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Human Influence or Natural Differentiation in Food Composition of four Amphibian Species from Histria Fortress, Romania?

***Severus-Daniel Covaciu-Marcov*[1*], *Diana Cupşa*[1], *Sara Ferenți*[1], *Anamaria David*[1], *Nicoleta Dimancea*[2]**

[1]University of Oradea, Faculty of Sciences, Department of Biology; Str. Universității, No.1, 410087, Oradea, Romania
[2]University of Oradea, Faculty of History, Geography and International Relations, Environmental Geography, Str. Universității, No.1, 410087, Oradea, Romania

Abstract: We analysed food composition of four amphibian species (*Pelobates syriacus*, *Pelobates fuscus, Hyla arborea* and *Epidalea* (*Bufo*) *viridis*) in the conditions of an extremely rich and homogeneous trophic offer, represented by crowded Nematocera at the lights from Histria fortress. From the four species, three mainly consumed Nematocera, thus being opportunistic predators. However, *E. viridis* mostly fed on ants, thus indicating a trophic selectivity. Human activity affects differently the frogs, thus *H. arborea* hunts on the museum's walls, therefore consuming more flying preys, while *P. fuscus* hides under vestiges of the fortress. Except from *P. syriacus,* all of the species appear to be drawn by the artificial lights. Thus, human activity determines the high consumption of Nematocera, the lights captivating both the preys and the majority of the predators. *E. viridis* presents the most distinct food, due to the supremacy of ants, while *P. fuscus* and *H. arborea* record the most similar trophic spectrum. *P. syriacus* registers the highest food diversity, consuming the largest amounts of prey taxa, and is the only one that hunted in both the illuminated and non-illuminated, more natural areas from the proximity.

Key words: food diversity, microhabitats, human activity, Nematocera, trophic strategy

Introduction

It is known that amphibians are opportunistic hunters, feeding on the most abundant trophic resources from their environment (Bisa *et al.* 2007, Çiçek *et al.* 2007, Yu *et al.* 2009, Sas *et al.* 2009, Solé *et al.* 2009, de Paula-Lima *et al*. 2010). However, we tried to verify these facts once again taking advantage of a special occasion. We happened to be at Histria fortress in an evening when Nematocera: Chironomidae was fully displaying their swarm, in the air being at least several hundreds of thousands of individuals. Four amphibian species were present at the fortress: *Pelobates syriacus* Boettger, 1889*, Pelobates fuscus* (Laurenti, 1768), *Hyla arborea* (Linnaeus, 1758) and *Epidalea* (*Bufo*) *viridis* (Laurenti, 1768) hunting near the lights which attracted the Nematocera. Therefore, we had the opportunity to test to what extent this swarm of Nematocera influences the feeding of the amphibians. Theoretically, in this context, the food of the frogs was supposed to be homogeneous regardless the species and composed almost exclusively of Nematocera. In Romania, data about food composition exist only in three of these four species:

*Corresponding author: scovaciu@uoradea.ro

H. arborea (Cogălniceanu *et al.* 2000, Kovács *et al*. 2007, David *et al*. 2009), *P. fuscus* (Cogălniceanu *et al*. 1998, Nicoară *et al.* 2005) and *E. viridis* (Tesio, Teodorescu 1999, Covaciu-Marcov *et al*. 2005 a, David *et al.* 2008, Ferenţi *et al*. 2009).

The amphibians are important consumers in both the aquatic and terrestrial habitats, the quantitative information about their role in the ecosystems being very valuable (Whiles *et al.* 2006). Data of this kind are very rare in most of the parts from Romania, country in which the knowledge regarding the biodiversity and its status lack at European level (Hartel *et al.* 2008). Feeding information is crucial in understanding the life history, population fluctuations or the impact of habitat modifications on the amphibians (Anderson *et al*. 1999). These data are more essential in the endangered areas (Santos *et al*. 2004). The anthropogenic impact is present in Histria fortress at different levels for at least 2700 years.

The objective of this study is to compare the food composition of the four species from Histria fortress, in the context of an extremely quantitative rich, but uniform trophic offer, represented by Chironomidae. We propose to approach the following directions: 1. determining food composition, 2. establishing differences between food of the four species, 3. discerning trophic strategies.

Materials and Methods

The study took place in the night of 21/22 august 2005. The studied area is situated in the Eastern part of Romania, in Dobrudzha. Histria fortress is one of the oldest localities of Romania, being established over approximately 2700 years ago, being used as a port of the Black Sea. At present, in the area there is an archaeological site consisting of ruins of the fortress and museum building in front of which are exposed different vestiges. Near by there is an asphalted parking place. In the parking and in front of the museum there are several pillars of public lighting. The lights drew the Nematocera, which practically embraced the pillars and formed a vivid carpet. The whole site is surrounded by kilometres of natural land composed of lagoons and channels intersected by sand dunes. We captured the frogs by hand during an interval of an hour, at 23-24 p.m. We investigated a surface of approximately 200 m², including both the illuminated areas from the parking place and those in front of the museum, as well as the non- illuminated neighbouring ones, where the frogs were observed using a flashlight. On a whole, we captured 98 individuals (Table 1), but the populations, at least in the case of *P. syriacus*, are much larger. The four species had different distribution, *P. syriacus* being the only homogeneously spread in the investigated area. *H. arborea* was present on the walls of the museum in illuminated areas, *P. fuscus* in the zone of vestiges near the museum and *E. viridis* near the lights from parking space and museum. The capturing of the frogs stopped after an hour, in order to prevent the digestion of the preys.

The stomach contents were drawn using the stomach flushing method (Solé *et al.* 2005), the animals being afterwards released. The samples were deposited in test tubes, preserved in formaldehyde and determined in the laboratory. The food composition was evaluated by percentage abundance (%A) and frequency of occurrence (%f). The dietary diversity was estimated with Shannon-Wiener (1949) diversity index (H) and the similarity with Sorensen index (S). With the Sorensen index we compared the feeding web of all individuals from the same species, calculating a mean value for each randomisation. The niche overlap was estimated using Pianka's index (1973). The result of this index was transformed in a tree diagram, which shows the trophic linkage between the four species.

Results

All of the analysed individuals presented stomach content, feeding during the time of study. The feeding of all the four species was very intensive, both the maximum and average number of preys/individual being very high (Table 1).

In the case of *E. viridis,* the extremely high value is because of ant presence, while the other species have lower values than this one, but however very high, which were determined by Nematocera: Chironomidae.

Table 1. Number of analyzed individuals, maximum and average number of prey/individual, food diversity and similarity of the food consumed by individuals from the same species.

Species	***P. syriacus***	***P. fuscus***	***H. arborea***	***E. viridis***
No. of individuals	32	15	17	34
Maximum No. of prey/individual	72	29	38	186
Average No. of prey/individual	31.21	10.6	18.41	50.88
Diversity (H)	1.440	1.106	0.776	1.127
Similarity (Sorensen's S) mean	0.49	0.46	0.57	0.48

All of the preys were terrestrial invertebrates, both flying and non-flying. In the stomach contents of all of the four amphibians we determined 26 prey taxa (in case of some taxa we separated the larvae from the adults), together with vegetal fragments (Table 2). In the case of *P. syriacus* we also identified inorganic elements.

The differences between the four species are obvious with concern to the frequency of vegetal consumption as well as regarding the animal prey consumption. Thus, *P. syriacus* consumed the highest number of prey taxa (21) and *P. fuscus* the lowest one (12). From the 27 taxa, all of the four species consumed eight.

From the prey taxa, the Nematocera: Chironomidae registers high values in both the percentage amount and frequency of consumption. The ants and the Coleopterans also register higher values. The rest of the prey taxa have in general low amounts, but in some of the cases their frequency is relatively high (Table 2). For example, in the case of *P. syriacus* gastropods represent only 1.7% from the total of preys, while 34.37% of the frogs ingested them. The consumed snails had large dimensions (even 1 cm long slugs); at least the slugs were easily digested due to the lack of a hard cover. At the same time, unlike Nematocera: Chironomidae, the snails are trophic resource available for a longer period.

P. syriacus registers the maximum food diversity (H=1.440), while *H. arborea* the minimum one (H=0.776). On the contrary, *H. arborea* records the most obvious similarity between the individuals regarding the consumed preys (Table 1), due to the huge number of Chironomidae and to the fact that they consume a low amount of prey taxa. Analysing the trophic niche overlap, we can observe some important differences between the four species (Fig. 1).

Discussion

Three of the four studied species (*P. syriacus, P. fuscus* and *H. arborea*) mainly consumed Nematocera: Chironomidae. These were the most abundant possible preys in the feeding territory of frogs. The primarily Nematocera consumption underlines the opportunistic character of feeding of these frogs. In addition, it indicates the human influence, because the high amount of consumed Nematocera is a result of the presence of lights, which draw impressive quantities of Nematocera, easing the contact between the predator and its prey. Yet, there are certain differences between the food of the four species. In the case of *E. viridis* Nematocera do not hold a majority. *P. syriacus*, a large-sized species, registers the highest trophic diversity, consuming large sized preys (*Scolopendra* sp., Coleoptera: Cerambycidae). *P. syriacus* also consumed small-sized preys such as Acarians, despite its size. It is known that frogs, including the large ones, can consume in certain conditions extremely small preys (Covaciu-Marcov *et al*. 2005b, Cicort-Lucaciu 2009). The size of the frogs is not, however, the main factor which determines the differences in the trophic spectrum.

The differences that appear in the food of the four species are established by the used hunting territory, the usage of distinct microhabitats leading to avoiding food competition (Lima, Magnusson 1998). This determines the available prey offer, thus modifying the trophic spectrum. *H. arborea* has the most distinct hunting territory, the species hunting on the walls of the museum, at over 1 m above ground level, thus having direct access to the flying preys, such as the Nematocera. *H. arborea* is the only species which consumed Neuroptera and Brahicera. Beside these, the spiders appear in a large amount, which in

Table 2. Percentage abundance (%A) and frequency of occurrence (%f) of stomachs containing vegetal fragments and prey taxa.

The parameter analyzed	**percentage abundance (%A)**				**frequency of occurrence (%f)**			
Species	*P. syr.*	*P. fus.*	*H. arb*	*E. vir.*	*P. syr.*	*P. fus.*	*H. arb*	*E. vir.*
Vegetal fragments	-	-	-	-	78.12	46.66	35.29	70.58
Gastropoda	1.7	0.62	0.95	0.23	34.37	6.66	17.64	11.76
Acarina	0.2	-	-	-	6.25	-	-	-
Araneae	2.6	3.14	12.46	0.8	43.75	26.66	82.35	35.29
Crustacea Isopoda	12.11	5.03	0.31	2.77	53.12	26.66	5.88	47.05
Chilopoda undet.	-	-	-	0.8	-	-	-	11.76
Chilop. Scolopendra	0.3	-	-	-	9.37	-	-	-
Dermaptera	-	-	0.31	-	-	-	5.88	-
Isoptera	-	-	-	0.11	-	-	-	5.88
Heteroptera	0.2	0.62	0.31	-	6.25	6.66	5.88	17.64
Homoptera Cicadina	0.1	0.62	-	0.34	3.12	6.66	-	-
Coleoptera total	**6.8**	**9.43**	**1.91**	**4.5**	**87.5**	**53.33**	**23.52**	**82.35**
Coleopt. undet.	2.5	5.03	1.27	1.15	40.62	33.33	17.64	35.29
Coleopt. Carabidae	0.8	-	0.31	0.8	18.75	-	5.88	35.29
Coleopt. Stafilinidae	0.5	-	-	0.34	6.25	-	-	17.64
Coleopt. Scarabeidae	0.9	0.62	0.31	0.57	28.12	6.66	5.88	23.52
Coleopt.Cerambycidae	0.1	-	-	-	3.12	-	-	-
Coleopt.Curculionidae	1.8	3.14	-	1.5	34.37	26.66	-	58.82
Coleopt. Crizomelidae	0.2	0.62	-	0.11	6.25	6.66	-	5.88
Orthoptera	0.2	-	-	0.11	6.25	-	-	5.88
Neuroptera Crisopae	-	-	0.31	-			5.88	-
Nematoc. Chironomidae	52.75	69.81	79.55	37.22	96.87	93.33	100	94.11
Brahicera imago	-	-	0.95	-		-	11.76	-
Brahicera larvae	-	-	0.31	-		-	5.88	-
Lepidoptera imago	0.3	0.62	0.31	0.23	9.37	6.66	5.88	11.76
Lepidoptera larvae	0.1	-	0.95	0.23	3.12	-	17.64	11.76
Hymenopt. Formicidae	22.12	10.06	1.27	52.48	78.12	53.33	17.64	88.23
Hymenopt. Vespidae	0.1	-	-	-	3.12	-	-	-
Hymenoptera undet.	0.4	-	-	0.11	12.5	-	-	5.88

turn are advantaged by the wall, in which they can bind their cobwebs or take shelter in its cracks. On the contrary, the terrestrial preys, such as ants appear only by chance in the case of *H. arborea*.

In comparison to *H. arborea, E. viridis* consumed fewer amounts of flying preys, and even the most accessible trophic resource, Nematocera, register lower values. However, in the case of *E. viridis* Nematocera record higher values than in the cases of other populations (Covaciu-Marcov *et al*. 2005a, David *et al.* 2008). In the case of *E. viridis* the terrestrial preys, (Carabidae, Isopoda and ants) are emphasized. The preference of the species for ants seems to be a general one, being recorded in other studies as well (Nicoară *et al*. 2005, Ferenţi *et al*. 2009). The lower weight of the Nematocera in the case of *E. viridis*, despite the fact that all of the individuals were captured in illuminated areas, indicates the existence of a trophic selectivity. Even in the presence of an extremely rich trophic offer, *E. viridis* continues to preferentially search and feed on its favourite prey, ants. The preference towards ants is most likely due to the usage of formic acid at the venom production (Jones *et al*. 1999).

Our results suggest that *E. viridis* is adapted to hunt on the ground, consuming non-flying preys, *H. arborea* above the ground, hunting on flying preys and both *Pelobates* species seem to be indiffer-

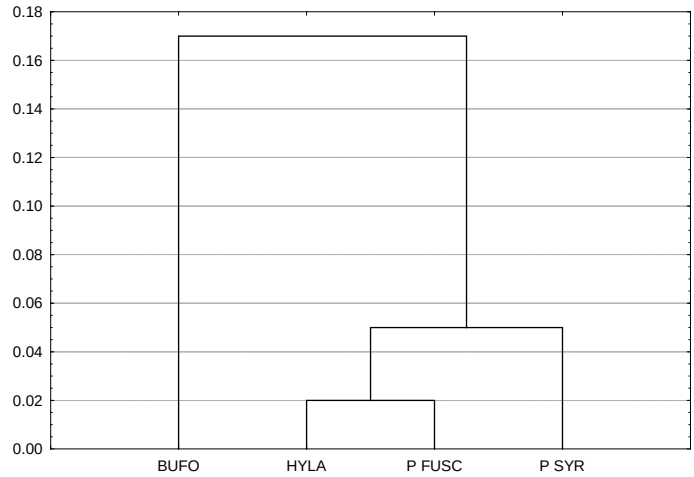

Fig. 1. Trophic niche overlap after Pianka's index (Bufo – *Epidalea viridis*, Hyla – *Hyla arborea,* P fusc – *Pelobates fuscus*, P syr – *Pelobates syriacus*).

ent, being adjusted to hunt both flying and no flying preys. Differences determined by the hunting territory, also exist between both Pelobates species. *P. fuscus* was extremely localized, being present only in the vestige zone in front of the museum, where it took shelter at the slightest danger. The fact was probably a consequence of the presence of refuges offered by the vestiges, but also of the fact that the vegetation was very rare and short in the area, *P. fuscus* hunting better in open areas with short vegetation (Tobias *et al.* 2001). The wider hunting territory allows *P. syriacus* to consume more prey categories and meanwhile not to consume so many Nematocera. The reduced Nematocera amount in its case is a consequence of the vaster hunting territory, because they were very numerous in the case of the individuals captured under the lights. The Nematocera were also present, but in a smaller amount, in the case of captured individuals from darker areas.

The presence of the Nematocera is obvious in the studied area, but humans indirectly, through the presence of the lights, determined them to focus and become accessible to the frogs in huge quantities. The lights indicate the clearest influence of humans on the feeding of the amphibians, which determine the agglomeration of the Nematocera, these being dominant in the food, or having very high amounts. Their overwhelming presence in the environment is a natural phenomenon, but humans modify their accessibility.

The differences between the trophic spectrums of the four species are both natural as well as human-influenced. The least dependent species on anthropogenic activities are *E. viridis* and *P. syriacus*. Despite the fact that it hunts in artificial zones, in the lights, *E. viridis* maintains its natural hunting skills, focussing on the terrestrial preys and being less interested in the flying ones regardless their abundance. *P. syriacus* is the only species that shows an indifference towards the anthropogenic areas, hunting in the same manner both in this situation and in the natural ones. Humans influence the feeding activity of *H. arborea* and *P. fuscus*. Thus, *H. arborea* uses the museum as hunting and hiding territory, being twice advantaged by the construction. In other areas, the species has high prey diversity because it exploits the habitat both horizontally and vertically (Kovács *et al*. 2007). Here however, it strictly limits to the horizontal component, not coming down on the ground, thus the food diversity is reduced. Although it leads to the decreasing of trophic diversity, the artificial habitat can, however be regarded as an advantage for the species in an area which lacks wood vegetation, therefore normally missing the vertical component. *P. fuscus* is encouraged by both the lights, which draw the preys, and the ancient vestiges under which it shelters.

Hunting territory also influences the presence of vegetal fragments in the food of frogs. Vegetal fragments have the highest frequency in the case of species which consumed more prey taxa and have high food diversity, namely *P. syriacus* and *E. viridis*. *H. arborea* hunts on the walls and therefore cannot really swallow grass. On the contrary, *P. syriacus* and

E. viridis hunt non-flying preys, in dense grass areas, having the opportunity to swallow most of the grass fragments. These data underline the accidental consumption of vegetal parts, which were swallowed together with the prey (Sas *et al*. 2007, Mollov 2008).

In the case of the four species it is difficult to establish the trophic strategy because of the episodic 'contamination' with Nematocera. Since the consumption of some reduced-sized preys indicates the utilization of an active foraging type of strategy (Duellman, Trueb 1986), one can consider that all of the four species use this kind of strategy. Although this finding is just a surface image induced by Nematocera, which even though they are many and small, in this case they rather determined a sit and wait type of tactic, being so numerous in the light area that practically the amphibians need not have to move. Only in the case of *E. viridis* does the selectivity towards the ants indicate the usage of the active foraging strategy. Finally, the data actually indicate just the high trophic plasticity of frogs, which change strategies depending on the real conditions from the environment.

H. arborea presents the highest similarities of the consumed preys. This species consumes the fewest prey taxa, thus the individuals approximately feed on the same type of preys. Although in this case the feeding of the species is much more intensive than the one recorded in Western Romania, the food diversity is still more reduced (Kovács *et al*. 2007). *H. arborea* and *P. fuscus* have the most similar trophic niches while *E. viridis* has the most different one from all of the species. The fact is determined by the distinct trophic strategy used by *E. viridis* and by the selectivity of the species towards ants. Although *H. arborea* has a different prey spectrum, it comes very similar to that of *P. fuscus* because of high Nematocera consumption.

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Антропогенно влияние или естествена диференциация в състава на храните на четири вида земноводни от крепостта Хистрия, Румъния?

С. Д. Ковачиу-Марков, Д. Купша, С. Ференти, А. Давид, Н. Диманцеа

(Резюме)

Анализиран е хранителният състав на четири вида земноводни (*Pelobates syriacus*, *Pelobates fuscus*, *Hyla arborea* и *Epidalea* (*Bufo*) *viridis*) в условията на много богата хомогенна трофична среда, представена от много Nematocera при осветлението на крепостта Хистрия. От четирите вида, три основно консумират Nematocera, защото са опортюнистични хищници. Въпреки това, *E. viridis* се хранят най-вече с мравки, което е показателно за хранителна избирателност. Човешката дейност се отразява по различен начин на жабите, като *H. arborea* лови по стените на музея и поради това – повече летящи жертви, докато *P. fuscus* се крие под останките на крепостта. С изключение на *P. syriacus*, всички видове ловят на изкуствено осветление. По този начин, човешката дейност определя висока консумация на Nematocera. Светлините привличат както плячката, така и по-голямата част от хищниците. *E. viridis* има най-разнообразен трофичен спектър, в който преобладават мравките. *P. fuscus* и *H. arborea* имат подобен хранителен спектър. *P. syriacus* има най-високо хранително разнообразие, консумира най-разнообразни жертви, и е единственият вид, който ловува както в осветените, така и в неосветените, близки околности.