2009). The overall aim of this approach is to identify conservation areas that will collectively achieve all the conservation targets for current and projected distributions (Hannah *et al.*, 2002; Williams *et al.*, 2005). Because many species are poor dispersers and are unlikely to colonize distant areas, a fundamental key factor to consider in the planning phase is to gauge a level of connectivity so as to allow species to move reliably to new suitable conditions (Williams *et al.*, 2005).

Despite the imperilled status of amphibians, these vertebrates are not as well represented in conservation studies as are other, less threatened, taxonomic groups (Brito, 2008; Felton *et al.*, 2009), and they are often neglected during conservation planning (Rodriguez *et al.*, 2004; Pawar *et al.*, 2007). The Amphibian Conservation Action Plan indicates as one of the most important priority for amphibian conservation the establishment of additional conservation areas in order to include the distribution ranges of threatened species not protected by the current systems (Gascon *et al.*, 2007). In addition, long-term conservation of amphibians in highly fragmented landscapes will require access to other areas that are currently climatically unsuitable but which are predicted to become suitable in the future (Hannah *et al.*, 2002; Araújo *et al.*, 2004; Heller and Zavaleta, 2009). The identification of conservation units that include and connect several ecosystems will be crucial to maintain amphibians diversity at broad spatial scales, alongside the conservation of micro-habitats that allow the protection of micro-endemic and rare species (Young *et al.*, 2004; Ocha-Ocha *et al.*, 2009). Thus, there is a need to evaluate the current amphibian diversity protection to determine where new protected areas should be established for securing a long term complete coverage and providing interconnectivity between protected area units (Ocha-Ocha *et al.*, 2009).

1.5 The study of climate change effects on species distribution

Faced with ongoing anthropogenic climate change (IPCC, 2007), accurate predictions of the current and potential future ranges of species are essential for managing biological diversity. One tool to forecast how species will respond to climate change is ecological niche modeling. Here, a relationship between species occurrence and a series of environmental variables, such as climatic variables and other predictors, is established and then projected onto estimated future values of these variables (Guisan and Thuiller, 2005). There are several mathematical approaches for modeling distributions (Elith *et al.*, 2006; Heikkinen *et al.*, 2006) under a similar conceptual framework. However, the variability in the predictions among these models (Thuiller, 2004) raised concerns about their application to conservation planning (Loiselle *et al.*, 2003). The use of a framework of ensemble forecasting permit to incorporated variability between alternate projections into the prioritization process (Thuiller, 2004; Araújo and New, 2007; Marmion *et al.*, 2008).

An innovative improvement of species distribution models for predicting the potential effects of climate change on species distribution can come from the integration with genetic analyses, focusing on *taxa* phylogeny or phylogeography (Habel *et al.*, 2010). Several studies have observed that *taxa* below the level of species can vary in the degree to which they differ from one another in the environmental space they occupy (Peterson and Holt, 2003; Murphy and Lovett-Doust, 2007; Pyron and Burbrink, 2009). The existence of ecotypes and locally adapted populations suggests genetically-based geographic variation in physiological traits that convey adaptation to climate and other aspects of environment (Savolainen *et al.*, 2007). Geographic variation in environmental tolerances suggests that regional populations should be modeled to capture these environmental dependencies (Loehle and LeBlanc, 1996). The implications of not accounting of population and phylogeographic structure for modeling species distributions, inferring niche structure or predicting the effects of climate change on species distributions are little studied (Pfenninger *et al.*, 2007). Nonetheless, the preservation of genetic diversity and unique lineages within species in a rapidly changing world is considered as a key aspect in conservation biology (Allendorf and Luikart, 2006).

Whether a widely distributed species should be modeled in a single comprehensive model or a set of models that represent multiple taxonomic units below the species level is relevant for three reasons. First, distribution models that are developed by pooling across subspecies or lineages within species may obscure the potential that these lineages occupy distinct niches, something that suggests idiosyncratic response of sub-specific lineages to changing climate. This might happen because a comprehensive model could smooth across environmental response curves of specific lineages. Such a model might not describe well the niche of a specific lineage that is adapted to a specific set of climatic conditions (Osborne *et al.*, 2007, Elith and Leathwick, 2009). Second, a species model could, alternatively, overestimate the species-level response to climate change when, in fact, only a few populations within the species could exhibit such a response. Third, the portion of a species range that is occupied by different subspecies or lineages can vary greatly or be roughly comparable. Isolated populations that account for a small fraction of species observations might have little influence on a species distribution model when the total number of observations is much larger. However, these isolated populations might contribute disproportionately to the response of species to changing climate, potentially representing the surviving portion of the species.

1.6 Overview of the thesis aims

The overall goal of this thesis is to contribute at understanding climate change impacts on amphibian diversity, specifically by pursuing the following objectives:

- i.* estimating the potential contributions of climate change to past amphibian declines across Italy;
- ii.* predicting the potential range shifts of amphibian species belonging to Italian fauna in response to future changing climate;
- iii.* identifying the range shifts' implications for conservation practices, by evaluating if the current network of reserves in Italy would be able to protect amphibian species in the future conditions;

- iv. testing if the inclusion of phylogenetic information in modelling exercises might improve prediction of climate change impacts on species distribution.

The following main questions have been addressed and constituted the basis for this research.

Why are amphibians declining? Do causative factors vary according to spatial resolution? Amphibians display a non-random geographical pattern of decline. By utilizing a spatial approach, both Chapters 2 and 3 aimed at gaining a better understanding of the factors producing amphibian declines in last decades, especially focusing on the climatic change hypothesis. In Chapter 2 I analyzed 19 amphibian species encompassing the whole Italian territory with a spatial resolution of 10 km. The study described in Chapter 3 focused on a smaller area, in central Italy, utilizing an higher resolution amphibian dataset. As the complex interactions between land use and climate change make it difficult to attribute biodiversity changes to either one of these drivers (Sala *et al.*, 2000), in both studies, a multi-factorial approach was applied, testing contemporarily several potential determinants of declines.

Which amphibian species will be more threatened by climatic change? Which areas in Italy will be more impacted by species loss? In Chapter 4 I forecasted amphibian range shifts in Italy through an ensemble niche modelling exercise, utilizing two carbon emission scenarios (A1FI and B1). Dispersal limitation of species were included considering two options, either no dispersal or a species-specific dispersal rate proportional to the intrinsic species vagility. Resulting projections allowed at identifying regions where the largest number of extinction events is expected and species that will be likely most threatened by climate change (e.g., Thuiller *et al.*, 2005, Araújo *et al.*, 2006, Huntley *et al.*, 2008).

Which places are the most important for amphibian diversity conservation? Are they included in the Italian network of protected areas? Could the network be improved to protect amphibians on the long run? The study presented in Chapter 4 further aimed at analyzing the efficacy of the Italian reserve network for protecting amphibian diversity in a climate change scenario. Current distributions and future predictions of amphibian range shifts were

used to perform gap and irreplaceability analyses. Additionally, spatial options for filling gaps in the reserve system are supplied in order to improve efficiency in the long run.

Can we improve our predictions of climate change impact on species distribution? How different evolutionary lineages belonging to the same taxa are affected by climatic change? The main tools used for determining species risk under climate change and to set conservation priorities rely on predictive models. The study described in Appendix D tested how taxonomic resolution influences predictions of the extent of suitable climatic conditions for species currently and under future scenarios. To address the issue, two data sets on species distributions were assembled that together include cases where species have been split into two sister species, have recognized subspecies, or have been formed by down-grading several species to the subspecies level. Results can help reduce uncertainty when predicting climate change impacts and setting conservation priorities.

Global warming and biodiversity: Evidence of climate-linked amphibian declines in Italy

2.1 Introduction

Here our primary goal is to clarify whether human-induced climatic shifts contributed to amphibian declines in Italy during the final decades of the 20th century. We do this by analyzing spatial patterns of population disappearances countrywide. Our approach is based on the recognition of the spatial effects that environmental alterations can have in determining geographic patterns of declines. Testing the hypothesis that global warming has already caused amphibian declines is challenging because many factors could act synergistically with climate in complex ways (e.g. Pounds, 2001), and studies involving single factors cannot address such interactions (Kiesecker *et al.*, 2001). Thus, our approach, similarly to previous studies (Stallard, 2001; Davidson *et al.*, 2002; La Marca *et al.*, 2005), is not focused on the climate hypothesis only, but considers multiple contemporary factors as potential explanations for amphibian decline. To the best of our knowledge, few studies have demonstrated the detrimental role of climate change, by itself or in association with other stressors, in the decline of amphibian populations in Europe (Bosch *et al.*, 2007; Di Rosa *et al.*, 2007; Reading, 2007).

We also examined other potential culprits for amphibian decline in Italy. Firstly we tested the habitat alteration hypothesis: in Europe, anthropogenic activities degrading native habitats, both by structural modifications and pollutant releases, are the most obvious factors contributing to amphibian population disappearances (Blaustein and Kiesecker, 2002; Beebee and Griffiths, 2005). Additionally, we considered the hypothesis that exposure to high levels of solar radiation had contributed to declines. Solar radiation could influence amphibian persistence in complex ways, enhancing the effects of other stressors (e.g. aridity, extreme temperatures), and/or by means of its UV-B component (e.g. Blaustein *et al.*, 1995; Blaustein and Kiesecker, 2002; Bancroft *et al.*, 2008). In fact, ultraviolet radiation

is part of the natural electromagnetic spectrum emitted by the sun, and about 10% of the ultraviolet radiation (UV-B - 280-315 nm) that impacts the Earth's atmosphere also reaches the surface, with an absolute intensity proportional to the local irradiation. Our analyses excluded some potentially important factors, such as invasive species, over-exploitation, and EIDs, because the available data do not allow detailed definitions of their geographic patterns. Among EIDs, the most widely recognized is chytridiomycosis, which is caused by the chytrid fungus *Batrachochytrium dendrobatidis*. The declines we studied all occurred prior to the known outbreaks of chytridiomycosis in Italy. In fact, it was first observed in this country only in 2003, even though the chytrid have been present there in an apparently nonpathogenic form since 1999 (Di Rosa *et al.*, 2007).

Multi-factorial studies on amphibian disappearances are largely lacking in Europe. In this region declines have conventionally been ascribed to the well-documented anthropogenic assault on wetlands habitats during 1970s and 1980s (e.g. Kuzmin, 1994; Alford and Richards, 1999; Stuart *et al.*, 2004). Moreover, the plight of Italy's amphibians is poorly known. Until now, the only broad assessment of threats was that of Andreone and Luiselli (2000), which was based on natural history parameters.

This study was based on patterns of presence in a geographic grid for 19 species. Grid-squares in which presence had previously been documented, but was not reconfirmed after a specific threshold year, were considered to represent declines (DECLINE), while the cell where species persisted were considered to harbor stable populations (STABLE). Using a GIS-based approach, we calculated, for each cell, the mean values – or shift in mean values – of different parameters, used as proxies for the three factors (Table 1, Figure 1). The measures of these parameters were entered as predictors in specific autocovariate models fitted on grid-square status (see Appendix A for a detailed description of the species and variables data sets and for the statistical methods applied). Our study represents the first attempt to identify causes of amphibian declines throughout Italy. The spatial analysis improves our understanding of human impacts, and may therefore help in formulating conservation strategies.

Table 1 – Variables used as proxies of the decline hypotheses

ABBREVIATION	VARIABLE DESCRIPTION
DD MEAN *	annual number of dry days - mean values for the period 1961-90
DD VAR *	annual number of dry days - shift between decades 1961-70 and 1981-90
T VAR *	annual mean temperature - shift between decades 1961-70 and 1981-90
P VAR *	annual cumulate precipitation - shift between decades 1961-70 and 1981-90
AGR §	percentage of surface in each UTM grid cell of agricultural land cover
UR §	percentage of surface in each UTM grid cell of urban land cover
IR °	horizontal irradiation

* Climatic predictors; § Habitat alteration predictors; § Solar radiation predictors

2.2 Results

The status of DECLINE was assigned to 1908 records (17.04%), and a status of STABLE was assigned to 9286 data (82.96%) (Table 2 and Figure 1h). Our criterion for assessing species status (STABLE / DECLINE) was supported by the estimation of extinction probability in a subsample of cells (Mann-Whitney U test: $Z = 2.654$, $p = 0.008$), highlighting the reliability of our characterization of species status for the whole dataset.

The rate of local decline was very different among species (Table 2). A large fraction of the amphibian species (52.63%) had 15-20% DECLINE cells. Species having the fewest DECLINE cells were the “green” frogs (*Pelophylax* spp.), the Italian newt (*Lissotriton italicus*), the Italian stream frog (*Rana italica*), and the common toad (*Bufo bufo*), which were declining in a relatively low percentage of squares (<15%). On the contrary, highest rate of local decline (over 30%) was observed for the common spadefoot toad (*Pelobates fuscus*), Apennine yellow-bellied toad (*Bombina pachypus*), and painted frog (*D. pictus*). Slightly lower rates of decline (over 20%) were found for the Alpine salamander (*Salamandra atra*) and the Italian crested newt (*Triturus carnifex*).

For all species, autocovariate models allowed us to identify at least one significant predictor associated with decline among the

considered variables (Table 3). The autocovariate variable was retained by the stepwise procedure in the final model for all but two species (*D. pictus* and *S. atra*). For 26.32% of the species, population survivor was negatively related to only one factor, whereas for the remaining species it exhibited such relationships with multiple factors.

Among the variables representing the factors we considered, those pertaining to habitat alteration were often important as expected. Surprisingly, however, the climatic variables appeared to be just as important (Table 3). The declines of 14 species (73.68%) were associated with at least one climatic variable (Table 3). Moreover, for *D. pictus*, *Mesotriton alpestris*, and *H. intermedia*, only climatic variables were significantly associated with population declines (Table 3). Likewise, the declines of 14 species were associated with at least one land use variable, and the decline of *B. pachypus*, *S. salamandra*, and *R. latastei* was congruent with the habitat alteration hypothesis only.

Overall, the variables that were significantly related to the largest of declines were UR (associated with decline of 11 species), T VAR (associated with decline of eight species), and P VAR (associated with decline of seven species). The variable UR was by far the one most strongly related to the decline of five species, being the most important or only variable associated with population disappearances (Table 3). Slightly less important was P VAR, which was the most important predictor for four species, while T VAR had the greatest explanatory importance for two species only (Table 3). The variables DD VAR, DD MEAN, and AG had similar overall importance, being retained in the final model of four to five species. They were the most important predictors for three, two, and one species respectively (Table 3). The irradiation (IR variable) was retained in models of only three species, but in two of these species (*S. atra* and *Salamandrina* spp.) it was the most important predictor (Table 3).

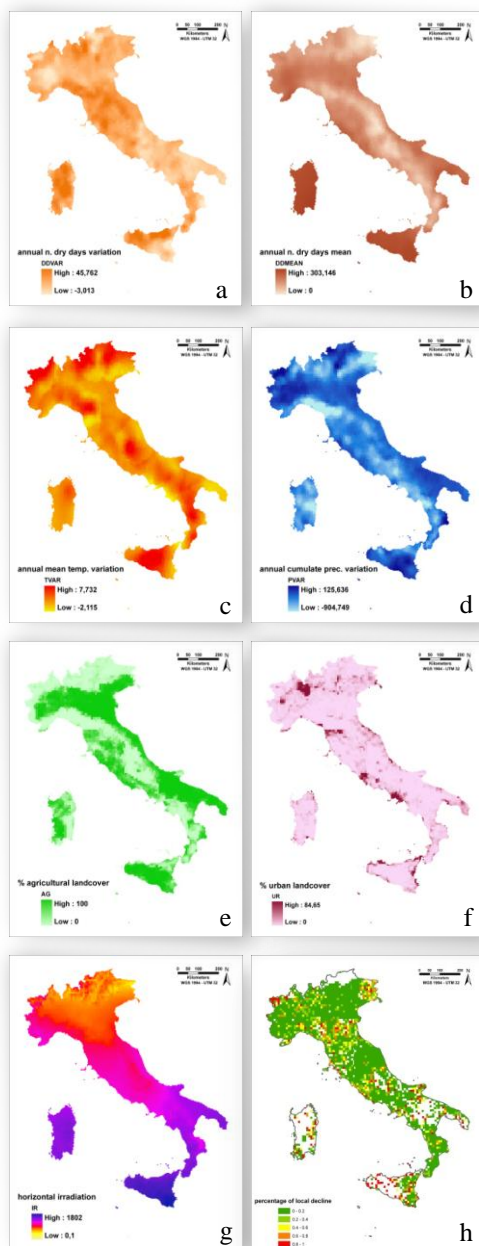


Figure 1 - Predictors and patterns of decline
 (a-g) Environmental surfaces utilized as proxies of the decline hypotheses:
 a) DD VAR (days);
 b) DD MEAN (days);
 c) T VAR ($^{\circ}\text{C}$);
 d) P VAR (mm);
 e) AG (percentage);
 f) UR (percentage);
 g) IR (J/m^2).
 (h) Percentage of declining species per cell.
 See Appendix A for variable descriptions

Table 2 - Amphibian local decline Total number of occupied cells (STABLE + DECLINE), and rate of local decline (DECLINE / [STABLE + DECLINE]) per species

SPECIES	TOT NO OF OCCUPATED CELLS	RATE OF LOCAL DECLINE
<i>Pelobates fuscus</i>	46	54.35
<i>Bombina pachypus</i>	309	37.54
<i>Discoglossus pictus</i>	53	32.08
<i>Salamandra atra</i>	60	23.33
<i>Triturus carnifex</i>	901	20.31
<i>Bombina variegata</i>	151	19.21
<i>Salamandrina</i> spp.	253	18.97
<i>Salamandra salamandra</i>	604	18.87
<i>Rana latastei</i>	232	18.53
<i>Lissotriton vulgaris</i>	697	18.36
<i>Mesotriton alpestris</i>	303	18.15
<i>Rana dalmatina</i>	757	16.91
<i>Hyla intermedia</i>	1012	16.01
<i>Rana temporaria</i>	446	15.92
<i>Pseudepidalea</i> spp.	812	15.89
<i>Bufo bufo</i>	1720	14.19
<i>Rana italica</i>	538	13.57
<i>Lissotriton italicus</i>	249	12.85
<i>Pelophylax</i> spp.	1639	12.20

Considering phylogenetic lineages, the two major clades – salamanders and anurans – showed similar patterns of association between variables and declines. In fact, none of the variables had significantly different influence on species declines in the two groups (Chi-Square Tests, $p > 0.05$ for all variables). Moreover no phylogenetic signal was evidenced in terms of number of predictors retained in the final models (Mann-Whitney U test: $U = 41.5$, $p > 0.05$).

Table 3 – Autocovariate models. Significant variables selected for the final model and Δ AIC resulted when each predictor is removed from the final model. Δ AIC indicates each predictor's relative importance in the model. Δ AIC of the most important predictor for each species is evidenced in bold (see text and Table 1 for details and variable abbreviations)

SPECIES	Δ AIC						
	DD VAR	DD MEAN	T VAR	P VAR	AG	UR	IR
<i>Pelobates fuscus</i>	-	1.4	-	0.084	1.37	-	-
<i>Bombina pachyopus</i>	-	-	-	-	4.21	-	-
<i>Discoglossus pictus</i>	-	-	2.84	0.17	-	-	-
<i>Salamandrina atra</i>	1.14	-	-	-	-	-	1.22
<i>Triturus carnifex</i>	-	2.5	2.71	0.58	-	4.28	-
<i>Bombina variegata</i>	1.13	-	1.03	7.28	-	2.39	-
<i>Salamandrina</i> spp.	-	-	-	-	-	5.04	7.88
<i>Salamandrina salamandra</i>	-	-	-	-	-	40.67	-
<i>Rana latastei</i>	-	-	-	-	-	2.72	-
<i>Lissotriton vulgaris</i>	-	-	0.6	3.74	3.27	2.22	-
<i>Mesotriton alpestris</i>	-	-	-	1.38	-	-	-
<i>Rana dalmatina</i>	3.35	-	-	-	-	3.2	-
<i>Hyla intermedia</i>	-	-	1.22	-	-	-	-
<i>Rana temporaria</i>	-	0.98	-	-	-	-	0.3
<i>Pseudoeuplatlea</i> spp.	3.1	-	-	-	1.04	-	-
<i>Bufo bufo</i>	-	-	1.24	6.06	-	2.36	-
<i>Rana italica</i>	-	-	5.14	-	-	8.76	-
<i>Lissotriton italicus</i>	-	-	-	-	0.55	1.84	-
<i>Pelophylax</i> spp.	81.1	54.8	69.3	-	-	1.26	-

Overall, the analysis of spatial patterns of Italian amphibian declines showed some regions with widespread population reduction of numerous species (Figure 1h). In the Eastern Alps we detected population reduction of several species (i.e. *B. variegata*, *M. alpestris*, *R. temporaria*, and *Salamandra atra*). In the Northern Apennine area a general population decline for *Bufo bufo*, *Pelophylax* spp. and *R. italica* occurred, and in North-Western Sicily a local reduction of *B. bufo*, *Pelophylax* spp., and *D. pictus* populations was seen. These areas were characterized by particularly severe climatic shifts (Figure 1 a-d) between the periods we examined. Regional declines of certain species (*T. carnifex*, *Bombina variegata*, *S. salamandra*, *R. latastei*.) were detected close to large cities such as Rome, Milan, Brescia, and Turin.

2.3 Discussion

Climate change hypothesis

Our results demonstrate that complex influences of several factors associated with amphibian population declines vary across species and areas. In testing the climate hypothesis, we considered two principal aspects of climate affecting amphibians: the decrease of water availability in space and time, and the increase in temperature. A number of climatologic studies (e.g. Brunetti *et al.*, 2004, 2006; Toreti and Desiato, 2008) described a positive trend for mean temperature, a reduction of number of wet days, and an increase in precipitation intensity all over Italy during the last century. These trends occurred mainly in the last 50 years, supporting the hypothesis of human-induced climatic shift. Our results show that these patterns agree with the observed spatial association between climatic changes and the occurred losses. The climatic shifts were important even for those species with the lowest rates of decline, suggesting that these species may be quite vulnerable to ongoing climatic changes in the region. This represents important evidence of global warming's impacts on amphibians in a region where declines were nearly always been ascribed to habitat loss (e.g. Kuzmin, 1994; Stuart *et al.*, 2004).

How do our findings compare with other studies? Davidson *et al.* (2002) argued that climate change was not important in the decline of eight amphibian species in California. The disparity between their

findings and ours could reflect a difference in climatic changes between California and Italy, or in the sensitivity of species involved. Also, the purely geographic proxies adopted by Davidson *et al.* (2002) may have been inadequate for analyzing the complex interplay of factors. For example, if climate interacts with diseases, the resulting altitudinal pattern of declines will depend in large measure on the natural history of the hosts and pathogens, and may not follow the simple, classic prediction of a shift to higher altitudes. On the contrary our results are congruent with those of Pounds *et al.* (1999) who found associations between increasing numbers of dry days, related to increasing precipitation variability, and species extinctions. Our results are also similar to those described by McMenamin *et al.* (2008), who demonstrated that decreasing annual precipitation and increasing temperatures during the warmest months of the year altered the landscape and the local amphibian communities in the Yellowstone National Park.

It should be pointed out that our findings do not indicate of an exclusive role of climate shifts in the observed declines, but rather they show that climate change is an additional serious threat contributing to the current amphibian crisis. Indeed global climate change may often act together with other agents (Pounds *et al.*, 2007). For instance, it may contribute to amphibian declines by promoting outbreaks of EIDs (climate-linked epidemic hypothesis) (e.g. Pounds *et al.*, 2006; Bosch *et al.*, 2007; Di Rosa *et al.*, 2007).

Habitat alteration hypothesis

Habitat alteration is widely recognized as the primary cause of amphibian population declines worldwide (e.g. Sala *et al.*, 2000; Gardner *et al.*, 2007). Our results show that human land use patterns, in particular changes linked to urbanization, have substantially contributed to amphibian declines in Italy. Not only did major cities represent centers of decline for a number of species, but all around Italy high levels of urbanization in the cells were consistent with the reduction of many amphibian populations. The sensitivity classification of Andreone and Luiselli (2000) is consistent with our results. In fact, for all the species that Andreone and Luiselli classified as “virtually un-adaptable to altered environments” we found an association between the local decline and urban land-use. We also observed fewer population declines associated with

agriculture than with urban land use, suggesting that this type of habitat alteration in Italy is less detrimental to the batrachofauna. The five species we found to be affected by agricultural land use were also categorized by Andreone and Luiselli as particularly vulnerable to area fragmentation (levels 2 and 3 of their classification). In light of this congruence, one can hypothesize that the high percentage of agricultural land use in these cells worsened the effect of range fragmentation, driving populations towards extinction.

Solar irradiation hypothesis

Of the factors we considered, incident solar radiation appeared to be the least important in amphibian declines in Italy. Irradiation, though probably not a direct cause of mortality, may have been important role in association with other stressors. In fact, it is possible that where the irradiation variable was significant, it acted by enhancing the effect of other stressors. For example the increase in sub-lethal exposures of UV-B radiation might increase the susceptibility of amphibians to contaminants, pathogens, or changes in climate (Pounds, 2001; Blaustein and Kiesecker, 2002). In addition the observed effects of irradiation variable could actually be due to interactions of solar irradiance with other parameters rather than to UV-B directly. For instance, high solar irradiance combined with the climatic shifts may lead to stronger climatic stress.

Implication for conservation strategies

Our work has multiple management implications. Classifying species risk and identifying the causes of past declines permits planning of more useful and effective management procedures for preserving species in the future. On the other hand, the identification of spatial hot-spots of decline can be very useful for directing protection efforts. We identified *Pelobates fuscus*, *B. pachypus*, and *D. pictus* as the most threatened Italian amphibian species. *P. fuscus* is a typical lowland inhabitant and it is classified by Temple and Cox (2009) as Least Concern. The Italian subspecies (*P. fuscus insubricus*) is reported to be declining significantly in most of its distribution (IUCN, 2008) in agreement with our detection of an extensive decline in its Italian range. On the contrary, Andreone and Luiselli (2000) predicted a low degree of vulnerability for the

common spadefoot toad. The Apennine yellow-bellied toad is considered as Endangered by Temple and Cox (2009), because it has experienced a decline in almost all of its range. According to our results this species is known to be threatened by loss and fragmentation of wetland habitat (Temple and Cox, 2009). Moreover, fungal infections by *Batrachochytrium dendrobatidis* were recently reported in *Bombina pachypus* populations (Stagni *et al.*, 2004). Similarly, the painted frog is an ecologically generalist species inhabiting many different habitats and is listed as Least Concern (Temple and Cox, 2009). GAA reports that Sicilian populations appear to be locally threatened, but not especially endangered, while our results demonstrated its rarefaction in a large fraction of occupied cells. Thus, we suggest that further investigations and that the planning of large-scale conservation programs for this species in Italy should be implemented. This kind of initiatives would be also helpful for improving and update the distribution data, which are relatively poor in this area. We demonstrated an association between the ongoing climatic alteration and the *D. pictus* population disappearance. In fact, climate change was extremely severe in its Italian range, in particular as variation of the number of dry days (Figure 2a), producing dry conditions limiting for most amphibian species. As regards potential hot-spots of decline, common patterns of population declines could be observed in some areas particularly affected by climatic changes and urban development. In light of our results we encourage planning of specific measures focused on these areas for optimizing the conservation efforts.

Human-provoked amphibian decline in central Italy and the efficacy of protected areas

3.1 Introduction

The main goal of this research was to understand the consequences of human land use disturbances for different amphibian species in a Mediterranean area in central Italy (Latium region). A secondary goal was to assess whether species declines are related to the climatic shifts that occurred in Italy in recent decades by considering climatic variables which have influenced amphibian persistence (D'Amen and Bombi, 2009). Finally, we aimed at determining if and to what extent the existing protected areas have been effective for species protection in recent decades in the study region. Indeed, although protected areas inhibit habitat destruction and direct human disturbance, they can be less adequate for protecting species from other stressors such as chemical pollution, and, above all, climatic shift conditions.

To achieve these goals, we began by examining changes in species abundance at the regional scale, and then, we devised a spatial approach based on the identification of the spatial effects of environmental alterations in determining the geographic patterns of declines observed in recent decades (Davidson *et al.*, 2002; D'Amen and Bombi, 2009). We used Generalized Additive Models (GAMs) to investigate the association between the state (stable/decline) of amphibian populations in 5×5 km cells in central Italy and proxies of different typology of anthropogenic stressors, climatic variables, and protection measures (Table 4). This analysis is relevant because no efficient conservation measures can be planned if there is an incomplete understanding of the causes leading to declines (see Appendix B1 for a detailed description of the species and variables data sets and of the statistical methods applied).

Table 4 - Predictors descriptions and abbreviations

ABBREVIATION	VARIABLE DESCRIPTION
<i>ART</i> *	Artificial surfaces
<i>URB</i> *	Urban fabric
<i>ICT</i> *	Industrial, commercial and transport units
<i>MDC</i> *	Mine, dump and construction sited
<i>STR</i> *	Total length of street coverage
<i>ART VEG</i> *	Artificial non-agricultural vegetated areas
<i>AGR</i> +	Agricultural areas
<i>ARA</i> +	Arable land
<i>P CRO</i> +	Permanent crops
<i>PAS</i> +	Pastures
<i>HET AGR</i> +	Heterogeneous agricultural land
<i>CLC VAR</i>	Land cover change
<i>HII</i>	Human Influence Index
<i>T VAR</i> §	Mean annual temperature variation
<i>P VAR</i> §	Cumulate annual precipitation variation
<i>DD VAR</i> §	Annual number of dry days variation
<i>PA</i>	Protected areas

* Urban predictors; + Agricultural predictors; § Climatic predictors

3.2 Results

Our analyses showed a local decline in Latium for all the considered species with 23-24% DECLINE cells. The species having the highest rate of decline is the smooth newt (*Lissotriton vulgaris*) (44.12%), while the Italian tree frog (*Hyla intermedia*) is declining in the lowest percentage of squares (23.73%) (Table 5).

Some of the variables considered were not significant in univariate models or were not retained after stepwise procedure in the final model for any species: *ART*, *AGR*, *MDC*, and all of the quadratic terms of climatic variables. For all species, statistically significant non-linear relationships were found between declines and at least

one variable (Table 6). Species persistence in the cells was negatively related with a maximum of four predictors (*i.e.* the common toad, *Bufo bufo*, the Italian tree frog, the smooth newt, the green frog, *Pelophylax* spp., the northern spectacled salamander, *Salamandrina perspicillata*). The percentage of protected areas was a significant predictor only for two species (the northern spectacled salamander, and the agile frog, *Rana dalmatina*).

Overall, the decline of eight species was associated with at least one agricultural predictor, and for six species the decline was associated with proxies of habitat destruction and alteration due to the urban and industrial fabric. Climatic variation predictors were retained in the final model of six species (Table 6). A relationship between the Human Impact Index, and amphibian decline was found for five species (the smooth newt, the Italian stream frog, *Rana italica*, the common toad, the green frog, and the Italian tree frog). On the contrary, land cover change was a significant predictor of declines for only one species (the Italian tree frog) (Table 6).

When considering the urban and agricultural predictors separately, we can observe a plain divergence: among the variables representing urban and industrial use, *URB* was the most important, and it was associated with the decline of four species (the smooth newt, the northern spectacled salamander, the Apennine yellow-bellied toad, *Bombina pachypus*, and the Italian tree frog), while the others (*ICT*, *STR*, and *ART VEG*) were significant for one species only (the green toad, *Bufo viridis* complex, the northern spectacled salamander, and the green frog, respectively) or not significant at all (*MDC*). In contrast, all variables linked to agricultural practices had a comparable importance, except for *P CRO* which was significant for the Italian crested newt, *Triturus carnifex*, only. Among climatic variables one predictor (*DD VAR*) was associated with the declines of five species (the smooth newt, the Italian crested newt, the green frog, the agile frog, and the Italian tree frog), while *P VAR* and *T VAR* were both significant predictors for the common toad only (Table 6).

Focusing only on the most important predictors of species decline, those pertaining to climate had the highest Δ AIC for 40% of the species (the Italian crested newt, the common toad, the green frog, and the agile frog). Urban predictors were the most significant predictors for three species (the smooth newt, the green toad, and the

Apennine yellow-bellied toad). The Human Impact Index was the most important predictor for two species (the Italian stream frog, and the Italian tree frog), while variables pertaining to the group of agricultural practices were the most important predictors for one species only (the northern spectacled salamander). The percentage of protected areas per square and the variation of land cover never had the highest Δ AIC score (Table 6).

Table 5 - Amphibian local decline in Latium Number of stable and decline cells and rate of local decline per species

SPECIES	DECLINE SQUARES	TOTAL SQUARES	RATE OF DECLINE (%)
<i>Lissotriton vulgaris</i>	45	102	44.12
<i>Rana italica</i>	31	76	40.79
<i>Triturus carnifex</i>	33	84	39.29
<i>Bufo bufo</i>	49	129	37.98
<i>Salamandrina perspicillata</i>	16	52	30.77
<i>Bombina pachypus</i>	11	37	29.73
<i>Bufo viridis</i> complex	9	33	27.27
<i>Phelophylax</i> spp.	36	133	27.07
<i>Rana dalmatina</i>	9	34	26.47
<i>Hyla intermedia</i>	14	59	23.73

We graphically combined our results of the species rate of decline and of the total number of significant negative predictors (Figure 2). For this visualization we assumed the threshold of 35% reduction in regional distribution for considering a species deserving of high conservation interest, setting the x and y axes intersect at that point. The graphic approach divides the ten species into four groups characterized by differential categories of risk (Figure 2). Three species (the common toad, the smooth newt, and the Italian stream frog) showed the most critical situation because they are both affected by a high number of stressors and their population has already been reduced in more than 35% of their regional distribution (Figure 2, up-right dial). Conversely, based on the same parameters, the green toad and the Apennine yellow bellied toad had the mildest

Table 6 - General Additive Models results Final model AIC value and significant variables selected with their relative importance. Δ AIC resulted when each predictor is removed from the final model. Δ AIC indicate each predictor's relative importance in the model. Δ AIC of the most important predictor for each species is evidenced in bold

Species	Final model AIC	Δ AIC													
		URB	ICT	STR	ART VEG	ARA	P CRO	PAS	HET AGR	CLC VAR	HII	T VAR	P VAR	DD VAR	PA
<i>Lissotriton vulgaris</i>	138.10	4.25						1.93			1.61			0.19	
<i>Rana italica</i>	85.19							5.89	2.85		6.00				
<i>Triurus carnifex</i>	108.03						1.77							6.41	
<i>Bufo bufo</i>	159.42					4.09					3.09	3.09	7.22		
<i>Salamandrina perspicillata</i>	54.14	5.43		6.34				4.81	13.35					0.53	
<i>Bombina pachypus</i>	47.64	3.26							1.44						
<i>Bufo viridis</i> complex	34.13		0.00												
<i>Phelophylax</i> spp.	135.24				2.55	7.15					11.62			19.05	
<i>Rana dalmatina</i>	26.11					6.86								15.20	6.54
<i>Hyla intermedia</i>	47.34	3.66								0.60	16.48			7.73	

condition in this region (Figure 2, low-left dial). More composite situations emerge for the other species. Notwithstanding the high rate of decline, the Italian crested newt could be protected because few factors affect population viability (Figure 2, low-right dial). In contrast, species that currently show lower declining rates, may need more complex conservation measures because several stressors affect their survival (i.e. the green frog, the northern spectacled salamander, Italian tree frog and the agile frog) (Figure 2 up-left dial).

3.3 Discussion

Declining of amphibians in central Italy

Our results show that several amphibian species in the Latium region in Italy have suffered recent declines. According to our results, two species are of particular concern because their rate of local decline is higher than 40%: the smooth newt and the Italian stream frog. Both species are classified as least concern in the IUCN Red List (Temple and Cox, 2009) and did not show a high rate of decline at the national level (D'Amen and Bombi, 2009). However, their decline in Latium deserves high conservation attention because it may signal a more serious problem for the species' future persistence.

The rate of local decline that we calculated for amphibian species in the study area is clearly related to our choice of the statistical method. There are numerous methods that provide a probabilistic basis for the persistence hypothesis based on sighting data (Solow, 1993a, 1993b; Burgman, *et al.* 1995; McCarthy, 1998; Solow and Roberts, 2003). Each method makes particular assumptions about the sighting record; since the performance is sensitive to deviations from these assumptions, the choice of the method should be based on the characteristic of the sighting record utilized (Solow and Roberts, 2003; Rivadeneira *et al.*, 2009). In our dataset we cannot a priori define a univocal form of the sighting rate for all cells. Indeed, many of the local disappearances of amphibians in the study region may occur due to gradual declines as a result of habitat loss, fragmentation and degradation. On the contrary, others would take place more rapidly for several causes. For instance, human activities such as wetland drainage for building purposes may produce an immediate drop in population size.

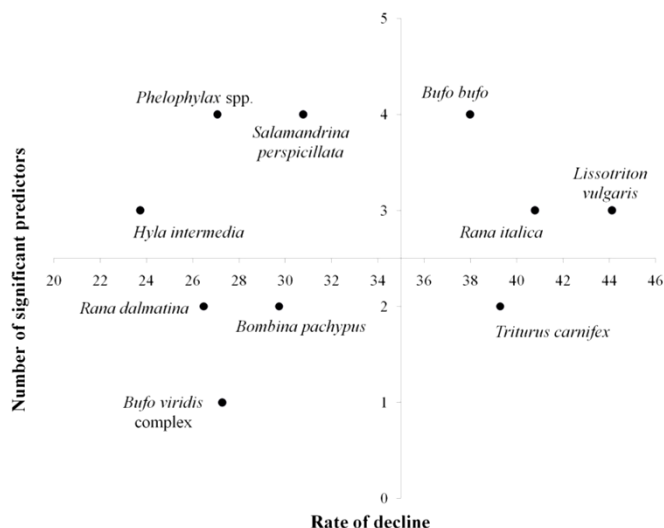


Figure 2 - Differential categories of conservation requirement for amphibians in Latium

Climatic events such as severe drought in the arid coastal areas can also be responsible for such rapid declines: a total reproductive failure in a single year may cause a severe drop in population size of short-lived species (Stewart, 1995). Moreover, exposure to amphibian chytridiomycosis or other parasites in high altitude populations can produce massive deaths in short periods (e.g. Bosh *et al.*, 2007). The non-parametric test that we applied works well when no information on population stability is known for each calculation (Solow and Roberts, 2003; Solow, 2005). The robustness of the nonparametric test is expected to come at the cost of lower power than the correct parametric test; despite this problem, the test has been applied to several groups of animals and plants (e.g. Solow and Roberts, 2003; van Der Ree and McCarthy, 2005; Farnsworth and Ogurcak, 2006; Duffy *et al.*, 2009).

Landscape and climate disturbance on amphibians

Our analyses of habitat destruction and alteration, as measured by different categories of urban and agricultural land use, allowed us to draw significant conclusions. For all species the amount of urban

and agricultural land use was associated with the observed declines and the Human Impact Index was an important predictor for half of the species. This supports the notion that human pressure is often inconsistent with the environmental requirements of amphibians (Atauri and de Lucio, 2001; Sala *et al.*, 2000; Davidson *et al.*, 2002; Hazell, 2003; Cushman, 2006; Gardner *et al.*, 2007). Urban areas are inhospitable habitats for amphibians because their natural habitats are converted into roads, houses, and industrial areas. Even if wetlands are preserved in urban areas, often in parks, they experience high human use and are excavated and stocked with fish (Delis *et al.*, 1996; Knutson *et al.*, 1999). The strength of habitat alteration effect varies among species not only according to the intensity of the disturbance, but also to intrinsic biological factors (Davidson *et al.*, 2002). The species that we found strongly associated with surrounding urban land use (the smooth newt and the northern spectacled salamander) are very sensitive to altered habitats. Accordingly, Andreone and Luiselli (2000) ranked these species ‘virtually inadapted to altered environments’, basing their classification on these species’ ecological constraints and distribution. Human density may have a direct impact on populations’ survival not only by means of urbanization, but also in more subtle ways, such as overexploitation for food, pets, medicinal and biological supply markets (Stuart *et al.*, 2004). This negative influence is consistent also with our results because the intensity of human activities, represented by the HII variable, was an important explanatory power for five species, particularly for the Italian stream frog and the Italian tree frog.

The probability of amphibian stable populations was significantly reduced by high agricultural surrounding land cover (80% of the species). Our results showed that, in the study area, not only permanent and intensive farming, but also more heterogeneous agricultural areas were associated with declines. Interestingly, although agricultural variables were a contributing factor, the strongest association with declines was shown only for the northern spectacled salamander. This result suggests that agricultural practices may act with other stressors, even if they are not a main driver of population declines. For instance, sub-lethal exposures of contaminants, due to water drift from cultivated areas, might increase the susceptibility of amphibians to other stressors as predation, UV-B radiation, pathogens, or changes in climate

(Pounds, 2001; Relyea and Mills, 2001; Relyea, 2003). In addition, as hypothesized by D'Amen and Bombi (2009), the high percentage of agricultural land use may exacerbate the effect of range fragmentation, driving populations towards extinction.

The spatial pattern of decline was consistent with the influence of climate change for six out of ten species. The growing number of dry days, which reflects increasing precipitation variability and aridity, was the most important factor related to amphibian local decline in central Italy. Our findings are congruent with those of other authors both in Central and North America (Pounds *et al.*, 1999; McMenamin *et al.*, 2008). Some ecological traits can explain the lack of a significant relationship between declines and climatic predictors for the remaining species. The northern spectacled salamander and the Italian stream frog are characteristic of forested habitats where they breed in oxygenate low running stream, which are less influenced by precipitation intensity with respect to other typologies of water bodies. The green toad is one of the most adaptable amphibians of the Palearctic, and is more tolerant to dry conditions than many other species (Gasc *et al.*, 1997). The Apennine yellow bellied toad utilizes temporary water bodies in multiple distinct periods of calling and oviposition which alternate with non-reproductive activity (Guarino *et al.*, 1998). This reproductive plasticity probably allows *Bombina pachypus* populations to use the temporary pools preferred for breeding, thus successfully overcoming climate fluctuations. Our results do not necessarily imply a direct mortality caused by climatic stressors. Climate may produce well documented synergistic effects with diseases, such as fungal infections, or with UV-B radiation (Kiesecker *et al.*, 2001; Simoncelli *et al.*, 2005; Kriger *et al.*, 2007). It is important to note that climatic change is expected to intensify in the coming decades, so that the detrimental effect shown in this paper will worsen, which will lead to massive range contractions, especially in the southwest of Europe (Araújo *et al.*, 2006; IPCC, 2007). Therefore, it will be particularly important to account for climate-linked threats to species conservation for planning long-term management programs of amphibian populations.

Spatial scales dependency

The causes of decline illustrated in this paper, are specific to the landscape resolution, while other processes can be highlighted across a different range of scales (Cushman and McGarigal, 2003). This trend is clearly shown when comparing our results with those obtained for the same species at a coarser resolution (10 km × 10 km) and at smaller spatial scale (Italy) by D'Amen and Bombi (2009). All but one species (the Italian green frog) were associated with landscape predictors at both scales. However, if we consider separately the effect of artificial and agricultural land cover some discrepancies emerge. Indeed, D'Amen and Bombi (2009) found fewer declines associated with agricultural than urban predictors, while at the regional scale we observed an opposite trend. In particular, only two species (the smooth newt and the Apennine yellow bellied toad) were negatively affected by agriculture at both scales of analyses and three species (the smooth newt, the northern spectacled salamander, and the green frog) by urban fabric. In contrast, the climatic results are generally in agreement with the country-scale research since all species identified as influenced by climate variations at the regional level were also associated with at least one climatic variable at the national level.

This comparison suggests a scale dependence of different categories of disturbance. The signal obtained by urban fabric disturbance emerges mainly at a large scale analysis because the urbanization activities completely eliminate suitable habitats for amphibians, thus extirpating entire populations. On the other hand, agriculture appears to be the main determinant of species declines at a finer resolution because it acts locally, producing habitat fragmentation and increasing the risk of population isolation. Climate change is likely to be a key factor at a multiple resolutions. Since many facets of the biology and ecology of amphibians are closely related to temperature and moisture, any modification of the climatic factors, measured at a different resolution, leads to an alteration of the phenology, breeding activity and demography for the more sensitive species (Duellman and Trueb, 1986; Angilletta, *et al.*, 2002).

Efficacy of protected areas

We discovered a negative association between species decline and abundance of protected areas only for two species. It means that the current network of nature reserves in Latium has been inadequate for safeguarding amphibian populations. This inefficient protection can be due to conservation strategies that safeguard single populations and reproduction sites, rather than landscapes. Because quality of habitat near breeding wetlands is important to most amphibians, it is necessary to consider the surrounding landscape in protection strategies. Efforts concentrated on narrow terrestrial buffers are likely to be inadequate (Herrmann *et al.*, 2005) because wetland fragmentation and water pollution can affect amphibian populations inhabiting small protected areas. Moreover, even in large and pristine reserves, other stressors arise as climatic conditions shift. An impressive example is the amphibian decline occurring in Yellowstone National Park (USA). This Park is one of the best-preserved ecosystems on our planet, where species have been actively protected longer than anywhere else on Earth. In Yellowstone, climatic change, and in particular droughts, have already disrupted wetland ecosystem equilibrium leading to massive amphibian population disappearances (McMenamin *et al.*, 2008). Similarly, we can hypothesize that growing arid conditions in Latium greatly damaged wetland system, even if well protected from more direct human stressors.

Will climate change reduce the efficacy of protected areas for amphibian conservation in Italy?

4.1 Introduction

The goal of this paper is to analyze the efficacy of the Italian reserve network for protecting amphibian diversity in a climate change scenario. We assessed how local amphibian biology and patterns of predicted climate change could act together to affect extinction risk and protected-area effectiveness over the mid 21st century. Our approach is based on ensemble niche modelling estimate of potential range shifts under two carbon emission scenarios (A1FI and B1) and two dispersal assumptions. The predicted distributions were used to perform gap and irreplaceability analyses.

We introduced a novel and flexible approach for conservation target definition to consider complex factors surrounding the effects of climate change. In particular, we incrementally adjusted targets for amphibian species conservation in response to the predicted change in species range size. We used these targets to determine under current and future climatic conditions the extent to which species are underrepresented in existing protected areas. We determined whether the current system of protected areas matches the most-valuable sites, taking into consideration the predicted effects of climate change on the distribution of suitable areas for amphibians. Additionally, we analyzed spatial options for filling gaps in the reserve system using an approach based on the principle of irreplaceability (Pressey *et al.*, 1994; Coetzee *et al.*, 2009). Such a framework could help to allocate limited conservation funds to priority areas, limiting investment in areas where conservation initiatives are likely inefficient in the long run. (see Appendix C for a detailed description of the species and variables data sets and of the statistical methods applied).

In the European context, Italy harbours a relatively diverse amphibian fauna (Gasc *et al.*, 1997; Bologna, 2004; Temple and Cox, 2009). In Italy, studies have separately addressed the

effectiveness of protected areas for terrestrial vertebrates and the potential for detrimental climate change impacts on amphibians. Maiorano *et al.* (2006, 2007) evaluated the capacity of Italian national and Natura 2000 protected areas to conserve vertebrate biodiversity under current climatic conditions. These authors found that existing protected areas are often insufficient to conserve current patterns of biodiversity in Italy. Similar results exist for other European regions (Hopkinson *et al.*, 2000; Dimitrakopoulos *et al.*, 2004; Araújo *et al.*, 2007). Finally, doubling current CO₂ levels will likely result in range contraction for those amphibian species that occur in mountainous and Mediterranean areas (Girardello *et al.* 2009). However, we know of no study that has focussed on Italy to investigate how extinction risk of amphibians is associated with the potential inadequacy of reserves systems under climate change.

4.2 Results

Predicted changes in species distributions

All modelling techniques provided good performance, with median AUC values between 0.83 and 0.9 (lower quartile: $0.79 < \text{AUC} < 0.85$; upper quartile: $0.88 < \text{AUC} < 0.93$). We find that Generalized Boosted Regression Models, Multivariate Adaptive Regression Splines, and Random Forest are the best performing models, with $\text{AUC} > 0.9$ for 10 species out 22 (Supplementary Materials: Table C3). Consensus models show diverse responses of species range to alternative climatic scenarios and dispersal assumptions (Table 7). Under a no-dispersal assumption (NO-DISP), all but two species are projected to lose suitable habitat in the future (median loss of 51%) (Figure 3). Losses are generally higher under the A1FI than under the B1 climate scenario although this difference is not statistically significant ($U=220$; $p=0.6055$. Mann-Whitney U Test; Table 7). Under a dispersal assumption (DISP), the consensus models forecast a reduction of the range size of 60% and 50% of the species under A1FI and B1 scenarios respectively (Table 7; Figure 4). We predict that eight species will suffer range reduction larger than 20% of current distribution, irrespective of climate change and dispersal scenario. One species, *Pelobates fuscus* is predicted to lose all suitable habitat under the A1FI scenario and absence of dispersal (Table 7). On the other hand, under the dispersal assumption, the

consensus forecasts for four species show an increase in the number of cells with suitable climate (Table 7).

Gap analysis

Using a 40% coverage threshold for matching reserves to grid cells, Nationally Designed Protected Areas (NPAs) and Overall Protected Areas (OPAs) occupy 282 and 617 cells respectively (Supplementary Materials: Figure C1). All of the species we considered are currently represented in both NPA and OPA protected areas. Nine species met their targets in OPAs, but none do when we restrict the analysis to NPAs (Table 8). In addition, more than half of the partial gap species (59% and 62% with NPAs and OPAs respectively) meet less than 50% of their respective targets (Table 8; Figure 5). The predicted number of species for which conservation targets are met decreases in the future independent of dispersal or climate change scenario (Tables 9, 10). Considering NPAs, some amphibians are predicted to become totally gap species, disappearing from all of the currently protected map cells. At the same time, the number of species that meet their target with OPAs will decrease and, if no-dispersal is assumed, one species will be completely absent in OPAs. In particular, *Pelobates fuscus*, *Pseudepidalea viridis*, and *Rana latastei* are only marginally represented in reserves (Table 8), each being protected in less than four cells by NPAs (Supplementary Materials: Table C4). Their representation is expected to decline in the future, when they will be completely unrepresented in NPAs, independent from the considered scenario (Supplementary Materials: Table C5 and C6). Moreover, if no dispersal is assumed, no map cells with suitable climate for *Pelobates fuscus* will be protected by OPAs, while *Pseudepidalea viridis* and *R. latastei* will be protected by OPAs in five cells or fewer (Supplementary Materials: Table C5).

Table 7 – Predicted species range change Current range extent (number of occupied cells), and percentage of predicted change in the future climatic conditions (according to A1FI and B1 socio-economic scenarios), under different dispersal assumptions

Species	Current range extent	Percentage of predicted range change			
		NO-DISP		DISP	
		A1FI	B1	A1FI	B1
<i>Bombina pachypus</i>	339	-40.24	-33.14	30.47	46.75
<i>Bombina variegata</i>	166	-75.32	-60.76	-40.51	-13.29
<i>Discoglossus pictus</i>	127	-1.64	-4.10	79.51	73.77
<i>Discoglossus sardus</i>	41	-73.17	-46.34	-29.27	39.02
<i>Hyla intermedia</i>	1110	-3.33	-6.94	128.11	99.10
<i>Hyla sarda</i>	58	-21.82	0.00	256.36	392.73
<i>Lissotriton italicus</i>	255	-14.12	-10.20	47.06	54.90
<i>Lissotriton vulgaris</i>	738	-50.27	-56.64	-20.19	-30.49
<i>Mesotriton alpestris</i>	351	-46.15	-46.72	-4.27	-1.99
<i>Pelobates fuscus</i>	64	-100.00	-92.19	-68.75	-29.69
<i>Pseudepidalea balearica</i>	638	-5.96	-10.19	257.84	203.13
<i>Pseudepidalea sicula</i>	99	0.00	0.00	167.35	162.24
<i>Pseudepidalea viridis</i>	109	-52.29	-51.38	-1.83	-0.92
<i>Rana dalmatina</i>	810	-50.74	-57.04	-20.12	-31.11
<i>Rana italica</i>	577	-44.89	-44.71	2.95	2.25
<i>Rana latastei</i>	235	-55.74	-55.32	-33.19	-31.06
<i>Rana temporaria</i>	514	-51.58	-42.53	-34.11	-20.21
<i>Salamandra atra</i>	82	-95.00	-63.75	-91.25	-36.25
<i>Salamandra salamandra</i>	680	-63.07	-54.25	-41.67	-29.58
<i>Salamandrina perspicillata</i>	242	-51.65	-57.02	33.47	20.66
<i>Salamandrina terdigitata</i>	53	-96.15	-73.08	-90.38	0.00
<i>Triturus carnifex</i>	958	-77.24	-71.82	-56.16	-49.90

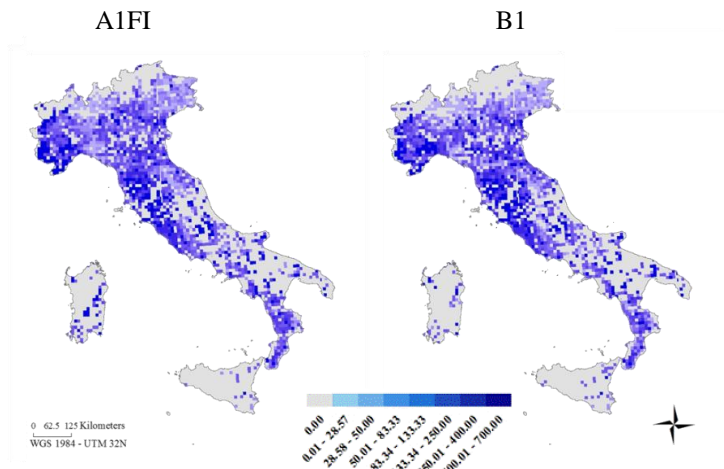


Figure 3 - Percentage of species loss per pixel according to two climatic scenarios It is calculated as the percentage of species lost respect to the current species richness in that pixel

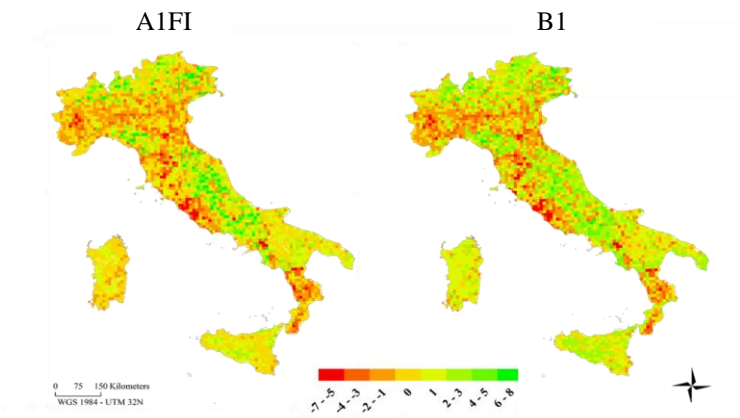


Figure 4 - Species variation per pixel under a dispersal assumption It is calculated as the number of species which are currently absent but predicted to move to the given pixel minus number of species predicted to disappear from the given pixel

Table 8 – Current percentages of target met by each species in NPAs and OPAs Values of 100% are represented by a dash. Values lower than 50% are presented in italics and percentages equal to 0 are highlighted in bold. (ext = species that are projected to become extinct under the future conditions)

Species	NPAs	OPAs
<i>Bombina pachypus</i>	71.99	-
<i>Bombina variegata</i>	9.35	38.74
<i>Discoglossus pictus</i>	20.34	29.73
<i>Discoglossus sardus</i>	13.47	43.78
<i>Hyla intermedia</i>	77.48	-
<i>Hyla sarda</i>	10.51	39.4
<i>Lissotriton italicus</i>	61.97	-
<i>Lissotriton vulgaris</i>	39.96	-
<i>Mesotriton alpestris</i>	25.25	83.24
<i>Pelobates fuscus</i>	2.45	7.36
<i>Pseudepidalea balearica</i>	35.88	76.77
<i>Pseudepidalea sicula</i>	18.3	36.6
<i>Pseudepidalea viridis</i>	1.72	25.81
<i>Rana dalmatina</i>	63.84	-
<i>Rana italica</i>	81.03	-
<i>Rana latastei</i>	3.33	14.44
<i>Rana temporaria</i>	53.28	-
<i>Salamandra atra</i>	26.93	97.35
<i>Salamandra salamandra</i>	67.92	-
<i>Salamandrina perspicillata</i>	33.95	82.14
<i>Salamandrina terdigitata</i>	72.78	92.37
<i>Triturus carnifex</i>	86.68	-

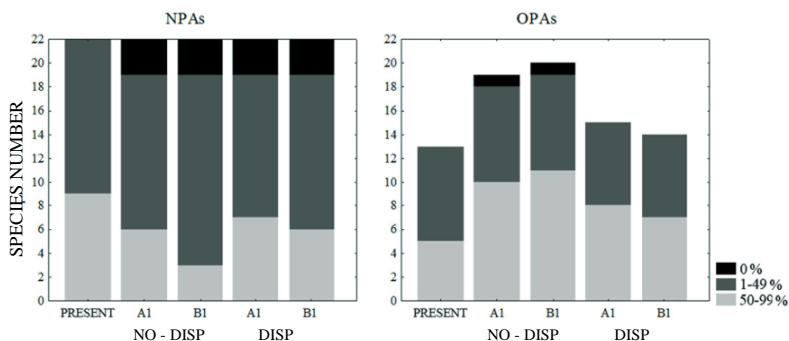


Figure 5 - Species representation. Number of gap and partially gap species in the current conditions and in the future scenarios, considering only NPAs and the OPAs. Different blocks colors represent amounts of target met

Irreplaceability analysis

Considering no cells as protected, the areas of highest values of irreplaceability for amphibian conservation are found on the island of Sardinia and the lowlands of North-Eastern continental Italy (most irreplaceable 1% of cells; Figure 6; see Supplementary Materials Figure C2 for place name locations). Secondary regions of high irreplaceability are present on Sicily and on the Tyrrhenian side of Southern Italy. The inclusion of Nationally Designed Protected areas (NPAs) in GAP analysis reduces irreplaceability scores for cells along the Italian peninsula and on the Tyrrhenian side of Southern Italy. Other peak areas of irreplaceability remain almost unmodified (Figure 6b). The addition of Natura 2000 areas (EPAs) had a strong positive effect, but the pattern of change is still similar to when no protected cells are included (Figure 6c), with the most irreplaceable 1% of cells still being unprotected in Sardinia and in North-Eastern continental Italy.

Under future conditions, both climate scenarios, and with no cells considered as protected, Sardinia and Sicily remain in the future as important as they are currently (Figure 7). The map cells on the Tyrrhenian side of Southern Italy increase in irreplaceability, as do cells in the mountainous areas of central and eastern Alps and, secondarily, in the central Apennine (Figure 7). If the presence of all

protected areas (OPAs) is considered, the irreplaceability maps calculated for future conditions represent the focal areas where new reserves should be designed for the long-term conservation of amphibians. The geographic pattern of these areas is similar among predictions based on the four socio-economic and dispersal assumptions: Sardinia, the lowlands of North-Eastern continental Italy and the central Alpine foothills are primary important areas and hold the most irreplaceable 1% of cells (Figure 8). Sicily and the Tyrrhenian side of Southern Italy represent further peaks of irreplaceability, and the central and northern Apennine become important in projections that assume no dispersal occurs (Figures 7a,c and 8a,c).

Representativeness in protected areas

The comparison of mean irreplaceability values of protected map cells with values calculated from 5000 sets of randomly selected map cells reveals that presently the entire network of Italian reserves (OPAs) and its components separately (Nationally Designed Protected areas [NPAs] and the Natura 2000 sites not overlapping with the former [EPAs]) protect sites with greater irreplaceability than that expected by chance (Table 11). This is also true under projections of future climate (both A1FI and B1) assuming no dispersal occurs. To the contrary, assuming dispersal occurs and under both A1FI and B1 scenarios, cells with NPAs are not more irreplaceable in the future than cells selected at random, and cells with EPAs are only marginally more irreplaceable than randomly chosen, unprotected cells. Nevertheless, Italian reserves have non-random differences in terms of mean irreplaceability respect to unprotected cells under future scenarios (both A1FI and B1) if they are considered as a whole (OPAs) (Table 11).

Table 9 - Percentages of target met by each species in NPAs and OPAs under two climate change scenarios and a dispersal assumption Values larger than 100% are represented by a dash. Values lower than 50% are presented in italics and percentages equal to 0 are highlighted in bold. (ext = species that are projected to become extinct under the future conditions)

Species	A1FI		B1	
	NPAs	OPAs	NPAs	OPAs
<i>Bombina pachypus</i>	77.62	-	84.21	-
<i>Bombina variegata</i>	18.93	52.99	17.93	55.29
<i>Discoglossus pictus</i>	24.17	34.52	19.89	30.42
<i>Discoglossus sardus</i>	30.48	65.31	21.27	63.81
<i>Hyla intermedia</i>	64.62	-	64.88	-
<i>Hyla sarda</i>	1.76	21.11	7.56	36.1
<i>Lissotriton italicus</i>	68.57	-	72.84	-
<i>Lissotriton vulgaris</i>	28.37	70.1	27.07	62.6
<i>Mesotriton alpestris</i>	46.57	-	42.4	-
<i>Pelobates fuscus</i>	0	23.13	0	12.59
<i>Pseudepidalea balearica</i>	61.32	-	51.19	-
<i>Pseudepidalea sicula</i>	23.21	39.04	23.42	39.39
<i>Pseudepidalea viridis</i>	0	17.41	0	13.85
<i>Rana dalmatina</i>	36.74	90.18	33.5	84.58
<i>Rana italica</i>	60.9	-	61.75	-
<i>Rana latastei</i>	0	13.79	0	13.55
<i>Rana temporaria</i>	53.74	-	54.66	-
<i>Salamandra atra</i>	42.86	42.86	23.01	69.04
<i>Salamandra salamandra</i>	31.61	96.69	34.21	-
<i>Salamandrina perspicillata</i>	34.74	75.26	35.19	76.42
<i>Salamandrina terdigitata</i>	60	60	42.56	51.07
<i>Triturus carnifex</i>	23.85	63.6	13.69	48.78

Table 10 - Percentages of target met by each species in NPAs and OPAs under climate change scenarios and a no-dispersal assumption Values larger than 100% are represented by a dash. Values lower than 50% are presented in *italics* and percentages equal to 0 are highlighted in **bold**. (ext = species that are projected to become extinct under the future conditions)

Species	A1FI		B1	
	NPAs	OPAs	NPAs	OPAs
<i>Bombina pachypus</i>	62.39	97.19	67.96	-
<i>Bombina variegata</i>	<i>13.97</i>	<i>38.43</i>	<i>15.04</i>	<i>42.61</i>
<i>Discoglossus pictus</i>	<i>21.06</i>	<i>29.16</i>	<i>18.1</i>	<i>26.33</i>
<i>Discoglossus sardus</i>	<i>37.05</i>	<i>64.84</i>	<i>21.49</i>	<i>64.48</i>
<i>Hyla intermedia</i>	64.31	-	62.92	-
<i>Hyla sarda</i>	<i>3.25</i>	<i>29.28</i>	<i>10.91</i>	<i>40.9</i>
<i>Lissotriton italicus</i>	54.09	88.61	57.39	91.15
<i>Lissotriton vulgaris</i>	<i>21.18</i>	<i>48.81</i>	<i>21.31</i>	<i>47.45</i>
<i>Mesotriton alpestris</i>	<i>24.86</i>	<i>74.59</i>	<i>22.51</i>	<i>77.52</i>
<i>Pelobates fuscus</i>	ext	ext	0	0
<i>Pseudepidalea balearica</i>	<i>27.52</i>	<i>63.39</i>	<i>24.24</i>	<i>55.16</i>
<i>Pseudepidalea sicula</i>	<i>18.42</i>	<i>36.84</i>	<i>18.42</i>	<i>36.84</i>
<i>Pseudepidalea viridis</i>	0	<i>14.19</i>	0	<i>11.2</i>
<i>Rana dalmatina</i>	<i>25.1</i>	<i>59.17</i>	<i>24.39</i>	<i>60.04</i>
<i>Rana italica</i>	<i>42.72</i>	<i>81.55</i>	<i>47.51</i>	<i>85.33</i>
<i>Rana latastei</i>	0	<i>7.09</i>	0	<i>5.29</i>
<i>Rana temporaria</i>	<i>49.4</i>	-	<i>49.71</i>	-
<i>Salamandra atra</i>	75	75	<i>26.13</i>	<i>87.09</i>
<i>Salamandra salamandra</i>	<i>16.99</i>	<i>63.43</i>	<i>18.43</i>	<i>64.51</i>
<i>Salamandrina perspicillata</i>	<i>27.98</i>	<i>64.19</i>	<i>30.14</i>	<i>70.92</i>
<i>Salamandrina terdigitata</i>	-	-	<i>45.87</i>	<i>61.16</i>
<i>Triturus carnifex</i>	<i>6.92</i>	<i>31.14</i>	<i>6.24</i>	<i>30.18</i>

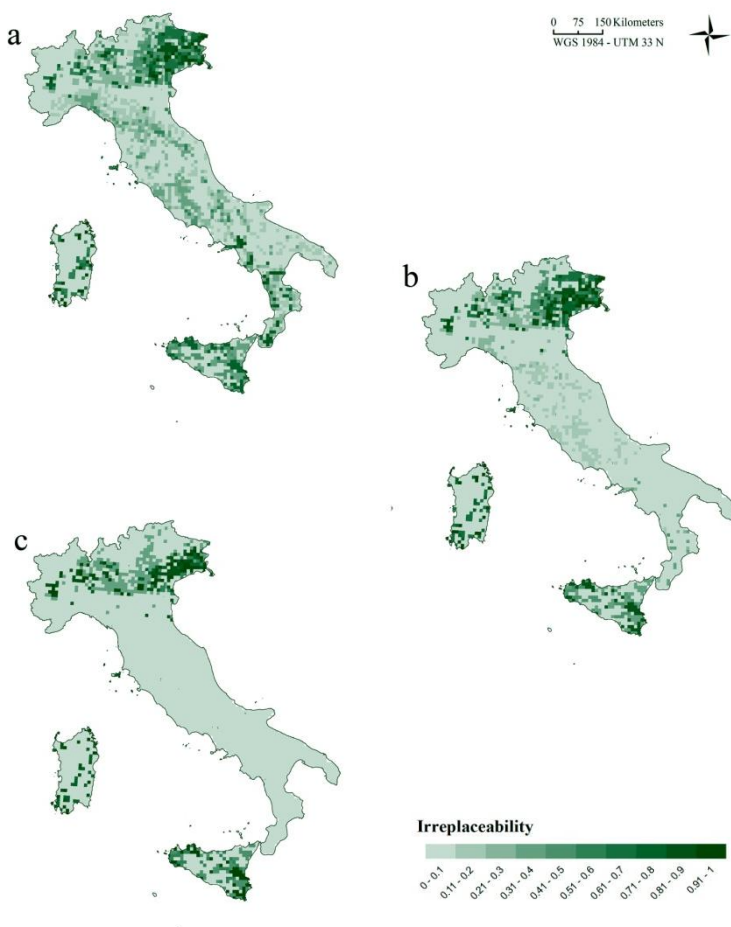


Figure 6 - Current irreplaceability patterns They were calculated by considering a) no pre-existent protected areas, b) nationally designed protected areas, c) all the protected areas network comprising Natura 2000 sites

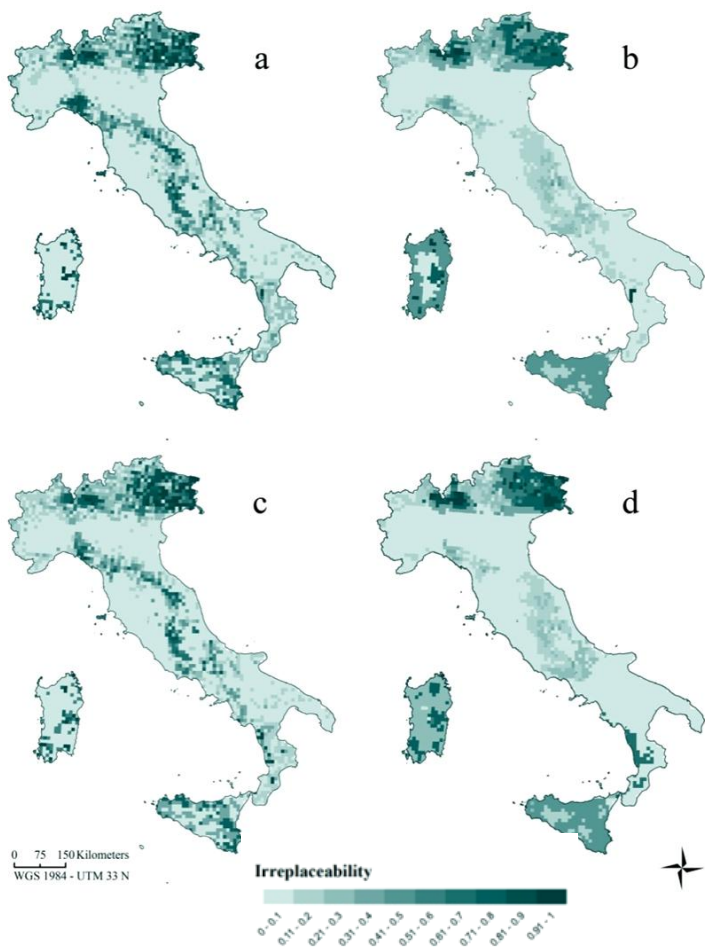


Figure 7 - Future conservation values of the Italian territory for amphibians Irreplaceability values were calculated considering all cells as unreserved, according to two emission scenarios (AIFI: a and b and B1: c and d) and two dispersal assumption (no-dispersal: a and c and dispersal: b and d)

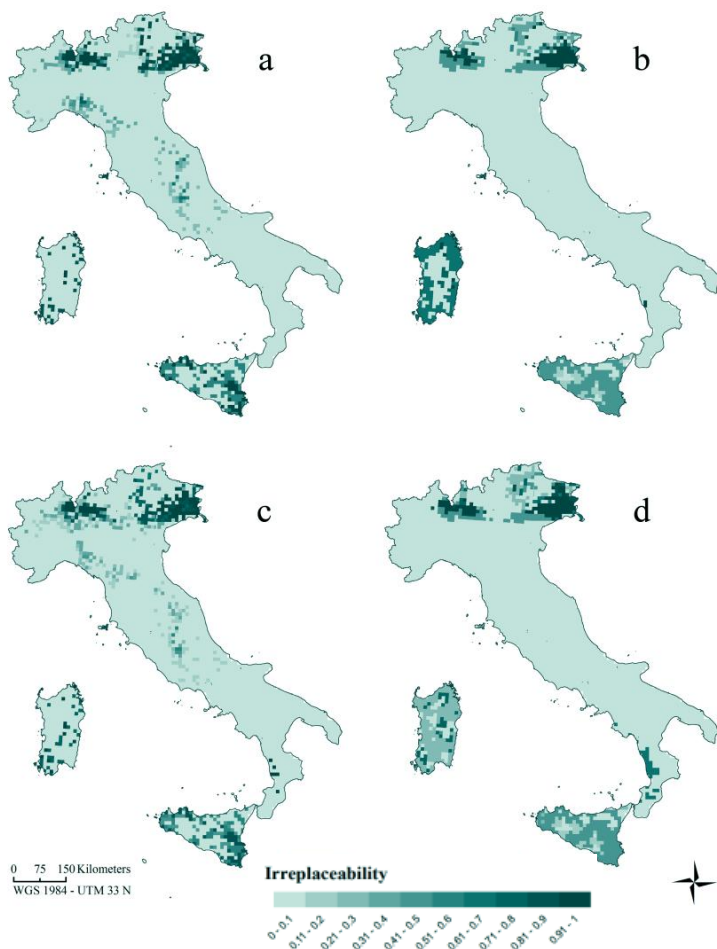


Figure 8 - Future irreplaceability patterns They were calculated by considering the existing OPAs, based on a consensus forecast in different climatic scenarios (A1FI: a and b and B1: c and d) and dispersal assumptions (no-dispersal: a and c, and dispersal: b and d)

Table 11 - Non-randomness test of differences between the mean irreplaceability value in protected cells (M_{obs}) and in 5000 random cell selections in the whole grid (M_{sim}). Irreplaceability was calculated by considering all cells as unprotected for NPAs and OPAs, and taking into account the existence of NPAs for EPAs. Non-random differences were assumed when $P_{Mobs \geq Msim} \leq 0.05$. In italic we presented significant values after Bonferroni correction

		NPAs	EPAs	OPAs	
PRESENT		M_{obs}	0.249	0.206	0.251
		M_{sim}	0.18	0.155	0.18
		$P_{Mobs \geq Msim}$	> 0.001	0.001	> 0.001
NO DISP	AIFI	M_{obs}	0.254	0.2	0.248
		M_{sim}	0.184	0.158	0.184
		$P_{Mobs \geq Msim}$	> 0.001	0.001	> 0.001
	BI	M_{obs}	0.256	0.225	0.261
		M_{sim}	0.186	0.16	0.186
		$P_{Mobs \geq Msim}$	> 0.001	> 0.001	> 0.001
DISP	AIFI	M_{obs}	0.202	0.164	0.209
		M_{sim}	0.18	0.156	0.18
		$P_{Mobs \geq Msim}$	0.045	0.277	0.001
	BI	M_{obs}	0.202	0.189	0.217
		M_{sim}	0.181	0.159	0.181
		$P_{Mobs \geq Msim}$	0.063	0.012	> 0.001

4.3 Discussion

Range modifications are predicted for all amphibian species under future climatic conditions and these changes will affect the degree to which species are represented in the Italian protected area network. Our results showed that under current conditions the existing network does not represent neither the entirety of amphibian diversity nor its geographic pattern. This inadequacy will get even worse on the long-term, when range shifts and reductions due to climate change will lead at decreasing species representation over the entire protected area system. We identified some areas currently highly irreplaceable and that will maintain their importance under all future climate scenarios. The inclusion of such areas in the network can produce an improvement of the network ability in protecting amphibians on the long-term.

Extinction risk driven by climate change

Predictions of range modification for amphibian species in Italy are clearly dependent on assumptions of dispersal. Under both alternative emission scenarios, if species are allowed to disperse, consensus models show potential eastward and upward range shifts for many species both in the peninsula and in northern Italy. Similar directions of species range shifts have already been documented in Europe and elsewhere for other groups (e.g. Parmesan *et al.*, 1999; Erasmus *et al.*, 2002). However, as there is high uncertainty about the ability of amphibians to successfully navigate highly fragmented landscapes, which leaves the assumption of no-dispersal representing the most realistic scenario for all species (Smith and Green, 2005; Araújo *et al.*, 2006). Under this assumption, the loss of predicted suitable habitat (70% or more) is forecasted for almost all species. The protection of the areas that remain suitable over the long term is the most prudent approach for conservation planning (Bakkens *et al.*, 2002; Williams *et al.*, 2005).

Predictions under different emission scenarios and dispersal assumptions agree in indicating that the species most sensitive to climate change are *Pelobates fuscus*, *Salamandrina terdigitata*, *Salamandra atra*, and *Triturus carnifex*. Among those, Girardello *et al.* (2009) forecasted a large range reduction for the first two species only. This discrepancy with the present results is explained by the

different modelling approach (a single technique) and climatic scenario ($2\times$ CO₂ climate scenario) used by Girardello *et al.*. The four species we identified are either endemic or subendemic at various taxonomic levels in Italy, yet are classified as Least Concern in the most recent Red List of European Amphibians (Temple and Cox, 2009). As reduction of distributional area is consistently a good predictor of extinction risk (Koopowitz *et al.*, 1994), our forecasts suggest increasing danger of extinction for these species by the middle of the current century. This supports the idea that the incorporation of vulnerability to climate change should be an important component of IUCN Red List assessments (Bomhard *et al.*, 2005; Coetzee *et al.*, 2009).

A specific comment is required for the Italian subspecies *P. fuscus insubricus*, which is endemic to the Po river plain of northern Italy and already recognized as a highly threatened *taxon* (Andreone and Luiselli, 2000; D'Amen and Bombi, 2009; Agasyan *et al.*, 2010). We predict this subspecies to lose all suitable habitats in Italy by the mid twenty-first century under the assumption of no dispersal, in agreement with Girardello *et al.* (2009). Moreover, it is particularly difficult for this toad to move through the environmental matrix because of the substantial anthropogenic impact in the Po river plain. The ability of the species to disperse to follow the shifting location of suitable environmental conditions is of crucial importance for any management initiative for the species. The creation of specific corridors that could allow this toad to disperse to areas with appropriate climate would facilitate its conservation. However, "assisted colonization" (*sensu* Hoegh-Guldberg *et al.*, 2008) could be necessary for preventing the extinction of this endemic *taxon*.

Current amphibian representation in Italian protected areas

The currently protected sites are on average more irreplaceable than remaining areas. Nevertheless, from gap and irreplaceability analyses it emerges that the existing network does not represent all amphibian species adequately under current conditions. This discrepancy can be explained by the site-specific strategy based on the direct and detailed knowledge of local diversity, which historically drove reserve selection in Italy (Maiorano *et al.*, 2007). The combination of NPAs and Natura 2000 sites is essential for improving representation of amphibian, but targets based on

geographic range extent are, nevertheless, achieved only for 40% of species. In fact, even if the Natura 2000 sites greatly increase the extent of protection geographically, the most irreplaceable areas for amphibian conservation (Sardinia, Sicily and the north-eastern Italy) are not integrated satisfactorily into the Italian reserve network. This lack of representation is of interest, considering that most of the species we did not consider are distributed in Sardinia (five species) and in north-eastern Italy (two species). The result is that the current reserve network is comprehensively inefficient and represents neither the entirety of amphibian diversity nor its geographic pattern.

Our results are in general accordance with earlier analyses of current park efficacy in Italy and that addressed all vertebrate classes (Maiorano *et al.*, 2006, 2007). Maiorano *et al.* (2006) demonstrated that amphibians are one of the least protected taxonomic groups, with the highest absolute number of total-gap species. Interestingly, Maiorano *et al.* (2006) discovered high values of irreplaceability for some areas that we noted as particularly irreplaceable for amphibians. Particularly important is the case of Sardinia where, even considering Natura 2000 implementation, the number of protected areas is extremely low and none coincide with areas of the highest diversity of either vertebrates (Maiorano *et al.*, 2006, 2007) or amphibians. To the contrary, by considering total vertebrate diversity, Sicily did not emerge in Maiorano *et al.* (2006, 2007) as an area of high irreplaceability, while this region is of great importance if one considers the conservation of amphibian diversity. Both previous analyses of Italian reserve efficacy (Maiorano *et al.*, 2006, 2007) did not consider the likely impacts of climate change on biodiversity distribution patterns, thus the priority sites selected for reserve system implementation do not account for the protection of vertebrates long-term climatic refugia. Future research should address the effects of climate change on the projected species representation for each vertebrate class in the Italian reserve system.

Long-term efficacy of the Italian protected areas for amphibian conservation

Our study represents a first attempt to evaluate the long-term efficacy of the Italian protected areas for the conservation of amphibians. Our approach is based on niche modelling estimates of

potential range shift leading changes in species representation in protected areas. Other studies used this approach to predict a decrease in species representation for different *taxonomic* groups and localities (e.g. Araújo *et al.*, 2004; Hannah *et al.*, 2007; Coetzee *et al.*, 2009). Range shifts and reductions due to climate change will lead to a decrease in the efficacy of nationally designated protected areas in Italy. In particular, some species will be represented in the future in very few protected cells while three species are projected to lose all map cells with suitable climate if dispersal is assumed not to occur. Climate change is predicted to decrease the number of protected cells with suitable climate for many amphibian species over the entire protected area system. Under both future emission scenarios we examined, only when the OPAs are taken together as a whole are protected cells on average more irreplaceable than the remaining cells. This result underscores the importance of Natura 2000 sites in complementing the nationally designed protected areas for long-term conservation of amphibians. This is similar to what was found for the Iberian peninsula by Araújo *et al.* (2007), who determined that sites of community importance are priority areas for the implementation of national reserves networks.

We have located important areas that, if protected, could contribute to the conservation of amphibians in Italy. The areas we identified are not yet defined at a spatial resolution to permit the proposal of new park boundaries. New research at a finer spatial resolution is required for setting boundaries of new reserves that will complement existing ones. Interestingly, we identify some areas that are the most irreplaceable currently for conservation of amphibian species and which will maintain their importance under all future climate scenarios. In particular, the creation of new reserves in specific areas of Sardinia, north-eastern continental Italy, and Sicily would increase substantially the efficiency of the network of protected areas for ensuring the long term conservation of amphibians. Such implementation assumes greater importance in view of the national responsibility that Italy carries for species protection (Schmeller *et al.*, 2008). In fact both Italian main islands are focal region for the global survival of eight amphibian species (seven in Sardinia and one in Sicily), while the protection of north-eastern continental Italy is essential for the long term persistence of the endemic frog *Rana latastei*.

Synthesis and Conclusions

Mounting evidences indicate that global climate is changing, that biological responses to warming are under way, and that current conservation strategies will need to be revised for being effective in the face of future climate change (Hughes, 2000; Hannah *et al.*, 2002). The general subject of this thesis was to gain a better understanding of different aspects that can improve amphibian conservation in the context of climate change. Centering the attention on the Italian fauna, I have identified the proximate factors already affecting amphibians and I have documented the differences in effects across the country. Further, moving to a smaller extent and increasing the resolution of the analysis I showed a general agreement in results. Both studies revealed that climate change has been a major cause of population disappearances for multiple amphibian species in past decades. Next, I explored the potential consequences of future climatic shifts on species distributions in Italy. Range modifications were predicted for amphibians under future climatic conditions and the degree to which these changes will affect species representation in the Italian protected area network was estimated. Finally, this research illustrates how the inclusion of information on sub-*taxon* membership and phylogeographic structure in modeling exercises, when available, can improve the predictions of potential impacts of climate change on biodiversity.

5.1 Past amphibian declines in Italy

Improving our knowledge of the threats affecting species survival is an obvious, yet important, aspect that can help at preventing future loss of populations by developing mitigation strategies. The analysis of spatial patterns of decline is a powerful method for identifying multiple factors causing widespread disappearances and testing hypotheses associated with those declines. This thesis demonstrates complex influences of several factors associated with amphibian population declines varying across species and areas. In testing the climate hypothesis, we considered two principal aspects of climate affecting amphibians: the decrease of water availability in space and time, and the increase in temperature. In fact, all over Italy, an

increase of mean temperature and of precipitation intensity, and a reduction of number of wet days, were recorded mainly in the last 50 years (e.g. Brunetti *et al.*, 2004, 2006; Toreti and Desiato, 2008). Considering the national level (Chapter 2), factors pertaining to anthropogenic habitat alteration, especially linked to urbanization, were often important as expected. Interestingly, the association of declines with climatic variables appeared to be just as important. I identified a common pattern of disappearances in areas that have been especially affected by climatic changes. These findings strongly suggest that shifting climatic conditions are an additional serious threat already contributing to the current amphibian crisis, also in temperate regions. Thus, by identifying the most threatened species, geographical hot spots of decline, and the primary causes of decline, this work provides a basis for improving management and setting conservation priorities in Italy.

The analysis of the causes of amphibian declines at a smaller extent, utilizing a different data set (Chapter 3), generally confirms results obtained at the national level. Notably, all species identified as influenced by climate variations at the regional level were also associated with at least one climatic variable at the national level. A scale dependence of different categories of disturbance was detected considering the land use influence. While signal obtained by urban-fabric disturbance emerges mainly at a small-scale, agriculture appears to be the main determinant of species declines at a finer resolution. Likely, agriculture impacts locally, producing habitat fragmentation and increasing the risk of population isolation, while urbanization activities completely eliminate suitable habitats for amphibians, thus can be evidenced at lower resolution. The status of amphibians in the considered region (i.e. Latium) warrants greater attention than has been given to date, especially because the protection of breeding sites through small natural reserves proved to be inefficient. My findings are in accordance with the conclusion of Houlahan *et al.* (2006), in that wetlands cannot be managed in isolation. Neighbouring wetlands and neighbouring land use both need to be taken into account in land-use planning for conservation.

5.2 Future threats from global warming

By combining niche modeling and systematic area prioritization for amphibians encompassing the Italian territory, I explored the implications of reserve location for the conservation of species in the current climate and accounting for the effects of climate change (Chapter 5). The great complexity of natural systems suggests that there are fundamental limits to the accurate prediction of future species distributions (Pearson and Dawson, 2003). Nevertheless, the identification of consensus among multiple modelling techniques, as applied in this study, is one of the most promising approaches to adequately represent the likely impacts of climate change (Araújo and New, 2007; Coetzee *et al.*, 2009). A further improvement ensues from the inclusion of species dispersal ability, which reduces uncertainty in projections of species distribution shifts (Engler and Guisan, 2009). Moreover, adjusting conservation targets in response to variation in species range size, as proposed in this research, makes prioritization methods sufficiently flexible for anticipating climate change impacts and accounting for them in systematic reserve design. This adaptive approach is important because time is a key factor in implementing conservation action.

The early identification of areas that are to be threatened by processes such as climate change is crucial for planning effective countermeasures. I evidenced the north-eastern continental Italy, Sardinia, and secondarily Sicily for their high irreplaceability scores under present and future conditions. These are the most valuable spots for amphibian conservation in Italy. A key finding of my thesis is that such areas have not yet been considered in any process of reserve selection. My results can direct the selection process toward areas crucial for efficiently complementing the existing network, but new researches at a finer spatial resolution are required for setting boundaries of potential new reserves.

5.3 Improved methods to conserve effective evolutionary units

Species are made up of local populations that may be locally adapted to different portions of their range and such populations may have a different genetic constitution to those in another part. The more historically isolated the groups, the more likely they are to have distinct genetic attributes, different evolutionary potential, and

dissimilar responses to environmental alteration (Rogers *et al.*, 2010). The study here presented (Appendix D) analyzes these patterns of within-species variation in view of developing models for predicting the potential effects of climate change on species distributions. Including information on within-*taxon* genetic diversity in many cases modifies predicted effects of climate change on the species or adds detail on climate change effects that contrast between subspecies or sister species. Such differences emerge as the subclade models capture the climate-distribution relationship for groups of populations with similar evolutionary histories and shared selective regimes (Avise, 2000). This richness of additional facets in the potential response of *taxa* and sub-*taxa* to climate change suggests that phylogeographic information should be incorporated into modeling exercises that examine potential effects of climate change on species distribution, when available.

Accounting for the potential different response of distinct evolutionary lineages to climate change is fundamental when predictive models are used to inform conservation managers. The lack of including this information can lead to some lineages having little representation in the resulting clade model projections. This, on the one hand can provoke an underestimation of climate tolerances and overestimate effects of climate change on *taxon* range size. On the other hand, some lineages more threatened by climate change can be undetected, and consequently neglected in long-term plans for conservation. In addition, the use of phylogeographic information for conservation prioritization purposes can direct management plans around areas that harbor high genetic diversity (Rissler *et al.*, 2006).

5.4 Priorities for amphibian conservation in Italy

The comparison of observed amphibian declines and projections of species risk under future conditions highlights some conservation priorities. The common spadefoot toad, *Pelobates fuscus*, emerges from both analyses as the most threatened amphibian species in Italy. Not only it has the highest rate of local decline in past decades, but also it is projected to lose a considerable part of its current distribution irrespectively of climate change and dispersal scenarios. Under the A1FI scenario, it is the only species predicted to disappear from the present know range in Italy. *P. fuscus* is a fossorial amphibian that inhabits much of the European plain areas. The

populations spread in small isolated areas in the Po Valley in Northern Italy belong to a distinct subspecies *P. fuscus insubricus*. A recent phylogeographic study of the common spadefoot toad, showed that the Po Valley has the largest centre of genetic variability for this species (Crottini *et al.*, 2007). Notwithstanding the great value of the Italian populations, the gap analysis here presented claims that *P. fuscus insubricus* is only marginally represented in reserves. The conservation of this subspecies must necessarily be reinforced, improving the ecological conditions of his habitat, and taking into account future modification of hydrology and climate (Andreone and Luiselli, 2000).

Other species currently characterized by high local declines rates emerge as critically affected by future climate change. In particular, both *Salamandra atra* and *Triturus carnifex* were affected by past climate alteration and they are forecasted to lose a conspicuous portion of their current suitable habitat in the future (up to 95% and 77% respectively), regardless of climate scenarios and dispersal ability. In spite of this threats, both species are ranked as Least Concern in the IUCN Red Lists (IUCN, 2008). This emphasizes the need for threatened species lists criteria to incorporate vulnerability to climate change (Bomhard *et al.*, 2005; Coetzee *et al.*, 2009). Particular attention deserves the subspecies *S. a. aurorae* spread in north-east Italy and qualified for listing as Critically Endangered for its extremely small distribution (less than 50 square kilometers) (IUCN, 2008). The narrow distribution range of this subspecies did not allow the development of climate envelop models at the resolution utilized in this thesis. It is highly recommendable further, high resolution, studies focusing only on the subspecies *S. a. aurorae* to ascertain its potential responses to future climate change and to plan specific conservation measures.

Notably, future climate change will likely cause range contractions also for species that were not affected by the climatic variation occurred in past decades. The most prominent example is the Italian agile frog, *Rana latestei*, a lowland species, mainly spread in Northern Italy, judged as Vulnerable by the IUCN Red List (2008). As it requires forest in close proximity to breeding sites, this species is threatened by the destruction of much of the original humid deciduous forests of the Padano-Venetian plain. According to my results, population declines in past decades were not associated with

climate variables. Nonetheless, forecasts of future range shifts show for this species a loss of suitable habitat ranging from 31 to 56 %. This means that the species currently not sensitive to climate change may be placed at increasing risk due to further raise in temperature and dry conditions. *R. latastei* deserves a particular attention also because it is not adequately represented in Italian reserves and this situation will worsen in the future, when it will be protected only in five UTM cells encompassing the present range. Additional protective measures are indispensable for its conservation, also accounting for likely impacts of climate change.

5.5 Final remarks

Climate change is acting in an environment already threatened by other processes (Thomas *et al.*, 2004). For decades conservationists and managers have dealt with habitat fragmentation and degradation, introduced species and pathogens, hunting pressures, and toxins. These issues remain and many will interact and be compounded by climate change. Our reaction to climate-linked threats to biodiversity must be formulated with the complexity and synergism of these processes in mind (Sinclair *et al.*, 2010). Habitat conservation must remain a priority for amphibians because their usually small areas of occupancy make them more susceptible to extinction from habitat loss and degradation than other vertebrates (Gascon *et al.*, 2007). In addition, if the effects of climate change on amphibians include physiological stress, changing niche space, and a compulsion to move across a fragmented or hostile environment, then one of the best solutions to soften the impact is addressing the fundamental issues of habitat quality and connectivity, to maintain the basic processes of dispersal, local extinctions and recolonizations. (Sinclair *et al.*, 2010). Protected areas alone offer a limited defense against problems posed by rapid environmental change. In fact, the matrix of land uses surrounding protected areas provides a biophysical framework that both impacts core reserves and hampers biodiversity in transition. A new reserves prioritization is required that accounts for potential effects of climate change, both in terms of areas as well as taxonomic groups.

We are still learning about climate change. Some problems are likely to be surmountable through management, adaptation, or evolution, others are likely to be more intractable (Dundley, 2005). As most

amphibians are very poor disperser, optimistic estimates of natural movement may be insufficient for species to keep pace with climate change, even in a favorable environment. In this cases managers should considered drastic measures to assuring successful colonization of new suitable areas as assisted dispersal (Araújo *et al.*, 2004; Hoegh-Guldberg *et al.*, 2008; Maxted *et al.*, 2008). Given the finite resources available to tackle the current biodiversity crisis, it is important that the available time and money are allocated in a cost-effective manner. The results from the studies here presented have contributed to the identification of causative factors of amphibian declines at regional and national level in Italy and these results may be used to inform the direction of cost-effective conservation. Additional data emerged from this thesis can direct amphibian conservation policy in areas useful for efficiently protect species both in current and future conditions. Such approaches are essential in preventing a further loss of amphibian diversity.

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Acknowledgements

Firstly, I would like to express my gratitude to my supervisor Prof. Marco A. Bologna, for allowing me the freedom to pursue research of my choosing, providing continuous support and overall guidance.

I greatly appreciated the collaboration with Peter B. Pearman who is a great workmate and friend. I learned a lot from you, and I am very grateful for your valuable advices, constructive feedback, and friendly chats in front of a beer.

During my thesis I benefit from the experience and suggestions from many people. I especially was glad about the availability of Nicklaus Zimmermann who accept me in his unit for four months (LAND USE DYNAMIC UNIT - FORSCHUNGSANSTALT FÜR WALD, SCHNEE UND LANDSCHAFT WSL) and I would like to thank to all the unit members for sharing their knowledge and experience. I'm also grateful to Antoine Guisan, who allowed me to take part to his classes, in the Lausanne University, initiating me to the world of distribution species modelling. I'm in debt to Francesco Tancredi and Luigi Maiorano for their kind technical assistance and help with C-plan. Last but not least, thanks also to Luca Luiselli for his statistical classes, stimulating discussions, and pleasant breaks.

I would like to acknowledge Biancamaria Pietrangeli and the Italian ISTITUTO SUPERIORE PER LA PREVENZIONE E LA SICUREZZA DEL LAVORO - ISPEISL- for funding the research reported in Chapter 3. Moreover the completion of the Latium herpetological database utilized in this study was ensured by the efforts of many people, notably staff and student of the Animal Ecology Laboratory (DIPARTIMENTO DI BIOLOGIA AMBIENTALE, ROMA TRE UNIVERSITY) and their efforts are gratefully acknowledged.

Thanks to all board commission members and reviewers for agreeing examining this thesis.

I thank all my fellow PhD students (belonging to several cycles), who in the last three years have been part of my life, sharing enthusiasm, apprehension, and good time – tea breaks, butterfly catching, and pinball matches! And a particular big hug to my colleagues of XXIII cycle! I am also grateful to my WSL housemates for the great time spent in Zurich among snowfalls, Catan matches, and excellent Swiss chocolate and cheese (fondue and raclette!). Cristina A., Cristina M., Imelda, and Tania.

To end with, I thank the persons who have supported me longest – my family and my closest friends.

I want reserve a special acknowledge to Pier, who has always encouraged and inspired me. Thank you for your love and for being always there for me.

Publications in SCI journals

- Bombi P., **D'Amen M.**, *submitted*. Scaling down distribution maps from atlas data: a test of different approaches with virtual species. *Journal of Biogeography*
- Bombi P., Luiselli L., **D'Amen M.**, *submitted*. Defining priority areas for conservation of African freshwater turtles with a multi methods approach. *Diversity and Distribution*
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DOCTORAL SCHOOL IN BIOLOGY

Ph.D. course: Biodiversity and Ecosystem Analysis

XIII CYCLE

**Impacts of climate change on amphibians:
past declines, predicted trends, and future
perspectives**

SUPPLEMENTARY MATERIALS

Manuela D'Amen

A.A. 2010/2011

Tutor: Prof. Marco A. Bologna

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Appendix A

Global warming and biodiversity: Evidence of climate-linked amphibian declines in Italy

Materials and methods

Species data

We utilized presence data of Italian amphibians, available in the CKmap 5.3.8 software (Stoch, 2000-2005). These country-scale data were collected by hundreds of volunteers through field sampling, as part of regional atlas projects carried out across Italy in the last decades (e.g. Doria and Salvidio, 1994; Bologna *et al.*, 2000; Lo Valvo and Longo, 2001). The results of these regional projects have been combined to produce the national atlas (Sindaco *et al.*, 2006). The CKmap databank represents the largest, most authoritative, and most updated resource of faunistic knowledge in Italy, being composed of more than 500 000 records of approximately 10 000 terrestrial and freshwater species (Ruffo and Stoch, 2005). This database contains 12 585 records, representing the current knowledge of the distribution in Italy of the 36 amphibian species belonging to the Italian fauna. CKmap data indicate the species occurrence within the 3557 cells of the Universal Transverse Mercator (UTM, 10 × 10 km) grid that intersect the Italian territory. In each cell, one single record per species is reported. Each record is accompanied by ancillary information, and the year of last observation (YLO) in the cell is provided for 11 562 records.

Because no other dates but YLO are provided for each species record in any cell, we could not calculate the probability of extinction by utilizing statistical methods based on repeated sampling data (e.g. Solow, 1993; Solow and Roberts, 2003). Therefore, we devised a different approach utilizing, for each cell, the YLOs to define if the species experienced a population decline. For each species we assigned the attribute 'DECLINE' to each cell with YLO prior to a selected threshold year, and the attribute 'STABLE' to each cell with YLO more recent than the same threshold. This criterion implies that the category 'STABLE' applies to squares where species persisted after the threshold and also to cells colonized after the threshold. Therefore, this category represents suitable conditions that permit

either persistence or new colonization. On the contrary, the category ‘DECLINE’ represents unsuitable conditions associated with local species decline. The threshold year was chosen considering the field effort over time by exploring the frequency distribution of YLOs in all the cells. Sporadic YLO records (a total of 293) fell between 1780 and 1959, but most occurred between 1960 and 2004, with the maximum number (902) corresponding to 1994 (Figure A1). The peak of YLO (which corresponds to the peak of knowledge) was during the 1990s, and was due to the above mentioned regional survey activities. Based on this frequency distribution, we chose 1985 as threshold, because it is before the peak of knowledge, and it allows us to assume that each square had multiple surveys after 1985. If none of these surveys re-confirmed the species presence, this fact was taken as an indication of a declining trend or local extinction for the populations in the cell (Drayton and Primack, 1996; Shaffer *et al.*, 1998; Santos *et al.*, 2007). In addition, we removed from the dataset 368 cells that had no data for any species after 1985, meaning that they probably were not surveyed after the threshold. These steps decreased the probability that the non-observation of given species in the cells after the threshold was due to lack of knowledge, reducing the percentage of commission errors (i.e. false positive decline rate).

To assess the accuracy of our criterion for defining declines, we compared our resulting species status (STABLE / DECLINE) in a subsample of cells from the original dataset (approximately 4% of CKmap records) with the probability of a species’ extinction in the same cells. This probability was calculated using a second dataset (data source used by Bologna *et al.* [2000]) by repeated sampling data statistics (Solow, 1993) to assess the probability of decline. This second dataset contains the entire survey history for each cell. The probability of extinction calculated for STABLE records were compared with those calculated for DECLINE records with a Mann-Whitney U test.

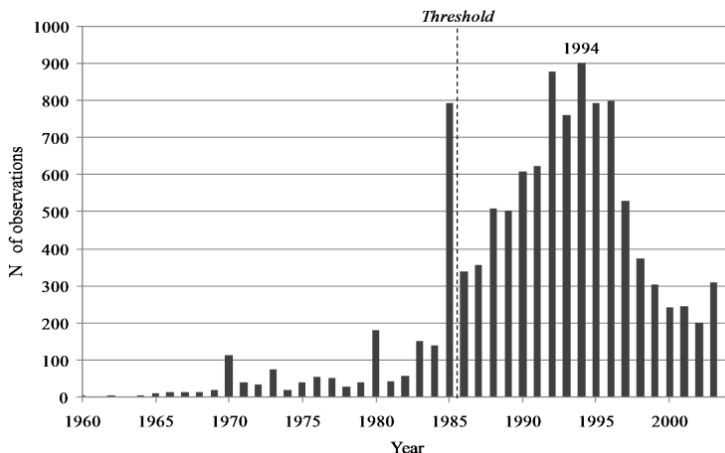


Figure A1 – Years of Last Observations (YLOs). Frequency distribution of the YLO per grid cell. The threshold was used for defining the species status (STABLE/DECLINE) in each cell (YLO after to [on the right of threshold in the graph] 1985: STABLE; YLO prior to [on the left in the graph] 1986: DECLINE)

To avoid biases caused by small sample size or underrepresented data, we excluded from the dataset species with less than ten records of each category (STABLE / DECLINE) (*Euproctus platycephalus*, *Salamandra lanzai*, *Speleomantes ambrosii*, *S. flavus*, *S. supramontis*, *Proteus anguinus*, *Discoglossus sardus*, *Hyla sarda*), alien species (*Lithobates catesbeianus*, *Xenopus laevis*), species marginally present in Italy with respect to their entire range (*Hyla arborea*, *H. meridionalis*, *Pelodytes punctatus*), and cave species (*Speleomantes* spp.). In addition, we adopted the species names as in “Amphibian Species of the World” (Frost, 2008) and we considered as single species those with problematic identification (*Pelophylax* spp.) or recent taxonomic splitting (*Salamandrina* spp., *Pseudepidalea* spp.). Thus, we analyzed a total of 19 species, out of which 12 anurans and 7 salamandrids.

Environmental data

To determine whether climate change, habitat alteration, and/or high levels of solar radiation were associated with amphibian declines, we

utilized nine environmental surfaces to represent these factors (Figure 1 a-g). Geographic pattern of climate change in Italy was defined using three variables that describe factors known to influence amphibian biology (e.g. Davidson *et al.*, 2002; Pounds *et al.*, 2006): annual number of dry days (DD), annual cumulate precipitation (P), and annual mean temperature (T). Annual mean values for these parameters were provided by the National System for collection, elaboration, and diffusion of Climatologic data of environmental Interest (SCIA, 2008). The SCIA databank contains thousands of climatic data on a monthly and annual basis derived from measures by hundreds of meteorological stations across Italy. Annual mean values of DD (d), P (mm), and T ($^{\circ}\text{C}$) for the period 1961-90 were downloaded and kriged in ArcGIS 9.2 (ESRI Inc., Redlands, CA, USA) to produce climatic surfaces representing their geographic variation across Italy, with a spatial resolution of 4×4 km. From these layers we calculated the mean values for the period 1961-90 (MEAN) and the shift between decades 1961-70 and 1981-90 (VAR). Finally, the six deriving climatic surfaces were re-sampled at the resolution of 10×10 km, utilizing the UTM grid as reference, by calculating the mean values per grid cell.

Habitat modification was quantified by calculating the percentage of surface in each UTM grid cell of agricultural (AG) and urban (UR) land cover classes (Figure 1 e-f). We utilized the Corine Land Cover 1990 data provided by the National Environmental Information System (SINAnet, 2008) of the Institute for Environmental Protection and Research (ISPRA), considering classes 1 (artificial surfaces) and 2 (agricultural surfaces) of the first level of land cover.

As a proxy of geographic differences in solar irradiation incidence, we utilized the map of horizontal irradiation (IR, J/m^2) available from Joint Research Center (Šúri *et al.*, 2007). We utilized data relative to the period 1981-90, with a spatial resolution map of 1×1 km. We re-sampled the map at the resolution of 10×10 km, by calculating the mean irradiation value for each UTM grid cell (Figure 1f). The values obtained for each of the considered variables, in every grid cell, were assigned to the relative faunistic data of all the considered species.

Statistical analyses

In order to classify the various species by their rate of local decline, for each species we calculated the percentage of DECLINE cells relative to the total number of occupied cells (i.e. STABLE + DECLINE). This approach potentially caused an underestimation of the real reduction rate, because the STABLE category includes not only the cells of persistence but also the newly colonized squares. On the other hand, the available dataset does not permit an estimation of the colonization rate, and therefore it is not possible to calculate the extent of species range change. Nevertheless, the ability of amphibians to undertake rapid large-scale migrations (such as at our spatial resolution) is doubtful (e.g. Araújo and Pearson, 2005; Smith and Green, 2005) and would be further inhibited by the high levels of habitat fragmentation in Italy (Araújo *et al.*, 2006). For this reason we assume that neo-colonized cells represent an unimportant fraction of the STABLE category and, therefore, that our rate of local decline is a good approximation of the real reduction rate.

Collinearity between environmental descriptors was avoided by excluding those variables strongly associated ($R^2 > 0.6$) with other parameters. Therefore, because P MEAN and T MEAN were correlated with, respectively, DD MEAN ($R^2 = 0.63$) and IR ($R^2 = 0.72$), the first two variables were excluded from all the following analyses.

In order to measure the influence of climate change, habitat modification, and solar radiation on amphibians' decline we fitted autocovariate models on cell status with a binomial probability distribution (STABLE [0] / DECLINE [1]) and a logit link, utilizing environmental variables as predictors. Autologistic models were elaborated in R 2.8.1 (R Development Core Team, 2008). This technique was selected after detecting spatial autocorrelation in cell status through the relative Moran's I correlograms (Moran, 1950; Legendre, 1993). The autocovariate model is a simple extension of generalized linear models (GLMs) (McCullagh and Nelder, 1989) built by adding a distance weighted function of neighboring response values (autocovariate) to model's explanatory variables. This approach accounted for spatial autocorrelation by estimating the degree to which the response variable at any one site was affected by the response values at surrounding sites (see Dormann *et al.*, 2007

for a review). For each square (i), the autocovariate (A) was calculated as:

$$A_i = \sum_{j=1}^8 w_{ij} y_j$$

where w_{ij} is the geographic weight of the eight closest neighboring cells (j) and y represents the response value at site j . The weights given to squares j is $w_{ij} = 1/h_{ij}$, where h_{ij} is the Euclidean distance between the centroids of squares i and j . The factor (y) assumes values -1 / 1 in the cell j according to its status (STABLE / DECLINE respectively) (Smith, 1994; Augustin *et al.*, 1996; Dormann *et al.*, 2007).

For each species, from an initial full model we selected the significant explanatory variables to be retained in the final model by applying a stepwise procedure based on the Akaike's Information Criterion (AIC; Akaike, 1974; Davidson *et al.*, 2002; Guerry and Hunter, 2002; Hermann *et al.*, 2005). This procedure works both backward and forward, by excluding and adding variables one by one from an initial full model, and comparing the resulting AIC values. The AIC score allows at measuring the fit of the models to the data (based on the likelihood) (Burnham and Anderson, 2002). The relative explanatory power of the retained predictors were then quantified as the change in the AIC score (Δ AIC) that resulted when one by one each predictor was dropped from the final model. Δ AIC is proportional to the relative contribution of each variable to the model, thus the higher is Δ AIC the higher the importance of each variable (e.g. Knapp *et al.*, 2003; McPherson and Jetz, 2007).

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Appendix B

Human-provoked amphibian decline in central Italy and the efficacy of protected areas

Materials and methods

Species data

We utilized presence data of 10 species of amphibians in Latium, central Italy (data source used by Bologna *et al.*, 2000) (Figure B1). This regional-scale dataset is derived from a compilation of bibliographic records of amphibians and field samplings spanning from 1970 to 2004. Field data were collected both opportunistically and through specific field campaigns by hundreds of volunteers within the regional atlas project. The data base is composed of 5723 amphibian records, accompanied by ancillary information as geographical coordinates and the observation year. On the basis of this information, all species records were referred to a 5 km × 5 km geographic grid resolution across the entire region, and the amphibian survey history for each grid cell was reconstructed.

Environmental data

We measured the percentage of different types of artificial and agricultural land use (Table 4) in each 5-km square based on digital 1:100,000-scale Corine Land Cover maps 2000 obtained from the National Environmental Information System (SINAnet) of the Institute for Environmental Protection and Research (ISPRA) (data available at <http://www.sinanet.apat.it/it/prodotti/mcgis/?searchterm=gis>). We also calculated the percentage of land use change between 1990 and 2000, and we measured the total length of streets in each cell. As proxy of the potential impact of human activity and density on amphibian survival we considered a synthetic index that measures the human influence on the Earth's land surface (Sanderson *et al.*, 2002). The Human Influence Index (HII) defines anthropogenic impact through geographic proxies at the global scale at a resolution of 1 km × 1 km. It was produced through an overlay of nine global data layers, which represent the variation across space of four

categories of factors presumed to exert an influence on ecosystems: population density, land transformation, accessibility, and electrical power infra structure (see Sanderson *et al.*, 2002 for a detailed description of each data set). The number of people in a given area is frequently cited as a primary cause of declines in species and ecosystems (Cincotta and Engelman, 2000), with higher human densities leading to higher levels of disturbance. Land transformation category, which includes layers of global land use, built-up centres, population settlements, streets and railways, is considered the greatest threat to biological diversity, resulting in loss and fragmentation of habitat in many different ecosystems (e.g. Vitousek, 1997). The human access category comprises layers of roads, major rivers, and coastlines. All ecosystems can be affected by the presence of access ways because these provide opportunities for several nature disrupting activities, e.g. hunting and extraction of resources, pollution and waste disposal (Gucinski *et al.*, 2001). Finally, since the increase of the human influence on ecosystems in the 20th century was fuelled by fossil energy, the HII also incorporates a power infrastructure proxy.

In order to explore the potential relationship between climate change and species declines we used the annual mean of three variables that describe climatic factors known to influence amphibian biology (e.g. Davidson *et al.*, 2001; Davidson *et al.*, 2002; Pounds *et al.*, 2006; McMenamin *et al.*, 2008): number of dry days, cumulate precipitation (mm), and temperature (°C). Annual mean values for these parameters were provided by the National System for the Collection, Elaboration, and Diffusion of Climatologic Data of Environmental Interest (SCIA) (data available at http://www.scia.sinanet.apat.it/sciaweb/scia_mappe.html). We downloaded the annual mean values of the considered variables for the 1961–2000 period, and we kriged them in ArcGIS 9.3 (ESRI Inc., Redlands, CA, USA) to produce climatic surfaces representing their geographic variation across Latium with 1 km × 1 km pixels. From these layers we calculated the shift in mean values between the decades 1961–1970 and 1991–2000 and we re-sampled these climatic surfaces at a 5 km × 5 km resolution by calculating the mean values per grid cell.

Finally we also included in our analyses the percentage of protected surface for each cell to estimate the effectiveness of the protected

areas for the conservation of amphibians. All areas belonging to the Natura 2000 network and National and Regional Natural Parks were considered (data available at <ftp://ftp.scn.minambiente.it/Cartografie/Natura2000>).

Statistical analyses

In order to assess amphibian species decline in every occupied grid square of the study area, we utilized sighting records from the regional data base described above. Only records with a definite year of sighting were considered for the calculations, whereas multiple records within the same year were excluded. Finally, a total of 297 cells were selected for the following analyses (the number of presence cells for each species is reported in Table 5) (Figure B1).

Several statistical methods have been developed to infer the probability of species persistence from museum or atlas records (e.g. Solow, 1993a, b; McCarthy, 1998; Solow and Roberts, 2003). The basic idea underlying these methods is that confidence in the continued existence of a species is greater the more recent the sighting (Solow and Roberts, 2003).



Figure B6 - Study region The grey grid indicates amphibian presence cells utilized for statistical analyses

In particular we utilized the Solow and Roberts (2003) non-parametric equation based on the most recent sightings and the end of the observation period, which makes minimal assumptions about the sighting rate (it assumes that the sighting rate is positive and smooth at the end of the observation period). Indeed, as we calculated the probability of persistence for ten species in more than two hundred grid cells, we could not assume a priori a common distribution for the sighting rate. The Solow and Roberts (2003) equation generates the following p -value for testing the null hypothesis (H_0 = the species remains extant at the time T [the total number of years from the first record]) against the alternative hypothesis (H_1 = the species is extinct):

$$p = \frac{t_n - t_{n-1}}{T - t_{n-1}}$$

where t_n is the number of years since the first record to the most recent record, and t_{n-1} is the number of years from the first record to the penultimate record. Small p -values suggest that the data are unlikely to occur if the species is still extant and consequently the p -value reflects the probability that another sighting will occur. Since this probability depends on variation of species abundance, we utilized this value to estimate local declines. We assumed that if the probability is $<15\%$, the abundance of the species is decreased so much to consider the species declining in the cell. Using this criterion for each species we attributed the category STABLE ($p \geq 0.15$) and DECLINE ($p < 0.15$) at all the occurrence squares. Moreover in order to rank the species by their rate of local decline, for each species we calculated the percentage of DECLINE cells relative to the total number of occupied cells (i.e. STABLE + DECLINE).

We explored the relationship of species cell status (STABLE [0] / DECLINE [1]) to the considered environmental variables by using Generalized Additive Modeling (GAM) technique with a binomial probability distribution and a logit link. GAMs are semi-parametric extensions of the traditional linear statistical models, where some predictors can be modelled non-parametrically in addition to linear and polynomial terms for other predictors. The only underlying assumption made is that the functions are additive and that the components are smooth. The strength of GAMs is their ability to

deal with highly non-linear and non-monotonic relationships between the response and the set of explanatory variables (Hastie and Tibshirani, 1986). For each species, we first tested all predictors individually in univariate GAMs (also quadratic effect was tested for climatic variables), and we retained as relevant for further analysis only those whose regression coefficient were significant at a p -value < 0.25 (Chi Square Test) (Hosmer and Lemeshow, 1989). We then entered all selected predictors in multivariate models, in which smoother terms were fitted with four and two degrees of freedom. Using a stepwise procedure, we determined the most parsimonious combination of predictors, and the most appropriate level of the ‘smoother’ terms, which produce the ‘best’ model in terms of the AIC statistic (Akaike’s Information Criterion) (Akaike, 1974). The AIC score allows to measure the fit of the models to the data (based on the likelihood) (Burnham and Anderson, 2002). The relative explanatory power of the retained predictors were then quantified as the change in the AIC score (Δ AIC) that resulted when each predictor was dropped one by one from the final model: the higher is Δ AIC the higher the importance of each variable (e.g. Knapp *et al.*, 2003; McPherson and Jetz, 2007). In all multivariate models collinearity between environmental descriptors was avoided by building separate models for those variables strongly associated ($R^2 > 0.6$) with other parameters, (i.e. ART is strongly correlated with URB [$R^2 = 0.94$] and STR [$R^2 = 0.86$], while AGR is correlated with ARA [$R^2 = 0.71$]). Among these alternative models Akaike Information Criterion was again used to select the best fit model.

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Will climate change reduce the efficacy of protected areas for amphibian conservation in Italy?

Materials and Methods

Species data set

We utilized presence data of Italian amphibians from CKmap 5.3.8 (Stoch, 2000–2005). This database reports species occurrence within the Universal Transverse Mercator (UTM, 10×10 km) grid that intersects the Italian territory (see Appendix A). We updated distribution information for newly recognized species using maps from IUCN Red List (see Frost, 2008 for a discussion; IUCN, 2009). To avoid biases caused by small sample sizes, we excluded from the analysis species with less than 20 records. At the same time, we also discarded those ubiquitous species for which we could not select a sufficient number of pseudo-absence points for model construction. Alien species, cave species, and species only marginally present in Italy with respect to their entire range were also excluded. As a result, we analyzed a total of 22 species, of which 20 are ranked by European Red List of Amphibians in the Low Concern category, while *Bombina pachypus* is considered Endangered, and *Rana latastei* Vulnerable (Temple and Cox, 2009). We adopted nomenclature following Frost (2008).

Environmental data sets

Niche-based models were calibrated using climatic, land use, and topographical predictors. Of 20 variables initially, we retained only those correlated at 0.70 or lower. Since the primary interest of this exercise is forecasting and not estimating model parameters, this level of correlation is not of concern (Legendre and Legendre, 1998). We used seven bioclimatic predictors: (1) annual mean temperature, (2) mean diurnal temperature range, (3) isothermality, (4) temperature annual range, (5) mean temperature of wettest quarter, (6) precipitation of warmest quarter, and (7) precipitation of coldest quarter. Current values for these variables were derived from the WorldClim database (Hijmans *et al.*, 2005), which are climate grids for 1950–2000 with a resolution of 30 arc-seconds (~1km). Potential

future values for the time interval 2041-2070 were derived from the 10 arc-minute resolution climate grids of Mitchell *et al.* (2004) for the IPCC scenarios A1FI and B1 and the HadCM3 circulation model, which is widely used for predicting climate change effects on fauna distribution in Europe (e.g. Araújo *et al.*, 2004; Araújo *et al.*, 2006; Araújo and Luoto, 2007). We chose A1FI and B1 to capture some of the uncertainty surrounding future projections, since they are based on contrasting story lines covering the range of possible demographic, socio-economic and technological changes thought to influence greenhouse gas emissions. To produce high-resolution climate scenarios, the 10 arc-minute grids were expressed as anomalies to the 1950-2000 period of WorldClim, interpolated to 30 arc-seconds resolution and then recombined with the corresponding grids from the WorldClim data set.

The effects of existing land use on habitat availability were accounted for by including two land cover variables, the percentage of forested surface and herbaceous vegetation (Corine Land Cover IV, SINAnet, 2008). Finally, topographic information was included in the models as the prevalent exposition direction in a continuous north-south gradient ranging between 1 and -1, derived from digital elevation model (75 m resolution). All variables were prepared in a geographical information system (ArcMap 9.2) and rescaled to the UTM resolution (10×10 km) to the extent of Italy.

Reserves data set

Data on the location of existing protected areas in Italy are available from the national Ministry for the Environment (<ftp://ftp.scn.minambiente.it/Cartografie>). These data comprise boundaries of Nationally Designed Protected areas (NPAs) and sites included in the European Natura 2000 network. NPAs consist of 774 parks that were founded by national or local administrations before 2004, with a total surface of approximately 29,400 km² (slightly less than 10% of the country's surface). The Natura 2000 network consists of 2885 sites, which are largely overlapping with NPAs and increase the protected surface by 34,700 km² (Natura 2000 areas not overlapping NPAs are hereafter defined as EPAs). Thus, the Overall Protected Areas (OPAs) cover a total surface of 64,100 km² (21% of Italy) (Gambino and Negrini, 2002).

The problem of matching reserve boundaries with species distribution is common when dealing with national atlases datasets at coarse resolution (Araújo, 1999; Araújo, 2004). In these cases, a threshold is needed to determine whether reserves should be considered present or absent in a grid cell. For solving this issue we tested different thresholds (from zero to 100% with intervals of 10) and chose the value that resulted in selection of a number of cells with a total surface equal to the total surface of Italian protected areas (considering independently NPAs and OPAs). We considered as protected any cell with a proportion of park coverage larger than 40% (Figure C1). This is consistent with Araújo (2004), who observed a plateau in species accumulation curves with the use of a 40% coverage threshold to assign reserves to grid cells.

Niche modelling

We modelled species distributions using eight different techniques available in the R-based BIOMOD package (Thuiller, 2003; R Development Core Team 2008): Generalized Linear Models (GLM; McCullagh and Nelder, 1989), Generalized Additive Models (GAM; Hastie and Tibshirani, 1990), Classification Tree Algorithms (CTA; Breiman *et al.*, 1984), Artificial Neural Networks (ANN; Ripley, 1996), Mixture Discriminant Analysis (MDA; Hastie and Tibshirani, 1996), Multivariate Adaptive Regression Splines (MARS; Friedman, 1991), Generalized Boosted Regression Models (GBM; Friedman, 2001), and Random Forest (RF; Breiman, 2001). Pseudo-absence points for each species were generated by a random selection among the grid cells where the species was not reported, maintaining a prevalence of 50% (Liu *et al.*, 2005). The performance of each model was determined with ten-fold cross-validation of AUC (Fielding and Bell, 1997). Subsequently, we derived projections for the future climatic scenarios A1FI and B1.

We computed a consensus of single-model projections for each scenario using the weighted average approach, which increases significantly the accuracy of species distribution forecasts (Araújo and New, 2007; Marmion *et al.*, 2008). We utilized the AUC scores to weight the corresponding models and included in the consensus estimation only those models with an AUC score higher than 0.70 (Swets, 1988). In particular, the habitat suitability of the i th grid cell (WA_i) was calculated as:

$$WA_i = \frac{\sum_j (AUC_j \times p_{ji})}{\sum_j AUC_j}$$

where AUC_j is the validation score of the model elaborated with the j th technique and p_{ji} is the probability of presence for the considered species as predicted by j th model. We transformed these probability values into presence/absence maps by using an optimal ROC threshold (Cantor *et al.*, 1999), which optimizes the percentage of correctly predicted presences and absences (Liu *et al.*, 2005).

Individual dispersal capability might severely restrict the ability of populations, and consequently of whole species, to track suitable climatic conditions geographically (Guisan and Thuiller, 2005; Massot *et al.*, 2008). Consequently, we accounted for specific ability of range shift by considering species-specific dispersal limitations derived from the literature of the species, or a closely related species (Hannah *et al.*, 2007; Table C1). This value represents a realistic measure of the intrinsic ability of a species to shift its distribution in response to climate change. In deriving future projections, the application of the dispersal constraint allowed us to distinguish between a potentially suitable area, and a potentially colonizable area (*sensu* Engler and Guisan, 2009). In particular, each species could occupy suitable cells within a dispersal radius from current observations according to the vagility of the species. As species range shifts are also influenced by extrinsic factors, i.e. the highly fragmented landscape, we considered a second, more-restrictive scenario of no-dispersal in order to estimate the proportion of current habitat that remains suitable under future conditions. Potential distributional shifts were measured as the difference in the total number of grid cells occupied currently and under each of the future climate change scenarios.

Table C9- Greatest migration distances registered for the considered species, or closely related taxa, calculated migration distance by 2060, and bibliographical references For species with several references we used an average value (References: 1 Hartel, 2008; 2 Clausnitzer and Clausnitzer, 1984; 3 Stumpel and Hanekamp, 1986; 4 Carlson and Edenhamn, 2000; 5 Vos et al., 2000; 6 Warwick, 1949; 7 Bell, 1977; 8 Dolmen, 1981; 9 Griffiths, 1984; 10 Joly and Grolet, 1996; 11 Perret et al., 2003; 12 Hels, 2002; 13, Moore, 1954; 14 Haapanen, 1974; 15 Parker and Gittins, 1979; 16 Sinsch, 1988; 17 Hartel et al., 2009; 18 Seitz et al., 1992; 19 Schmidt et al., 2007; 20 Angelini et al., 2007; 21 Schabetsberger et al., 2004)

Species	Max distance recordered (m)	Estimate migration distance by 2060 (m)	References
<i>Bombina pachypus</i>	250	15000	1
<i>Bombina variegata</i>	250	15000	1
<i>Discoglossus pictus</i>	250	15000	1
<i>Discoglossus sardus</i>	250	15000	1
<i>Hyla intermedia</i>	12600	756000	2, 3, 4, 5
<i>Hyla sarda</i>	12600	756000	2, 3, 4, 5
<i>Lissotriton italicus</i>	182	10920	6, 7, 8, 9
<i>Lissotriton vulgaris</i>	182	10920	6, 7, 8, 9
<i>Mesotriton alpestris</i>	350	21000	10, 11
<i>Pelobates fuscus</i>	500	30000	12
<i>Pseudepidalea balearica</i>	3621	217260	13, 14, 15, 16
<i>Pseudepidalea sicula</i>	3621	217260	13, 14, 15, 16
<i>Pseudepidalea viridis</i>	3621	217260	13, 14, 15, 16
<i>Rana dalmatina</i>	188	11280	17
<i>Rana italica</i>	460	27600	18
<i>Rana latastei</i>	188	11280	18
<i>Rana temporaria</i>	460	27600	18
<i>Salamandra atra</i>	319	19140	19
<i>Salamandra salamandra</i>	319	19140	19
<i>Salamandrina perspicillata</i>	500	30000	20
<i>Salamandrina terdigitata</i>	500	30000	20
<i>Triturus carnifex</i>	300	18000	21

Criteria for species conservation targets

We established the conservation target for species conservation for use in both gap and irreplaceability analyses on the basis of species-specific extent of occurrence, following Rodrigues *et al.* (2004). We defined 10 cells (1000 km²) as the minimum area needed for species viability. For those species with an initial range of less than 10 cells, we set the conservation target to 100% of the current range size. On the other hand, we set a 10% conservation target for very widespread species (>1000 cells [$>10^6$ km²]), as this percentage represents approximately the total Italian surface covered by NPAs. Targets for all species with intermediate range sizes were calculated by interpolating the extreme range size targets using a linear regression on the log-transformed number of initially occupied cells (Table C2).

Because our criterion for setting species conservation targets relied on range extents, we adjusted conservation targets on the basis of range size alteration that is predicted to take place with climate change. This flexible approach permits future modification of species conservation requirements to account for climate change. For instance, a species might be currently distributed to few cells and, therefore, have a very high conservation target. If this species will have more suitable habitat in the future, and will enlarge its distribution accordingly, the relative target should be proportionally reduced in order to use efficiently limited funds for conservation (Table C2).

Gap and irreplaceability analyses

In gap analysis species distributions are compared to the distribution of conservation areas and the degree of species representation in the reserve network is determined (Jennings, 2000). We considered those species not represented in any protected area to be gap species, while species that met only a portion of their conservation target were considered partial gap species (Rodrigues *et al.*, 2004).

To measure relative conservation importance of different map cells, we estimated the irreplaceability value of each grid cell using the C-Plan Systematic Conservation Planning System, Version 4 (Pressey *et al.*, 2009). Simply defined, the irreplaceability of a map cell is the degree to which the cell is required in a reserve network in order to achieve established conservation targets (Pressey *et al.*, 1994). To

avoid an intractable exact calculation, irreplaceability was estimated as the number of combinations of sites that include the focal site and meet conservation targets, but which would not meet the targets if the focal site were removed. This estimation was done using a predictive approach based on the central limit theorem (Ferrier *et al.*, 2000). Finally, irreplaceability values were rescaled between 0 and 1: values close to 1 indicate difficult to replace sites, potentially containing species endemic to those sites, while values close to 0 indicate easily replaceable sites, ones containing only widely distributed species. We predicted the irreplaceability of each cell using current species occurrences and using future potential distributions under different climate and dispersal scenarios. In addition, we considered three alternative conservation systems (i.e. no reserves, NPAs, and OPAs) for assessing the relative contribution of existing reserve networks.

Test of park efficacy

In order to evaluate the effectiveness of the existing Italian national park system (OPAs) and its components (NPAs and EPAs), we compared the mean irreplaceability value of cells in conservation networks to the mean value expected in cells randomly selected regardless of their conservation status (Araújo *et al.*, 2007). We calculated the probability that the observed mean value for protected cells differed from a random mean value by comparing the observed mean value to the distribution formed by 5000 random selections of a number of grid cells equal to the number of protected cells (Gotelli, 2000). For present and future scenarios, we tested whether OPAs and NPAs have higher irreplaceability than the remaining map cells, i.e. with all protected areas excluded. Additionally, we estimated in an irreplaceability analysis the contribution of the new Natura 2000 sites designation to the representativeness of the existing reserve system. Because the existence of reserves alters the estimate of the irreplaceability of map cells and, therefore, the potential contribution of all the “unreserved” sites, we recalculated the irreplaceability values considering the existence of NPAs for testing EPAs selection for present and long term conservation.

Table C10 - Specific conservation targets calculated for present conditions and for alternative dispersal and climate change scenarios

SPECIES	PRESENT	NO-DISP		DISP	
		A1FI	B1	A1FI	B1
<i>Bombina pachypus</i>	106	83	88	115	118
<i>Bombina variegata</i>	75	29	40	53	67
<i>Discoglossus pictus</i>	64	62	61	87	85
<i>Discoglossus sardus</i>	30	11	19	23	38
<i>Hyla intermedia</i>	111	107	103	274	228
<i>Hyla sarda</i>	38	31	37	114	119
<i>Lissotriton italicus</i>	94	87	89	109	111
<i>Lissotriton vulgaris</i>	118	109	103	120	118
<i>Mesotriton alpestris</i>	107	80	80	105	106
<i>Pelobates fuscus</i>	41	ext	5	17	32
<i>Pseudepidalea balearica</i>	120	120	120	228	193
<i>Pseudepidalea sicula</i>	55	54	54	95	94
<i>Pseudepidalea viridis</i>	58	35	36	57	58
<i>Rana dalmatina</i>	114	112	107	120	119
<i>Rana italica</i>	120	103	103	120	120
<i>Rana latastei</i>	90	56	57	73	74
<i>Rana temporaria</i>	118	89	97	102	110
<i>Salamandra atra</i>	48	4	23	7	35
<i>Salamandra salamandra</i>	119	88	98	108	114
<i>Salamandrina perspicillata</i>	91	61	56	104	99
<i>Salamandrina terdigitata</i>	36	2	13	5	35
<i>Triturus carnifex</i>	104	87	96	113	117

Table C11 - Cross validated AUC values for each species model techniques

SPECIES	Cross Validated AUC values							
	ANN	CTA	GAM	GBM	GLM	MARS	MDA	RF
<i>Bombina pachypus</i>	0.848	0.791	0.863	0.865	0.86	0.866	0.85	0.894
<i>Bombina variegata</i>	0.903	0.89	0.92	0.93	0.903	0.919	0.896	0.919
<i>Discoglossus pictus</i>	0.953	0.887	0.88	0.946	0.884	0.907	0.918	0.958
<i>Discoglossus sardus</i>	-	0.875	0.891	0.867	0.84	0.938	0.875	0.914
<i>Hyla intermedia</i>	0.735	0.761	0.766	0.775	0.765	0.776	0.739	0.794
<i>Hyla sarda</i>	0.938	0.873	0.915	0.911	0.847	0.915	0.896	0.938
<i>Lissotriton italicus</i>	0.823	0.784	0.85	0.853	0.829	0.857	0.834	0.888
<i>Lissotriton vulgaris</i>	0.778	0.799	0.843	0.826	0.835	0.842	0.82	0.853
<i>Mesotriton alpestris</i>	0.905	0.885	0.947	0.941	0.921	0.931	0.907	0.93
<i>Pelobates fuscus</i>	0.923	0.796	0.952	0.936	0.931	0.965	0.936	0.949
<i>Pseudepidalea balearica</i>	0.746	0.775	0.827	0.819	0.826	0.81	0.802	0.808
<i>Pseudepidalea sicula</i>	0.958	0.915	0.966	0.949	0.944	0.972	0.926	0.974
<i>Pseudepidalea viridis</i>	0.944	0.949	0.942	0.982	0.839	0.933	0.927	0.983
<i>Rana dalmatina</i>	0.754	0.727	0.812	0.823	0.812	0.815	0.777	0.827
<i>Rana italica</i>	0.855	0.853	0.903	0.897	0.898	0.891	0.866	0.91
<i>Rana latastei</i>	0.931	0.877	0.914	0.918	0.908	0.91	0.879	0.9
<i>Rana temporaria</i>	0.893	0.878	0.897	0.891	0.897	0.89	0.89	0.853
<i>Salamandra atra</i>	0.871	0.824	0.891	0.902	0.902	0.922	0.87	0.891
<i>Salamandra salamandra</i>	0.806	0.798	0.828	0.829	0.814	0.802	0.795	0.725
<i>Salamandrina perspicillata</i>	0.813	0.829	0.897	0.902	0.904	0.893	0.887	0.9
<i>Salamandrina terdigitata</i>	0.861	0.8	0.936	0.873	0.859	0.898	0.932	0.932
<i>Triturus carnifex</i>	0.752	0.767	0.813	0.816	0.811	0.814	0.795	0.803

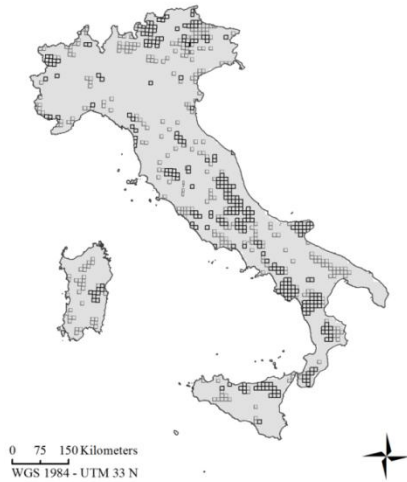


Figure C1 - Cells with at least 40% of their surface protected. NPAs (black) and EPAs (grey)



Figure C2 - Place name locations discussed in the text

Table C12 - Specific range extent (total number of occupied cells) and occupied cells with NPAs and OPAs per species at present

SPECIES	Range	Protected range	
		NPAs	OPAs
<i>Bombina pachypus</i>	339	76	125
<i>Bombina variegata</i>	166	7	29
<i>Discoglossus pictus</i>	127	13	19
<i>Discoglossus sardus</i>	41	4	13
<i>Hyla intermedia</i>	1110	86	180
<i>Hyla sarda</i>	58	4	15
<i>Lissotriton italicus</i>	255	58	94
<i>Lissotriton vulgaris</i>	738	47	123
<i>Mesotriton alpestris</i>	351	27	89
<i>Pelobates fuscus</i>	64	1	3
<i>Pseudepidalea balearica</i>	638	43	92
<i>Pseudepidalea sicula</i>	99	10	20
<i>Pseudepidalea viridis</i>	109	1	15
<i>Rana dalmatina</i>	810	73	142
<i>Rana italica</i>	577	97	168
<i>Rana latastei</i>	235	3	13
<i>Rana temporaria</i>	514	63	157
<i>Salamandra atra</i>	82	13	47
<i>Salamandra salamandra</i>	680	81	165
<i>Salamandrina perspicillata</i>	242	31	75
<i>Salamandrina terdigitata</i>	53	26	33
<i>Triturus carnifex</i>	958	90	185

Table C13 - Specific range extent (total number of occupied cells) and occupied cells with NPAs and OPAs per species under alternative climate change scenarios if dispersal is not allowed Extents smaller than ten cells are evidenced in italics and values equal to 0 are highlighted in bold

SPECIES	NO-DISP A1FI			NO-DISP B1		
	Range	Protected range		Range	Protected range	
		NPAs	OPAs		NPAs	OPAs
<i>Bombina pachypus</i>	202	52	81	226	60	91
<i>Bombina variegata</i>	39	4	11	62	6	17
<i>Discoglossus pictus</i>	120	13	18	117	11	16
<i>Discoglossus sardus</i>	11	4	7	22	4	12
<i>Hyla intermedia</i>	1073	69	162	1033	65	151
<i>Hyla sarda</i>	43	1	9	55	4	15
<i>Lissotriton italicus</i>	219	47	77	229	51	81
<i>Lissotriton vulgaris</i>	367	23	53	320	22	49
<i>Mesotriton alpestris</i>	189	20	60	187	18	62
<i>Pelobates fuscus</i>	0	0	0	5	0	0
<i>Pseudepidalea balearica</i>	600	33	76	573	29	66
<i>Pseudepidalea sicula</i>	98	10	20	98	10	20
<i>Pseudepidalea viridis</i>	52	0	5	53	0	4
<i>Rana dalmatina</i>	399	28	66	348	26	64
<i>Rana italica</i>	318	44	84	319	49	88
<i>Rana latastei</i>	104	0	4	105	0	3
<i>Rana temporaria</i>	230	44	95	273	48	107
<i>Salamandra atra</i>	4	3	3	29	6	20
<i>Salamandra salamandra</i>	226	15	56	280	18	63
<i>Salamandrina perspicillata</i>	117	17	39	104	17	40
<i>Salamandrina terdigitata</i>	2	2	2	14	6	8
<i>Triturus carnifex</i>	218	6	27	270	6	29

Table C14 - Specific range extent (total number of occupied cells) and occupied cells with NPAs and OPAs per species under alternative climate change scenarios if dispersal is allowed Extents smaller than ten cells are evidenced in italics and values equal to 0 are highlighted in bold

SPECIES	DISP A1FI			DISP B1		
	Range	Protected range		Range	Protected range	
		NPAs	OPAs		NPAs	OPAs
<i>Bombina pachypus</i>	441	89	144	496	99	156
<i>Bombina variegata</i>	94	<i>10</i>	28	137	12	37
<i>Discoglossus pictus</i>	219	21	30	212	17	26
<i>Discoglossus sardus</i>	29	7	15	57	8	24
<i>Hyla intermedia</i>	2532	177	397	2210	148	320
<i>Hyla sarda</i>	196	2	24	271	9	43
<i>Lissotriton italicus</i>	375	75	120	395	81	127
<i>Lissotriton vulgaris</i>	589	34	84	513	32	74
<i>Mesotriton alpestris</i>	336	49	107	344	45	111
<i>Pelobates fuscus</i>	20	0	<i>4</i>	45	0	<i>4</i>
<i>Pseudepidalea balearica</i>	2283	140	342	1934	99	258
<i>Pseudepidalea sicula</i>	262	22	37	257	22	37
<i>Pseudepidalea viridis</i>	107	0	<i>10</i>	108	0	8
<i>Rana dalmatina</i>	647	44	108	558	40	101
<i>Rana italica</i>	594	73	141	590	74	138
<i>Rana latastei</i>	157	0	<i>10</i>	162	0	<i>10</i>
<i>Rana temporaria</i>	313	55	123	379	60	139
<i>Salamandra atra</i>	7	3	3	51	8	24
<i>Salamandra salamandra</i>	357	34	104	431	39	115
<i>Salamandrina perspicillata</i>	323	36	78	292	35	76
<i>Salamandrina terdigitata</i>	5	3	3	52	15	18
<i>Triturus carnifex</i>	420	27	72	480	16	57

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Within-taxon niche structure: niche conservatism, divergence and predicted effects of climate change¹

Introduction

Here, we explore how *taxonomic* resolution influences predictions of the extent of suitable climatic conditions for species currently and under future scenarios. We are interested in whether biologically meaningful differences (10% or more) in predicted remaining suitable area arise as a result of the taxonomic resolution that is used in modeling the relationship between climate and distribution. We use data on two sets of vertebrates, a selection of breeding bird species in North America and several species of the European herpetofauna. The chosen *taxa*, clearly not a random selection from their respective continental faunas, supply examples of either polymorphic species with established subspecies taxonomy or small clades of sister species distinguished based on molecular evidence. We develop ecological niche models using a machine-learning algorithm that implements boosted regression trees. A general circulation model of climate provides data on potential future climatic conditions as may develop under the A2 scenario detailed by the Intergovernmental Panel on Climate Change (IPCC, 2007) third assessment.

We are primarily interested in determining whether the taxonomic resolution of lineages influences predictions of climate change impacts on the future distributions of these lineages. We investigate three conflicting hypotheses: first, we might expect species pairs in the European herpetofauna to show little niche overlap because of the accumulated ecological differences that may accompany

¹ Based on: Pearman P.B., D'Amen M., Graham C., Thuiller W., Zimmermann N.E., 2010. Within-taxon niche structure: Niche conservatism, divergence and predicted effects of climate change. *Ecography*. doi: 10.1111/j.1600-0587.2010.06443.x –The first two authors contributed equally to the research and writing of the manuscript. The idea for this manuscript arose at the workshop “The Utility of Species Distribution Models as Tools for Assessing Impacts of Global Change” at Riederalp, Switzerland (August 2008).

speciation. In contrast and second, we expect subspecies to show great niche similarity because the taxonomic standing of subspecies suggests that they are genetically and ecologically less differentiated than *bone fide* species. Third, we could conjecture that there should be little niche differentiation among sibling species and subspecies because of the coarse resolution of the European dataset and the observation that sister species generally show little niche differentiation (Peterson *et al.*, 1999). Our results suggest that an understanding of the phylogeographic structure within small clades or among closely related lineages provides an alternative perspective on potential responses of *taxa* to climate change. This can help reduce uncertainty when predicting climate change impacts on species potential distributions.

Methods

Terminology, study taxa and distribution data

We address the relationship between taxonomic resolution and predictive species distribution modeling using sister species and groups of subspecies. Sister species are dichotomous clades while the designation of subspecies within species defines groups that can vary greatly in both morphological and genetic distinctiveness. To avoid proliferation of terminology, we refer to models derived from observations pooled from various *taxa*, which may be across sister species or subspecies, as ‘clade models’. We refer to models that are derived from single *taxa*, either single species or single subspecies, as subclade models.

Herpetofauna of Europe. The herpetofauna of Europe provided a well-studied group of vertebrates, here referred to as herptiles. We primarily considered *taxa* for which taxonomic revision has upgraded sub-species to sister species based on molecular phylogenetic analysis (Table D1). We extracted published distribution data from the Atlas of Amphibians and Reptiles in Europe (Gasc *et al.*, 1997). This large-scale atlas summarized, in a standardized way, data from national atlas projects, from the literature and from museum collections. Species presence in this atlas was reported within the 3869 50 × 50 km Universal Transverse Mercator (UTM) cells that intersected Europe. Because of changes in taxonomic status of several *taxa* occurred after publication of the

Atlas, we updated the distribution information for the newly recognized species using national atlases (Pleguezuelos *et al.* 2002, Loureiro *et al.* 2008), published papers (Arntzen *et al.* 2007) and unpublished government reports, while maintaining the atlas resolution. We assumed that atlas cells where species were not noted as present represent true absences. While this likely introduced a small amount of error into the models, it was unlikely that these errors varied systematically between sister species or subspecies.

Breeding Birds in North America. The assemblage of breeding birds in North America provided several cases in which widely distributed species demonstrated well-developed population structure or geographically structured morphological variation. This included cases where: (1) subspecies were recognized based on stable color polymorphisms and/or morphological variation or (2) subspecies were recognized based on regional to continental patterns of genetic and morphological characteristics (Table D2). Data on the distribution of these *taxa* in the United States and Canada came from the download web site of the North American Breeding Bird Survey (BBS, USGS Patuxent Wildlife Research Center 2009). We selected three of four *taxa* because they were formerly recognized as species that were subsequently downgraded to subspecies in order to form one polytypic species. These included *Junco hyemalis* (Dark-eyed Junco), *Colaptes auratus* (Northern Flicker) and *Dendroica coronata* (Yellow-rumped Warbler, Table D2). We excluded *Junco hyemalis* subspecies *J. h. aikenii* and *J. h. mearnsi* because of the small number of occurrences that they presented, six and twenty-two respectively. Workers who survey BBS routes were encouraged to report to which archaic species the observed forms of these species belong (D. Ziolkowski, pers. com.). We also elected to analyze BBS occurrence data on *Passerella iliaca* (Fox Sparrow) because recent work showed marked geographic structure in mtDNA variability in this species (Zink 1994, 2008). We assembled species observation data from 4514 BBS routes that were surveyed at least once between and including the years 1997-2008. We used the available route coordinates as the location for all observations associated with any particular survey route.

Table D1 - Lineages, species and sub-species of European herpetofauna
(References 1 Arntzen et al. 2007, Steinfartz et al. 2007; 2 Steinfartz et al. 2007, Themudo and Arntzen 2007; 3 Yu et al. 2007; 4 Garcia-Paris et al. 2003; 5 van der Kuyl et al. 2002; 6 Arnold et al. 2007, Bohme et al. 2007)

Clade English	Clade	Level	Sub-clade taxa	Sub-clade taxa English	Level
1. Italian crested newt	<i>Triturus carnifex</i>	species, archaic			
			<i>T. carnifex</i>	Italian crested newt	species
			<i>T.macedonicus</i>	Macedonian crested Newt	"
2. Marbled newt	<i>Triturus marmoratus</i>	species, archaic			
			<i>T. marmoratus</i>	Marbled newt	species
			<i>T. pygmaeus</i>	Southern Marbled newt	"
3. Yellow- bellied toad	<i>Bombina variegata</i>	species, archaic			
			<i>B. variegata</i>	Yellow- bellied toad	species
			<i>B. pachypus</i>	Apennine yellow-bellied toad	"
4. Parsley Frog	<i>Pelodytes punctatus</i>	species, archaic			
			<i>P. punctatus</i>	Parsley frog	species
			<i>P. ibericus</i>	Iberian Parsley frog	"
5. Hermann's Tortoise	<i>Testudo hermanni</i>	species			
			<i>T. h. hermanni</i>	Western Hermann's tortoise	sub species
			<i>T. h. boettgeri</i>	Eastern Hermann's tortoise	"
6. European Green Lizard	<i>Lacerta viridis</i>	species, archaic			
			<i>L. bilineata</i>	Western Green lizard	species
			<i>L. viridis</i>	European Green Lizard	"

Table D2 - Lineages, species and sub-species of Birds in North America
(References 1 Zink and Weckstein 2003; 2 Fletcher and Moore 1992; 3 Sibley 2000; 4 Mila et al. 2007)

Clade English	Clade	Level	Sub-clade taxa	Sub-clade taxa (English)	Level
1. Fox Sparrow	<i>Passerella iliaca</i>	species	-	Red	sub specific groups
			-	Slate-colored	"
			-	Sooty	"
			-	Thick-billed	"
2. Northern Flicker	<i>Colaptes auratus</i>	species	<i>C. a. auratus</i>	Yellow-shafted Flicker	sub species
			<i>C. a. cafer</i>	Red-shafted Flicker	"
3. Dark-eyed Junco	<i>Junco hyemalis</i>	species	<i>J. h. hyemalis</i>	Slate-colored Junco	sub species
			<i>J. h. aikenii</i>	White-winged Junco	"
			<i>J. h. oreganus</i>	Oregon Junco	sub species group
			<i>J. h. mearnsi</i>	Pink-sided Junco	sub species
			<i>J. h. caniceps</i>	Gray-headed Junco	"
4. Yellow-rumped warbler	<i>Dendroica coronata</i>	species	<i>D. c. coronata</i>	Myrtle Warbler	sub species
			<i>D. c. auduboni</i>	Audubon's Warbler	"

Climate data

All data for current conditions and potential future climates were obtained from the WorldClim website (<http://www.worldclim.org/download>, Hijmans *et al.* 2005). We used climate data from the HadCM3 general circulation model for the A2a future scenario. Climate data layers at 10-minute resolution were trimmed to the extent of North America and then re-projected in the Albers equal area projection with ArcInfo and ArcMap 9.3 (ESRI 1999-2008), using bilinear interpolation to produce a final resolution of 18.8 km. At this resolution, the coordinates for neighboring BBS transects only rarely occur in the same pixel. We used the time slices for 2020, 2050, and 2080 that were available on the WorldClim website, but because trends in predicted extent of suitable climate were temporally consistent, we only present analyses for current conditions and 2080. Current and future climates of Europe were estimated as for North America, except that the working resolution was 50×50 km to coincide with atlas data on the European herpetofauna.

Ecological niche modeling

Niche models were developed using the algorithm for generalized boosted regression trees in the R statistical package ‘gbm’, available at <http://www.cran.r-project.org>. We chose this method because it (1) can fit non-linear models to complex data, (2) performed comparatively well when models were used to predict species distributions as functions of independent datasets (Elith *et al.* 2006), (3) had been shown to model species-environment relationships accurately (Elith and Graham 2009) and (4) avoided over-fitting through use of additional functions, available in the gbm package (R Development Core Team 2009). In building gbm models, one generally develops a model object using a large number (several thousands) of regression trees. Subsequently, we determined an optimal number of these trees to use in projecting the model into geographic space, using (“cv”) in the R functions gbm() and gbm.perf() to avoid over-fitting.

Climate variables and model development

Nineteen bioclimatic variables were available as part of the WorldClim datasets (Hijmans *et al.* 2005). From these variables we calculated growing degree days above 0.5 °C and second quarter precipitation. These 21 variables can demonstrate substantial correlation, depending on the geographical location and extent of the area under consideration. We avoided using correlated variables for calibrating gbm models by defining multiple alternate sets of variables that displayed mutual correlations of < 0.7 . There were six alternative parameterizations for fitting models to breeding bird data and four for fitting data on European reptiles and amphibians. For each subspecies, species and clade we chose the model with the highest ten-fold, cross-validated AUC value for further analysis (Table D3, D4). For each species and clade we compared the geographic distribution of suitable climate resulting from both the clade model and the union of the corresponding subclade models. We examined these patterns using several criteria for establishing a threshold value for constructing a binary prediction of *taxon* distribution (Liu *et al.* 2005). We tabulated the number of currently occupied sites that are predicted to retain suitable climatic conditions in the future under the models. We also examined the degree to which predicted range for one *taxon*, developed as one subclade model, coincided with predicted range for a related *taxon*, developed as another subclade model, i.e. ‘cross-prediction’. Finally, we considered the area that was predicted by one or more subclade models to have suitable climatic conditions to be an ‘aggregate’ model for the distribution of the clade (or species in the case that subclade models represent subspecies).

Niche occupancy

We examined graphically the niche for each *taxon*, as estimated by clade and subclade models, along each of the environmental variables. Our objective here was to compare probabilities of occurrence of related *taxa*, including clades, species and subspecies. We focused on the distribution of the niche along the environmental variables that most influenced the gbm models for each *taxon* to help us understand potential differences in the predicted distributions that were generated by clade and subclade models (results not shown). But because each *taxon* also varied in prevalence in the training

datasets, we compared species probabilities of occurrence along environmental gradients in a way that would not be biased by differences in prevalence, as follows.

For each *taxon*, we considered the best-performing gbm model and projected it to climate values across the same map from which the training data came. We then standardized the probabilities of occurrence by dividing the probability value associated with each map cell by the sum of probabilities of across the entire map. This allowed construction of a density function for each *taxon*, the area under which sums to unity (Warren *et al.* 2008), as

$$1 = \sum_{j=1}^M p_j$$

where M is the number of map cells and p_j is the probability of occurrence of the species in the particular map cell j . These standardized values represented the modeled probability that an occurrence actually coincides with a particular cell and are dependent on the total number of map cells and the frequency distribution of the values of predictor variables. Because the density function of each *taxon* is standardized to sum to unity, these standardized distributions were comparable across *taxa*. The validity of these relationships depended on the assumption that the prevalences of the species in the data with which the model was trained and across the cells of the map are identical, i.e. that the training data came from a random sample taken from the area represented by the map.

We assigned the standardized map cell values to 100 uniform-width bins that spanned the range of values of each environmental variable that was used in the models. The total probability density corresponding to the map cells that were assigned to a particular bin equaled:

$$d_i = \sum_{j=1}^{m_b} p_j$$

where d_i is the proportion of probability density that is contributed by the map cells, numbering m_b , that have values of the

environmental variable that fall within the interval corresponding to bin b .

We divided the accumulated density within each bin (the d_i 's) by the corresponding number of map cells assigned to the bin to remove the dependence of accumulated bin density on the frequency of the corresponding map cells. We used a Gaussian kernel (Mouillot *et al.* 2005) to produce for each *taxon* and variable a smoothed distribution of mean probabilities of occurrence along the 100 intervals. Thus standardized, the distribution of average probability of occurrence along an environmental gradient is comparable among species and lineages that vary in prevalence.

Composite clade models

We used the standardized bin values to compare niche estimates that were produced by subclade and clade models. We also produced a model that summarized the niche across all subclade models. To do this, we first observed that each subclade model was developed independently of other subclade models. Thus, the standardized probabilities of occurrence of subclades in each map cell were statistically independent. We further assumed that no other biologically relevant processes influenced the probability of co-occurrence of related *taxa*. We then took advantage of the fact that the standardized bin values represent the mean probability that the species in question will be found in each map cell within a bin. The quantity one minus this probability is the probability that the species will be found in map cells corresponding to other bins. To construct an analog of the clade model from the subclade models, we calculated the mean probability of occurrence of at least one of the related *taxa* for the map cells represented by each bin as:

$$P_b(x) = 1 - \prod_{i=1}^N (1 - P(x_i))$$

where $P_b(x)$ is the average probability of the clade occurring in the sites represented in bin b , N is the number of *taxa* (and subclade models) and $P(x_i)$ is the mean probability of occurrence of *taxon* across the map cells represented by bin b . We call this model the ‘composite’ model, either for a clade of two sister species or for a species with multiple subspecies.

Results

Models of the distributions of birds and herptiles displayed AUC values consistently above 0.9 (Table D3, D4). In both the bird and herptile datasets, AUC values were marginally higher for subclade models (0.97 ± 0.005 ; mean \pm std. error) than they were for models that were trained on data pooled across *taxa* (clade models 0.95 ± 0.02), but the average magnitude of the difference was not significant (Pairwise Wilcoxon test, $N=10$, $P=0.24$). Nonetheless, it was unusual that AUC was higher for subclade models for 9 of the 10 species (Binomial Test, $P=0.01$). For herptiles, we found no evidence of a correlation between model fit (measured by cross-validated AUC) and *taxon* prevalence ($r_s = -0.34$, $n=18$, $P=0.16$). For birds, there was a weak correlation between model fit and *taxon* prevalence ($r_s = -0.56$, $n=15$, $P=0.03$). However, this correlation became non-significant when the observation for *Colaptes auratus* (clade model) was omitted from the analysis. As a clade, *C. auratus* demonstrated the greatest prevalence and the lowest AUC value of all clade models for birds.

Current predicted geographic distributions

Several of the clades of both European herptiles and North American birds demonstrated notable differentiation at the subclade level in the current geographic location of modeled suitable climate. For the herptiles, subclade models for each of the sister species showed little or no cross-prediction (Figure D1). Thus, there was substantial difference in niche occupancy among sister species.

In North American birds, models for subspecies of *Colaptes auratus* and *Dendroica coronata* did not substantially cross-predict the range of the other subspecies of the same species. In contrast, models of the subspecies of *Passerella iliaca* cross-predicted extensively, as did the models of the subspecies of *Junco hyemalis* (Figure D2).

Table D3 - Cross validated AUC for different parameterizations (European herpetofauna)

<i>taxon</i>	Cross validated AUC			
	1	2	3	4
<i>Bombina variegata</i> (clade)	0.971	0.957	0.952	0.951
<i>Bombina variegata</i>	0.978	0.965	0.955	0.968
<i>Bombina pachypus</i>	0.984	0.96	0.97	0.98
<i>Lacerta viridis</i> (clade)	0.96	0.952	0.943	0.944
<i>Lacerta viridis</i>	0.965	0.953	0.939	0.945
<i>Lacerta bilineata</i>	0.987	0.972	0.968	0.936
<i>Pelodytes punctatus</i> (clade)	0.983	0.978	0.96	0.973
<i>Pelodytes punctatus</i>	0.981	0.973	0.957	0.969
<i>Pelodytes ibericus</i>	0.976	0.97	0.969	0.97
<i>Testudo hermanni</i>	0.975	0.947	0.94	0.949
<i>Testudo hermanni hermanni</i>	0.978	0.976	0.943	0.955
<i>Testudo hermanni boettgeri</i>	0.976	0.96	0.958	0.952
<i>Triturus carnifex</i> (clade)	0.976	0.968	0.94	0.963
<i>Triturus carnifex</i>	0.978	0.967	0.974	0.959
<i>Triturus macedonicus</i>	0.988	0.972	0.967	0.97
<i>Triturus marmoratus</i> (clade)	0.993	0.988	0.957	0.984
<i>Triturus marmoratus</i>	0.993	0.98	0.974	0.988
<i>Triturus pygmaeus</i>	0.996	0.994	0.97	0.92

Table D4 - Cross validated AUC for different parameterizations (North American Birds)

taxon	Cross validated AUC					
	1	2	3	4	5	6
<i>Colaptes auratus</i>	0.821	0.816	0.819	0.819	0.815	0.821
<i>Colaptes auratus cafer</i>	0.954	0.958	0.955	0.955	0.959	0.956
<i>Colaptes auratus auratus</i>	0.929	0.932	0.932	0.927	0.932	0.929
<i>Dendroica coronata</i>	0.912	0.912	0.911	0.907	0.907	0.906
<i>Dendroica coronata auduboni</i>	0.948	0.954	0.953	0.949	0.953	0.949
<i>Dendroica coronata coronata</i>	0.946	0.948	0.944	0.944	0.945	0.941
<i>Junco hyemalis</i>	0.93	0.93	0.927	0.932	0.932	0.929
<i>Junco hyemalis caniceps</i>	0.943	0.927	0.93	0.944	0.925	0.925
<i>Junco hyemalis hyemalis</i>	0.946	0.948	0.942	0.944	0.948	0.944
<i>Junco hyemalis oreganus</i>	0.977	0.974	0.98	0.978	0.973	0.981
<i>Passerella iliaca</i>	0.938	0.937	0.938	0.933	0.935	0.933
<i>Passerella iliaca</i> , red group	0.969	0.971	0.954	0.966	0.956	0.953
<i>Passerella iliaca</i> , slate group	0.925	0.943	0.925	0.925	0.938	0.941
<i>Passerella iliaca</i> , sooty group	0.922	0.906	0.908	0.947	0.932	0.934
<i>Passerella iliaca</i> , thickbill group	0.939	0.893	0.972	0.95	0.933	0.979

Results on cross-prediction by subclade models were largely robust to choice of threshold for converting probability values into binomial (presence-absence) maps of predicted distributions, both for birds and herptiles species. Because of this general consistency among threshold criteria, we further consider and report only distributions based on the maximized True Skill Statistic (TSS) criterion (Allouche *et al.* 2006).

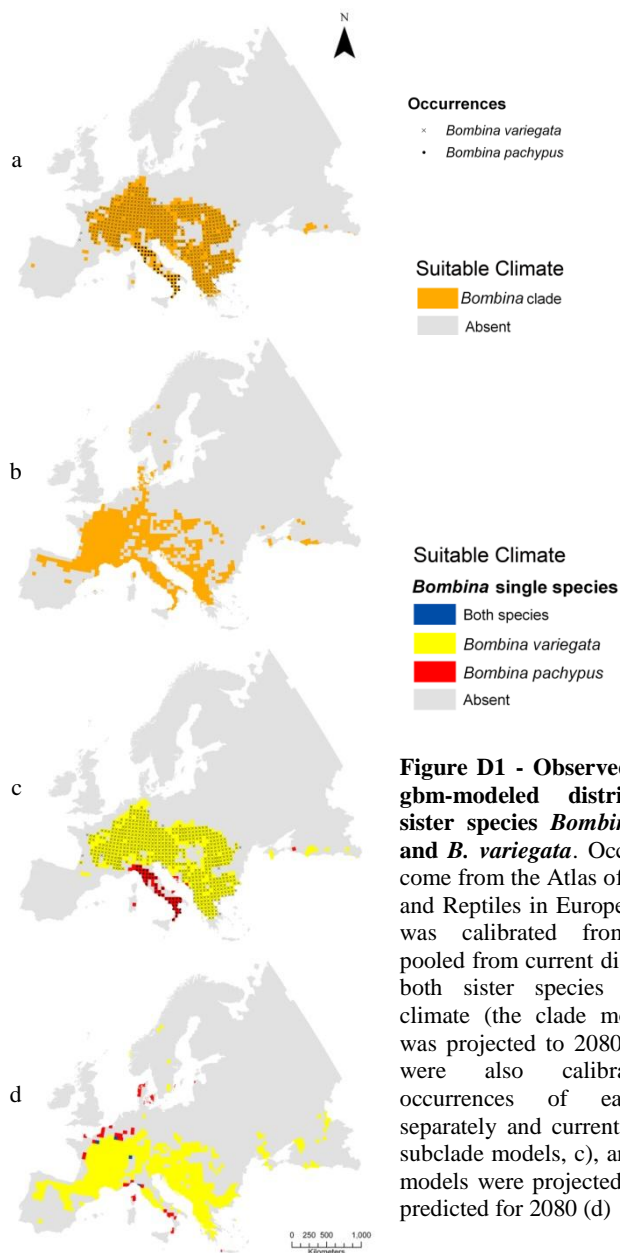
Predictions of clade models versus aggregated subclade models

The predicted distributions from clade models and aggregated (i.e. geographic union of) subclade models coincide to varying degrees. Aggregated subclade models predict larger suitable areas for European herptile *taxa* under current climatic conditions than do clade models in all but *Pelodytes punctatus* and *Triturus carnifex* clades (Figures D3a, D4a). In all cases, the extents of these differences are small, less than 9% (Table D5). The extent of suitable

area predicted by aggregated subclade and clade models for species in the *T. carnifex* clade are nearly identical (difference of 0.55%; Figure D4a and Table D5). In North American birds, the aggregated subclade models predict larger areas of currently suitable climatic conditions than do the clade models. For example, much of the area around the hybrid zone between the two subspecies of *C. auratus* is predicted habitable by one or the other subclade models, but not by the clade model (Figure D5a). For *C. auratus*, as well as for the other avian species, the subclade models predict suitable climate in areas that extend beyond and peripheral to the area that is predicted suitable by the clade model (Figures D5a, D6a).

Climate change and the persistence of suitable conditions

For amphibians and reptiles, clade models predict that more currently occupied sites will remain suitable in the future than does the aggregate of subclade models, sometimes by up to a factor of two (Table D6). Within clades, however, *taxa* often differ in the degree to which the predictions of persistent suitable conditions made by aggregated subclade models differ from the predictions of the corresponding clade model. For example, the clade model for *Testudo hermanni* predicts more than twice the number of sites remain climatically suitable for *T. hermanni hermanni* than does the subclade model for this subspecies itself (Table D6). Further, all clade models for herptiles predict the persistence of suitable conditions at as many or more sites, for all subclades collectively, than do the corresponding aggregated subclade models. For example, the clade model for *Lacerta viridis* predicts twice as many sites remain suitable than does the aggregate of subclade models (Table D6). In contrast, three out of four clade models for the North American birds predict the persistence of suitable climatic conditions at fewer sites than does the aggregate of subclade models (Table D7).



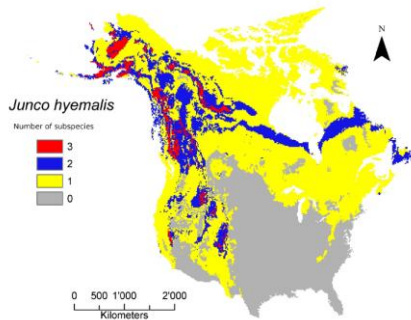


Figure D2 - Modeled current distributions of three subspecies of *Junco hyemalis*, showing extent of cross prediction among subclade models

Total predicted suitable area

The aggregate of subclade models predicts more extensive areas of suitable climate currently than the clade models for four of six herptile clades (Table D5). Under modeled climate at 2080, aggregates of subclade models predict greater suitable area than clade models for three of six herptile clades (Figures D3b, D4b; Table D5). In comparison, for three of the four species of North American birds we studied, subclade models predict greater extent of suitable area at year 2000, compared to the predictions of clade models (Figures D5b, D6b; Table D5). For the year 2080, aggregate models predicted greater extent of suitable habitat for all four avian species than do clade models. For example, in *Passerella iliaca* subclade models predict greater habitat suitability in the southern portion of the range by 2080 than does the clade model.

Clade and aggregated subclade models for herptiles agree in predicting the trend (i. e. increase or decrease) of change of potential habitat by 2080 (Table D8). However, subclade models for four of six herptile clades show contrasting responses to shifting climatic conditions. For example, the clade model of *Triturus marmoratus* clade and the aggregated subclade model both predict a reduction of habitat suitability by 2080 for the entire clade (Table D8). Nonetheless, the subclade model for the species *Triturus pygmaeus*, one of the two clade members, forecasts an increase in suitable area for this species (Table D8), while the extent of suitable habitat of the

clade as a whole decreases. Similarly, clade and aggregate models agree on the trend in extent of suitable habitat for three of the four bird clades. However, within each clade, subclade models predict that *taxa* will experience opposite trends in extent of suitable climate by the year 2080 (Table D8 and D9).

Niche differences within clades and species

The trend for subclade models to predict greater climatic suitability of peripheral geographic areas, mainly under current conditions, was also reflected in the probability of map cell occupancy as a function of climatic gradients. In our data, subclade models sometimes predict greater probability of occurrence towards the extremes of environmental gradients than do clade models. This pattern is exemplified by *Lacerta viridis* and *L. bilineata*, which have higher probability of occurrence at greater and smaller current temperature seasonality values, respectively, than the corresponding clade model (Figure D7). Similarly, in North American birds, the subclade model of *Colaptes auratus auratus* demonstrates greater probability of occurrence at both low and high extremes of growing degree-days than does the clade model (Figure D8). In contrast, the great proportion of probability density of the subclade model for *Dendroica coronata auduboni* is found at higher values of mean temperature of the driest quarter than indicated for the clade model of *Dendroica coronata*.

The niches modeled by clade models and the composite models are similar across herptile species (Figure D9). Often, the peak of probability of occurrence is slightly higher for the clade model than for the composite model. On the contrary, in birds the niche as determined from clade models often differs from the niche as determined from the subclade-based composite models. For instance, the composite model for the entire species for *Colaptes auratus*, indicates that subclade models overall predict marginally greater probability of occurrence at lower values of growing degree-days than does the clade model (Figure D10). In contrast, the composite subclade-based model for *Dendroica coronata* shows higher probability of occurrence at high values of the mean temperature of the driest quarter of the year than does the clade model.

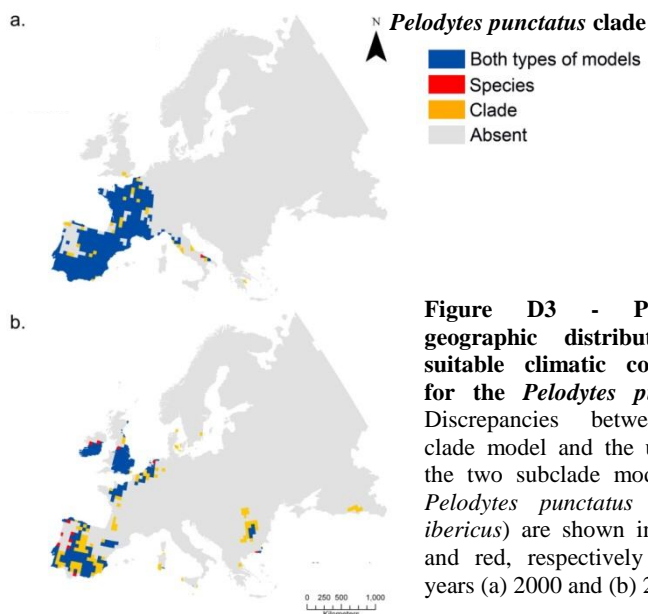


Figure D3 - Predicted geographic distribution of suitable climatic conditions for the *Pelodytes punctatus*
Discrepancies between the clade model and the union of the two subclade models (for *Pelodytes punctatus* and *P. ibericus*) are shown in orange and red, respectively for the years (a) 2000 and (b) 2080.

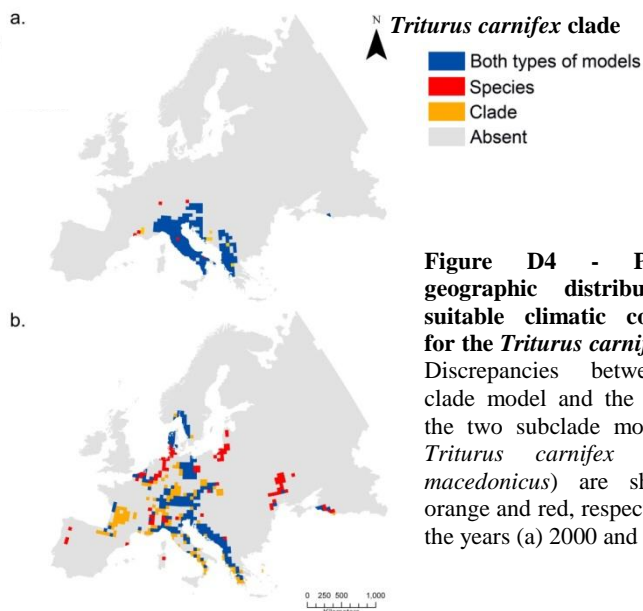


Figure D4 - Predicted geographic distribution of suitable climatic conditions for the *Triturus carnifex* clade
Discrepancies between the clade model and the union of the two subclade models (for *Triturus carnifex* and *T. macedonicus*) are shown in orange and red, respectively for the years (a) 2000 and (b) 2080

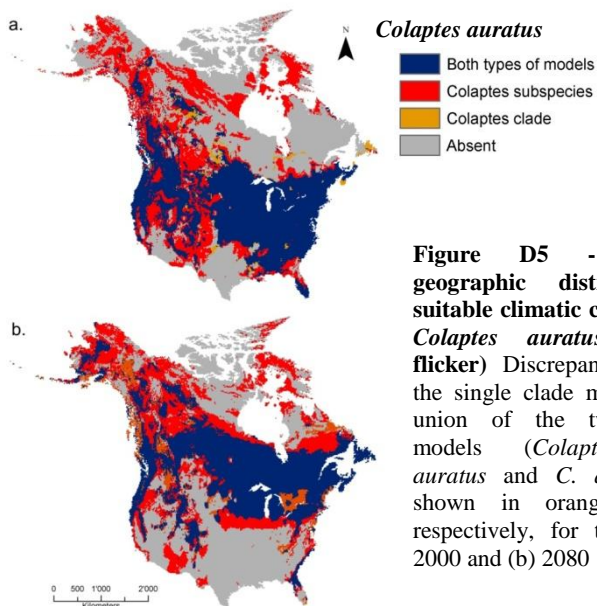


Figure D5 - Predicted geographic distribution of suitable climatic conditions for *Colaptes auratus* (northern flicker) Discrepancies between the single clade model and the union of the two subclade models (*Colaptes auratus auratus* and *C. a. cafer*) are shown in orange and red, respectively, for the years (a) 2000 and (b) 2080

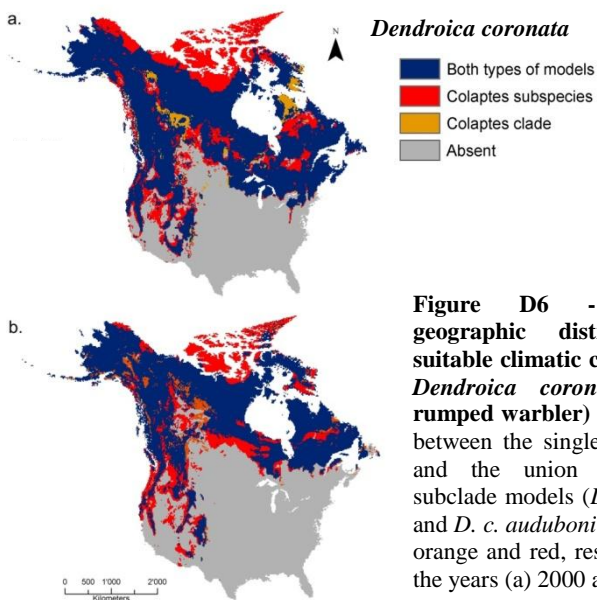


Figure D6 - Predicted geographic distribution of suitable climatic conditions for *Dendroica coronata* (yellow-rumped warbler) Discrepancies between the single clade model and the union of the two subclade models (*D. c. coronata* and *D. c. auduboni*) are shown in orange and red, respectively, for the years (a) 2000 and (b) 2080

Discussion

We chose two datasets for analysis because of our familiarity with them and the intuition that, because of their differences in geographic location, spatial resolution, taxonomic focus, and the evolutionary distinctness of the *taxa*, they could display distinct tendencies in terms of the degree of niche differentiation among *taxa* and potential impacts for predicting the effects of climate change. Further, the results of previous studies have demonstrated that closely related *taxa* can show a range of degrees of niche divergence and that this is to be expected given the diversity of species and their ecologies (Wiens and Graham 2005). For example, niche models of closely related species can show substantial similarity, to such a degree that closely related species pairs from diverse *taxa* can sometimes mutually predict distribution limits of putative sister species (Peterson *et al.* 1999).

Table D5 - Comparison of difference in suitable area extent predicted by aggregated subclade and clade models for the years 2000 and 2080

	SCM-CM		Difference (%)	
	2000	2080	2000	2080
<i>Bombina variegata</i> (clade)	25	57	3.54	8.18
<i>Lacerta viridis</i> (clade)	50	-52	5.80	-4.11
<i>Pelodytes punctatus</i> (clade)	-32	-102	-8.31	-48.80
<i>Testudo hermanni</i> (clade)	27	151	8.63	12.02
<i>Triturus carnifex</i> (clade)	-1	-49	-0.55	-16.28
<i>Triturus marmoratus</i> (clade)	15	60	4.64	19.54
<i>Colaptes auratus</i> ('clade')	12 760	10 675	39.57	33.96
<i>Dendroica coronata</i> ('clade')	7901	5768	21.76	20.12
<i>Junco hyemalis</i> ('clade')	9191	10 451	24.17	34.86
<i>Passerella iliaca</i> ('clade')	-210	962	-0.68	4.70

SCM: aggregated subclade model, in number of raster cells.

CM: clade model, in number of raster cells.

Difference: $100 \times (\text{SCM} - \text{CM}) / \text{SCM}$.

Table D6 - Persistence of suitable conditions in initially occupied cells, according to clade model and sub-clade models (European herpetofauna)

Predicted taxa	Occurrences	Sub-clade models				Clade-level model				clade 2080/ subclade 2080†	Model AUC††
		2000		2080		2000		2080			
		predicted	%**	predicted	%	predicted	%	predicted	%		
<i>Bombina variegata</i> (clade)	577	565	97.92	354	61.35	563	97.57	354	61.35	1	0.971
<i>Bombina pachypus</i>	43	43	100	7	16.28	42	97.67	37	86.05	5.29	0.984
<i>Bombina variegata</i>	534	522	97.75	347	64.98	521	97.57	317	59.36	0.91	0.978
<i>Lacerta viridis</i> (clade)	722	707	97.92	227	31.44	697	96.54	459	63.57	2.02	0.96
<i>Lacerta viridis</i>	345	335	97.1	164	47.54	326	94.49	150	43.48	0.91	0.965
<i>Lacerta bilineata</i>	377	372	98.67	63	16.71	371	98.41	309	81.96	4.9	0.987
<i>Pelodytes punctatus</i> (clade)	325	323	99.38	66	20.31	323	99.38	130	40	1.97	0.983
<i>Pelodytes punctatus</i>	273	271	99.27	35	12.82	271	99.27	88	32.23	2.51	0.981
<i>Pelodytes ibericus</i>	52	52	100	31	59.62	52	100	42	80.77	1.35	0.976
<i>Testudo hermanni</i> (clade)	208	207	99.52	149	71.63	206	99.04	163	78.37	1.09	0.975
<i>Testudo hermanni hermanni</i>	59	59	100	27	45.76	59	100	55	93.22	2.04	0.978
<i>Testudo hermanni boettgeri</i>	149	148	99.33	122	81.88	147	98.66	108	72.48	0.89	0.976
<i>Triturus camiflex</i> (clade)	169	168	99.41	61	36.09	168	99.41	91	53.85	1.49	0.976
<i>Triturus camiflex</i>	125	124	99.2	44	35.2	124	99.2	67	53.6	1.52	0.978
<i>Triturus macedonicus</i>	44	44	100	17	38.64	44	100	24	54.55	1.41	0.988
<i>Triturus marmoratus</i> (clade)	294	293	99.66	97	32.99	293	99.66	137	46.6	1.41	0.993
<i>Triturus marmoratus</i>	212	211	99.53	34	16.04	211	99.53	82	38.68	2.41	0.993
<i>Triturus pygmaeus</i>	82	82	100	63	76.83	82	100	55	67.07	0.87	0.996
Mean across herpetofauna taxa, excluding clade values	252.71	188.58	99.24	79.5	42.69	187.5	98.73	111.17	63.62		
Standard error	46.17	45.08	0.28	27.61	7.05	44.81	0.46	28.94	5.72		

** Sensitivity is the proportion (or percent) of occurrence points that are correctly predicted.

† This number is the ratio of the number of map cells that still have suitable climate for the *taxon* in the year 2080, as predicted by clade models and sub-clade models.

††Model AUC was calculated by evaluating clade models on all observations. AUC for subclade models was calculated using presences of the subclade group and considering all other cells as absences.

Table D7 - Persistence of suitable conditions in initially occupied cells, according to clade model and sub-clade models (North American birds)

Predicted taxa	Occurrences	Sub-clade models				Clade-level model				clade 2080/ subclade 2080†	Model AUC ^{††}
		2000		2080		2000		2080			
		predicted	%**	predicted	%	predicted	%	predicted	%		
<i>Colaptes auratus</i> (clade)	3501	3181	90.86	2053	2839	2839	81.09	1758	50.21	0.86	0.821
<i>Colaptes auratus auratus</i>	2507	2250	89.75	1430	2105	2105	83.96	1299	51.81	0.91	0.932
<i>Colaptes auratus cafer</i>	994	931	93.66	623	734	734	73.84	459	46.18	0.74	0.959
<i>Dendroica coronata</i> (clade)	1409	1297	92.05	534	1241	1241	88.08	442	31.37	0.83	0.912
<i>Dendroica coronata</i>											
<i>auduboni</i>	571	533	93.35	442	483	483	84.59	244	42.73	0.55	0.954
<i>Dendroica coronata coronata</i>	838	764	91.17	92	758	758	90.45	198	23.63	2.15	0.948
<i>Junco hyemalis</i> (clade)*	1568	1479	94.32	659	1423	1423	90.75	479	30.55	0.73	0.932
<i>Junco hyemalis caniceps</i>	161	154	95.65	105	136	136	84.47	76	47.2	0.72	0.944
<i>Junco hyemalis hyemalis</i>	876	820	93.61	186	785	785	89.61	164	18.72	0.88	0.948
<i>Junco hyemalis oreganus</i>	531	505	95.1	368	502	502	94.54	239	45.01	0.65	0.981
<i>Passerella iliaca</i> (clade)	408	393	96.32	149	391	391	95.83	206	50.49	1.38	0.938
red group	148	139	93.92	36	146	146	98.65	82	55.41	2.28	0.971
slate group	157	152	96.82	51	145	145	92.36	60	38.22	1.18	0.943
sooty group	38	37	97.37	9	38	38	100	27	71.05	3	0.947
thickbill group	65	65	100	53	62	62	95.38	37	56.92	0.7	0.979
Mean across bird taxa, excluding clade values	683.47	577.27	94.58	308.64	535.8	535.82	89.81	262.27	45.17		
Standard error	253.72	193.78	0.86	127.1	179.7	179.74	2.31	110.47	4.45		

* The designation of species as a clade assumes that the group of subspecies shown here, and potentially other subspecies that were not modeled, form a monophyletic group. This is likely, but has not been determined with molecular data, for *Junco hyemalis* subspecies. Means and standard errors were calculated before rounding values to two decimal places

** Sensitivity is the proportion (or percent) of occurrence points that are correctly predicted.

† This number is the ratio of the number of map cells that still have suitable climate for the *taxon* in the year 2080, as predicted by clade models and sub-clade models.

†† Model AUC was calculated by evaluating clade models on all observations. AUC for subclade models was calculated using presences of the subclade group and considering all other cells as absences.

Table D8 - Predicted number of suitable raster cells for herptiles *taxa* at 2000 and 2080, and percentage of future range change

<i>taxon</i>	Predicted geographic distribution in number of raster cells		
	2000	2080	Range change (%)
<i>Bombina variegata</i>	651	654	0.46
<i>Bombina pachypus</i>	56	47	-16.07
<i>Bombina variegata</i> SCM	707	697	-1.41
<i>Bombina variegata</i> CM	682	640	-6.16
<i>Lacerta viridis</i>	475	971	104.42
<i>Lacerta bilineata</i>	393	315	-19.85
<i>Lacerta viridis</i> SCM	862	1264	46.64
<i>Lacerta viridis</i> CM	812	1316	62.07
<i>Pelodytes punctatus</i>	342	142	-58.48
<i>Pelodytes ibericus</i>	52	67	28.85
<i>Pelodytes punctatus</i> SCM	385	209	-45.71
<i>Pelodytes punctatus</i> CM	417	311	-25.42
<i>Testudo hermanni hermanni</i>	102	150	47.06
<i>Testudo hermanni boettgeri</i>	211	1136	438.39
<i>Testudo hermanni</i> SCM	313	1256	301.28
<i>Testudo hermanni</i> CM	286	1105	286.36
<i>Triturus carnifex</i>	139	233	67.63
<i>Triturus macedonicus</i>	44	70	59.09
<i>Triturus carnifex</i> SCM	183	301	64.48
<i>Triturus carnifex</i> CM	184	350	90.22
<i>Triturus marmoratus</i>	227	99	-56.39
<i>Triturus pygmaeus</i>	102	208	103.92
<i>Triturus marmoratus</i> SCM	323	307	-4.95
<i>Triturus marmoratus</i> CM	308	247	-19.81

SCM: aggregated subclade models.

CM: clade model.

Table D9 - Predicted number of suitable raster cells for birds *taxa* at 2000 and 2080, and percentage of future range change

<i>taxon</i>	Predicted geographic distribution in number of raster cells		
	2000	2080	Range change (%)
<i>Colaptes auratus auratus</i>	20 782	22 138	6.52
<i>Colaptes auratus cafer</i>	11 884	9732	-18.11
<i>Colaptes auratus</i> SCM	32 243	31 433	-2.51
<i>Colaptes auratus</i> CM	19 483	20 758	6.54
<i>Dendroica coronata auduboni</i>	8988	9981	11.05
<i>Dendroica coronata coronata</i>	29 871	20 604	-31.02
<i>Dendroica coronata</i> SCM	36 303	28 670	-21.03
<i>Dendroica coronata</i> CM	28 402	22 902	-19.36
<i>Junco hyemalis caniceps</i>	9169	9460	3.17
<i>Junco hyemalis hyemalis</i>	30 902	22 413	-27.47
<i>Junco hyemalis oreganus</i>	8419	7839	-6.89
<i>Junco hyemalis</i> SCM	38 023	29 979	-21.15
<i>Junco hyemalis</i> CM	28 832	19 528	-32.27
<i>Passerella iliaca</i> red group	24 902	13 253	-46.78
<i>Passerella iliaca</i> slate group	15 228	22 413	47.18
<i>Passerella iliaca</i> sooty group	15 648	7033	-55.05
<i>Passerella iliaca</i> thickbill group	2894	2585	-10.68
<i>Passerella iliaca</i> SCM	30 842	20 468	-33.64
<i>Passerella iliaca</i> CM	31 052	19 506	-37.18

SCM: aggregated subclade model

CM: clade model

Notes: The use designation of species as a clade assumes that the group of subspecies shown here, and potentially other subspecies that were not modeled, form a monophyletic group. This is likely, but has not been determined with molecular data, for *Junco hyemalis* subspecies

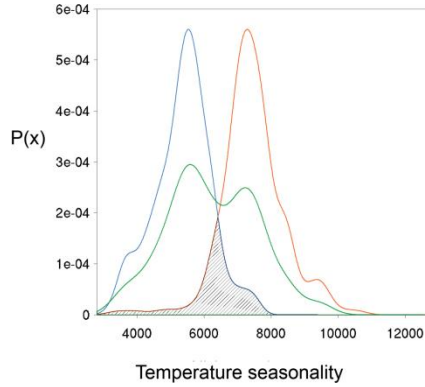


Figure D7 - Niche dimension (mean probability density) along the variable temperature seasonality, measured as 100 times the standard deviation of 12 monthly temperature means Data are the smoothed mean bin values of standardized probability of occurrence in map cells. The green line represents the *Lacerta* clade niche model. The red and blue lines represent the subclade models constructed respectively for *L. viridis* and *L. bilineata*

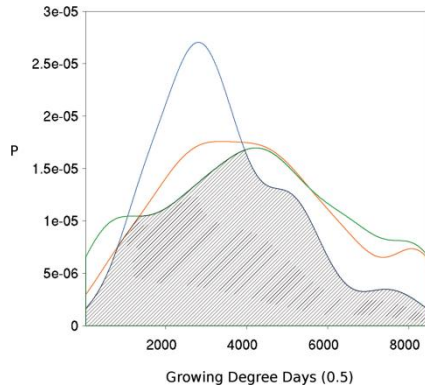


Figure D8 - Probability distribution (mean probability density) for occupancy of map cells by *Colaptes auratus* (clade) and two subspecies, as a function of growing degree days above 0.5°C The orange line is *Colaptes auratus* niche model. The blue and green lines are the subclade models constructed respectively for *C. a. cafer* and *C. a. auratus*

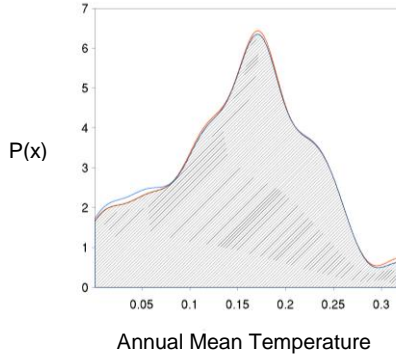


Figure D9 - Niche dimension (mean probability density) along the variable annual mean temperature ($^{\circ}\text{C} \times 0.01$) The red line is the *Bombina* clade niche model. The blue line is the analogous composite model constructed from the niche models of the two corresponding subclade models

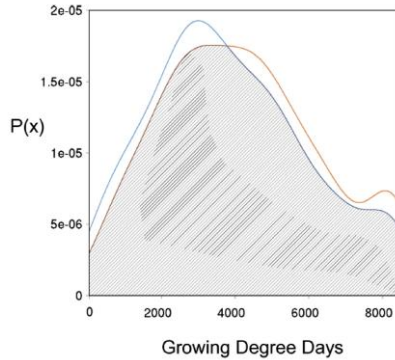


Figure D10 - Niche dimension (mean probability density) of *Colaptes auratus* along the variable growing degree days (above 0.5°C) The orange line is the clade niche model. The blue line is the analogous composite model constructed from the niche models of the two corresponding subclades

In other cases, niche modeling has revealed divergent climatic affinities among very closely related species (Rice *et al.* 2003, Graham *et al.* 2004b, Knouft *et al.* 2006). In general, our data support the existence of a broad range in the degree of similarity in the niche characteristics of closely related *taxa*. The niche differences we identify here between closely related *taxa* substantially influence predictions of climate change impacts on species future potential distributions.

Clade and subclade models can differ in their portrayal of the potential effects of climate change. This occurs when subclades occupy different niches and is well exemplified by *Colaptes auratus*. Clade models can smooth across the climate-distribution relationships that are identified by subclade models (e.g., *Lacerta* clade, Figure D7; *Dendroica* clade, Figure D9). Further, subclade models can predict suitable areas that are geographically peripheral to areas predicted suitable by clade models (e.g. Junco clade). When a *taxon* has strong phylogeographic structure, models can underestimate climate tolerances and overestimate effects of climate change on *taxon* range size, as shown for seven of the 10 study *taxa* (Table D5). On the other hand, subclade models can detect lineage-specific effects of climate change that are absent in clade models. For example, distinguishing between two *Bombina* species suggests that some lineages are likely more threatened by climate change than are others, and that this is not detected by clade models (Figure D1). Together, these results suggest that models that integrate phylogeographic and subspecies structure can provide an important additional perspective on the potential effects of climate change on species distribution.

Our results support the hypothesis that sister species can show marked niche differentiation. For example, models of the sister species *Triturus carnifex* and *T. macedonicus* show no geographical overlap in the distribution of suitable climatic conditions. Similarly, models of *Lacerta viridis* and *L. bilineata*, and *Bombina variagata* and *B. pachypus* show little overlap in the distribution of suitable climate. We also found substantial niche differences for the subspecies of the woodpecker, *Colaptes auratus*, and the warbler *Dendroica coronata*. However, models for three broadly distributed subspecies of *Junco hyemalis* demonstrated cross-prediction for their geographic ranges, which is in conflict with their generally exclusive

distributions. This variability is in agreement with Peterson and Holt (2003), who found variation among subspecies in the degree to which models of one subspecies cross predicted the distribution of other subspecies. Substantial differences in the niches occupied by subspecies may occur without strong reproductive isolation, as in the case of *Colaptes auratus* (Moore 1987, Moore *et al.* 1991). Deep and persistent phylogeographic divisions and accompanying differences in niche occupancy suggest that ecological niche models should take these biological characteristics into account. This paper has demonstrated two ways to approach the construction of niche models and predicted distributions when such divisions exist.

It remains unclear whether intra-specific variation in niche occupancy is due to differences in the ‘available’ environmental variation among regions, adaptation to local conditions with concomitant maladaptation to foreign conditions, or other causes. Murphy and Lovett-Doust (2007) conclude that niche occupancy varies among regions in the broadly distributed tree *Gleditsia triacanthos* (Fabaceae), but that this is primarily due to regional differences in the availability of environments. In contrast, experimental studies have repeatedly found differences in performance among populations of trees grown in differing environments. These differences suggest that populations are often best adapted to the local environment (Rehfeldt *et al.* 1999, Rweyongeza *et al.* 2007, Savolainen *et al.* 2007) and that genotype-by-environment interactions are common in widespread species. While ecological correlates of intraspecific niche differentiation may be elusive (McPherson and Jetz 2007), deep phylogeographic divisions within species indicate the potential for intraspecific niche differentiation and suggest the need for definitive field experiments.

Biotic interactions

Another factor that could potentially influence niche occupancy of phylogeographic lineages of a species or clade is geographic variation in the strength of biotic interactions. Incorporation of variables to represent the distribution of potential competing or facilitating species frequently leads to improvement in the performance of species distribution models (Elith and Leathwick 2009, Meier *et al.* 2010). This suggests that niche differences among subspecies or sister species might arise in part through the

differential interaction of these *taxa* with additional species. In this case, studies that take advantage of phylogeographic structure to construct subclade models can examine benefits in the form of improved model fit that could arise from incorporating information on the distribution of potentially interacting species. An approach that models subclade distributions, as we have done here, allows for the possibility that biotic interactions could affect subclades to different degrees.

Composite niche models and climate change

Models to describe the relationship of climate to the distribution of subspecies and regional portions of widely distributed species could make sense if three conditions were met: (1) a biological basis for regionalization, such as distinct geographic lineages and population genetic subdivision, (2) evidence for local or regional adaptation, and (3) evidence for geographic structure in tradeoffs that result from local adaptation to environment. Some of these conditions, although time consuming and expensive to evaluate, have been reported for species in a variety of unrelated higher *taxa*. Dozens of experimental studies of the effect of provenance on tree growth, survival and reproduction suggest geographic variation in traits that might be important to mediating population response to climate change (reviewed in Savolainen *et al.* 2007). Variation in timing of reproduction among populations of bird species in regions with distinct climates and habitats can be genetically based (Blondel *et al.* 1990, Lambrechts and Dias 1993), respond to selection (Bradshaw and Holzapfel 2006, 2008) and can create fitness costs in newly established populations (Van Der Jeugd *et al.* 2009). Similarly in larval anurans, variation in season length can select for divergent growth curves as a function of temperature (Berven *et al.* 1979, Berven 1982). Further, when some geographically-restricted *taxa* (subspecies or lineages) below the species level have low prevalence, relative to other related *taxa*, these narrowly distributed *taxa* can contribute little to modeled distribution at the level of the species. Development of predicted distribution based on a composite niche model gives greater weight to the contribution of these *taxa* to the niche occupancy displayed by a species. This approach deserves closer inspection and further development.

Data limitation and potential bias

A number of potential sources of spurious influence and bias deserve recognition. We recognize that one aspect of several models of avian distribution in North America is predicted presence in the high arctic, something that is unrealistic for these species. This likely occurs in part because we used only climate data and no land cover variables. Further, despite being the best available data, the BBS dataset includes few survey routes in the high arctic, so that there may be insufficient absence data to constrain distribution in this extremely cold environment. This artifact would likely have been lessened had we additionally constrained the models by the presence of forest vegetation, but this would have been arbitrary had not a full range of land cover data been comprehensively included in the study. In any case, each modeling exercise is limited to a finite number of variables. The results of comparisons, such as those reported here, could potentially be limited to the variables that we used, as in any correlative analysis. Subsequent research can examine an extended set of environmental and landcover variables, but limitations in the northern extent of BBS data will likely remain.

We summarized the geographic location of each 39.4 km BBS route by using a single geographic location to locate the species occurrences found along the entire BBS route. Through much of the North American Midwest, northern Canada, coastal regions and eastern provinces and states, the error that this introduces into the occurrence-climate relationships is surely minimal. Climate variation along survey routes in these areas is small in comparison to the climate gradients that exist over the continental extent of the entire collection of survey routes. In areas of greater topographical diversity, our practice may have introduced error. This is unlikely to have influenced our results substantially for two reasons. First, we used climate estimates with a cell size of 10 minutes, or roughly 20 km at the temperate latitudes of our study. Climate averaging within these cells is roughly at the same resolution as the location data for survey routes. Secondly, BBS survey routes are almost exclusively along roads and thus are biased against extremes of high elevation. The climatic conditions of topographically diverse areas may be poorly represented in climate data that have a resolution of multiple kilometers. In the herptile dataset, it is unlikely that all atlas cells have been equally well surveyed. Habitat affinities of herptiles likely

guide surveys, however, and we assume that inaccuracies are not critical to our interpretation of the patterns we have elucidated.

Final remark

The analyses that we present demonstrate that subclade models, here representing either species or subspecies, can consistently, if only marginally, outperform clade models as measured by 10-fold cross-validated AUC values. More importantly, subclade models capture the climate-distribution relationship for groups of populations with similar evolutionary histories and shared selective regimes (Avice 2000). Incorporation of this information when modeling species can substantially alter the predicted effects of climate change on the potential distribution of species. We have shown that there are a number of ways to combine subclade models to reflect on the distributions of entire species and that these methods can vary substantially in their predictions for future species distributions. Modeling of species distribution sometimes involves pooling data from groups of populations with very different demographic and evolutionary history. Although this has been advocated as a method for dealing with populations with divergent demographic and evolutionary histories (Broennimann and Guisan 2008), this practice needs further study and refinement. Differential representation of segments of subspecies or phylogeographic lineages in species occurrence data sets can lead to some lineages having little representation in the resulting species model unless appropriate measures are taken.

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