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Amphibians of the genus *Rana* in the steppe zone of Ukraine: population status, distribution, and ecological features

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abstract

Frogs of the genus *Rana* Linnaeus, 1758 are represented in the steppe zone of Ukraine by their southernmost populations. The distribution of brown frogs in the steppe zone of Ukraine is closely related to the floodplain forests of major rivers (Dnipro, Dnister, Danube, Southern Bug, Oril, Samara, Donets, etc.). The paper presents for the first time data on the distribution, ecological and population features, habitats, specifics of reproduction and development of brown frogs. New habitats of *R. arvalis* were found in the floodplain habitats of the lower reaches of the Dnipro River in Kherson Oblast and populations were confirmed in Dnipropetrovsk and Kirovohrad oblasts. The southern border is intrazonal. The new southernmost localities are described from Kherson Oblast (the villages of Pravi Solontsi and Krynky). The habitats belong to forest type habitats (G). The mean abundance of frogs is 5–10 individuals per 1 km of route and can reach 50 individuals per 10 m2 during the spawning season (Black Forest Reserve, Kirovohrad Oblast). The breeding season is in late March to early April at air temperatures of +3–4 to 8–12°C and water temperatures of +7–9°C. The mating process is short. The Dnipro isolates of *R. arvalis* may be of relict nature, form a series of refugia and be of important historical and indicator value. Given the overall reduction in the range of *R. arvalis* within its core area, active populations in the south can be valuable reserves for the conservation of the species and the sustainability of steppe biodiversity. Two other species, *R. temporaria* and *R. dalmatina*, were not found and their habitat within the steppe zone has not yet been confirmed. As typical forest species, they are common closer to the forest-steppe zone. Given the current state of brown frog populations in the steppe zone of Ukraine, their low numbers and isolation, they need regional protection along with their habitats. The issue of determining the origin of the identified populations in order to form an idea of the historical and genetic value of the southernmost populations of brown frogs in Ukraine remains promising.

Земноводні роду *Rana* степової зони України: стан популяцій, поширення та особливості екології

Наталія Сурядна, Галина Микитинець

Резюме. Жаби роду *Rana* Linnaeus, 1758 представлені у степовій зоні України своїми найпівденнішими популяціями. Поширення бурих жаб у степовій зоні України тісно пов'язане з заплавними лісами крупних річок (Дніпро, Дністер, Дунай, Південний Буг, Оріль, Самара, Сіверський Донець та ін.). В роботі вперше наводяться дані про поширення, еколого-популяційні особливості, оселища, специфіка розмноження та розвитку бурих жаб. Виявлені нові місця мешкання *R. arvalis* в заплавних біотопах пониззя Дніпра Херсонської області та підтверджені популяції в Дніпропетровській та Кіровоградській областях. Південна межа виду має інтрозональний характер. Нові найпівденніші локальні місця описані з Херсонщини (с. Праві Солонці та с. Кринки, Олешшя). Оселища відносяться до біотопів лісового типу (G). Чисельність в середньому складає 5–10 особин на 1 км маршруту і може досягати 50 ос. на 10 м^2 у період нересту (урочище «Чорний Ліс», Кіровоградщина). Період розмноження — кінець березня або початок квітня при температурі повітря +3–4 до 8–12°С, води +7–9°С. Парування короткотривале. Середня кількість ікринок в кладці — 1236 штук. Придніпровські ізоляти, можуть мати реліктову природу, формувати серію рефугіумів та мати важливе історичне та індикаторне значення. Враховуючи загальне скорочення ареалу *R. arvalis* в межах основного ареалу, дієві популяцій півдня можуть бути цінними резерватами збереження виду та сталості біорізноманіття степової зони. Два інших види — *R. temporaria* та *R. dalmatina* не знайдені і їх мешкання в межах степової зони нами поки не підтверджене. Як типові лісові види поширені ближче до лісостепової зони. Враховуючи сучасний стан популяцій бурих жаб степової зони України, їх малочисельність і ізольованість, вони потребують регіональної охорони разом з оселищами. Нові знахідки, узагальнені та підтверджені існуючі місця мешкання, дозволили розширити наші уявлення про ареал та просторову специфіку бурих жаб степової зони. Перспективним залишається питання визначення походження південних популяцій методами фітогеографії задля формування уявлення про історико-генетичну цінність південних популяцій бурих жаб України.

Ключові слова: *Rana,* межі поширення, рефугіуми, біотопи, розмноження, популяційні особливості.

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Introduction

The dynamics of habitats in time and space, relict populations, ancestral species, probable refugia or simply isolates—all these gain new significance as relevant findings are expanded. Range boundaries and the presence of typically forest-dwelling species in the steppe always arouse interest in their evolutionary biogeography and prospects for further existence [Zagorodniuk 2019; 2020]. Such habitats can be important population niches or reserves for endangered species with a pronounced tendency to shrink their ranges, as well as an important core of species and genetic diversity.

The trend of amphibian range reduction and extinction is threatening worldwide. Forty-one percent (35–50%) of amphibian species are threatened with extinction, and almost 50% of species show a decline in numbers [Sillero *et al.* 2014]. The alleged causes of this decline are related to anthropogenic impacts, habitat degradation, climate change, and infectious diseases of amphibians [Stuart *et al.* 2004; Araújo *et al.* 2006; Grant *et al.* 2016].

One of them is the genus *Rana* Linnaeus, 1758, which in Ukraine is represented by three species: *Rana temporaria* Linnaeus, 1758, *Rana arvalis* Nilsson, 1842 and *Rana dalmatina* (Fitzinger in Bonaparte, 1839). The southern limit of distribution of these species is in the steppe zone with local populations that may have unique origins or currently unknown routes of dispersal.

The first mention of brown frogs in the steppe zone is given in the works of O. Brauner [Brauner 1903, 1906]. However, the latest information on amphibians of Ukraine [Pysanets 2014] indicates that grass and quick frogs are practically absent in the steppe zone, and the sharp-toothed frog was recorded locally in the lower reaches of the Dnipro River, without identifying specific locations and with the assumption that they are extinct.

The earliest occurrences of brown frogs, in particular *R. arvalis* in Eastern Europe, are also reported from steppe faunas, while later occurrences belong to both typical forest and steppe faunas. This may indicate the historical value of steppe populations. There is evidence of a high level of genetic variation in *R. arvalis*, which is typical for areas that correspond to refugia [Babik *et al.* 2004; Roček & Šandera 2008].

Brown frog populations, their ecological characteristics, development, habitat features and distribution are studied by our colleagues from other parts of the range within Ukraine [Tkachenko 2007; Smirnov & Skilsky 2011; Smirnov & Buchko 2018].

The aim of the present study is to describe, confirm and summarise the distribution pattern, ecological features of brown frog populations, and to find out the habitat specificity of the studied species in the steppe zone of Ukraine.

Study area

The northern boundary of the steppe zone, according to the physical and geographical zoning [Marynych & Shyshchenko 2006], runs along the line of the following settlements: north of Velyka Mykhailivka, Shyriaieve (Odesa Oblast) through Pervomaisk (Mykolaiv Oblast), north of Novoukrainka, Kropyvnytskyi through Znamianka (Kirovohrad Oblast), Onufriivka along the Vorskla River to Kobeliaky and Novi Sanzhary (Poltava Oblast), north of Krasnohrad through Balakliia (Kharkiv Oblast) along the Oskil River to the state border. The boundary of the steppe zone was refined on the basis of geobotanical zoning [Didukh & Sheliag-Sosonko 2003].

The present research covers almost the entire steppe zone, which includes several administrative regions of Ukraine, namely most of Odesa, Mykolaiv, Kherson, Zaporizhzhia, Dnipropetrovsk, Donetsk, Luhansk, southern part of Kirovohrad, Poltava, and Kharkiv oblasts. The main focus was on areas of the steppe zone where the remnants of natural forests along the valleys of the Dnipro, Oril, Samara, Dnister, Danube, Southern Bug, and Donets rivers and the remaining forests on the border with the forest-steppe were preserved.

Materials and Methods

The period of research was quite long, starting in the late 1990s. Standard field research methods were used in the work [Pysanets & Suriadna 2007]. The main emphasis was on *R. arvalis* populations, as they were found and studied in this work. The characteristics of *R. temporaria* and *R. dalmatina* are summarised from the literature.

The material of the work comprised 220 individuals, including 88 (27 ♂, 45 ♀, 16 juv.) of *R. arvalis* recorded by us in Kherson, Dnipropetrovsk, and Kirovohrad oblasts, as well as 132 individuals of all three species from the collections of the Department of Zoology at the National Museum of Natural History, NAS of Ukraine (NMNH-z) [Mykytynets 2010; Suriadna & Pysanets 2010]. We also recorded 14 clutches and a total of more than 22 thousand eggs. Materials from open databases (https://ukrbin.com (UkrBIN), https://www.inaturalist.org (iNaturalist)), oral reports of colleagues and literature data were used. The detailed cadastral description of the finds is provided in accordance with the current administrative division of Ukraine (Figs 1–2). Mapping was performed using QGIS.

Several approaches were used to count clutches, depending on the conditions and circumstances. Most often, pairs were captured, then the eggs laid in the morning were counted, the animals were released and the clutches returned to the water bodies. This is how we analysed 10 clutches from the Black Forest tract (Kirovohrad Oblast). When clutches were found in small clusters, they were carefully removed from the water and the number of eggs was counted (near the village of Mykolaivka, Dnipropetrovsk Oblast). We also used the volumetric weight method, in particular, weighed the entire mass of eggs laid, counted the number of eggs per 100 g of clutch and recalculated the approximate number of eggs for the entire weight of the clutch (near the village of Pravi Solontsi, Kherson Oblast). All frogs and clutches were carefully returned to the place of discovery. Egg development,

metamorphosis, hatching percentage, mortality, and other parameters were studied in the laboratory. The development of *R. arvalis* was monitored for three months from 23 March to early June 2017 (n = 471 eggs). The frogs that underwent metamorphosis were returned to the natural environment.

The habitat characterisation was based on the classification of habitats of the steppe zone of Ukraine with a brief description and corresponding codes [Didukh *et al.* 2020]. Information on phenology and water parameters (air, water, soil temperature, pH, and salinity) were determined using standard alcohol thermometers and an AZ-86021 oximeter/pH meter.

Cadastral characteristics of the study material and survey data

Rana arvalis Nilsson, 1842 (see: Fig. 1)

Original data: *Dnipropetrovsk Oblast,* Dnipro Raion: (*1*) near Pidhorodne, date of registration—11.04.2007, time of registration—15.00–16.00, 3 specimens (3♂), coordinates—48.566833, 35.013056 (hereafter accordingly); (*2*) near Obukhivka, 11–12.04.2007, 10.00–11.00, 2 (1♀, 1♂), 48.535833, 34.810350; (*3*) Dnipro–Oril Nature Reserve, near Obukhivka, 07.04.2012, 14.00–15.00, 2 (2♂), 48.521500, 34.817000; (*4*) near Mykolaivka, 09.04.2012, 10.00–11.00, 1 (1♂), one clutch of 2865 eggs, 48.520189, 34.734725. *Kirovohrad Oblast,* Kropyvnytskyi Raion: (*5–7*) Black Forest protected tract, near Vodiane: (*5*) 19–20.05.2012, 4 (1♀, 2♂, 1 juv), 48.774991, 32.545702; (*6*) 06–07.04.2013, 20.00–21.00, 29 (10♀, 19♂), 10 clutches (consisting of 887, 914, 918, 1153, 1197, 1213, 1280, 1447, 1579, and 1774 eggs, respectively), 48.774444, 32.545556; (*7*) 07.04.2013, 10.00–11.00, 48.776694, 32.543000. *Kherson Oblast,* Kherson Raion, near Pravi Solontsi: (*8*) 03–04.11.2013, 16 (6♀, 2♂, 8 juv), 46.576139, 32.655778; (*9*) 03–04.10.2016, 13.00–14.00, 9 (1♀, 3♂, 5 juv), 46.568611, 32.661917; (*10*) 23.03.2017, 20.00–22.00; 05.11.2021, 14.00–15.00, 6 (2♀, 5♂), several clutches with a total volume of up to 6.5–7.5 thousand eggs, 46.567333, 32.657417; (*11*) 06–07.05.2017, 9 (3♀, 4♂, 2 juv), 46.567984, 32.660623; (*12*) near Krynky, 04.10.2016, 6 (3♀, 3♂), 46.744500, 33.098917.

Literature, collections, and other data: *Dnipropetrovsk Oblast,* Novomoskovsk Raion: (*13*) near Vasylivka (Samara Forest), 6–7.04.2013, 48.704219, 35.540652 (Facebook message); (*34*) near Kozachy Gai [NMNH-z], 48.411468, 35.634329. Dnipro Raion: (*32*) near Obukhivka, Dnipro–Oril Nature Reserve [NMNH-z], 48.517178, 34.817089; (*33*) Dnipro City, 48.467939, 35.10205 [Bulakhov 2005]; *Luhansk Oblast*, Siverodonetsk Raion: (*14*) Holykove, 06.06.2010, 49.145048, 38.170731 (iNaturalist); (*15*) Lake Kleshnia, near Syrotyne, June 2007, 48.901719, 38.512866 (personal message from Igor Zagorodniuk). *Donetsk Oblast*, Kramatorsk Raion, Holy Mountains National Nature Park [UkrBIN]: (*16*) near Pyskunivka, 48.884937, 37.861476; (*17*) near Pryshyb, 49.020573, 37.666530; (*18*) near Sosnove, 49.063282, 37.499010; (*19*) near Staryi Karavan, 48.921884, 37.763798; (*20*) near Sviatohirsk, 49.026977, 37.546771. *Kharkiv Oblast,* Izium Raion: (*21*) near Synycheno, 49.101428, 37.409904 [UkrBIN]. *Mykolaiv Oblast*, Pervomaisk Raion: (*22*) Pervomaisk, 48.046996, 30.847182 [Goncharenko 2002; Pysanets 2014]; (*23*) near Kryve Ozero, 47.945834, 30.342572 [NMNH-z]; (*24*) near Krymka, 47.934234, 30.760798 [Goncharenko 2002, NMNH-z]. *Odesa Oblast,* Izmail Raion: (*25*) Danube Delta, 45.427761, 29.492952 [Kotenko 1999; Pysanets 2007, 2014]. *Kherson Oblast,* Kherson Raion: (*26*) near Antonivka, 46.673975, 32.775929 [NMNH-z]; (*27*) near Bilozerka, 46.639897, 32.449660 [NMNH-z]; (*28*) near Hola Prystan, asphalt plant, 16.04.2004, 46.505962, 32.483901 [NMNH-z, Suriadna & Pysanets 2010; Pysanets 2014]. *Poltava Oblast*, Kremenchuk Raion: (*29*) Gunky, 49.247500, 33.565556 [Manilo & Radchenko 2008]; Poltava Raion: (*30*) basin of the Vorskla River near Bilyky, 49.251111, 34.282222 [NMNH-z]; *Kirovohrad Oblast,* Kropyvnytskyi Raion: (*31*) near Znamianka, Black Forest protected tract, 48.735859, 32.586865 [NMNH-z].

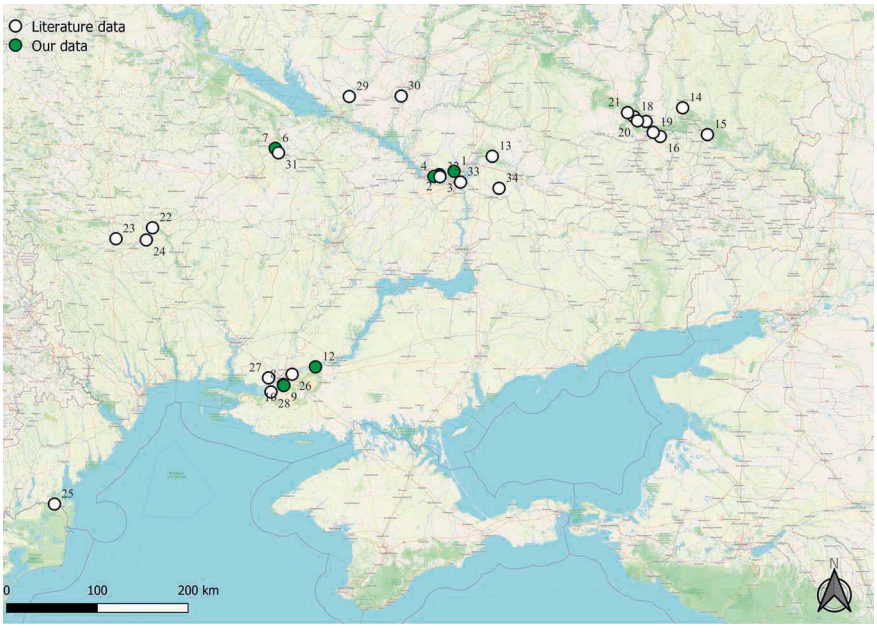

Fig. 1. Record localities of *Rana arvalis* in the steppe zone of Ukraine (notation in the text).

Рис. 1. Місця знахідок *Rana arvalis* в межах степової зони України (позначення в тексті).

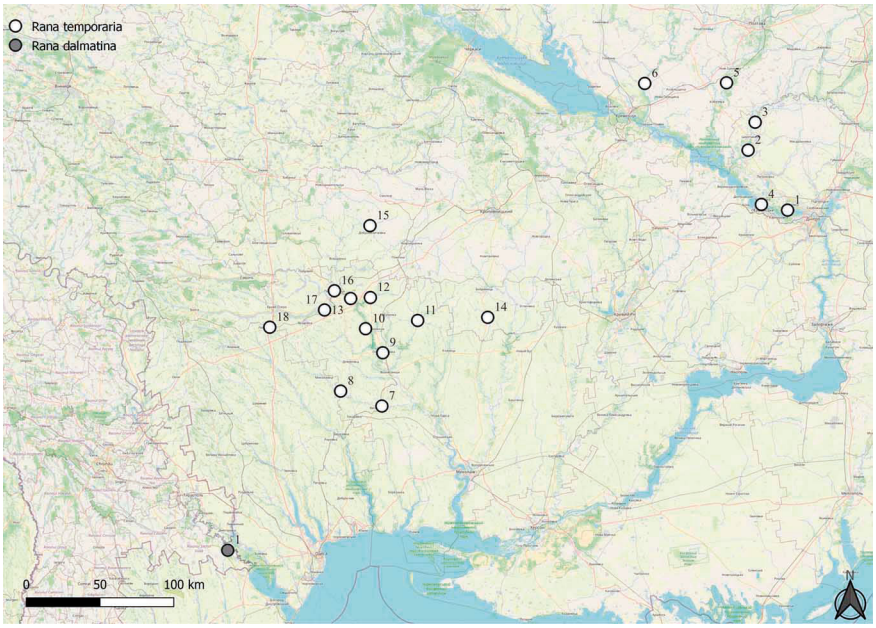

Fig. 2. Record localities of *R. temporaria* and *R. dalmatina* in the steppe zone of Ukraine (notation in the text).

Рис. 2. Місця знахідок *R. temporaria* та *R. dalmatina* в межах степової зони України (позначення в тексті).

Rana temporaria (Linnaeus, 1758) (Fig. 2)

Original data: we do not have our own data from the steppe zone.

Literature, collections, and other data: *Dnipropetrovsk Oblast,* Dnipro Raion: (*1*) Dnipro–Oril Nature Reserve, 48.517178, 34.817089 [Bulakhov *et al.* 2007]; (*2*) near Mohyliv, 48.864237, 34.471122 [Bulakhov *et al.* 2007]; (*3*) valley of the Oril River near Babaikivky, 49.025167, 34.533063 [Bulakhov *et al.* 2007]; (*4*) the upper reaches of the Zaporizhzhia (Dnipro) Reservoir, 48.550373, 34.587888 [Bulakhov *et al.* 2007]; *Poltava Oblast,* Poltava Raion: (*5*) basin of the Vorskla River Bilyky, 49.251111, 34.282222 [NMNH-z]; Kremenchuk Raion: (*6*) Hunky, 49.247500, 33.565556 [Pysanets 2014]; *Mykolaiv Oblast*, Voznesensk Raion: (*7*) valley of the Chichiklia River near Mykolaivka, 47.387778, 30.975556 [Goncharenko 2002; Pysanets 2014]; (*8*) Chichiklia River, 47.455698, 30.901650 [Goncharenko 2002]; (*9*) Oleksandrivka, 47.682337, 31.271123 [Goncharenko 2002]; (*10*) Bohdanivka, 47.824183, 31.120038 [Goncharenko 2002]; (*11*) Bratske, 47.872529, 31.576594 [Goncharenko 2002]; Arbuzyn Raion: (*12*) Blagodatne, 48.007299, 31.162140 [Goncharenko 2002]; (*13*) basin of the Southern Bug River, 48.001607, 30.989536 [Goncharenko 2002]. *Kirovohrad Oblast:* (*14*) Hromokliia River, 47.891054, 32.190148 [Goncharenko 2002]; (*15*) Novoukrainsk Raion, 10 km north of Dobrovelychkivka, 48.427056, 31.158837 [NMNH-z]; Pervomaisk Raion: (*16*) Pervomaisk, 48.046996, 30.847182 [Goncharenko 2002; Pysanets 2014]; (*17*) near Krymka, 47.934234, 30.760798 [Goncharenko 2002]. *Odesa Oblast*, Podilsk Raion: (*18*) Liubashivka [Goncharenko 2002].

Rana dalmatina (Fitzinger in Bonaparte, 1839) (see: Fig. 2)

Original data: no data available from the steppe zone.

Literature, collections, and other data: (*1*) Moldova, Ştefan Vodă District, near Olănești, 46.504135, 29.912574 [Reminnyi 2007; Pysanets & Reminnyi 2008; Pysanets 2014].

Results

New finds of brown frogs in the steppe zone in southern Ukraine, which may play an important role in the evolutionary history of the genus, are identified and described. The existence of other habitats of brown frog populations in the steppe zone was reliably confirmed. The ecological features are described, the distribution pattern is determined, and the southern limits of the species' range are analysed. The paper presents important data from primary surveys.

Description of findings of brown frogs in the steppe zone of Ukraine

Long-term expedition surveys allowed us to discover *R. arvalis* in floodplain forests of the lower Dnipro River. This article describes these southernmost locations for the first time. In autumn 2013, we found *R. arvalis* in the vicinity of Pravi Solontsi in Kherson Oblast. This was confirmed in October 2016, spring 2017, and November 2021 (Fig. 1, *8–11*). They are found mainly in floodplain forest

habitats, in litter or in dense herbaceous vegetation (Fig. 3, top row). Several spawning grounds with clutches were also found here, measuring approximately 50 x 20 m and up to 0.5 m deep. Depending on the season, different water levels were observed. We found *R. arvalis* on each expedition, including during spawning, indicating the relative resilience of this southern population.

The second, no less important southern find of *R. arvalis* is an island between the Krynka and Konka rivers near the village of Krynky. In October 2016, six individuals were found here on the forest floor (Fig. 1, *12*; Fig. 3, second row). Over the next four years (2017–2020), we were unable to find them here. According to Y. Roman (pers. com.), he first found *R. arvalis* in Krynky in 1999.

Important *R. arvalis* sites to the north were also confirmed. In 2007–2012, single individuals of *R. arvalis* were found in four localities in Dnipropetrovsk Oblast (Fig. 3, third row). As it was early April, all of them were in water bodies with abundant vegetation. Mostly males were counted (7 out of 8 individuals). Frogs were observed during the day. The size of the lakes was 50 × 40 m, 300 × 50 m, 0.3–0.7 m deep with thickets of duckweed, cattail, reeds, and bush amorpha. Woody vegetation around the waterbodies includes pine, willow, poplar, birch, and oak. A spawning pond with clutches and completely burnt reeds was found near the Dnipro–Oril Nature Reserve.

We confirmed the findings of *R. arvalis* in late May 2012 in the Black Forest tract northwest of the town of Znamianka, Kirovohrad Oblast. In particular, several individuals were found on the forest floor, on land in a dense oak forest. A year later, in early April 2013, mass emergence from wintering and reproduction of *R. arvalis* (spawning, mating pairs, and clutches) was observed here. This is a large spawning pond of more than 500 m^2 in an oak forest, 0.5–1 m deep, with riparian thickets of elderberry, willow, and maple (see: Fig. 3, bottom row). In total, more than 50 individuals were recorded.

According to the classification of habitats of the steppe zone of Ukraine, the identified habitats of *R. arvalis* belong to forest type (G). Mostly it is G1—deciduous forests [Didukh 2020]. Biotopes near Pravi Solontsi, Kherson Oblast: G:1.132—Alder eutrophic swampy forests (Alnetea glutinosae); G:1.133—Ash and alder forests (Alnion incanae) on eluvial sediments. In Krynky: G:1.112—Short-floodplain willow forests with white willow (*Salix alba*). In Dnipropetrovsk Oblast: G:1.112—Short-floodplain willow forests with white willow (*Salix alba*); G:1.113—Floodplain forests with white poplar (*Populus alba*) and sedge (*Populus nigra*); G:1.217—Floodplain oak forests. In Kirovohrad Oblast: G:1.225—Immoral hornbeam-oak forests (Lamio maculatae–Carpinetum betuli). It should be clarified that on land, frogs can be found in dry and flooded meadows, among ferns and sedges near waterbodies on high bumps, on the forest floor, in grass, in blackberries, in fallen leaves on trails and even on unpaved forest roads that are little used. Some spawning habitats are located near dirt and even asphalt roads. These are almost universally large ponds with stagnant or slowly flowing water with a lot of vegetation, fallen old trees, shrubs and a small amount of reeds.

We have not yet found *Rana temporaria* and *R. dalmatina* in the steppe zone of Ukraine, but their findings have been described by other researchers, which requires some generalisation.

Rana temporaria occurs close to the forest-steppe zone (Fig. 2). The southernmost record locality of this species in the steppe zone is the village of Mykolaivka, valley of the Chychyklia River [Goncharenko 2002].

The distribution of *R. dalmatina* was long considered to be limited to Transcarpathia (most finds in the plain part). But later the species was found in the basin of the Dnister River [Pysanets 2014]. According to Yevhen Pysanets, the closest record locality to the steppe zone of Ukraine is in the south of Moldova on the border with Odesa Oblast, near the village of Olănești (see: Fig. 2).

Ecological features of brown frogs in the steppe zone

We describe the population and ecological features of brown frogs in the steppe zone using the southernmost populations of *R. arvalis* as an example, which is important both for clarifying the range boundaries and for understanding the possible ways of the species' origin.

Fig. 3. *Rana arvalis* and its habitats in the steppe zone of Ukraine: upper row—floodplain forest near Pravi Solontsi, Kherson Oblast; second row—Krynky, Kherson Oblast; third row—near Pidhorodne, Dnipropetrovsk Oblast; bottom row—Black Forest protected tract, Kirovohrad Oblast.

Рис. 3. *Rana arvalis* та біотопи у степовій зоні Україні: верхній ряд — заплавний ліс с. Праві Солонці Херсонська обл., другий ряд — с. Кринки Херсонська обл.; третій ряд — окол. м. Підгородне Дніпропетровська обл.; нижній ряд — урочище «Чорний ліс», Кіровоградська обл.

An important record locality is the vicinity of the village of Pravi Solontsi in Kherson Oblast, where *R. arvalis* was found. Here we observed a rather early start of the breeding season. We observed spawning on 23 March at a daytime air temperature of 11.5°C and water temperature of 9°C. The group clutch contained up to 7.5 thousand eggs. The average air temperature during all observations was 19°C in autumn, 17°C in spring, and 14°C of water. The mean water salinity was 2.36‰, and the mean pH was 7.4. The most common co-occurring species in the studied waterbodies were *Bombina bombina, Pelobates fuscus, Lissotriton vulgaris, Triturus dobrogicus, Hyla orientalis*, and *Pelophylax* (*Pelophylax ridibundus, Pelophylax esculentus,* and *Pelophylax lessonae*). Among reptiles, the most commonly found were the grass snake (*Natrix natrix*) and the dice snake (*N. tessellata*), and the European pond turtle (*Emys orbicularis*).

High numbers of *R. arvalis* were observed during spawning. Dozens of individuals were counted per 10 m^2 of water surface (Black Forest protected tract, Kirovohrad Oblast) (Fig. 4). In late May, the abundance was 5 individuals per 1 km. In autumn, on land in habitats of Kherson Oblast, from 6 (Krynky) to 10 individuals (Pravi Solontsi) per 500 m were found. As for the sex structure, males outnumbered females in spawning waters. During ground surveys (especially in autumn), more females and juveniles were observed.

Spawning ponds may be under anthropogenic pressure. Such a clutch (2865 eggs) was observed in the vicinity of Mykolaivka, Dnipropetrovsk Oblast, with a male *R. arvalis* nearby. The average air temperature during the survey period was 13.5°C and water temperature was 12.8°C, slightly lower than in Kherson Oblast. Water salinity was 1.78‰, pH 7.8. Co-occurrence was noted with *Bombina bombina, Hyla orientalis* with clutches, *Lisotriton vulgaris, Pelobates fuscus* with clutches*, Pelophylax esculentus,* and *Pelophylax ridibundus*.

In the Black Forest tract, given the spawning season, active animals were observed during the day, evening and night, both on land, in oak forests, and in spawning ponds. The number of eggs was counted in ten pairs of *R. arvalis* that were planted. A total of 12 362 eggs were counted, with an average of 1236 eggs per clutch (min 887, max 1774). The average air temperature in April was 3–4°C and water temperature was 6–7°C; in May 21°C and 18–20°C, respectively. The average water salinity was 2.03‰, pH 7.3. *R. arvalis* was found here together with *Bufo bufo, Pelobates fuscus*, *Hyla orientalis, Pelophylax lessonae,* and *Pelophylax esculentus.*

In summary, the breeding season of *R. arvalis* begins in late March (Kherson Oblast)–early April (Dnipropetrovsk and Kirovohrad oblasts) at air temperatures of 3–4 to 8–12°C and water temperatures of 7–9°C. Spawning ponds are usually shallow (0.3–0.5 m, up to a maximum of 0.7–0.9 m). Active frogs and their clutches are found in spring in water bodies at air temperatures of 11–16°C and water temperatures of 12–15°C. On land, frogs were observed at air temperatures of 21–24°C in spring and 14–23°C in autumn. The salinity and pH of the spawning water bodies showed a relatively wide range of values from 1.08 to 6.7‰ and 6.6 to 8.8, respectively.

Mating is extensive, short, and eggs are numerous, presented in large portions from different pairs (see: Fig. 4). Males make loud gurgling sounds during mating, which makes it possible to detect them by ear. After laying eggs, the frogs quickly leave the waterbodies. Active spawning of *R. arvalis* can last only a few days. For example, on 5 April 2013, the spawning of *R. arvalis* in the Dnipro–Oril Nature Reserve had already ended, but on 6–7 April, 200 km north (Black Forest tract), it was still ongoing. Sometimes individual males remain near the clutches for some time.

The development of *R. arvalis* (from Pravi Solontsi) was monitored for three months, from 23 March to June 2017 in the laboratory. A total of 471 eggs were collected from natural clutches. Eggs began to develop rapidly on 26 March (Fig. 5, photo 1). A few days later, 464 tadpoles emerged from the eggs, which was 98.5%. From 3 April, the tadpoles were already active and well fed (fed with boiled dandelion leaves) (Fig. 5, photo 2). The growth of larvae was rather slow, and they doubled in size in 20 days before 10 May. After 8 days (18 May), the hind limbs began to appear, and after 10–15 days (27–30 May) most tadpoles had limbs. This is approximately 60–65 days of development (Fig. 5,

Fig. 4. Clutches and breeding process of *Rana arvalis* in the steppe zone of Ukraine.
Рис. 4. Кладки та розмноження *Rana arvalis* у степовій зоні України.

photo 3). On 1–2 June, the first 20 tadpoles completed their metamorphosis, and within a week all of them had finished. The development after hatching lasted 68–78 days, and the hatching rate was 98.5% (464 tadpoles out of 471 eggs). Metamorphosis begins simultaneously and is completed within 2–3 weeks. Mortality during the metamorphosis was significant (up to 20%). The body length of fry immediately after metamorphosis was about 15 mm. Frogs that had undergone metamorphosis were returned to their natural habitat.

Ecological features, reproduction, growth and development, and other population characteristics of *R. temporaria* and *R. dalmatina* within their range are described in detail in the literature [Goncharenko 2002; Tkachenko 2007; Smirnov & Skilsky 2011; Pysanets 2014; Smirnov & Buchko 2018].

Analysis of the southern limit of brown frogs distribution in Ukraine

The analysis of the findings allowed us to outline the distribution of brown frogs in the steppe zone of Ukraine and to establish some regularities. The spatial assessment revealed a certain intrazonal pattern for *R. arvalis*, which is manifested in the penetration of the species deep into the steppe along the remnants of the wide river valleys of the Dnipro, Southern Bug, and Donets, with the presence of isolated populations in the lower reaches of the Dnipro and Danube (see Fig. 1). We can assume that this azonality is closely related to the floodplain-forest complexes that are biotopically characteristic of this species. The southernmost populations in Kherson Oblast are located near the city of Hola Prystan, the village of Pravi Solontsi, and the village of Krynky. An unconfirmed finding is known from Odesa Oblast. To the north, the spec ies is observed in forest habitats of Mykolaiv, Kirovohrad, and Dnipropetrovsk oblasts and further to the north-east in Kharkiv, Luhansk, and Donetsk oblasts.

Fig. 5. Larval stages of *Rana arvalis*: (*1*) early development; (*2*) tadpoles on day 5 to 7; (*3*) young frogs on day 60 to 65).
Рис. 5. Стадії личинкового розвитку *Rana arvalis*: (*1*) початок розвитку; (*2*) пуголовки на 5–7 добу; (*3*) молоді жаби на 60–65 добу.

The southern limit of *R. temporaria* distribution (see Fig. 2) in the steppe zone is concentrated in the middle part of the Southern Bug and Dnipro basins. This may be due to the fact that these areas have been studied in more detail. That is why we have so many record localities here, which, in our opinion, is not enough to outline the distribution boundary. At the same time, we can talk about several key southern localities where we can draw a conditional 'line'. In Mykolaiv Oblast, it is the Chychyklia River and the Gromokleya River, and further in Dnipropetrovsk Oblast, the upper reaches of the Zaporizhzhia (Dnipro) Reservoir and the Dnipro–Oril Nature Reserve.

Regarding the distribution of *R. dalmatina* in the steppe zone, according to the literature, there is only one reliable southernmost locality outside Ukraine (see Fig. 2). The distribution of *R. dalmatina* is quite specific in Ukraine and occupies its western part (Carpathian region). The steppe zone can only partially be included in the southern limit of the range in Odesa Oblast. If new finds are made, the steppe zone may include a significant part of the eastern edge of the species, particularly along the border with Moldova.

Discussion

Description of findings of brown frogs in the steppe zone of Ukraine

The distribution of brown frogs in the steppe zone of Ukraine is closely related to the floodplain forests of major rivers (Dnipro, Dnister, Danube, Donets, Southern Bug, Oril, Samara, etc.) and forests (Black Forest tract, Samara Forest).

The sharp-toothed frog lives in the forest zone (inhabiting deciduous, mixed, and pine forests), as well as in the forest-steppe and steppe zones, floodplains along river banks and even in semi-deserts, thus demonstrating wide ecological plasticity and genetic variability [Babik *et al.* 2004; Roček & Šandera 2008]. There is evidence that genetic variation is characteristic of areas corresponding to refugia [Babik *et al.* 2004], which is important given the presence of our Lower Dnipro isolated populations.

The locality of *R. arvalis* in the Dnipro floodplains near Kherson was reported by O. O. Brauner [Brauner 1906]. Finds near Antonivka, a village near Kherson, were reported by other authors [Tarashchuk 1984; Peskov *et al.* 2004; Manilo & Radchenko 2008]. The western edge of the range, which partially passes through the Netherlands, is also characterised by the presence of isolated populations [Van Delft & Creemers 2008].

The collection NMNH-z includes collections of S. Tarashchuk from the vicinity of Antonivka, where two males of *R. arvalis* were found on 2 April 1979. In the same year, on 30 August, 101 individuals of *R. arvalis* (51 males, 38 females, and 12 young) were recorded in the same area. This is a fairly large population. For two more years in a row, *R. arvalis* was found in the same floodplain forest. In April 1980, 6 adult males were found, and on 10 April 1981, 10 adults (7 females and 3 juveniles) were found. Later, *R. arvalis* was not found in Antonivka. Decrease in numbers and reduction of *R. arvalis* range is observed within the core area of its habitat [Smirnov & Skilsky 2011].

In the lower reaches of the Dnipro, in particular in the floodplains below Kherson, on the left bank islands between the Old Dnipro, Hola Prystan Spit and Chaika, and on Velykyi Potiomkinskyi Island, *R. arvalis* has been recorded annually since 1960 [Semenov 1980]. The cited author suggests that the species probably reaches the Dnipro Estuary, where floodplain habitats end and there is no salty water. We were unable to detect *R. arvalis* in these areas. It can be assumed that *R. arvalis* was indeed abundant and widespread here at that time. Since the late 1970s, the steppe zone has been subjected to significant anthropogenic impact, which has negatively affected the abundance and spatial structure of this species. Deliberate drainage and climate change, habitat fragmentation have been and remain a serious threat to the species' populations. Only hard-to-reach, constantly flooded areas have allowed some populations to survive at the southern edge of the range. Intensive searches along the entire Dnipro riverbed should be continued to verify whether these are indeed local populations or whether they are likely to inhabit all floodplain habitats of the Dnipro in the steppe zone of Ukraine, which is necessary to further substantiate the intrazonality of the southern distribution boundary.

Populations of *R. arvalis* have been repeatedly recorded in Dnipropetrovsk Oblast. We were able to confirm the presence of *R. arvalis* in the Dnipro–Oril Nature Reserve and found new localities near Dnipro City. In Mykolaiv, Donetsk, Poltava, Kharkiv, and Luhansk oblasts, we failed to find the moor frog, but according to the literature and collection materials we can talk about the current distribution of the species in the steppe zone. The most interesting locality is in Odesa Oblast. It was identified by T. I. Kotenko [1999] at the mouth of the Danube, and these findings are also referred to by Y. M. Pysanets [2014] (see Fig. 1). However, this record locality has not yet been confirmed, and it may be of important historical and biogeographical value.

The issue of determining the origin of southern populations of *R. arvalis* using modern molecular genetic methods and phylogeography remains certainly promising. For example, the reconstruction of the same phylogeography of *R. temporaria* and *R. dalmatina* showed spatial specificity. This is an early expansion of the range of *R. temporaria*, based on genetic diversity, while the same process in *R. dalmatina* is likely to be a more recent event, as evidenced by its genetic uniformity, which was manifested in extremely low heterozygosity values throughout the range, including potential refugial areas [Vences *et al.* 2013]. In contrast, *R. arvalis*, as we noted above, is characterised by genetic diversity in likely refugia [Roček & Šandera 2008]. Therefore, such contradictory data require confirmation and further research. Perhaps this is where the ancestral population with unique genetic diversity or uniformity lives, or perhaps this is where one of the mysterious refugia of the ancestral population of *R. arvalis* from the postglacial era is located.

Ecological features of brown frogs in the steppe zone

The ecological characteristics of southern brown frogs are important for understanding the general patterns of population status and development throughout the entire range. The ecological and population characteristics of *R. arvalis* in the lower reaches of the Dnipro River are described in detail by Semenov [1980]. The author notes rather high numbers of *R. arvalis* in the 1970s and 1980s. Thus, in October 1970 and 1977, 15–20 individuals of *R. arvalis* were recorded on a 100-meter strip, mainly in this year in a ratio of 1 : 6–7. Compared to our data, these are rather high numbers for the lower reaches of the Dnipro River in this particular year. The cited author notes the presence of rather distinct dark temporal spots, and does not mention an important diagnostic feature of brown frogs—the Λ–shaped spot. It should be noted that adults of this year's *Pelophylax esculentus* also have temporal spots, as we previously reported [Mykytynets & Suriadna 2007]. Such *P. esculentus* may be more abundant in these areas and difficult to identify. Unfortunately, it is not possible to compare this now, but when talking about the distribution of certain species, it is necessary to rely on several basic diagnostic features.

The population of *R. arvalis* from Antonivka, Kherson Oblast, was numerous and has been mentioned several times. The collection NMNH-z contains more than 100 specimens of this species from this location. This population was studied by a well-known herpetologist S. Tarashchuk [Tarashchuk 1984]. The cited author notes that 10 to 25 individuals were found on a 3-metre strip of a 100-metre route. According to our data, along a longer route (500 m), we found 6 (Krynky) to 10 individuals (Pravi Solontsi). More numerous *R. arvalis* spawned further north (Black Forest tract, Kirovohrad Oblast). However, in May the number of individuals decreased markedly (5 individuals per 1 km). In Dnipropetrovsk Oblast, single individuals were recorded (see cadastre to Fig. 1). *R. arvalis* is scarce in the Pobuzhzhia region [Goncharenko 2002]. It is most often found in floodplain tree and shrub communities (24–29 specimens/ha). We can assume that *R. arvalis* was indeed quite abundant, but today we observe a sharp decrease in abundance, both in the south and within the main range [Smirnov & Skilsky 2011]. However, the specifics of reproduction and development of southern populations described by us indicate their relative stability against the background of a decline in numbers since the 1960s.

As for the sex structure, S. Tarashchuk [1984] noted a ratio of males to females of about 1 : 1. In contrast, we noted that males are more common in spawning waters, and females are more common during land-based surveys. Therefore, when comparing the sex structure, we should focus on the seasonal activity of *R. arvalis*.

Many of the aquatic habitats of *R. arvalis* in the Netherlands, especially bogs, are characterised by low pH. This problem was particularly acute in the 1980s and 1990s. As a result, the water bodies are too acidic for breeding—when the pH is below 4.5, breeding is rare. For this reason, *R. arvalis* has disappeared from many habitats [Van Delft & Creemers 2008]. Therefore, controlling the pH in spawning ponds is important, and according to our studies, the lowest pH is 6.6, which indicates favourable conditions for spawning and requires special conservation measures.

Comparing the data on the development of *R. arvalis*, we can say the following. The breeding period in our observations is somewhat longer, beginning in late March, while according to the literature, mating begins in the first half of April [Tarashchuk 1984]. We also have larger clutches and a longer period of metamorphosis in laboratory conditions [Tkachenko 2007]. These results may indicate a certain plasticity of the species, which preserves and increases its resistance properties under conditions of different quality impacts. But at the same time, it can be extremely vulnerable, which is manifested in a sharp decrease in numbers and makes it impossible to even detect it in natural conditions for a long period of time.

Analysis of the southern limit of brown frogs distribution in Ukraine

The steppe zone of Ukraine is the southern edge of the wide geographic range of the genus *Rana*. The nature of the boundary is ambiguous and can be zonal, intrazonal, isolated populations, or isolated finds of individual species far from the main range. The distribution of *R. arvalis* extends from north-eastern France and northern Belgium in the west to the Southern Urals, northern Kazakhstan and Altai in the east (124°E and 60°N in Yakutia), and reaches northern China. Its distribution from south to north is equally wide, stretching from the Pannonian lowlands and corresponding latitudes in Europe and northern Kazakhstan in Asia to the northern parts of both continents (up to 69° in Finland), avoiding the Alps and higher elevations of the Carpathians and Scandinavia [Babik *et al.* 2004; Roček & Šandera 2008].

Regarding the southern limit of *R. arvalis* in Ukraine, there is no single reasonable opinion. According to Y. Pysanets, it enters the steppe zone to the mouth of the Dnipro (Hola Prystan), then to the right bank—to the village of Antonivka, then to the Southern Bug without specifying a certain locality, and the lower Danube is separately noted as an isolated population [Pysanets 2014: Fig. 19.1]. According to G. E. Goncharenko, the species reaches the Black Sea coast, but does not reach the Crimea, then spreads north to the lower reaches of the Dnipro and east through the lower reaches of the Don [Goncharenko 2002]. Opinions are somewhat contradictory regarding the 'descent' or 'ascent' of the sharp-toothed frog. Semenov [1980] notes that the southern limit of the range of *R. arvalis* runs along the Dnipro at least 20 km below Kherson (probably inhabiting the islands) and on the left bank, probably to the south-western outskirts of Hola Prystan.

Our additional findings and analysis of the literature suggest that *R. arvalis*, penetrating the steppe along river valleys, forms the azonal character of the southern limit of its distribution. Some isolates, especially those from the Dnipro River, may be relict in nature, form a series of refugia, in particular, play a key role in the formation of the fauna of southern Ukraine and are of important historical and indicator value.

The range of *R. temporaria* extends from the Pyrenees to the Urals. In the north it reaches the coast of the Barents and White seas. In the east, it reaches the Komi Republic. The distribution map of *R. temporaria* [Pysanets 2014: Fig. 20.1] is identical to that of *R. arvalis*. In this monograph, Y. Pysanets notes that the southern limit of *R. temporaria* distribution in Ukraine passes through the north of Odesa Oblast, with the map showing a southern isolated population and the species

distributed to the lower reaches of the Dnipro River in Kherson Oblast. Further north, it is found in Mykolaiv, Zaporizhzhia, central parts of Dnipropetrovsk (Dnipro–Oril Nature Reserve), and Kharkiv oblasts. The southern limit of the species in Ukraine is also mentioned in the work of G. E. Goncharenko, where it is noted that the border runs along the middle reaches of the Dnister and Dnipro [Goncharenko 2002]. She notes that *R. temporaria* is found everywhere in the Pobuzhzhia region.

In Dnipropetrovsk Oblast, *R. temporaria* is recorded for the Dnipro–Oril Nature Reserve. It is also recorded in the upper reaches of the Zaporizhzhia Reservoir (left bank, opposite Kamianske and in the valley of the Oril River from Babanivka to Mohyliv in Tsarychanka Raion), but the authors point out that there have been no new records in the last 25 years and that the species is probably extinct [Bulakhov *et al.* 2007]. The occurrence of *R. temporaria* in Zaporizhzhia Oblast requires reliable confirmation. The map of the geographic range of *R. temporaria* [Pysanets 2014: Fig. 20.1] should be treated with caution.

We cannot discuss confidently the nature of the southern limit of distribution. The findings analysed here are likely to be the most thoroughly studied areas. Here we can preliminarily talk about natural zonation for *R. temporaria* and broader habitat preferences, unlike *R. arvalis*, which is more inclined to floodplain and forest habitats. The southern limit of distribution of *R. temporaria* on the left-bank part needs to be clarified. To understand whether the species can continue to live in the steppe zone or whether it is spread northwards immediately after the middle part of the Dnipro basin.

There are currently no records of *R. dalmatina* in the steppe zone of Ukraine. Given the species' range, which occupies western Ukraine [Pysanets 2014: Fig. 18.2], the southern limit can be only partially represented here. The eastern limit will be more widely represented here if this species is found in Odesa Oblast along the border with Moldova.

According to the literature, *R. dalmatina* is gradually expanding its range in Ukraine and is found far beyond the Transcarpathian Lowland, which is the currently recognised distribution range of the species in Ukraine [Smirnov & Buchko 2018].

Given the above, we can talk about a decrease in the numbers and range of *R. arvalis*. There is a relatively stable situation with the numbers and range of *R. temporaria*, although it is necessary to monitor the status of populations and clarify the southern limit of distribution, especially in the eastern part of the steppe zone. It is important to expand the range of *R. dalmatina*, and thus increase its numbers within Ukraine, which may contribute to its wider distribution in the steppe zone.

Conclusions

The study revealed certain trends in the distribution of frogs of the genus *Rana* in the steppe zone of Ukraine, which is important for understanding the current state of populations of forest-dwelling species on the edge of their range.

The detected local breeding populations of *R. arvalis* in Kherson Oblast (Pravi Solontsi and Krynky) and the confirmed populations in Dnipropetrovsk and Kirovohrad oblasts allow us to suggest the stability of populations and their value for the biodiversity of southern Ukraine, especially on the background of the overall decline in the *R. arvalis* range.

The numbers of *R. arvalis* are low but stable. Activity and reproduction in the studied populations begin in March. They are active until the end of November. High productivity and favourable conditions for development are observed.

The distribution of *R. arvalis* is closely associated with floodplain forests of large rivers and remnants of forests. The southern limit of *R. arvalis* is probably intrazonal. The species penetrates deep into the steppe along the remnants of the wide river valleys of the Dnipro, Southern Bug, and Donets, with isolated populations in the lower reaches of the Dnipro and Danube, which may be relict in nature and form a series of refugia.

The other two species, *R. temporaria* and *R. dalmatina*, have not been confirmed, but the generalised data on the distribution of these species both in the steppe zone and on the border with the forest-steppe allowed us to assess the importance of further searches and additional research.

Given the current status of southern populations of brown frogs, their low numbers and isolation, they require regional protection along with their habitats.

The need to determine the origin of southern populations using molecular genetic methods is intended to consider the historical value of brown frog populations in the steppe zone of Ukraine.

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