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 *Pelophyla xiperciz* (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 pipyis* (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.

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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 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.

<table>
<thead>
<tr>
<th>Point indications</th>
<th>Geographic coordinates</th>
<th>Date</th>
<th>Conductivity</th>
<th>Salinity</th>
</tr>
</thead>
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<td>14.08.2010</td>
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<td>14.08</td>
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<td>23.08.2010</td>
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</tr>
<tr>
<td>Point SWB</td>
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<td>30.08.2010</td>
<td>13.70</td>
<td>8.1</td>
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<tr>
<td>Point E1 (east 1)</td>
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<td>2.2</td>
</tr>
<tr>
<td>Point E1</td>
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<td>5.52</td>
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</tr>
<tr>
<td>Point S (south)</td>
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<tr>
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<tr>
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<td>4.89</td>
<td>2.6</td>
</tr>
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</table>

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.

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
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.

![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.](image-url)
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., Schoenoplectus 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 51 cm. 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 conductivity.
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.

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