# Life Cycle of Tree Frogs (*Hyla savygnyi*) in Semi-Arid Habitats in Northern Israel

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# Abstract

The life cycle of tree frogs (*Hyla savygnvi*) in localities of various habitats in northern Israel in the Upper Galilee and Golan Heights (annual rainfall range of 500-1,000 mm) and the Hula Valley, ranging from 212 to 740 m above sea level (ASL), was studied. Tree frogs were observed around winter rain pools, springs and streams. Fifty-one different breeding places were monitored. Only ponds and springs having stable non-flowing water are used by tree frogs for reproduction and are where larvae can metamorphose. The male call in the breeding places attracts the female, who then comes to the male, and breeding occurs underwater. 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, the Golan Heights and the Hula Valley. In various breeding places, larvae grow between April and June. Apparently tree frog larvae adapt to breeding places where stable water is found, such as in ponds or springs, but not to breeding places with flowing water such as streams or rivers. Various Anuran and Urodela larvae were found in the same breeding sites where the larvae of H. savygnyi were found, including Pseudepidalea Viridis, Rana bedriagae, Pelobates syriacus, Salamandra infraimmaculata and Triturs vittatus. Following metamorphosis, H. savygnyi adapt to terrestrial life in semi-arid habitats, and is found on plants or in hiding places under rocks and in holes to prevent dehydration particularly during hot and dry weather. The ability to change color helped the frogs to hide in various substrates. The rate of water loss of terrestrial H. savygnyi during dehydration is around 50% of body weight. Plasma osmolality increased from 200 mOsm/kg to about 450 mOsm/kg, which helped the tree frog to survive in semi-arid habitats.

# 1. Introduction

The distribution, morphology, ecology and taxonomy of tree frogs (Hyla savygnyi) in the Middle East have been studied quite extensively (Degani, Nagar, & Yom-Din, 2012; Gomez, Gauthier, & Lengagne, 2011; Grach, 2007; Gvoždík, 2010; Stock, 2008). The high genetic diversity in relatively small areas (Degani, Nagar, & Yom-Din, 2012) is making it difficult to study its phylogeography and taxa. Since it is very interesting to study this species and its adaption to various habitats and large range of distribution, the location of tree frogs must be defined in detail and might vary in met population. Nevertheless (Gvoždík, 2010), who carried out extensive mtDNA studies, suggested that H. savygnyi can be found in Israel and the Golan Heights, and that their geographic distribution also includes Jordan, Syria and Lebanon. However, other species might also be found in Israel, e.g., H. felixarabica sp. nov. (Gvoždík, 2010). Based on morphological characterizations, coloration and call structure, H. heinzsteinitzi is located at three sites in a small area in the Judean Hills within a 6x13 km range at altitudes of 730-895 m above sea level (ASL) (Grach, 2007). The Hyla genus represents various color changes (Degani, 2013; Nielsen, 1980). Most of the work dealing with color changes of the Hyla genus has been done on the European species, H. arborea (Nielsen, 1980) and more information has been published compared to H. savygnyi. Many aspects of the life cycle of the Hyla genus especially in Europe have been studied and published. The species belonging to the Hyla genus is nocturnal whereby the males display a vocal sac and dark flank stripe, which both vary in coloration (Gomez et al., 2009; Grach, 2007). The life cycle of *H. arborea* was 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 on the line of the towns Oshmyany-Uzda-Slutsk) to southern Russia. From there, the margin runs south and south-eastwards approximately along the line of the Bryansk Province-Kursk Province west

of the Byelgorod Province (Shebekino District), then southwards in the Ukraine from Kharkov City to the Dnepropetrovsk Province and the Donetsk Province. In Crimea, the frog is found along the southern shores and extreme northwest of the peninsula. The frog (H. arborea) is active primarily in the evening and at night, when it descends to the ground from vegetation to forage and rehydrate. During the autumn, it migrates to hibernate in Europe. Hibernation in Europe takes place from September-December to February-early May on land (in soil, burrows, heaps of stones and holes in trees). Reproduction occurs in places at different times in Europe 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 (Tarkhnishvili, 1999). Females enter pools after the males and leave immediately after breeding. There is varying information on the number of eggs laid per female. A clutch contains about 200-1,000 eggs deposited in portions, usually in the form of small rounded clumps containing from 3 to 100 eggs. The H. arborea clutch contains 200-1,000 eggs deposited in a few clumps of 270-315 eggs over several hours, after which the female leaves the pool (Tarkhnishvili, 1999). Metamorphosis occurs from June to September, depending on the geographic position of a locality. In some cases, the larvae overwinter and complete transformation the following summer. H. arborea preys on various invertebrates, mainly insects. Its ability for long leaps makes it possible to forage on fast-flying insects, which comprise a considerable proportion of its food. The frogs forage on land. During the breeding season, adults forage periodically on the shore and on high plant stems above the water surface.

Information on the life cycle of *H. savygnyi*, whose distribution in the eastern Mediterranean is less clear, shows that it is found in southeast Anatolian regions of Turkey and Israel (Degani, Nagar, & Yom-Din, 2012; Stock et al., 2008). Many aspects of *H. savygnyi* distribution (Degani & Kaplan, 1999; Stock et al., 2012) related to its life cycle have been studied, e.g., larvae growth at the breeding site and complete metamorphosis (Degani, 1982; Degani, 1986; Goldberg, Eviatar, & Degani, 2009), adult behavior (Degani, 2013), genetic variation of different populations (Degani, Nagar, & Yom-Din, 2012; Nevo, 1979), physiology (Degani, 1984) and morphology (Degani, 2013; Kalayci, 2015). Adaption of the life cycle to various habitats in northern on the southern border of its distribution in northern Israel is not well described.

The current study examines the life cycle and adaption of different breeding sites to the terrestrial environment of the tree frog (*H. savygnyi*) and provides more information about the adaption of this species to semi-arid conditions.

# 2. Materials and Methods

## 2.1 Study Area

The study was carried out over four consecutive decades (1979-2014). Different aquatic habitats were dispersed over an area of approximately 1,400 km<sup>2</sup> in northern Israel, including northern and eastern Israel, the Golan Heights and the Hula Valley (Figure 1). The habitats included ponds, rock pool holes, springs and streams that were stable or flowing bodies, and in which water was available part or all year-round. The unpredictable breeding places such as ponds, which were flooded during the autumn when rainfall began, gradually dried out between the late winter months and early summer.

## 2.2 Sampling

The water body was examined by nets (pore size  $450 \ \mu$ m) from a depth of approximately 10-30 cm, as previously described by (Degani & Kaplan, 1999). Rain pools and ponds in Israel that were filled by rain held the water for 3-4 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 Terrestrial Adaption

Adult *H. savignyi* were collected from the Sasa Pond (Degani, 1982) during the breeding period in February-March. The experiments were conducted in desiccators over dry silica gel (0-5 relative humidity [RH]) at 10°C. Upper limits of water loss were determined when the frogs lost their coordination. Five adults were used in each experiment. The adults were weighed using Mettler H 315 balance until the frogs (10%, 20% and 30%) reached their upper limit of water loss. Twelve adult tree frogs were dehydrated to different percentages of body weight to establish the effect of dehydration on concentration of both plasma and muscle. Blood samples were taken from the dehydrated frogs by puncturing the heart using a 1 ml syringe washed with lithium heparin as previously described (Degani, 1984). The blood samples were centrifuged immediately and the plasma frozen for later analysis. Sodium, potassium and chloride concentrations (Degani, 1981) and urea in the plasma were determined as described by (Degani, Silanikove, & Shkolnik, 1984).



Figure 1. Various habitats of *Hyla savignyi*: Sp1, Bald Spring; Sp3, Navoraya Spring; Po2, Kash Pond; Po3, Dovev Pond; Po4, Matityahu Pond; Po5, Lehavot Pond; Po6, Sasa Pond; Po7, Fara Pond; and Po8, Raihania Pond

Water Body	Type of Body	Area of Breeding Places in Northern Israel	Larva of <i>Hyla savignyi</i> Found	Larvae of Amphibia
Nimrod Pond	Winter pond	Northern Golan Heights		Si, Bv
Nimrod Spring	Continuous spring	Northern Golan Heights		Bv
Barn Pond	Winter pond	Hula Valley		Bv
Shunit Spring	Seasonal spring	Hula Valley		Bv
North Pond	Winter pond	Hula Valley		Bv
South K.S. Pond	Winter pond	Hula Valley		Bv
Nahalin Spring	Seasonal spring	Naftali Heights	Hs	Bv
Hula Pond	Permanent pond	Hula Valley		Bv, Rb
Sal Pond	Winter pond	Central Golan Heights	Hs	Rb
Katzarin Pond	Winter pond	Central Golan Heights		Tv
Paras Pond	Winter pond	Central Golan Heights	Hs	Tv, Bv
Surman Pond	Winter pond	Central Golan Heights	Hs	Bv
Oil Pipe Pond	Winter pond	Central Golan Heights		Bv, Hs, Rb
Gamla Pond	Winter pond	Central Golan Heights	Hs	Bv
Tel Bazak Pond	Winter pond	Central Golan Heights		Bv, Hs
Darach Pond	Winter pond	Central Golan Heights		Bv
Sbina Pond	Winter pond	Central Golan Heights		Bv, Hs, Rb
Bageria Pond	Winter pond	Southern Golan Heights		Rb
Irsim Pond	Winter pond	Southern Golan Heights		Rb
Irism Pond North	Winter pond	Southern Golan Heights		
Nov Pond North	Winter pond	Southern Golan Heights		Rb
Hispin Pond	Winter pond	Southern Golan Heights		Bv
Shachar Pond	Winter pond	Northern Israel		Tv, Bv, Hs
Kash Pond	Winter pond	Northern Israel	Hs	Bv
Dalton Pond 1	Winter pond	Upper Galilee	Hs	Bv
Dalton Pond 2	Winter pond	Upper Galilee	Hs	Bv
Gush Halav Pond	Winter pond	Upper Galilee S		Si, Bv
Bechania Pond	Winter pond	Upper Galilee		
Sasa Pond	Winter pond	Upper Galilee	Hs	Si, Tv, Bv, Ps
Humama Spring	Continuous spring	Upper Galilee		Si
Zakin Sprin	Continuous spring	Upper Galilee		Si
Bet Jan Reservoir	Permanent pond	Upper Galilee		Si
Fasuta Pond	Winter pond	Western Galilee		Si
Menachem Pond	Winter pond	Western Galilee	Hs	Tv
Shetula Pond	Winter pond	Western Galilee		
Maron Pond	Winter pond	Western Galilee	Hs	Si
Sumara Pond	Winter pond	Western Galilee		Si
Tel Arad Pond	Winter pond	Western Galilee	Hs	Si, Tv
Sasa Rock Pool	Rock pool (all year)	Upper Galilee		Si
Tel Dan	Continuous stream	Northern Galilee		Si
Balad Spring	Spring	Carmel mountain	Hs	Si
Navoraya Spring	Spring	Hula Valley	Hs	Si, Rb
Maalot Rock Pool	Rock Pool	Upper Galilee		Si

Table 1. Distribution of *Hyla savignyi* and other amphibian larvae in the aquatic system that were examined in northern Israel: Si - Salamandra infraimmaculata; Tv - Triturus vittatus vittatus; Hs - Hyla savignyi; Bv - Bufo viridis; Ps - Pelobates syriacus; Rb - Rana bedriaga

Water Body	Type of Body	Area of Breeding Places in Northern Israel	Larva of <i>Hyla savignyi</i> Found	Larvae of Found Amphibia	
Manof Pool	Pool	Misgave	Misgave Hs		
Kash Pool	Pool	Hula Valley	Hula Valley Hs S		
Dovev Pool	Winter pond	Upper Galilee Hs		Tv, Bv, Ps, Si	
Matityahu Pool	Pool	Upper Galilee			
Lehavot Pool	Pool	Hula Valley	Hs	Rb	
Fara Pool	Pool	Northern Galilee	Hs	Si, Tv, Bv,	
				Rb, Ps	
Raihaniya Pool	Winter pond	Upper Galilee		Bv, Ps	
Elrom Pond	Winter pond	Northern Golan Heigh	nts	Ps	

#### 2.4 Biotic and Abiotic Water Parameters

Water quality testing during larval growth, which was described in detail in the chapter on water quality, included dissolved oxygen (%), water temperature (°C), pH, electrical conductivity ( $\mu$ s/cm), ammonium concentration (mg/l), nitrate concentration (mg/l), chlorophyll *a* concentration (mg/l), water volume (m<sup>3</sup>) and aquatic invertebrates-number of taxa and biomass ( $\mu$ g/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) (Degani, 2013), chlorophyll *a* concentration (mg/L–1) extracted from GF/F filters in 90% ethanol (Degani, 2013), and ammonium concentrations (NH<sub>4</sub>, mg/L–1) carried out using kits based on color changes. Color changes were measured using a dedicated spectrophotometer (NOVA 60, Merck; Germany) (Degani, 2013).

## 2.5 Terrestrial Behavior Exterminate

*H. savignyi* was observed from plants beneath stones in the Sasa Pond in northern Israel (Degani, 1982; Degani, 1986) during the spring and summer (March to June) (Degani, 1984). The breeding places and colors of the frogs (Degani, 2013) were determined.

## 3. Statistical Analysis

The range of biotic and abiotic parameters of aquatic habitats was calculated, and average water parameters were analyzed by one-way analysis of variance (ANOVA), with the level of significance between groups set at p < 0.05 (ANOVA) (Tukey test).

## 4. Results

Tree frogs (*Hyla savygnyi*) are found in northern Israel from the north in the Upper Galilee and Golan Heights (annual rainfall range of 500-1000 mm) (Figure 1), to the southern coastal plain (annual rainfall of about 250 mm). Among the 51 different breeding places of amphibians, ponds and springs (Table 1) are used by tree frogs for reproduction, and metamorphosed populations were found around these breeding places. Water in the breeding places of tree frogs is available for two or more, and up to six, months and is sometimes available all year-round.

The life cycle of tree frogs is presented in Figure 2. Males are the first to arrive at the pond and call for females. Call structure has a short rise time, as described by (Grach, 2007). The females then arrive, and breeding occurs underwater. 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. The amplexus of tree frogs involves the male holding the female above the front legs, then they swim together, and the female lays 2 to 4 lies of eggs (Figure 2).

The time for the larvae to grow and complete metamorphosis is presented in Figure 2. Tree frogs can breed several times during the warm season, usually when there is a drop in barometric pressure, two or more inches of rainfall, and in the darkness of night.



Figure 2. Life cycle of tree frogs (*Hyla savygnyi*): mature tree frogs (A); reproduction behavior (B); eggs (C); larvae different states of growth and metamorphosis (D); and juveniles (E)

Larvae growth and complex metamorphosis take place in northern Israel during the summer and early autumn until October. Larvae growth in various breeding places is presented in Figure 3. There is no information available on the adult terrestrial activity of *H. savygnyi*. Considering the spatial distribution of rain pools and the dispersal ability of juvenile adults of only about 2-4 km, it seems that most of the population is isolated.



Figure 3. Growth rate of *Hyla savignyi* larvae in various habitats: Sp1, Bald Spring; Sp3, Navoraya Spring; Po2, Kash Pond; Po3, Dovev Pond; Po4, Matityahu Pond; Po5, Lehavot Pond; Po6, Sasa Pond; Po7, Fara Pond; and Po8, Raihania Pond

The times where *H. savingnyi* larval species were in the different breeding places are affected by ecological conditions and water quality (Table 2).

In various breeding places, the larvae growth period is between April and June.

Most of the habitats where tree frog larvae growth occurs are ponds. Some springs in which water collected in the pool are also used by tree frogs (Figure 1). Apparently tree frogs do not adapt to flowing water such as in streams.

Table 2. Overlap times when *Hyla savingnyi* larval species were in different habitats with other amphibian larvae.  $\mathbf{Sp} - \mathbf{Springs}; \mathbf{St} - \mathbf{Streams}; \mathbf{Pi} - \mathbf{Rock}$  pool holes;  $\mathbf{Po} - \mathbf{Pools}$ 

Site	Species	Months when Hyla savingnyi larval were in breeding places									
Site		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
Sp1	Hs							•	•	•	
Sp2	Hs									•	
Po1	Hs							•	•		
Po2	Hs							•	•		
Po3	Hs							•	•		
Po4	Hs						•	•	•		
Po5	Hs						•	•	•		
Po6	Hs						•	•	•		
Po7	Hs						•	•	•		

The range of water quality in the breeding places of tree frogs is presented in Table 3. According to the results, the temperature ranges from 10°C to 25°C and oxygen from 1 to 18 mg/L in green water, with different concentrations of chlorophyll a from 0.1 up to 122 mg/L.

Table 3. The range of abiotic parameters of water at various breeding sites where *Hyla savingnyi* larvae grow and complete metamorphosis

Abiotic Parameter	Minimum	Maximum
Water temperature, °C	10.5	25.4
Oxygen concertation, mg/L	1.0	18.6
Electronics conductivity, us/cm	270.0	1732.5
Ammonia, mg/L	0	1.3
pH	7.2	9.8
Chlorophyll a, mg/L	0.1	122

Following metamorphosis, *H. savingnyi* moves to terrestrial life in semi-arid habitats. The frogs are found on plants or in hiding places under rocks and in holes to prevent dehydration particularly during hot and dry weather. The rate of water loss of terrestrial *H. savingnyi* is presented in Figure 4. At dehydration of around 50% body weight, plasma osmolality increases from 200 mOsm/kg to about 450 mOsm/kg.

The main ions affecting plasma concentration during dehydration are  $Na^+$  and  $Cl^-$ . However, a low urea accumulation was found (Table 4).

-	% Dehydration	Plasma mOsm/Kg mean ± SD	$ \begin{array}{l} Plasma \ Na^+ \ mM/L \\ mean \pm \ SD \end{array} $	Plasma Cl <sup>-</sup> mM/L mean ± SD	Urea mM/L mean $\pm$ SD
-	0 - 10	$200 \pm 10$	$110 \pm 7$	$101 \pm 10.2$	$20.3\pm5.2$
	10 - 20	$330\pm41$	$168\pm40$	$153\pm 32$	$30.4\pm 4.2$
	20 - 30	$437 \pm \! 30.3$	$208\pm46$	$194\pm51$	$37.5\pm 9.1$

Table 4. Plasma composition of tree frogs during dehydration



Figure 4. Plasma concentration during water body loss of Hyla savingnyi

During terrestrial life, *H. savingnyi* can be found in different substrates, trees, plants grass and various soils. The frogs are relatively small in size. The juveniles completing metamorphosis weigh  $301 \pm 55$  mg, and the adults measure 3-4 cm. During terrestrial life, *H. savingnyi* has the ability to change color, which is affected by substrates. In general, when the frogs are found on trees or plants their color is green and changes to brown and black according to the substrate (Figure 5).



Figure 5. Mature different colored H. savingnyi in various places in the habitats

## 5. Discussion

The current study described the life cycle and adaption to terrestrial life of tree frogs (*H. savygnyi*) in northern Israel, adding information to studies carried out on this species of the Middle East tree frog (Degani, Nagar, & Yom-Din, 2012; Gomez, Gauthier, & Lengagne., 2011; Grach, 2007; Gvoždík, 2010; Stock, 2008). The results of the current study are in agreement with previous studies, whereby the breeding places of *H. savygnyi* include various stable water bodies, including winter pools in which water is available at least two or more months (Degani, 1999) and springs (Figure 1). This finding shows that no differences exist between breeding places of *H. savygnyi* and *H. arborea* (Tarkhnishvili, 1999) in which reproduction occurs in small stagnant water bodies (ponds and puddles, some of which are very small) and slowly flowing brooks with dense herbaceous and shrub vegetation (Kuzmin., 1999). This is a relatively short period of larvae growth and complete metamorphosis, representing

adaption to xeric habits where many of the breeding places dry up after several months (Degani & Kaplan, 1999). In comparison to other amphibian species, shorter periods of growth and complete metamorphosis were found in northern Israel for *Salamandra infraimmaculata* (Degani, 1982; Degani, 1986), *Triturus vittatus vittatus* (Pearlson & Degani, 2007), *Pelobates syriacus* (Degani, 2015) and *Rana bedriaga* (Degani, 1982; Degani, 1986). Only the larvae of *Pseudepidalea viridis* might have a shorter period in breeding places (Degani, 1982; Degani, 1986; Goldberg, Nevo, & Degani, 2012). In northern Israel, the larvae of *H. savygnyi* were found during the spring and beginning of summer when temperatures increase in the breeding places.

H. savygnyi lives in much drier landscapes than H. arborea schelkownikowi, including steppes, deserts and semideserts (Gvozdik, Moravec, Klutsch, & Kotlik, 2010). Therefore, its adaption to terrestrial life is very important. Following metamorphosis, H. savygnyi lives mainly near water bodies, in wet sites, oases, gardens, bushlands and mountain forests. However, during the summer time and on hot days, the tree frogs found hiding places under stones or in holes in the ground. During this time, H. savygnvi is in danger of dehydration. The frogs' relatively small body and skin that does not prevent water loss, as was found in this study of H. savygnyi, is in agreement with previous findings (Degani, 1984) whereby tree frogs have to find different breeding places to adapt to semiarid habitats. The ability to lose 50% of their body weight and increase plasma concentration, as described in the current study, are ways of helping them to survive in terrestrial life. The only amphibian species that is more adapted to terrestrial life than H. savygnyi is Pseudepidalea viridis (Degani, Silanikove, & Shkolnik, 1984). P. viridis has the ability to survive with high plasma osmolality by urea accumulation, which is not found in H. savygnvi. The high genetic and color diversity in relatively small areas (Degani, Nagar, & Yom-Din, 2012; Degani, 2013) might help this species to adapt to different environments found along the southern border of its distribution that includes Mediterranean semi-arid and arid environments. The results of this study along with a previous study (Degani, Nagar, & Yom-Din, 2012; Degani, 2013) support the hypothesis that the ability to change color and plant preference depends on adaptability to different habitats and different substrates. These phenomena help H. savygnvi to silence and prevent carnivores if they are found in the different substrates. Accordingly, H. savygnvi may even be found at long distances from water bodies in xeric environments, such as rocky slopes and in xerophytic bushes (Tarkhnishvili, 1999).

In conclusion, the life cycle of the tree frog (*Hyla savygnyi*) in semi-arid habitats is affected by various phenomena, e.g., unpredictable breeding places, short periods of larvae growth and complete metamorphosis, the ability to change color affected by various substrates, and high percentage of water loss, which help it survive in semi-arid habitats.

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