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Diet of The North African Green Frog *Pelophylax saharicus* in The Wetlands of Edough (north- eastern of Algeria)

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ABSTRACT

The diet of the North African green frog *Pelophylax saharicus* was studied with the aim of exploring the variation in diet among males and females. Food data were recovered by analyzing stomach contents. The field study was carried out at the Edough Peninsula in northeastern Algeria. The frog diet was studied by categorizing122 animal fragments in the stomachs of 19 males and 25 females. Frogs ate a wide variety of invertebrates. The diet of *P. saharicus* was dominated by four prey categories (Coleoptera, Hymenoptera, Odonata, and Diptera). The food spectrum consisted mainly of insects, which represent more than 85% of the diet. Hymenoptera and beetles are particularly important with 19% and 34%, respectively. Annelids represent 7%, Arachnids 4% and Gastropods 3% of the prey eaten by *P. saharicus*.

INTRODUCTION

Northern Africa is an area known as a biodiversity hotspot **(De Jong, 1998; Mayers *et al.*, 2000; Sanmartín, 2003; Véla & Benhouhou, 2007; Cuttelod *et al.*, 2008; Amor *et al.*, 2010; Bouzid *et al.*, 2017; Dinis *et al.*, 2019)**. Its geographical position between the two barriers, the Mediterranean Sea in the north and the Sahara desert in the south, strongly influences the distribution of biodiversity and determines the rates of amphibians' endemism **(Schleich *et al.*, 1996; Bouazza & Rihane, 2021)**. Several studies confirm that this region has served as a glacial refuge for several animals, particularly the amphibians **(Plötner, 1998; Garcia-Paris *et al.*, 2003; Carranza & Arnold, 2004; Fromhage *et al.*, 2004)**. The Edough massif, located in the North-East of Algeria is the best example of this biodiversity **(Médail & Quézel, 1999; Véla *et al.*, 2007; Bouzid *et al.*, 2017)**. Despite the ecological and biological importance that occupies these areas, little is known about terrestrial fauna due to the lack of information on the distribution and taxonomy of endemic species **(Amor *et al.*, 2010)**.

Climate is the primary factor controlling the distribution of organisms and large-scale wealth patterns **(Nix, 1982; Currie, 2001; Guisan & Thuiller, 2005)**.

Regions with a Mediterranean climate are characterized by low amounts of annual precipitation and a rare seasonal cycle with mild and humid winters and hot, dry summers **(De Smet & Smith, 2001; Bougdah & Amira, 2017; Amira & Bougdah, 2018; Dong *et al*., 2021)**.

According to studies of **DeMenocal (2004)** and **Schuster *et al.* (2006)**, North Africa has steadily moved from wetter climatic conditions to drier climatic conditions. These climatic changes have direct effects on the different types of habitats **(Rognon, 1989; Le Houerou 1997; Drake *et al.*, 2011)**, and it affects terrestrial and aquatic flora and fauna.

Temporary ponds are typical and unique habitats in the Mediterranean region. They play an important heritage role in biological diversity. However, they are fragile and threatened by both climate changes and human activities **(Rhazi *et al.*, 2006)**. Amphibians play an important role as an agent of bioindicators of environmental changes **(Rohman *et al.*, 2020)**. These changes include climate change, water pollution, native habitat, and the introduction of exotic species **(Izza & Kurniawan, 2014)**. All these factors affect amphibians and lead to progressive population declines **(Alford & Richards, 1999; Reading, 2007)**. According to **IUCN (2015)**, about 33% of amphibian species are threatened.

The green frog or *Pelophylax* belongs to the family of Ranidae, and is widely spread in all the parts of Algeria **(Poiret, 1789; Pellegrin, 1927; Olivier, 1984; Salvador, 1996)**. The North African green frog is the most frequent amphibian, the most abundant, the most ubiquitous although it is exposed to several threats such as rivers drying, the fragmentation of biotopes, collection and also the appearance of various cases of malformations linked to agriculture **(Amor *et al.*, 2010; Benhassine & Nouira, 2012)**.

Understanding the position of amphibians in the food chain is extremely important because the composition of their diet can be a significant indicator of the quality of their habitat **(Rohman *et al.*, 2020)**. In addition, amphibians are important components of ecosystems. On the one hand, they provide direct energy flow from invertebrates to higher trophic levels. In the other hand, they can be important aquatic and terrestrial consumers **(Cupsa *et al.*, 2007)**.

In this context, the trophic status of the green frog *Pelophylax saharicus* was addressed to fill the lack and the scarcity of information on the diet of this endemic species of North Africa.

MATERIALS AND METHODS

1. Study area

This study was carried out in ponds located at the Edough massif in the North-East of Algeria. The Edough is boarded by the Mediterranean Sea in the North, the national road number 44 to the asouth, the agglomeration of Annaba in the East and the Iron Cape in

the West (Fig. 1). The sampling sites were chosen with precision according to two fundamental criteria: the density of the species studied and the accessibility to the selected sites and (Table 1).

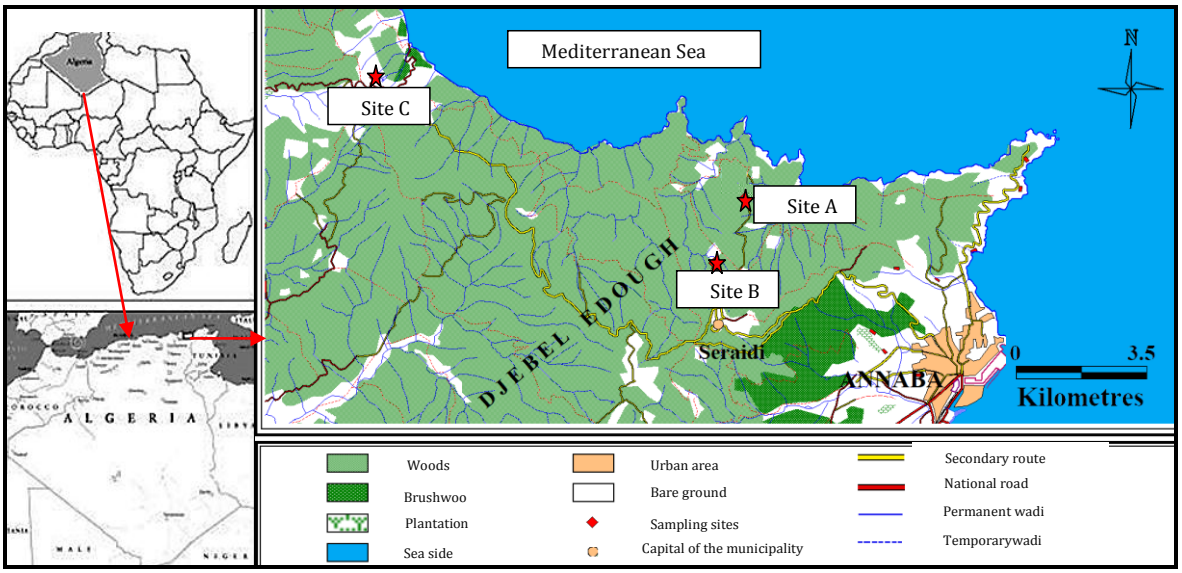

Fig. 1. Location of the study site and sampling areas (Source: the National Institute of Cartography Hussein Dey Algiers modified)

The sampling sites are located at altitude, in a forest environment dominated by cork oak. These are temporary callitriche ponds for the first two sites and buttercup ponds for site 3. These are small areas (<20 m^2) and occupy endorheic depressions, and the water supply is limited directly to precipitation. The first site (A), the "Daouret Zlazel ", is located between the coordinates (WMA;36° 56 '8640" N, 7°40'9710'E), the second (B) is called "Qoba Sidi El Khedayer "and located at (WMA;36°55'3650"N, 7°40'2740"E) and the third site (C) is named "El-Golla" and located between coordinates (WMA;36°58'8270"N, 7°32'83.70E) (Table 1).

Table 1. Main characteristics of the studied sites

Site		Geographic coordinate	Surface area (m^2)	Depth (m)	Altitude (m)	Plant
Site A	Daouret Zlazel	36° 56 ' 51.77" N, 7° 40’ 58.38' E	6	1.20	290	- *Callitriche obstungifolia* - *Chara vulgaris* - *Myriophulum alternifolium*
Site B	Qoba Sidi El Khedayer	36° 55' 36.41" N, °40' 20.18" E	13	1.00	492	- *Calattriche obstungifolia* - *Lemna minor* - *Chara vulgaris*
Site C	El-Golla (Ain Barabr)	36° 58' 82.70" N, 7° 32’ 83.70 E	15	1.50	412	- *Ranunculus aquatilis* - *Cératophylum submersum* - *Convolvulus arvensis* - *Juncus acutus*

2. Sampling

The sampling was carried out during the spring period; March, April and May 2020. Our study focused on 66 frogs including 22 males and 44 females of *P. saharicus.* The capture of the specimens studied was carried out either by immobilization with a powerful flashlight and a dip net, or by searching under tree trunks or in puddles or along the banks. The captured frogs were immediately received cooling anesthesia at 4°C.

In the laboratory, the morphometric parameters, viz. physical size (SVL: snout-urostyle length), weight, lengths and widths of the snout and length of the hind legs were measured. The frogs were then dissected to identify the sex and recover the contents of the digestive tracts. This manipulation allowed us to sort and separate the different elements including head, legs, wings, thorax, mandibles, cerci, elytra and vegetation. These items were then classified into taxonomic categories up to order. The entomological guide of **Dierl and Ring (1992)** and **Roy (2003)** were used for the identification of prey.

3. Data analysis

The data collected was processed by various complementary methods and analyses were made based on gender.

a) Relative abundance or relative frequency (n%): the relative abundance or frequency of capture or consumption is defined as the number of a given item or food category out of the total number of food items ingested .

n % = ni/Nt X 100

ni: this is the number of individuals of the species or category considered.

Nt: the total number of all prey items.

b) Degree of presence or frequency of occurrence (F%): the frequency of occurrence, expresses the number of appearances of each item or food category out of the total number of stomachs analyzed.

F % = Na/Nt X 100

Na: the number of appearances

Nt: the total number of stomachs analyzed.

4. Statistical analysis

The obtained results were statistically processed using descriptive statistics and test –t of Student to compare morphometric parameters between males and females and to compare prey abundances between sexes to detect intraspecific differences in the use of food resources. The data were statistically analysed using Staistica 10. software.

RESULTS

1. Demographic structure of the population

A sampling of P. saharicus in the wetlands of the Edough Mountain allowed the capture of 66 individuals including 22 males and 44 females, with a sex ratio of around 2 (**Table 2**).

Table 2. The number of frogs sampled and sex ratio in *P. saharicus*populations.

	Male	**Female**	**Population**	**Sex-ratio**
Abundance	22	44	66	2

2. Morphometric parameters

The morphometric measurements taken on 66 adult individuals (44 females and 22 males) of *P. saharicus* showed that the population of the Edough massif presents an average weight between 17 g and 68 g (mean = 32.60 g), the length of the body (snout-cloaca) varies between 53.59 mm and 90.7 mm (mean =66.07 mm). Regarding the measurements of the muzzle, its length varies between 14.35 mm and 30.04 mm while its width is between 16.84mm and 28.53mm. The length of the hind legs varies slightly between the right and left sides. It is on average 97 mm for both.

Table 3. Morphometric parameters of *P. saharicus* populations at Edough.

		Weight (g)	**SVL (mm)**	**Muzzle length (mm)**	**Snout width (mm)**	**Hind legs Right (mm)**	**Hind legs Left (mm)**
Female (N=44)	Mean	36.17	66.94	20.67	21.76	96.17	97.50
	Sd	16.68	9.23	3.47	2.84	16.37	12.23
	Min	18	53.42	12.99	16.84	129.89	125.51
	Max	68	90.7	30.04	28.53	28.44	75.25
Male (N=22)	Mean	26.44	65.26	21.26	23.03	98.63	96.20
	Sd	5.45	4.27	2.36	1.93	7.04	8.58
	Min	17	55.92	18.61	19.77	109.3	109.01
	Max	38	73.24	27.47	26.16	78.88	75.08
Female + male (N=66)	Mean	320	667	211	222	982	968
	Sd	11.50	7.99	2.67	2.84	10.74	11.16
	Min	17	53.59	14.35	16.84	129.89	125.51
	Max	68	90.7	30.04	28.53	78.31	75.08

There is a dimorphism between males and females for some of the measured parameters (**Table 3**). The weight is much higher in the female (t= 3.11, P = 0.002) and

the width of the muzzle is significantly greater in the male (t=-1.98, P = 0.05). Concerning the other parameters, we did not note any significant differences, neither between the length of the SVL body (t=-0.94, P > 0.05) nor between that of the legs (t=-0.46 on the right, t =-0.79 on the right, P > 0.05) and for the length of the muzzle (length: t=-1.98, P > 0.05).

3. Diet of the North African green frog (*P. saharicus*)

We analyzed the stomach contents of 44 frogs (19 males and 25 females), and the total number of prey amounts to 122 prey. Examination of the food spectrum shows great diversity in the choice and consumption of prey available in the environment (**Table 4**).

The prey consumed belongs to three phyllum (Arthropoda, Annelida, and Mollusca), four classes (Insecta, Arachnida, Oligochaeta, and Gastropoda,) and 14 orders (Diptera, Coleoptera, Hymenoptera, Orthoptera, Odonata, Trichoptera, Dermatoptera, Lepidoptera, Heteroptera, Hemiptera, Blattoptera, Araneae and Haplotaxida, and Basommatophora. Arthropods are dominant with 89.34% of the diet (**Table 4**).

Table 4. Taxonomic characterization of the diet of *P. saharicus*.

Phyllum	Class	Ordre	% Ordre	% Class	% Phyllum
Arthropoda	Insecta	Diptera	9.01	85.24	89.34
		Coleoptera	27		
		Hymenoptera	22		
		Orthoptera	2.45		
		Odonata	10.65		
		Trichoptera	0.81		
		Dermaptera	4.09		
		Lepidoptera	3.27		
		Heteroptera	0.81		
		Hemiptera	2.45		
		Blattoptera	0.81		
	Arachnida	Araneae	4.09	4.09	
Annelida	Oligochaeta	Haplotaxida	7.37	7.37	7.37
Mollusca	Gastropoda	Basommatophora	3.27	3.27	3.27

3.1. Abundance and frequencies of prey

The diet of frogs contains both aquatic and terrestrial prey. The average number of prey per frog is 2.77 prey with an average of 2.88 prey for females and 2.63 for males. The maximum number of prey recorded in a frog is 8 in the site of El-Golla in March. For this analysis, we counted frogs whose stomachs contained at least one prey item.

In terms of abundance, insects dominate the ingested population with 104 prey, or 85.24% (Tables 4). The other categories are poorly represented, Arachnids with 5 preys,

Annelids with 9 preys and molluscs with 4 preys. Finally, plant debris is present in all the frogs examined, but we did not count them with animal prey (**Table 5**). A number of 72 preys were counted in the females and 50 in the males. There are no significant differences between the abundance of prey in the two sexes (t= -0.76, P>0.05).

Table 5. .*P. saharicus*diet composition

Category of Preys	**Females (n=25)**			**Males (n=19)**			**Females + Males (n=44)**		
	n	**n (%)**	**F (%)**	**n**	**n (%)**	**F (%)**	**n**	**n (%)**	**F (%)**
Diptera	2	2.77	12	9	18	31.57	11	9.01	20.45
Coleoptera	17	23.61	36	17	34	57.89	34	27	45.45
Hymenoptera	15	20.83	48	13	26	42.10	28	22	45.45
Orthoptera	2	2.77	8	1	2	5.26	3	2.45	6.81
Odonata	11	15.27	28	2	4	5.26	13	10.65	18.18
Trichoptera	0	0	0	1	2	5.26	1	0.81	2.27
Dermaptera	4	5.55	12	1	2	5.26	5	4.09	9.09
Lepidoptera	3	4.16	12	1	2	5.26	4	3.27	9.09
Heteroptera	1	1.38	4	0	0	0	1	0.81	2.27
Hemiptera	2	2.77	8	1	2	10.52	3	2.45	9.09
Blattoptera	1	1.38	4	0	0	0	1	0.81	2.27
Araneae	2	2.77	8	3	6	15.78	5	4.09	6.81
Haplotaxida	8	11.11	24	1	2	5.26	9	7.37	15.90
Basommatopha	4	5.55	8	0	0	0	4	3.27	4.54

(N: Abundance, fi%: Relative frequency, F%: Frequency of occurrence)

The analysis of relative frequencies reveals that among insects, Coleoptera and Hymenoptera are the most consumed, with 27% respectively for the former and 22% for the latter (Table 4). They are followed by Odonates (Larvae) and Diptera with 10.65 and 9.01% of the diet. The other categories occupy between 1 and 7% of the prey consumed. The least present categories are Tricoptera, Heteoptera and Blattoptera with 0.81%.Regarding the other categories, Spiders represent about 4% of the prey consumed, Gasterodes 3.27% and Annelids about 7%.Taking into account the frequencies of occurrence of various food items, we find that Coleoptera and Hymenoptera were found in more than 45% of the sampled frogs. Diptera and Odonates are present in the stomachs of almost 20% of frogs and Annelids (Haplotaxida) in about 15% of frogs (**Table 4**).

3.2. Monthly change in prey consumed

The evolution of the population of prey in the stomachs of frogs indicates that March is the most abundant with 58 preys, while April is the least abundant with 29 preys. During May, there is an increase in the number of prey reaching 42. For male individuals, the most abundant month is March (37 preys) and the least abundant is May (5 preys). On the other handfor females May is the most abundant (37 preys) and the least abundant is April (20 preys) (**Figure 2**).

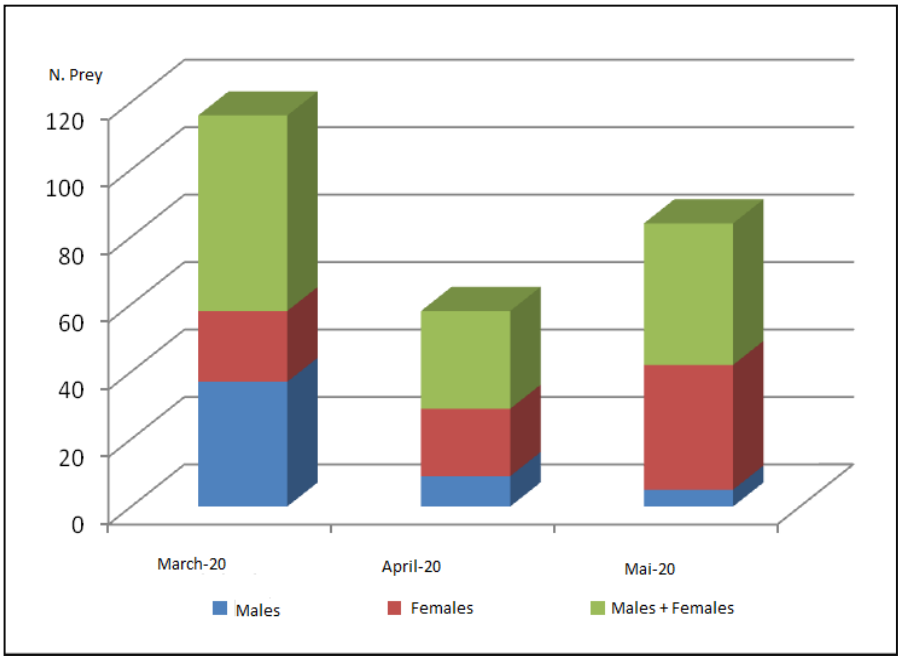

Figure 2. Monthly variation in the diet of *P. saharicus*.

The obtained results show that the Coleoptera, the Diptera and the Hymenoptera are the orders present during the 3 months of the study as well as, to a lesser extent, the Lepidoptera and the Dermaptera. The most regular group is that of Coleoptera with 14 individuals in March and 11 individuals in April and May.

Some categories of prey were only found for a single month. These are Trichoptera and Orthoptera present only during the month of March, Blattoptera, Hemiptera and Heteroptera present only in April.

DISCUSSION

This study aims to understand the diversity of the Edough massif, which is one of the most precious ecosystems in Annaba. This region, known for its forest environments and its high rate of endemism, is one of the most important in the country in terms of biodiversity (**Toubal, 2009; Hamel *et al.*, 2013; Brahim bounab *et al.*, 2020**).

The species present at the temporary ponds are often rare and threatened because they are adapted to particular living conditions, this is the case of the frogs, which frequent those of the Edough which are sometimes flooded and sometimes dry (**Allem *et al.*, 2017).**

Our sampling began in March 2020 and lasted until May. It allowed the random sampling of 66 individuals, 42 females and 22 males at the level of 3 temporary ponds. The sex ratio is in favour of females and depends on several factors, including the

sampling period and season, the sampling technique and the population itself (**Toft, 1980; Kovács & Török, 1995).**

The results relating to the composition of *P. saharicus diet*,during the 3 months of the study, show that it is very diversified and similar tothose of of **Bellakhal *et al.* (2010)** on *P. saharicus* and **Benhassine & Nouira (2009)** on *Pelopylax saharica* and *Discoglossus pictus* **Cicek & Mermer (2006); Cicek, (2007); Ruchin & Ryzhov (2002)** on *Rana ridibunda*.

P. Saharicus with 104 preys ingested, which is equivalent to 85.24%. Coleoptera and Hymenoptera are the most common, followed by Odonata and Diptera. The other categories are poorly represented: the arachnids represent 3% prey, the annelids with 9% prey, and the molluscs with only 4% prey.

Our results are consistent with results reported on the population of *P. saharicus* at Lake Tonga in El-Kala National Park, where Insects dominate with 59.93%, Arachnids with 16.89%, and Gastropods with 1.37% **Khiari (2017).** Moreover, **Bellakhal *et al.* (2010),** report similar results with a diet composed of 86.2% Insects, 7.11% Arachnids, and 1.21% Gastropods.

According to the frequencies of occurrence, we find that Coleoptera and Hymenoptera were found in more than 45% of the sampled frogs. Diptera and odonates in 20% of frogs and Annelids (Haplotaxida) in about 15% of frogs.

The diet of *P. saharicus* is composed of both terrestrial and aquatic prey and this was found at all three sites, although the proportion of aquatic prey is relatively low.

Studies on the diet of Ranidae have revealed that they feed exclusively on terrestrial prey **Jenssen & Klimstra, (1966); Beschkov, (1970); Whitaker *et al.*, (1981); Da Silva *et al.* (1989)** while others have shown the existence of aquatic prey in the diet such as amphibians, gastropods and crustaceans (**Benhassine & Nouira 2009**).

The diet of a species mostly reflects the conditions in which it lives. In our case, the three studied sites have similar characteristics. Studies on Ranids have revealed that prey choice is linked to the availability and the soil characteristics of the surrounding environment **(Whitaker *et al.*, 1981; Duellman & Trueb, 1986; Hirai & Matsui, 1999; 2000; Ortega *et al.*, 2016).** Furthermore, according to **Mendelev (1974)** and **Low & Török (1998)**, Ranidae consumes prey in proportion to their relative abundance in the surrounding environment.

The temporal evolution of the diet of the green frog in the Edough region during the study season is not very clear. The differences are probably due to the progressive change in the composition of the invertebrates at the level of the pools.

Some authors have revealed that adult green frogs can consume in addition to a wide variety of invertebrates (mainly insects) vertebrates, such as amphibians or lizards

(Benhassine & Nouira, 2009).In our case, we did not find vertebrate prey but vegetation is present.

Numerous plants debris was foundin the stomach. This suggests that they are present accidentally, ingested during prey attack, or selected by frogs to facilitatethe prey digestion or for use as a medicinal plant. Information on plant consumption contributes to the understanding of behavioral patterns. Plant additions to the diet of tropical frogs have become more frequent in recent years. **Da Silva *et al.* (1989)** demonstrated that *Xenohyla fruncata* consumes bromeliad fruits and seeds particularly during the dry season, when invertebrates are less abundant.

CONCLUSION

The present work is part of more developed research on the ecology of amphibians in the Edough region. The results of this preliminary study provide the first data on the regional food of green frogs in this region of Algeria. They clearly show the character of the opportunistic predator of the species. Complementary studies throughout the year and on several sites with the estimation of potential prey biomasses in the sites are recommended.

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All authors have read and agreed to the published version of the manuscript.

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