INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-9, Issue-1 Jan-Mar-2019 Coden:IJPAJX-CAS-USA, Copyrights@2019 ISSN-2231-4490

Received: 25th Feb-2019 Revised: 25th Mar-2019 Accepted: 26th Mar-2019

DOI: 10.21276/Ijpaes http://dx.doi.org/10.21276/ijpaes Research Article

ECOLOGICAL AND GENETIC VARIATION OF THE DISTRIBUTION OF VARIOUS SPECIES OF AMPHIBIANS AT THE SOUTHERN BORDER OF THEIR DISTRIBUTION

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ABSTRACT : The current mini-review describes the distribution of amphibian species in terms of their adaptation from Mediterranean to desert climates. According to the data collected in this mini-review and from some unpublished data, it was found that the adaptation of amphibian species from arid to Mediterranean climates was highest for *Bufovariabilis*, followed by *Triturusvittatus*,*Hylasavignyi*, *Pelobatessyriacus*, *Rana bedriagae* and *Latonia nigriventer*. Many parameters affecting adaptation to different habitats have been described, including aquatic and terrestrial habitats. Among them, the most important parameters affecting semi-arid and arid habitats are the large number of tadpoles, the short growth and complete metamorphosis period, finding hiding places to prevent dehydration, and physiological adaptation to accumulate urea in the body fluid. A quality model is suggested to show the adaptation of various amphibian species to habitats at the southern border of its distribution.
Keywords: Bioindicator;Environmental pollution;Genetic markers; RAPD PCR; Amphibian populations

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INTRODUCTION

Amphibian species populations are important bioindicators for the environment affected by human development and pollution [1, 2]. Israel is developing very rapidly both in terms of agriculture and urban expansion. Seven amphibian species exist in Israel: two are Urodaela – banded newt, *Triturusvittatus*Litvinchuk et al. [3] synonym *Ommatotritonvittatus*, which is found in Israel, Lebanon, Jourdan, Syria, Turkey and Iraq, and fire salamander, *Salamandrainfraimmaculata*[4],which is distributed in Israel, Lebanon, Jordan, Syria, Turkey and Iran. Both of these species belong to the Salamandrinae family. Five Anuran species also survive in Israel: tree frog, *Hylasavignyi*(Hylidae family), synonym *H. arborea*[5, 6],which is found in Yemen, Jordan, southern Syria and extreme north-eastern Israel; green toad, *Bufoviridis* (Bufonidae family) synonym *PseudepidaleaViridis*[7, 8], 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 they have also penetrated into urban areas; water frog,*Rana bedriagae* (Ranidae family) synonym *Rana ridibunda*[9, 10]; spadefoot, *Pelobatessyriacus* (Pelobatidae family) [11-14], whose wide distribution belongs to the Pelobatidae family in part of Europe and East and West Asia, including North Syria, Israel, North Iraq, Iran, Asia Minor and Caucasus; and Hula painted frog, *Latonia nigriventer* (Alytidae family) [15, 16] belonging to the Alytidae family (this is an endemic species found in the Hula reserve).

All these amphibian species are found at the southern border of their distribution at relatively extreme conditions. In Israel and other places, amphibians breed in different aquatic habitats, including temporary ponds, semi-permanent ponds, spring and streams, where water is available all year round or part of the year [11, 12, 17-24]. Many factors affect the aquatic phase of amphibians [25].

Israel offers mainly xeric habitats, unusual for amphibians, and constitutes the southeastern range of distribution of these species at the extreme condition of these species [8, 11, 22, 26]. Hence, amphibian larvae occupy a very narrow and specific ecological niche in the region, and are under severe pressure from predators and other biotic and abiotic factors [12, 27]. To define the range of water quality in which tadpoles of various species can grow and complete metamorphosis, it is not only important to gain knowledge about those species for their protection but it also may serve as a bioindicator for environmental pollution. The adaptation of amphibians to various habitats at the southern border of their distribution in Israel in a relatively small area is examined by genetic variation in different areas. Genetic markers have been used to study the variation among different populations. All of the seven amphibian species in Israel belong to a different genus, and some belong to different families and orders [11, 21, 28]. Various methods are used to study the genetic variation of these species and their adaptation to different habitats or geographical distribution. A relatively large number of studies on genetic variation have been carried out for *S. infraimmaculata* using the enzyme system loci [29] DNA polymerase chain reaction (RAPD PCR) [30], mitochondrial DNA analysis [13, 31-33]. Amplified Fragment Length Polymorphism (AFLP) [34], gene expression microarrays [35], microsatellites [36, 37] and RNA excretion [38]. Some of these methods can be applied to other species in order to study the genetic variation among various habitats in Israel, but not all of them. The variation among populations in Israel was studied for *T. vittatusvittatus*[26, 33, 39], Pearson*H. savignyi*[5], *B. viridis* (*P. Viridis*) [8, 42], *R. bedriagae*[43] and *Latonia nigriventer*[44].
The purpose of this mini-review and unpublished data is to examine the different adaptations of seven species of amphibians in semi-arid habitats at the southern border of their distribution in both aquatic and terrestrial habitats. The hypothesis examined in the present study is that the adaptation to different areas of amphibians at the southern border of distribution is mainly by metamorphosis phases and not aquatic phases.

MATERIALS AND METHODS

Sampling

The breeding sites of amphibian populations in Israel (Figure1) at both terrestrial and aquatic phases from north to south were examined in different samplings, namely, near various water bodies including winter pools, pits, springs and streams, and the locations and various amphibian larvae of these places were defined. Tadpoles were collected with a net (pore size 450 μm) from a depth of approximately 10-40 cm every two weeks during larvae growth and after complete metamorphosis [11, 12], and their species were identified as previously described [17]. The metamorphosed animals were collected mainly during the winter and spring near the breeding places as previously described [45-48].

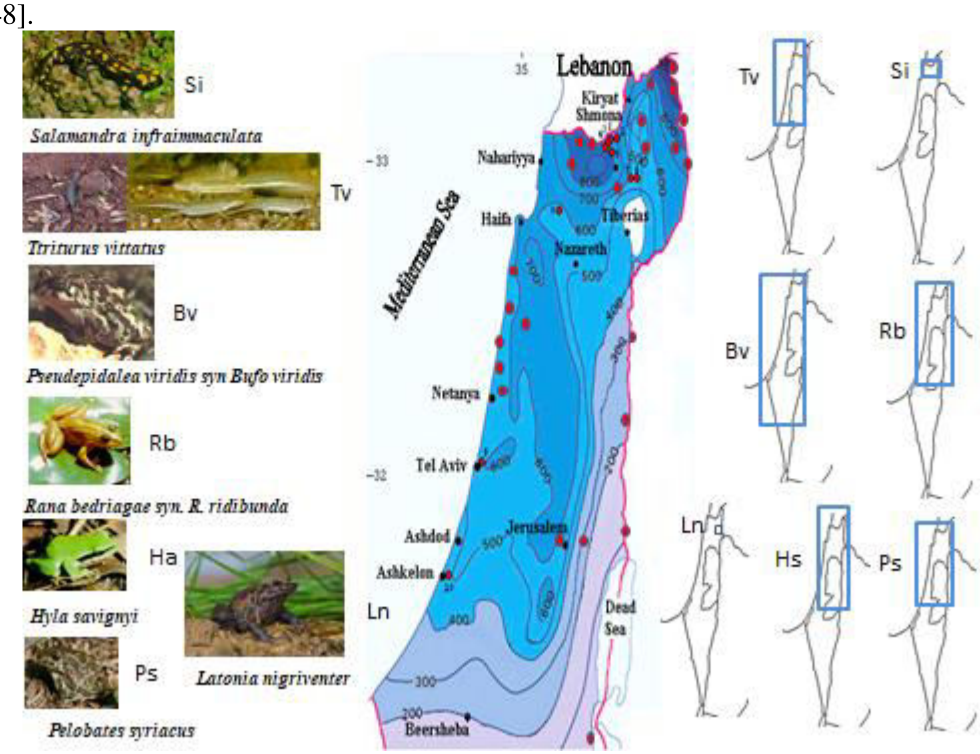

Figure 1:Various breeding places in Israel where theamphibians' tadpoles were collected and identified in Israel.

Water quality, including pH, ammonium concentration, electrical conductivity (EC), temperature, relative oxygen content (O_2) and pH [49], genetic variation [13, 40, 44], were measured. The evolutionary history tree was derived using the Neighbor-Joining method(Saitou and Nei, 1987). The phylogenetic tree was done as previously described [51]. Phylogenetic analyses were conducted using MEGA4 [51].

RESULTS

The seven species of amphibians were found in various habitats in Israel. Two belong to Urodela – *Triturusvittatus* and fire salamander,*Salamandrainfraimmaculata*,and five are Anuran species, including the tree frog, *Hylasavignyi*, the green toad, *Bufoviridis* (*Pseudepidaleaviridis*), the water frog,*Rana bedriagae*, the spadefoot, *Pelobatessyriacus* and the Hula painted frog (*Latonia nigriventer*) (Figure 1). *Latonia nigriventer*and *S. infraimmaculata* are distributed in small areas compared to all the other amphibian species. *B. viridis* and *T. vittatus*are distributed in large areas. *B. viridis* adapted to a wide range of areas in Israel and also survived in desert environments. In the ecological conditions of the examined larvae in the breeding sites shown in Figures 2 to 8, the conditions are not extreme different between species, and some species adapted to different types of breeding sites (ponds, spring and streams) in the same area [1, 5, 18, 52-60]. However, there are different conditions among breeding sites in the area of the species distribution of larvae habitats. Some species were studied in more detail, e.g., *S. infraimmaculata*, which are bred in all kinds of water bodies such as springs, streams, winter ponds and rock poles (Figure2) [61].

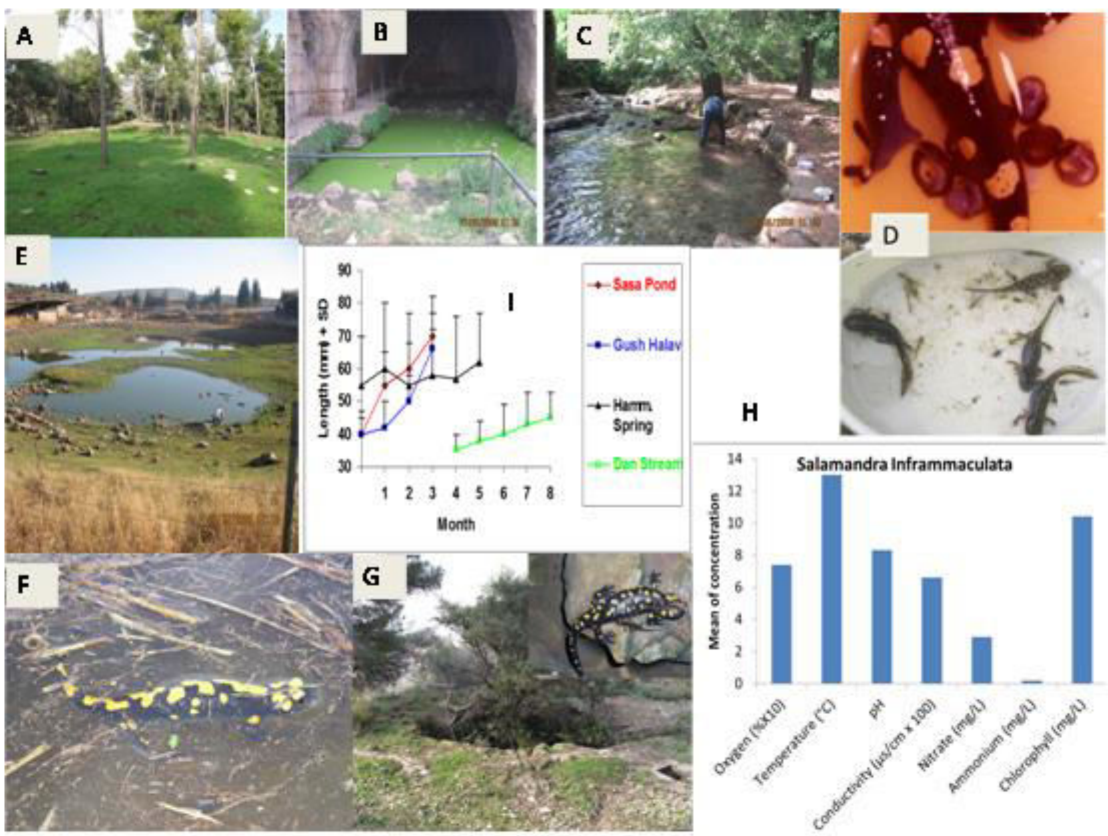

Figure 2:Presentation of ecological terrestrial and aquatic habitats of *S. infraimmaculata* including niches of larvae in northern Israel with respect to five ecological characteristics – oxygen (mg/L), ammonium ([mg/L]/2), temperature (°C), conductivity ([uS/cm] ×100) and pH. The different areas in Israel (see Figure1) are: A, terrestrial habitats, Western Galilee; B, breeding place, Nimrod pit, Harmon mountain; C, breeding place, Tel-Dan stream, northern Israel; D, oviposition and large development of larvae *S. infraimmaculata*; E, Raihaniya pond, Lower Galilee; F, female *S. infraimmaculata*oviposition in Sasa pond, Upper Galilee; and G, Maalot pit, Western Galilee. H, ecological niches of water where *S. infraimmaculata* tadpoles are found in northern Israel.I, measurement of tadpoles grown in different breeding sites in the Upper Galilee.

The oviposition of larvae in most populations took time, from November to December, and totaled 90-130; the oviposition in permanent springs totaled 68-72 [62]. On the other hand, some species such as *T. vittatus* (Figure3) [36] and *B. viridis* (Figure4) Degani et al. [40] used only relatively specific breeding sites and unpredictable breeding places in winter (ponds, rock pools and streams). Both these species are part of a wider distribution; they adapted to relatively extreme conditions and were also found in all areas of other amphibian distributions (Figure1) [11]. Both *T. vittatus* and *B. viridis*lay eggs and have external fertilization [20].*T. vittatus* deposit between 18-68 eggs on plants or rock surfaces [59] and the number of eggs per female of *B. viridis* spawning is much greater (5,687-17,602) [8, 63].

Both species have a relatively short period of larval growth, and complete metamorphosis takes place over a short period [11, 12, 16, 64]. It is very difficult to compare larval growth in natural habitats so it is suggested examining habitats having extensive larval growth and development [16]. The only differences in this habitat are the temperature in the ponds and the growth period of *B. viridis* in winter, and the time and cold water of *T. vittatus* in spring and summer [16].

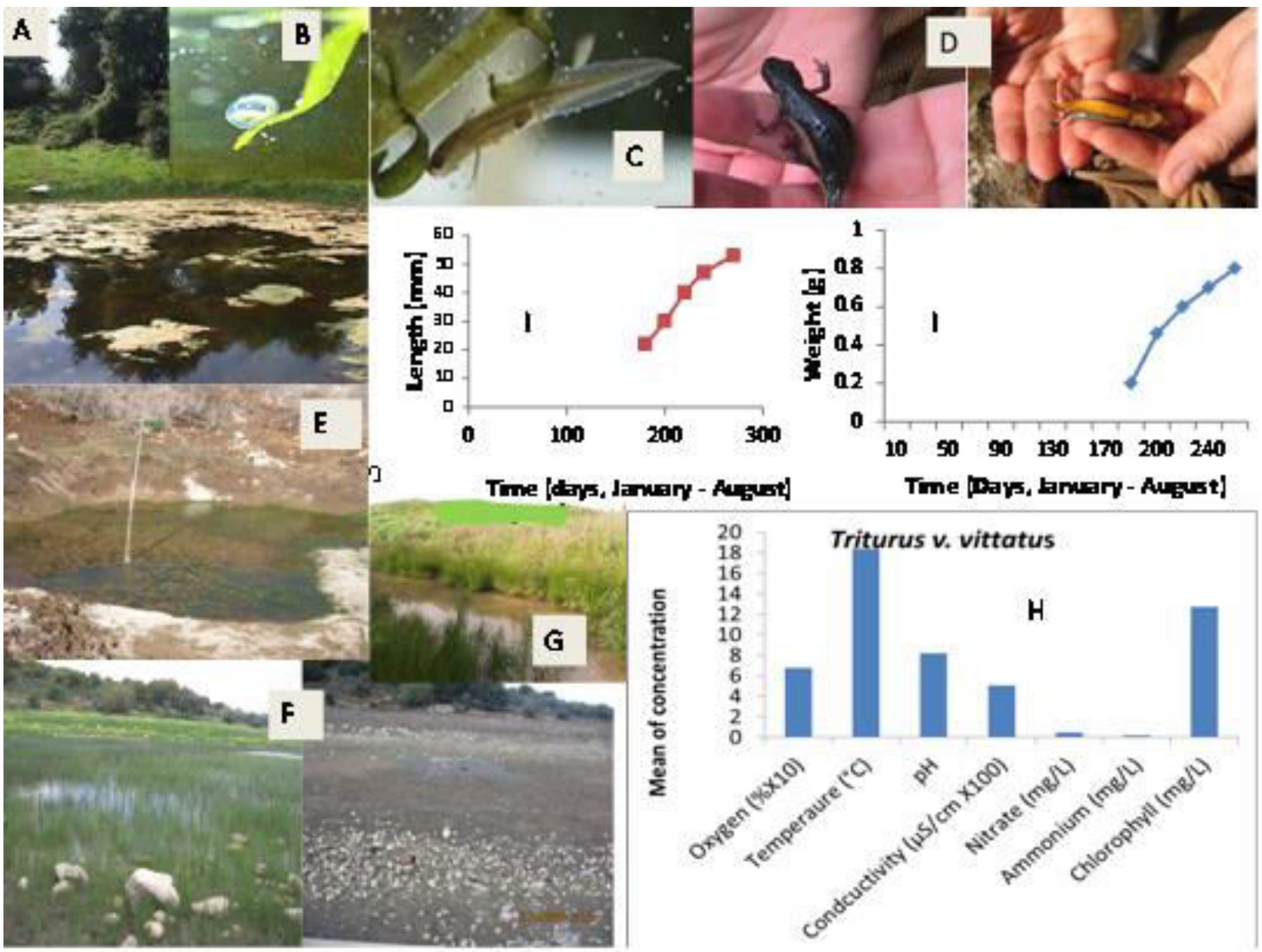

Figure 3:Presentation of ecological terrestrial and aquatic habitats of *Triturusvittatus* including niches of larvae in northern Israel with respect to five ecological characteristics – oxygen (mg/L), ammonium ([mg/L]/2), temperature (°C), conductivity ([uS/cm] ×100) and pH.A, winter pond in Upper Galilee. B, eggs. C, larvae newt. D, adult female's terrestrial newt. E and F (Sasa in winter and summer) winter ponds in Upper Galilee.H, water qualities of *Triturusvittatus* tadpoles in northern Israel.

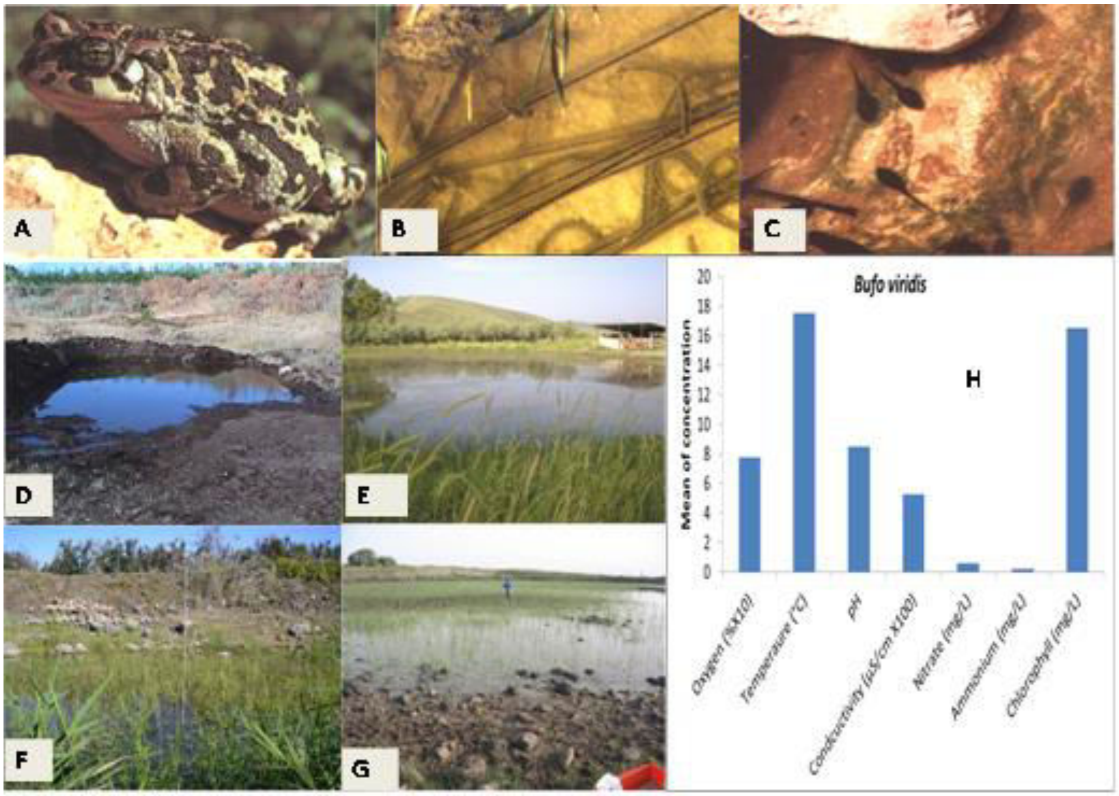

Figure 4:Presentation of ecological terrestrial and aquatic habitats of *Bufoviridis* (Syn.*PseudepidaleaViridis*) including niches of larvae in northern Israel with respect to five ecological characteristics – oxygen (mg/L), ammonium ([mg/L]/2), temperature (°C), conductivity ([uS/cm] ×100) and pH. Larval growth (weight and length) and complete metamorphosis in winter ponds in the Upper Galilee (Sasa pond) are presented. A, Adult *Bufoviridis*. B, eggs of *Bufoviridis*. C, tadpoles of *Bufoviridis*.D, winter pond before drying up.E, winter pond in Upper Galilee.F, Fara pond.G, Kash pond (Hula valley). H, ecological niches of *Bufoviridis* larvae in northern Israel. I, measurement of tadpoles grown in winter ponds in Upper Galilee.

H. savignyi larvae are also found in unpredictable habitats, many of which are winter ponds (Figure5). Like in the larvae of *T. vittatus*and *B. viridis*, a short growth period and complete metamorphosis occurs between 1-2 months [11, 12, 16, 64], and they can use the same breeding places as *T. vittatus*and *B. viridis*if the water conditions are suitable from winter to summer.

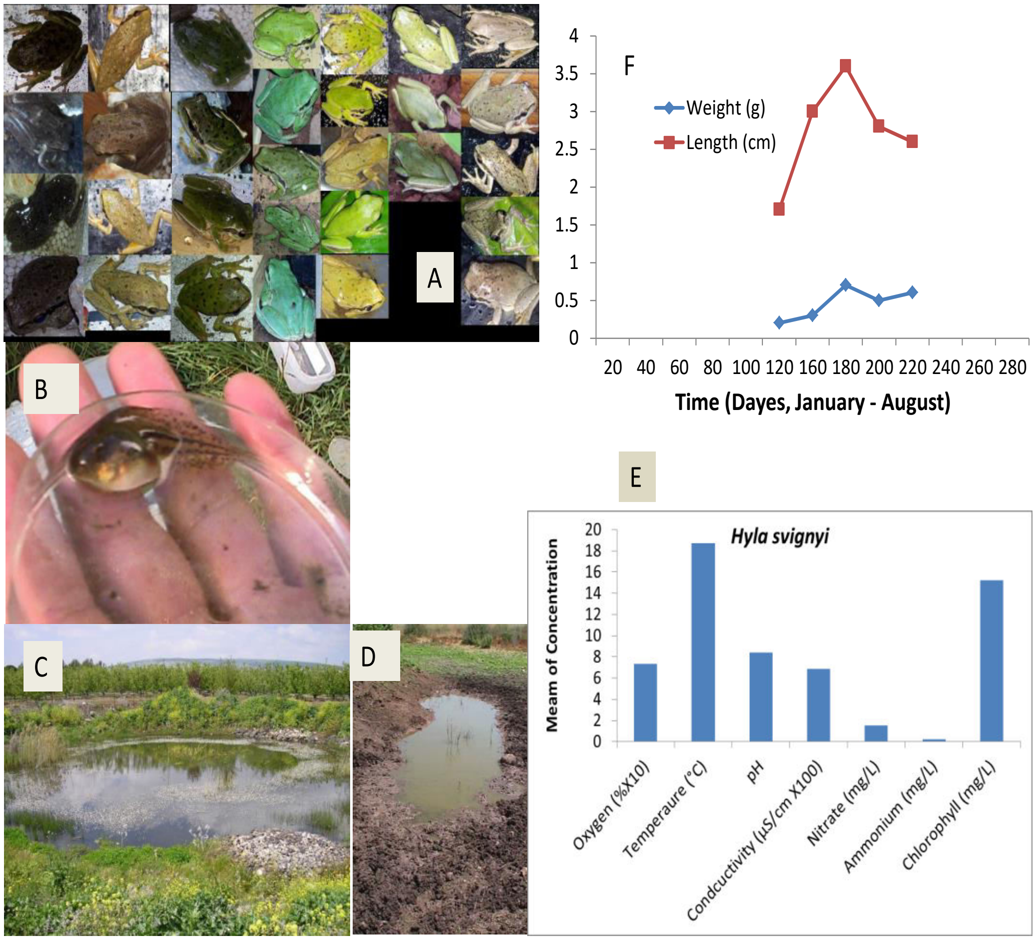

Figure 5:Presentation of ecological terrestrial and aquatic habitats of *Hylasavignyi* include niches of larvae in northern Israel with respect to five ecological characteristics – oxygen (mg/L), ammonium ([mg/L]/2), temperature (°C), conductivity ([uS/cm] ×100) and pH.A, adult *Hylasavignyi* with different colors as recorded in northern Israel.B, tadpoles of *Hylasavignyi*, C and D, Lahavot pond. E, ecological niches of *Rana bedriagae* larvae in northern Israel. F, measurements of tadpoles grown in a winter pond (Sasa) in Upper Galilee.

The larval growth period of *R. bedriagae* (Figure6) and *P. syriacus* (Figure7) is relatively long, and complete metamorphosis lasts for over four months from winter to summer; tadpoles are found at these breeding places [16].
The studies on amphibian larvae interactions regarding growth and complete metamorphosis may show specific niches for each species. The studies were carried out in winter ponds in the Upper Galilee (Figure 9) [16, 62] and in many breeding sites in northern Israel [11, 12].
Based on this information, the tadpoles are found in specific niches. For example, the tadpoles of *T. vittatus*and fire salamander, *S. infraimmaculata*, which followed the same diet, were found at different times in the ponds, and water temperatures differed during the growth periods: *S. infraimmaculata* during the winter and *T. vittatus* during the spring and beginning of summer (Figure 9). The tadpoles of *S. infraimmaculata, T. vittatus* and *Rana bedriagae* were found at the bottom of the pond most of the time; *H. savignyi* were found throughout the pond and were usually sedentary compared to *P. syriacus*,which moved up and down constantly [16].

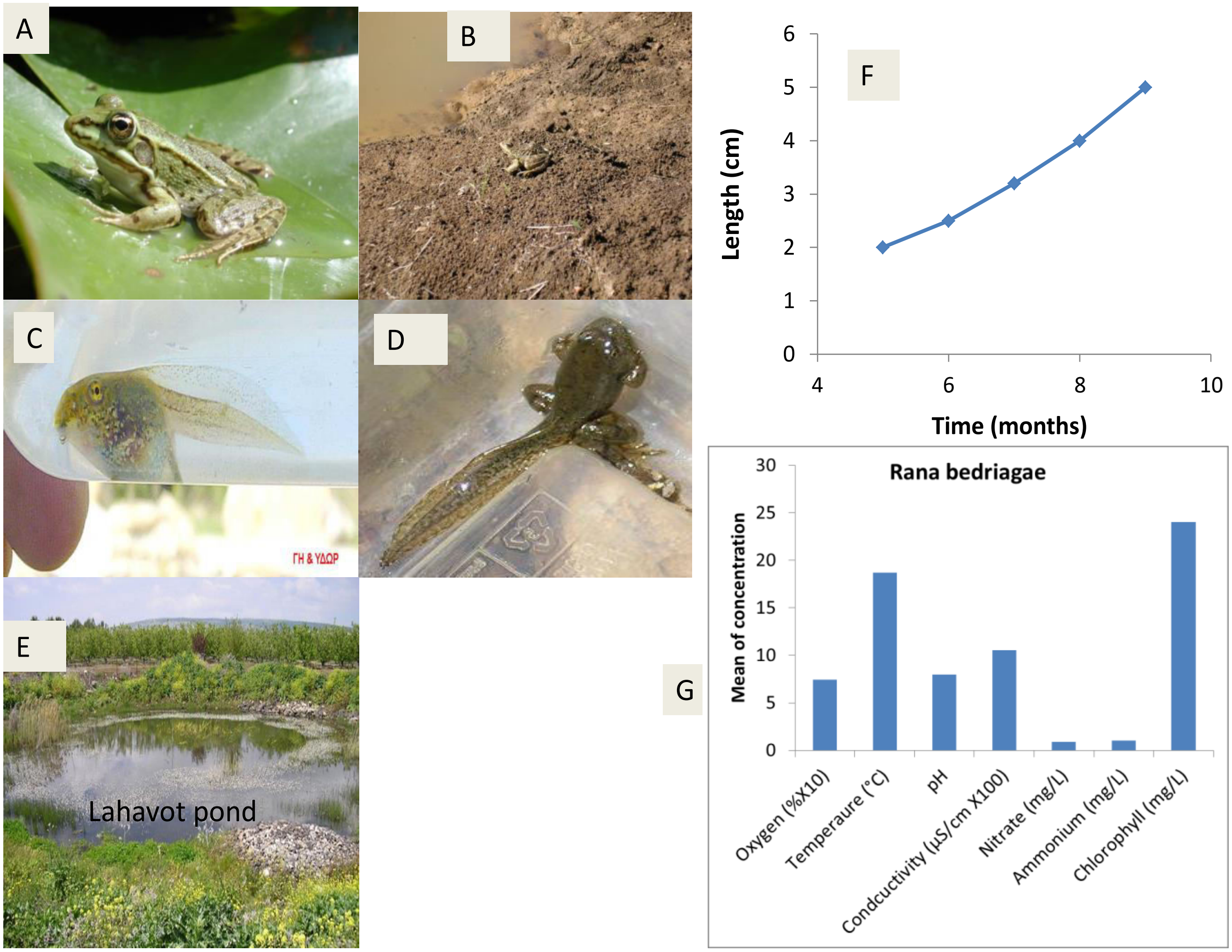

Figure 6:Presentation of ecological terrestrial and aquatic habitats of *Rana bedriagae*including niches of larvae in northern Israel with respect to five ecological characteristics – oxygen (mg/L), ammonium ([mg/L]/2), temperature (°C), conductivity ([uS/cm] ×100) and pH.A, adult *Rana bedriagae*.B, juvenile *R. bedriagae*, C, *R. bedriagae* tadpoles.D, *R. bedriagae* during metamorphosis.E, Lahavot pond in Hula valley.F, larval growth in Lahavot pond. G, ecological niches of *R. bedriagae* tadpoles in northern Israel.

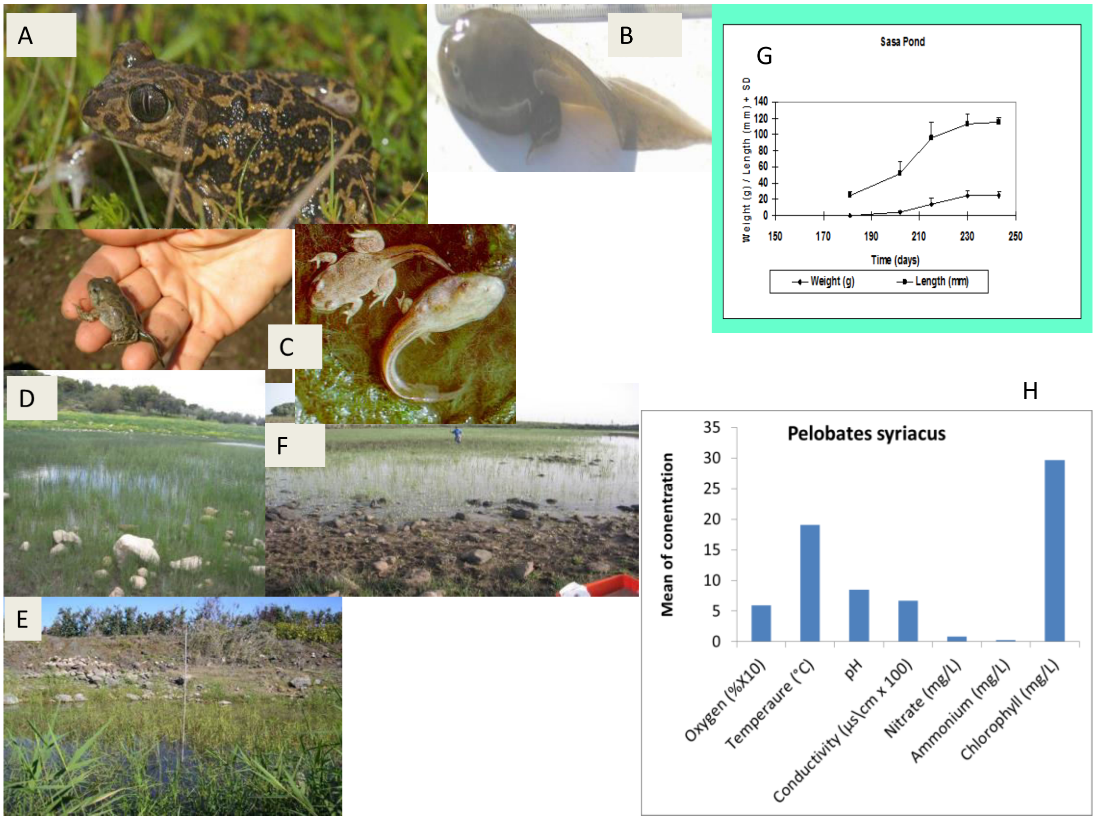

Figure 7:Presentation of ecological terrestrial and aquatic habitats of *Pelobatessyriacus* include niches of tadpoles in northern Israel with respect to five ecological characteristics – oxygen (mg/L), ammonium ([mg/L]/2), temperature (°C), conductivity ([uS/cm] ×100) and pH.A, adult *P. syriacus*, B. *P. syriacus* tadpole.C, tadpole under metamorphosis.D, E and F, breeding sites (northern Israel) in ponds of *P. syriacus*.G, larval growth in a pond (Sasa) from spring and summer in Upper Galilee.H, ecological niches of tadpoles in northern Israel.

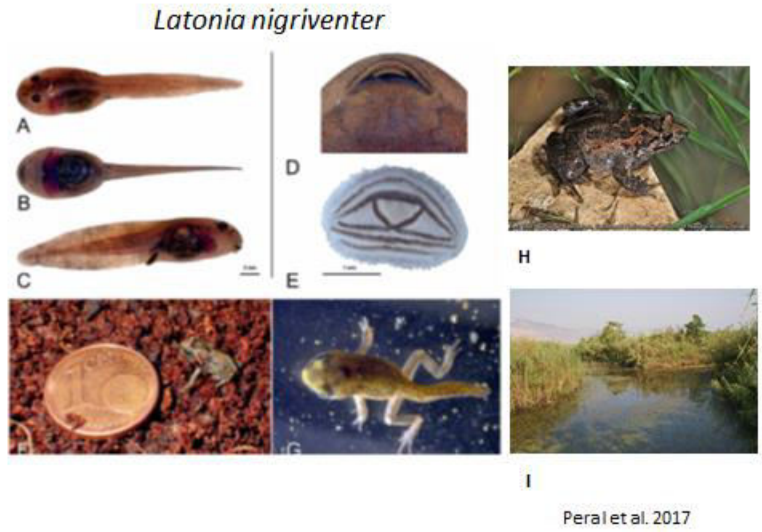

Figure 8:Presentation of ecological terrestrial and aquatic habitats of *Latonia nigriventer*including niches of larvae in different areas in northern Israel (see Figure1) [64]. A-E are*Latonia nigriventer* tadpoles [26]. G, tadpole under metamorphosis. F, juvenile.H, adult.I, breeding site.

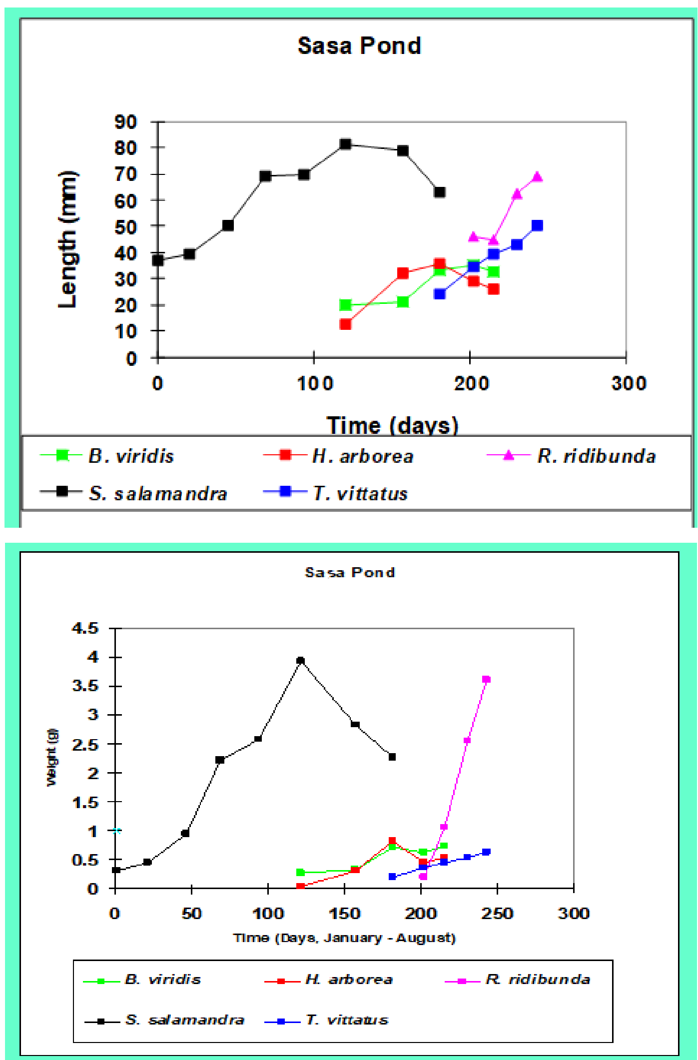

Figure 9:The growth of various amphibians in a winter pond (Sasa) in the Upper Galilee from January to August [16].

Relatively fewer studies were carried out to examine amphibian adaptation after metamorphosis to terrestrial life compared to tadpoles in Israel.*B. viridis*, which has the largest distribution (Figure1), penetrated into desert and arid environments and showed very high adaptation [65-69] through their ability to store water in the urine bladder and accumulate urea in the plasma compared to other species such as *T. vittatus*, *P. syriacus*[70, 71], *S. infraimmaculata*[53, 72, 73], *R. bedriagae*[65] and *H. savignyi* (Figure10) [74].

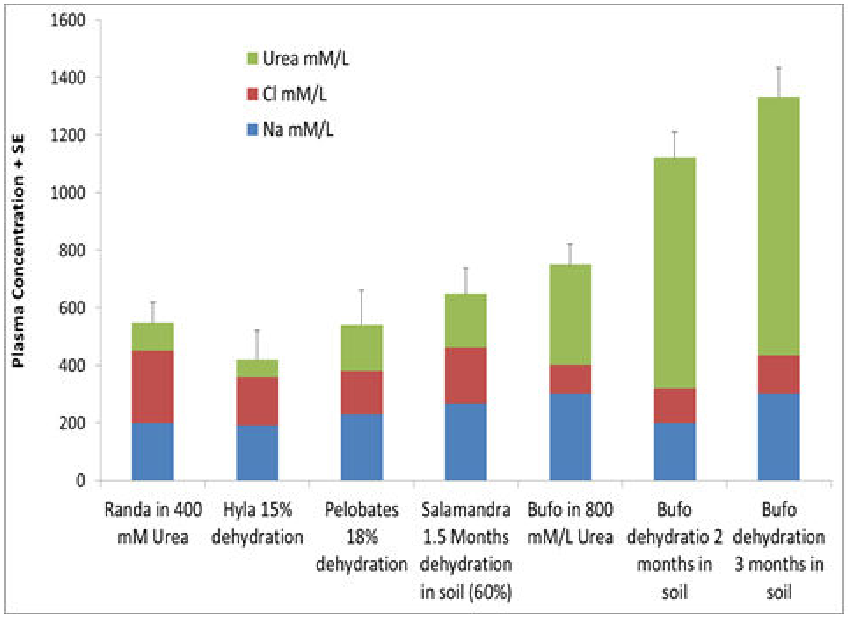

Figure 10:Plasma composition and concentration of various mature amphibians during the environment starch [65, 67, 68, 70, 72, 74].

The amphibian skin does not prevent dehydration, and the ability to survive in terrestrial life depends on physiological adaptation ability, e.g., plasma concentration. Based on the information collected regarding the ability to survive, high plasma concentrations might be related to the ability to accumulate urea and water in the bladder (Figure10). The ability to accumulate urea is related to a high adaptation to dry conditions, as was found in *B. viridis*[66].

Another ability to adapt to terrestrial life is environmental behavior, involving finding hiding places and getting past relatively long dry seasons. Most amphibian species in Israel, except for *R. bedriagae*, are active at night, and they find hiding places at a relatively high soil humidity and negative photo taxis.

Once metamorphosis is complete, the amphibians become terrestrial animals and are in danger of dehydration. The environmental behavior in finding a hiding place is an important adaptation to prevent dehydration and survive. The subject of finding hiding places was studied in various species and it was based on moisture, light and temperature, or even digging in the soil, as was described in *P. syriacus*[7, 75] (Figure11). Environmental behavior was studied in various amphibian species at the southern border of their distribution: *S. infraimmaculata*[76-78], *T. vittatus*[19, 36, 79] and *P. syriacus*[7, 75] (Figure11). In all of these species, the effected to hiding places is to seek high humidity in the soil and dark.

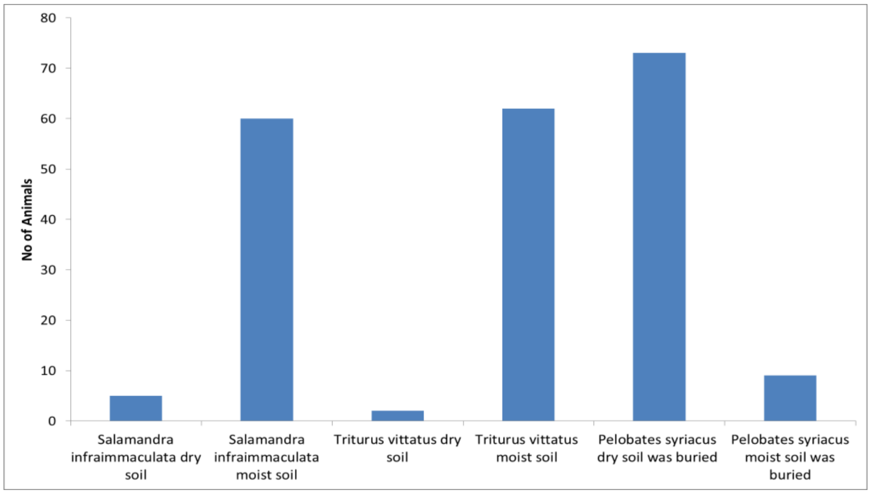

Figure 11:The behavioral response to the substrate of *Salamandrainfraimmaculata, Triturusvittatus* and *Pelobatessyriacus*.

Thephylogenetic tree of amphibian species based on the Cytochrome *b* (Cyt b) sequence is presented in Figure 12. A maximum probability calculated accordingly [47]shows differences among various species whereby all the Anura species belong to different families and the two Urodela species belong to one Salamandrinae family. The phylogenetic tree (Figure12)is support the this bring closer between those species. The variation among individuals in the same species is higher in *B. viridis* and *T. vittatus* than in other species found in Israel. Those species seem to have very high adaptation to terrestrial life and extreme variation among habitats.

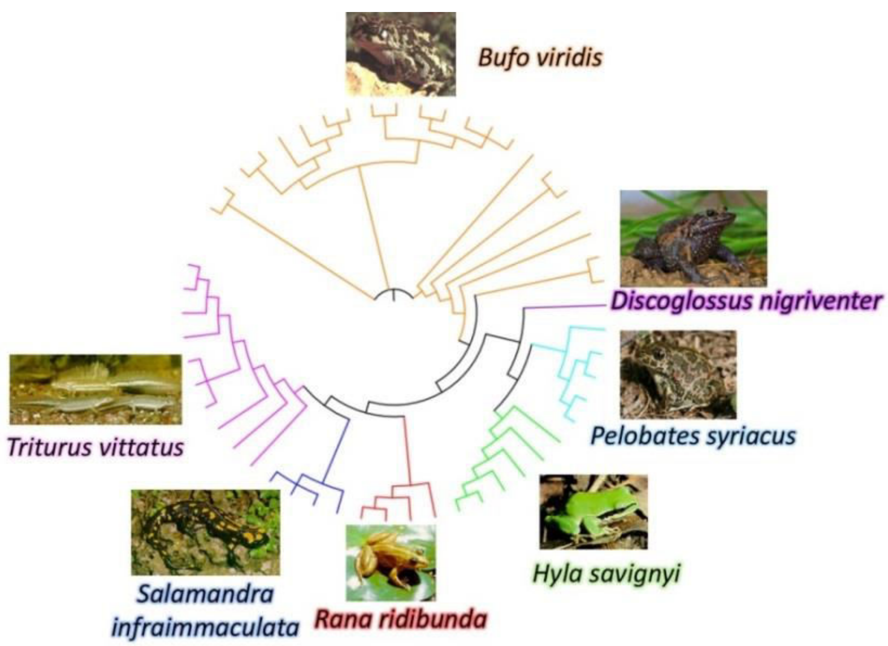

Figure 12:The phylogenetic tree species of amphibians – nucleotide similarity and divergence of Cyt bsequences from one Israeli site and from three other countries: Greek, Iran and Turkey (Table 1).

Table 1:The source of Cyt b sequences used for alignment and the phylogenetic tree.

GenBank accession no. and references	Name	Location
(Goldberg et al., 2011a)	Bufoviridis	Matityahu rev comp
=	Bufoviridis	Fara
=	Bufoviridis	Herzliya
=	Bufoviridis	Hadera
=	Bufoviridis	Bet Zayit
=	Bufoviridis	Hulon B
=	Bufoviridis	Shafdan
=	Bufoviridis	Kziv rev comp
=	Bufoviridis	Jerusalem
=	Bufoviridis	Ga’ash
=	Bufoviridis	Naaran
=	Bufoviridis	Orvim
=	Bufoviridis	Raihaniya
=	Bufoviridis	Nahalit
=	Bufoviridis	Hulon A
=	Bufoviridis	Kash
=	Bufoviridis	Manof
=	Bufoviridis	Bika
=	Bufoviridis	Afeka
=	Bufoviridis	Hermon
=	Bufoviridis	Hazeva
=	Bufoviridis	Masade
KC867706.1	Discoglossusnigriventer	Lake Hula
FJ595201.1	Pelobatessyriacus	Kash pond
FJ595200.1	Pelobatessyriacus	Fara pond
FJ595202.1	Pelobatessyriacus	Raihaniya pond
FJ595203.1	Pelobatessyriacus	Sasa pond
FJ595199.1	Pelobatessyriacus	Elrom pond
FJ595189.1	Hylasavignyi	Dir-Hanna pond
FJ595193.1	Hylasavignyi	Jauda spring
FJ595195.1	Hylasavignyi	Matityahu pond
FJ595191.1	Hylasavignyi	Fara pond
FJ595194.1	Hylasavignyi	Leshem pond
FJ595197.1	Hylasavignyi	Sasa pond
DQ474161	Rana ridibunda	Greece, Thrace (Nestos R.)
DQ474160	Rana ridibunda	Greece, Thrace (Kotili)
DQ474162	Rana ridibunda	Greece, Thrace (Therma)
DQ474163	Rana ridibunda	Greece, Thrace (Dadia)
EU852738.1	Salamandrainfraimmaculata	Tel Dan
EU852731.1	Salamandrainfraimmaculata	Balad
EU852736.1	Salamandrainfraimmaculata	Matityahu
EU852735.1	Salamandrainfraimmaculata	Manof
(Pearlson et al., 2010)	Triturusvittatus	Berekhya
=	Triturusvittatus	Afeka
=	Triturusvittatus	Nahalit
=	Triturusvittatus	Pharaa
=	Triturusvittatus	Matityahu Q.
=	Triturusvittatus	Amiad
=	Triturusvittatus	Leshem
=	Triturusvittatus	Jaudha
=	Triturusvittatus	Dovev
=	Triturusvittatus	Kash

DISCUSSION AND CONCLUSION

The adaptation of seven amphibian species in various areas in Israel changes from Mediterranean to desert climates. According to these results, a hypothesis is suggested that characteristicsamong the various parameters affect amphibian adaptation to semi-arid environments. In conclusion, all the data show that *B. variabilis* adapted most to the terrestrial habitat and extreme conditions, and after it, *T. vittatus, H. savignyi*, *P. syriacus*, *S. infraimmaculata*, *R. ridibunda* and *D. nigriventer*, respectively. However, considerably more studies must be carried out in order to support this hypothesis due to the fact that many parameters are involved in amphibian adaptation to arid and semi-arid habitats [5, 7, 8, 35, 36, 40, 57, 61, 68, 75, 77, 82, 83, 84]. When trying to summarize the adaptation to arid and semi-arid habitats of amphibians based on those species, the idea is supported that adaptation must take place in both terrestrial and aquatic phases. Two species, *B. variabilis* and *T. vittatus*, may show high adaptation to arid environments. In both these species, the breeding places are winter pools and other unpredictable habitats, and the growth period and complete metamorphosis are relatively short compared to other amphibian species [8, 23, 58,71]. The physiological adaptation of *B. variabilis* to arid environments [9, 68, 69, 85], requires considerably more studies compared to *T. vittatus*[86, 87]. It seems that the physiological adaptation of *B. variabilis* to extreme conditions is by urea accumulation in the blood [74].

The other amphibians, *H. savignyi*, *P. syriacus*, *S. infraimmaculata*, *R. ridibunda* and *D. nigriventer*,adapt less to extreme conditions, however, only a few studies on physiological adaptation have been carried out for those species and it is very difficult to compare between them. In *S. infraimmaculata*, a different physiological adaptation between the populations in semi-arid habitats was found compared to moist habitats where water is available all year round [17, 53, 72, 73, 87]. Another Anuran species might exhibit a different strategy of adaptation to terrestrial life that could help them survive in semi-arid habitats. Among them, environmental behavior is very important. *P. syriacus*dig into the soil to prevent dehydration [8, 75, 88] and *H. savignyi*might find hiding places among plants or in the ground and change their color according to the substrate [5, 89].

In comparing the phylogenetic tree of Cyt sequences, the high genetic variation found in the species *B. variabilis* and *T. vittatus* (Figure12) enabled them to adapt better to extreme conditions than the other species. These species are distributed in wider areas in Israel than the other species.

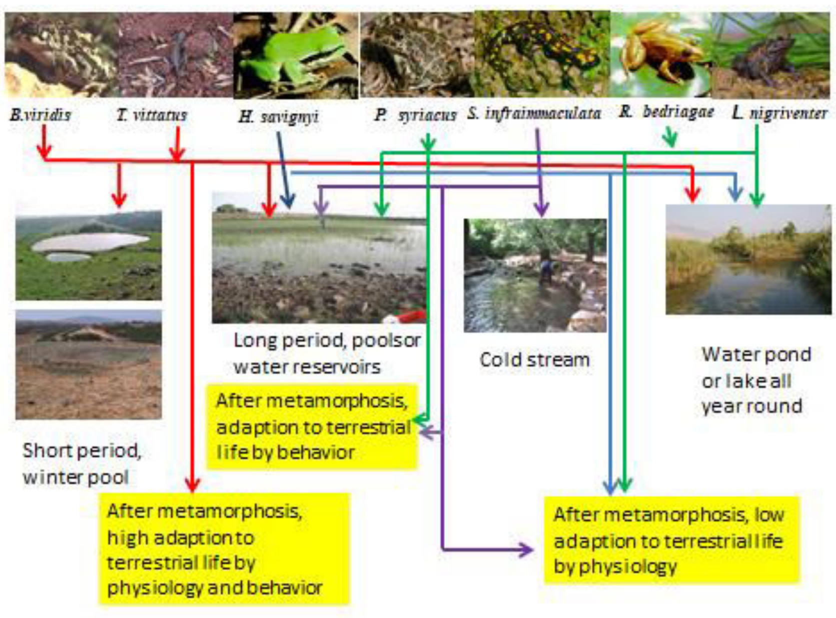

Figure 13:Suggested model of comparison among the various amphibian species.

In summary, a quality model is suggested to show the adaptation of various amphibian species to habitats at the southern border of their distribution(Figure13).

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INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences