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Data from a five year monitoring on Green frogs (*Pelophylax esculentus* complex) at the Black sea coast of north Bulgaria

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Abstract. In a previous study, the authors had predicted that the local green frog population had developed adaptations to live and breed in mixoligohaline waters, however the registered in 2010 mass migration of the adults into the sea near the Shablenska Tuzla lagoon was an isolated event, conditioned by the correlated impact of defined climatic factors. To support that hypothesis, the site was an object of five-year monitoring. Additionally, other three regions with similar topographic characteristics - the Black sea shores near Durankulak lake, Ezeretz lake and the Bolata bay - were investigated. During the period of the field surveys and according to data from fishers and biologists working in the region, no further mass migration of frogs in the sea waters was detected. An isolated observation of a specimen, swimming in the sea at Bolata bay (water with conductivity of 19.55 mS/cm), leads to the conclusion that the adult green frogs from the local population are able to inhabit the sea waters, but other factors like the sea surf prevent them to colonise the sea shores.

Key words: marsh frog, edible frog, halinity tolerance, habitat shift, limnetic ecology, herpetology.

Introduction

The green frogs from the *Pelophylax esculentus* complex represent one of the main components of the limnetic herpetofauna in Bulgaria. Because of the high population density and abundance of the species, they are crucially important for the trophic web (see Beshkov, 2007). According to recent data (Stojanov et al. 2011, Tzankov & Popgeorgiev 2014, Lukanov et al. 2014) two of the species from the complex - the marsh frog *Pelophylax ridibundus* (Pallas 1771) and the edible frog *Pelophylax esculentus* (Linnaeus 1758) - inhabit the territory of Northeast Bulgaria. In the country, the hybridogenic kleptospecies *P. esculentus* (for overview see Holenweg 2001) was found in the water basin of the Danube river and the Durankulak lake (data in Beškov & Beron 1964, Beshkov 1965, Batshvarov & Popov 1973, Undziyan 2000, Beshkov & Nanev 2002, Stojanov et al. 2011). Only recently, the species was recorded to inhabit other water basins near the Black sea coast – Shablenska Tuzla lagoon (Natchev et al. 2011) and probably even more to the south (*P.* cf. *esculentus* in Stojanov et al. 2011).

The third species from the complex – the pool frog *Pelophylax lessonae* (Camerano 1882) was indirectly confirmed as a part of the Bulgarian fauna from Beshkov (1965) for lake Srebarna, but not confirmed later (Biserkov & Naumov 2012, Tzankov & Popgeorgiev 2014). Recently it was found nearby town Oryahovo by means of bioacoustics studies (Tzankov & Popgeorgiev 2014).

It is known that the green frogs from genus *Pelophylax* are able to live and breed even in sites which are highly polluted by industrial wastes and effluent waters (for overview see Bodin et al. 2013, Zhelev et al. 2014). These species are able to inhabit waters with increased salinity (Hemmer & Kadel 1980, Kuzmin 1999, Plötner 2005, Covaciu-Marcov et al. 2006). According to Litvinchuk et al. (2015) in the region of Kaliningrad, *P. ridibundus* can be found in brackish water bodies (4.7 - 6.7 ‰). For the frogs from *P. esculentus* complex was reported that they even enter sea waters (Kuzmin et al. 2008, Natchev et al. 2011). A mass invasion of green frogs in the waters of the sea was detected in the summer of 2010 in the region of Shablenska Tuzla (Natchev et al. 2011). In their study the authors explain that phenomenon by coincidence of climatic factors and predict that the mass migration of frogs from the *P. esculentus* complex in the Black sea was an isolated and occasional event with regional impact.

The main goal of the present study is to verify that hypothesis by five year monitoring on the region of the frog invasion which was reported in Natchev et al. (2011). Parallel to the main field surveys, in the region were located other three sites where migration of green frogs into the sea may occur.

Material and methods

The marsh and the edible frogs have very similar ecologic demands as well as have similar position in the trophic web in Bulgaria. Both species inhabit permanent or temporary water basins with stagnant water, but they are also able to handle streams and can be found in rivers too (Stojanov et al. 2011). Comparative studies concerning the feeding spectrum of the three species from the *P. esculentus* complex in Bulgaria are lacking. Data provided for populations living in synthopy in Serbia revealed a relatively great dietary overlap - Pianka's index 0.76-0.85 (Paunović et al. 2010).

The monitoring of the sites in the region of Shabla municipality, where potential migration of the frogs into sea water may occur, started in the summer of 2010 and was carried out for the period of five years. Four localities were selected as objects of the survey (Fig. 1):

1. The sand beach in the region of Shabla and Ezeretz lakes (43.583184°N, 28.572520°E);
2. The sand beach in the region of Durankulak lake (43.680310°N, 28.561302°E);
3. The Bolata bay (43.385041°N, 28.470878°E);
4. The sand beach in the region of Shablenska Tuzla lagoon (43.561151°N, 28.591644°E);

All of the four sites are in range of 40 km and possess similar topographic characteristics as the site of the previously described migration into the sea. In the four localities, near the sea coats, there are

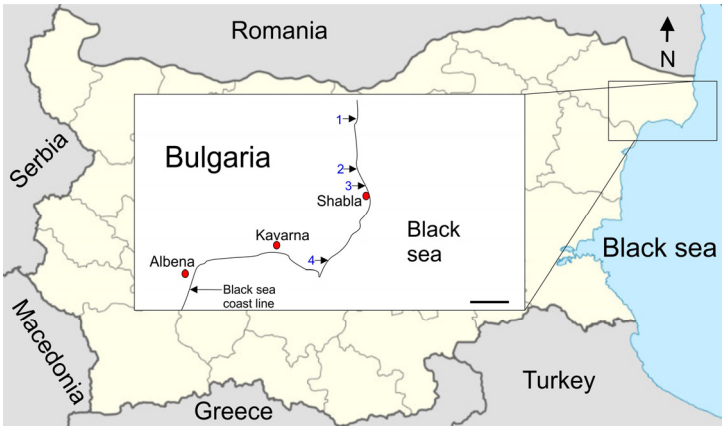

Figure 1. Schematic map of the coast line in the region of Albena, Kavarna and Shabla (NE-Bulgaria). The red dots are indicating the named settlement in the region. The numbers and the short arrows are indicating the position of: 1 - Durankulak lake; 2 - Ezeretz lake; 3 - Shabenska tuzla lagoon; 4 - Bolata bay. The image is based on a product of mapsof.net. Scale bar 10 km.

permanent water basins which are inhabited by dense breeding populations of marsh frogs with single occurrence of edible frogs. There are no physical obstacles or barriers which may prevent the frogs to enter the sea waters and in all four localities there are plenty of temporary puddles which dry out when the rainfalls level drops.

The field surveys were carried from August to October (in 2010) and in the spring and in summer months from the beginning of April until the end of September (from 2011 to 2015). The sites were visited in irregular periods of two to three weeks.

During the surveys were investigated the presence of adult frogs, tadpoles and eggs both in the water bodies as well as in the sea. The water conductivity was measured by using of WTW Cond 330i conductivity controller (analyser) with electrochemical sensor "WTW TetraCon 325" (WTW Gmbh, Weilheim, Germany) and Testo 240 with sensor Testo Term Type 10. The sensors were cleaned in bi-distillate water after every measurement. Categorization of the water salinity was made according to the Vinece system (1959) and that of Brischoux and Kornilev (2014) for Black sea. Topographic recordings were performed by GPS hand-held systems Garmin Colorado 300, Garmnin Etrex (10 and 30), and Garmin Oregon (Garmin International Inc., Kansas, USA). The position of the investigated four large water basins is indicated on Fig. 1.

Mating and other signals were recorded by using a Zoom H1 (Zoom North America, New York). The audio-recording was performed in April and May fife time every year during the monitoring. The signals were always documented for one hour after the sun down in four following days at every one of the four investigated areas. During the monitoring no adult frogs, larvae, eggs or other animals were harmed.

Results

For the larger water basins (except the sea), increases of the water halinity over 5 mS/cm was registered only in the Shablenska Tuzla lagoon. The salinity of the other permanent freshwater basins remained in the range of the oligohaline (brackish) waters. The puddles which were investigated (see Fig. 2a) were oligo- to mesohaline. Permanent and temporary fresh water bodies with conductivity of over 5 mS/cm were densely inhabited by adult animals and tentatively determined tadpoles from *P. esculentus* complex (see Figs 2 b,c). The frogs used such bodies also to spawn.

During the whole period of the monitoring, we observed and documented only one isolated case of single subadult specimens swimming in the Black sea - at the Bolata bay in the spring of 2013 (Fig. 3). The frog was trapped, investigated and released. The animal was very active and in good condition – no visible damage or injuries were observed. The

Figure 2. Numerous oligo- and mesohaline puddles were scattered around the main water basins; a, mixoligohaline puddle in the reed belt of Shablenska tuzla lagoon; b, tadpole that could be tentatively attributed to *P. esculentus* complex inhabiting a mixoligohaline puddle with water conductivity of 6.65 mS/cm; c, adult green frogs permanently inhabiting a mesohaline puddle with water conductivity over 14 mS/cm;

Figure 3. Subadult *P. ridibundus* swimming freely in the Black sea at the Bolata bay - water conductivity 19.55 mS/cm;

Figure 4. Temporary water basin at the beach of Bolata bay densly inhabitet by tadpoles of *Pelobates* spp. and *Bufotes viridis* complex;

conductivity of the water direct at the spot was 19.55 mS/cm.

In the springs of 2013 and 2015 at the Bolata bay, direct on the beach was detected a temporary water basin with a diameter of approximately 19 m (in 2013) and 26 m (in 2015). The puddle was located very close to the sea (see Figs 1 and 4) and repetitive measurements of the water conductivity were in ranges between 1.8 and 4.2 mS/cm. In this particular water body were observed and documented numerous frogs larvae of *Bufotes viridis* complex and *Pelobates* spp. (see Fig. 5). No eggs or larvae of green water frogs were documented in that puddle in springs of 2013 and 2015.

The sound analysis of more than 112 hours of signal recording revealed that all calls which we were able to identify as a signal coming from a single male can be identified as produced by *P. ridibundus*. Mating signals of *P. esculentus* and *P. lessonae* were not identified, nor as solitary calling males or in the group signals.

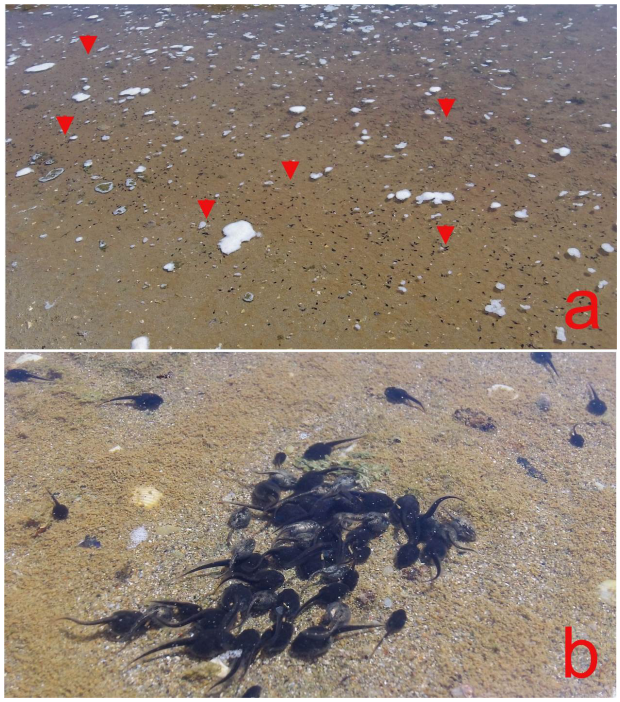

Figure 5. Tadpoles of *Pelobates* spp. and *Bufotes viridis* complex inhabiting water basin with conductivity under 5 mS/cm: a, general view of the water surface – the arrowheads are indicating single tadpoles; b, detailed view of the tadpoles.

Discussion

Our results indicate, that the local marsh and edible frogs are adapted to live and breed in oligo- to mesohaline waters and the increased conductivity of the water is not affecting dramatically the density of the population. Interestingly, no other anuran species or newts [in the particular case *Lissotriton vulgaris* and *Triturus dobrogicus* (see Stojanov et al. 2011)] were detected to live or spawn in mesohaline basins.

Massive migration of the green frogs in the waters of Black sea was detected only in the days between 12 - 16 August 2010 (see Natchev et al. 2011). This event suggests that despite the adults from the local populations of green frogs are well adapted to waters with increased water salinity, they do not enter frequently the sea. Individual migrations of frogs in the sea waters are obviously fairly rare. Our results confirm only one occasion of a subadult green frog swimming in sea waters with conductivity of about 20 mS/cm. Zamfirescu & Surugiu (2001) described a similar observation from Dobrogea Black sea coast at the channel to Mangalia lake where adult *Pelophylax ridibundus* and *Bufotes viridis* were observed in water with salinity that vary in large ranges (1,75-15,17 g/l).

In the water body represented on Figs 1, 4 and 5 we observed and documented numerous frog larvae of *Bufotes viridis* complex and *Pelobates* spp. Most probably the Speadtoad larvae belonged to *P. syraicus* as this species tolerate higher osmotic stress than *P. fuscus* as demonstrated in the preliminary research of Stănescu et al. (2014). The puddle was virtually boiling from the activity of the tadpoles, which indicate the good condition of the larvae. Because of the position of the puddle between the sea and the local fresh water Bolata lake, the halinity of the water varied in some ranges during the larvae development. Our measurements indicate that *Bufotes* and *Pelobates* larvae from the local populations can tolerate temporary changes in the water halinity, however we did not found larvae of these species in waters with conductivity above 5 mS/cm.

On the base of the results from the five year monitoring in the four localities, we conclude that the green frogs from the investigated populations have developed adaptation to live and breed in waters with conductivity of over 5 mS/cm. The eggs are covered in not permeable gelatinous capsule and the embryos can develop in the waters wit increased sa-

linity, but even more vulnerable larvae (see Gomez-Mestre et al. 2004) survive in oligohaline to mixo-oligohaline puddles. Postmetamorphic (subadult and adult) green frog specimens are able to tolerate the halinity even of the sea waters. As no other amphibians were detected to live in mesohaline waters, the adaptation of the frogs from *P. esculentus* complex to withstand osmotic pressure can be regarded as an evolutionary advantage. However, the mass migration of green frogs into the waters of Black sea, recorded in August 2010 was an isolated phenomenon, caused by coincidence of climatic factors as predicted from Natchev et al. (2011). During the period of the monitoring, only a single individual was detected to enter the sea waters. We propose that the green frogs are able to tolerate the halinity of the sea water, but the main factor which prevents the colonisation of the Black sea shores is the water surf.

In attempt to register the presence of *Pelophylax lessonae* we recorded the mating calls of the green frogs at all investigated sites. The species was not detected during the whole period of the monitoring. However, no call records can be attributed to *P.* kl. *esculentus*. Thus, we conclude that a R-E system population inhabits the studied sites, with only females' *P.* kl. *esculentus* being presented. This system was rarely observed throughout the *P. esculentus* complex distribution (Rybacki & Berger 2001). It is possible that pool frogs inhabit the region, still further investigations are needed to confirm and document their persistence, as well as that of males of *P.* kl. *esculentus*. As the presence of *P. lessonae* in Bulgaria was currently confirmed via acoustic identification, further analysis of the frog's calling signals coming from this region are demanded.

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