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Research Article

Post-invasion spread of Chinese sleeper (*Perccottus glenii*) in the Lower Danube drainage (Budjak region of Ukraine)

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Abstract

The Chinese sleeper *Perccottus glenii* (Actinopterygii: Odontobutidae), is an invasive alien species (IAS) of fish that has been included into the European IAS list of Union Concern. The present study aims to describe the current distribution of Chinese sleeper in the Ukrainian Lower Danube drainage. Fish assemblage monitoring took place at 70 localities inside the Budjak historical region in south-western Ukraine over a twenty-year period (2001–2021), with Chinese sleeper occurrence confirmed at 24 localities. Our data not only confirm the spread of invasive Chinese sleeper within the Budjak region, but also outside the Danube basin, including the Sasyk Lagoon (2 ind.) and several overgrown banks along sea foreshore. Considering that Chinese sleeper are presently found in waterbodies intensively used as fisheries, in river forefront marshes and at one site outside the Danube basin, it is highly plausible that further expansion of this fish species will be observed in the near future.

Key words: invasive alien species, IAS of European concern list, odontobutids, non-native range expansion

Introduction

Invasive Alien Species (IAS) are a growing problem worldwide, with many having been shown to have unexpected and unwanted impacts on native biodiversity and ecosystem services (chemical, physical or structural impact on ecosystems), local economies and/or human health (Mooney and Hobbs 2000; Roy et al. 2019; Gallardo et al. 2019). The main adverse impacts on native species noted to date are competition, predation, hybridisation, disease transmission, parasitism, poisoning/toxicity, bio-fouling, grazing/herbivory/browsing and negative interactions with other IAS (Blackburn et al. 2014; Roy et al. 2015). The European Strategy on IAS (Council of Europe 2003), finalised in 2003, utilises four main criteria to derive a ranked list of IAS within the European Union, i.e. likelihood of arrival, likelihood of establishment, likelihood of spread post-invasion and potential impact on biodiversity (Roy et al. 2015). The IAS list of Union Concern is updated every two years based on risk assessments proposed by the EU member states (European Commission 2014).

Recent studies have confirmed that the estuaries of large European rivers are common sites of biological introduction and invasion (Paavola et al. 2005; Nehring 2006; Leuven et al. 2017; Vilizzi et al. 2021). This is especially true of the Elbe Estuary, with 31 alien species, and the Rhine delta zone, with 42 species (Nehring 2006; Leuven et al. 2009). This is also true for the lower reaches of the River Danube and its delta (Bănăduc et al. 2016; Alexandrov et al. 2007), with 20 non-indigenous fish species registered within the Danube basin, six of which have been found in the delta zone (Zorić et al. 2014; Năstase et al. 2017). These aquatic biological invasions have been shown to have a strong negative impact on natural protected areas within the Danube delta zone (Kahl 2018). The Budjak region of Ukraine is situated in the southern part of Bessarabia, part of the interfluve between two of Europe's largest river basins, the Danube (with the Prut as a main tributary) and the Dniester. Owing to human activities and water management within the region, a number of aquatic IAS have increased their ranges by spreading from one basin to the other, starting with the pumpkinseed sunfish *Lepomis gibbosus* (L., 1758), which spread from the Danube to the Dniester at the beginning of the 20th century (Pavlov and Bilko 1962; Zambriborshch and Shumilo 1953). Among the best known of these IAS is the Chinese pond mussel, *Sinanodonta woodiana* (I. Lea, 1834) (Bivalvia, Unionidae), which was first recorded in the Danube-Sasyk Canal in 1999 (Yurishinets and Korniushin 2001) and is now common throughout the Dniester basin (Munjiu et al. 2020). Another, the oriental river prawn, *Macrobrachium nipponense* (De Haan, 1849) (Decapoda: Palaemonidae), was first introduced into the Cuciurgan Reservoir in the Dniester basin, on the border between Ukraine and Moldova (Son et al. 2013), and is now recorded at several localities in the Danube delta (Zhmud et al. 2021; Yuryshynets et al. 2022).

An important invasive fish species included in the IAS list of Union Concern (European Commission 2017) is the Chinese sleeper, *Perccottus glenii* Dybowski, 1877 (Actinopterygii: Odontobutidae), which has previously been shown to have a strong negative impact on the aquatic ecosystems it has invaded, including competition with local fauna, predation and eventual formation of monospecific communities (Reshetnikov 2013; Rakauskas et al. 2016; Pupina et al. 2018; Kutsokon et al. 2021b). Generally speaking, the Chinese sleeper avoids lotic habitats, instead showing a preference for lentic waters, i.e. small ponds and marshes (Berg 1949), particularly those with heavy vegetation cover (Reshetnikov 2013; Rakauskas et al. 2016; Kutsokon 2017), with adults preferring deeper areas and young tending to inhabit shallow zones (Berg 1949). This species shows a wide tolerance, being resistant to oxygen deficiency, moderate chemical pollution and significant temperature drops (Chai et al. 2020), and can survive in water bodies of low-productivity (Kutsokon et al. 2021b), not least due to its wide-spectrum, omnivorous feeding style, with a general preference shown for zooplankton in juvenile stages switching to benthos and smaller fish (including cases of cannibalism) in later stages (Berg 1949; Koščo et al. 2008; Rau et al. 2017).

The Chinese sleeper is native to China, northwestern Korea, Far Eastern Russia and northern Sakhalin (Mori 1936; Bogutskaya et al. 2008), but has spread in Central and Eastern Europe, primarily through transportation of aquacultural fish stocks since 1970–1971 (Reshetnikov 2004; Kutsokon 2017). The species' range in the Danube is presently restricted to the Middle and Lower reaches, including the river basins of some tributaries, e.g. the Tisza river basin (Koščo et al. 2003; Jurajda et al. 2006; Hegediš et al. 2007; Ćaleta et al. 2011; Covaciu-Marcov et al. 2011, 2017; Kutsokon 2017). There is also an isolated population in the Upper Danube basin in Germany, where it inhabits several lakes and streams in the Naab river basin (north tributary of the Danube), though it remains absent in the German sector of the Danube (Nehring and Steinhof 2015). The existing Danube basin Chinese sleeper populations are related to the Carpathian population, first introduced from China to the Upper Dniester basin in Ukraine (Grabowska et al. 2020).

The Carpathian Chinese sleeper population inhabits water courses of Central and (partly) Eastern Europe (Grabowska et al. 2020), including water bodies in western Ukraine and the Middle Dnieper and Southern Bug basins (Kutsokon 2017). In south-western Ukraine, it is not yet found in the River Tyligul and tributaries of the Southern Bug, Kodyma and Savranka (Kutsokon et al. 2021a), though it is present in other parts of the Dniester and Southern Bug basins, with high population numbers at some localities (Kutsokon 2017; Moshu and Chiriac 2011). The Chinese sleeper was first registered in the Romanian section of the Danube delta, with individuals found in the Ukrainian stretch soon after (Năstase 2007; Kvach 2012). Outside of the delta, the species has also been registered in Lake Kartal, at sites along the main stretch of the Ukrainian Danube and in the Moldavian part of Lake Kahul (Moshu and Chiriac 2011; Kvach et al. 2020). In recent years, the species has also spread within the Ukrainian delta zone and is now also recorded in the Dnieper Estuary (Kutsokon 2017; Kvach et al. 2016) and in the brackish Gulf of Yahorlyk in the Black Sea (Kvach et al. 2021).

It is quite likely that the Danube delta region, including lakes within the delta system, could act as a source of Chinese sleeper invasion into neighbouring aquatic systems, as has previously been shown for the pumpkinseed sunfish, which was first recorded in the Ukrainian part of the Danube delta at the beginning of 20th century (Pavlov and Bilko 1962) and went on to form the source population for invasion of the River Dniester (Zambriborshch and Shumilo 1953). The aim of this study, therefore, was to provide an accurate assessment of the current distribution of Chinese sleeper within the Lower Ukrainian Danube region, thereby providing a solid baseline for future invasion studies.

Table 1. Chinese sleeper (*Perccottus glenii*) sampling localities in the Budjak region of Ukraine.

Drainage	Water system	Waterbody	Coordinates	Sampling date/period
River Danube	Kahul system	Lake Kahul	45.387191, 28.440749; 45.380525, 28.409547	2019
		Viketa Canal	51.262260, 30.264536	2019
	Kartal system	Lake Kartal	45.312319, 28.501303; 45.288267, 28.519610; 45.298047, 28.504401; 45.306262, 28.507050; 45.288381, 28.521044	2019; 2019; 2020; 2021
		Fisheries ponds	45.291886, 28.480744	2019-2021
		Prorva Canal	45.290455, 28.464393	2021
		Canal between Kyshovata River and Danube	45.269482, 28.527310	2021
	Yalpuh-Kuhurlui system	Lake Yalpuh	45.351735, 28.669589; 45.345554, 28.675258	2019–2020
		Lake Kuhurlui	45.262318, 28.644076; 45.329600, 28.651655	2001; 2019
		Repida Canal	45.333020, 28.776909	2009
		Polder Canal	45.253721, 28.631740; 45.261901, 28.592700; 45.256378, 28.597220; 45.255472, 28.593950; 45.271544, 28.563499; 45.265950, 28.560956; 45.263936, 28.567389; 45.263526, 28.583486	2001
	Lake Katlabuh	Canal to fisheries ponds	45.339978, 28.952689	2020
	Lake Kytay	Kyrhyzh-Kytay River	46.004740, 29.052210	2009
	Lower Danube	Lower Danube	45.246555, 28.632922	2001
	Danube Delta	Tataru Island	45.343855, 28.994607	2015
		Kiliya Branch	45.353618, 28.988335	2015
		Yermakiv Island	45.428250, 29.467972	2020
		Bazarchuk Bay	45.417331, 29.563387; 45.418194, 29.558194	2017; 2020
		Canals in Vylkove	45.408806, 29.583336; 45.414917, 29.585118; 45.414066, 29.585097; 45.414406, 29.586296; 45.408632, 29.583742; 45.396360, 29.613681; 45.414406, 29.586296; 45.414917, 29.585118; 45.393955, 29.610915	2011; 2012; 2013; 2017; 2019; 2020; 2020; 2021
		Pond in Vylkove	45.400233, 29.589574	2013
		Ochakivske Branch	45.390083, 29.598579	2011; 2012; 2013; 2017
		Solonyi Kut	45.472539, 29.651048	2021
		Ankudinove Branch	45.411164, 29.755557; 45.412002, 29.760428; 45.407076, 29.762446	2021
		Novostambulske Branch	45.337402, 29.780972; 45.335265, 29.768431; 45.340681, 29.757446; 45.340981, 29.757610; 45.339561, 29.769068	2020; 2021
		Skhidne Branch	45.304253, 29.752112; 45.304015, 29.750373; 45.304126, 29.753417; 45.304352, 29.753723	2020; 2021;
		Anakin Kut	45.281137, 29.733213	2021
		Tsyhanka Branch	45.246654, 29.741176	2020
		Starostambulske Branch	45.221745, 29.752027; 45.223311, 29.730146	2020
Sasyk Lagoon	Sasyk Lagoon	Sasyk Lagoon	45.414361, 29.584917; 45.540616, 29.653965; 45.414361, 29.584917; 45.792694, 29.690769	2013; 2018
		Kohylnyk River	46.283897, 29.034407	2009
River Dniester	Dniester delta	Lower Dniester	46.429498, 30.171425	2007
		Turunchuk River	46.487642, 30.145060	2013
	Dniester Estuary	Dniester Estuary	46,275510, 30,358162; 46.328607, 30.103166	2011

Materials and methods

Fish assemblage monitoring took place inside the Budjak historical region in south-western Ukraine and was based on data collected over a twenty-year period, from 2001 to the present (Table 1). The study region has a climate characterised as temperate semi-arid with sea influence, having an average annual temperature of 11 °C (22 °C in July and −1 °C in January), with 340–450 mm average annual precipitation. The southern part of the

Budjak region is situated in the drainage of the Kilia Branch of the Danube, which transports approximately 58% of the water and sediment discharge of the Danube at this point (Bondar and Panin 2001). The river drainage has a pH of 7.35–8.28, a salinity of 0.21–0.23‰, and the summer oxygen content ranges from 5.59–10.14 mg/l. The bottom sediments are manly grey silt, with areas of sand, silty sand and, in Anakin Kut Bay, black silt (Lyashenko and Zorina-Sakharova 2012). The Danube lakes include the Kahul, Kartal, Yalpuh, Kuhurlui, Katlabuh and Kytai, which connect to the Kilia Branch via canals. The lakes are generally shallow, with a mean depth of 0.7–2.2 m and maximum depths up to 7.0 m (Shvebs and Igoshin 2003), with oligohaline waters (0.5–1.0‰), the salinity dependant on Danube water inflow. The Sasyk Lagoon is a natural water body situated at the confluence of two steppe rivers, the Kohylnyk and Sarata. Prior to 1978, the lagoon was a shallow brackish water basin (av. depth 1.5–2.0 m) connected to the Black Sea; however, in the 1980s, the lagoon was separated from the Black Sea by a dam and influx of water from the Danube via an artificial canal gradually reduced its salinity (Kharchenko et al. 1990). The quality of the reservoir's aquatic environment and its water balance is now highly dependent on the operation of an irrigation system and, especially, on inflow of freshwater through the Danube-Sasyk Canal (Kharchenko et al. 1990; Vasenko and Lungu 2005), which keeps total mineralisation levels in the reservoir relatively steady at around 1.5–2.0‰ (Vasenko and Lungu 2005). The sediments are manly silty sands, with shells in the southern part (Liashenko and Zorina-Sakharova 2017).

A total of 70 freshwater localities were examined for presence of Chinese sleeper, including sites along the main arm of the Danube, within the Danube delta zone, in lakes along the Lower Danube, canals and rivers of the Lower Danube basin and the Dniester delta and its estuary (Table 1). In each case, the sampling localities were mapped using QGis software, the geographic coordinates noted, and all fish caught at the site identified and counted. Later, the data obtained were divided into three groups depending on sampling period, the first including reports prior to the first official registration of Chinese sleeper in 2011 (see Kvach 2012), allowing a more accurate estimation of first date of introduction, and two five-year groups representing reports after 2011, i.e. 2012–2016 and 2017–2021. At three localities, monitoring was undertaken over longer periods, i.e. a fisheries pond near Lake Kartal (45.291886; 28.480744; annual sampling between 2012 and 2021); a canal at Vylkove, near the Danube Biosphere Reserve Office (45.408806; 29.583336; annually 2011–2021); and the Ochakivske branch river (45.390083; 29.598579; annually 2011–2013 and 2017).

In all cases, sampling sites were categorised under the EUNIS classification system (Davies et al. 2004), e.g. C1.3: permanent eutrophic lakes, ponds and pools; C2.3: permanent non-tidal, smooth-flowing watercourses; anthropogenic water bodies (artificial canals, gravel pits and aquaculture ponds)

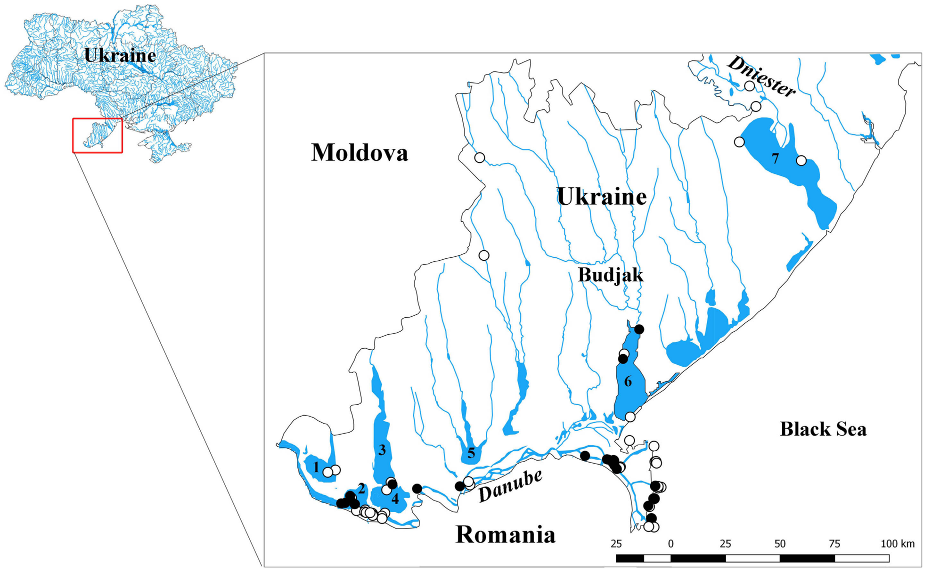

Figure 1. Map of Ukraine, with Chinese sleeper (*Perccottus glenii*) sampling localities in the Budjak region indicated. Confirmed catches are marked as black spots and absence as white spots. Individual water bodies are marked with numbers: 1 = Lake Kahul, 2 = Lake Kartal, 3 = Lake Yalpuh, 4 = Lake Kuhurlui, 5 = Lake Katlabuh, 6 = Sasyk Lagoon, 7 = Dniester Estuary.

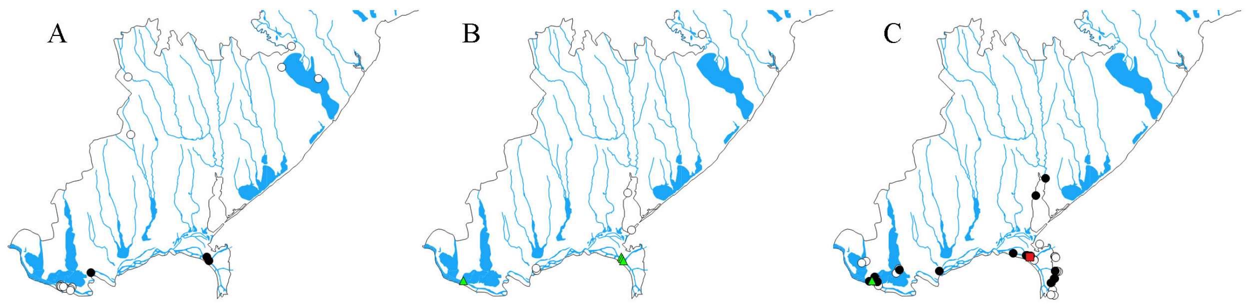

Figure 2. Confirmed findings of Chinese sleeper (*Perccottus glenii*) A) before 2011, B) 2012–2016, C) 2017–2021; white spots = absence, black spots = sporadic findings, green triangles = common, red square = numerous (see Figure 1 for definition).

J5.3: highly artificial non-saline standing waters; J5.4: highly artificial non-saline running waters. A range of sampling gear was used during sampling, to ensure the most efficient sampling of individual sites. Exploration samples of possible habitats were obtained using a fishing drag equipped with a 10-mm mesh net towed behind a motor boat (up to 5 km/h, 50–100 m per sample), while 6-mm mesh dipnets were used along shallow beaches and at depths up to 1.2 m from boats at vegetated localities. A 6-mm mesh, 8-m beach seine was used to trawling a minimum of 100 m along shallow beaches without vegetation. In lakes, sampling was restricted to multimesh 6.25–55 mm mesh gillnets (1.5 m high, 30 m long), using European Standard methodology EN 14757:2015 (European Standard 2015), with 3–6 mm mesh fyke-nets also used at localities free of vegetation, both gill-nets and fyke-nets being left for 12 hours overnight.

Results

Chinese sleeper occurrence was confirmed at 24 of the 70 localities examined (Figures 1, 2), including three localities in the Lake Kartal system,

Table 2. Chinese sleeper (*Perccottus glenii*) registration localities in the Budjak region of Ukraine. Fish density represented as numerous = > 100 ind, common = 10–100 ind, and sporadic = < 10 ind.

Water system	Waterbody	Fish density
Lake Kartal	Lake Kartal	numerous
	Fisheries ponds	numerous
	Prorva Canal	sporadic
Lakes Yalpuh and Kuhurlui	Lake Yalpuh	sporadic
	Repida Canal	sporadic
Lake Katlabuh	Canal to ponds	sporadic
Danube Delta	Yermakiv Island	sporadic
	Bazarchuk Bay	common
	Canals in Vylkove	numerous
	Pond in Vylkove	common
	Ochakivske Branch	common
	Novostambulske Branch	sporadic
	Skhidne Branch	sporadic
	Anakin Kut	sporadic
Sasyk Lagoon	Northern part	sporadic
	Western part	sporadic

two in the Yalpuh-Kuhurlui system, one in the Lake Katlabuh system, eight sites in the Danube delta and two in the Sasyk Lagoon (Table 2). Forty-six sites had no Chinese sleeper occurrence, including sites in the Dniester delta system and along the rivers Kyrhyzh-Kytai and Kohylnyk. At two of the long-term monitoring sites (fisheries pond near Lake Kartal, Vylkove canal near the Danube Biosphere Reserve Office), Chinese sleeper populations remained high (more than 100 ind.) during each survey year (Table 2), while numbers increased from sporadic in 2011 to common in 2012–2013/2017 on the Ochakivske branch. At no locality were numbers observed declining or populations disappearing over the study period.

At three study localities, the Chinese sleeper had been registered prior to 2012, with one report from the Ripida Canal (connecting Lake Kuhurlui with the Danube) in 2009 and two further reports from 2011, one from canals in Vylkove and one from the Ochakivske Branch. Between 2012 and 2016, the species was also recorded in aquacultural ponds along the southern shore of Lake Kartal, as well as canals and a pond in the City of Vylkove. Over the last five years, the species has been registered in Lake Kartal and the Prorva Canal (connecting Lake Kartal with the Danube), Lake Yalpuh, a canal connecting Lake Katlabuh with aquacultural ponds, and several localities in the Danube delta, i.e. Yermakiv Island, Bazarchuk Bay, the Novostambulske and Skhidne branches, Anakin Kut and the Sasyk Lagoon.

Discussion

In this study, we compared data gathered over the past twenty years to examine the spread of invasive Chinese sleeper along the lower reaches of the Ukrainian Danube. Based on the analysis of observations dating back to 2001, we can now confirm the first finding of Chinese sleeper in the Ukrainian part of the Danube basin as 01.09.2009 in the Repida Canal, a date two years earlier than the official registering of the species in canals around Vylkove in 2011 (Kvach 2012). The Repida Canal is situated ca. 30 km from Lake Fortuna, where the fish was first registered for the Danube delta in 2007 (Năstase 2007). Somewhat surprisingly, we found clear evidence that the species is spreading outside of the Danube basin, with the presence of Chinese sleeper confirmed at several localities along the Black Sea shore, along the overgrown banks of the Novostambulske and Skhidne branches and in Anakin Kut Bay. This, along with its presence in the Gulf of Yahorlyk (Kvach et al. 2021), not only confirms the species' ability to colonise brackish and salt waters, but also raises the potential of further spread from the Danube basin into other rivers emptying onto the sea coast. The only confirmed registration outside the Danube basin was in the Sasyk Lagoon, where the species was found sporadically at two sites on the northern and western shores in those areas with lowest mineralisation levels (Ivanova 2021), i.e. in the upper part, close to the river inflow, and the western part, close to the mouth of the Danube-Sasyk Canal. While not confirmed, it is likely that the fish were introduced into the lagoon through the canal. This limited distribution within the oligohaline lagoon suggests that the species is living under pessimum conditions, though this is unlikely to be due to salinity or hypoxia as the Chinese sleeper is highly tolerant to these factors (Ruchin et al. 2004; Chai et al. 2020). Instead, it is more likely that these sporadic findings reflect the early occurrence of the species in the lagoon. Furthermore, it is possible that further spread of the species further north of the Danube delta is being limited by predation, owing to the common occurrence of sander (*Sander lucioperca* (L., 1758)), one of the main commercial component in the lagoon fisheries (Yurasov and Kuzmina 2017), or perhaps a lack of suitable habitat, as sandy and clay bottoms tend to prevail in the lagoon. Indeed, the different habitat types found throughout the study range are likely to be the main factor dictating differences in the numbers of Chinese sleeper at the different study locations. Thus, in muddy freshwater lakes and canals, fish density tended to be higher, with the species "common" or "numerous" in sites such as Lake Kartal or the canals in Vylkove. While the species was also common on the Ochakiv branch, numbers were somewhat lower along several other branches of the delta (e.g. Novostambulske, Skhidne), though these probably represent newer populations. Sporadic findings were also registered in delta bays (e.g. Solonyi Kut, Anakin), which lack muddy bottoms (usually the preferred habitat) and have unstable salinity levels. As juvenile Chinese sleeper are tolerant to salinity levels up to 10‰ (Ruchin et al. 2004), however, this is unlikely to be the main factor limiting their further spread; instead, once again, these are likely to represent early colonisers in these habitats.

Historically, the first introductions of Chinese sleeper are likely to have been the result of human activities, with later spread also occurring through local transfer by anglers, aquacultural "hitchhiking" (Kutsokon 2017), or releases from aquariums (Reshetnikov 2004, 2013; Kutsokon 2017), with its occurrence in the Sasyk Lagoon almost certainly the result of human transfer. On the other hand, there is also clear evidence of "natural" post-invasion spread in the Budjak region, i.e. through natural range expansion via the riverine flow of the Danube and related water courses. As in its native range, and some other parts of its non-native range, the Chinese sleeper in the Budjak region tends to prefer slow flowing or lentic waterbodies with well-developed aquatic vegetation (Koščo et al. 2003; Reshetnikov 2013; Kutsokon 2017), habitats that were largely supplied by artificial canals in this study. Many of the lakes in the Budjak region of the Lower Danube basin (e.g. lakes Kartal, Yalpuh, Kuhurlui and Katlabuh) are connected to the Danube by numerous canals, as is Sasyk Lagoon, and these canals have all acted as routes of introduction. In the Danube delta itself, the Chinese sleeper has also been recorded in small bays and marshes overgrown with aquatic plants, away from the main flows of the Danube branches. The Chinese sleeper is currently absent from the northern part of the study area, as well as the lower reaches of the Dniester and the South Podolia region of Ukraine (Kutsokon et al. 2021a). It is possible that the further spread of this species is currently being constrained by the relative lack of available waterbodies in the region, with many of the water basins becoming isolated in summer as water levels drop in summer.

As the Chinese sleeper has now been found in waterbodies subject to intensive fisheries activity (e.g. the Yalpuh-Kuhurlui lake system and Katlabuh lake), along river forefront marshes (e.g. the Novostambulske and Skhidne branches, Anakin Kut Bay) and in the Sasyk Lagoon outside the Danube basin, it is quite plausible that further expansion of the species will be observed in the very near future. As the species is known to have expanded its range naturally along artificial waterways and as an aquacultural "hitchhiker", we suggest that further monitoring of such waterbodies is needed in southern Ukraine in order to control and manage future invasion processes.

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