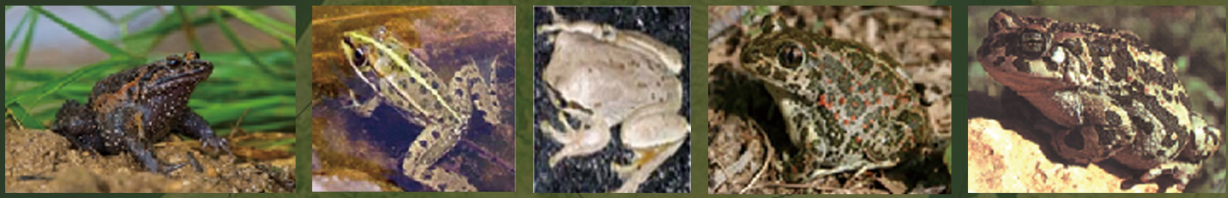
Biological Adaptations of Anuran Species across Diverse Habitats, Spanning Mediterranean to Desert Climates

Prof. Gad Degani



Scientific Research Publishing

Biological Adaptations of Anuran Species across Diverse Habitats, Spanning Mediterranean to Desert Climates

Prof. Gad Degani Ph.D.
Email: gad@migal.org.il
Phone: 972-505586706
www.migal.org.il

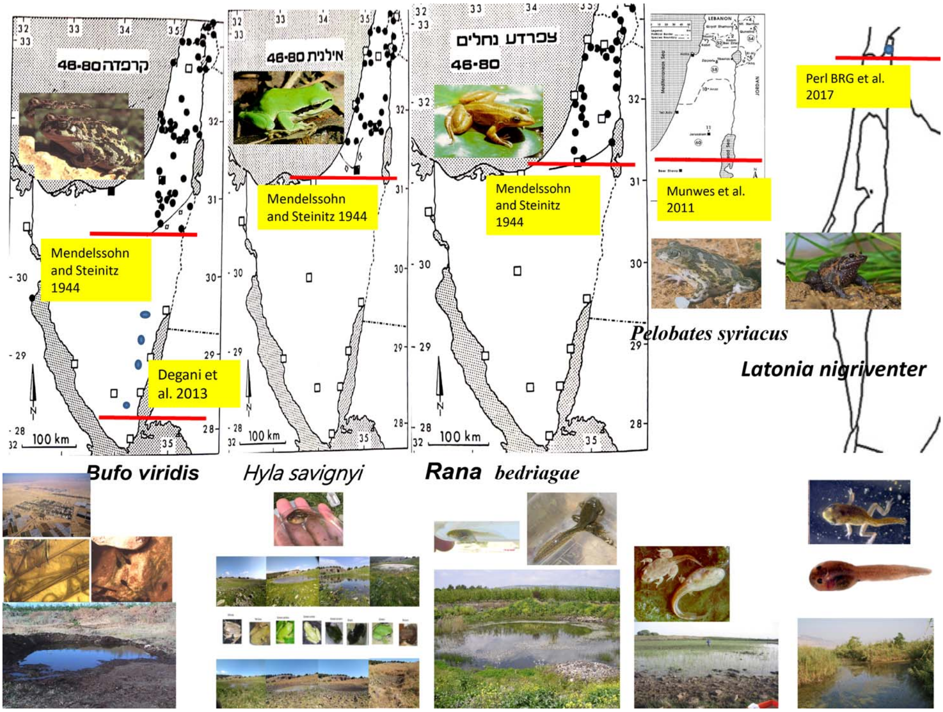
MIGAL Galilee Research Institute LTD.
Tel-Hai College

Scientific Research Publishing

Biological Adaptations of Anuran Species across Diverse Habitats, Spanning Mediterranean to Desert Climates

Published by
Scientific Research Publishing, Inc.
ISBN: 978-1-64997-800-4
Word Count: 92,000 words
Publication Date: 2024-03
http://www.scirp.org

Graphical Abstract



Acknowledgements

I would like to thank the Ph.D. students working in my laboratory who contributed directly or indirectly to this book: Dr. Gal Levy, Dr. Karen Jackson, Dr. Svetlana Yom Din, Dr. Tzchori Itai, Dr. David Kushnirove, Dr. Avshalom Hurvitz, Dr. Oren Pearlson, and Dr. Tali Goldberg; and the M.Sc. students Meital Ezagouri, Lior Sahder, and Dali David; my colleagues at MIGAL and Tel-Hai Academic College, Prof. Dani Bercovich, Dr. Doron Goldberg, and Dr. Ari Meerson.

Contents

1. Class Amphibia

Table 1. Amphibians in Israel.

Name	Systematic name	Synonyms
Southern banded newt	*Ommatotriton vittatus*	*Ommatotriton vittatus*, *Triturus vittatus* (found in Turkey, Syria, and Israel) and *O. ciliensis* (found in Turkey)
Near Eastern fire salamander	*Salamandra infraimmaculata*	*S. i. orientalis*, *S. i. infraimmaculata*
Green toad	*Bufotes sitibundus*	The genus *Bufo* (*Bufo viridis*) was changed to the genus *Pseudepidalea*. In 2010, it was shown that *Pseudepidalea* is a junior synonym of *Bufotes*. *Bufoviridis*, Laurenti, 1768 was changed to *Bufotes sitibundus*
Eastern spadefoot toad	*Pelobates syriacus*	
Middle East tree frog	*Hyla savignyi*	*H. arborea*
Levant water frog	*Pelophylax bedriagae*	*Rana ridibunda*, *Rana bedriagae*
Hula painted frog	*Latonia nigriventer*	

The earliest amphibians are thought to have appeared on earth in the Devonian period, almost 359 to 416 million years ago, whereas the true frogs, toads and salamanders are estimated to have appeared around 145 to 200 million years ago during the Jurassic period. The early amphibians probably evolved from freshwater fish Sarcopterygii, which possessed lungs and used gills as their primary means of acquiring oxygen.

Amphibians are cold-blooded vertebrates, the first four-legged animals (tetrapods) on land. In most cases, amphibians have well-defined limbs and feet, a supportive rib cage, and a neck that enables the skull to rotate.

The class Amphibia contains over 6,300 known species, 54% of which live in the neotropical region. They cannot tolerate the high salt content of sea water and are therefore the only vertebrates not to have colonized marine habitats (Capula, 1989). The class is divided into three orders. The largest, Anura, is divided into approximately 30 families and more than 4,500 species, ranging in size from a few millimeters to a couple of feet in length, and found in nearly every niche on earth. They have four limbs, the hind limbs typically being larger and modified for leaping or climbing. Most are external fertilizers. These species are also vocal, making sounds that range from squeaks to barks. In lentic environments, anuran breeding occurs in water bodies ranging from ephemeral pools to large permanent lakes (Richter-Boix et al., 2006). Most species deposit their eggs or live larvae in aquatic environments; these go through a larval phase (tadpoles) characterized by intensive morphological transformation—metamorphosis—to the juvenile stage, with an adult appearance; they then continue growing until the reproductive stage.

The order Urodela (Caudata) comprises about 500 tailed species arranged in 10 taxonomic families. They are found in all of the world's temperate zones, except Australia, Antarctica and most of Africa. They are known as the "tailed amphibians" because all species possess a tail that is approximately equal to the length of their body and that confers their ability to swim. Typical caudates have four limbs of similar size that are used for terrestrial walking. Many salamanders have a biphasic lifecycle consisting of an aquatic larval form with external gills and a metamorphosed terrestrial adult form that breathes through lungs and/or moist skin. Some species do not metamorphose, retaining a larval appearance throughout their lives, whereas other species lack an aquatic larval stage and hatch on land as terrestrial forms that resemble miniature adults.

The order Apoda includes approximately 174 known species. They are characterized by long, worm-like segmented bodies and are found mostly in parts of South America, Africa and South Asia. These amphibians have shortened tails, small (nearly functionless) eyes and no legs. They resemble eels or earthworms and live underground and in aquatic habitats.

Amphibians' reproductive stage can be described by the site of egg deposition, egg and clutch characteristics, type and duration of embryonic and larval development, and type of parental care, if any (Duellman and Trueb, 1986). Many amphibians are tied to aquatic habitats for reproduction. Others reproduce on land, underground, or in trees, even in temperate zones. The Near Eastern fire salamander, *Salamandra infraimmaculata*, displays ovoviviparity, whereby embryos developing inside eggs are retained in the mother's body until they are ready to hatch.

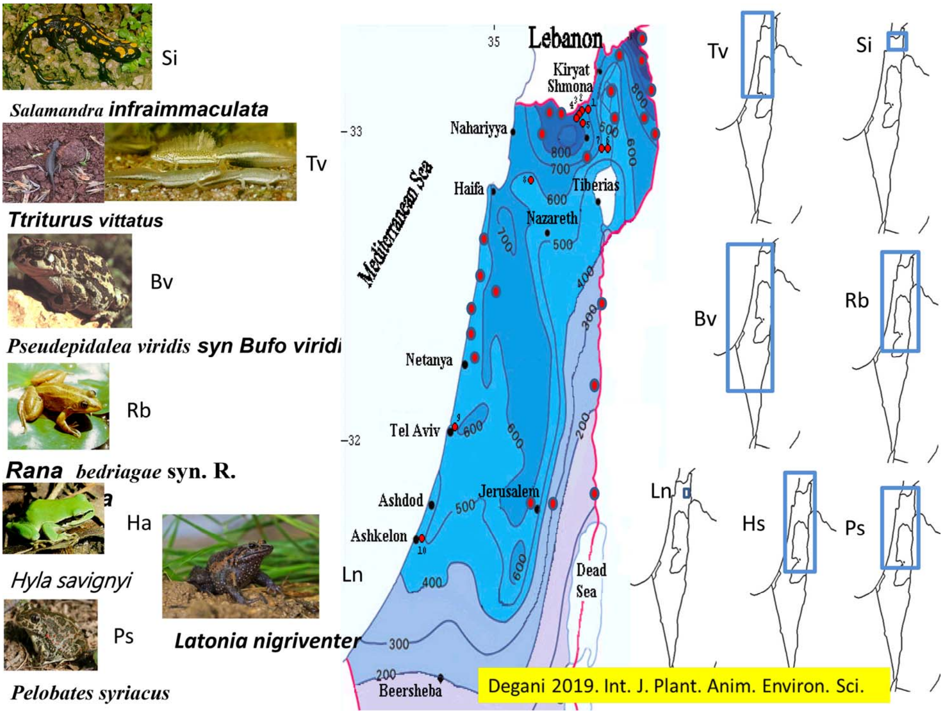
Israel, which is developing very rapidly with respect to both agriculture and urban expansion, has seven amphibian species: two members of the Urodela—the southern banded newt *Triturus vittatus* (Litvinchuk et al., 2005), synonym *Ommatotriton vittatus*, which is found in Israel, Lebanon, Jourdan, Syria, Turkey and Iraq, and *Salamandra infraimmaculata* (Degani, 2019b; Steinfartz et al., 2000), which is distributed in Israel, Lebanon, Jordan, Syria, Turkey and Iran. Both of these species belong to the family Salamandrinae; and five anuran species—the Middle East tree frog *Hyla savignyi* (family Hylidae), synonym *H. arborea* (Gvozdik et al., 2010), which is found in Yemen, Jordan, southern Syria and extreme northeastern Israel; the green toad *Bufo viridis* (family Bufonidae), synonym *Pseudepidalea viridis* (Degani, 2019a), which is found throughout many countries in Europe in different habitats, most of them relatively dry, as well as in Israel, including mountainous areas, semi-arid and arid habitats, and urban areas; the Levant water frog *Pelophylax bedriagae* (family Ranidae), synonyms *Rana ridibunda*, *Rana bedriagae* (Degani and Mendelssohn, 1983; Schneider et al., 1992); the eastern spadefoot toad *Pelobates syriacus* (family Pelobatidae) (Degani and Kaplan, 1999; Gomez-Rodrıguez et al., 2009; Mendelssohn and Steinitz, 1943), with wide distribution in parts of Europe and East and West Asia, including northern Syria, Israel, northern Iraq, Iran, Asia Minor and the Caucasus; and the Hula painted frog *Latonia nigriventer* (family Alytidae) (Degani, 1982, 1986a, 2019a; Perl et al., 2017), which is endemic to the Hula reserve in Israel.

2. Amphibians in Israel

Amphibian populations play a crucial role as indicators of the environmental impact of human development and pollution (Blaustein and Johnson, 2003; McCallum, 2007). The seven amphibian species found in Israel are at the southern border of their distribution and live under relatively extreme conditions. They breed in various aquatic habitats, including temporary and semi-permanent ponds, springs, and streams, where water availability varies throughout the year (**Figure 1**, **Table 2**) (Degani, 2019a). The aquatic phase of amphibians is influenced by numerous factors (Pearlson, 2012).

Israel offers predominantly xeric habitats, which are not the usual amphibian niche (Degani, 2019a). Consequently, amphibian larvae occupy a narrow and specific ecological niche in the region, facing significant pressures from predators and other biotic and abiotic factors (Veith, 1994). Understanding the water quality range required for tadpoles of different species to grow and complete metamorphosis is crucial for species conservation and as a bioindicator of environmental pollution. Genetic variation has been examined to assess the adaptation of amphibians to different habitats and geographical distributions in Israel. Genetic markers, including enzyme system loci, random amplified polymorphic DNA polymerase chain reaction (RAPD PCR), mitochondrial (mt) DNA analysis, amplified fragment length polymorphism (AFLP) (Arntzen et al., 2015), gene-expression microarrays, microsatellites, and RNA extraction, have been utilized to study genetic variation in *S. infraimmaculata* (Blank and Blaustein, 2014). Some of these methods can be applied to other species to study genetic variation among different habitats in Israel, although not all methods are suitable. Genetic variation among populations in Israel has been investigated for *T. vittatus* (Degani et al., 2013a). Amphibian species in semi-arid habitats at the southern border of their distribution exhibit various adaptations in both aquatic and terrestrial environments (Degani, 2019a). Seven species, including

H. savignyi, *B. viridis*, *Pelophylax bedriagae*, and *L. nigriventer*, have been studied to understand their genetic variation and adaptation to different areas in Israel (Degani, 2019a). It has been hypothesized that the primary mode of adaptation for these amphibians in different regions involves the metamorphosis phases rather than the aquatic ones (Degani, 2019a).



Degani 2019. Int. J. Plant. Anim. Environ. Sci.

Figure 1. Diverse breeding sites across Israel from which tadpoles of amphibians have been collected and identified (Degani, 2019a).

Table 2. Comprehensive overview of the specific locations and environments where the larvae of amphibians have been discovered in the northern region of Israel (Degani and Kaplan, 1999).

Label	Name	Type	Longitude	Latitude	Height (m.a.s.l.)	Amphibian Larvae
Sp1	Balad	Spring	32°43'13"N	35°04'17"E	446	Si, Hi
Sp2	Humema	Spring	33°00'28"N	35°23'43"E	900	Si
Sp3	Navoraya	Spring	32°59'47"N	35°30'39"E	663	Si
Si1	Tel-Dan	Stream	33°14'53"N	35°39'10"E	190	Si
Pi1	Maalot	Rock pool hole	33°00'06"N	35°42'48"E	596	Si, Bv
Pi2	Nimrod	Rock pool	33°15'05"N	35°42'48"E	760	Si, Hs, Rb
Po1	Manof	Pool	33°50'58"N	35°13'52"E	340	Si, Bv
Po2	Kash	Pool	33°01'47"N	35°25'29"E	809	Tv, Hs, Bv, Ps
Po3	Dovev	Pool	33°03'05"N	35°24'54"E	765	Si,T v, Hs, Bv, Ps
Po4	Matityahu	Pool	33°04'04"N	35°27'18"E	665	Tv, Hs, Bv, Ps
Po5	Lehavot	Pool	33°09'13"N	35°38'28"E	665	Hs, R
Po6	Sasa	Pool	33°01'58"N	35°23'30"E	665	Si, Rb, Tv, Hs, Bv, Ps
Po7	Fara	Pool	33°03'58"N	35°27'39"E	665	Si, Rb, Tv, Hs, Bv, Ps

m.a.s.l.—meters above sea level; Sp—springs; St—Streams; Pi—Rock pool holes; Po—Pools; *Si—Salamandra infraimmculata*; *Tv—Triturus vittatus*; *Hs—Hyla savignyi*; *Bv—Bufo viridis*; *Ps—Pelobates syriacus*; *Rb—Rana bedriaga.*

3. Green Toads

3.1 Classification

The green toad is classified in the kingdom Animalia, phylum Chordata, class Amphibia, order Anura, family Bufonidae, genus and species *Bufotes viridis* (now *Bufotes sitbndus*). It is important to mention that in 2006 (Haas, 2003), the genus name of this species was modified from *Bufo* to *Bufotes* due to genetic and morphological differences observed among various species previously grouped under the genus *Bufo*.

3.2 Distribution

The green toad has widespread distribution, predominantly in the Old World (Arntzen and García-París, 1995). The family Bufonidae is known for its high species diversity, with over 350 species distributed across various regions (Frost, 2021). The green toad thrives in diverse habitats (Cummingham and Cherry, 2004). Its distribution range spans Europe—excluding the British Isles and parts of western Europe beyond the Rhine River—Sicily, Crete, Kazakhstan, Tajikistan, and the Kerman Province of Iran (Degani et al., 2013a). Isolated populations of green toad have also been observed in the Balearic Islands, Sardinia, and Corsica. In North Africa, it can be found along the coastal regions of the northwestern Sahara, encompassing western Morocco and Algeria to western Libya (Degani et al., 2013a). On the Arabian Peninsula, it is present in the southern Hadhramaut region (Duellman, 1993), and in southwestern Asia, it is likely to be found in proximity to Afghanistan and the Altai Mountains in Russia, Mongolia, and Western Xinjiang, China.

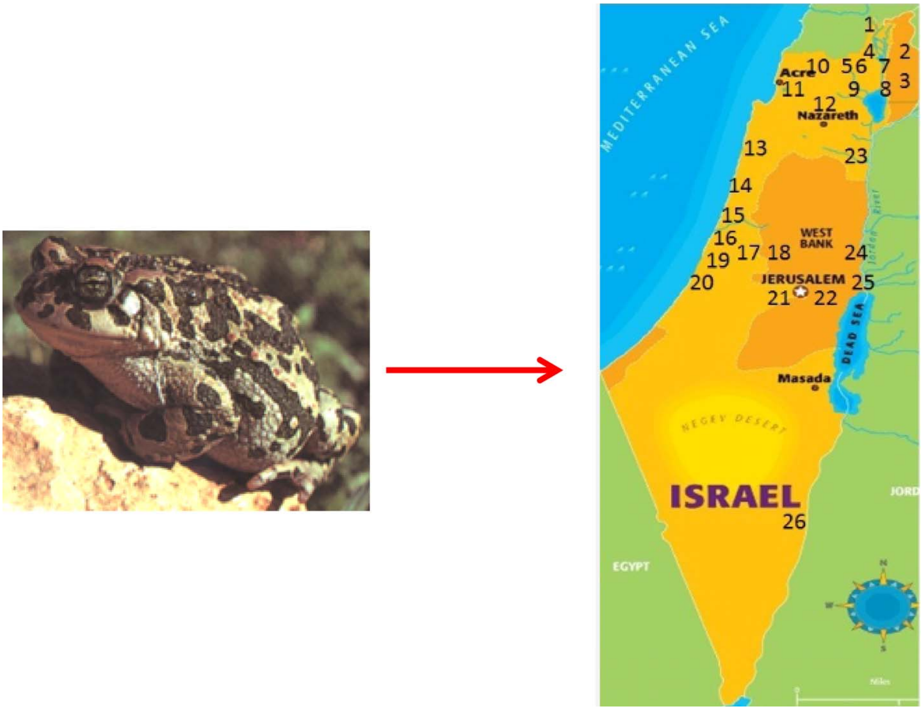


Figure 2. Distribution of the green toad in Israel (Degani, 2016).

3.3 Adult morphology

While the green toad is not commonly found in the northern part of its range, it does form dense populations in certain areas that have been influenced by human activities, with their numbers in anthropogenic habitats often surpassing those in neighboring natural ones. The presence of burrows created by burrowing rodents can further contribute to the higher density of green toad populations. In suitable habitats, the abundance of green toads can exceed 100 individuals per 100 square meters. During the metamorphosis stage, the density of toadlets can reach several dozens of individuals per square meter. In arid regions, the distribution of green toads is less uniform, with dense populations found in oases that are separated by dry areas where the toads cannot thrive (Degani, 2019a). Green toads are known for their remarkable heat tolerance, up to around 40°C. They are also quite resilient to desiccation, only dying when they lose approximately 50% of their body water. To combat dry conditions, the toads regularly seek out water bodies during the night to rehydrate. They are primarily active at twilight and at night, taking shelter during the daytime. However, during the reproductive period, they become active during the day. On hot days, the toads often find refuge in shallow water. In addition, it is typical for the toads to migrate considerable distances—2 to 5 km away from their breeding ponds. Green toads hibernate on land, although in some cases they may choose to hibernate in water bodies such as streams, ditches, or wells. They can hibernate individually or in groups. The timing of hibernation varies significantly across their range, depending on altitude and latitude. In the southern parts of their distribution, hibernation is often absent, and the toads remain active throughout the year. In contrast, aestivation, a period of reduced activity under hot and dry conditions, is believed to occur in southern desert regions. The reproductive period also exhibits considerable variation, occurring between February and July in different parts of their range. The southern areas tend to have the longest reproductive period, lasting approximately 170 days, whereas the duration of development before metamorphosis is shortest, typically 21 to 25 days. Spawning takes place in a diverse range of water bodies, including ponds, swamps, lakes, streams and river pools, reservoirs, ditches, and puddles. The preferred spawning sites are generally not deeper than 50 cm and can be either freshwater or saline. The green toad employs two mating strategies: active female choice by competing males and active male choice by females. The mating process involves pectoral amplexus, and assortative mating, where individuals with similar traits mate preferentially, has been observed. A single clutch of eggs can contain anywhere between 2,000 and 30,000 eggs arranged in 1 - 2 rows (**Figure 3**). The spawn is deposited in two strings measuring 2 to 7 m in length. Metamorphosis occurs from spring through summer, with the timing depending on the latitude and altitude of the population. Mass appearance of newly metamorphosed juveniles is common, often resulting in pond shores being covered with thousands of toadlets that disperse shortly after metamorphosis. Sometimes, migrating juveniles form large groups, moving together as a cohesive band (Degani, 2019a).

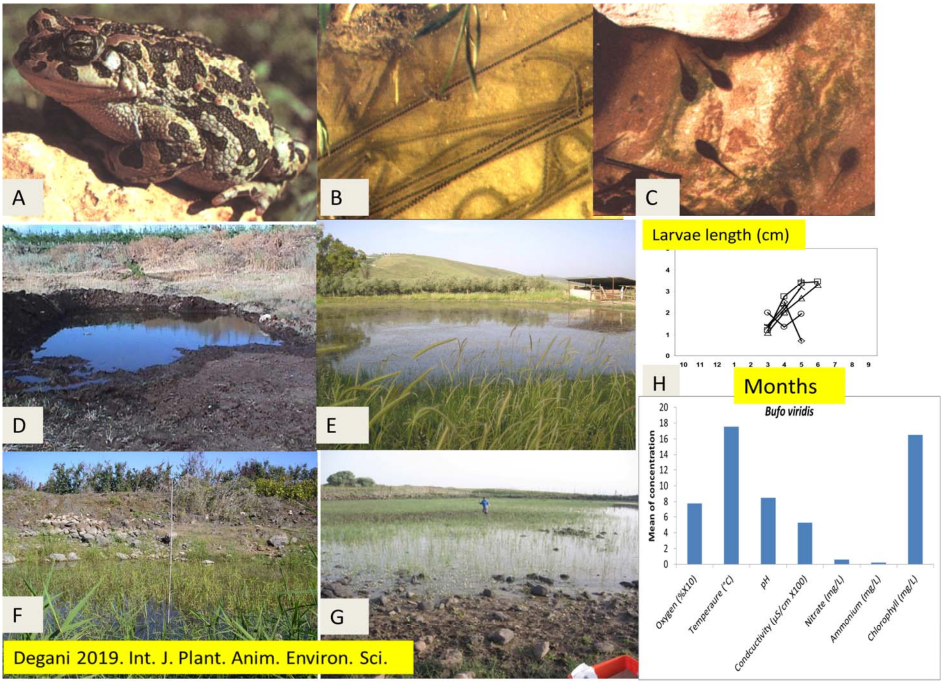


Figure 3. The green toad in northern Israel. (A) Adult green toad. (B) Green toad eggs. (C) Green toad tadpoles. (D-G) Green toad habitats. (D) Winter pond before drying up. (E) Winter pond in the Upper Galilee. (F) Fara pond. (G) Kash pond in the Hula valley. (H) Key ecological characteristics of the habitats (Degani, 2016).

3.4 Variations and adaptability

In Israel, the green toad displays significant variation and adaptability across its habitat range. It is considered one of the most versatile amphibians in the Palearctic region. It can be found in various ecosystems, including forests, forest steppes, steppes, semi-deserts, and deserts. Unlike many other amphibians, the green toad exhibits a high tolerance for dry conditions (Degani, 2016). It is capable of thriving in both wet and dry environments. In forested areas, this species tends to inhabit open spaces and bushlands, often far from water sources. However, in the arid regions of the southern part of its range, the green toad primarily occupies moist areas such as oases, shores of irrigation ditches, and lakes. It also takes advantage of irrigation ditches and channels as routes for dispersal.

3.5 Life history

Green toads have a wide distribution in Israel, ranging from the northern regions of the Upper Galilee and the Golan Heights (Degani, 2016) to the southern coastal plain and the desert. Among the 51 identified breeding sites for amphibians, ponds are the most commonly used by green toads (Degani, 2016), and they breed in most of the examined breeding places in northern Israel (Degani, 2016; Degani and Kaplan, 1999; Goldberg et al., 2012). The availability of water in these breeding sites varies from 1 month to year-round. The length of time taken for tadpoles to grow and metamorphose into toads depends on the specific region. The life cycle of green toads is depicted in **Figure 4**, with males being the first to arrive at the pond and initiate mating calls. Females subsequently arrive and the mating process, known as amplexus, takes place with the couple swimming in the water. The female lays eggs and the male fertilizes them. The breeding season in Israel varies with location, geography, and ecological conditions (Degani, 1982; Goldberg et al., 2012). It occurs in March-April in the north—Upper Galilee and Golan Heights, and earlier in the lower and central regions. In central Israel, breeding takes place during the winter. During amplexus, the male grasps the female above her back legs, and they swim together while the female lays 2 to 4 clutches of eggs (**Figure 4**). The number of eggs ranges from 5,000 to 17,000 per female. Larval growth and complex metamorphosis occur in northern Israel toward the end of winter and throughout spring, specifically from March to June (**Table 3**), whereas in southern Israel, they occur earlier, during the winter. The rate of tadpole growth varies among ponds and also fluctuates from year to year depending on ecological conditions (**Figure 5**) (Degani, 2016). In northern Israel, the growth and metamorphosis of green toads takes place within a range of 1 to 5 months. Green toads have the ability to colonize new pools and ponds where water is available for a relatively short period, making it challenging to monitor the distance of their movement from breeding sites.

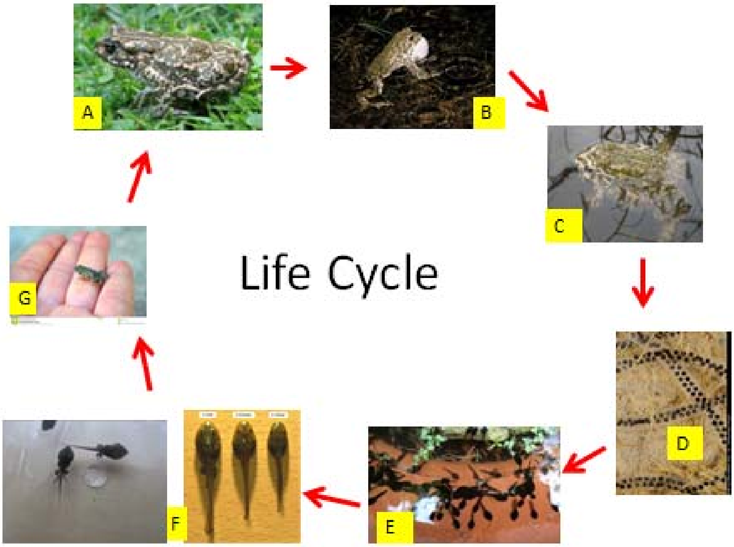


Figure 4. Life cycle of the green toad. (A) Mature frogs. (B, C) Reproductive behavior. (D) Eggs. (E, F) Larvae in different stages of growth and metamorphosis. (G) Juveniles (Degani, 2016).

Table 3. The periods of green toad tadpole growth in ponds.

Breeding places	Periods when eggs and larvae are found
Po2 Kash pond	March to June
Po4 Matityahu pond	April to May
Po6 Sasa pool	March to June
Po7 Fara pool	March to June
Po8 Rihaniya pool	March to May

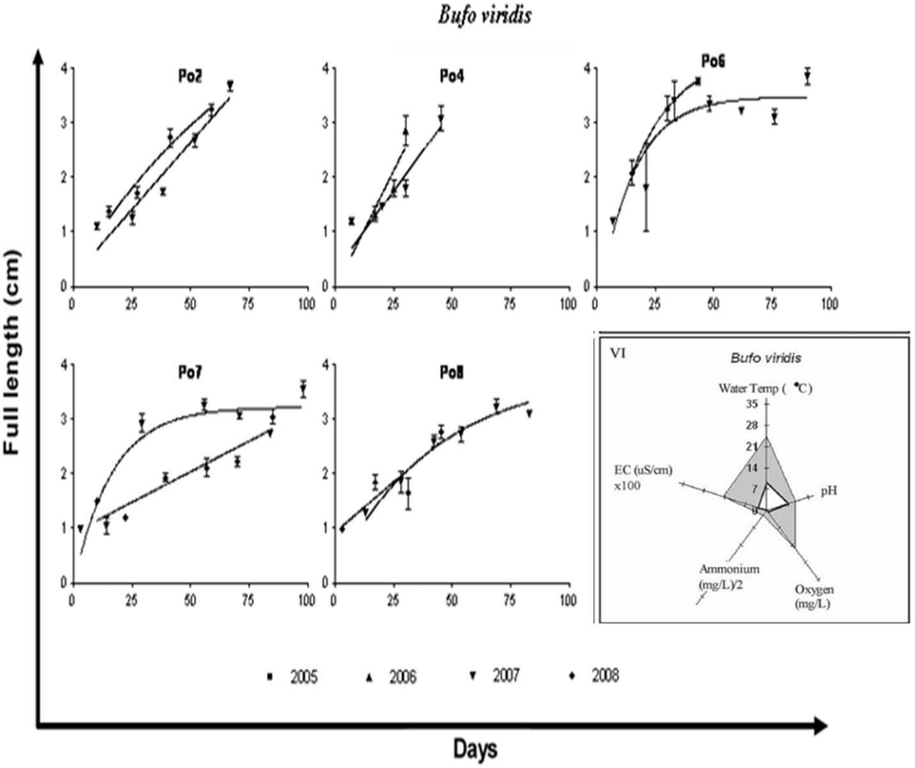


Figure 5. Green toad tadpole growth in various ponds (Degani, 2016). Locations of habitats where young amphibians were investigated include Kash Pond (Po2), Matityahu Pond (Po4), Sasa Pond (Po6), Fara Pool (Po7), and Raihaniya Pool (Po8). Five ecological characteristics were examined: oxygen, ammonium, temperature, conductivity and pH (Figure 3).

The maximum estimated lifespan for green toads is 7 to 10 years, although it may vary among different populations in the Caucasus region. The tadpoles consume detritus and algae, moving toward the shore during the daytime and to greater depths in the evening. They also consume small amounts of animal matter, such as protozoa, rotatoria, and microcrustaceans. Newly metamorphosed toadlets prey upon springtails (Collembola), beetles (Coleoptera), mites (Acarina), and flies (Diptera). Adult green toads feed primarily on crawling invertebrates, including spiders and beetles. Occasionally, small amounts of aquatic invertebrates are found in the stomachs of individuals captured in the spring along pond shores. However, most toads do not feed during their breeding migrations. Like other toad species, green toads display mirmecophagy, i.e., they consume ants as a significant component of their diet as adults, but not as juveniles. This feeding behavior develops after metamorphosis and may be associated with age-related changes in their foraging strategy.

3.6 Adaptation

The adaptation of green toads to terrestrial life involves several physiological and anatomical changes urea accumulation play a significant role in this process. Green toads, like many amphibians, metamorphose from aquatic tadpoles to terrestrial adults (Degani et al., 1984). During this process, they undergo a series of changes that allow them to transition from an aquatic to a terrestrial lifestyle. These changes include the development of lungs for breathing air, the growth of limbs for locomotion on land, and skin modifications to prevent dehydration. Urea accumulation, on the other hand, is primarily associated with the ability of some animals to tolerate high levels of urea in their bodies. Urea is a waste product of protein metabolism and is typically excreted in the urine. Some animals, such as certain fish and marine reptiles, have adaptations that allow them to accumulate and tolerate high concentrations of urea in their tissues, which helps them survive in environments with limited water availability, such as marine or desert environments (Degani, 2016).

Instead, these toads have evolved other mechanisms to prevent dehydration and maintain water balance on land. Their skin contains specialized glands that secrete mucus, which helps retain moisture and reduce water loss. They also have behavioral adaptations, such as seeking out damp areas and burrowing into the ground during periods of dryness. In an experiment conducted during the summer (Degani, 2016), green toads were subjected to slow dehydration while being kept on soil, and the accumulation of urea in the plasma was the main factor affecting plasma osmolality (**Figure 6**).

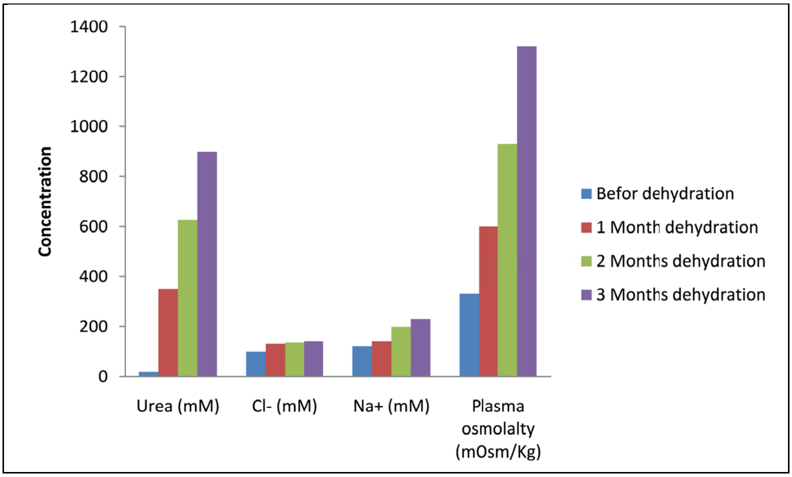


Figure 6. Mean blood plasma concentration and composition during green toads' slow dehydration on soil (Degani, 2016).

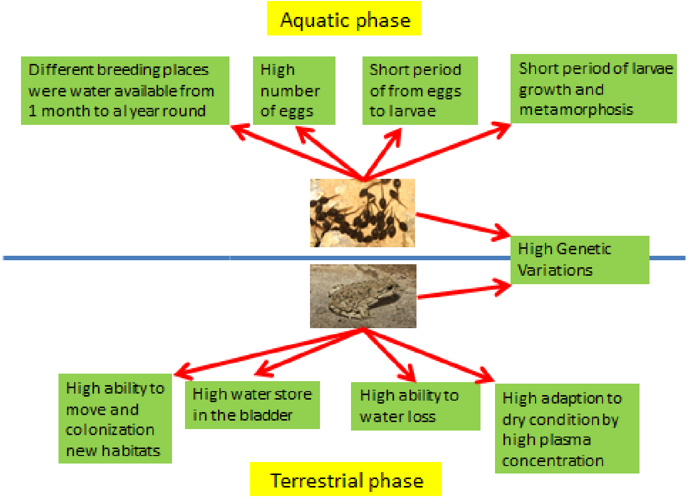


Figure 7. Proposed model for the adaptation of green toads to semi-arid and arid environments, which involves their ability to adapt to both aquatic and terrestrial habitats (Degani, 2016).

Degani (2016) focused on the adaptation of green toads to semi-arid habitats in northern Israel, and discussed their specific needs during their aquatic phase, such as the availability of breeding places with water throughout the year or for at least 1 month. The study further highlighted the high number of eggs produced by each female and the short period of growth and metamorphosis. **Figure 7** shows a suggested model for the adaptation of green toads to both aquatic and terrestrial life under arid or semi-arid conditions. This model helps visualize the ways in which green toads have evolved to thrive in challenging environments with limited water resources.

3.7 Genetic differences between populations in different environments

Green toads' genetic diversity was studied, focusing on populations from various breeding locations in Israel, and in Egypt, Turkey, Iran, and Germany (considered as outgroups for comparison) (Degani et al., 2013a). DNA sequences of the 269-bp D-loop (control region) and 262-bp cytochrome b (*Cyt b*) were compared. A 96.7% similarity index, according to Wilbur and Lipman (1983), was found for the D-loop alignment with the consensus fragment (GenBank accession no. AF190253) and a 97.3% similarity index was found for the *Cyt b* alignment with the consensus fragment (GenBank accession no. L10982).

Variations in the nucleotide sequences of the control region among different populations of green toad in Israel ranged from 0% to 2.7% (**Table 4**). When comparing the Israeli populations with those in Egypt, Turkey, Iran, and Germany, the differences ranged from 1.2% to 8.2%. The most significant disparities, with percentages ranging from 7.0% to 8.2%, were observed between the German and Israeli populations (**Table 4**).

The *Cyt b* nucleotide sequences differed among populations in Israel, ranging from 0% to 3.5% (**Table 4**). Comparing the Israeli populations to populations outside of Israel—Egypt, Turkey, Iran, and Germany, average *Cyt b* nucleotide sequence genetic variability ranged from 0.5% to 5.5%. The most substantial differences were observed between the Egyptian and German populations and the Israeli populations, with a range of 3.0% to 5.5% (Degani et al., 2013a).

Table 4. Range of nucleotide variations in the D-loop (control region) and *Cyt b* fragments among green toad populations, as determined using DNASTAR software (Degani et al., 2013a). There were 24 habitats in Israel, and 1 each for the other 4 countries (Figures 2 and 3).

	Site	Percept variation among population in Israel	Percept variation among population in Israel
		Control region	*Cyt* b
1	Hermon	0.6 - 2.4	0 - 1.5
2	Masade	0 - 1.8	0 - 2.5
3	Orvim	0 - 1.5	0 - 2.5
4	Nahalit	0 - 1.5	0 - 2.5
5	Fara	0.3 - 2.1	0 - 2.5
6	Matityahu	0 - 1.8	0 - 2.5
7	Raihania	0.3 - 1.5	0 - 2.5
8	Kash	0 - 1.8	0 - 2.5
11	Kziv	0.6 - 2.7	0 - 3.5
12	Manof	0 - 1.8	0 - 2.5
13	Hedera	0 - 1.8	0 - 3.5
14	Gaash	0 - 1.8	0 - 3.5
15	Herzelia	0.6 - 2.1	0 - 2.5
16	Afeka	1.5 - 2.7	0 - 2.5
17	Hulon A	0.3 - 2.1	0 - 2.5
18	Hulon B	0.9 - 2.7	0 - 2.5
19	Shafdan	0.6 - 2.4	0 - 2.5

20	Palmahim	0 - 18	0 - 2.5
21	Ben-Zayit	0 - 1.5	0 - 2.5
22	Jerusalem	0.6 - 2.4	0 - 2.5
23	Gidron Mount	0.3 - 1.5	0 - 1.5
24	Naaran	0.3 - 2.4	0 - 2.5
25	Ein-Fara	0.3 - 2.5	0 - 2.5
26	Hezeva	0 - 1.8	0.5 - 3.0
27	Egypt	1.6 - 2.8	3.0 - 5.5
28	Turkey	1.7 - 3.2	0.5 - 3.0
29	Iran	1.2 - 3.0	0.5 - 3.0
30	Germany	7.0 - 8.2	3.0 - 5.5

D-loop analysis of haplotypes consisted of individuals from at least two distinct areas (**Figure 8**). Haplotypes 3, 5, and 8 collectively made up approximately 50% of all samples. Haplotypes 3 and 8 were common across almost all regions in Israel, whereas haplotype 5 was more prevalent in the eastern populations. It is worth noting that the outgroup populations were exclusively characterized by independent haplotypes. There was no clearly discernible geographical pattern.

Table 5. Latitude and longitude of 30 green toad breeding sites.

Site and zone			Longitude	Latitude
Northern Israel	1	Israel_Hermon	33˚17'29"N	35˚45'13"E
	2	Israel_Masade	33˚13'59"N	35˚45'09"E
	3	Israel_Orvin	33˚09'29"N	35˚40'15"E
	4	Israel _Nahalit	33˚04'56"N	35˚27'48"E
	5	Israel_Fara	33˚03'58"N	35˚27'39"E

	6	Israel_Matityahu	33°04'04"N	35°27'18"E
	7	Israel_Raihaniya	33°03'01"N	35°29'10"E
	8	Israel_Kash	33°01'47"N	35°29'26"E
	9	Israel_Jahudha	32°56'42"N	35°36'49"E
	10	Israel_Sasa	33°01'58"N	35°23'30"E
	11	Israel_Kaiv	33°02'40"N	35°14'38"E
	12	Israel_Manof	33°50'58"N	35°13'52"E
Central Israel	13	Israel_Hedera	32°26'29"N	34°54'09"E
	14	Israel_Gaash	32°13'39"N	34°49'31"E
	15	Israel_Herzela	32°11'22"N	34°48'30"E
	16	Israel_Afeka	32°07'06"N	34°49'21"E
	17	Israel_Hulon A	32°01'08"N	34°47'40"E
	18	Israel_Hulon B	32°00'07"N	34°45'31"E
	19	Israel_Shafdan	31°56'30"N	34°44'42"E
	20	Israel_Palmahim	31°55'52"N	34°42'26"E
	21	Israel_Bet-Zayit	31°46'55"N	35°09'39"E
	22	Israel_Jerusalem-Mammilla	31°46'34"N	35°13'25"E
Southern and eastern Israel	23	Israel_Gidron Mount	32°06'37"N	35°29'25"E
	24	Israel_Naaran	31°54'26"N	35°28'04"E
	25	Israel_Ein Fara (Wadi Kelt)	31°47'43"N	35°20'15"E
	26	Israel_Hazeva	30°46'03"N	35°16'42"E
Sites outside of Israel	27	Egypt	31°00'49"N	29°49'22"E
	28	Turkey	39°39'41"N	33°25'01"E
	29	Iran	38°53'20"N	45°09'53"E
	30	Germany	50°21'35"N	07°35'52"E

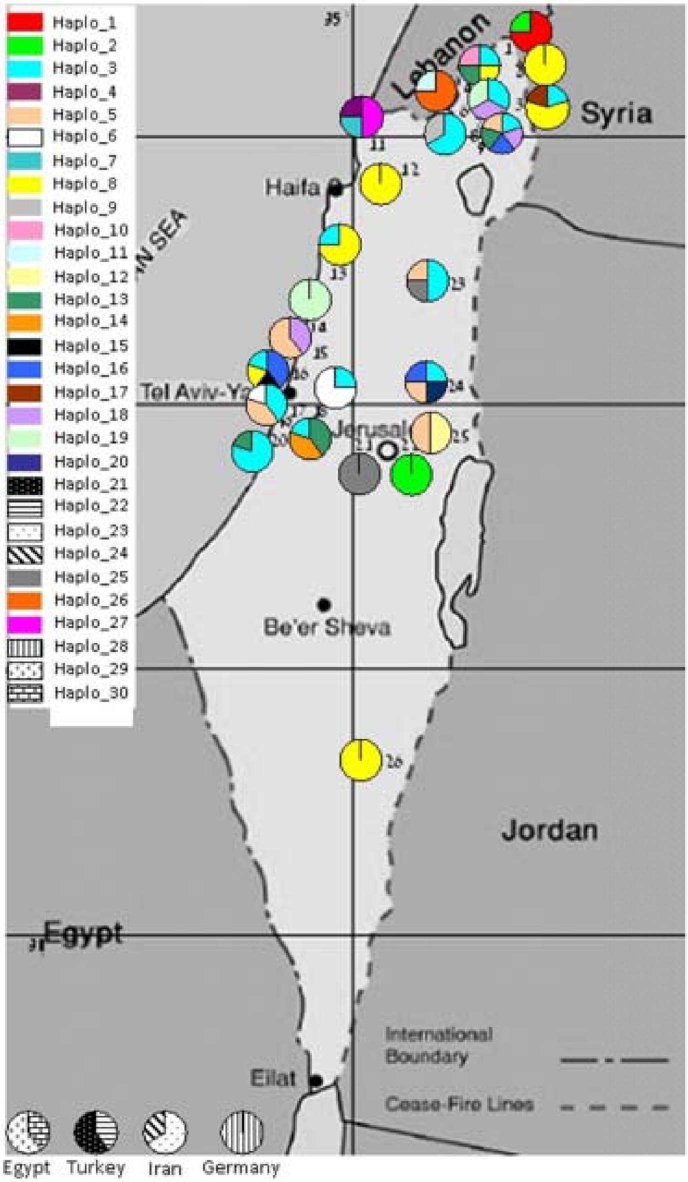


Figure 8. Haplotype frequencies of green toad populations across Israel according to D-loop analysis. Site names and localities are given in Table 5 (Degani et al., 2013a).

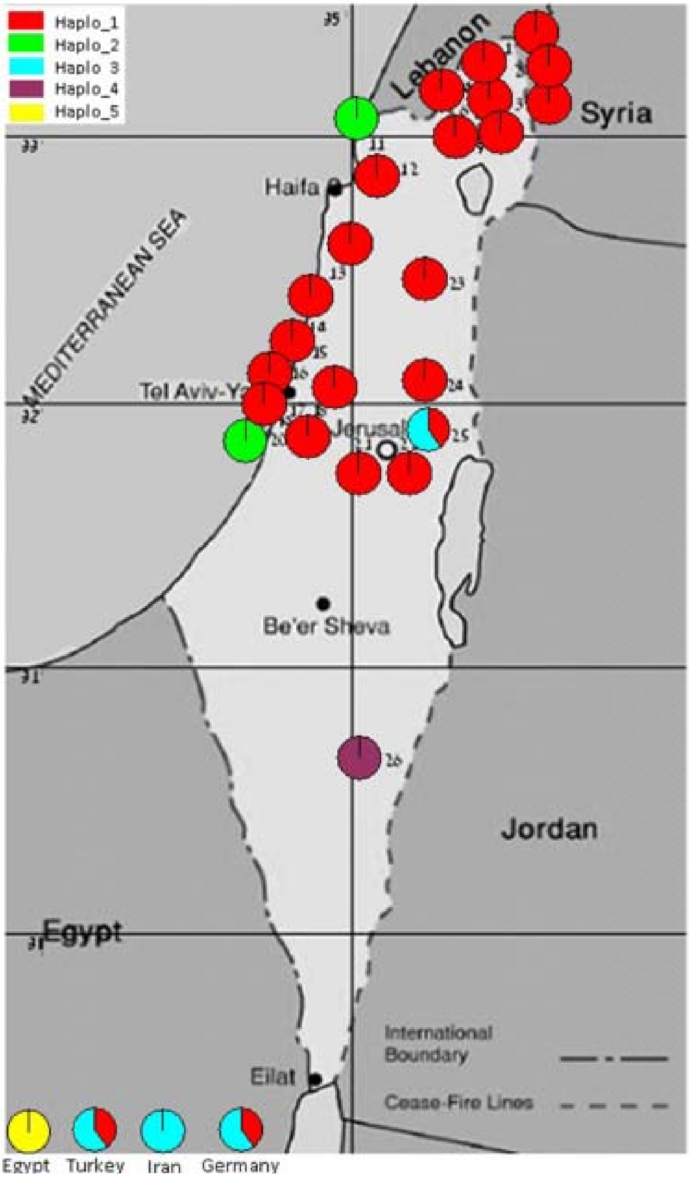


Figure 9. Frequencies of haplotypes in green toad populations across Israel based on analysis of *Cyt b*. The names of the sites and their corresponding geographical details can be found in Table 5 (Degani et al., 2013a).

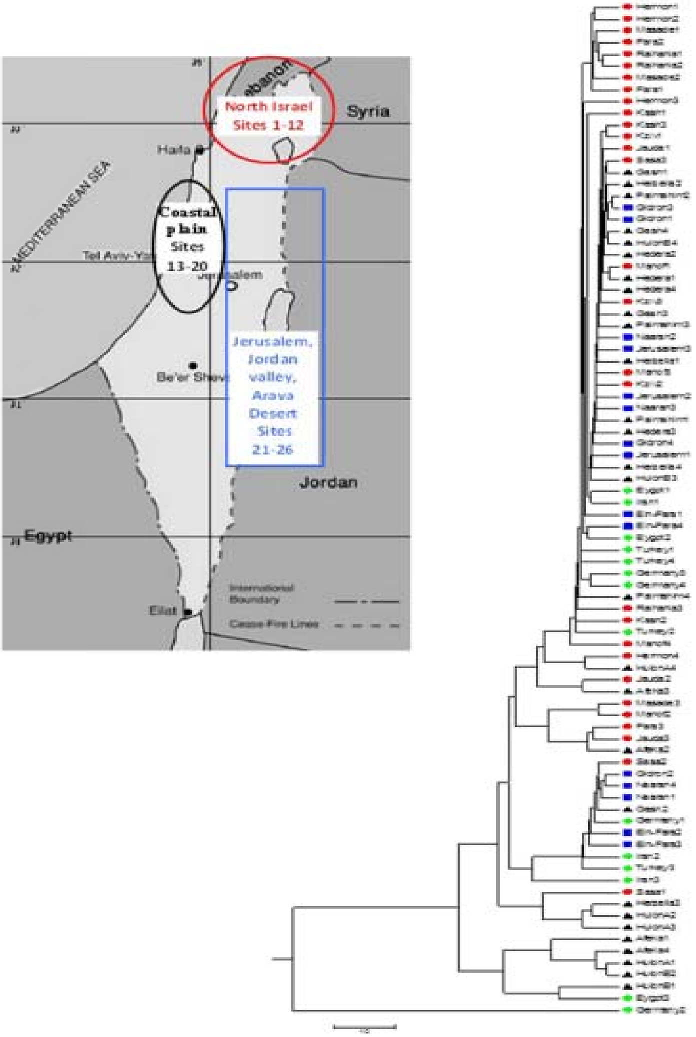


Figure 10. Dendrogram of genetic relatedness of sampled green toad individuals constructed with the MEGA5 program (Tamura et al., 2011), and estimated from AFLP analysis of 508 polymorphic loci. Numbers on the right indicate the number of individuals sampled from each population. Symbol on the left represents the region in Israel: red circle, northern Israel (sites 1 to 12); black triangle, central Israel (sites 13 to 20); blue square, southern and eastern Israel (sites 21 to 26); green diamond, sites outside of Israel (sites 27 to 30) (Degani et al., 2013a).

GenAlEx analysis revealed that the *Cyt b* samples were comprised of five haplotypes (**Figure 9**), with a total of 117 individuals. Haplotype 1 was the most prevalent (76.9% of individuals), and it was distributed across populations in northern, central, and southern Israel. Haplotypes 2 and 3 were fixed, meaning that they were present in all 13 and 10 individual sequences, respectively. Haplotypes 4 and 5 were each found in only one sample. The analysis of *Cyt b* fragments showed relatively limited variation among green toad populations. However, when comparing the *Cyt b* sequences of populations 25 and 26, the southernmost and easternmost populations, respectively, distinct clusters were observed that were not present in other populations.

Bayesian analysis of genetic population structure revealed that all individuals belonged to a single cluster, indicating their high similarity and lack of significant differences from other groups. The dendrogram in **Figure 10** was constructed using a coefficient matrix of genetic distances among all sampled green toad individuals. It shows that the genetic relationships among individuals were only moderately correlated with their geographical locations.

Analysis of three molecular markers (D-loop, *Cyt b*, and RAPD) in different populations of green toads in Israel exposed to highly diverse climates revealed significant genetic similarity, with the exception of populations located on the ecological edges of Mount Hermon and the desert regions. The AFLP analysis yielded less conclusive results, likely due to the limited number of individuals sampled (Degani et al., 2013a; Medina et al., 2006). Therefore, future studies on bufonids in Israel should aim to increase sample size in order to accurately identify the true ecological correlates of these species.

4. Tree Frogs

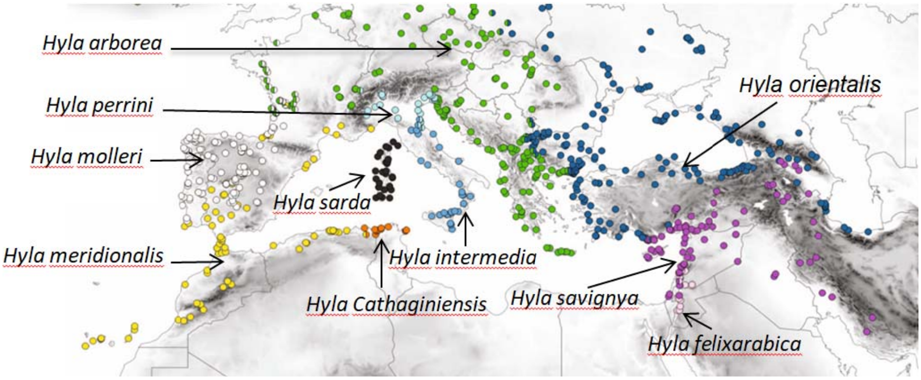
4.1 Classification

The taxonomy of tree frogs in the Middle East has not been fully agreed upon, despite extensive study of cell structure and morphology, and a combination of mitochondrial and nuclear genes (Degani, 2016; Grach et al., 2007; Gvozdik et al., 2010; Stöck et al., 2008). The high genetic diversity in relatively small areas (Degani, 2016) makes it difficult to study phylogeography and taxa using molecular markers. Gvozdik et al. (2010) carried out extensive mtDNA studies and suggested that the tree frog *Hyla savignyi* is found in Israel in the Golan Heights, and that its geographical distribution also includes Jordan, Syria and Lebanon. However, other species might also be found in Israel, such as *Hyla felixarabica* sp. nov. Grach et al. (2007) described the endemic population of another species in Israel, *Hyla heinzsteinitzi*, based on morphological characterization, coloration and cell structure. *H. heinzsteinitzi* is located at three sites in a small area (6 × 13 km^2) in the Judean Hills at altitudes of 730 - 895 m above sea level. The tree frog is one of the most important amphibians in northern Israel. Many nocturnal anuran species are colored (see review by Gomez-Rodrıguez et al., 2009) and exhibit a large repertoire of visual displays. The various colors of tree frogs in Israel are presented in **Figure 11**. The dominant color is green, but with large variation, from dark green to bright green. Variation is also found for the other colors (black, brown and white) (Degani, 2013). Most studies on the colors of tree frogs have addressed the European species, *Hyla arborea* (Nielsen, 1980), with more information published than for *H. savignyi*.

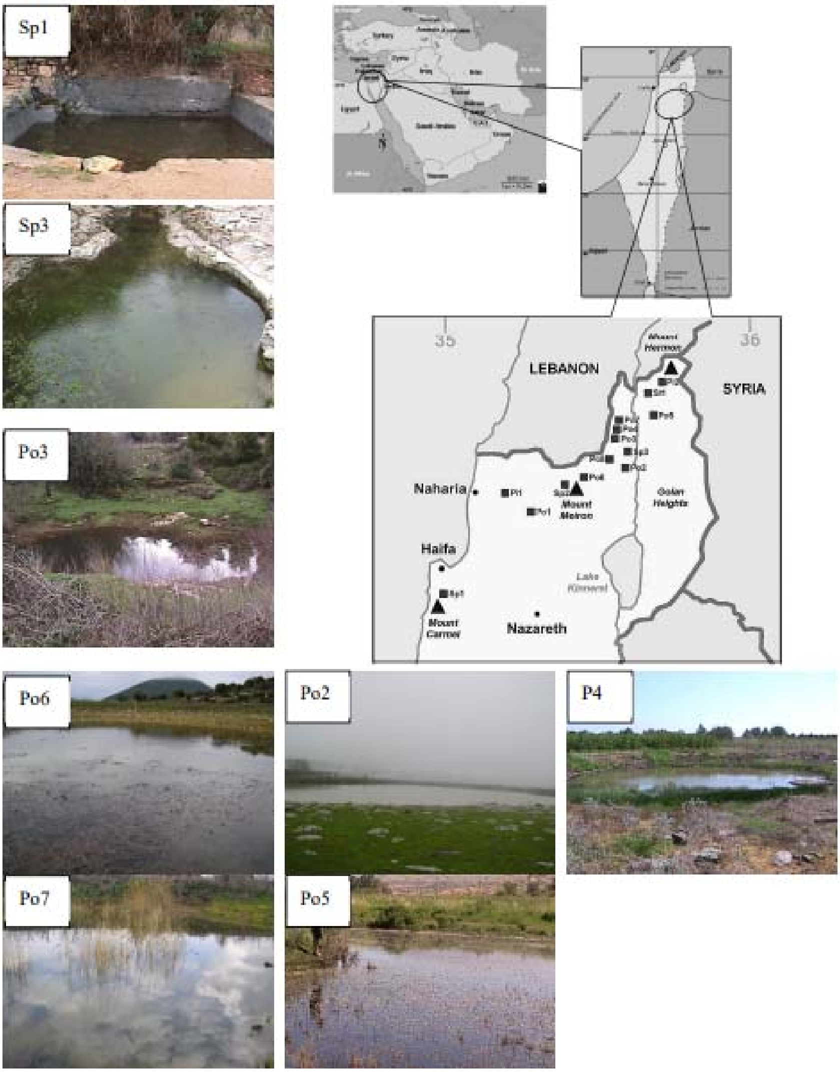
Figure 11. Different colors of the tree frog *Hyla savignyi* (Degani, 2016).

4.2 Distribution

Many aspects of the *Hyla* life cycle, especially in Europe, have been studied and published. *Hyla* species are nocturnal, and the males display a vocal sac and a dark flank stripe, both varying in coloration (Gomez-Rodrıguez et al., 2009; Grach et al., 2007). The life cycle of *H. arborea* has been described in detail in its wide distribution throughout Europe: Denmark (Aarhus and Jutland), Sweden (Scania) and the southern shore of the Baltic Sea in Lithuania (Vilnius City), through Byelorussia (approximately along the line joining the towns Oshmyany-



(A)



(B)

Figure 12. (A) Distribution of tree frogs in the western Palearctic region combining information from 25 research studies (Dufresnes et al., 2020). (B) Various habitats of the tree frog *Hyla savignyi* in northern Israel: Sp1, Bald Spring; Sp3, Navoraya Spring; Po2, Kash Pond; Po3, Dovev Pond; Po4, Matityahu Pond; Po5, Lehavot Pond; Po6, Sasa Pond; Po7, Fara Pond (Degani, 2016).

Uzda-Slutsk) to southern Russia. From there, the margin runs south and southeastward approximately along the line of the Bryansk Province-Kursk Province west of the Byelgorod Province (Shebekino District), then southward in the Ukraine from Kharkov City to the Dnepropetrovsk Province and the Donetsk Province. In Crimea, *H. arborea* is found along the southern shores and extreme northwest of the peninsula. It is active primarily in the evening and at night, when it descends from the vegetation to forage and rehydrate on the ground. During the autumn, from September-December to February-early May, it migrates to Europe to hibernate. Hibernation takes place on land (in soil burrows, heaps of stones and holes in trees). Reproduction occurs at different times from April to May, but sometimes in March, June or even late July. As a rule, more males are found in breeding pools than females. Females enter the pools after the males and leave immediately after breeding.

4.3 Life cycle

The life cycle of *Hyla savignyi* in various habitats in northern Israel—the Upper Galilee and Golan Heights (annual rainfall range of 500 - 1,000 mm) and the Hula valley, and the surrounding mountains ranging from 212 to 740 m above sea level—was studied. Tree frogs were observed around winter rain pools, springs and streams. Fifty-one different breeding places were monitored. Only ponds and springs with stable non-flowing water are used by tree frogs for reproduction and for metamorphosis of the larvae (**Figure 12**). The male call attracts the female to the breeding site, and breeding occurs underwater (Degani, 2016). The time of breeding in Israel varies according to location and geographical and ecological conditions. It takes place in March-April in northern Israel. The larvae grow between April and June. Tree frog larvae seem to be adapted to breeding places with still water, such as ponds or springs, but not flowing water such as streams or rivers.

Information on the number of eggs laid per female varies. A clutch contains about 200 - 1,000 eggs, usually deposited in small rounded clumps of 3 to 100 eggs each over several hours, after which the female leaves the pool (Degani, 2016). Metamorphosis occurs from June to September, depending on the geographical location. In some cases, the larvae overwinter and complete transformation the following summer. The tree frog preys on various invertebrates, mainly insects. Its ability to take long leaps allows it to forage for fast-flying insects, which comprise a considerable proportion of its diet. During the breeding season, adults forage periodically on the shore and on high plant stems above the water surface.

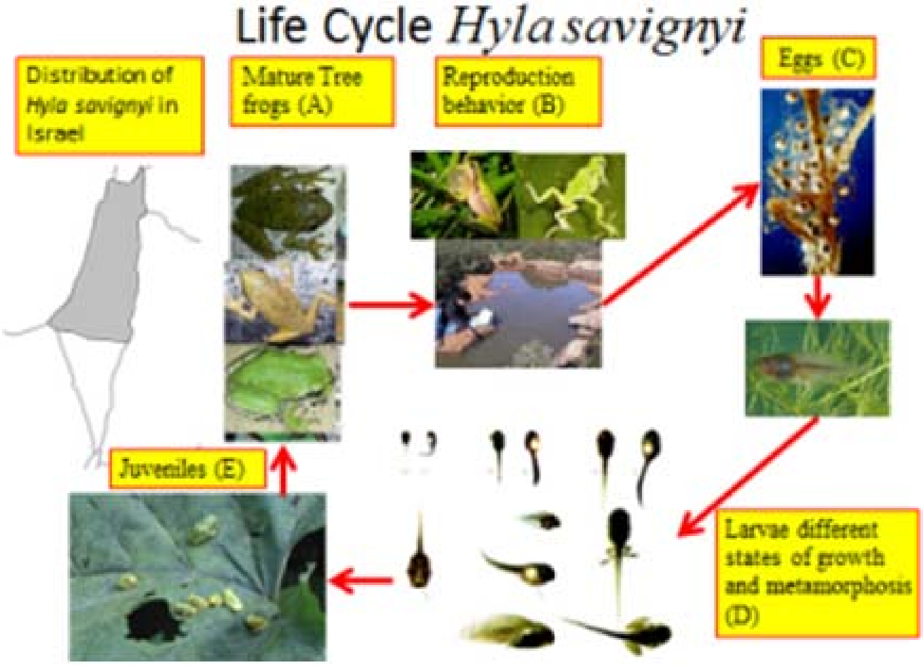


Figure 13. Life cycle of the tree frog *Hyla savignyi.* (A) Mature tree frogs. (B) Reproductive behavior. (C) Eggs. (D) Larvae at different stages of growth and metamorphosis. (E) Juveniles (Degani, 2016).

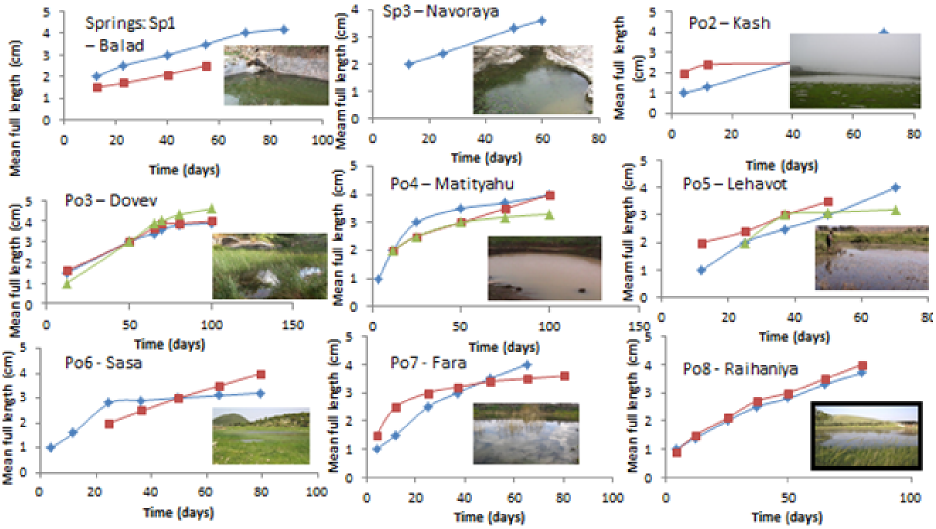


Figure 14. Growth rate of tree frog (*H. savignyi*) larvae in various habitats: Sp1, Balad Spring; Sp3, Navoraya Spring; Po2, Kash Pond; Po3, Dovev Pond; Po4, Matityahu Pond; Po5, Lehavot Pond; Po6, Sasa Pond; Po7, Fara Pond; and Po8, Raihaniya Pond (Degani, 2016, 2022).

4.4 Frog color and substrate preference

Despite intense interest in the color variations of the *Hyla* genus, relatively little is known about how these variations affect their selection of substrate, described mainly by Degani and Biton (2013).

Most studies on *Hyla* color preferences focus on sexual interactions and males' selection of females (Grach et al., 2007; Gomez-Rodrıguez et al., 2009). The ability to change color while adapting to different habitats, especially in the case of relatively small frogs such as *Hyla* (Degani and Biton, 2013; Stöck et al., 2008), differs from that of other species of tree frog that change color, e.g., the Pacific tree frog (*Pseudacris regilla*) (Grismer, 2002). This supports the notion that the ability to change color and plant preferences depend on adaptability to different habitats. In Israel, *H. savignyi* tree frogs reproduce in many unpredictable habitats where the water dries up during the summer and there are only a few rainy months each year, and where all of the grass dries up and the ground turns brown or black (Degani and Kaplan, 1999; Goldberg et al., 2009b). Degani and Biton (2013) found that tree frogs prefer green plant substrates. However, what is less known is that tree frog color changes according to substrate color (Degani and Biton, 2013; Stöck et al., 2008), suggesting that crypsis (i.e., background matching) is not a only function of physiological color change, but physiological color change may also be used for hydro- and/or thermoregulation. *H. savignyi* is abundant in northern and central Israel and can be found throughout the entire year, including the hot, dry summers. In summer, while the tree frog clings to leaves with circulating air, the danger of dehydration increases (Warburg, 1971, 1992). I suggested that the function of the intricate dorsal pattern of *Hyla ornatissima* serves to protect the animal from predation, camouflaging it on the leaves of the trees where the animals live. During the hot, dry summer, the tree frogs seek hiding places on the ground to prevent dehydration. *H. savignyi* survives in xeric habitats by being able to tolerate high rates of water loss and to rapidly replenish water. This is due to their ability to tolerate a decrease in the volume of plasma and an increase in hematocrit (Degani, 2022). They are also able to tolerate an increase in plasma and muscle osmolality with increased levels of NaCl, K^+ and Cl^- (Degani and Warburg, 1984). During the summer, many of the ponds where *H. savignyi* breeds dry up (Degani and Kaplan, 1999; Goldberg et al., 2009a, 2012); annual plants (therophytes) die and the color of the ground changes from green to brown or black (Degani, 2022). Only green perennial plants grow during the summer in Israel. Their microclimate is more suitable for *H. savignyi* with respect to preventing dehydration during dry periods (Degani and Warburg, 1984). A high preference is exhibited by *H. savignyi* for green plants, which can help the frog endure dry conditions. Thus, *H. savignyi*'s ability to change color for camouflage as it moves to different substrates is a very important adaption mechanism in xeric habitats where the color of the environment varies between seasons.

To analyze and characterize tree frog behavior, 30 mature tree frogs were collected in Moshav Haspin, which is located in the southern Golan Heights (longitude 274522, latitude 750012 and altitude 420 m above sea level) from November 15 - 20, 2011. They were kept in an aquarium measuring 180 cm x 60 cm x 60 cm covered by a net and divided into four parts (45 cm), with different substrates made of white stones, basalt stones and black soil, brown clay stones and green plants (**Figure 15**). Frogs chose the green vegetated part of the aquarium over the other substrates.

Figure 15. Aquarium with different colored substrates (Degani and Biton, 2013).

The preference for substrate color is shown in **Figure 16**. The change in substrate color preference changed mainly for the green and white colors during the experimental period. The percentage of frogs that selected the green substrate increased from 39% to 68%, whereas that of those selecting the white substrate decreased from 43% to 18%. However, the preference for the black and brown substrates was low and did not change considerably during the experimental period. There was a strong correlation between substrate color and frog color. The color of the tree frog was affected by the substrate: 87.5% of the white frogs chose the white substrate, 83% of the brown frogs chose the brown substrate, 82% of the black frogs chose the black substrate, and 95% of the green frogs chose the green substrate. There were no significant differences between night and day in choice of substrate color.

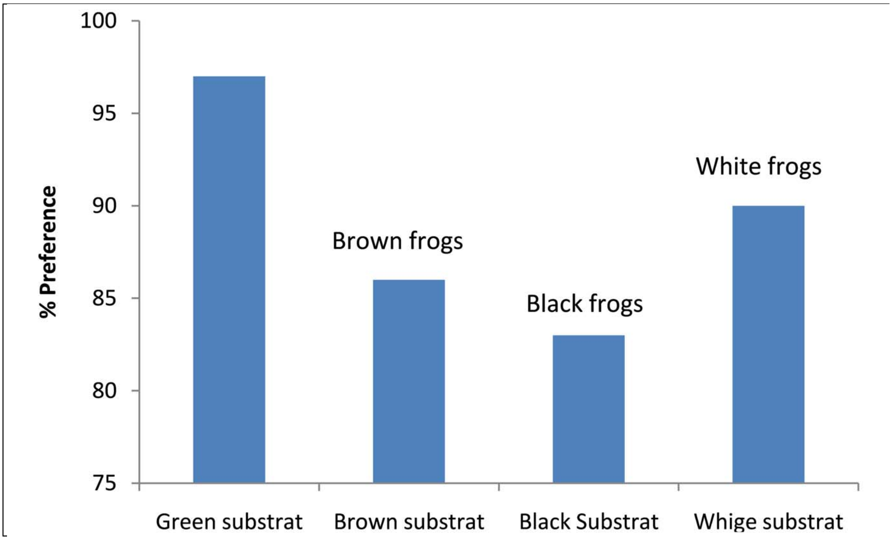


Figure 16. The different colors of *Hyla* and their preference for substrate color (Degani and Biton, 2013).

4.5 Tree frogs in unstable habitats

The tree frog's body colors vary with habitats and seasons, to match the colors present in their surroundings. **Figure 17** provides examples of the color changes observed in the tree frog habitat. In winter and early spring, various shades of green can be seen in and around water bodies. Over time, the colors transition from light green to brown or even black. Throughout the year, the tree frogs exhibit colors that blend harmoniously with their environment (**Figure 17**) (Degani, 2022).

During the breeding season, when the tree frogs migrate to the ponds, the winter pond area is lush with green vegetation, and the tree frog colors match those shades of green. As the winter ponds dry up, the tree frog colors change to brown and black to blend in with the new habitat colors (**Figure 18**) (Degani, 2022).

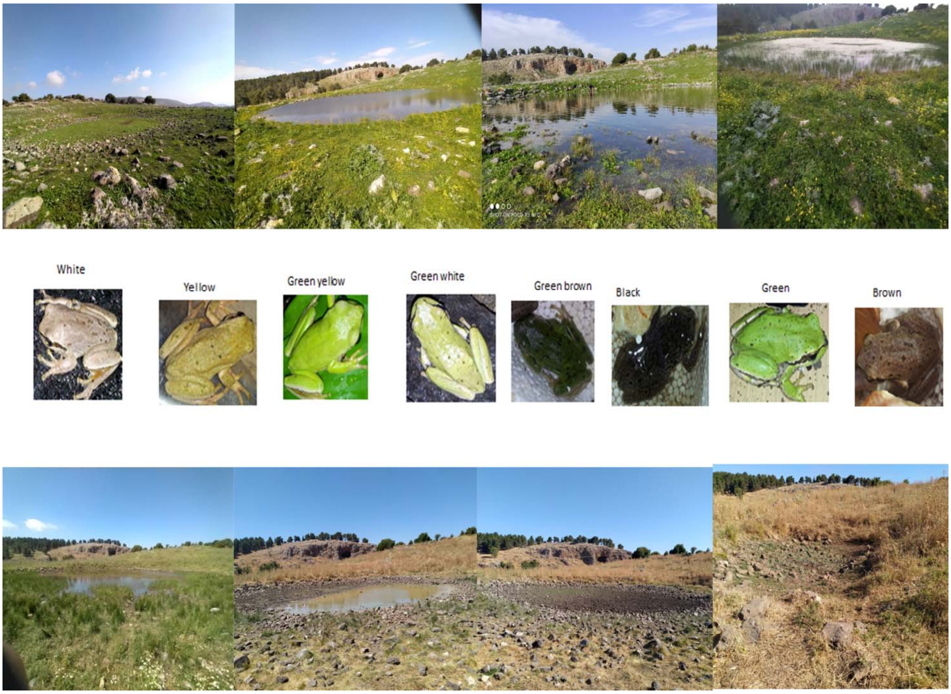


Figure 17. The winter pond, located at Nahalit Pool (35˚27'48"E 33˚04'56"N, elevation 665 m above sea level), and its surrounding area undergo notable changes in color and are inhabited by tree frogs that exhibit matching hues throughout the winter and summer seasons.

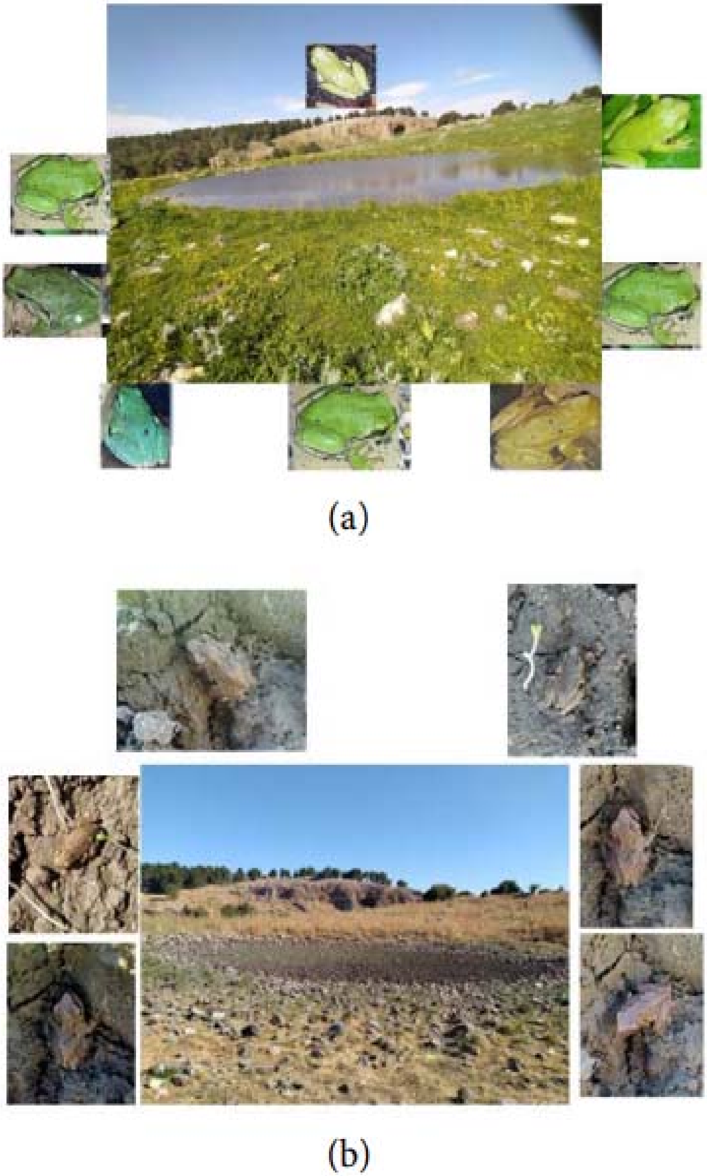


Figure 18. (a) In the winter, the pond area is covered with green (22 Feb 2021). (b) The dried pond, 24 May 2021 (Degani, 2022). Brown and black tree frogs were found in the dry pond, between and underneath the stones.

4.6 Adaptation to a semi-desert habitat: water loss and tolerance to changes in plasma ion and urea contents

Tree frogs are abundant in northern and central Israel, and can be found throughout the year, including the hot and dry summers. In summer, they are at risk of dehydration. The frog is small, and its skin does not prevent water loss. The ability to adapt to a habitat like that in Israel involves changes in plasma concentrations of ions and urea (Degani and Warburg, 1984). Ion (Na^+, K^+, Cl^-) and urea concentrations in the plasma and tissues of dehydrated frogs were compared, and the rates of dehydration and limits of water loss as well as rehydration in both juvenile and adult frogs were studied. An attempt was made to establish relationships between the water content of the plasma and tissue ion and urea concentrations (Degani, 2016).

The rate of evaporative water loss in the juvenile tree frog was significantly higher than that in adults. Juveniles reached their limit of water loss after 4 days (at 10˚C and 0 - 5% relative humidity), whereas adults reached this point after about 2 weeks. The maximum limit of water loss was 4.57% for adults and about 35% for juveniles. In both adults and juveniles, most of the water uptake took place after 2 hours. There were no significant differences between the two groups after that period.

Plasma concentration increased following dehydration as shown by the linear correlation between body water loss and increased plasma concentration (**Figure 19**). Maximum plasma concentration was 510 mOsm/kg in dehydrated frogs (which lost 4.5% of their body weight due to muscle dehydration). During the increase in plasma concentration the hematocrit rose to 65% (**Figure 19**).

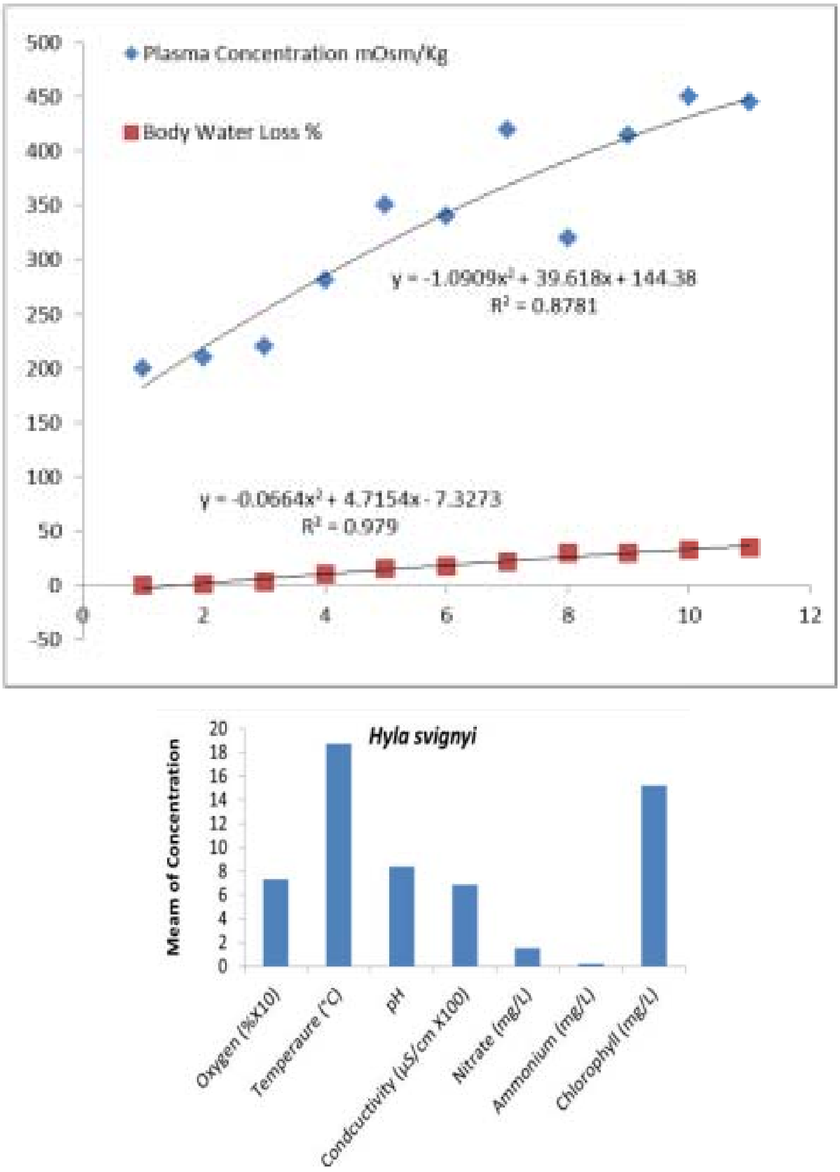


Figure 19. Change of osmolarity in blood plasma of *Hyla* during dehydration. C = 8.8B + 199.2 (C = plasma concentration in mOsm/kg. B = body weight loss. r = 0.92; *P* < 0.001) (Degani, 2016).

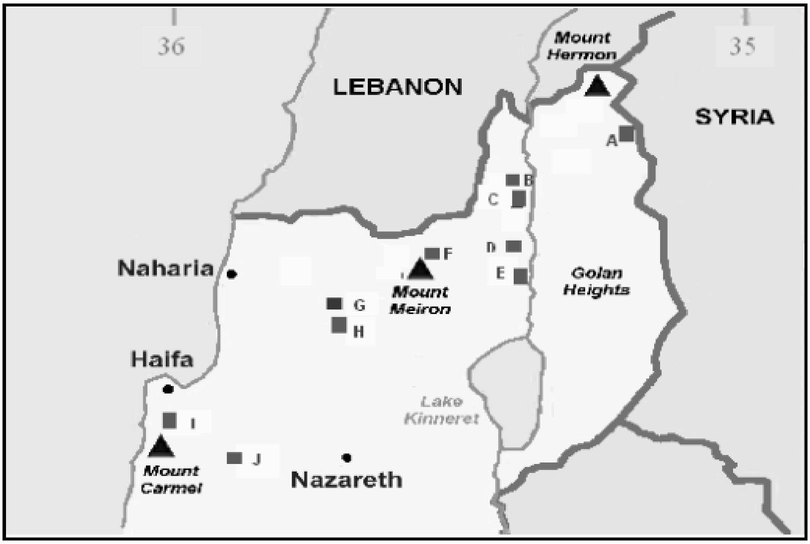
4.7 Molecular genetic diversity across breeding sites in northern Israel

The genus *Hyla* includes 31 distinct species found in the Holarctic regions, which include North America, Asia, Europe, and the northern part of Africa. These species are further classified into five species groups: *H. arborea*, *H. cinerea*, *H. versicolor*, *H. savignyi* and *H. eximia* (Degani et al., 2012).

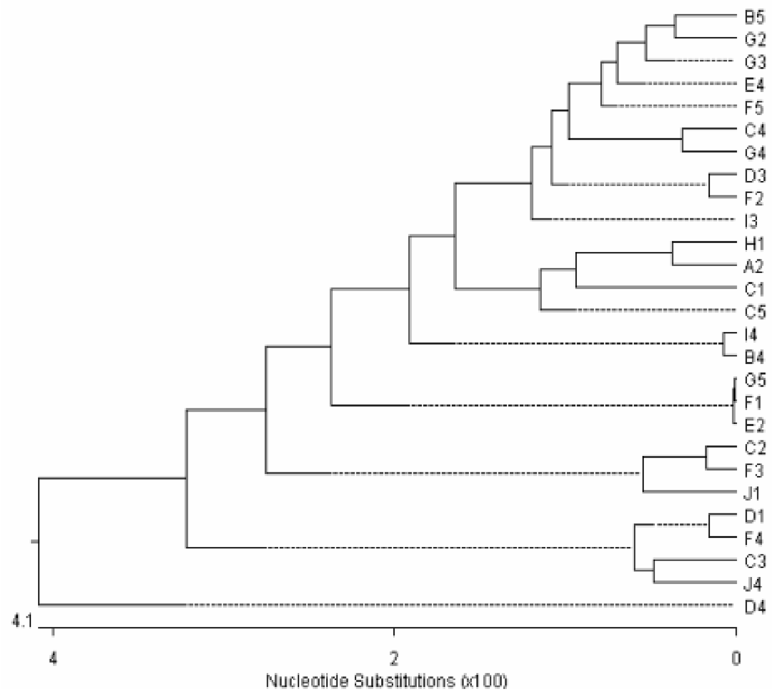
In a comprehensive study conducted by Stöck et al. (2008), mitochondrial and nuclear markers were employed to analyze the various *Hyla* species and subspecies across circum-Mediterranean areas. Their investigation in the Middle East revealed the presence of *H. savignyi* in Cyprus, where it thrives in well-illuminated habitats characterized by broad-leafed and mixed forests, bush and shrub lands, meadows, gardens, vineyards, orchards, parks, lakeshores, and low riparian vegetation. These frogs predominantly inhabit arboreal environments, residing on bushes, shrubs, and trees. In Israel, the taxonomy of three frog species, ranging from *H. arborea* to *H. savignyi*, was distinguished through the analysis of mtDNA and nuclear DNA fragments (Stöck et al., 2008). Andersen et al. (2004) investigated the population structure of the European tree frog, *H. arborea*, in Denmark, considering suitable habitats and population sizes. Their study utilized the genetic variation of 12 polymorphic DNA microsatellites.

To investigate the molecular DNA variation in *H. savignyi*, we collected samples from multiple breeding sites in northern Israel. We employed various genetic techniques, including DNA extraction, amplification of specific genetic markers, and sequencing. *Cyt b* and *12S* were amplified for sequencing and used for the assessment of genetic variation by RAPD PCR (Degani et al., 2012). The DNA fragments' nucleotide sequences were deciphered from a 255-bp clone of *Cyt b* and a 320-bp clone of *12S* (**Figure 20**). Within the *Cyt b* fragment, there were variations at nucleotide positions 64, 124, 143, 145, and 175 among populations residing in different breeding sites. A detailed breakdown of these variations can be found in Degani et al. (2012). The investigation of *Cyt b* variation among populations of *H. savignyi* from distinct breeding sites revealed a comparable scenario. Using Arlequin software, the analysis of nine sequences indicated a high degree of genetic similarity, with gene identity ranging from 98.4% to 100%. The examination of *Cyt b* revealed that the genetic variation within individual *H. savignyi* specimens is evenly distributed among the populations. While the genetic variation within populations was found to account for 30% of the total, an even higher significant variation of 69.74% was observed among all populations.

To assess DNA variation of *H. savignyi* at different breeding sites, the primers OPA-16 and OPA-18 were employed, as illustrated in **Figure 21**. The OPA-16 primer revealed 5 to 14 identical bands, and OPA-18 yielded 7 to 14 identical bands. The number of common bands ranged from 0 to 7 with OPA-16 and from 0 to 8 with OPA-18. Notably, certain populations exhibited markedly low similarity when compared to the rest of the populations, such as those from Leshem Pond and Jauda Spring when analyzed using the OPA-16 primer (**Figure 21**).



(A)



(B)

Figure 20. (A) The 10 examined breeding sites colonized by *Hyla savignyi*. A—Elrom Pond, B—Fara Pond, C—Matityahu Pond, D—Navoraya Spring, E—Jauda Spring, F—Sasa Pond, G—Leshem Pond, H—Dir-Hanna Pond, I—Balad Spring, J—Gahar Stream. (B) Unrooted phylogenetic tree of the partial *12S* fragment based on nucleotide sequences of *H. savignyi*. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events. The phylogenetic tree was constructed using the MegAlign program (DNASTAR) by the CLUSTALW method. The branch length represents the evolutionary distance (Degani et al., 2012).

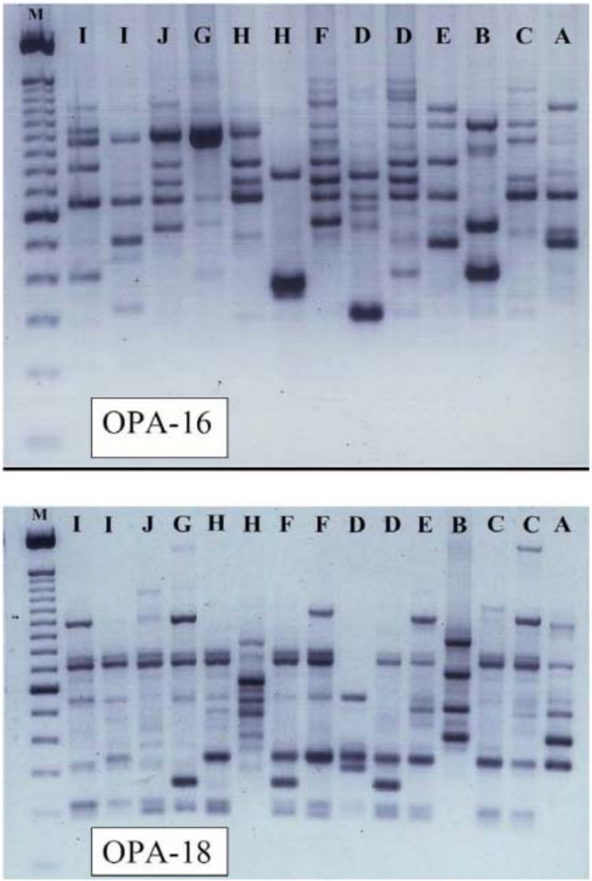


Figure 21. RAPD PCR results using primers OPA-16 and OPA-18. A—Elrom Pond, B—Fara Pond, C—Matityahu Pond, D—Navoraya Spring, E—Jauda Spring, F—Sasa Pond, G—Leshem Pond, H—Dir-Hanna Pond, I—Balad Spring, J—Gahar Stream.

5. Eastern Spadefoot Toad

5.1 Classification and description

Pelobates syriacus, commonly known as the eastern spadefoot toad, can be classified based on its unique characteristics. Unlike other toads, it lacks a tympanic membrane, and has smooth skin and an ossified sternum. Its eyes have vertical pupils, and it has well-developed webbing between its toes. One distinctive feature is the presence of large and spade-shaped inner metatarsal tubercles in its hind feet. Spadefoot toad species tend to be larger in size than other toads. In addition, their vomerine teeth series is longer and more divided, with a narrower gap. The inner metatarsal tubercle is quite large and yellowish in color. The webbing between the toes exhibits prominent incisions. The frontal area between the eyes is not particularly pronounced. The dorsal coloration of these toads typically ranges from yellowish to gray, adorned with large dark-greenish spots. The ventral surface is whitish-gray and lacks any discernible pattern (Degani, 2015).

5.2 Distribution

Taxonomy and distribution of *Pelobates* species is presented in **Figure 22**. The evolutionary relationships and geographical spread of different *Pelobates* species were studied by Dufresnes et al. (2020) using phylogenomic analysis. The distribution information was derived from updated records combined with genetic data, and a map was created based on these findings. According to this research, *Pelobates vespertinus* has a wide distribution range, extending to Kazakhstan and Siberia in the eastern regions. The area study by Degani (2015) described the *Pelobates* species in Israel (**Figure 22**). Spadefoot toads are found in various regions, including Azerbaijan, Iran, Iraq, Israel, Lebanon, Russia, Syria and Turkey (*P. syriacus*), and Bulgaria, Greece and Romania (*P. balcani-*

cus), demonstrating their wide distribution across different countries (Degani, 2013) (**Figure 22**).

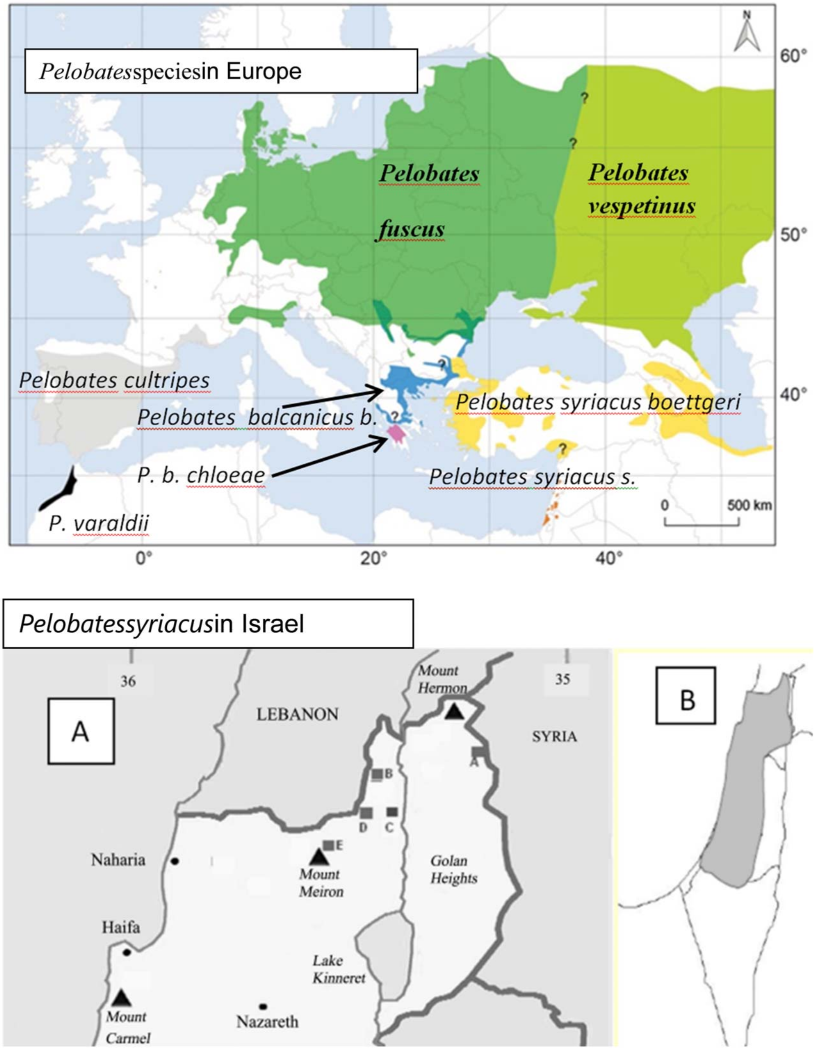


Figure 22. Distribution of *Pelobates* species in Europe (Dufresnes et al., 2020) and *Pelobates syriacus* distribution in Israel (Degani, 2015). A—Elrom Pond, B—Fara Pond, C—Kash Pond, D—Raihaniya Pond, E—Sasa Pond.

5.3 Life cycle

The secretive nature of the spadefoot toad, characterized by its nocturnal activity, faint underwater breeding call, and hidden appearance, has resulted in a lack of comprehensive information about its life history (Degani, 2015). In addition, the genus *Pelobates* is found in various climates, which influence its life cycle. Despite these challenges, some fundamental biological traits are shared among the species, which can aid in filling the knowledge gaps specifically for *P. syriacus* in Israel. A detailed account of the eastern spadefoot toad's habitats, life cycle, growth of larvae, burrowing behavior, and adaptation to terrestrial environments in Israel can be found in Degani (2015). The findings are based on extensive observations and data collected over a period of more than 30 years in the country's northern region (**Figure 23**).

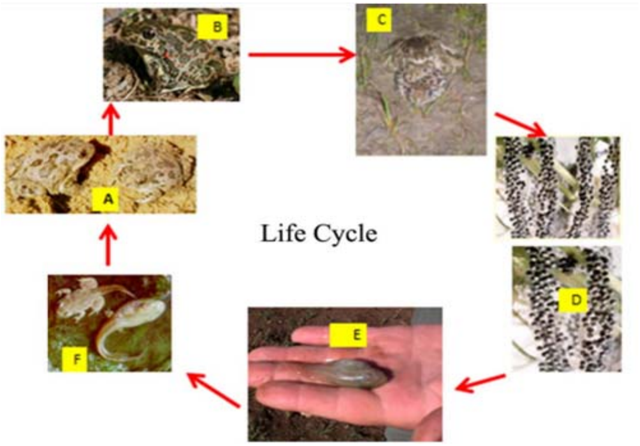


Figure 23. The life cycle of ***Pelobates syriacus*** can be divided into several stages. (A) Juveniles. (B) Mature females. (C) Amplexus, which refers to the mating embrace of the male and female during the reproductive process (Cogălniceanu et al., 2013). Following successful mating (D) the female lays eggs, which are then typically deposited in water bodies such as ponds or temporary pools. (E) The eggs develop into tadpoles. As the tadpoles grow and develop, they undergo metamorphosis (F). This stage marks the transition from an aquatic lifestyle to a terrestrial one.

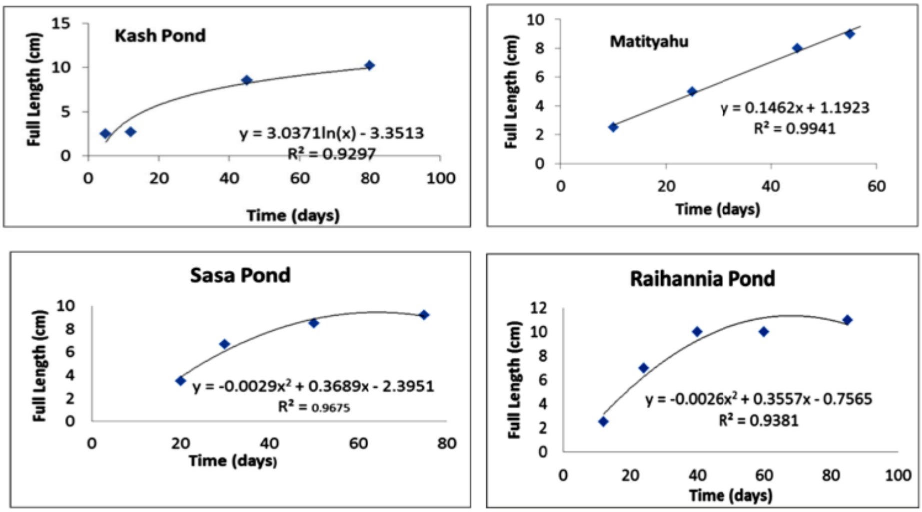


Figure 24. The growth rates of *Pelobates syriacus* larvae are depicted in the graph, where the Y-axis represents the average length of the larvae and the X-axis represents the duration of time, in days, that the larvae spend in the water body until they undergo metamorphosis (Degani, 2015).

The breeding process begins with male toads arriving at the pond and emitting a distinctive mating call, resembling the sound of a crow, known as a "wonk." The females then arrive, and breeding takes place in the water. The timing of breeding in Israel varies based on geographical and ecological factors, with March-April being the breeding period in northern Israel—Upper Galilee, and the Golan Heights, whereas in lower and central Israel, it occurs earlier, even during the winter. During amplexus, the male grasps the female above her hind legs, and they swim together as a unit. The female then deposits 2 to 4 clusters of eggs (**Figure 23**). The number of eggs can vary significantly, ranging from 2,000 to 10,000 per female. Larval growth and the complex process of metamorphosis take place in northern Israel from summer to early autumn, typically until October. **Figure 24** provides an overview of larval growth in different breeding locations (Degani, 1982; Goldberg et al., 2009a, 2012). Unfortunately, there is a lack of information regarding the adult terrestrial activity of eastern spadefoot toads. However, considering the distribution of rain pools and the limited dispersal range of juvenile adults, estimated to be around 2 to 4 km, it appears that the population is predominantly isolated.

5.4 Terrestrial activity

A brief overview of the adult terrestrial activity of the spadefoot toad reveals interesting patterns. One study indicated a noteworthy negative correlation between the percentage of toads engaged in burrowing behavior and soil moisture levels (Degani and Carmali, 1988). In soil with low moisture content (40%), a relatively high percentage of toads (73%) were observed to be buried, whereas a significantly lower percentage (9%) was found in soil with a high moisture level (100%) (**Figures 25** and **26**).

Soil moisture can have a significant effect on the burrowing behavior of *P. syriacus*. These toads are semi-fossorial, meaning that they spend a significant portion of their lives underground, especially during periods of adverse environmental conditions or aestivation (**Figures 25** and **26**). Soil moisture can influence the burrowing behavior of spadefoot toads by: (i) facilitating burrowing: adequate soil moisture provides the necessary conditions for toads to excavate burrows effectively. Moist soil is easier to manipulate and dig through than dry or compacted soil. Spadefoot toads have specialized adaptations on their hind feet, including a spade-like structure, which allows them to efficiently dig burrows. Moist soil enables them to use this adaptation more effectively; (ii) maintaining burrow integrity: burrows dug by spadefoot toads serve as a shelter from extreme temperatures, predators, and desiccation. Moist soil helps maintain the structural integrity of the burrow walls, preventing collapse or erosion due to weather conditions or disturbances. If the soil is too dry, the burrow walls may become unstable, potentially endangering the toads. Spadefoot toads often burrow during periods of aestivation—a dormant state during hot and dry periods. Aestivation helps them conserve water and energy. Adequate soil moisture is crucial for toads to dig deep enough burrows to find a suitable microenvironment with higher humidity and lower temperatures. Moist soil conditions are essential for their successful aestivation.

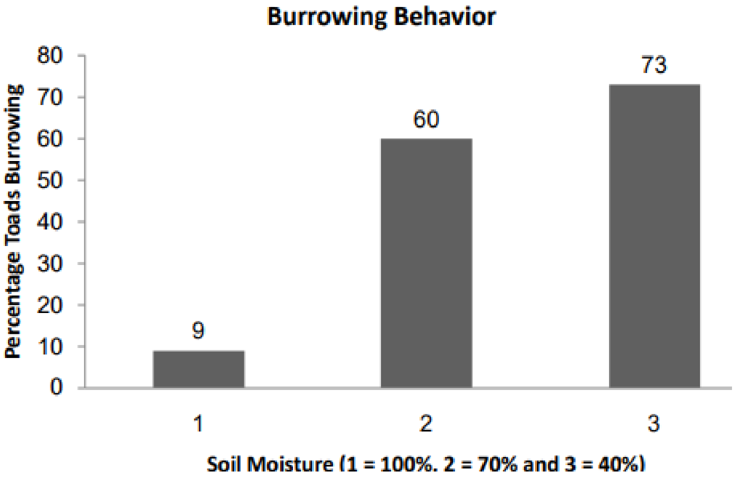


Figure 25. The behavior of burrowing spadefoot toads is influenced by soil moisture. Burrowing spadefoot toads, like earthworms, termites, and certain types of insects, have different preferences and adaptations to soil moisture levels (Degani and Carmali, 1988).

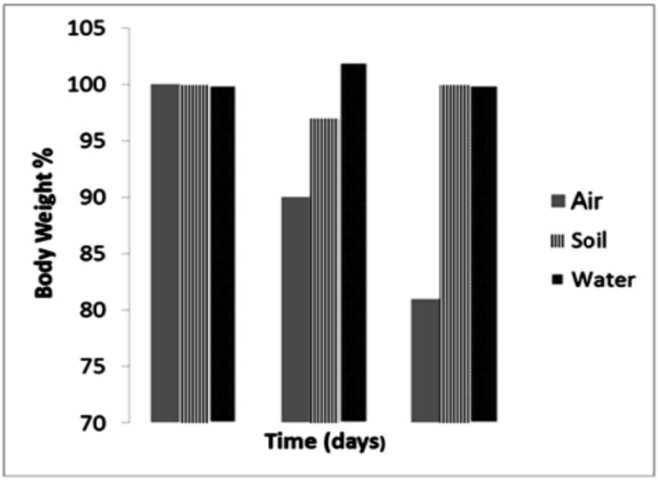


Figure 26. Spadefoot toads experience desiccation under various conditions: exposed to the atmosphere and nestled within the earthy confines of a soil burrow (Degani, 2015).

5.5 Reproduction

Spadefoot toads typically breed in temporary pools or ponds formed by rainwater. Soil moisture plays a critical role in determining the availability of breeding habitats. Sufficient soil moisture allows for the formation and maintenance of these temporary water bodies, which are crucial for successful reproduction and the development of the tadpoles. It is important to note that the specific preferences and behaviors of spadefoot toads can vary based on regional and local conditions. Factors such as temperature, rainfall patterns, and soil composition can also influence their burrowing behavior in conjunction with soil moisture levels. Research studies focusing on local populations of spadefoot toads can provide more precise insights into their specific responses to soil moisture variations in a particular habitat.

5.6 Dehydration in air

When spadefoot toads are exposed to dry air for an extended period, they experience dehydration. Their skin, which is typically moist and permeable to water, starts to lose moisture rapidly. This can lead to several physiological changes in the toad's body. For instance, the toad's skin may become dry, making it less efficient at gas exchange and potentially impairing its ability to breathe. Dehydration can also affect the toad's metabolic processes, leading to a decrease in overall activity and potentially causing physiological stress. In extreme cases, severe dehydration can be fatal to spadefoot toads (**Figure 27**).

5.7 Burrowing in soil

Spadefoot toads have the ability to burrow into the soil, especially during periods of dryness or drought. Burrowing helps them find moister environments and escape the dehydrating effects of the air. When buried in the soil, the toads reduce water loss through their skin due to the higher humidity and moisture content in the ground. The soil provides insulation and a more stable microclimate, reducing the rate of water loss and preventing excessive dehydration.

This adaptation allows spadefoot toads to survive while waiting for more favorable conditions.

5.8 Over-hydration

Although spadefoot toads are adapted to both terrestrial and aquatic environments, they are not fully aquatic species. If a spadefoot toad is submerged in water for a prolonged period, it may experience negative effects due to over-hydration. The toad's skin, which usually plays a role in water absorption, may become oversaturated and less efficient at gas exchange. Extended periods in water can also lead to imbalances in the toad's electrolyte levels, affecting its overall health and physiology. While some species of toads have adaptations to tolerate aquatic conditions for short periods, continuous submersion can be detrimental to spadefoot toads.

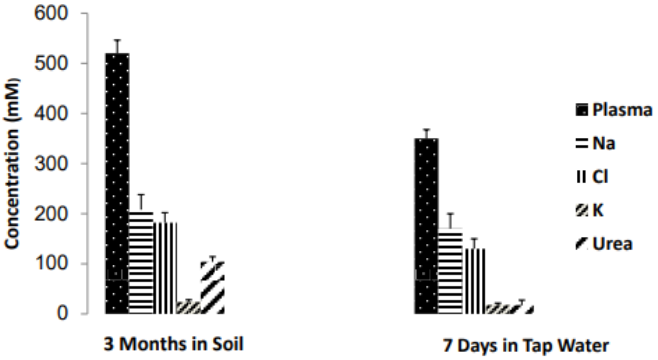
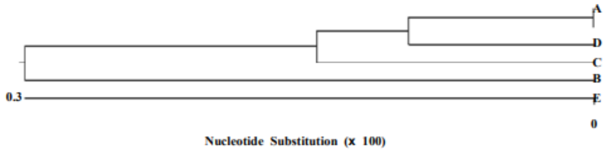


Figure 27. Alterations in the plasma ion and urea composition were observed in young *Pelobates syriacus* individuals inhabiting subterranean tunnels and aquatic environments (Degani, 2015).

5.9 Genetic differences in different populations within breeding locations

Research was conducted to examine the genetic characteristics of eastern spadefoot toads dwelling in different habitats and elevations in the northern region of Israel (**Figure 22**). The study involved PCR amplification of *Cyt b* and *12S*, enabling an analysis of genetic variation based on five DNA polymorphisms and the utilization of RAPD PCR (Degani, 2013). Looking at the nucleotide sequences of the mtDNA fragments from a 460-bp clone of *Cyt b* and a 380-bp clone of *12S*, no genetic variations were found among the populations for *12S*. Analysis of the five sequences using Arlequin software revealed a high degree of genetic similarity among the populations, ranging from 98.7% to 99.6% in terms of gene identity (Degani, 2013) (**Figure 28**). Munwes et al. (2010) hypothesized two distinct communities of eastern spadefoot toad tadpoles in the Upper Galilee.



D	C	B	A	E	
99.3	99.3	99.1	98.9	-	E
99.6	99.1	98.7	-	-	A
99.1	99.6	-	-	-	B
99.6	-	-	-	-	C
-	-	-	-	-	D

Raihania Pond	Kash Pond	Fara Pond	El-Rom Pond	Sasa Pond	
99.3	99.3	99.1	98.9	-	Sasa Pond
99.6	99.1	98.7	-	-	El-Rom Pond
99.1	99.6	-	-	-	Fara Pond
99.6	-	-	-	-	Kash Pond
-	-	-	-	-	Raihania Pond

Figure 28. Unrooted phylogenetic tree and percent identity of the partial *Cyt b* fragment based on the nucleotide sequence of *Pelobates syriacus*. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events. The phylogenetic tree was constructed using the MegAlign program (DNASTAR) by the CLUSTALW method. The branch length represents the evolutionary distance. A—Elrom Pond, B—Fara Pond, C—Kash Pond, D—Raihaniya Pond and E—Sasa Pond.

The populations of Elrom Pond, located at the highest altitude, and Fara Pond, situated at the lowest altitude, exhibited the lowest levels of genetic similarity compared to other populations (**Figure 28**). This was determined by analyzing the DNA variation among *P. syriacus* populations from different breeding sites using the OPA-4 primer. The level of band sharing ranged from 0.92 to 1.00. Specifically, the similarity between the Elrom Pond population and the populations of Kash Pond, Raihaniya Pond, and Sasa Pond was relatively low (0.92). Similarly, the Fara Pond population, at the lowest altitude, showed low similarity with the populations of Kash Pond, Raihaniya Pond, and Sasa Pond (0.92). Similar results were obtained with the OPA-3 primer. The lowest level of similarity (0.93) was observed between the populations of Elrom Pond and Fara Pond, in comparison to the other ponds (Kash Pond, Raihaniya Pond, and Sasa Pond) (Degani, 2013) (**Figure 28**).

Munwes et al. (2010) used the eastern spadefoot toad in Israel as a model organism to investigate certain hypotheses concerning population genetics and network theory. Their findings contradicted most of the predictions made by the "abundant center" model, which suggests that peripheral populations should exhibit lower population density and genetic diversity than core populations. Moreover, they suggested that dispersal between core and peripheral populations should be asymmetrical, primarily directed outward from the core population; however, they did not observe such a trend in their study. Their data did not support the hypothesis of no or non-linear change in genetic diversity toward the range edge. On the contrary, their results aligned with Fisher's hypothesis (presented in The Genetical Theory of Natural Selection in 1930), which proposes an increase in genetic variability from the core to the edge of a species' distribution. Munwes et al. (2010) attributed this finding to the significantly harsher climatic and abiotic conditions experienced by both tadpoles and post-metamorphic individuals at the distribution edge over multiple generations. Importantly, Munwes et al.'s (2010) results have substantial implications for the conservation of this critically endangered species in Israel.

We identified two distinct communities that are genetically connected through two specific rain pools in the Upper Galilee. A detailed understanding of the spatial subdivision of this species is crucial for future management and restoration efforts concerning temporary wetlands in Israel. Cohen et al. (2021) studied the genetic diversity and composition of the eastern spadefoot toad (*P. syriacus*) population influenced by temporal variations occurring over short and long periods of time. They put forth the hypothesis that the allelic makeup within populations would undergo changes from year to year, aligning with short-term fluctuations in environmental conditions. Interestingly, the impact of short-term climate variations on genetic composition has scarcely been explored in the literature, and the significance of annual climatic fluctuations remains largely unexplored. Cohen et al.'s (2021) study revealed that climatic variations between successive years, particularly in terms of rainfall amounts and number of rainy days, can significantly modify both the allelic composition and diversity observed in microsatellites. They proposed that environmental selection, which is subject to fluctuations, varies across different regions globally, with its strongest effects expected in areas characterized by unstable short-term climatic conditions.

6. *Water Frogs*

6.1 Classification and description

The Levant water frog *Pelophylax bedriagae*, formerly known as *Rana ridibunda*, is a common water frog of medium size. The length from the tip of its snout to its vent varies between 42.5 and 89.5 mm in males, and between 43.0 and 95.2 mm in females. However, the range of snout-vent lengths can differ depending on the geographical location. When viewed from the side, the snout is pointed, whereas from the top it appears sub-elliptical to triangular. The length of the head is slightly greater than its width. The nostrils are directed toward the back and are located approximately halfway between the snout and the eyes. The distance between the nostril and the eye is slightly longer than the distance between the two nostrils. The eyes, positioned on the upper part of the body, are relatively large and have horizontally elongated pupils. The space between the eyes is narrower than the diameter of each eye. The eardrum is prominent and about 10% smaller in diameter than the eye; it has a slightly pear-like shape. A noticeable fold extends above the eardrum and merges with two folds on the upper sides of the body. Male frogs possess paired vocal sacs (Bam-E-Zar et al., 2019; Disi and Amr, 2010).

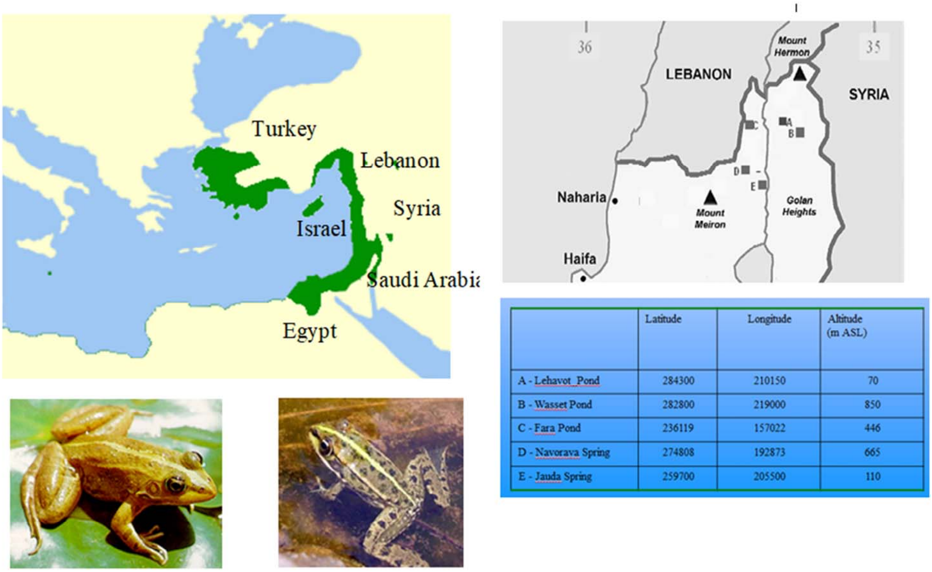
The taxonomic reassessment of Middle Eastern water frogs includes an Israeli study (Sinsch and Schneider, 1999), in which eight morphometric features of water frogs from 14 localities in Turkey, Syria, Jordan and Israel were compared with those of *R. ridibunda* in Kazakhstan, Armenia and Greece (Thrace). The study sites included the typical localities of *R. ridibunda*, *Rana r. caralitana*, *Rana esculenta* var. *bedriagae* and *Rana levantina*. Multivariate comparisons (principal component analysis, discriminant analysis) based on the log10-transformed variables demonstrated that only two taxa differed significantly in size and shape. By applying a morphospecies criterion, *R. ridibunda* was found to be represented exclusively by the three reference populations, whereas all other populations (in Turkey, Syria, Jordan and Israel) were of the same species—*Pelophylax* (*Rana*) *bedriagae*. Data collected on the Levant water frog in northern Israel and on other species of amphibians have been published in various papers (Degani, 1982; Degani and Kaplan, 1999). Its structure and mating call variations in Israel were studied by Nevo and Beiles (1988), and the genetic differences between Israeli and Greek populations were documented by Nevo and Filippucci (1988).

6.2 Distribution

Pelophylax bedriagae is a species of true frog native to the Balkan Peninsula in southeastern Europe. Its distribution in Israel is shown in **Figure 29** (Schneider et al., 1992). Because of the great difference between the details and the dispersion in Europe, its names changed until molecular tools entered into systematics (Bam-E-Zar et al., 2019; Disi and Amr, 2010).

Kyriakopoulou-Sklavounou et al. (2008) conducted a study on the physical and genetic differences among three populations of Levant water frogs in northern Greece. They used specific physical measurements and electrophoresis of enzymes with varying forms. The overall findings suggested that these differences were a result of distinct selection pressures on the physical characteristics, which included the presence of a closely related species in the same habitat. This phenomenon was not observed in the genetic analysis of the enzymes.

Plötner et al. (2008) indicated that Anatolian marsh frogs cannot be classified as *Pelophylax bedriagae* based on mtDNA techniques. Furthermore, they described the relationship between *R. ridibunda* and *Rana lessonae* frogs in Europe. It was found that natural hybridizations occur between the DNA of *R. ridibunda* and *R. lessonae*, as observed by Berger (1970) and Degani et al. (2013b).



	Latitude	Longitude	Altitude (m ASL)
A - Lehavot_Pond	284300	210150	70
B - Wasset Pond	282800	219000	850
C - Fara Pond	236119	157022	446
D - Navorava Spring	274808	192873	665
E - Jauda Spring	259700	205500	110

Figure 29. Distribution of *Pelophylax bedriagae* (Degani et al., 2013b).

6.3 Life cycle

Limited information is available regarding the ecology and life cycle of the Levant water frog in Israel, as discussed in previous studies (Degani, 1982; Degani and Kaplan, 1999). Research has focused on investigating the ecological and biological factors in breeding sites inhabited by amphibian larvae in northern Israel, which include winter ponds, rock pools, springs, and streams (Degani, 1982). The breeding season typically occurs in May and June, although some larvae may persist in the water throughout the summer and undergo metamorphosis toward either the end of summer or the beginning of winter. However, published information is scarce on the ecological characteristics of the water bodies where *Pelophylax bedriagae* live and reproduce.

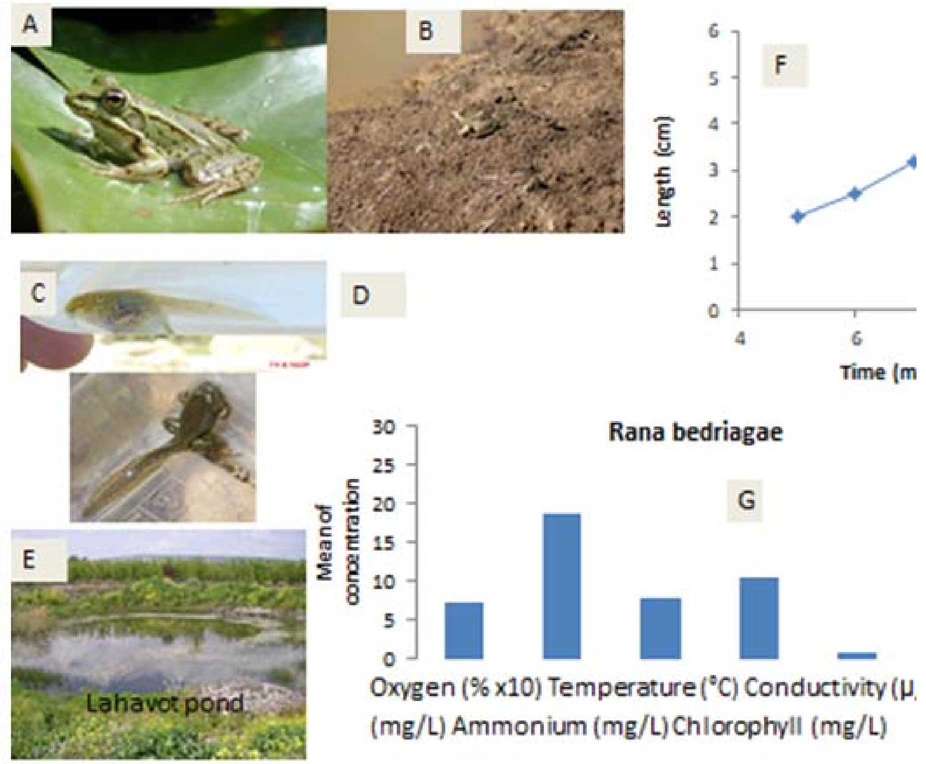


Figure 30. Different terrestrial and aquatic habitats of ***Pelophylax bedriagae*** in northern Israel. (A) Adults, which live on land. (B) Juveniles. (C) Tadpoles. (D) Metamorphosis stage. (E) Lahavot pond in Hula valley where the species can be found. (F) Growth of ***Pelophylax bedriagae.*** (G) Ecological characteristics of the habitats: oxygen level, ammonium concentration, temperature, conductivity, and pH (Degani, 2019a).

6.4 Different larval growth periods among ponds

Figure 31 displays the growth of *Pelophylax bedriagae* larvae in different ponds. The duration of larval growth varies across ponds and in different years. In most ponds, larval growth occurs during the summer months, specifically from May to September (Degani et al., 2013b). However, in the Navoraya Spring (northern Israel), larvae are found throughout the year (**Figure 31**).

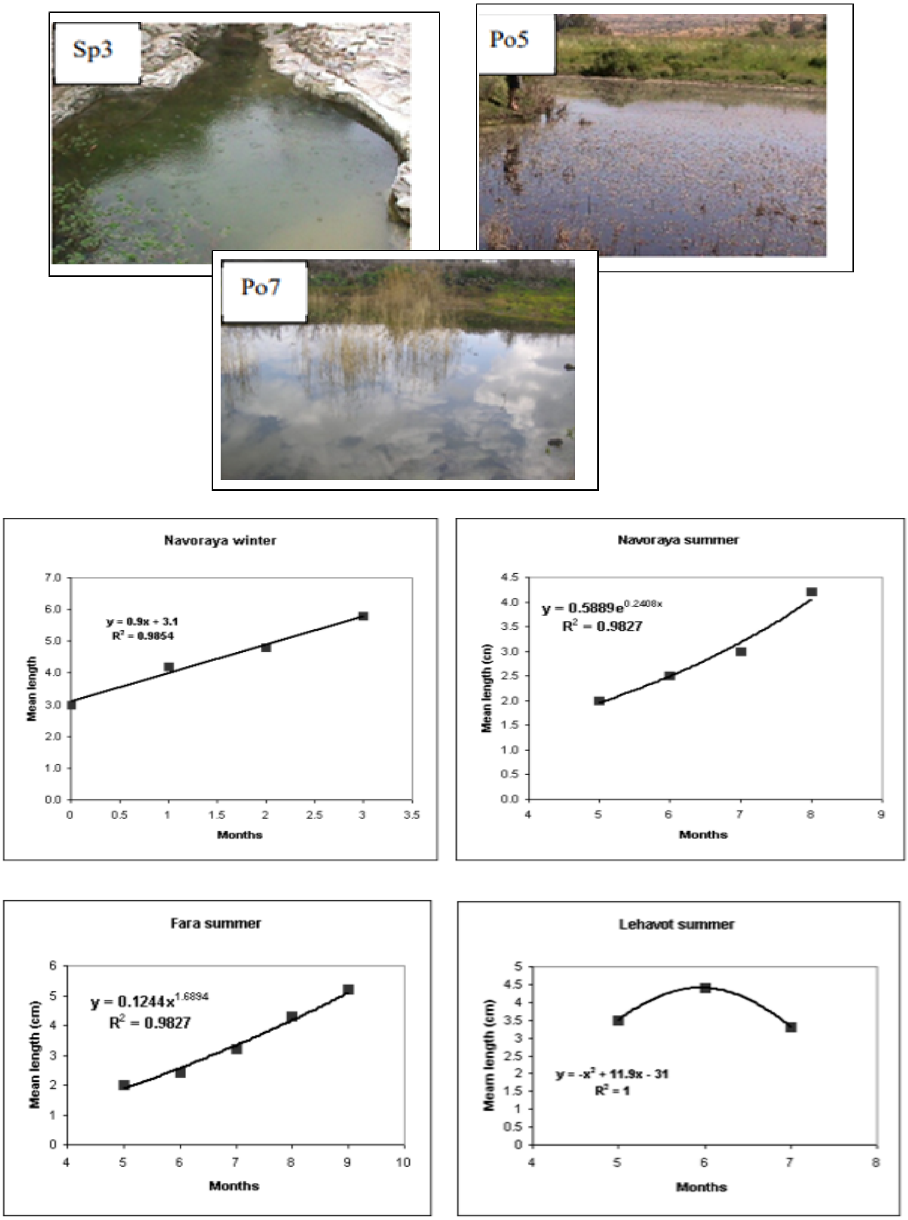


Figure 31. Larval growth of *Pelophylax bedriagae* at different breeding sites. Sp3, Navoraya Spring; Po5, Lehavot Pond; Po7, Fara Pond (Degani et al., 2013b).

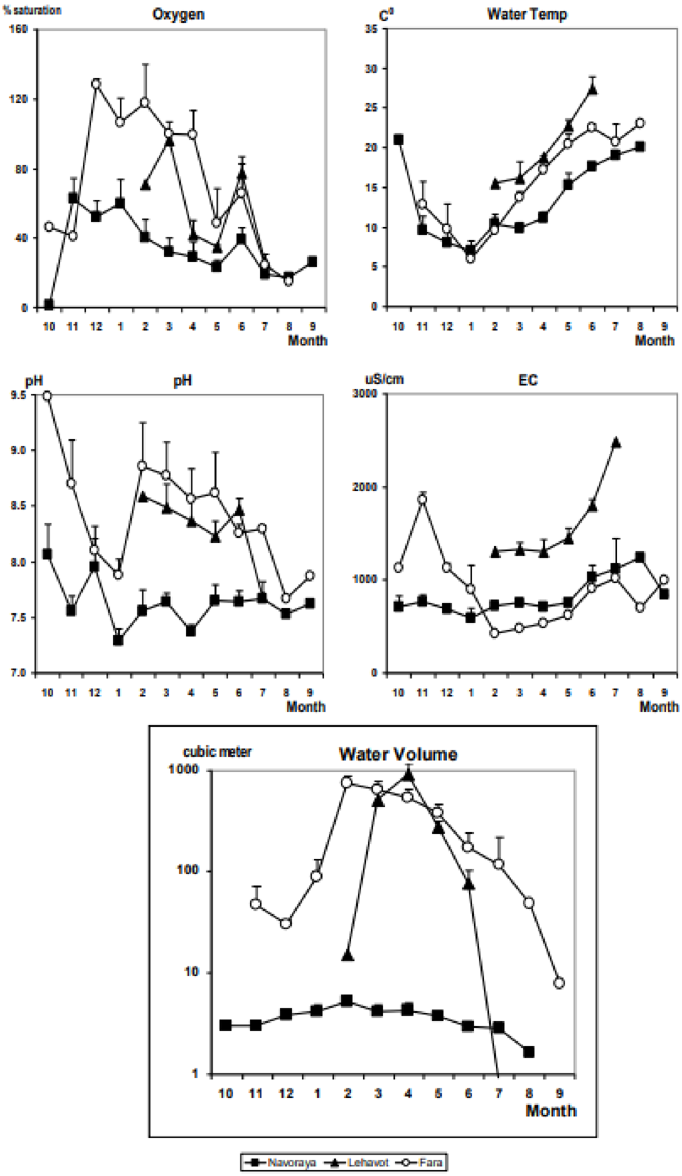


Figure 32. Water parameters in the different breeding sites where *Pelophylax bedriagae* larvae thrive and undergo complete metamorphosis (Degani et al., 2013b).

The growth patterns of larvae differ across breeding sites, ranging from winter to summer. The growth rate equation for Navoraya Spring during the winter was calculated as $Y = 0.9X + 3.1$, with an R^2 value of 0.9854. In the summer, the growth rate equation was $Y = 0.5889e0.2488x$, with an R^2 value of 0.9827. For Fara Pond during the summer, the growth rate equation was $Y = 0.1244X\ 1.6984$, with an R^2 value of 0.9827. Lastly, in the Lehavot Pond during the summer, the growth rate equation was $Y = -X2 + 11.9X - 31$, with an R^2 value of 1 (**Figure 31**) (Degani et al., 2013b). **Figure 32** illustrates the water quality parameters in the three different ponds where *Pelophylax bedriagae* larvae were present. In Lehavot and Fara Ponds, water was available from winter until the end of summer, at which point the ponds dried up. However, in Navoraya Spring, water was available year-round (**Figures 31** and **32**) (Degani et al., 2013b).

The ponds underwent a change in size, decreasing from 1000 m^3 of water to 0 m^3, whereas the spring maintained a constant water volume of 3 to 5 m^3. As the seasons progressed from winter to spring and into summer, there was a notable increase in temperatures across the different water bodies, with a range of 5 to 30°C (**Figure 32**). The rate at which the water temperature changed was higher in the ponds than in the spring. However, no significant differences in temperature were observed among the water bodies during the 4 years of the study (Degani et al., 2013b). The concentrations of dissolved oxygen varied between 0% and 120% saturation, being significantly highest during the winter and decreasing throughout the summer.

6.5 Comparison of green toads' and water frogs' adaptations to habitat conditions

6.5.1 Differential urea tolerance and osmoregulation

Many land-dwelling amphibians demonstrate the ability to adapt to different osmotic environments. The accumulation of urea as a solute in both intra- and extracellular fluids appears to be crucial for their adaptation to high salinity or terrestrial life. Several studies, including Degani (1985), have highlighted the significance of urea accumulation in regulating osmotic concentrations. Urea serves as a major solute for adjusting the overall osmotic balance. During dehydration, when urine production decreases, there is an increase in urea accumulation in the body fluids (Degani, 1985). Moreover, net urea synthesis may contribute to this enhanced accumulation of urea (Degani, 1985).

When urea is present in low concentrations (0 - 50 mM) in the plasma, it is evenly distributed throughout the body fluids due to its hydrophilic nature and lack of charge. However, there is limited knowledge regarding the distribution of urea, both extracellular and intracellular, when it accumulates to high concentrations (300 - 900 mM). This phenomenon has been observed in certain terrestrial amphibians such as *Scaphiopus couchi*, *Ambystoma tigrinum*, *Salamandra salamandra* (Degani, 1981b), and green toads (Degani et al., 1984). Further research is needed to understand the specific mechanisms and patterns of urea distribution under these conditions.

When body fluids reach high concentrations, muscle excitability decreases rapidly, and the muscles undergo significant swelling. In addition, urea can denature cell proteins and when present, it prevents the coagulation of isolated native proteins. *Bufo viridis* is a green toad species that primarily inhabits terrestrial environments, whereas *Rana ridibunda* is a semi-aquatic species found in Europe and North Africa, occupying different habitats (Warburg, 1971). Variations in these two species' adaptation to high concentrations of urea solution were examined. In the case of the green toad, which undergoes saline adaptation and prolonged dehydration in soil, urea accumulation in the plasma was observed. On the other hand, no urea accumulation was found during saline adaptation in the semi-aquatic frog (Degani et al., 1984). The green toads exhibited tolerance to urea solutions of up to 800 mM. However, when exposed to a concentration of 900 mM, the toads became severely emaciated, and the experiment had to be terminated. Interestingly, when subjected to a 600 mM urea solution, three out of five frogs died. Both species maintained hyperosmotic blood levels during the experiment. It should be noted that the concentration of urea in the plasma was consistently lower than that in the surrounding medium, as depicted in **Figure 33** (Degani et al., 1984).

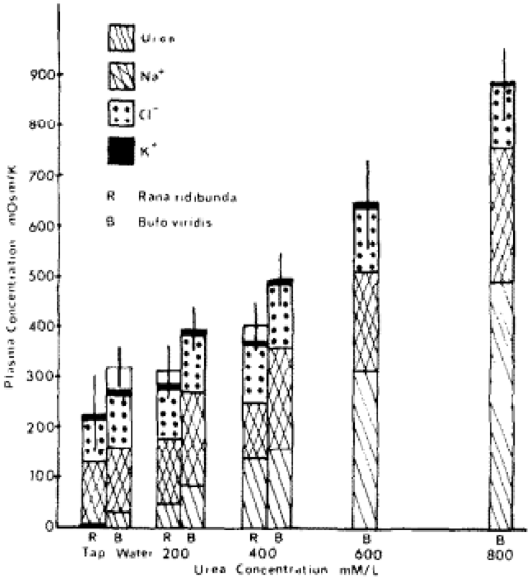


Figure 33. During the process of acclimation to urea solutions, its concentration and that of other constituents in the plasma were examined in the green toad (*Bufo viridis*) and the semi-aquatic frog *Rana ridibunda* (Degani et al., 1984).

In all tested media, the blood plasma of the semi-aquatic frogs consistently exhibited lower concentrations of urea than the green toads under equivalent medium concentrations (**Figure 33**). As the frogs acclimated to media with varying urea concentrations, the urea concentration in their plasma increased. In tap water, urea constituted approximately 10% of the total osmotic concentration in toads and only 2% in frogs. However, in a medium containing 400 mM urea, the urea concentration in the plasma accounted for 31% of the total osmotic concentration in the toads and 39% in the frogs. The highest recorded urea concentration in the toads' blood plasma reached 493 mM (56%) during acclimation to an 800 mM urea solution. The plasma osmolality of both toads and frogs exhibited a linear correlation with the urea concentration in the plasma (**Figure 34**). Under normal conditions with tap water, NaCl was the primary osmotically active component in the blood of both toads and frogs. The concentrations of plasma Na^+ and Cl^- were consistently higher in the toads compared to the frogs in most of the tested urea media, and these differences were statistically significant. The muscle tissue of the green toad experienced less weight loss than that of the semi-aquatic frog in the presence of both 300 mM and 500 mM urea. Furthermore, the concentration of urea in the muscle tissue of green toads was lower than that in the frogs, particularly when the muscle tissue was exposed to high urea concentrations ranging from 400 mM to 800 mM.

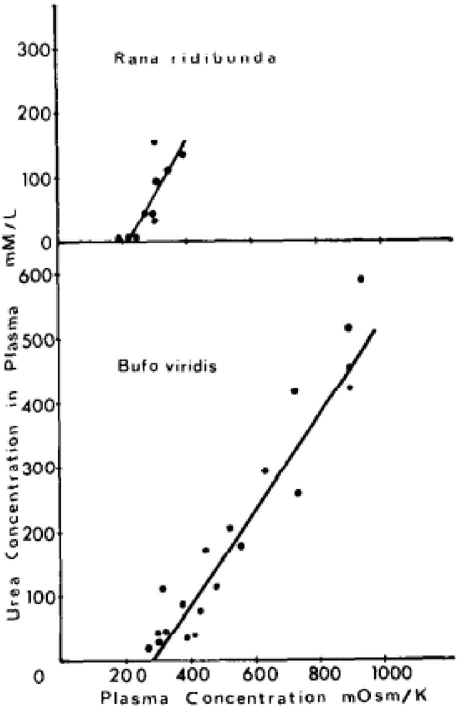


Figure 34. Relationship between plasma osmolality and urea concentration in the plasma.

6.5.2 Comparison of oxygen utilization by the land-dwelling green toads and the semi-aquatic water frogs

In arid environments with high summer temperatures and low humidity, the terrestrial green toad species faces the risk of dehydration, and they are therefore less active during the summer than they are in temperate climates. To cope with these harsh conditions, they take refuge underground and remain inactive for several months (Degani et al., 1984). During this period, low oxygen consumption is an advantage. However, in all amphibians, high temperatures generally lead to an increase in metabolic rate (Degani, 1983). On the other hand, the semi-aquatic frog species faces a less severe problem in its habitat. It remains active and breeds during the spring and summer (Degani, 1982).

To gain a deeper understanding of how green toads endure extended periods of inactivity in the scorching and arid summer season, a comparative analysis was conducted on oxygen consumption and CO_2 production in both green toads and the water frogs before and after dehydration. The purpose was to investigate the survival mechanisms employed by green toads under these challenging conditions (Degani, 1983). Both species, when hydrated or dehydrated, exhibit metabolic patterns that could be represented by two distinct lines. At temperatures below approximately 24°C, there was a linear phase with a slope close to zero (**Figures 35-36**) (Degani, 1983).

The subsequent phase is characterized by a curvilinear pattern, with each species achieving its respective asymptote differently, depending on hydration status. When hydrated, both species display the same rate of oxygen consumption to a temperature of around 27°C (**Figure 35**). However, at higher temperatures, the metabolic rate of the green toad is lower than that of the water frog. The asymptote for the toad is approximately 38°C, whereas the frog's asymptote is around 36°C (Degani, 1983). At higher temperatures, the frog consistently exhibits a higher level of CO_2 production compared to the toad (**Figure 36**) (Degani, 1983). When dehydrated, both species exhibit similar metabolic patterns (**Figures 37** and **38**).

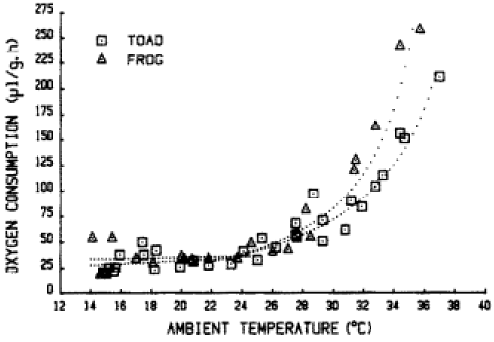


Figure 35. Oxygen utilization by hydrated green toad and water frog.

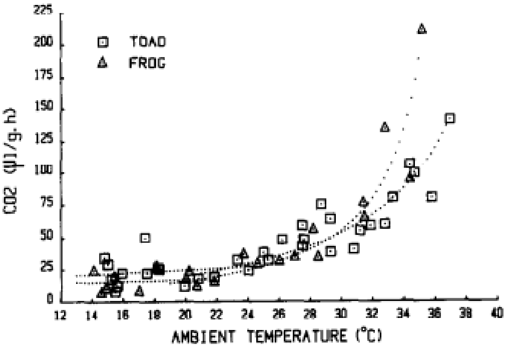


Figure 36. CO_2 production by hydrated green toad and water frog.

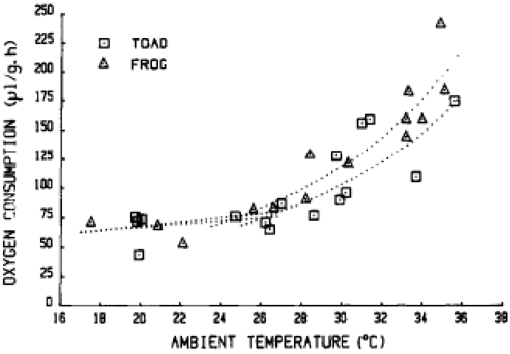


Figure 37. Oxygen consumption of dehydrated green toad and water frog.

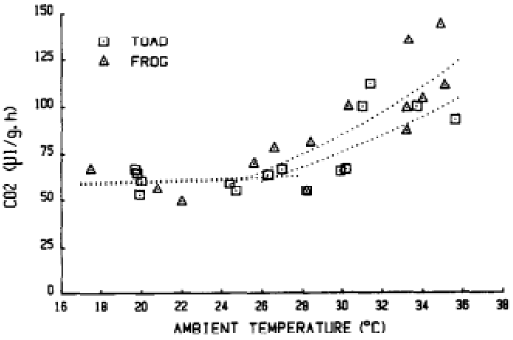
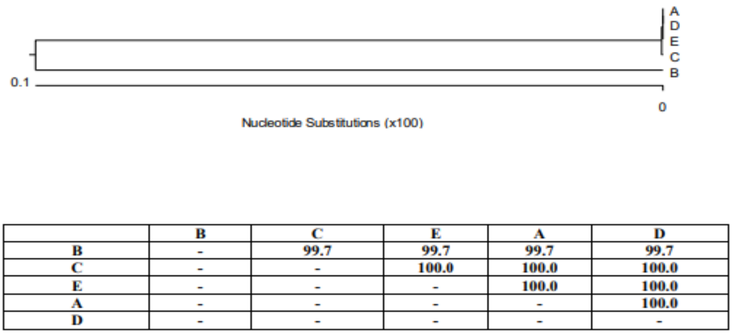


Figure 38. CO_2 production of dehydrated green toad and water frog.

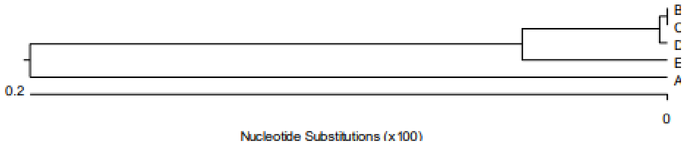
6.6 Variations in mitochondrial genes *12S* and *16S* in water frogs from various breeding sites in northern Israel

To examine genetic variations, the mitochondrial genes *12S* and *16S* of the water frog *Pelophylax bedriagae* were amplified and sequenced from various breeding sites in northern Israel (Degani, 2013). Genetic variation in the nucleus was further assessed by RAPD PCR. The DNA fragments' nucleotide sequences were determined from a 367-bp clone of *12S* (**Figure 39**) and a 525-bp clone of *16S* (**Figure 40**). Among the different breeding sites, the *12S* fragment exhibited variation in nucleotide 331 (Degani, 2013).



	B	C	E	A	D
B	-	99.7	99.7	99.7	99.7
C	-	-	100.0	100.0	100.0
E	-	-	-	100.0	100.0
A	-	-	-	-	100.0
D	-	-	-	-	-

Figure 39. Nucleotide variation in the mitochondrial gene *12S* among populations of *Pelophylax bedriagae* from various breeding sites in northern Israel. Evolutionary history was determined by neighbor-joining method (Degani, 2013).



	B	C	E	A	D
B	-	100.0	99.8	99.6	100.0
C	-	-	99.8	99.6	100.0
E	-	-	-	99.6	99.8
A	-	-	-	-	99.6
D	-	-	-	-	-

Figure 40. Nucleotide variation in the mitochondrial gene *16S* among populations of *Pelophylax bedriagae* from various breeding sites in northern Israel. Evolutionary history was determined by neighbor-joining method.

Using Arlequin software, analysis of the *12S* sequences revealed a high genetic similarity among the five populations, ranging from 99.7% to 100% (**Figure 39**). On the other hand, the *16S* gene sequences showed slight variations among the breeding site populations in Israel, with a genetic similarity ranging from 99.6% to 100% (**Figure 40**) (Degani, 2013).

7. Hula Painted Frog

7.1 Classification and description

The painted frogs of the genus *Discoglossus*, which are part of the family Alytidae, are among the most ancient group of frogs. Their origins can be traced back to the Jurassic period. The Hula painted frog, initially categorized within this genus, was recognized as such until 2013. However, recent studies involving genetic and skeletal analyses have revealed that this species is actually closely related to a group consisting of all of the other *Discoglossus* species. Consequently, it was reclassified and assigned to the genus *Latonia*. *Latonia* is a genus of giant frogs that existed during the Miocene through Pleistocene epochs, but was then presumed to have become extinct (Bina Perl et al., 2017). The unique position of *Latonia nigriventer* as the sole surviving species of an ancient clade highlights the need for special efforts to safeguard its existence, as it represents a significant portion of alytid phylogenetic diversity. However, very little is currently known about the natural history of this remarkable frog, which was only recently rediscovered and remains one of the rarest amphibians in the world (Bina Perl et al., 2017). Aside from some brief descriptions of its tadpole and adult forms (Mendelssohn and Steinitz, 1943), the available information about its sister group, *Discoglossus*, has been the sole reference for speculations on *L. nigriventer*'s natural history.

7.2 Life cycle

The Hula painted frog is a critically endangered species of frog endemic to the Hula valley in northern Israel, thought to be extinct until its rediscovery in 2011 (**Figure 41**). Although not much is known about its specific life cycle, we can make some general assumptions based on what is known about other frog species (Bina Perl et al., 2017).

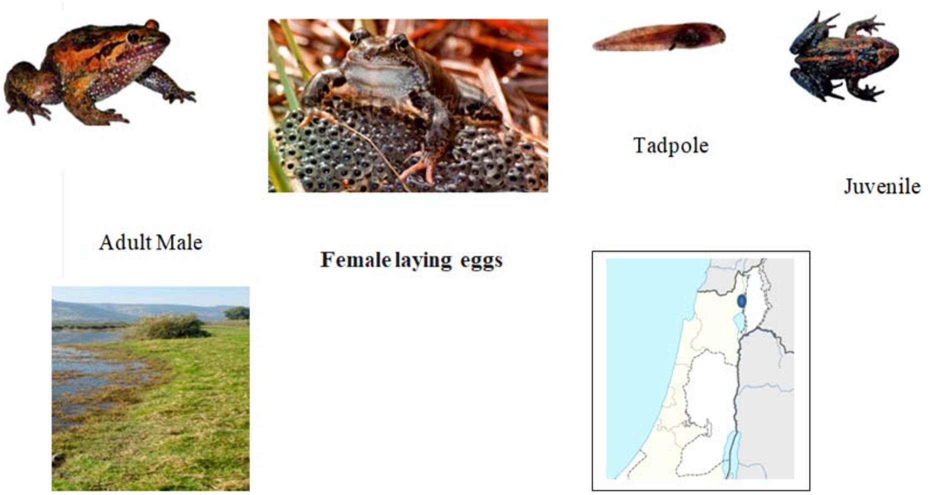


Figure 41. The life cycle of *Latonia nigriventer*, also known as the Hula painted frog (Bina Perl et al., 2017).

1. Egg Stage: The life cycle typically begins with the female laying eggs in a suitable aquatic habitat. The Hula painted frog likely lays its eggs in shallow water bodies such as ponds, pools, or marshes (**Figure 41**).
2. Tadpole Stage: After the eggs are laid, they hatch into tadpoles. Tadpoles are aquatic and have gills to breathe underwater. They undergo a process of metamorphosis where they gradually develop limbs, lose their gills, and grow lungs for breathing air. The duration of the tadpole stage can vary depending on environmental conditions and species, but it generally lasts for several weeks to a few months (**Figure 41**).
3. Metamorphosis: Once the tadpoles have completed their development, they metamorphose into juvenile frogs. During this process, their tails shrink, their hind limbs develop, and their bodies adapt for terrestrial life. The exact duration of metamorphosis can also vary but typically takes a few weeks (**Figure 41**).
4. Juvenile and Adult Stage: Once the metamorphosis is complete, the juvenile frogs leave the water and begin their life on land. They develop their

adult coloration and markings over time. As adults, Hula painted frogs are typically small to medium-sized, measuring around 4 - 6 cm in length (**Figure 41**).

It is important to note that the information available on the specific life cycle of the Hula painted frog is limited due to its rarity and critically endangered status. Researchers and conservationists continue to study and monitor the species to gather more information and develop conservation strategies to protect it.

7.3 Population genetics analysis of the recently rediscovered Hula painted frog reveals high genetic diversity and low inbreeding

The Hula painted frog is considered extremely rare and is poorly understood. Given its limited ability to disperse, the significant disruption of its habitat, and its low population size, conservation efforts are urgently needed. A genetic analysis of this species was conducted, using 18 newly developed microsatellite markers and four different models. The effective population size (Ne) was calculated using a sample of 125 Hula painted frog individuals collected from a single location. The estimates of potentially reproducing adults in this population (Nad) were determined through a capture-recapture study involving 118 adult Hula painted frogs captured at the same site. Surprisingly, the data indicated that despite Nad estimates ranging from approximately 234 to 244 and Ne estimates ranging from about 16.6 to 35.8, the species displays remarkably high genetic diversity (observed heterozygosity = 0.771) and a low inbreeding coefficient (FIS = −0.018). This perplexing result could potentially be explained by two hypotheses: genetic rescue from unknown Hula painted frog populations in close proximity, or recent interbreeding of genetically distinct subpopulations. Regardless of which scenario is correct, the original locations of these populations still need to be determined (Bina Perl et al., 2018).

8. Diversity of Biological, Ecological and Genetic Characteristics, and Adaptation among Amphibian Species in Israel

8.1 Amphibian larval growth in various habitats

The larvae of amphibians inhabit diverse aquatic environments throughout the year, corresponding to their respective breeding seasons (Degani, 1982; Degani and Kaplan, 1999; Goldberg et al., 2009a). The larvae of the Near Eastern fire salamander (*S. infraimmaculata*) have been observed in various breeding sites located at higher elevations in northern Israel. These larvae are present in streams, springs, and pits from October to August, and in ponds from November to April. While the Middle East tree frog *H. savignyi* primarily occupies winter ponds, it is also observed in some springs. The Levant water frog *Pelophylax bedriagae* is found in winter ponds and springs, while larvae of the green toad *P. syriacus* and the southern banded newt *T. vittatus* have been discovered exclusively in winter ponds.

Based on the given information, the tadpoles have specific niches. For instance, the tadpoles of *T. vittatus* and *S. infraimmaculata*, which have similar diets, were observed at different times in the ponds, and the water temperatures varied during their growth periods. *S. infraimmaculata* tadpoles were found during the winter, whereas *T. vittatus* tadpoles appeared in spring and early summer. The tadpoles of *S. infraimmaculata*, *T. vittatus*, and the Levant water frog were predominantly found at the bottom of the pond, whereas *H. savignyi* tadpoles were distributed throughout the pond and usually displayed little movement compared to *P. syriacus*, which constantly moved up and down.

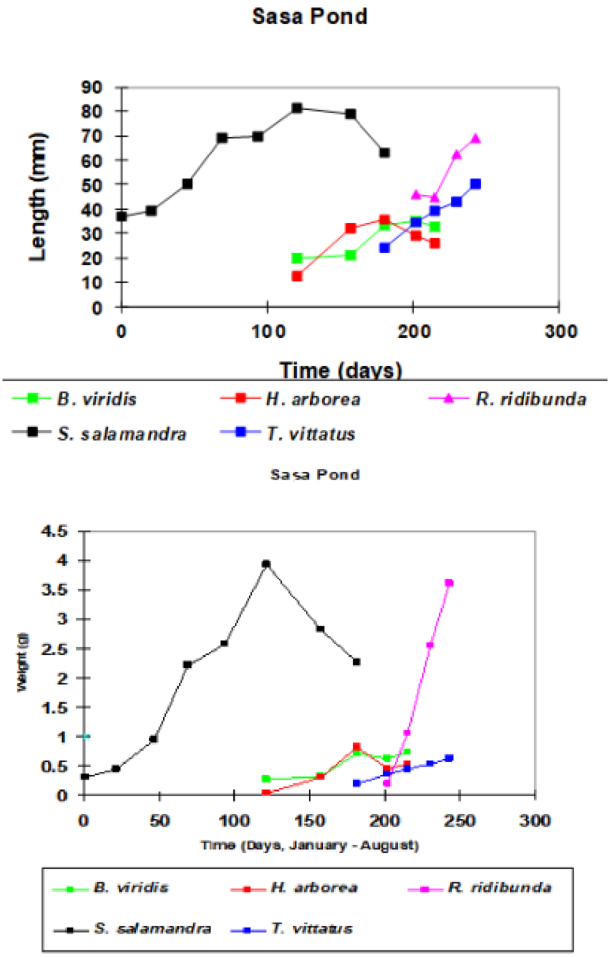


Figure 42. Growth of various amphibians in a winter pond (Sasa) in the Upper Galilee from January to August (Degani, 2019a).

No differences were found in the range of ecological niches of larvae from six amphibian species in northern Israel by measuring five ecological characteristics. *S. infraimmaculata* and green toads were detected in ponds with temperatures ranging from 1.5 - 24˚C, whereas all other larvae were observed at temperatures above this range, up to 34˚C. No dramatic differences were found between the various larvae with respect to oxygen concentration or conductivity in the water (**Figure 43**).

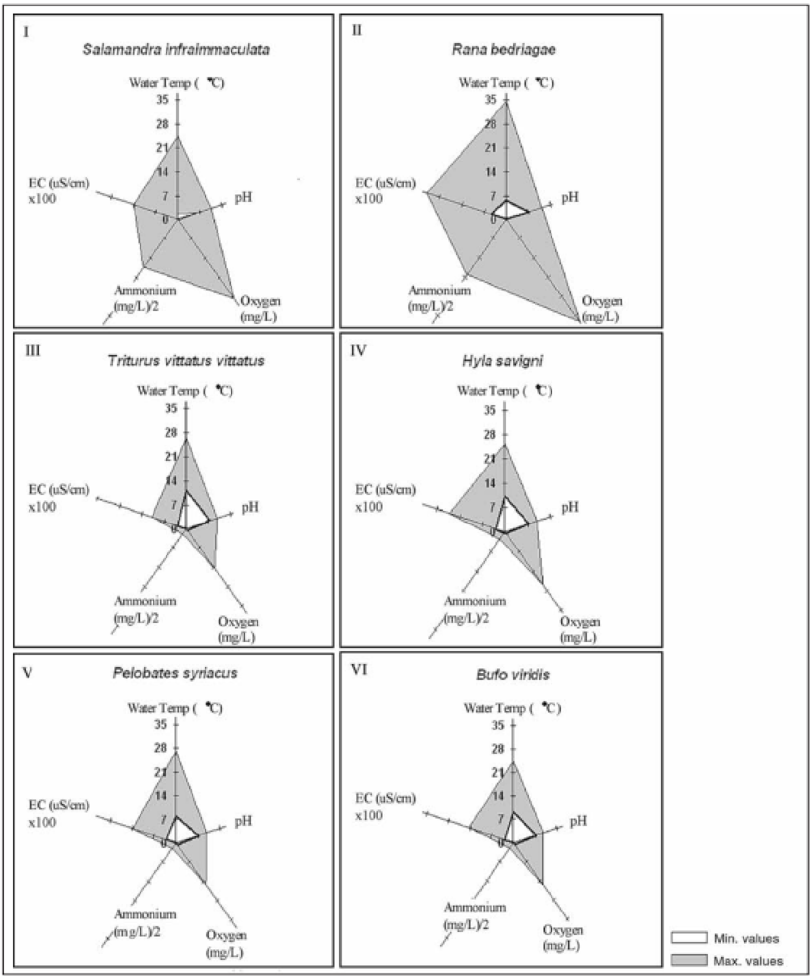


Figure 43. Ecological niches of larvae from six amphibian species in northern Israel, taking into account five ecological characteristics: oxygen levels, ammonium concentration, temperature, conductivity, and pH levels. The figure is constructed based on the coordinates of various sites in relation to these five ecological variables (Goldberg et al., 2009a).

8.2 Adaptations of amphibian species

There have been relatively few studies conducted in Israel on the adaptation of amphibians to terrestrial life after metamorphosis, as compared to studies focusing on tadpoles. Among the various species, the green toad (*B. viridis*) has the widest geographical distribution, and it has successfully colonized desert and arid environments. These frogs have demonstrated remarkable adaptability by their unique ability to store water in their urinary bladder and accumulate urea in their plasma. In comparison to other species—*T. vittatus*, *P. syriacus*, *S. infraimmaculata*, *Pelophylax bedriagae*, and *H. savignyi*, the green toad shows a superior capacity for water retention and urea accumulation (**Figure 43**) (Degani, 2019a).

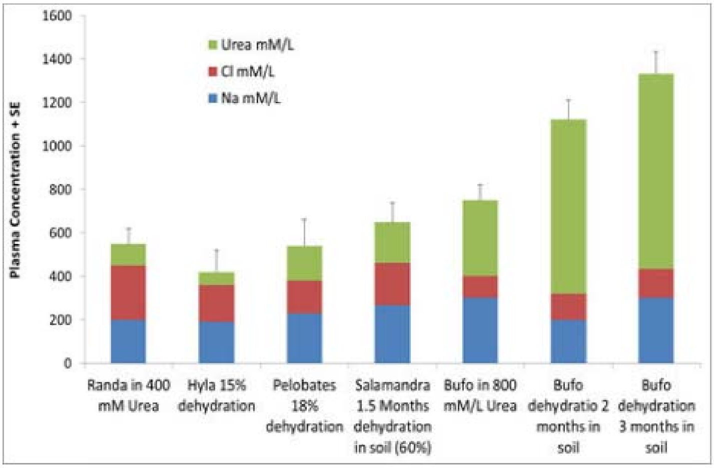


Figure 44. During the starch period, the composition and concentration of plasma in mature amphibians vary (Degani, 1981a, 1981b, 1985, 1986b, 2019a; Degani and Warburg, 1984; Hoffman and Katz, 1989; Hoffmann, 2010; Katz et al., 1984).

The skin of amphibians cannot prevent dehydration, and their ability to thrive in terrestrial environments relies on physiological adaptations, such as plasma concentration. From the gathered information on survival capabilities, it appears that high plasma concentrations can be associated with the capacity to accumulate urea and water in the bladder (**Figure 44**). The ability to store urea is connected to a strong adaptation to dry conditions, as observed with green toads (Degani et al., 1984).

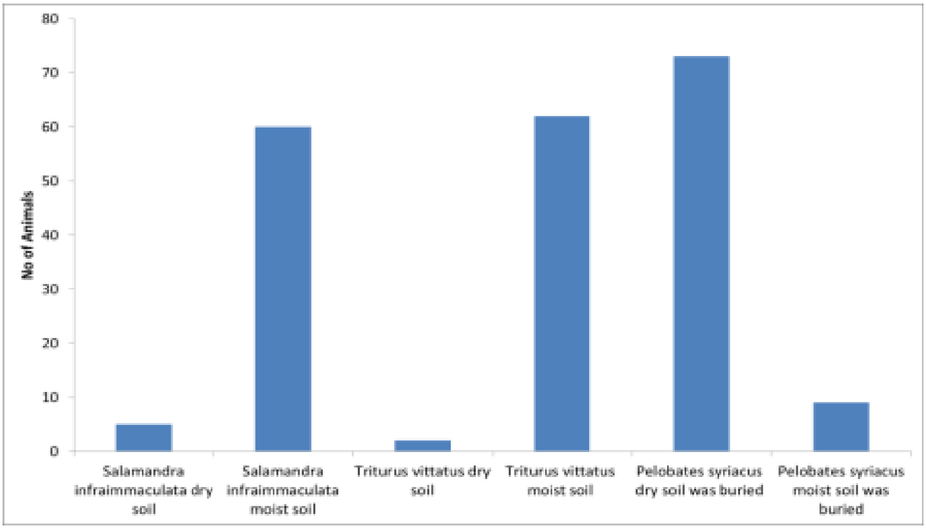


Figure 45. The behavior of *Salamandra infraimmaculata*, *Triturus vittatus*, and *Pelobates syriacus* in response to different substrates.

Another crucial adaptation for amphibian survival on land is their environmental behavior, which involves locating hiding places and enduring extended dry seasons. In Israel, most amphibian species, except for *Pelophylax bedriagae*, are predominantly active during the night. They seek out hiding places with relatively high soil humidity and exhibit negative phototaxis. Once metamorphosis is complete, amphibians transition into terrestrial animals and become vulnerable to dehydration. Finding suitable hiding places through environmental behavior is a critical adaptation for preventing dehydration and ensuring their survival. An investigation of hiding place selection has been conducted on various species, focusing on factors such as moisture, light, temperature, and even soil digging, as observed with *P. syriacus* (**Figure 45**) (Degani, 2015; Degani and Carmali, 1988). Environmental behavior has been studied in multiple amphibian species at the southern edge of their distribution, including *S. infraimmaculata*, *T. vittatus* and *P. syriacus* (Degani, 2015; Degani and Carmali, 1988) (**Figure 45**). For all of these species, the primary criterion for selecting hiding places is a preference for high soil humidity and darkness (Degani, 2019b).

8.3 Phylogenetic tree of amphibian species in Israel based on *Cyt* b

Figure 46 illustrates the evolutionary relationships of amphibian species based on their genetic makeup, specifically the *Cyt b* gene sequence. The tree was constructed using a method developed by Goldberg and colleagues in 2009, which calculates the maximum probability of the relationships. The tree reveals distinct differences among the species, particularly in the Anura group where each species belongs to a different family. On the other hand, the two Urodela species are grouped together in the family Salamandrinae. This phylogenetic tree, as presented in **Figure 46** and discussed by Degani in 2019 (Degani, 2019a; Goldberg et al., 2009b), provides support for the notion that these species are closely related. Notably, there is significant variation within individual species, with the highest levels found in green toads and *T. vittatus* compared to the other species found in Israel. These particular species exhibit a remarkable adaptation to life on land, showcasing extreme variations across different habitats. This information is based on research conducted by Goldberg et al. (2009b).

The research conducted by Degani (2019a) focused on the adaptation of seven amphibian species in different areas of Israel, transitioning from Mediterranean to desert climates. Based on the findings, a hypothesis was proposed suggesting that various characteristics play a role in amphibian adaptation to semiarid environments. The data indicated that the green toad *B. variabilis* (*B. sitibundus*) demonstrates the highest level of adaptation to terrestrial habitats and extreme conditions, followed by the southern banded newt *T. vittatus*, the tree frog *H. savignyi*, the spadefoot toad *P. syriacus*, the fire salamander *S. infraim-*

maculata, the water frog *R. ridibunda* (*Pelophylax bedriagae*), and the Hula painted frog *L. nigriventer*. However, further studies are necessary to support this hypothesis since multiple parameters are involved in amphibian adaptation to arid and semi-arid habitats. Degani's review in 2019a explored various aspects of adaptations observed in different anuran species. In summarizing the adaptation of amphibians to arid and semi-arid habitats based on the mentioned species, it becomes evident that adaptation needs to occur in both the terrestrial and aquatic phases. Two species, *B. variabilis and T. vittatus*, exhibit notable adaptation to arid environments. These species tend to breed in winter pools and other unpredictable habitats, and their growth and metamorphosis periods are relatively short compared to other amphibian species. Further studies are required to better understand the physiological adaptation of the green toad to arid environments, particularly in relation to urea accumulation in the blood. Limited research has been conducted on the physiological adaptation of *H. savignyi*, *P. syriacus*, *S. infraimmaculata*, the water frog, and *L. nigriventer* to extreme conditions, making it challenging to draw comparisons between them (Degani, 2019a).

In the case of *S. infraimmaculata*, researchers have discovered distinct physiological adaptations in populations inhabiting semi-arid habitats vs. moist habitats where water is available throughout the year. Previous studies (Degani, 2019a) have shed light on this differentiation. It is possible that other anuran species employ different strategies to adapt to terrestrial life, aiding their survival in semi-arid habitats. Environmental behavior plays a crucial role in this regard. For instance, *P. syriacus* demonstrates the ability to burrow into the soil to prevent dehydration (Degani, 2019a). On the other hand, *H. savignyi* may seek hiding places among plants or in the ground and alter its coloration to match the surrounding substrate (Degani, 2019a).

Furthermore, when comparing the phylogenetic tree constructed based on *Cyt b* sequences, it becomes apparent that the green toad *B. variabilis* and newt *T. vittatus* exhibit high genetic variation (**Figure 46**). This genetic diversity potentially contributes to their superior adaptation to extreme conditions compared to other species. These particular species are also distributed across wider areas in Israel than the other species mentioned (Degani, 2019a).

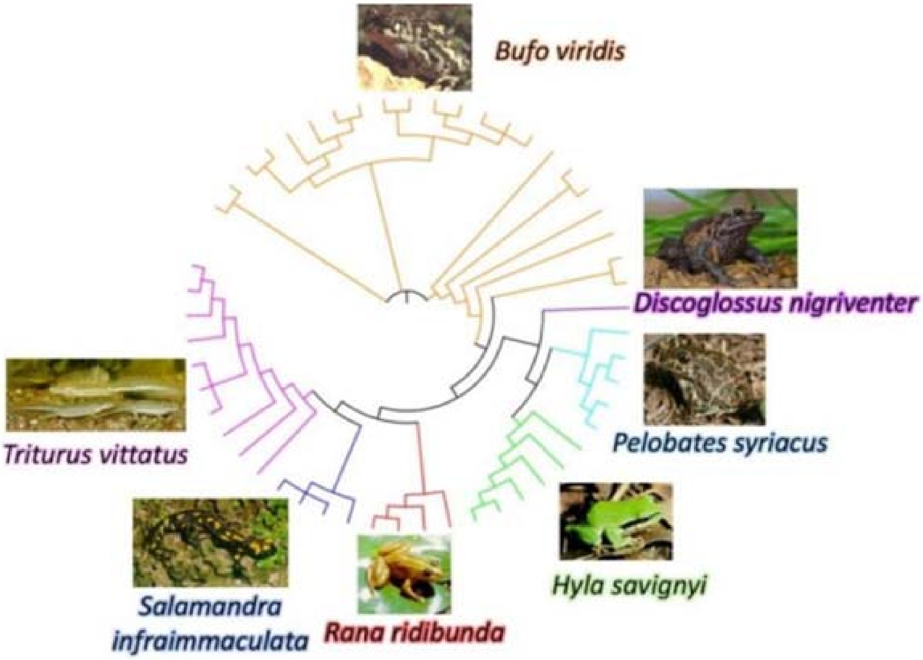


Figure 46. Phylogenetic tree focusing on various species of amphibians and their levels of *Cyt b* sequence similarity. The data used for this analysis were collected from four different locations: an Israeli site, and sites located in Greece, Iran, and Turkey (Degani, 2019a).

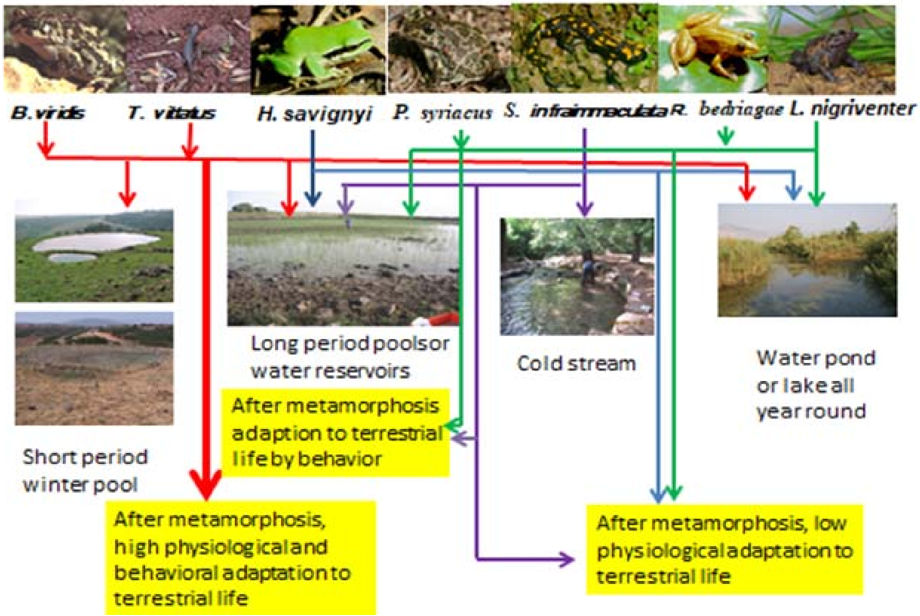


Figure 47. Framework for comparing the adaptation of different amphibian species specifically in habitats situated at the southern edge of their distribution, showing how they adapt to the unique conditions found in these particular habitats.

References

Andersen, L.W., Fog, K., Damgaard, C., 2004. Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). Biocontrol Science 271, 1293-1302.

Arntzen, J.W., Beukema, W., Galis, F., Ivanovic, A., 2015. Vertebral number is highly evolvable in salamanders and newts (family Salamandridae) and variably associated with climatic parameters. Contributions to Zoology 84, 85-113.

Arntzen, J.W., García-París, M., 1995. Morphological and allozyme studies of Iberian green toads (*Bufo viridis* subgroup): systematic and evolutionary implications. Journal of Evolutionary Biology 8, 695-710.

Bam-E-Zar, F., Fathinia, B., Shafaei-Pour, A., 2019. Trophology of Levant Green Frog, *Pelophylax bedriagae* (Amphibia: Anura: Ranidae) in Choram Township, Iran. North-Western Journal of Zoology 15, 168-174.

Berger, L., 1970. Some characteristics of the crosses within *Rana esculenta* complex in postlarval development. Annales Zoologici Fennici 27, 373-416.

Bina Perl, R.G., Gafny, S., Malka, Y., Renan, S., Woodhams, D.C., Rollins-Smith, L., Pask, J.D., Bletz, M.C., Geffen, E., Vences, M., 2017. Natural history and conservation of the rediscovered Hula painted frog, *Latonia nigriventer*. Contributions to Zoology 86, 11-37.

Bina Perl, R.G., Gefen, E., Malka, Y., Barocas, A., Renan, S., Vences, M., Gafny, G., 2018. Population genetic analysis of the recently rediscovered Hula painted frog (*Latonia nigriventer*) reveals high genetic diversity and low inbreeding. Scientific Reports 8, 5588.

Blank, L., Blaustein, L., 2014. A multi-scale analysis of breeding site characteristics of the endangered fire salamander (*Salamandra infraimmaculata*) at its extreme southern range limit. Hydrobiologia 726, 229-244.

Blaustein, A.R., Johnson, P.T.J., 2003. The complexity of deformed amphibians. Frontiers in Ecology and the Environment 1, 87-94.

Capula, M., 1989. Simon and Schuster's Guide to Reptiles and Amphibians of the World. Simon and Schuster Inc.

Cogălniceanu, D., Székely, P., Iosif, R., Székely, D., Stănescu, F., 2013. Life history and conservation of spadefoot toads (genus *Pelobates*) in Romania. FrogLog 21, 24-26.

Cohen, O., Ram, Y., Hadany, L., Gafny, S., Geffen, E., 2021. Annual climatic fluctuations and short-term genetic variation in the eastern spadefoot toad. Scientific Reports 11, 13514.

Cummingham, M., Cherry, I., 2004. Molecular systematics of African 20-chromosome toads (Anura: *Bufonidae*). Molecular Phylogenetics and Evolution 32, 671-685.

Degani, G., 1981a. The adaptation of *Salamandra salamandra* (L.) from different habitats to terrestrial life. British Journal of Herpetology 6, 169-172.

Degani, G., 1981b. Salinity tolerance and osmoregulation in *Salamandra salamandra* (L.) from different populations. Journal of Comparative Physiology 145, 133-137.

Degani, G., 1982. Amphibian tadpole interaction in a winter pond. Hydrobiologia 96, 3-8.

Degani, G., 1983. Oxygen consumption rates of *Salamandra salamandra* (L.) from different habitats. British Journal of Herpetology 6, 281-283.

Degani, G., 1985. Urea tolerance and osmoregulation in *Bufo viridis* and *Rana ridibunda*. Comparative Biochemistry and Physiology A 82, 833-836.

Degani, G., 1986a. Growth and behavior of six species of amphibian larvae in a winter pond in Israel. Hydrobiologia 140, 5-10.

Degani, G., 1986b. Plasma protein and morphology of *Salamandra salamandra* in Israel. Amphibia-Reptilia 7, 105-114.

Degani, G., 2013. Genetic variation among various populations of spadefoot toads (*Pelobates syriacus*, Boettger, 1869) at breeding sites in northern Israel. Advances in Biological Chemistry 3, 440-447.

Degani, G., 2015. The habitats, burrowing behavior, physiology adaptation and life cycle of Spadefoot toads (*Pelobates syriacus*, Boettger, 1869) at the southern limit of its distribution in Israel. Open Journal of Animal Sciences 5, 249-257.

Degani, G., 2016. The habitats, life cycle and physiological adaptation of Green toad (*Pseudepidalea viridis*) in northern Israel. Internal Journal of Scientific Research 5, 113-117.

Degani, G., 2019a. Ecological and genetic variation of the distribution of various species of amphibians at the southern border of their distribution. International Journal of Plant, Animal and Environmental Sciences 9, 21-41.

Degani, G., 2019b. The Fire Salamandra (*Salamandra infraimmaculata*) and the Banded Newt (*Triturus vittatus*) along the Southern Border of Their Distribution. Scientific Research Publishing Inc.

Degani, G., 2022. Changes in Tree frog (*Hyla savignyi*) coloration in unstable habitats at the southern border of its distribution. Open Journal of Animal Sciences 12, 68-75.

Degani, G., Biton, E., 2013. Tree frog (*Hyla savygnyi*) color and substrate preference. American Open Animal Science Journal 1, 31-39.

Degani, G., Carmali, D., 1988. Burrowing behavior of *Pelobates syriacus* biology of behaviour 13, 22-29.

Degani, G., Goldberg, T., Gazith, A., Elorom, E., Nevo, E., 2013a. The DNA variation of *Pseudepidalea viridis* (Syn. *Bufo viridis*) from various habitats. Zoological Studies 52, 1-15.

Degani, G., Goldberg, T., Yom-Din, S., 2013b. The ecology and variation in

DNA of *Rana bedreagae* from various breeding site in North Israel. Research Open Journal of Animal Sciences 1, 1-14.

Degani, G., Kaplan, D., 1999. Distribution of amphibian larvae in Israeli habitats with changeable water availability. Hydrobiologia 405, 49-56.

Degani, G., Mendelssohn, H., 1983. The habitats, distribution and life history of *Triturus vittatus vittatus* (Jenyns) in the Mount Meron area (Upper Galilee, Israel). British Journal of Herpetology 6, 317-319.

Degani, G., Nagar, R., Yom-Din, S., 2012. Molecular DNA variation in *Hyla felixarabica* from various breeding sites in northern Israel. Herpetologica Romanica 6, 51-67.

Degani, G., Silanikove, N., Shkolnik, A., 1984. Adaptation of Green toad (*Bufo viridis*) to terrestrial life by urea accumulation. Comparative Biochemistry and Physiology 77A, 585-587.

Degani, G., Warburg, M.R., 1984. Changes in concentrations of ions and urea in both plasma and muscle tissue in dehydrated hylid anuran. Comparative Biochemistry and Physiology Part A: Physiology 77, 357-360.

Disi, M., Amr, Z.S., 2010. Morphometrics, distribution and ecology of the amphibians in Jordan. Vertebrate Zoology 60, 147-162.

Duellman, W., 1993. Amphibian Species of the World: Additions and Corrections. Lawrence, University of Kansas.

Duellman, W.E., Trueb, L., 1986. Biology of Amphibians. McGraw-Hill.

Dufresnes, C., Berroneau, M., Dubey, S., Litvinchuk, S.N., Perrin, N., 2020. The effect of phylogeographic history on species boundaries: a comparative framework in *Hyla* tree frogs. Scientific Reports 10, 5502.

Frost, D.R., 2021. Amphibian species of the world: an online reference. Version 6.2. Electronic Database. American Museum of Natural History New York.

Goldberg, T., Nevo, E., Degani, G., 2009a. Breeding site selection according to suitability for amphibian larval growth under various ecological conditions in the semi-arid zone of northern Israel. Ecologia Mediterranea 35, 65-74.

Goldberg, T., Nevo, E., Degani, G., 2012. Amphibian larval in various water bodies in the semi-arid zone. Zoological Studies 51, 345-361.

Goldberg, T., Pearlson, O., Nevo, E., Degani, G., 2009b. Sequence analysis of mitochondrial DNA in *Salamandra infraimmaculata* larvae from populations in northern Israel. South American Journal of Herpetology 4, 268-274.

Gomez-Rodrıguez, C., Dıaz-Paniagua, C., Serrano, L., Florencio, M., Portheault, A., 2009. Mediterranean temporary ponds as amphibian breeding habitats: the importance of preserving pond networks. Aquatic Ecology 43, 1179-1191.

Grach, A., Plesser, Y., Werner, Y.L., Gasith, A., 2007. A new, sibling, tree frog from Jerusalem (Amphibia: Anura: Hylidae). Journal of Natural History 41, 709-728.

Grismer, L.L., 2002. Amphibians and Reptiles of Baja California, Including Its Pacific Islands and the Islands of the Sea of Cortes. University of California Press.

Gvozdik, V., Moravec, J., Klutsch, C., Kotlik, P., 2010. Phylogeography of the middle eastern tree frogs (*Hyla*, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. Molecular Phylogenetics and Evolution 55, 1146-1166.

Haas, A., 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). Cladistics 19, 23-90.

Hoffman, J., Katz, U., 1989. The ecological significance of burrowing behaviour in the toad *Bufo viridis*. Oecologia 81, 510-513.

Hoffmann, M., 2010. The impact of conservation on the status of the world's vertebrates. Science 330, 1503-1509.

Katz, U., Degani, G., Gabbay, S., 1984. Acclimation of the euryhaline toad *Bufo viridis* to hyperosmotic solution (NaCl, urea and mannitol). Journal of Experimental Biology 108, 403-409.

Kyriakopoulou-Sklavounou, P., Sjogren, P., Jansson, H., 2008. Discordant genetic and morphological differentiation between three *Rana ridibunda* populations from northern Greece. Hereditas 115, 51-61.

Litvinchuk, S.N., Zuiderwijk, A., Borkin, L.J., Rosanov, J.M., 2005. Taxonomic status of *Triturus vittatus* (Amphibia: Salamandridae) in western Turkey: trunk vertebrae count, genome size and allozyme data. Amphibia-Reptilia 26, 305-323.

McCallum, M.L., 2007. Amphibian decline or extinction? Current declines dwarf background extinction rate. Journal of Herpetology 41, 483-491.

Medina, R.F., Barbosa, P., Christman, M., Battisti, A., 2006. Number of individuals and molecular markers to use in genetic differentiation studies. Molecular Ecology Notes 6, 1010-1013.

Mendelssohn, H., Steinitz, H., 1943. A new frog from Palestine. Copeia 1943, 231-233.

Munwes, I., Geffen, E., Roll, U., Friedmann, A., Daya, A., Tikochinski, Y., Gafny, S., 2010. The change in genetic diversity down the core-edge gradient in the eastern spadefoot toad (*Pelobates syriacus*). Molecular Ecology 19, 2675-2689.

Nevo, E., Beiles, A., 1988. Genetic parallelism of protein polymorphism in nature: ecological test of the neutral theory of molecular evolution. Biological Journal of the Linnean Society 35, 229-245.

Nevo, E., Filippucci, M.G., 1988. Genetic differentiation between Israeli and Greek population of the marsh frog, *Rana ridibunda*. Zoologischer Anzeiger 221, 418-424.

Nielsen, H.I., 1980. Color and color adaptation of the European tree frog *Hyla arborea*. Journal of Experimental Zoology 211, 143-151.

Pearlson, O., 2012. Ecology and genetic variance of the banded newt *Triturus vittatus vittatus* in northern Israel. PhD thesis, University of Haifa, Israel.

Perl, B.R.G., Gafny, S., Malka, Y., Renan, S., Woodhams, D.C., Louise Rollins-Smith, L., Pask, D.L., Bletz, C.M., Geffen, C.E., Vences, M., 2017. Natural history and conservation of the rediscovered Hula painted frog, *Latonia nigriventer.* Contributions to Zoology 86, 11-37.

Plötner, J., Uzzell, T., Beerli, P., Spolsky, C., Ohst, T., Litvinchuk, S.N., Guex, G.D., Reyer, H.U., Hotz, H., 2008. Widespread unidirectional transfer of mitochondrial DNA: a case in western Palaearctic water frogs. Journal of Evolutionay Biology 21, 668-681.

Richter-Boix, A., Llorente, G.A., Montori, A., 2006. A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient. Evolutionary Ecology 8, 1139-1154.

Schneider, H., Sinsch, U., Nevo, E., 1992. The lake frogs in Israel represent a new species. Zoologischer Anzeiger 228, 97-106.

Sinsch, U., Schneider, H., 1999. Taxonomic reassessment of Middle Eastern water frogs: morphological variation among populations considered as *Rana ridibunda*, *R. bedriagae* or *R. levantina*. Journal of Zoological Systematics and Evolutionary Research 37, 67-74.

Steinfartz, S., Veith, M., Tautz, D., 2000. Mitochondrial sequence analysis of *Salamandra* taxa suggests old splits of major lineages and postglacial recolonizations of central Europe from distinct source populations of *Salamandra salamandra*. Molecular Ecology 9, 397-410.

Stöck, M., Sicilia, A., Belfiore, N.M., Buckley, D., Lo Brutto, S., Lo Valvo, M., Arculeo, M., 2008. Post-Messinian evolutionary relationships across the Sicilian channel: mitochondrial and nuclear markers link a new green toad from Sicily to African relatives. BMC Evolutionary Biology 8, 56.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular

Biology and Evolution 28, 2731-2739.

Veith, M., 1994. Morphological, molecular and life history variations in *Salamandra salamandra* (L.). Mertensiella 4, 355-397.

Warburg, M.R., 1971. The water economy of Israel amphibians: the Urodeles *Triturus vittatus* (Jenyns) and *Salamandra salamandra* (L.). Comparative Biochemistry and Physiology Part A: Physiology 40, 1055-1063.

Warburg, M., 1992. Breeding patterns in fringe population of fire salamanders, *Salamandra salamandra*. Journal of Herpetology 2, 54-58.

Wilbur, W.J., Lipman, D.J., 1983. Rapid similarity searches of nucleic acid and protein data banks. Proceedings of the National Academy of Sciences of the United States of America 80, 726-730.

$5.00
ISBN 978-1-64997-800-4
50500>
9 781649 978004