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Land use preferences and distribution of *Pelophylax spp.* in Bosnia and Herzegovina

Master´s thesis

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Declaration

I hereby declare that I have authored this master’s thesis carrying the name „ Land use preferences and distribution of *Pelophylax spp*. in Bosnia and Herzegovina“ independently under the guidance of my supervisor and cosupervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master’s thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 21.04.2023.

Vrhovac Borina

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Land use preferences and distribution of *Pelophylax spp.* in Bosnia and Herzegovina

Summary:

Thesis topic "Land Use Preferences and Distribution of *Pelophylax spp*. in Bosnia and Herzegovina," aimed to identify the preferred habitats of *Pelophylax kurtmuelleri*, *Pelophylax ridibundus*, and their hybrids and develop a model to predict their occurrence based on land use type in Bosnia and Herzegovina. *Pelophylax spp.* individuals were collected between 2018 and 2019 and molecularly identified in Vrhovac et al., 2020. Data on the composition of Corine Land Cover types across 18 research locations within a buffer zone were obtained using ArcGIS software. R Software was used to analyse preferred habitats of *P. kurtmuelleri, P. ridibundus* and their hybrids, as well as predicting the occurrence of chosen taxon's based on land use type data for Bosnia and Herzegovina and the kernel density map of samples occurrences. The study found that a large-scale analysis of land use preferences did not have strong statistically significant differences between the selected taxa and land use type. It was suggested that future studies should analyze data on a smaller scale of land use and with an increased amount of samples for each taxon. However, the hybrid of *P. ridibundus* and *P. kurtmulleri*, as well as *P. kurtmulleri*, have shown a degree of superiority in comparison to *P. ridibundus*, based on their distribution, diversity, and preference of the CLC types they have been identified on. These findings may contribute as insight to further research on the genetic and ecological aspects of *Pelophylax* species, potentially leading to a better understanding of their complex nature.

Keywords: land use preferences, distribution, *Pelophylax spp.,* Bosnia and Herzegovina, conservation planning, ArcGIS,statistical analysis, R

Content

1 Introduction

The genus *Pelophylax spp.*, as described by Fitzinger in 1843, includes a group of aquatic frogs commonly known as the Palearctic water frogs. These frogs are found across a wide geographical range, spanning from northern Africa to the Far East in Asia (Akin et al. 2010).

Because of their need for a steady supply of water and the fact that they can be found in a diverse range of locations, rivers and lakes are among the preferred locations where they occur (Jablonski et al., 2021). The spatial distribution of *Pelophylax spp.* is subject to various factors, including climate variables such as temperature and precipitation (Enriquez-Urzelai et al., 2019; Ortiz-Yusty et al., 2013; Kim et al., 2021), topographical features (Kuzmin et al., 2004; Yang et al., 2001; Liu et al., 2010), and land use patterns (Mikulcek & Pisut, 2012; Mikulicek et al., 2014; Wei et al., 2021). The distribution of organisms can be significantly affected by alterations in land use patterns and habitat quality, (Wei et al., 2021). The alteration of water quality and the availability of appropriate breeding habitats can be attributed to urbanisation and agricultural practices (Gray et al., 2002; Gagne & Fahrig, 2007).

The findings of various studies suggest that *Pelophylax* species display a wide range of habitat preferences, encompassing both large water bodies with dense emergent vegetation and smaller water bodies with sparse vegetation (Mikulcek & Pisut, 2012; Mikulicek et al., 2014). The emergence of certain species, including *P. ridibundus*, can be attributed to anthropogenic activities such as the expansion of small bodies of water and the construction of artificial waterways. Consequently, the displacement of two populations of native water frogs, namely *P. lessonae* and *P. esculentus*, has taken place. The study indicated that *P. lessonae* and *P. esculentus* exhibit a preference for habitats with minimal human impact, whereas *P. ridibundus* is predominantly observed in densely populated regions such as artificial gravel pits within the surveyed locality (Mikulcek et al., 2014).

The adaptability of *P. ridibundus* to varying ecological conditions and its specific habitat preferences enable its survival and flourishing in ecological systems that have been affected by human activity. The expansion of *P. ridibundus* and the decline in other water frog populations can be attributed to their adaptability to different habitats, including artificial aquatic environments. Moreover, the hybrid *P. esculentus* was found to occupy various habitats such as marshes, sandpits, and dead river arms, similar to its paretal species, *P. lessonae*. The reported occurrence could potentially be attributed to the hybridogenetic system and the dependence on their host species for reproduction, as well as their specific habitat preferences (Schenck & Vrijenhoek, 1989; Bergen et al., 1997; Vrijenhoek & Pfeiler, 1997; Plénet et al., 2000; Negovetic et al., 2001; Doeringsfeld et al., 2004).

The distribution and habitat preferences of hybrids can be impacted by a combination of parental species traits and environmental conditions. Hybridization, the interbreeding of different species, can have an impact on the land use preferences and distribution of *Pelophylax spp*. (Gunther, 1974; Rybacki & Berger, 1994 Pagano et al,. 2001; Holenweg Peter et al., 2002; Plenet et al., 2005; Hotz et al., 2008; Mikulicek et al., 2014). The success of the hybrid species and its sexual host varied depending on the environment, according to a study by Plenet et al. (2005). Furthermore, the hybrid species did not exhibit dominance over its sexual competitors in environmental settings. There could be potential benefits to hybrids forming, but also risks of introgression to native species.

Frogs, like other amphibians, are recognized for their increased vulnerability due to their physical limitations and limited movement, making them valuable indicators of environmental changes (Hof et al., 2011; Wake & Vredenbug, 2008; Li et al., 2013). Alterations in land use patterns at the landscape scale can have both direct and indirect impacts on the populations of amphibians (Findlay & Houlahan 1997; Green, 1997; Lehtinen et al., 1999; Pope et al., 2000; Johnson et al., 2002; Welsh et al.,, 2002).

Understanding land use preferences can serve as a significant factor in the distribution of species and may have an impact on the formulation of conservation strategies, whether for the species or their respective ecosystems.

2 Scientific hypothesis and aims of the thesis

My current study provides an ecological insight into these species and their hybrid, as well as possible implications for species and genetic conservation, either of native species populations or hybridogenetic water frog complexes. In order to conserve genetic diversity as part of natural resources, it is important to understand to what extent it is lost. This thesis can help us address effective conservation strategies to prevent diversity loss.

The main objectives of this research are to:

(1) **identify the preferred habitats of *P. kurtmuelleri, P. ridibundus* and their hybrids**

(2) **develop and test a model to predict occurrence based on land type of *P. kurtmuelleri, P. ridibundus* and their hybrids based on habitat suitability in Bosnia and Herzegovina.**

3 Literature research

A necessary literature research was conducted in addition to the study's primary materials and methods in order to assist in answering the study's main objectives. It involved gathering, synthesizing, and analyzing various data and viewpoints from numerous scientific researches into the subject of the research, the genus *Pelophylax*, as well as the taxonomic groups to which it belongs, in relation to previously understood land use preferences and their distribution, appropriately. The literature pertaining to the factors, both environmental and genetic, that may explain population dynamics of water frogs and their potential impacts on distribution and land use preferences of *Pelophylax spp*. was also thoroughly reviewed and presented. We were able to deduce the discussion and conclusions by combining this information with the outcomes of the methodologies we used. A comprehensive viewpoint is presented in both the discussion and the conclusion.

3.1 Habitat preferences and distribution patterns

3.1.1 Class Amphibia (Blainville, 1816)

Approximately 365 million years ago, amphibians began their evolutionary and phylogenetic history (Carroll, 1992). Amphibians are the descendants of Triadobatrachus, the first terrestrial vertebrate, and they lie between fish and reptiles on the evolutionary tree. In terms of both morphology and physiology, they are clearly distinct from all other terrestrial vertebrates (Hillman et al., 2009). The species exhibits extensive niche diversification at various altitudinal ranges and is found in tropical, subtropical, and temperate biomes along distinct elevational gradients (Duellman & Trueb, 1986; Duellman & Sweet, 1999).

They obtained their name from the Greek words "amphi" and "bios," which means "living a double life." Their life cycle is delineated by two distinct phases, each of which encompasses a specific developmental phases. The physiological characteristics of amphibians, as described by Toledo and Jared (1993), have been observed to impose limitations on the distribution of anurans. However, despite these limitations, anurans have diversified and adapted to occupy various niches, including terrestrial, aquatic (both lentic and lotic), scansorial (arboreal, phytotelms, rock outcrops), and fossorial (leaf litter, organic top soil) habitats in both the Old and New Worlds Pitt et al., 2017; Wells, 2019; Crnobrnja-Isailović et al., 2022).

Aquatic environments are necessary for the majority of European amphibians, particularly for breeding purposes (Gasc J-P et al., 1997; Arnold & Oven 2002; Speybroeck et al., 2016). A positive correlation has been observed between small aquatic habitats and the majority of amphibian species identified in Bosnia and Herzegovina (Đurović et al., 1979; Lelo et al., 2015; Adrović, 2015). The importance of ponds as small aquatic habitats for amphibians has been widely recognized (Curado et al., 2011). Therefore, amphibian diversity is greatest where precipitation is abundant and/or evaporation is low (Duellman & Trueb, 1986). Environments with the ideal proportions of humidity, warmth, and light are necessary for life of amphibians (Pough, 2007). Selection of wet or moist habitats minimise the dehydration risk associated with terrestrial movement for amphibian species, especially in open canopy habitats (Mazerolle & Desrochers, 2005). The ability to migrate through unfavourable terrestrial habitats may be greatly facilitated by vegetation and microhabitats that conserve moisture and provide shade (Patrick et al., 2006; Earl & Semlitsch, 2015). Dry open-canopy habitats, e.g. clear cuts, grasslands, or agricultural fields, are generally avoided by many amphibian species (Rothermel & Semlitsch, 2002; Johansson et al., 2005; Rittenhouse & Semlitsch, 2009; Todd et al., 2009; Semlitsch et al., 2009; Popescu & Hunter, 2011; Harper et al., 2015). Certain types of habitats are particularly important for facilitating the movement of amphibians through their environment dispersal (Baldwin et al., 2006; Pitt et al., 2017).

3.1.2 Order Anura (Fischer von Waldheim, 1813)

Anurans exhibit the most extensive geographical range among all amphibians, displaying the highest diversity in the oriental, neotropical, and afrotropical regions (Duellman & Sweet, 1999; Sweet & Pianka, 2007). The group exhibits significant heterogeneity, as evidenced by their widespread distribution, spanning from arid regions to tropical forests. According to Pough et al. (2009), the physical characteristics of a frog, such as its shape and size, are influenced by its habitat and its feeding habits.

3.1.3 Family Ranidae (Rafinesque, 1814)

Green frogs and brown frogs are the two native frog species found in in the European region. Both specimens have elongated, slim bodies, a small waist, and generally smooth skin. Although they can swim well in the water, they move about on land by jumping (Hollman, 1998). Green frogs are often observed coexisting with other frogs in larger populations as a result of their frequent vocalizations. They are predominantly observed in the vicinity of aquatic environments. According to Nowakowski et al. (2010), urban environments can also be inhabited due to their higher resistance to anthropogenic alterations. Brown frogs exhibit a greater inclination towards terrestrial behaviour and are comparatively less vocal than green frogs. Additionally, they tend to inhabit moist environments (Arnold & Ovenden, 2004).

3.1.4 Genus *Pelophylax* (Fitzinger, 1843)

The green frogs, genus *Pelophylax,* also referred to as Palearctic water frogs, are divided into western (European) and eastern populations. Even though the western portion of the genus Pelophylax, which belongs to the Ranidae family, has undergone the most research, its evolutionary relationships have not yet been fully clarified (Lymberakis et al., 2007).

The physiological characteristics of the skin of green frogs make them susceptible to arid habitats and isolation resulting from unfavorable environmental factors such as oceans or deserts. A large majority of viable freshwater habitats are occupied by green frogs (Beerli et al., 1996) and distance to permanent waters should also be expected to be low (Rittenhouse & Semlitsch, 2007), since some species like within this genus are highly water dependent. Some species from this genus live in low altitude wetlands like rice fields (Hirai & Matsui, 2002), riparian zones, and vegetative filter strips alongside streams and lakes (Munoz-Carpena et al., 1993). The positive trend for habitat use within high wood volumes of Norway spruce also indicate a preference for pool frog (*P. lessonae*) movements through mature forests, similar to what has been shown for other amphibian species (Todd et al., 2009). The edible frog

(*Pelophylax esculentus*) have been shown to use hedgerows in agricultural landscapes to orient towards ponds (Mazerolle & Vos, 2006).

The well-known distribution of species today and their genetic diversity are a result of the Tertiary's complicated geologic history and the Quaternary's climatic cycles (Lymberakis et al., 2007). At the end of the Messinian crisis, which occurred between 5.5 and 5.3 million years ago, it is thought that the Mediterranean salinity basin was filled, isolating some populations of green frogs (Crete, Cyprus) from those on land (Anatolia, Peloponnese, Levant), as well as from those in Spain and North Africa. The isolation of the green frog populations can be understood as a coordinated process occurring over the entire Mediterranean basin after the Messinian crisis, when the Mediterranean rapidly flooded (Akn et al., 2010). The southern Mediterranean peninsulas—the Iberian, Apennine, and Balkan—were also important places of refuge during glacial periods, besides the one mostly described in central Europe (Hewitt, 2000; Taberlet et al., 1998; Stewart & Lister, 2001).

Being a genetically and phylogenetically complex group of vertebrates, they are important for biogeographic studies as well as the process of speciation (Plötner, 2010). The process of diversification that occurred during the Miocene epoch, approximately 15 million years ago, resulted in the emergence of three distinct lineages of the common green frog species within the European region. These are the *ridibunda/bedrigae* line, the *lessonae* line, and the *perezi* line (Lymberakis et al., 2007).

The Balkans are home to six species. The small green frog P. *lessonae* (Camarano, 1882) and the large green frog *P. kl. esculentus* (Linnaeus, 1758) are found throughout most of Europe, and the southern extent of their range extends into Bosnia and Herzegovina's northern region (Zimić & Ćurić, 2014; Lelo et al., 2015; Vrhovac et al., 2020), usually found present in ponds, springs and fishponds (Obratil, 1980/1981). The entire Balkan peninsula is home to marsh frog *P. ridibundus* (Pallas, 1771), while albanian green frog *P. shqipericus* (Hotz et al., 1987) is distributed in Albania, thought lowlands of the Adriatic coast of southern Montenegro and western Albania (Gunther, 2004; Plötner 2005; Vucić et al., 2018) with recorded data in Bosnia and Herzegovina (Vrhovac et al., 2020). *P. epeiroticus* (Schneider et al., 1984) occupies the western coast of Greece and the southern part of Albania (Schneider et al., 1984; Sofianidou & Schneider, 1989; Schneider & Haxhiu, 1992), while *P. kurtmuelleri* (Gayda, 1940) lives in Greece, the southern parts of neighbouring Balkan countries (Plötner, 2005; Speybroeck et al., 2016), as well as Bosnia and Herzegovina (Vrhovac et al., 2020).

3.1.4.1 *Pelophylax ridibundus* (Pallas, 1771) - the Marsh frog

The largest native frog in Europe is the green marsh frog, or *Pelophylax ridibundus*. With some specimens exceeding 17 cm, it can grow to a maximum body length of 15 cm (Džukić et al., 2003). It has a snout that is somewhat pointed, slightly to moderately warty skin texture. The body has black or dark patterns and is often green or olive green color. Males often have gray vocal sacs, and the tops of their thighs have marblings of grey, white, or light olive. There are dorsolateral folds and a dorsal stripe on occasion. Different sizes and colors of the same species are widespread because the environment favors variety (Speybroeck et al., 2016). The metatarsal tubercle on the foot is soft, slightly sloping, and small, measuring up to one-fourth the length of the great toe of the hind leg. They can be heard vocalizing throughout the summer in addition to the late spring mating season. Marshes inhabit sparsely vegetated water bodies with high dissolved oxygen levels, such as lakes, river backwaters, gravel pits, and canals. It frequently inhabits alluvial lowlands and exhibits greater flood tolerance (Pagano et al., 2001 a,b). In fishery ponds, gravel pits, and dead river arms, *Pelophylax ridibundu*s was the most prevalent and also obtained the highest dominance (Mulicek et al., 2014). It can be found at altitudes of up to 2,000 meters above sea level. It is territorial, go into hibernation in water, or it buries itself in the water bodies's muck. It can live up to eleven years at most (Arnold & Ovenden, 2002). The area extends from Kazakhstan in the east through all of Western, Central, and Eastern Europe. Figure 1. shows how the green pond frog is distributed geographically throughout BiH (Lelo et al., 2015; Lelo & Zimić, 2020; Vrhovac et al., 2020).

Figure 1. *Pelophylax ridibundus* and geographical distribution in Bosnia and Herzegovina. Retrieved from BHHU-ATRA (2023). Large green frog: *Pelophylax ridibundus*. <http://bhhuatra.com> Bosnian-Herzegovinian Herpetological Association Atra. Retrieved: <20/02/2023 >

3.1.4.2 *Pelophylax kurtmuelleri* (Gayda, 1940) – the Balkan frog

The Balkan green frog, *Pelophylax kurtmuelleri*, previously known as *Rana balcanica* (a junior synonym, Dubois & Ohler, 1994) is a semiaquatic species that is common in Greece and the southern parts of neighbouring Balkan countries (Sofianidou, 1997). There are genetic evidence for the occurrence of the Balkan water frog, *Pelophylax kurtmuelleri*, the Czech Republic, Switzerland, France, Italy, Ukraine, and some regions of Russia (Lanza 1962; Bellati et al., 2013; Laghi et al., 2013; Plötner et al., 2015; Dufresnes et al., 2017, 2018; Bisconti et al., 2019; Ivanov, 2019; Vershinin et al., 2019), despite the fact that the native range of the frog is restricted to the Balkan Peninsula (Figure x). Some authors have indicated the presence of alleles and/or haplotypes of the species in the Baltic Region in Latvia, Lithuania and Poland (Plötner et al., 2008; Hauswaldt et al., 2012; Kolenda et al., 2017). *P. kurtmuelleri* has been recorded for the first time in Bosnia and Herzegovina where it appears to be invasive based on its abundance, distribution (Figure 2.), and the formation of new hybrid zones with *P. ridibundus* (Vrhovac et al., 2020).

The taxonomic classification of *P. kurtmuelleri* remains a topic of debate and has not achieved universal acceptance. This taxon was accorded full species status based on phylogenetic inference based on DNA sequence data (Lymberakis et al., 2007; Pyron & Wiens, 2011; Plötner et al., 2012), cytogenetic variations, and centromeric hybridization pattern (Marracci et al., 2011), morphometrics (Gavrilović et al., 1999), bioacoustics (Schneider & Sinsch, 1992; Schneider et al., 1993; Lukanov et al., 2015) as well as hybridogenetic interactions (Hotz et al., 1985; Berger et al., 1994).

Certain authors assert that it represents a subspecies of *P. ridibundus* (Speybroeck et al., 2016; Jablonski, 2011). In the recent review of species list of the European herpetofauna updated by the Taxonomic Committee of the Societas Europaea Herpetologica (Speybroeck et al., 2020) *Pelophylax kurtmuelleri* has not been recognized at the species rank.

Based on the established ecological characteristics of the species, appropriate locations comprise of low-lying and elevated terrains, in close proximity to water bodies like rivers and streams, without regard to the specific habitat classification (Sacchi et al., 2011). It inhabits a variety of aquatic settings but mainly likes vast and open water basins. It can be found from 0 to 800 m above sea level in both its native and invasive range, however big populations do not occur over 600 m above sea level (Gasc et al., 1997; Sacchi et al., 2011). It lays its eggs in ecosystems with still or slow flowing water (Uzzell et al., 2009).

Although its population is now vast and steady and it has a Least Concern (LC) classification on the IUCN species list, it could be threatened by excessive commercial shooting (which is occurring in the Lake Skadar region) as well as pollution wetland habitats (Uzzell et al., 2009).

Figure 2. *Pelophylax kurtmuelleri* and geographical distribution in Bosnia and Herzegovina (Vrhovac et al., 2020)

The *P. kurtmuelleri,* also known as the southern *P. ridibundus,* presents a serious threat to the native water frogs of Western Europe when it is artificially moved by people (Quilodrán et al., 2015). This species has the potential to disrupt the hybrid genetic structures of *P. kl. esculentus*, which could result in the extinction of the local frog populations.

3.2 Factors affecting distribution patterns

Various effects on amphibian populations structure, density, and distribution patterns have been attributed to multiple factors, such as weather variables, for example temperature and precipitation (Enriquez-Urzelai et al., 2019; Ortiz-Yusty et al., 2013), climate change (Kim et al., 2021), topography (Kuzmin et al., 2004; Do et al., 2017; Song & Lee 2009; Yang et al., 2001, Liu et al., 2010), diseases (Pound et al., 2006; Pasmans et al., 2008; Picco & Collins 2008; Kolby et al., 2014), excessive exploitation and trade (Andreone et al., 2006; Warketin et al., 2009; Mohneke et al., 2010), contamination of the environment (Marques et al., 2013; Taiwo et al., 2014; Mahmood et al., 2016), land use (Findlay & Houlahan, 1997; Mikulcek & Pisut, 2012; Mikulicek et al., 2014; Wei et al., 2021;), and the introduction of non-native invasive species (Mikulcek & Pisut, 2012; Mikulicek et al., 2014).

Nearly half of all amphibian species are at risk of extinction, and about one-third of them are threatened with extinction globally (Blaustein et al., 1994; Collins & Storfer, 2003; Stuart et al., 2004; Beebee & Griffiths, 2005; Mendelson et al., 2006; McCallum, 2007).

3.2.1 Land use change, habitat fragmentation and loss

Anthropogenic disturbances can alter land use patterns that can have both direct and indirect impacts on population of small animals, especially amphibian populations (Wilbur, 1980; Green, 1997; Findlay & Houlahan, 1997; Lehtinen et al., 1999; Pope et al., 2000; Semlitsch, 2000; Welsh et al., 2002; Johnson et al., 2002).

Habitat degradation and fragmentation reduce the area available for the animals and reduce the overall diversity (Soulé, 1987; Blaustein et al., 1994; Fahrig, 2003; Collins & Storfer, 2003; Stuart et al., 2004), as well as the local population size (Shaffer, 1987) which may in turn reduce a species geographical distribution. Local population extinction can be another potential outcome (Dellis et al., 1996; Alford & Richards, 1999; Semlitsch & Boddie, 1998). Land conversions, for example, from forest to agricultural land can make forest-dwelling amphibians more vulnerable to climate change (Kafash et al., 2018). One of the factors that can cause habitat loss and degradation is urbanization (Hamer & McDonell, 2008). Research, that analyzed habitat fragmentation and habitat loss of pool frog (*Pelophylax lessonae*) habitats, either anthropogenic or natural, showed that it can affect landscape connectivity (Wikström et al., 2018).

Studies showed that agriculture has adverse effects on amphibian populations (Semlitsch, 2000). The way land is used and how extensively can have negative impacts on different species of frogs (Pellet et al., 2004; Aryal et al., 2020), especially due to local effect such as (e.g. road mortality, degraded roadside habitat) than to large-scale fragmentation effects (Marsh et al., 2017). For instance, in wetlands, agriculture can shorten the hydroperiod and increase sedimentation, which can have an influence on development time or larvae (Gray, 2002). The use of agricultural chemicals and pesticides has the potential to affect various ecological aspects, such as food web dynamics, community structure (Wilbur, 1997), the mobility and survival of amphibian larvae (Bridges, 1997; Bridges & Semlitsch, 2000; Relyea & Mills, 2001), and wetland productivity (Hanson et al., 1994).

Some amphibians may have a positive correlation with anthropogenic structures (Knutson et al., 1999; Kolozsvary & Swihart, 1999). For instance, some species like green toads, whose natural habitats are typically found in steppes and wild river food-plains, that have undergone degradation in various regions, are also widely recognized for their positive relation to occurrence in urban regions (Bogdan, 2014; Konowalik et al., 2020; Mazgajska & Mazgajska, 2020; Landler et al., 2023).

There are 37% of Europe's total amphibian species found on the Balkan peninsula, making it home to a large amount of amphibian diversity. According to Heatwole and Wilkinson (2015), this proportion is estimated to be over 30% in the countries of the Western Balkans. According to Heatwole & Wilkson (2015), habitat alteration, more notably its fragmentation, degradation, and destruction, has been linked to the loss of frog populations in Southern Europe and Turkey. This element has been acknowledged as a severe threat to amphibian populations in all Western Balkan nations.

The location and arrangement of suitable habitats for amphibians play a crucial role in determining how amphibian populations are organized in space. Understanding the reasons for the decline in amphibian populations requires analyzing how these habitats are arranged and how they interact together. Through the analysis of these factors, we can gain insight into how to protect and conserve amphibian populations in the future (Beebee & Griffiths, 2005). Knowing species distribution and habitat preferences can enable impact assessment studies of land use on the occurrence of specific species. This can facilitate the identification of strategies to enhance landscape management and amphibian preservation (Pellet et al., 2004).

3.2.2 Hybridogenetic complexes

Hybridogenesis is a reproductive process in which a hybrid offspring, possessing fertility, is produced through the mating of a hybrid with a parent species (Schultz, 1969). It is a significant contributor to diversity, especially adaptive diversity, and it sometimes results in the formation of entirely new species. In some cases, genetic barriers may be too robust, resulting in incompatible genomes. This can lead to the production of unviable hybrids that can occur due to the exclusion of one parental genome, which can prevent recombination or normal gametogenesis with recombination (Quilodrán et al., 2015). As a result, the parental lineages will maintain their genetic integrity, diverge further, and eventually recognize and avoid unsuitable sexual partners in cases where hybrids are not well-adapted to habitat conditions. In the event that hybrids are capable of acclimating to a given environment and producing progeny that are reproductively isolated from the parent species, a novel hybrid-derived species is generated (Christiansen & Reyer, 2009).

Two different hypotheses—the generalistic heterosis hypothesis and the habitat segregation hypothesis—are used to explain the success and evolutionary destiny of hybridogenetic lineages, which are offspring produced by breeding two genetically distinct populations or species.

According to the generalistic heterosis theory, the combination of various advantageous characteristics from each parent, such as improved disease resistance, increased genetic diversity (Simon et al., 2003) and improved physiological adaptations, results in hybrids being more fit than their purebred parents. As a result, hybridogenetic lineages may have better odds of surviving and reproducing than their purebred predecessors, which may account for their successful evolution (Baker, 1965; Schultz, 1971,1977; Lynch, 1984; Van Doninck et al., 2002).

The habitat segregation theory, on the other hand, contends that hybridogenetic lineages can coexist with their parental species by filling various niches within their common habitat (Moore, 1984; Case & Taper, 1986). With less rivalry for resources as a result of the habitat division, the hybrids can flourish in their own ecological niche (Vrijenhoek, 1978, 1979; Case & Taper, 1986).

In a study by Plenet et al. in 2005, it was found that the performance of the hybrid species *Rana esculenta* and its sexual host was found to be dependent on the occurring environmental conditions, and as a hybrid did not demonstrate a general capacity for adaptation across different environments.

These findings suggest that the success of hybrid species cannot be explained by the generalistic model of higher fitness due to genetic diversity. Instead, the results supported the habitat segregation hypothesis and other alternative concepts, which propose that hybrid species can coexist with their parental species by occupying different ecological niches within their shared habitat, thereby reducing competition and increasing their chances of survival and reproduction.

Both genetic and ecological mechanisms, particularly the generalistic heterosis hypothesis and the habitat segregation hypothesis, can be used to explain the evolutionary success and destiny of hybridogenetic lineages. These hypotheses emphasise the various elements that can affect the persistence and environmental adaptation of hybrid species.

3.2.2.1 Hybridogenesis of *Pelophylax spp.*

Pelophylax, a genus of amphibians found across Eurasia between the Atlantic and Pacific coasts, exhibits multiple hybrid forms that are produced through a process known as hybridogenesis. This involves the clonal transmission of one parental genome from generation to generation, while the other is discarded during meiosis (Tuner, 1974; Uzzell & Berger, 1975, Uzzell et al., 1977; Graf et al., 1977; Graf & Müller, 1979). All known hybridogenetic water frogs have one parental genome originating from the marsh frog *Pelophylax fortis* (previously referred to as *Pelophylax ridibundus* in earlier literature, but this is now recognized as an unrelated eastern species (Plötner & Ohst, 2001; Plötner, 2005). The other parental genome is either from the pool frog *Pelophylax lessonae* (Berger, 1968; Uzzell & Hotz, 1979) or the Iberian frog *Pelophylax perezi* (Graf et al., 1977; Graf & Pelaz, 1989). In the conventional sense, solely the nominal European form *P. f. fortis* is deemed to elicit genome elimination, whereas other species pairs that were examined do not exhibit such an effect (Hotz et al., 1985).

Pelophylax hybrid forms can persist independently and evolve into distinct evolutionary taxa if they possess environmental stability and competitive abilities against the parental species. This can occur once they have developed ecological differentiation and reproductive isolation. Polyploids already exhibit increased resistance to the low temperatures and distinctive physical traits in northern regions (Pruvost et al., 2013b; Kierzkowski et al., 2011). This was the case with *Pelophylax*'s most extensively documented hybridogenetic system is the edible frog (*P. esculentus,* genomes LF), which results from the hybridization of the European marsh frog *P. fortis* (previously referred to as *P. ridibundus* in literature) (genomes FF) and the European pool frog *P. lessonae lessonae* (genomes LL) (Dedukh et al., 2020).

The occurrence of hybrids can depend on the preferences of parental species for alternative habitats, but can also be influenced by the hybridogenetic system and the reproductive dependence of hybridogens on their host species (Mikulicek et al., 2014). The composition of water frog populations was strongly influenced by taxon-specific habitat preferences. While *P. ridibundus* was frequent and dominant in larger, less vegetated water bodies, like fishery ponds, gravelpits and dead river arms, *P. lessonae* showed preferences for smaller water bodies, like marshes and sandpits, where, however, its dominance was always lower in comparison to the other two forms. *P. esculentus* occupied similar habitats as *P. lessonae* (marshes, sandpits, dead river arms), but were occasionally found also in fishery ponds and gravelpits. Accordingly, these results were concordant with previous studies and highlighted preferences of parental species for alternative habitats as well as hybrid showing high ecological valency. The occupation of a broader spectrum of ecological niches by hybrid individuals, particularly if they maintain a greater degree of genetic diversity and adaptability through asexual or clonal reproduction, can potentially result in a competitive advantage (Gunther 1974; Rybacki & Berger 1994; Pagano et al. 2001a; Holenweg Peter et al. 2002; Plenet et al. 2005; Hotz et al. 2008). However, the occurrence of *P. esculentus* in the same habitats as *P. lessonae* might not reflect only the habitat preferences of the hybrids, but might be linked to the hybridogenetic system and reproductive dependence of hybridogens to their host species (Schenck & Vrijenhoek, 1989; Bergen et al., 1998; Vrijenhoek & Pfeiler, 1997; Plénet et al., 2000; Negovetic et al., 2001; Doeringsfeld et al., 2004). The coexistence of hybridogenetic lineages and parental species and/or their occupation of intermediate positions on ecological gradients can be explained with habitat segregation, frozen niche variation, and/or intermediate niche theories (Thibault, 1974; Moore, 1984; Vrijenhoek, 1979,1984; Case & Taper, 1986).

Various lineages of *Pelophylax* have been introduced beyond their indigenous habitats throughout Europe (Dufresnes & Dubey, 2020). Hybrids are known to possess superior fitness compared to their parents, because of their somatic heterozygosity (heterosis), particularly in hypoxic environments (Hotz et al., 1999).

3.3 Land use types in Bosnia and Herzegovina

Bosnia and Herzegovina is a country located in southeastern Europe, covering an area of 51 129 km2. BiH is made of two autonomous political entities of comparable size, namely the Federation of Bosnia and Herzegovina (FB&H) and the Republic of Srpska (RS), with an additional unit known as Brčko District that is administered by the local government.

The first study of land use coverage was done within the Corine Land Cover (CLC) project by the Geodetic Institute of Bosnia and Herzegovina. Three other analyzes of CLC change for 2006, 2012 and 2018 made on Faculty of Agricultural Food, University of Sarajevo (Čustović et al., 2006; 2012; 2018).

As per the Corine Land Cover (CLC) database from 2018, the predominant land cover category is that of forest vegetation and other natural surfaces, accounting for 64.4% of the total land area. The second most prevalent category is that of agricultural surfaces, comprising 33.1% of the land cover. The remaining portion of 2.4% is allocated to three categories, namely artificial surfaces (1.7%), water surfaces (0.7%), and wet areas category, specifically swamps (0.1%) (Čustović et al., 2020). The geographical area of Bosnia and Herzegovina comprises of moderate wetlands, which are distinguished by an average annual precipitation rate of around 1,250 mm (Crnobrnja-Isailović et al., 2022). Between 2000 to 2018, there was a net gain of 48 hectares in the wetland classification. There was an 853-hectare increase observed in the category of water bodies. The primary changes that were noted involved the conversion of forested regions into transitional woodland or shrubbery, which spanned a combined area of 23,882 hectares (Ljuša et al., 2021). In the Republic of Srpska, the CLC 2018 database indicates that the largest land coverage on the first level of classification is comprised of forest and semi-natural areas, accounting for 61.25%. Agricultural areas follow with 36.70%, while artificial surfaces represent a mere 1.3%. As second, the most prevalent classifications comprise of forest vegetation, accounting for 47.25%, and arable land, encompassing 26.91%. In relation to the third tier of categorization, the dominant vegetation in the area is that of broad-leaved forests, which accounts for 34.61% of the total coverage. Additionally, there are complex cultivation patterns covering 17.47% of the area, agricultural regions with significant natural vegetation covering 9.45%, mixed forests covering 7.58%, pastures covering 5.47%, coniferous forest vegetation covering 5.06%, sclerophyllous vegetation covering 4.92%, transitional woodland-shrub covering 4.19%, non-irrigated arable land covering 4.14%, and natural grasslands covering 3.24%. One of the surface covers that had a representation below 1% was the discontinuous urban area, which covered 0.97% (Drašković et al., 2020).

4 Methodology

Data on the occurrence of green frogs were collected during fieldwork in 2018 and 2019 from 18 localities in Bosnia and Herzegovina, as obtained from Vrhovac et., al 2020. Data samples from 117 green frog individuals have been selected using molecular identification (Vrhovac et. al, 2020) and three taxons have been chosen for further analysis:

- ***P. kurtmuelleri* with 43 samples across 13 locations,**
- ***P. ridibundus* with 34 samples across 7 locations and**
- ***their hybrid P. ridibundus x P.kurtmuelleri* with 43 samples across 13 locations.**

Data was organised in Excel and further analysed using ArcGIS software and statistical software R. Land-cover data for Bosnia and Herzegovina was taken from Corine Land Cover (CLC) 2018, Version 2020_20u1.

4.1 Study area and sampling data

From 2018 to 2019, between April and July, a collection of *Pelophylax spp*. individuals was conducted with authorization from the Federal Ministry of Environment and Tourism (Federation of B&H), as well as Ministry for the Spatial planning, the construction, and ecology of the Republika Srpska. GPS coordinates and DNA samples were gathered with a total of 127 individuals of green frogs from 18 locations (collection points) on territory of Bosnia and Herzegovina (Figure 3).

During the time of the fieldworks *Pelophylax spp.* individuals were captured by hand using medical gloves and "Meredov" net. A small part (5mm) of the toe above its base was removed, and samples were stored in tubes with 96% ethanol.

Figure 3. Habitat examples of collection data points. Fatničko polje (left side) and Dabarsko polje (right side).

4.2 Species identification

We tested the presence of *Pelophylax* taxa in whole B&H and SE Montenegro based on molecular analyses. Details on DNA identification can be found in Vrhovac et al., (2020). Out of 127 individuals of green frogs sampled across Bosnia and Herzegovina, 117 individuals were identified, using molecular analysis as *P. ridibundus*, *P. kurtmuelleri* and hybrid of *P. ridibundus x kurtmuelleri*. These selected *Pelophylax* taxons were used for further data analysis in this thesis to provide an ecological insight into these species and their hybrid and understand better how these complex hybridogenetic complexes cohabit in their examined land-use preferences (Figure 4; Appendix A1).

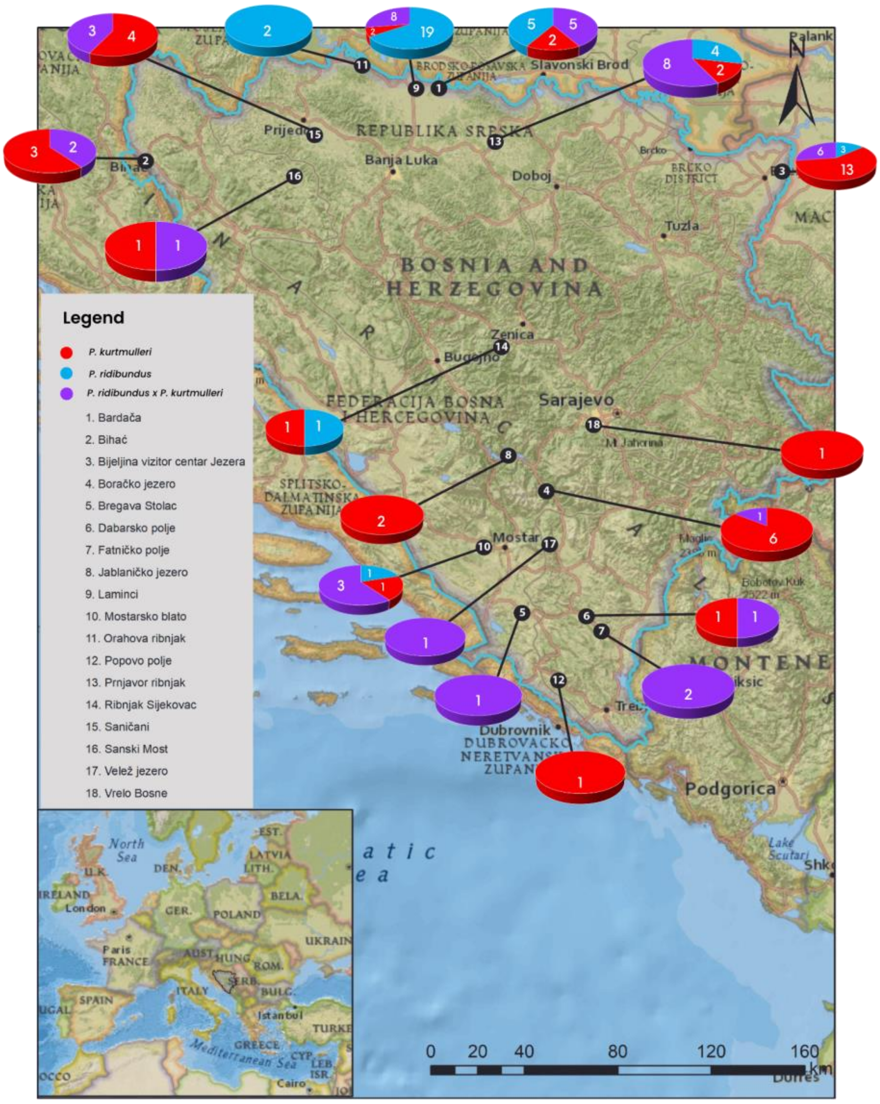

Figure 4 . Overview of chosen species, *P . ridibundus*, *P. kurtmulleri* and hybrid according to the molecular data (Vrhovac et al., 2020). Numbers on the map correspond to the localities according to the legend on the left side of the figure. The pie chart numbers represent the species' and hybrid' representations on the localities (data collection points) according to the legend in different colours.

4.3 Data analysis

4.3.1 ArcGIS

All the collected data was entered into Microsoft Excel. The data contains GPS coordinates with species and hybrids identified according to Vrhovac et al., 2020. Land-cover data for Bosnia and Herzegovina has been taken from Corine Land Cover (CLC) 2018, Version 2020_20u1.

For the purpose of identifying the preferred land use type of *P. kurtmuelleri, P. ridibundus* and their hybrids, the available data of sample GPS coordinates was analysed by ArcGIS software, where the database and 18 cartographic views were created. By creating a database and cartographic maps, new quantity data has been obtained.

To assess the habitat preferences of our target species and hybrid, we extracted 18 different locations (data points) where each taxon was found. The sum of the land-use types surrounding each data-point was extracted within a radius (buffer zone) of 500 m. As part of the buffer zone, from each data point, the Corina Land Cover was analysed with its land use categorization according to CLC label 3. Level-3 classes are required to be used with the European CLC datasets. On level 3, there are a total of 44 classes. The classes are structured with a focus on achieving visual interpretability, and their recognition frequently necessitates the human ability to abstract and integrate information. The process of identifying and evaluating objects captured in aerial or satellite images is commonly referred to as visual interpretation. The aforementioned approach was utilized in the establishment of the CLC database. The analysis is derived from the interpretation elements of the recorded landscape object. As a result, it is difficult to characterise them in a machine-readable manner. The classes obtained in the findings were described in accordance with the most recent version of the Nomenclature Guidelines (Kosztra et al., 2019).

The CLC types of each data point were rasterized and presented in a grid network utilizing 25 m2 tiles. This display format presents all individual CLC types with their respective percentage share, allowing the analysis of the examined area and the identification of each existing CLC type in the area. The analysis will also produce three maps of kernel density for each species and hybrid. These maps represent the density calculation around specific cells, allowing a better understanding of the distribution of the studied species and hybrids. Kernel density is a method used to estimate the probability density function of a set of data points. It helps to get a better understanding of the distribution of the data by providing an accurate representation of the overall density of the species and hybrids, providing valuable insights for future research.

4.3.2 R Software

A generalized linear model (GLM) was used to analyze land use effects on the occurrence of *P. kurtmuelleri, P. ridibundus* and their hybrid occurrences. These models helped in identifying the preferred habitats of *P. kurtmuelleri, P. ridibundus* and their hybrids, as well as predicting the occurrence of chosen taxon’s based on land use type data for Bosnia and Herzegovina.

The aim of the first analysis was to find out whether there is a statistically significant variation in the overall amount of land use types across selected taxonomic groups. The purpose was to obtain findings that would indicate whether certain taxon exhibit a greater preference for a higher number of Corine Land Cover types compared to others. As part of the analysis of the generalized linear model (GLM) a response variable (also known as as apendent variable) was established as sum of the land use types and explanatory variables (also known as independent variables or predictors) which were chosen taxon. In the sum of the land use types, CLC type categorized as water body was not used, as green frogs are highly water dependent and were found all in close proximity to or in water bodies. These data were Poisson distributed, as it is particularly well suited for modelling when the response variable is a count data, in this case the sum of land use types. For this GLM models, analysis of deviance based on likelihood ratio Chi-square tests was used to assess the significance using the ANOVA function. Chi-square test was used to get results for degrees of freedom (DF) and p value, that said the if overdispersion occurs or not.

Furthermore, the second analysis investigated the correlation between the overall number of individuals within each taxonomic group (subset of taxon) and the quantity of land use types. Generalized linear model (GLM) with Poisson distribution was used as well where a response was established as sum of the land use types and explanatory variables which was number of individuals within each taxonomic group. Quasi-Poisson regression has been used in this analysis as a variant of Poisson regression to model overdispersed count data and as output of the ANOVA function included the F-statistic, F value and p-value for the overall significance of the model.

In addition, ANOSIM function was used to test whether there is a significant difference among chosen taxons based on composition, the percentage share, of CLC types found in buffer zones of sample locations. This can be considered as a qualitative analysis as we tested composition of CLC types, where the aforementioned analysis can be considered as quantative since the sum of land use types were used. Distance matrix with Bray Curtise was specified amongst all types of CLC, based on total 117 indivudual sample values of chosen taxons. The distance was then tested using 9999 permutations, also using the Bray Curtis distance, after what we got the ANOSIM statistic R value and the significance value of p. Following same functions we tested if soil composition differ among localities to get ANOSIM statistic R value and the significance value of p.

The next step following ANOSIM was to apply NMDS analysis to visualize the data. Data was specified by % of CLC types found on researched area among taxons. CLC types names are used with abbreviations: w-water bodies, wc-water courses, ccp-complex cultivation patterns, pastures, blf-broad leaved forest, agr-land principally occupied by agriculture with significant areas of natural vegetation, duf-discontinuous urban fabric, tws-transitional woodland shrub, nial-non irrigated arable land, sv-sclerophyllous vegetation, im-inland marshes, mf-mixed forest, ng-natural grasslands. The analysis was to test if % of CLC types differ between taxons. CLC types that had 0% for some location and taxons, were replaced by lowest value (0.001) that can be registered for getting the statistical analysis. Distance matrix with Bray Curtise was used and to get best solution of stress value, stress function was optimized 100 times ("try"=100). The stress value can give us answer if distance between each CLC types in their percentage and each individual share good enough interpretation of data.

5 Results

The initial result consisted of obtaining maps that display the Corine Land Cover (CLC) categories on predetermined land use types, accompanied by the identification of species and hybrids at the corresponding data points. Additionally, a database was established during this process. The database encompasses the total number of Corine Land Cover (CLC) types located within a 500-meter buffer zone, alongside the proportionate distribution of each CLC type within the total area. During the R analysis, qualitative and quantitative results were obtained by utilizing data on land use types and the occurrence of *P. kurtmuelleri, P. ridibundus*, and their hybrid. The statistical analysis allowed us to comprehend the variations that clarified the taxons preferences towards particular land use types and their corresponding distribution patterns.

5.1 Land use types of locations

We produced a total of 18 maps obtaining CLC types each in a buffer zone of 500 m. To calculate the surface area of the buffer zone with a radius of 500 metres, we used the formula for the area of a circle: A = pi * r^2; constant pi=3.14; r is the radius of the circle 0.5 km. Therefore, the surface area of the buffer zone is 0.785 km2 and referred to as the “researched area” for each location. The total research area of 18 locations is 14.13 km2 (=0.785*18) On each map there are from two to four CLC types surrounding the data collection point. They are presented in percentage share in the buffer zone for each location. (Figure 5; Appendix B1-B8: the rest of CLC % maps of the researched area).

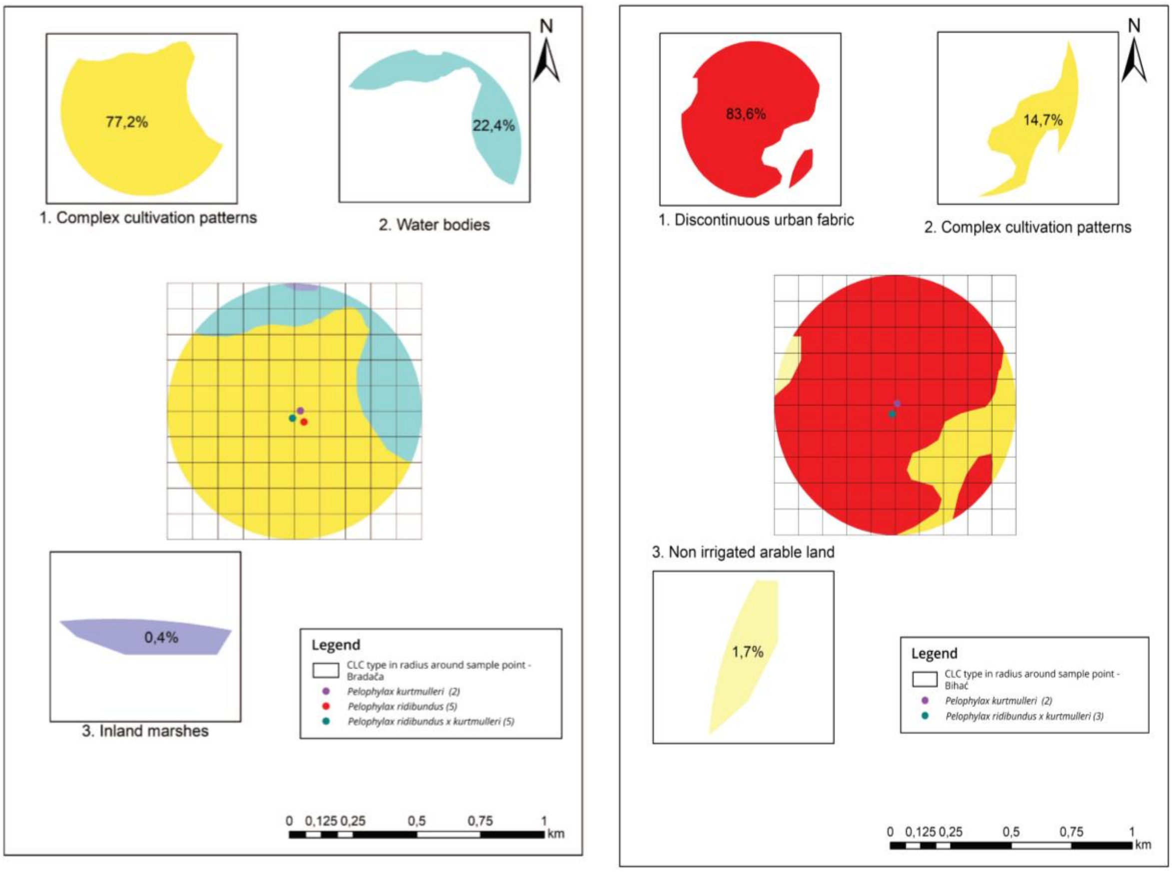

Figure 5. Example overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Bardača (left) and Bihać (right).

Total amount of CLC types found across 18 locations are 13 presented on Graph. X with their respective precentages: water bodies (16.80%), complex cultivation patterns (17.69%), pastures (14.19%), broad leaved forest (13.31%), land principally occupied by agriculture with significant areas of natural vegetation (9.51%), discontinuous urban fabric (8.13%), transitional woodland shrub (6.09%), non irrigated arable land (4.47%), sclerophyllous vegetation (3.67%), water courses (2.5 %), inland marshes (1.94%),mixed forest (1.63%), natural grasslands covers (0.10%).

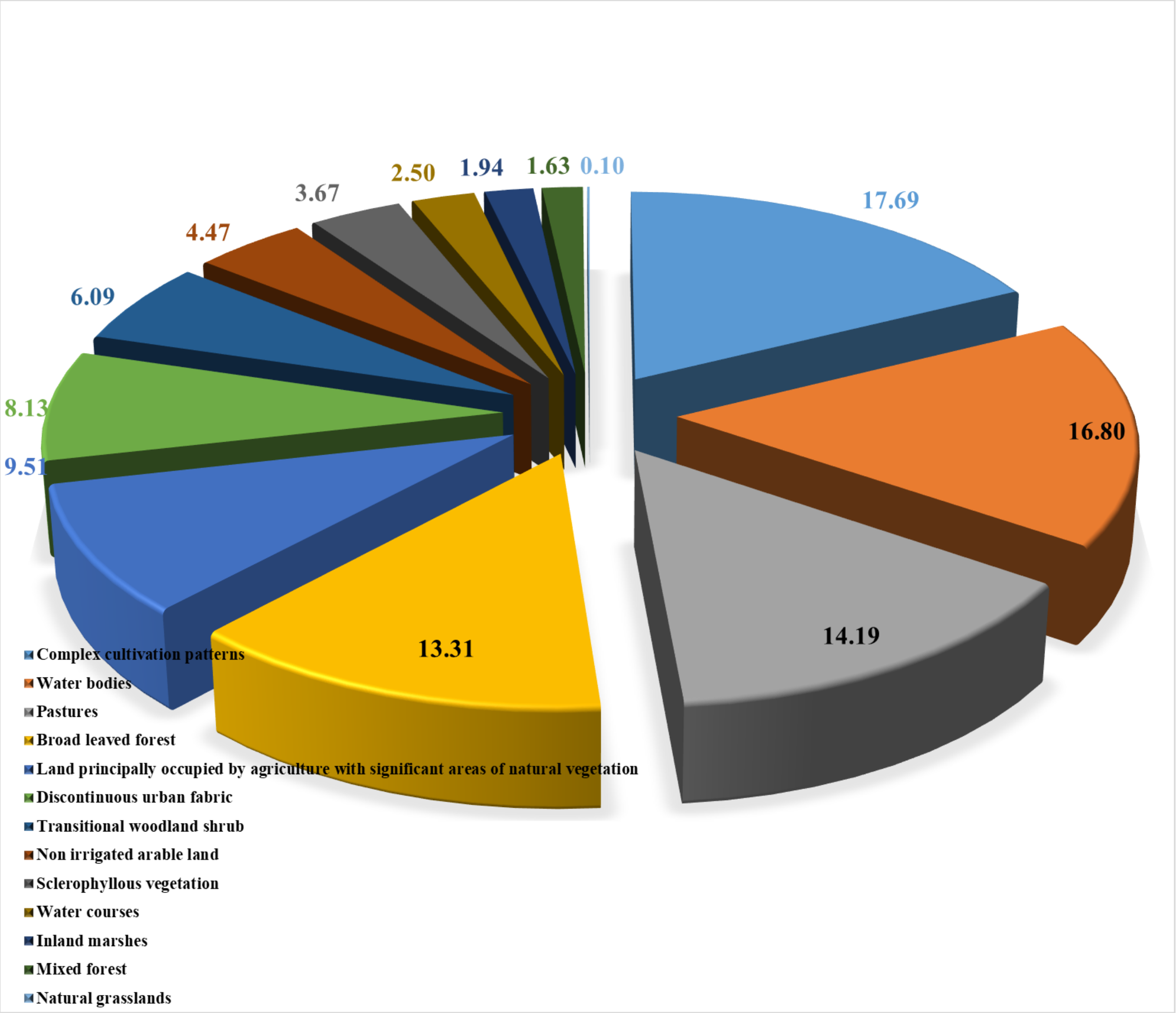

Figure 6. CLC types in % for all locations (the researched area)

The data presented in Figure 6 suggests that the CLC type characterized as "complex cultivation patterns" holds the highest prevalence across all research areas, with a percentage of 17.69%. The CLC type "water bodies" accounts for 16.80% of the total, ranking second in percentage. "Pastures" and "broad leaved forest" follow closely with 14.19% and 13.31%, respectively. The category of "natural grasslands" exhibits the smallest proportion of Corine Land Cover (CLCs), amounting to 0.10%.

5.2 Land-use preferences and distribution

5.2.1 ArcGis

The study investigated the land use-preference and distribution of three taxons, namely *P. ridibundus*, *P. kurtmulleri*, and their hybrid, within the researched area (locations where each species and hybrid were registered) (Figure 7).

The total research area for *P. ridibundus* was 5.495 km², and 8 types of Corine Land Cover (CLC) were identified. The most prevalent types were complex cultivation patterns (32.81%) and water courses (23.09%), followed by non-irrigated arable land (11.27%) and pastures (10.86%). Other CLC types identified were land principally occupied by agriculture with significant areas of natural vegetation (7.48%), broad leaved forest (7.44%), discontinuous urban fabric (2.1%), non irrigated arable land (11.27%) and inland marshes (4.98%).

For *P. kurtmulleri*, the total research area was 10.99 km², and 12 CLC types were identified. The most prevalent CLC type was water bodies (24.27%), followed by complex cultivation patterns (18.46%), and land principally occupied by agriculture with significant areas of natural vegetation (11.54%). The rest of CLC types were pastures (10.56%), discontinuous urban fabric (9.4%), broad leaved forest (8.73%), transitional woodland shrub (6.05%), non irrigated arable land (5.75%), inland marshes (2.49%), mixed forest (2.09%), water courses (0.55%), natural grasslands (0.13%).

The total research area for hybrid was 10.205 km², and 12 types of CLC were identified. The most frequently found CLC type were pastures (19.33%), followed by water bodies (19.07%) and complex cultivation patterns (17.45%). The remaining types of CLC found were broad leaved forest (13.76%), discontinuous urban fabric (10.12%), land principally occupied by agriculture with significant areas of natural vegetation (6.17%), sclerophyllous vegetation (5.08%), non- transitional woodland shrub (3.57 %), irrigated arable land (3.39%), inland marshes (1.36%), water courses (0.59%), natural grasslands (0.14%).

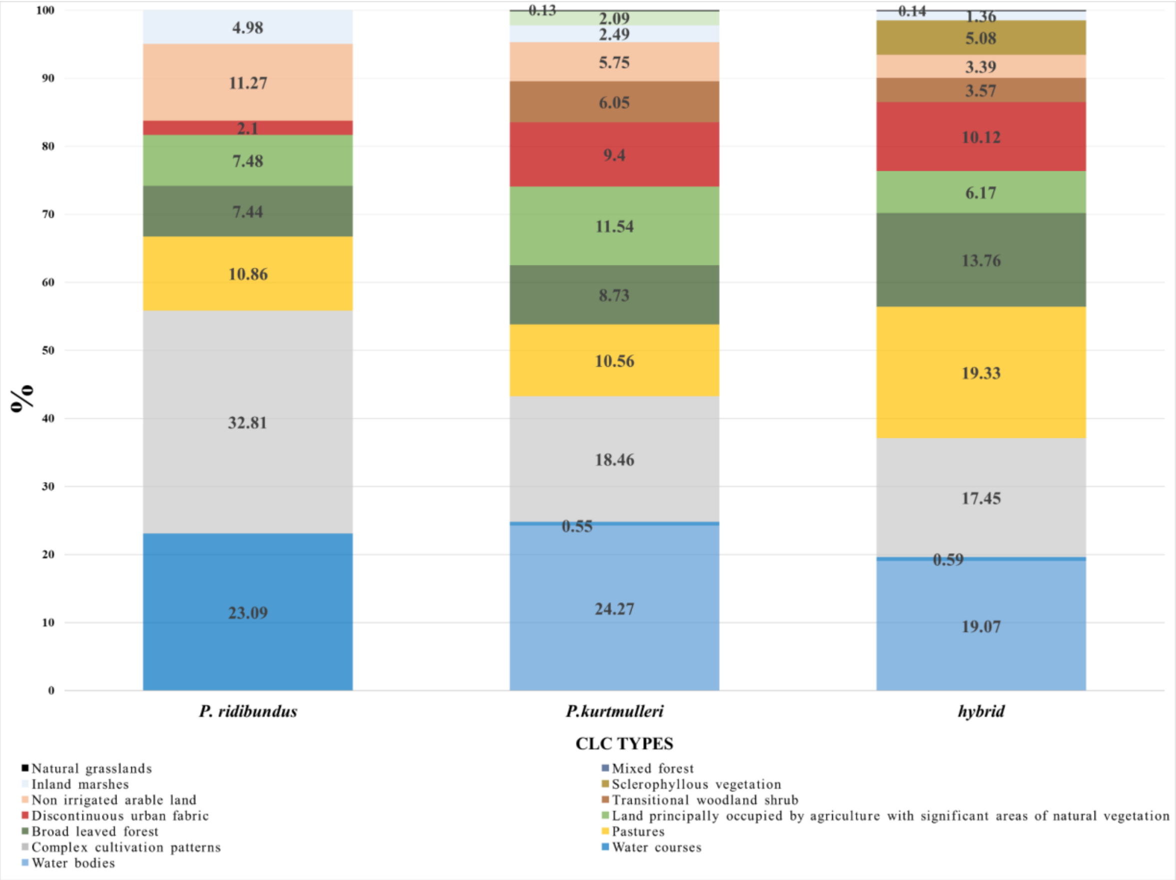

Figure 7. Overview of CLC types (%) found in buffer zone of each location for *P. ridibundus*, *P. kurtmulleri* and hybrid

From the Figure 7, we can see that most of the CLC types identified were found for all three taxons, but differ in percentage shares. Water bodies and water courses are present, as expected since all green frogs are water dependent. Complex cultivation patterns had a significant percentage share for *P. ridibundus* (32.81%), followed by *P. kurtmulleri* (18.46%) and the hybrid (17.45%). Hybrid has the highest percentage (19.33%) for the pastures and broad leaved forest (13.76%), while *P. kurtmulleri* and *P. ridibundus* have relatively similar lower share of these CLC types compared to hybrid. Land principally occupied by agriculture with significant areas of natural vegetation has *P. kurtmulleri* with the highest percentage (11.54%) followed by *P. ridibundus* (7.48%) and the hybrid (6.17%). Similar shares of discountinous urban fabric was found for hybrid and *P. kurtmulleri*, while *P. ridibundus* had the lowest share (2.1%) of this CLC type within its own researched area. *P. ridibundus* wasn't found on some CLC types, such as transitional woodland shrub, sclerophyllous vegetation, mixed forest, or natural grassland, and it was only present in trace amounts or wasn't found at all for other CLC types, such as *P. kurtmulleri* on sclerophyllous vegetation and hybrid on mixed forest.

The output of kernel density analysis is a density map (Figure 8) that represents the density of the probability distribution around each observation point, with values ranging from low to high density for each taxon.

The maximum surface value is observed at the specific location of the species or hybrid, and diminishes proportionally with increasing distance from that point. The surface value ultimately becomes zero at the distance equivalent to the search radius from the aforementioned point.

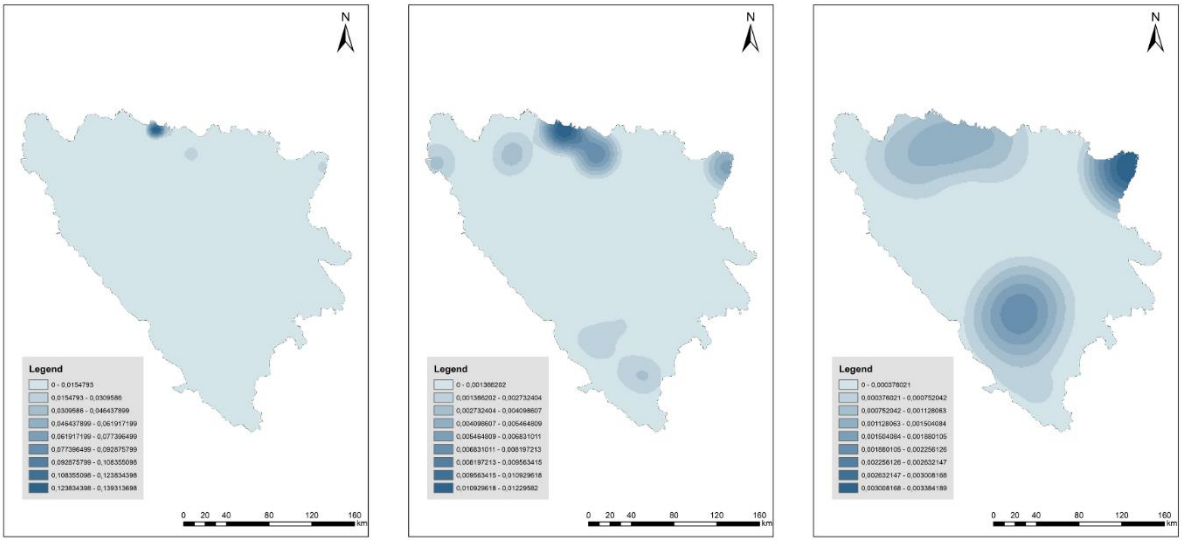

Figure 8. Kernel density map for *P. ridibundus*, hybrid & *P. kurtmuelleri*

5.2.2 R Software analysis

Based on the initial analysis, it was determined that there was no overdispersion ($p>0.05$; DF for $\chi 2=2$, $p=0.99$). This suggests that there was no significant variation in the number of land use types used among the taxa. Thus, it can be concluded that there was no statistically significant correlation observed between the number of land types and taxa. Subsequent analysis was conducted to examine whether the quantity of individuals within each taxonomic group can be accounted for by the number of land types, indicating a higher number of individuals on a greater number of land types. Following the identification of overdispersion of data through the Poisson distribution, we proceeded to specify quasipoisson and conducted a F test. The results obtained for each taxon were as follows: *P. kurtmulleri* ($p>0.05$; $F=0.3267$, $p=0.5782$), *P. ridibundus* ($p>0.05$; $F=2.2017$, $p=0.198$), hybrid ($p>0.05$; $F=0.0572$, $p=0.8153$), and for all taxons ($p>0.05$; $F=1.9$, $p=0.17$). The findings suggest that there is an equal distribution of individuals across different land use types, either with the same number of individuals per land use type or an equal number of individuals across all land types. The results indicate that the Quasi-Poisson regression model may offer some explanatory capacity, but the statistical significance of the model is insufficient to draw conclusive inferences regarding the association between the predictor variables and the response variable. Insufficient taxon samples have resulted in a limited number of observations.

The next analysis resulted in findings indicating the presence of a significant difference among selected taxonomic groups based on their composition or the percentage share of Corine Land Cover (CLC) types observed in buffer zones of the sample locations. The ANOSIM statistic R was calculated to be 0.06713 with a significance level (p) of 0.0013. The ANOSIM test generates a R value that ranges from -1 to 1. Higher values of R indicate greater dissimilarity between groups, while values closer to 0 indicate greater similarity. The results indicate a minimal distinction among the selected taxonomic groups, as determined by the CLC type composition observed in the buffer zones of the sampled sites. Following these results, next analysis was to determine if or how significantly % of CLC types differ amongst locations. Results showed that the composition of CLC types from buffer zone differ among 18 locations (permutation=9999; ANOSIM statistic $R=1$, $R>0$; significance value $p=0.0001$).

To visualize the similarity or dissimilarity between % of CLC types and individuals amongst selected taxons, using NMDS analysis we got two dimensions for interpretation and the stress value of 0.14. The stress value is a measure of how well the NMDS plot represents the original dissimilarity matrix. It ranges from 0 to 1, with values closer to 0 indicating a better fit. The stress value of 0.14 suggests a reasonable fit between the NMDS plot and the original dissimilarity matrix (Figure 9).

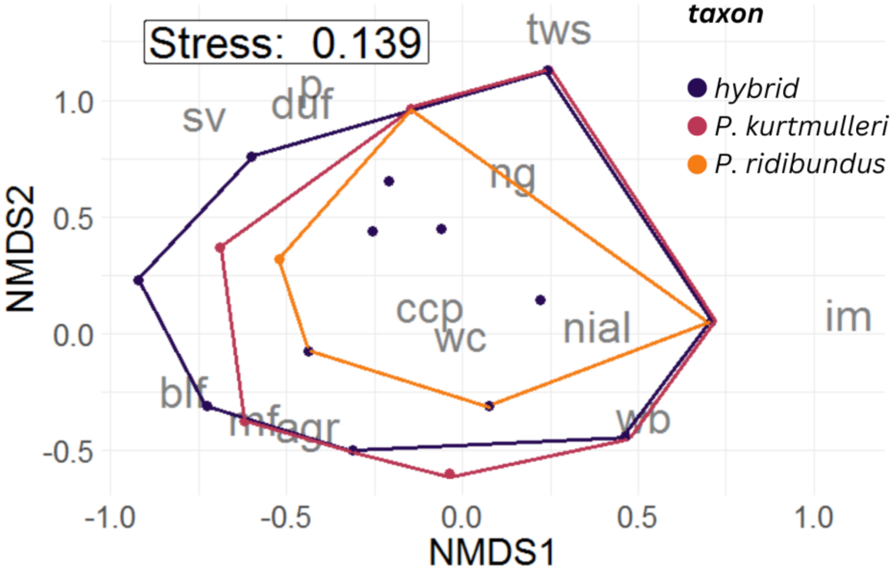

Figure 9. Convex hulls visually representing polygons that exist between sample locations. These polygons show the proportional use of each CLC type by *P. ridibundus*, *P. kurtmulleri*, and their hybrid.

The findings of the Non-metric Multidimensional Scaling (NMDS) analysis, as illustrated in Figure 8., suggest that the hybrid land use type demonstrates the most extensive coverage of the Convex Hull, encompassing a significant proportion of the Corine Land Cover (CLC) types. The aforementioned observation implies that the hybrid land use category is prevalent across a majority of the Corine Land Cover (CLC) types and is likely to manifest more frequently in specific land use types such as p-pastures, duf-discontinuous urban fabric, sv-sclerophyllous vegetation, and blf-broad-leaved forest. The findings indicate that P. kurtmulleri displayed a comparable trend to the hybrid land use type with regards to its occurrence across various CLC types. Specifically, it was observed to be present in CLC types such as mixed forest (mf), land primarily utilized for agriculture with substantial portions of natural vegetation (agr), and water bodies (wb). Conversely, *P. ridibundus* was predominantly associated with CLC types such as complex cultivation patterns (ccp), water courses (wc), non-irrigated arable land (nial), and natural grasslands (ng). On the other hand, P. ridibundus exhibited the least Convex Hull coverage.

6 Discussion

6.1 Land use types of researched area

The analysis of Corina Land Cover involved the categorization of land use based on the CLC label 3. The process of identifying and evaluating objects captured in aerial or satellite images for the analysis of CLC in Bosnia and Herzegovina requires the human ability to abstract and integrate information, as classes are structured with a focus on achieving visual interpretability. However, it is important to note that there may be certain details at each habitat that are not observed during this process (Čustović et al., 2006, 2012, 2018). As a result, the CLC types obtained for certain locations, which were the subject of study, exhibit some variations in comparison to the findings obtained through fieldwork. Water bodies or courses were absent in specific locations of sample collection within the 500 m buffer zone, despite all samples being taken in close proximity to or within water bodies. The green frog species exhibit a dependence on water and requires access to aquatic environments for the purpose of reproduction. This is evidenced by their frequent vocalizations and their tendency to coexist with larger populations of other frog species (Beerli et al., 1996; Rittenhouse & Semlitsch, 2007; Nowakowski et al., 2010). It is important to consider that the Corine Land Cover (CLC) measure may not provide a comprehensive representation of land use, and the absence of certain details during visual interpretation may impact the accurate percentage distribution of CLC types obtained in the findings. The CLC types, namely water bodies type (16.80%), water courses (2.5%), and inland marshes (1.94%) (Figure X's pie chart), may potentially exhibit a greater percentage share. This is due to the fact that every location where a sample of green frogs was recorded, water body or some wet area classification would be registered as a CLC type. Consequently, the proportion of the buffer zone occupied by other types of CLC would also be affected. For instance, in the Appendices, registered locations for collected samples at Fatničko polje and Dabarsko polje indicate the presence of four distinct CLC types. However, none of these types corresponds to water bodies, water courses, inland marshes, or other wet area classifications. In different areas of the researched area in FBiH and RS of Bosnia and Herzegovina, other CLC types were recorded. Complex cultivation patterns (17.69%), pastures (14.19%), and land principally occupied by agriculture with significant areas of natural vegetation (9.51%) were those that were found in larger percentages. According to data for RS in BiH, complex cultivation patterns occupy 17.47% of the area, pastures occupy 5.47%, and agricultural regions with substantial natural vegetation occupy 9.45% (Drašković et al., 2020). Approximately more than 30% of the land in BiH is used for agriculture (Čustović et al., 2020; Drašković et al., 2020). The findings indicate that the discontinuous urban fabric was observed in 8.13% of the researched areas. Specifically, the data for Bosnia and Herzegovina found that artificial surfaces accounted for 1.7%, as reported by Čustović et al. (2020), while in the Republika Srpska region, the proportion was less than 1%, according to Drašković et al. (2020).

According to Ljuša et al. (2021), the presence of transitional woodland shrub (6.09%) in forested regions can be attributed to the process of conversion into transitional woodland or shrubbery. The recorded data for the area of Republika Srpska in Bosnia and Herzegovina indicates that 4.19% of the land classified as transitional woodland-shrub. Anthropological alterations may provide an insights into the possible effects on land use preferences and distribution of the samples of studies samples and taxons. Results of CLC types in this study showing forest vegetation, natural surfaces and not as human influenced CLC types include: broad leaved forest of 13.31%, mixed forest (1.63%), non irrigated arable land 4.47%, sclerophyllous vegetation 3.67% and natural grasslands 0.10%. Forest vegetation and other natural surfaces, account for 64.4% of the total area in BiH (Čustović et al., 2020) and 61.25% for RS (Drašković et al., 2020). Second, the most prevalent classifications for RS in BiH accounts for forest vegetation (47.25%). The dominant vegetation was broad-leaved forests, which accounts for 34.61% of the total coverage, mixed forests 7.58 %, non irrigated arable land 4.14%, natural grasslands covers 3.24 %, sclerophyllous vegetation 4.92% (Drašković et al., 2020). Results and the literature review for area of BiH and specific area of RS both show a significant proportion of agricultural land use, with complex cultivation patterns, pastures, and agricultural regions with significant natural vegetation being the most prevalent classifications.

6.2 Land use preference and distribution

The findings found the absence of a significant correlation between taxonomic groups and land use factors. This can be also attributed due to insufficient taxon samples, what resulted in a limited number of observations.

There was a significant difference among the selected taxonomic groups based on their composition or the percentage share of CLC types from the buffer zones across 18 locations. This means that the CLC types observed in buffer zones of sample locations were different among different taxonomic groups, indicating that each taxonomic group had a unique preference for specific land use types. Subsequent to these findings, in an effort to gain an improved understanding of the reason that underlies the variation in CLC types across taxonomic groups, we carried out an assessment of the percentage of CLC types across 18 different locations. The initial analysis revealed that the variations in land use types observed among selected taxonomic groups were not restricted to the sampled sites alone, but rather exhibited a broader distribution across multiple locations. The data suggests that the results of the primary analysis exhibit a greater level of abstraction and relevance to a wider range of areas beyond the specific sample sites that were the subject of investigation in the research.

The diversity of CLC types across taxonomic groups and their potential association with specific land use types can be looked at within the Figure 7 and Figure 9. The habitat preferences of *Pelophylax* species are known to vary, with some preferring larger water bodies that have dense emergent vegetation, while others prefer smaller water bodies with minimal vegetation (Mikulcek & Pisut, 2012; Mikulicek et al., 2014). What we first found is that hybrid has the biggest diversity across the majority of CLC types recorded for all taxons. The occurrence of *P. kurtmulleri* across various CLC types exhibited a trend that was similar to that of the hybrid land use type, where *P. ridibundus* exhibited the least diversity of CLC types that it was registered on. One possible explanation for the relatively lower diversity of CLC types in comparison to *P. kurtmulleri* and hybrid species could be attributed to the smaller sample size of *P. ridibundus* that was collected, identified, and included in the analysis.

It is not accurate to equate CLC types with ecological niches, but they can be viewed as a useful proxy or analogue for ecological niches, especially when trying to understand the relationship between land use patterns and species distribution. However, it is important to keep in mind that ecological niches are complex and multifaceted, and cannot be fully captured by any single variable, including CLC types. While taking in account that CLC type can be viewed as one of the possible aspect of an ecological niche, interactions of hybrids with its parental species can be explained. Hybrid's diversity of CLC types can be explained as part of the generalistic heterosis theory and the habitat segregation theory. With increased genetic diversity hybrid (Moore, 1976; Simon et al., 2003) can be better fit to characteristic and habitats of various land use type as suggested in the results. That way they would have better odds in survining and reproducing (General-Purpose Genotype model; Baker, 1965; Schultz, 1971,1977; Lynch, 1984; Van Doninck et al., 2002) therefore being the most sampled and identified across the researched areas. On the other hand they could support the habitat segregation theory as well, as they could be filling various niches within the common habitat of parental species (Thibault, 1974; Case & Taper, 1986) and be able to be viable in their own ecological niche (Vrijenhoek, 1978, 1979; Bell, 1982; Case & Taper, 1986; Weeks, 1993). However, additional environmental factors (Plenet et al., 2005) would have to be taken into consideration and tested in addition to parental species in order to establish a more definitive stance regarding the hybrid hypothesis. If it has a general tolerance for different environments, then higher fitness due to genetic diversity, could be the case.

Preferences of certain land use types of hybrid and their presence on same CLC types as parental species might be as well linked to the their hybridogenetic system and reproductive dependence of hybridogenesis to their parental species (Schenck & Vrijenhoek, 1989; Bergen et al., 1997; Vrijenhoek & Pfeiler, 1997; Plénet et al., 2000; Negovetic et al., 2001; Doeringsfeld et al., 2004). Besides being prevalent across majority of CLC types found in this research for all taxons, it can be more frequently found close to CLC types such as pastures, discontinuous urban fabric, sclerophyllous vegetation, and broad-leaved forest.

P. ridibundus was mostly associated with complex cultivation patterns, non irrigated arable land amongst other CLC types such as pastures, land principally occupied by agriculture with significant areas of natural vegetation, broad leaved forest, discontinuous urban fabric, non irrigated arable land and inland marshes. Water courses are as well part of the CLC type it was found on confirms that *P. ridibundus* prefers habitats near water bodies and wetlands (Pagano et al., 2001; Mikulicek et al., 2014). In the study of Mikulicek et al., 2014, *P. ridibundus* had the ability to thrive in environments altered by human activity where *P. lessonae* and their hybrid *P. esculentus*, favoured habitats with minimal human interference.

In results of this thesis *P. kurtmulleri* has appeared to occupy most of the CLC types found for *P. ridibundus*, but specifically was observed to be present in CLC types such as mixed, fores, land primarily utilized for agriculture with substantial portions of natural vegetation and water bodies. This shows that *P. kurtmulleri* can thrive as well in habitats similar to *P. ridibundus* even if they are pressured by the man-made activities. Even though the taxonomic classification of *P. kurtmuelleri* remains a topic of debate and has not achieved universal acceptance (Speybroeck et al., 2020), results imply that it could be superior than *P. ridibundus,* that can be as well suggested in its occurance across BiH (Vrhovac et al., 2020). It's presence and invasive character can also have potential to disrupt the hybrid genetic structures of *P. kl. esculentus* also present in BiH (Vrhovac et al., 2020; Quilodrán et al., 2015), which could result in the extinction of the local frog populations (Quilodrán et al., 2015).

The kernel density representation of *P. kurtmulleri*, *P. ridibundus*, and their hybrid occurrences in Bosnia and Herzegovina (Figure 8), following the results of the CLC types with which they are most commonly associated, could offer insight into the areas for future research where they may be distributed. The analysis of species densities and occurrences in particular CLC types surrounding water bodies can provide indications of possible preferences and distribution patterns.

7 Conclusion

A large-scale analysis of land use preferences that included numerous locations throughout Bosnia and Herzegovina found not as strong statistically significant differences between the selected taxa and land use type. Therefore, it would be advised for future studies to analyse data with a smaller scale of land use and an increased amount of samples for each taxon. The hybrid of *P. ridibundus* and *P. kurtmulleri*, as well as *P. kurtmulleri*, have shown a degree of superiority in comparison to *P. ridibundus*, based on their distribution, diversity and preference of the CLC types they have been identified on. More detailed analyses, both genetic and ecological mechanisms, are needed to provide a closer answer to the impacts of their interactions and consequences. A re-evaluation is required regarding the genetic contamination of native species by foreign lineages. Rather than introducing novel hybridogenetic complexes, the introduction of modified versions of pre-existing systems may potentially disrupt the native *Pelophylax* assemblages in an undetected way. Early detection, accurate identification, and ongoing surveillance of hybrid expansions are necessary (Dufresnes et al., 2017). The use of models that can answer habitat suitabilities and preferences has been previously observed in predicting the expansion of invasive amphibians (Lobos et al., 2013). This approach could potentially contribute to the reduction of costs associated with the identification of individuals from invasive species, ultimately enhancing eradication efforts (Hester & Cacho, 2012).

The effective management of species that hybridize and exhibit physical similarity, such as *Pelophylax spp*., presents a notable challenge in regions of prolonged occurrence. This can be attributed to the possibility of the silent replacement of native populations by introduced species, which may occur without significant observable changes in demographic patterns.

These findings may contribute to further research on the genetic and ecological aspects of *Pelophylax* species, potentially leading to a better understanding of their complex nature.

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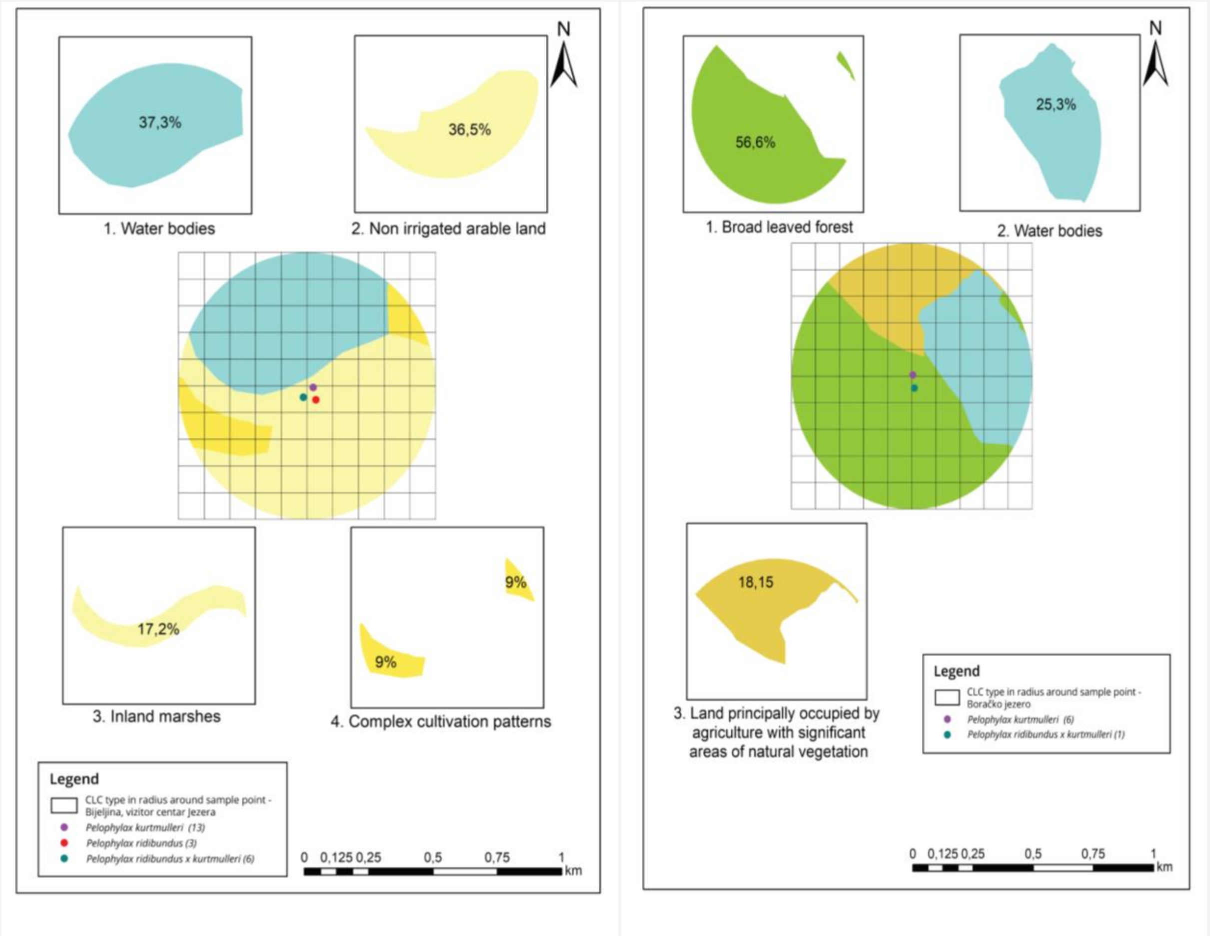
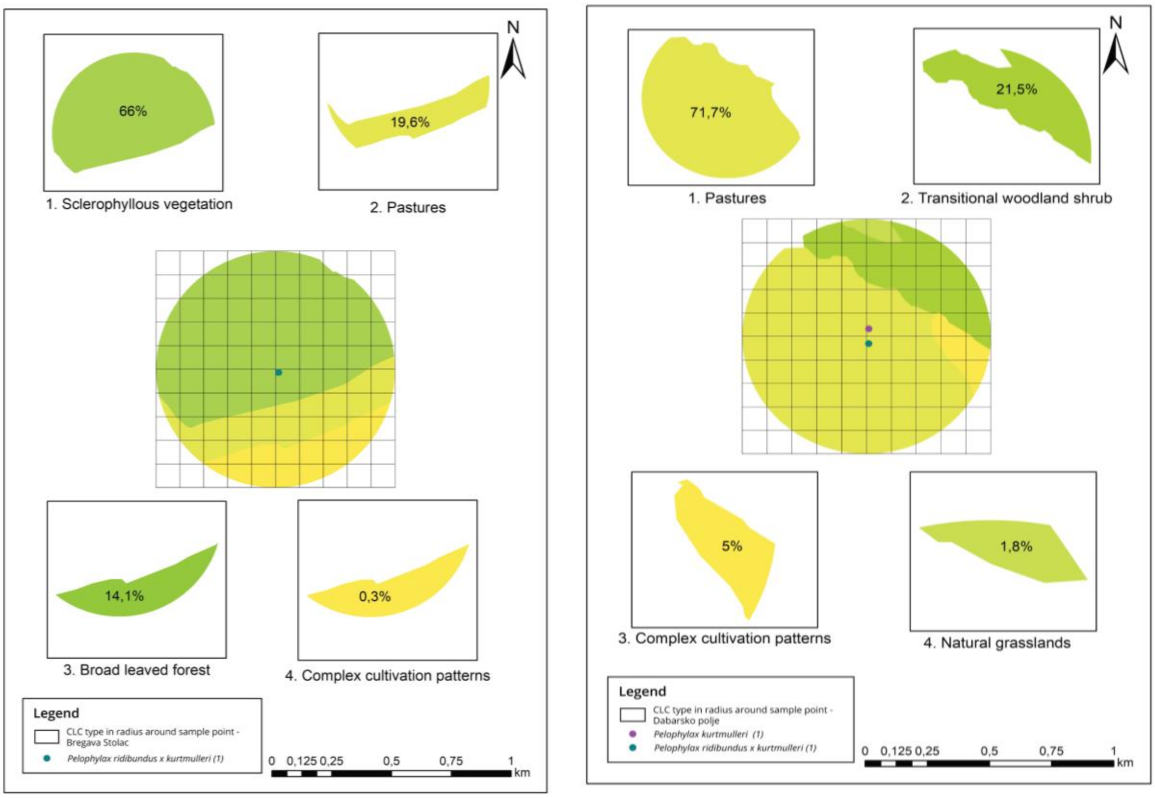
Appendices

Appendix A1. List of collection data points in BiH; Number of samples are under each taxon.

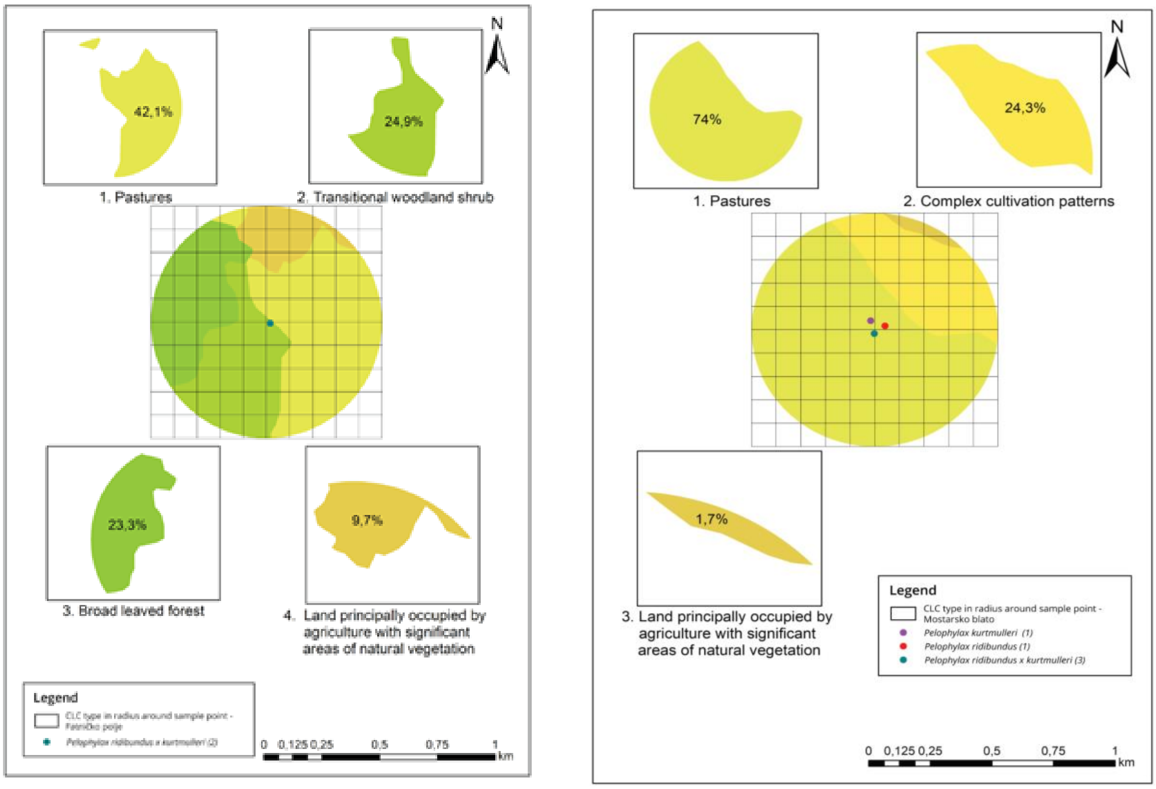
No.	**Date**	**Locations**	**Coordinates**	***P. ridibundus* (35)**	***P. kurtmulleri* (39)**	**hybrid (43)**
1.	20.5.2017.	Popovo polje	42.821 18.095	-	1	-
2.	23.03.2019.	Fatničko polje	43.015 18.322	-	-	2
3.	10.07.2018.	Dabarsko polje	43.074 18.238	-	1	1
4.	20.09.2018.	Stolac, rijeka Bregava	43.085 17.959	-	-	1
5.	09.07.2018.	Mostarsko blato	43.339 17.700	1	1	3
6.	01.08.2017.	Velež jezero	43.344 18.049	-	-	1
7.	15.08.2018.	Boračko jezero	43.551 18.032	-	6	1
8.	05.06.2018.	Jablaničko jezero	43.696 17.832	-	2	-
9.	07.05.2018.	Sarajevo, Vrelo Bosne	43.810 18.287	-	1	-
10.	01.08.2018.	Sanski most	44.764 16.672	-	1	1
11.	15.05.2018.	Bihać	44.805 15.864	-	2	3
12.	01.08.2018.	Prijedor, Saničani ribnjak	44.924 16.778	-	4	3
13.	19.05.2019.	Bijeljina, Vizitor centar Jezera	44.782 19.306	3	13	6
14.	15.08.2018.	Prnjavor ribnjak	44.901 17.753	4	2	8
15.	20.06.2018.	Bardača	45.105 17.449	5	2	5
16.	21.04.2019.	Sijekovac ribnjak	43.015 18.322	1	1	-
17.	21.04.2019.	Laminci	45.104 17.324	19	2	8
18.	15.4.2017.	Orahova ribnjak	45.198 17.028	2	-	-
Total number of all individuals:				**117**		



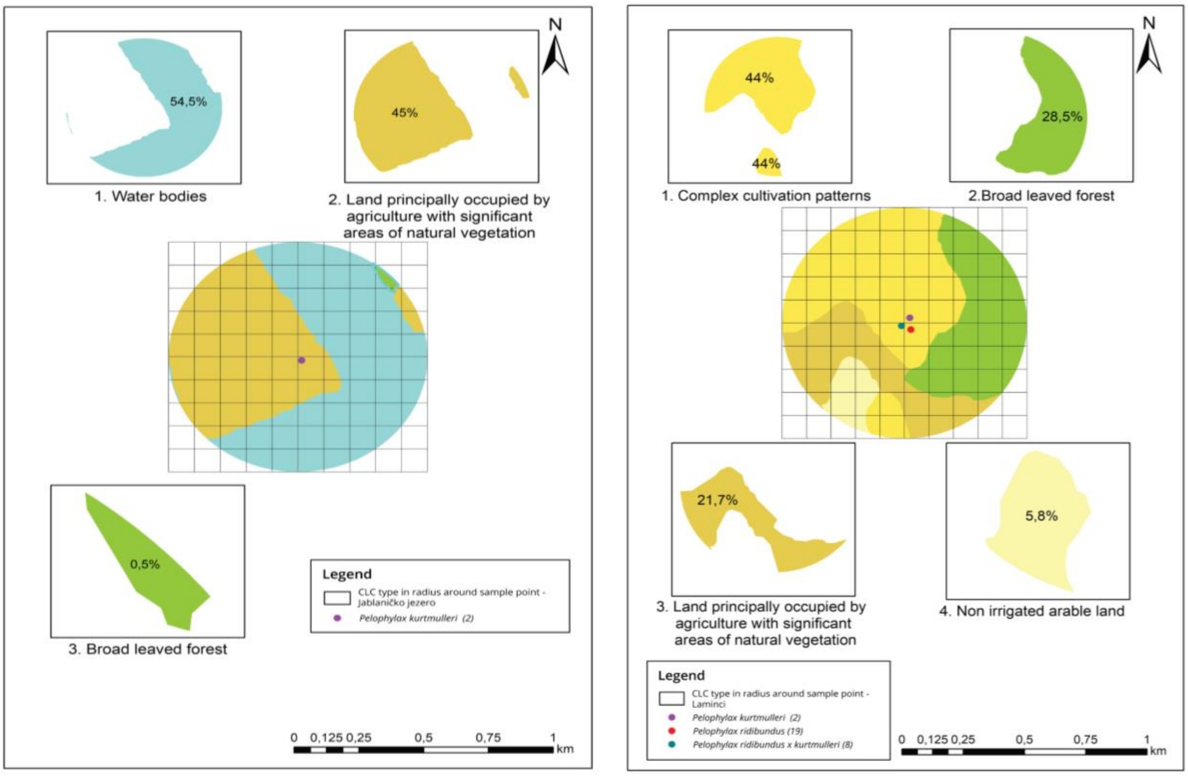
Appendix B1 Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Bijeljina, vizitor centar Jezera (left) and Boračko jezero (right).



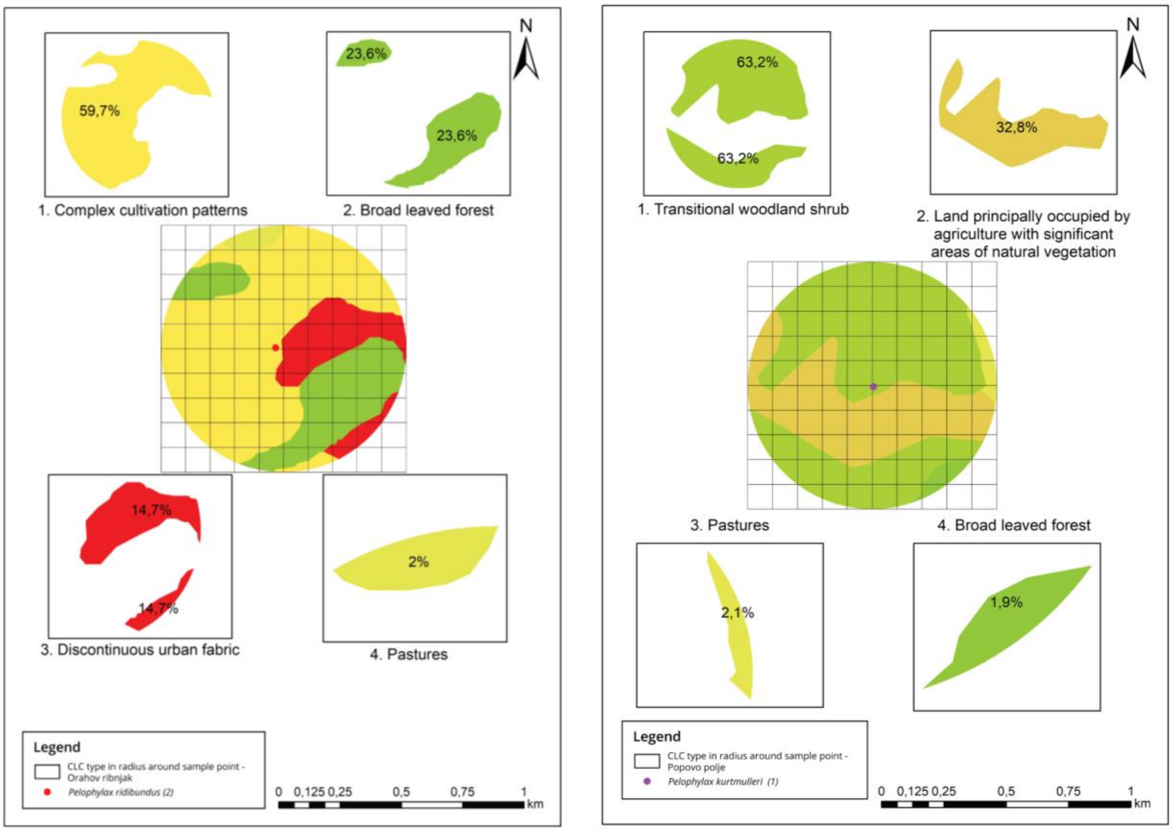
Appendix B2. Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Bregava, Stolac (left) and Dabarsko polje (right).



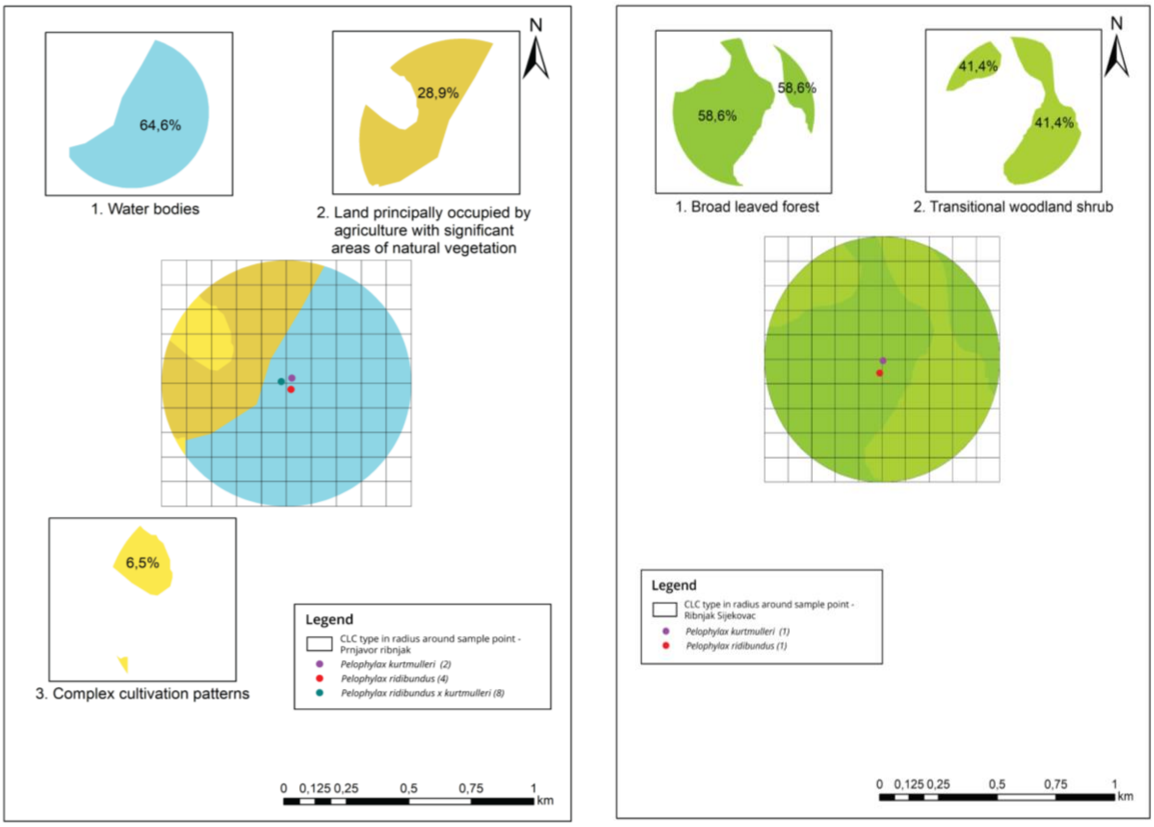
Appendix B3. Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Fatničko polje (left) and Mostarsko blato (right).



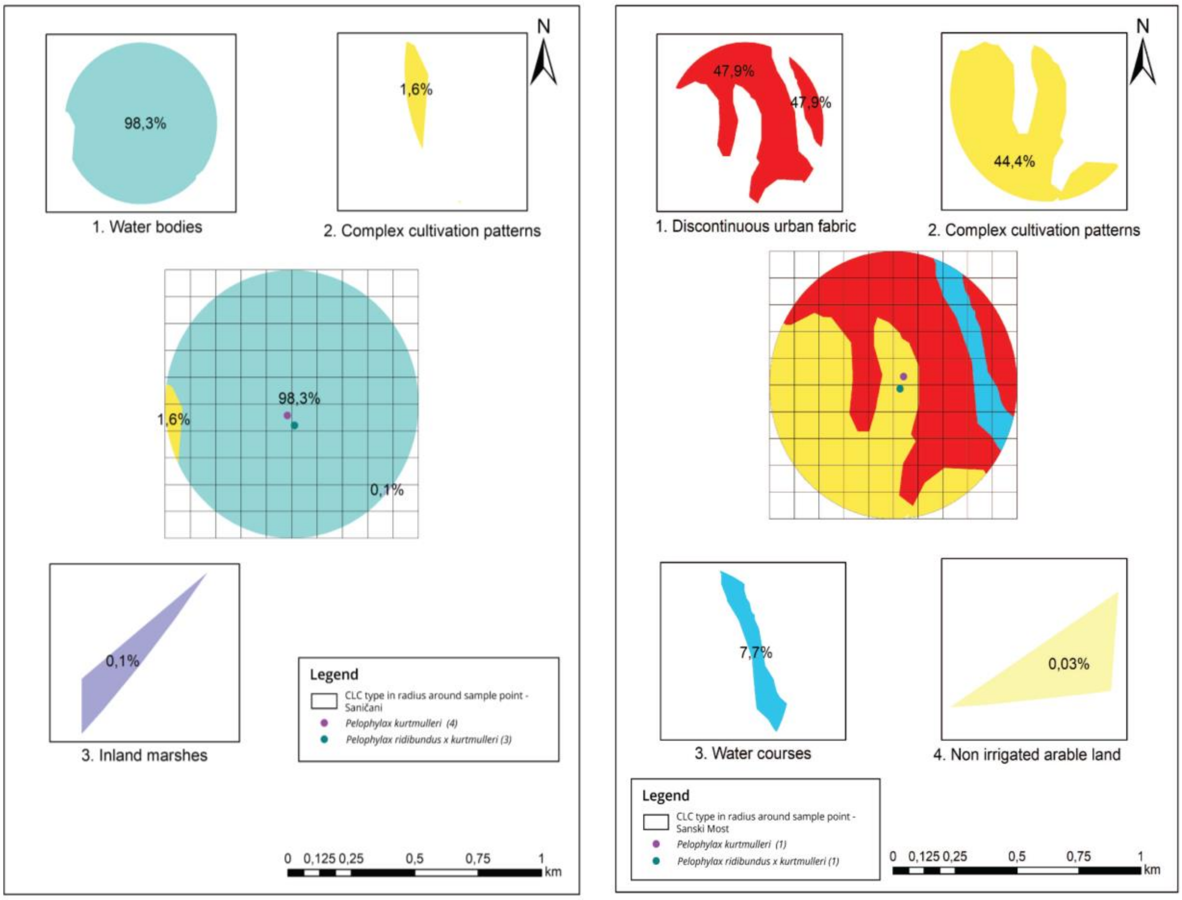
Appendix B4. Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Jablaničko jezero (left) and Laminci (right).



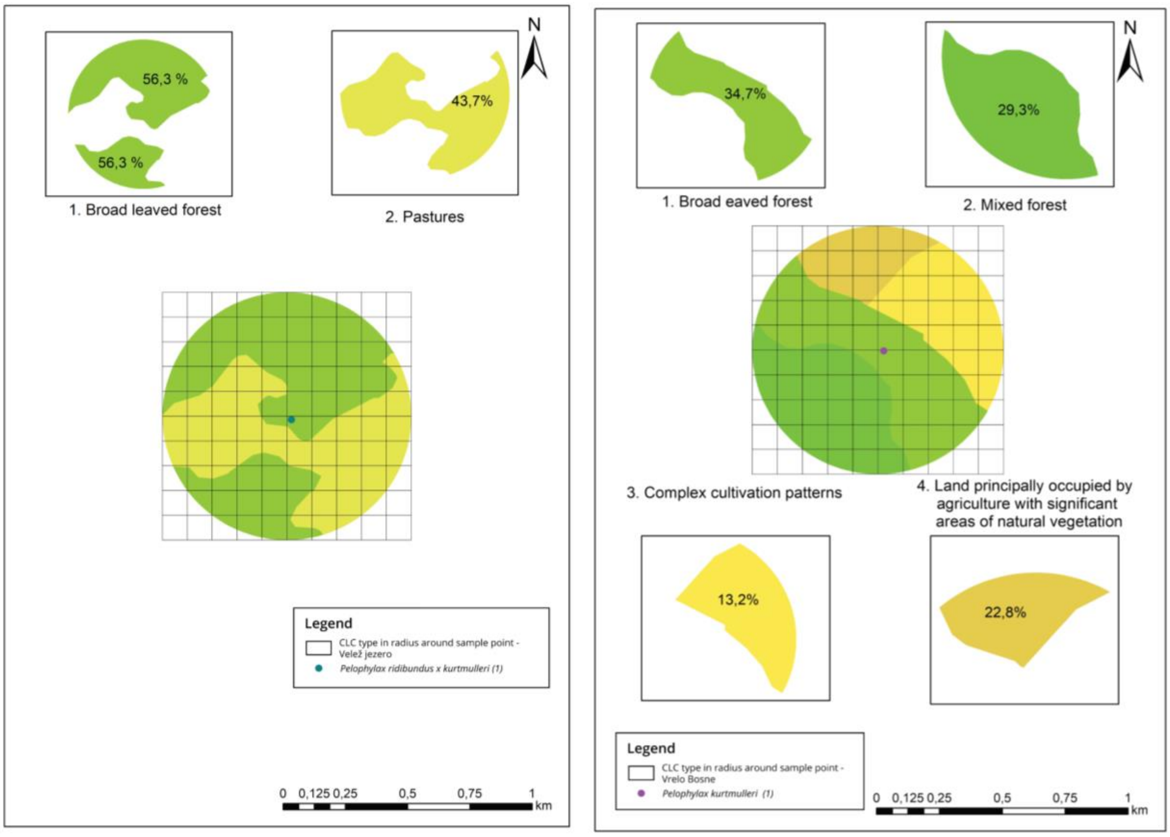
Appendix B5. Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Orahov ribnjak (left) and Popovo polje (right).



Appendix B6. Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Prnjavor ribnjak (left) and ribnjak Sijekovac(right).



Appendix B7. Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Saničani (left) and Sanski most (right).



Appendix B8. Overview of CLC types in buffer zone of 500m and species/hybrid found at the sample point data for Velež jezero (left) and Vrelo Bosne (right).