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The Habitats, Life Cycle and Physiological Adaptation of Green Toad (Pseudepidalea viridis) in Northern Israel

Science

KEYWORDS :

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ABSTRACT

Green toads (Pseudepidalea viridis) (synonym Bufo viridis) are distributed throughout Israel from the north in the Upper Galilee, the Golan Heights, and the southern coastal plain including the desert area. The present study described the life cycle and physiological adaptation of green toads to semi-arid habitats in northern Israel. The breeding places are mainly ponds where water is immovable or has a very low flow, and is available in very high variability from one month to all year round. The ability to use various water bodies is one of the ways green toads adapt to terrestrial life. The main adaptation phenomenon of green toads is explained by the large geographical distribution described in this paper involving a large number of eggs, short periods of tadpole growth, complete metamorphosis in the breeding places, physiological adaptation to water loss, and high plasma concentration through urea accumulations of the toads in the terrestrial phase. The proposed model explained the adaptation of green toads to various habitats, and its large distribution was represented to summarize its main adaptation character.

Introduction

Green toads (*Pseudepidalea viridis*) (synonym *Bufo viridis*) belong to the *Bufonidae* family, which is one of the most species-rich and widely distributed amphibian families (more than 350 species). *P. viridis* has survived in various habitats (Cunningham, 2004) and is distributed throughout Europe, east to Kazakhstan, Tajikistan, the Kerman province of Iran, Israel, Egypt and northern African countries (Fig. 1). Isolated populations exist on the Balearic Island, Sardinia and Corsica; in northern Africa, they are present along the coastal area of northwestern Sahara, from western Morocco and Algeria (including the Ahaggar Massif) to western Libya. Asian bufonids use partial sequences of mitochondrial DNA genes (Liu, 2000). Three samples of *P. viridis* from Armenia and Georgia have also been sequenced so that their sequences could be compared to the sequence of their sibling, tetraploid species, *Bufo danatensis.* The karyological and flow cytometric data from seven Eurasian populations have being analyzed by (Cavallo, 2002). Contrary to the information mentioned above, in the present study, we examined the genetic molecular variation among populations in the same region comprised of different climates, and found that the genetic variation correlated with the geographical distribution of the *P. viridis* species. Amphibians have been found to survive in relatively isolated populations associated with detached habitats, such as breeding ponds (Degani, 1999b). An analysis of this European *P. viridis* reproduction is described in detail (Castellano, 2004; Castellano, 1999a). In Israel, *P. viridis* (syn. *B. viridis*) is distributed throughout the country in different types of areas, including the desert (Nevo et al., 1975)i. The allozymic variation in proteins is encoded by 26 loci in *P. viridis* (Dessauer et al., 1975). Five hundred and seventeen specimens of *P. viridis* from 11 populations in Israel and one population from Vis Island in the Adriatic Sea were analyzed electrophoretically. The observed genetic variation suggested that selection for heterozygosity as an adaptive strategy operates in the ecologically variable environment in which green toads live (Dessauer et al., 1975). A comparison of Cyt b and D-loop fragments from Israeli sites with those from the four outgroups showed that an analysis of molecular variance (AMOVA) was greatest among regions (Degani, 2013b; Goldberg, 2011). Values of the proportion of total genetic variance among regions (PhiRT) in the Israeli sites were relatively low and not statistically significant. A cluster analysis of RAPD for classifying *P. viridis* revealed a subgroup comprising seven northernmost populations, and three populations near the southern and eastern deserts surrounding Israel (Degani, 2013b; Goldberg, 2011). The use of amplified fragment length polymorphism (AFLP) analysis defined all individuals in a single cluster. Variations in *P. viridis* according to the AMOVA test of Israeli sites using GenAl were 2% among regions, 8% among populations, and 90% within populations (Degani, 2013b; Goldberg, 2011). Many aspects of the life cycle of *P. viridis* were studied, including body size and calling variation (Castellano, 1999b), reproduction (Castellano, 2004; Kovacs, 2010; Ensabella, 2003) number of eggs per female (5687-17602) (Castellano, 2004), larvae growth (Degani, 1982; Degani, 1986) and migration to breeding sites ((Castellano, 2004; Kovacs, 2010; Ensabella, 2003).

Although many aspects of *P. viridis* in Israel were studied, e.g., biology (Degani, 1984b), physiology (Katz et al., 1984; Katz et al., 1986; Degani, 1985b; Degani, 1985a; Degani, 1984a), breeding, larvae growth and metamorphosis (Degani, 1982; Degani, 1986; Goldberg, 2009; Goldberg, 2012), conservation and genetic variation (Degani, 1999b) and morphology (Werner, 1988), the complete picture of adaptation to arid and semi-arid environments is not clear.

The aim of the present study is to describe the adaptation of *P. viridis* to semi-arid conditions in various habitats in northern Israel by life cycle, breeding behavior, larvae growth and physiological adaptation.

2. Materials and Methods

2.1. Study Area

The study was carried out over four consecutive decades (1979-2015). Different aquatic habitats were dispersed over an area of approximately 1,400 km^2 in northern Israel. The habitats included springs and streams that were stable water bodies where water was available year-round, rock pool holes, which were filled by rainwater and whose hydro periods were long (about 200 d/yr), and ponds, which were flooded during the autumn when rainfall began, and gradually dried out between the late winter months and early summer (Degani, 2015) (Fig. 1).

2.2. Sampling

The water body was examined using nets (pore size 450 μm) from a depth of approximately 10 - 30 cm, as described previously by (Degani, 1999b). Rain pools and ponds in Israel that were filled by rain held the water for 1 - 4 months before drying up; large ponds dried up after a period of 6 - 12 years. The elevation of the breeding places is between 0 to 1,000 m above sea level. The breeding places that were examined are presented in Table 1 and Figure 1.

2.3. Biotic and Abiotic Water Parameters

Water quality testing during larval growth, included dissolved oxygen (%), water temperature (˚C), pH, electrical conductivity (μs/cm), ammonium concentration (mg/l), nitrate concentration (mg/l), chlorophyll *a* concentration (mg/l), water volume (m^3) and aquatic invertebrates-number of taxa and biomass (μg/l). Water parameters were measured at a depth of 10 cm every two weeks during the period in which the pools were filling up. Temperature (˚C) and level of oxygen dissolved in water (dissolved oxygen concentration, mg/L−1 and oxygen saturation, %) were obtained by a hand-held oxygen meter (WTW, Oxi330 set, Germany) *in situ*, and one liter of water was sampled for the laboratory water quality tests, including pH (WTW, pH315i, Germany), electrical conductivity (EC, mS/cm−1) corrected to 25˚C (WTW, Multiline P4, Germany), chlorophyll *a* concentration (mg/L−1) extracted from GF/F filters in 90% ethanol (Goldberg, 2009), and ammonium concentrations (NH_4, mg/L−1) carried out using kits based on color changes. Color changes were measured using a dedicated spectrophotometer (NOVA 60, Merck; Germany) (Goldberg, 2009).

2.4. Terrestrial Adaptation

The experiment was conducted during the summer. The toads were maintained on moist soil in three containers (1 x 1 x 0.5), 10 adult toads per container, which gradually dried out during the three-month period. Three animals were removed from the dry soil monthly during the three-month period following dehydration. Blood samples were taken by heart puncture using a 1 ml syringe previously washed with lithium heparin (Degani, 1985b; Degani, 1981). The blood samples were then centrifuged immediately for 10 min at 300g, then treated with TCA (5% and centrifuged again for 15 n&r at 300g. Urea concentration was determined in the plasma water (Bruhmann, 1980). Sodium and potassium in the plasma were determined by atomic absorption (Model 130) and Cl^- by automatic titration (radiometer chloridetitrator), as previously described by (Degani, 1981).

Results

Green toads (*P. viridis*) are distributed throughout Israel from the north in the Upper Galilee, the Golan Heights (Fig. 1), and the southern coastal plain to the desert. Among the 51 different breeding places of amphibians, ponds (Table 1) are used the most for breeding by green toads. Green toads breed in most of the breeding places examined in northern Israel (Table 2).

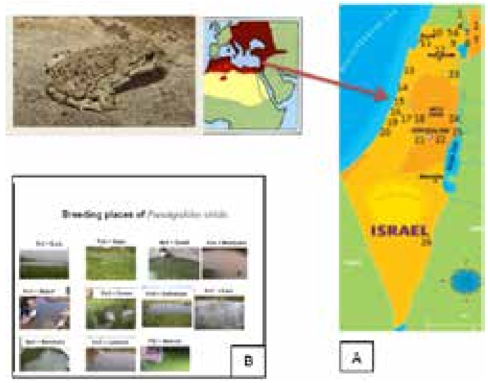

Figure 1. The distribution of green toads (*P. viridis*) in Israel. The numbers show places where adults or tadpoles are observed (A). The habitats are an example of breeding sites of green toads in northern Israel (B).

Table 1. The breeding sites and habitats in which amphibian larvae were found in northern Israel.

Label	Name	Type	Longitude	Latitude	Height (m.a.s.l.)	Amphi. Larvae
Sp1	Balad	Spring	32°43'13" N	35°04'17" E	446	Si, Hs
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
St1	Tel-Dan	Stream	33°14'53" N	35°39'10" E	190	Si
Pi1	Maalot	Rock pool hole	33°00'06" N	35°16'26" E	596	Si
Pi2	Nimrod	Rock pool hole	33°15'05" N	35°42'48" E	760	Si, Hs, Rb
Po1	Manof	Pool	32°50'58" N	35°13'52" E	340	Si, Bv
Po2	Kash	Pool	33°01'47" N	35°29'26" E	809	Tv, Hs, Bv, Ps
Po3	Dovev	Pool	33°03'05" N	35°24'54" E	765	Si, Tv, 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	70	*Hs, Rb*
Po6	Sasa	Pool	33°01'58" N	35°23'30" E	810	Si, Tv, Hs, Bv, Ps
Po7	Fara	Pool	33°03'58" N	35°27'39" E	676	Si, Tv, Hs, Bv, Ps, Rb
Po8	Raihaniya	Pool	33°03'01" N	35°29'10" E	665	Tv, Hs, Bv, Ps

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

Water is available in the breeding places of green toads for one month to all year round. The time for the tadpoles to grow and complete metamorphosis depends on the area in Israel (Table 1).

The life cycle of green toads is presented in Figure 2. Males are the first to arrive at the pond, sending out a mating call. The females then arrive, and the amplexus occurs with the couple swimming in the water, the female laying the eggs and the male fertilizing the eggs. Breeding time in Israel varies according to location and geographical and ecological conditions. It takes place in March-April in northern Israel, the Upper Galilee and the Golan Heights, and earlier in the lower locations and in central Israel. In central Israel, the breeding period is during the winter.

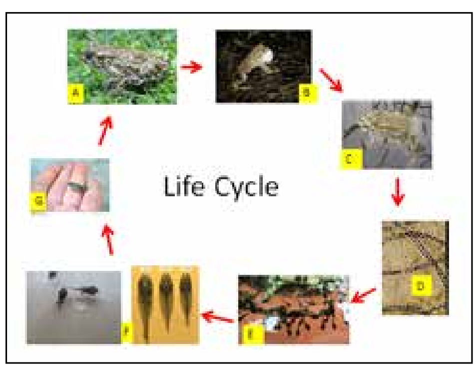

Figure 2. Life cycle of green toad (*Pseudepidalea viridis*): mature tree frogs (A); reproduction behavior (B,C); eggs (D); larvae different states of growth and metamorphosis (E,F); and juveniles (G).

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The amplexus of the green toad involves the male holding the female above the back legs, they swim together, and the female lays 2 to 4 lies of eggs (Fig. 2). The number of eggs differs, ranging between 5,000 to 17,000 per female. Larvae growth and complex metamorphosis take place in northern Israel at the end of winter and in spring from March to June (Table 2), however they occur earlier in the winter time in southern Israel. Tadpole growth in various breeding places is presented in Figure 3.

Table 2. The periods of green toad tadpole growth in ponds (picture of the ponds in Fig. 1).

Breeding Places	Periods When Eggs and Larvae Are Found
Po2 Kash Pond	Mach to June
Po4 Matityahu Pond	April to May
Po6 Sasa Pond	March to June
Po7 Fara Pool	March to June
Po8 Raihaniya Pool	March to May

The rate of tadpole growth in the various ponds differs and also changes from year to year according to ecological conditions (Fig. 3). The ecological conditions of the breeding places where tadpole growth and complete metamorphosis occur is presented in Table 3.

Figure 3. Green toad tadpole growth in various ponds.

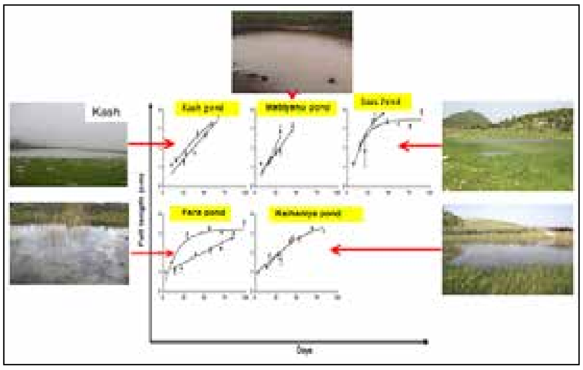

Table 2. Ecological abiotic parameters in breeding places at the time of tadpole growth and complete metamorphosis.

Abiotic Parameters	Range in Water During Tadpole Growth and Complete Metamorphosis
Temperature, °C	7-23
Conductivity, μS/cm	0.03-0.14
pH	7-7.5
Oxygen, mg/L	0.3-14
Ammonia, mg/L	0-1

In northern Israel, the metamorphosis of green toads occurred 1 - 5 kilometers (km) from the breeding places. However, green toads can colonize a new pool and ponds where water is available for a relatively short time, therefore it is very difficult to monitor the distance of movement from breeding places.

In the summer, green toads are found underground in hiding places, and during this time, plasma concentration increases significantly with urea concentration. During the experiment conducted in the summer, green toads were maintained on soil under slow dehydration, and urea accumulation in the plasma was the main parameter affecting plasma osmolality (Fig. 4). The osmolality of the plasma increased dramatically from 330 to 1320 mOsm/Kg by urea accumulation, changing in the plasma from 20 to 901 mM/L (Fig. 4).

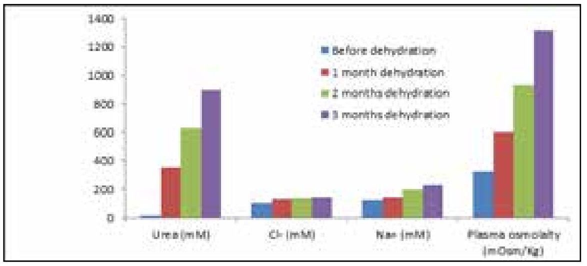

Figure 4. Mean of blood plasma concentration and composition of green toads' slow dehydration in soil.

Discussion

The amphibians have two phases of life, aquatic and testis. The majority of species undergo metamorphosis, moving from a larval stage (usually aquatic) through the development of limbs and lungs, to become terrestrial adults as we know in green toads. The present study described the habitats, life cycle and physiological adaptation of green toads (*P. viridis*) in northern Israel, suggesting an explanation of the wide distribution of the green toads species in relatively semi-arid or arid environments (Cunningham, 2004). The adaptation to semi-arid habitats in northern Israel as described in the present study is supported by many previous observations, and explained the adaptation of this species to arid and semi-arid habitats. The main adaptation of the aquatic phase involves: various breeding places where water is available between 1 month to all year round, a high number of eggs for each female, and a short period of growth and complete metamorphosis. Table 3 presents various breeding places of green toads in northern Israel from previous studies including other amphibian species. The results of the present study are supported by previous observations of different aspects of amphibian larvae in northern Israel, including green toads (Degani, 1982; Degani, 1986a; Degani, 1999a; Goldberg, 2009; Goldberg, 2012). Figure 5 illustrates the proposed model of adaptation of green toads to both aquatic and terrestrial life in arid or semi-arid conditions.

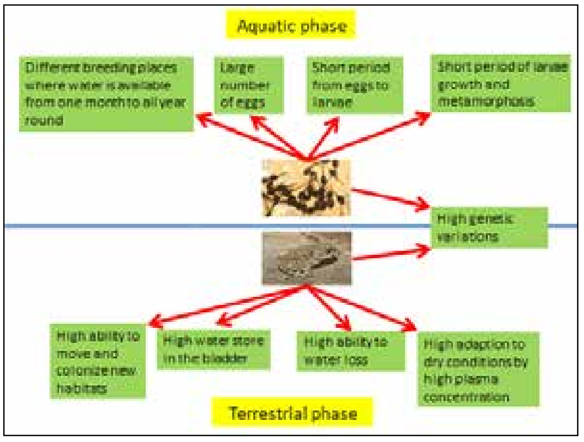

Figure 5. Suggested model of adaptation of green toads to both aquatic and terrestrial life in arid or semi-arid conditions.

The adaptation to a wide variety of breeding sites (Table 3), especially with a short period of water availability, a large numbers of eggs and a short period of larvae growth and complete metamorphosis that is supported by the present study and previous observations (Degani, 1982; Degani, 1986a; Degani, 1999a; Goldberg, 2009; Goldberg, 2012) may explain the large distribution in arid and semi-arid environments (Cunningham, 2004). According to various studies on green toads, the tadpoles are found in slow-flowing water bodies or immoveable breeding places such as ponds (Table 3). During the life cycle in unpredictable habitats in arid environments, genetic variation is an advantage. A previous study has shown a relationship to exist between genetic diversity and environmental variability (Nevo, 1984). Numerous studies have examined *P. viridis* genetic variations from different habitats in order to explain the adaptation to terrestrial life in various critical conditions throughout Israel and in Egypt, Turkey, Iran and Europe (out-groups) (Degani, 2013b; Nevo et al., 1975; Liu et al., 2000; Dessauer et al., 1975).

The ability of *P. viridis* to move long distances from breeding places and colonize new habitats also helps green toads to survive in various terrestrial habitats (Dessauer et al., 1975).

The adaptation to extreme environments shows that *P. viridis* adapts to terrestrial life (Katz et al., 1984; Katz et al., 1986; Degani, 1985b; Degani, 1985a; Degani, 1984a) as described in the present study, but also to water with high salinity (Katz, 1973; Katz, 1975). This adaptation is explained by the high plasma concentration of urea, Na^+ and Cl^- accumulation, as described in the present paper.

To summarize, all of the adaptations of green toad to semi-arid and arid environments during the life cycle in the suggested model are presented in Figure 5.

Table 3. The breeding sites where tadpoles of green toads are found in various studies in northern Israel (Degani, 1982; Degani, 1986a; Degani, 1999a; Goldberg, 2009; Goldberg, 2012; Degani, 2013; Degani, 2013a; Degani, 2013c; Degani, 2016; Degani, 2015).

Name of water body	Type of water body	Region in Israel	Larvae of amphybia
Nimrod Pond	Winter pond	Northern Golan Heights	Si, Pv
Nimrod Spring	Continuous spring	Northern Golan Heights	Pv
Barn Pond	Winter pond	Hula Valley	Pv
Shunit Spring	Seasonal spring	Hula Valley	pv
North Pond	Winter pond	Hula Valley	Pv
South K.S. Pond	Winter pond	Hula Valley	Pv
Hula Pond	Permanent pond	Hula Valley	Rb,Pv
Sal Pond	Winter pond	Central Golan Heights	Hs, Pv
Paras Pond	Winter pond	Central Golan Heights	*Hs Tv, Pv*
Surman Pond	Winter pond	Central Golan Heights	Pv Hs
Oil Pipe Pond	Winter pond	Central Golan Heights	*Pv, Hs, Rb*
Gamla Pond	W*inter pond*	*Central G*olan Heights	Hs, Pv
Tel Bazak Pond	Winter pond	Central Golan Heights	*Pv, Hs*
Darach Pond	Winter pond	Central Golan Heights	Pv
Sbina Pond	Winter pond	Central Golan Heights	*Hs, Rb, Pv*
Hispin Pond	Winter ponds	Southern Golan Height	Pv
Shachar Pond	Winter pond	Northern Israel	*Tv, Hs, Pv*
Kash Pond	Winter pond	Northern Israel	Hs, Pv
Dalton Pond 1	Winter pond	Upper Galilee	Hs, Pv
Dalton Pond 2	Winter pond	Upper Galilee	Hs, Pv
Gush Halav Pond	Winter pond	Upper Galilee	*Si, Pv*
Sasa Pond	Winter pond	Upper Galilee	Hs, Si, Tv, Ps, Pv

Pv - Pseudepidalea viridis, Hy - Hyla savignyi, Si - Salamandra infraimmaculata, Tv - Triturus vittatus vittatus, Ps - Pelobates syriacus, Rb - Rana bedriaga.

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