Herpetology Notes, volume 4: 347-351 (2011) (published online on 22 October 2011)

Green frog invasion in the Black Sea: habitat ecology of the *Pelophylax esculentus* complex (Anura, Amphibia) population in the region of Shablenska Tuzla lagoon in Bulgaria

Nikolay Natchev[1]*, Nikolay Tzankov[2] and Richard Gemel[3]

Because of their highly vascularised and permeable skins (Spotila and Berman, 1976), amphibians have a low capacity to handle increased salinity of the waters they inhabit. Nevertheless, a few species are able to live in brackish and even hypersaline waters (for overview see Neil, 1985; Chakko, 1968; Karraker, 2007; Sillero and Ribeiro, 2010). Reports on amphibians living under saline conditions are numerous, but only a few amphibians can survive prolonged exposure to sea water. Among anuran species, adaptations to elevated salinity differ considerably (Beebee, 1985; Shoemaker et al. 1992). One ranid species – the crab eating frog, *Fejervarya cancrivora* (Gravenhorst, 1829) – is considered to possess the highest salinity tolerance among amphibians. In this species, 50% of the larvae survived in up to 80% sea water – equivalent to 0.5%M Cl (Dunson, 1977).

In some euryhaline species only specific populations are adapted to survive in saline environments (Christman, 1974; Gomez-Mestre and Tejedo, 2004). In other species, like the green frog *Lithobates clamitans* (Latreille, 1801), local adaptation to elevated salinity was not determined (Karraker, 2007). Hemmer and Kadel (1980) report salinity tolerance of *Pelophylax perezi* (SEOANE, 1885) on the Balearic islands to be 1.1 to 1.3‰. Plötner (2005) gives a short compilation of salinity tolerance of *Pelophylax epeiroticus* (Schneider, Sofianidou and Kyriakopoulou-Sklvounou, 1984). This species from south western Greece can survive in salinity up to 6‰. According to Bentley and Schmidt-Nielsen (1971), the lethal effect of sea water on *Lithobates pipiens* (Schreber, 1782) is due to the effects of the sodium concentration *per se*, rather than to the osmotic effect of the solution. Another species, *Pelophylax ridibundus* (Pallas, 1771) also tolerates and is resistant to high water salinity – 0.9 to 8.3‰ (Kuzmin, 1999). Frogs of the *Pelophylax esculentus* complex are able to handle an increased sodium concentration and to withstand the osmotic gradient when inhabiting brackish sea waters (for overview see Kuzmin et al., 2008). Covaciu-Marcov et al. (2006) report occurrence of *P. ridibundus* in brackish waters in Romania. Accidental occurrences of single frogs or small groups were already observed in different places – estuary zones and lagoons along the Bulgarian coast line (pers. obs.).

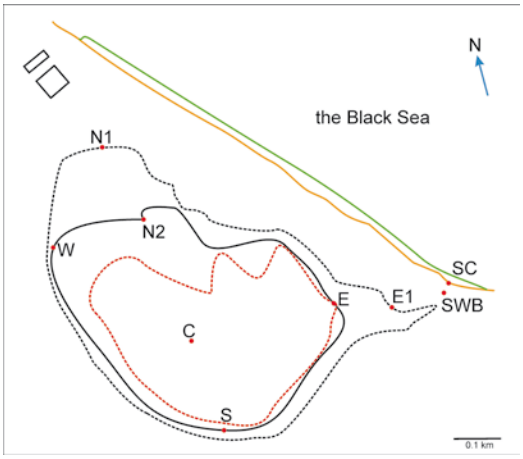

Figure 1. Schematic map of Shablenska Tuzla lagoon based on GPS data. Solid black lines represent the boundaries of the reeds free water surface of the lagoon in August 2010. The solid orange line represents the Black Sea coast. The area between the solid green and the orange lines represents the region in which green frogs were found swimming in sea waters. The dashed red line represents the boundaries of the water surfaces on 30.10.2007. The dashed black line represents the boundaries of the vegetation belt outside the lagoon shores. The red dots indicate the points from which water samples were collected.

1 University of Vienna, Department of Theoretical Biology, Althanstrasse 14, A-1090 Vienna, Austria; e-mail: nikolay.natchev@univie.ac.at
2 Museum of Natural History, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel blvd., Sofia, Bulgaria;
3 Natural History Museum Vienna. Burgring 7, A-1010 Vienna, Austria.
*Corresponding author.

Table 1. Geographic coordinates of the points from which water samples were taken and conductivity/salinity of the waters from the relevant water basins measured during the period of the investigation.

Point indications	Geographic coordinates	Date	Conductivity (mS/cm)	Salinity (‰)
Point SC (sea coast)	43°33'30.20"N / 28°35'48.99"E	14.08.2010	16.77	10.01
Point SWB (sea water basin)	43°33'29.43"N / 28°35'48.18"E	16.08.2010	14.08	8.00
Point SWB		23.08.2010	13.39	7.9
Point SWB		30.08.2010	13.70	8.1
Point E1 (east 1)	43°33'30.46"N / 28°35'42.36"E	16.08.2010	4.24	2.2
Point E1		23.08.2010	5.52	2.9
Point S (south)	43°33'26.09"N / 28°35'25.26"E	23.08.2010	4.77	2.6
Point W (west)	43°33'41.51"N / 28°35'15.29"E	23.08.2010	4.79	2.6
Point C (center)	43°33'32.53"N / 28°35'24.95"E	23.08.2010	4.86	2.6
Point E (east)	43°33'31.71"N / 28°35'38.93"E	23.08.2010	4.74	2.6
Point N1 (north 1)	43°33'41.54"N / 28°35'24.44"E	23.08.2010	5.51	2.9
Point N2 (north 2)	43°33'47.15"N / 28°35'22.48"E	23.08.2010	4.89	2.6

Figure 2. Water basins as habitats of two green frogs of *P. esculentus* complex in the region of Shablenska Tuzla: (a) Shablenska Tuzla lagoon; (b) total plan of the basin (point SWB), which was inhabited by adult specimens from both water frogs of *P. esculentus* complex; (c) distance between the shores of the SWB and the waters of the Black Sea was about 12 m.

On August 14th 2010, in the region of Shablenska Tuzla in Bulgaria (Fig. 1), a massive invasion of green frogs of the *Pelophylax esculentus* complex in the Black Sea was observed. According to the local people, the phenomenon started on August 12th 2010. The last observation of green frogs in sea water was made on August 16th 2010. During the four day period, numerous frogs were found swimming in the sea water and were also seen to rest on the sand beaches of the Black Sea coast. To our knowledge such a mass phenomenon was not previously recorded on the Bulgarian Black Sea coast and on Black sea coasts in general. According to local fishermen, such an "invasion" of frogs into the sea had never been seen before. Only adult animals were observed and collected in sea waters. Tadpoles or very young animals were not observed in the Black Sea. The maximal distance to which animals were seen to swim away from the coast was approximately 12 meters. Figure 1 shows the region in which the green frogs were observed in sea water. The animals were collected by the investigators by using different traps (see below) in the Black Sea along a distance of approximately 800 meters along the beach. The conductivity of the sea water on August 14th 2010 was 16.77 mS/cm (Tab. 1). During the first days in which green frogs were observed to swim in the Black Sea, the water was completely still. On August 16th 2010, a landswell appeared, but we were still able to observe a small number of animals in the sea. On that day we found 12 dead frogs on the shore (see Fig. 3a). The dead specimens were fixed in alcohol, sectioned, and examined under a dissection stereomicroscope (Wild M5A and Wild M420). There was no visible damage to the skin. The dissections revealed that there was no water in the lungs and no significant injuries of

Figure 3. Green frogs of the *P. esculentus* complex in the region of Shablenska Tuzla: (a) a dead specimen found on the Black Sea shores on 16.08.2010; (b) adult frogs from the local population of *P. esculentus* complex inhabiting basins with conductivity of the water about 14 mS/cm.

the internal organs were found.

Shablenska Tuzla is in the Dobruja microclimatic zone of the north Black Sea region. The average yearly temperature in the zone is 11°C and the average wind speed is about 3.5 m/s. The average wind speed is twice as high as the average wind speed on the south Black Sea coasts. The total sum of rainfall in the region of Shabla is one of the lowest in Bulgaria: 386-552 mm per year. Even in short periods of time, the sea water salinity at the Shabla sea coast can vary across a large range – from 13.8 to 18.6 ‰ (all data taken from Georgiev, 2003). Formerly Shablenska Tuzla was used for sea-salt production. The waters of the lagoon were mixopolyhaline during the spring periods and hyperhaline during summer-autumn periods (for overview see Stoyneva and Michev, 2007). Currently the water of the lagoon is brackish with salinity levels above concentrations typical of freshwaters (see Ortiz-Santaliestra et al., 2010). Stoyneva and Michev (2007) report that after the middle of the 1990s the water halinity was measured to be between 4 and 5.3 ‰ (mixoligohaline waters).

In the summer of 2010, we started studying the impact of the unusual heavy rainfalls in the region of the village of Gorun on the water salinity of the sea and other water basins in the region of Shablenska Tuzla. An important aim of our field research was to detect the relationship between the water salinity of the different water bodies and the presence (or absence) of eggs, tadpoles and adult frogs of the *P. esculentus* complex. Investigations on the habitat of the *P. esculentus* complex population in the region of Shablenska Tuzla were performed in the period from August 14th to September 9th 2010. For species determination, specimens were trapped on land as well as in water by using plastic containers and nets. The identification of the green frogs was made on the basis of leg/body ratio comparisons, the ratio between the length of the inner metatarsal tubercle and the length of the first toe in adults and some specific coloration traits (see Plötner, 2005, 2010). Specimens and habitat characteristics were documented using a Canon EOS 300D digital camera with a Canon Tele Zoom Lens 75-300 mm II (Canon Inc., Tokyo, Japan).

The boundaries of the lagoon in general and its reed-free water surface were mapped on the basis of GPS coordinates taken by a hand-held Garmin Colorado 300 GPS unit (Garmin International Inc., Kansas, USA) with an accuracy of 5 meters. For graphical representation of our geographic data, a "Google Maps" image from October 30th 2007 was used (see Fig. 1).

Water samples were taken from the locations represented in Fig. 1. The exact geographic coordinates of these points are listed in Tab. 1. The water samples from points "N", "S", "E", "W" and "C" were collected from a boat. The depth of the lagoon was measured at every water sample collection point. As the bottom of the lagoon is covered by a thick layer of mud, we measured the depth as the distance from the water surface to the superficial layer of sediment. On the basis of GPS coordinates, we were able to mark the boundaries of the lagoon and its open water surface. We compared the water surface (free of reeds) from August 2010 and October 2007 and found that in August 2010 the margins of the open water were much wider (see Fig. 1). Three of the points from which we took water samples in the summer of 2010 were beyond the lagoon in the autumn of 2007. The water depth was between 61 and 78 cm.

Shores of Shablenska Tuzla lagoon in the summer of 2010 were abundantly covered with vegetation (Fig. 2a). The predominant plants were *Phragmites australis*, *Typha* sp., *Schoenoplecus lacustris*, *Carex riparia*, and *Juncus maritimus*. During the whole period of the investigation, within this vegetation belt we found abundant small water bodies occupied by *Pelophylax* tadpoles of different development stages, juveniles and adult specimens. Interestingly, we were able to find some *Pelophylax* eggs. The conductivity of the water of such puddles was similar to that of the open water surface of the lagoon. The measurements at points "W", "N2", "S", "C" and "E" showed almost equal values (see Tab. 1). The conductivity of the water from all samples was measured by using "WTW Cond 330i" conductivity controller (analyser) with electrochemical sensor "WTW TetraCon 325" (WTW Gmbh, Weilheim, Germany). The sensor was cleaned in bi-distillate water after every measurement.

On the south-east side of the lagoon we found a partly dry "duct", which suggests that there is a temporary connection of the lagoon to the sea (Fig. 2b,c). Within this channel we found a water basin (here termed "Sea Water Basin" or "SWB") with a length of 24 meters and width of approximately 9 meters. The total shore length was approximately 84 meters and the water depth was between 38 and 51cm. This basin was inhabited predominantly by adult *P. ridibundus* frogs with sporadic occurrence of *P.* kl. *esculentus*. A large number of these frogs were swimming in the water or were resting on the shores in dense groups (see Fig. 3b). On some days we counted up to 324 specimens. Tadpoles and eggs were not found in this water basin. The conductivity of the water was more similar to that of the sea rather than that of the lagoon (see Tab. 1).

According to Beshkov (2007), *P. ridibundus* is the main component of the wetland herpetofauna in Bulgaria, and because of the high density of its populations, this species has an extremely important role in the trophic webs. In water basins along the Danube River, *P. ridibundus* occurs together with *P.* kl. *esculentus* (Beshkov, 2007). This habitat sharing of two green frogs of the *P. esculentus* complex was confirmed by our investigation of their population in the region of the brackish Shablenska Tuzla lagoon. Despite the relatively high salinity of the waters of the lagoon and the water of the abundant single puddles distributed within the reed belt, the frogs use these basins as breeding habitats. The embryos and tadpoles of the local population are able to develop in waters with conductivity above 5 mS/cm. Despite being densely inhabited by adult frogs, no eggs or young animals were found in the water body termed here "SWB" (see Figs. 1, 2b,c). Adult amphibians are more tolerant to elevated salinity than their embryos and larvae (see Gomez-Mestre et al., 2004). It is possible that only adult specimens can survive in water in which conductivity exceeds 13 mS/cm. An alternative explanation for the absence of non-adult specimens in the "SWB" is that in waters with salinity above defined levels, the *P. ridibundus* and *P.* kl. *esculentus* frogs from the local population do not spawn. Reproduction of *P. ridibundus* in marine water with low salinity, at 0.5 to 1 m. from the shore, is reported for a population inhabiting the Apsheron Peninsula of the Caspian Sea (Kuzmin, 1999).

The frog invasion in the waters of the Black Sea in the region of Shablenska Tuzla lagoon in the summer of 2010 lasted over 96 hours and numerous animals were found to live for more than a month in the "SWB". Adult frogs from the local population are able to live for prolonged periods in waters with conductivity even above 16 mS/cm. The salinity of the water is therefore not the limiting factor preventing the adult frogs of the *P. esculentus* complex from inhabiting the Black Sea shores. In our opinion, the main reason why the green frogs do not invade more frequently the sea waters is that the animals cannot handle the landswell. Our dissections revealed no significant injuries or other damage on the specimens found dead on the Black Sea shores. It is possible that the frogs were not able to swim in higher waves for a prolonged time and were thrown out on the beach where they dehydrated in the sun.

In conclusion, we argue that the invasion of the frogs of the *P. esculentus* complex in the Black Sea in the region of Shablenska Tuzla in August 2010 was an isolated natural event. Migration activities of *P. ridibundus* are known from hibernation habitat to aestivation locality (Heym, 1974). However, the dispersal described in the present study is clearly not related to hibernation. In our opinion this phenomenon is the result of the specific ecological conditions in the spring and the summer of 2010. Because of the heavy rainfalls (over 200 mm) on July 9th 2010 (information provided from the National Institute of Meteorology and Hydrology, Branch of Varna, Bulgarian Academy of Sciences), the conductivity of the lagoon waters dropped under 6 mS/cm, which may have had a positive effect on the successful development of the frogs' embryos and tadpoles. The population became denser and some adult animals were forced to occupy even water basins with high elevated

salinity. During the period between 12th and 16th August 2010, the sea waters were completely still and numerous adult frogs dispersed even into the Black Sea. Further studies will reveal whether populations of these species, which have entirely freshwater habitats, also possess the potential to tolerate elevated salinity of the water, or such adaptations have evolved independently within the groups which live near brackish water basins.

Acknowledgments. The authors are grateful to Georgy Georgiev, Danko Georgiev and Galin Angelov (members of Getia Pontica) as well as to Stefan Vladimirov for their support during the field research. "Dr. Josef Weisgram, Dr. Egon Heiss and Dr. Walter Lechner (all Vienna University) provided helpful comments on the ecological framework. We thank the National Institute of Meteorology and Hydrology, Branch of Varna (Bulgarian Academy of Sciences) for the information concerning the weather conditions in the region in 2010. Dr. Vesselin Budakov (Kliment Ohridski University, Sofia) is acknowledged for English improvement.

References

Beebee, T.J.C. (1985): Salt tolerance of Natterjack Toad (*Bufo calamita*) eggs and larvae from coastal and inland populations in Britain. Herpetol. Jour. **1**: 14-16.

Bentley, P.J., Schmidt-Nielsen, K. (1971): Acute effects of sea water on frogs (*Rana pipiens*). Comp. Biochem. Physiol. Part A. **40**: 547-548.

Beshkov, V. (2007): Amphibians and reptiles of non-lotic Bulgarian wetlands. In: Inventory of Bulgarian Wetlands and their Biodiversity, p. 211-214. Michev, T.M., Stoyneva, M.P., Eds., Publ. House Svetlostrouy, Sofia.

Chakko, G. (1968): Salinity tolerance in some South Indian Anurans. Proc. Indian Acad. Scien. B. **67**: 233-236.

Christman, S.P. (1974): Geographic variation for salt water tolerance in the frog *Rana sphenocephala*. Copeia. **1974**: 773-778.

Covaciu-Marcov, S.D., Ghira, I., Cicort-Lucaciu, A.S., Sas, I., Strugariu, A., Bogdan, H.V. (2006): Contributions to knowledge regarding the geographical distribution of the herpetofauna of Dobrudja, Romania. North-West. Jour. Zool. **2:** 88-125.

Dunson, W.A. (1977): Tolerance to high temperature and salinity by tadpoles of the Philippine frog *Rana cancrivora*. Copeia. **1977**: 375-378.

Georgiev, D. (2003): "Shablensko Ezero" Protected Area Management Plan. Bulgarian-Switzerland program for biodiversity conservation.

Gomez-Meste, I., Tejedo, M., Ramayo, E., Estepa, J. (2004): Developmental alterations and osmoregulatory physiology of a larval anuran under osmotic stress. Physiol. Biochem. Zool. **77**: 267-74.

Gomez-Mestre, I., Tejedo, M. (2004): Contrasting patterns of quantitative and neutral genetic variation in locally adapted populations of the Natterjack Toad, *Bufo calamita*. Evol. **58**: 2343-2352.

Hemmer, H., Kadel, B. (1980): Studien am Wasserfrosch - *Rana perezi* (SEOANE 1885) - der Balearen (Amphibia, Anura, Ranidae). Boll. Soco Hist. Nat. Balears. **24**: 55-70.

Heym, W.D. (1974): Studien zur Verbreitung, Ökologie und Ethologie der Grünfrösche in der mittleren und nördlichen Niederlausitz. Mitt. Zool. Mus. Berlin. **50**: 263-285.

Karraker, N. (2007): Are embryonic and larval green frogs (*Rana clamitans*) insensitive to road deicing salt? Herpetol. Conserv. Biol. **2**: 35-41.

Kuzmin, S. (1999): The Amphibians of the Former Soviet Union. 298 p. Pensoft, Sofia-Moscow.

Kuzmin, S., Tarkhnishvili, D., Ishchenko, V., Dujsebayeva, T., Tuniyev, B., Papenfuss, T., Beebee, T., Ugurtas, I.H., Sparreboom, M., Rastegar-Pouyani, N., Disi, A.M.M., Anderson, S., Denoël, M., Andreone, F. (2008). *Pelophylax ridibundus*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 15 February 2011.

Neill, W.T. (1958): The occurrence of amphibians and reptiles in salt-water areas and a bibliography. Bull. Marine Sci. Gulf Carib. **8**: 1-97.

Ortiz-Santaliestra M.E., Fernández-Benéitez. M.J., Marco. A., Lizana. M. (*2010*): Influence of ammonium nitrate on larval anti-predatory responses of two amphibians. Aquatic Toxicol. **99**: 198-204.

Plötner, J. (2005): Die westpaläarktischen Wasserfrösche, von Märtyrern der Wissenschaft zur biologischen Sensation. Beiheft der Zeitschrift für Feldherpetologie 9, 160 p. Bielefeld.

Plötner, J. (2010): Möglichkeiten und Grenzen morphologischer Methoden zur Artbestimmung bei europäischen Wasserfröschen (*Pelophylax esculentus*-Komplex). Zeitschrift für Feldherpetologie **17**: 129-147.

Shoemaker, V.H., Hillman, S.S., Hillyard, S.D., Jackson, D.C., McClanhan, L.L., Withers, P.C., Wygoda, M.L. (1992): Exchange of water, ions, and respiratory gases in terrestrial amphibians. In: Environmental physiology of the amphibians, p. 125-150. Feder, M.E., Burggren, W.W., Eds., The University of Chicago Press, Chicago.

Sillero, N., Ribeiro, R. (2010): Reproduction of *Pelophylax perezi* in brackish water in Porto (Portugal). Herpetol. Notes. **3**: 337-340.

Spotila, J.R., Berman, E.N. (1976): Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. Comp. Biochem. Physiol. Part A. **55**: 407-411.

Stoyneva, M.P., Michev, T.M. (2007): Shablenska Touzla – In: Inventory of Bulgarian Wetlands and their Biodiversity. Part 1: Non-Lotic Wetlands, IBW0218. Michev, T.M., Stoyneva, M.P., Eds., Publ. House Svetlostrouy, Sofia.

Accepted by Philip de Pous; Managing Editor: Luis Felipe Toledo