

Research Article

Caspian invaders vs. Ponto-Caspian locals – range expansion of invasive macroinvertebrates from the Volga Basin results in high biological pollution of the Lower Don River

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Abstract

Until the 2000s, faunal exchange between endemic faunas of the Caspian Sea basin and the Azov-Black Sea (aka Pontic) basin was asymmetrical, with fauna heading towards the Volga Delta and Caspian Sea from or via the Black and Azov Seas and little exchange in the opposite direction. This study is based on a hydrobiological survey of the Don River Basin conducted in August 2011 and reflects a period when three new Caspian invaders (*Dikerogammarus caspius*, *Adacna glabra*, and *Theodoxus major*) were already widely distributed in the study area. At the time of the expedition, of all the invasive species present, these three caused the greatest impact on local species and communities in the Don Basin. The greatest biological contamination was found in areas which had already had relatively favorable conditions for existing resident Ponto-Caspian communities. In contrast, those stations devoid of invaders were those under the most severe anthropogenic pressure or subject to estuarine conditions. This pattern was confirmed by biocontamination indices being significantly correlated with standard water quality indices. The situation in the studied area, involving both the Azov-Caspian exchange via the Volga-Don Canal and the eastward invasion of exotic species, such as *Ferrissia californica* and *Potamopyrgus antipodarum*, clearly identifies a single Ponto-Caspian Invasion Corridor that connects all three marine basins of the Ponto-Caspian region. The importance of recognising and accounting for regional differences when considering the monitoring of aquatic invasions is discussed.

Key words: alien species, biocontamination, biological invasion risk, Lower Don, Ponto-Caspian area, Volga-Don Canal

Introduction

During the Pliocene-Quaternary, the Azov-Black Sea basin and Caspian Sea basin were located immediately adjacent to one another and characterized

by similar ecological conditions and recurrent connections/isolations (Krijgsman et al. 2019). For many decades, the dominant “Caspian theory” claimed that the Azov-Black Sea relict fauna was only a depleted “sub-set” of the Caspian fauna, which had invaded the Azov-Black Sea region (Mordukhay-Boltovskoy 1960). However, it subsequently became clear that the evolution of relict faunas in the Ponto-Caspian Region was more complicated than previously supposed, with molecular biogeography studies revealing consecutive vicariance and gene flow events between the two basins, for a range of animal taxa over millions of years (Cristescu et al. 2004; Cristescu and Herbert 2005; Audzijonyte et al. 2006; Gelembiuk et al. 2006; Wilke et al. 2007).

It was assumed that in recent decades man-made connections would have enhanced the mutual faunal exchange of endemic faunas between the two basins. However, until the 2000s, this process has been strongly asymmetrical, with fauna invading the Volga Delta and Caspian Sea from or via the Black and Azov seas (Grigorovich et al. 2003) and little exchange in the opposite direction. Similarly, until the 2000s, there were no known invasions of Caspian basin macroinvertebrate endemics into the Black Sea basin (Grigorovich et al. 2002). This was related to the fact that only fauna from the Black Sea basin were used for experiments that preceded large-scale government programs aimed at deliberately introducing Ponto-Caspian macroinvertebrates into other areas of the former USSR. Later, the Black Sea estuaries and less frequently the Don Delta, were the sources for numerous intentional introductions of gammarids, mysids, and polychaetes into artificial dam reservoirs on the Dnieper and Pripyat rivers, while in contrast the Caspian Sea and Volga Basin were never used as sources (Karpevich 1968). Despite this, since the 2000s, information on the expansion of species from the Caspian Sea and Volga Delta both upstream to the Volga Reservoirs and to the Azov Sea Basin has started to accumulate (Shokhin et al. 2006; Lubina and Sayapin 2008; Nabozhenko 2008; Sonina and Filinova 2011; Zinchenko and Kurina 2011).

Prior to construction of the Tsimlyansk Reservoir, the rapids of the Tsimlyansk river-bend restrained the upstream movement of Ponto-Caspian species (Mordukhay-Boltovskoy 1960). Subsequently, construction of this reservoir and other reservoirs in the Manych sub-basin facilitated the range expansion of the relict communities consisting mainly of Ponto-Caspian species. The entire lengths and hydrological regimes of many large Ponto-Caspian rivers (Volga, Danube, Dnieper and Dniestr etc.) and their major tributaries, have been transformed by cascades of dams, but this is in contrast to the Don River where such construction has been confined solely to the lower part (Karpevich 1968). Therefore, the Lower Don River represents the first region where we can observe an expansion of Caspian invaders and their impacts on previously established, closely related Ponto-Caspian communities already resident in the region. The aim of our study

was to document, for the first time, the biological invasions of benthic macroinvertebrates caused by the Volga-Don Canal (VDC) connection of this river with the Caspian basin.

We use the ALARM Project approach to assess invasive alien species introductions via European inland waterways (Panov et al. 2009), which includes the following main components: selection of management assessment units within invasion network; assessment of “biological contamination” of the ecosystem, invasiveness and impact of alien species; and identification of main invasion gateways, corridors, and pathways of introduction.

In the present study, we define the “local” Ponto-Caspian species as those that are native to the Don River (both the endemics of the Azov-Black Sea basin and the species common to both the Azov and Caspian seas), and we define the “invaders” as those species that invaded from the Caspian Sea basin through the Volga-Don Canal.

At all stages of the analysis, we compared exotic transcontinental invaders and Caspian invaders. Known features of the interaction between Caspian invaders and related local Ponto-Caspian species were generalized. Problems of biological invasions’ monitoring in the nearby regions of potential distribution of Caspian invaders were identified and discussed.

Materials and methods

Study area

The Don River, at 1870 km long, is the fifth-longest river in Europe and discharges to the Taganrog Gulf of the Azov Sea. Its catchment area is 422 thousand km² and the valley and riverbed has a smooth longitudinal profile, which slopes gradually (mean gradient of 0.096 m/km) down to the mouth. For almost the entire length, the Don River has a developed valley with a wide floodplain, many branches (side channels with local name “erik”) and oxbow lakes. It reaches a width of 12–15 km in its lower course. Near the city of Kalach-on-Don, the valley is narrowed by spurs of the Middle-Russian and Volga uplands and in this short stretch, the floodplain is absent. The Don River valley is asymmetrical in profile, with a high and steep right margin, contrasting with a flat, low left margin. There are three terraces along the slopes of the valley, the valley bottom is filled with alluvial deposits, and the river channel itself is winding with numerous sandy shallow shoals.

For the next 130 km below the Tsimlyansk Dam, navigable water depth in the Don River is maintained by a sequence of three dam-and-ship-lock complexes: the Nikolayevsky, Konstantinovskiy, and the Kochetovskiy ship locks. The lower course of the Don River consists of five main stretches and sub-basins: the river upstream of Tsimlyansk Reservoir up to the mouth of the Ilovlya, the Tsimlyansk Reservoir, the river from the Tsimlyansk

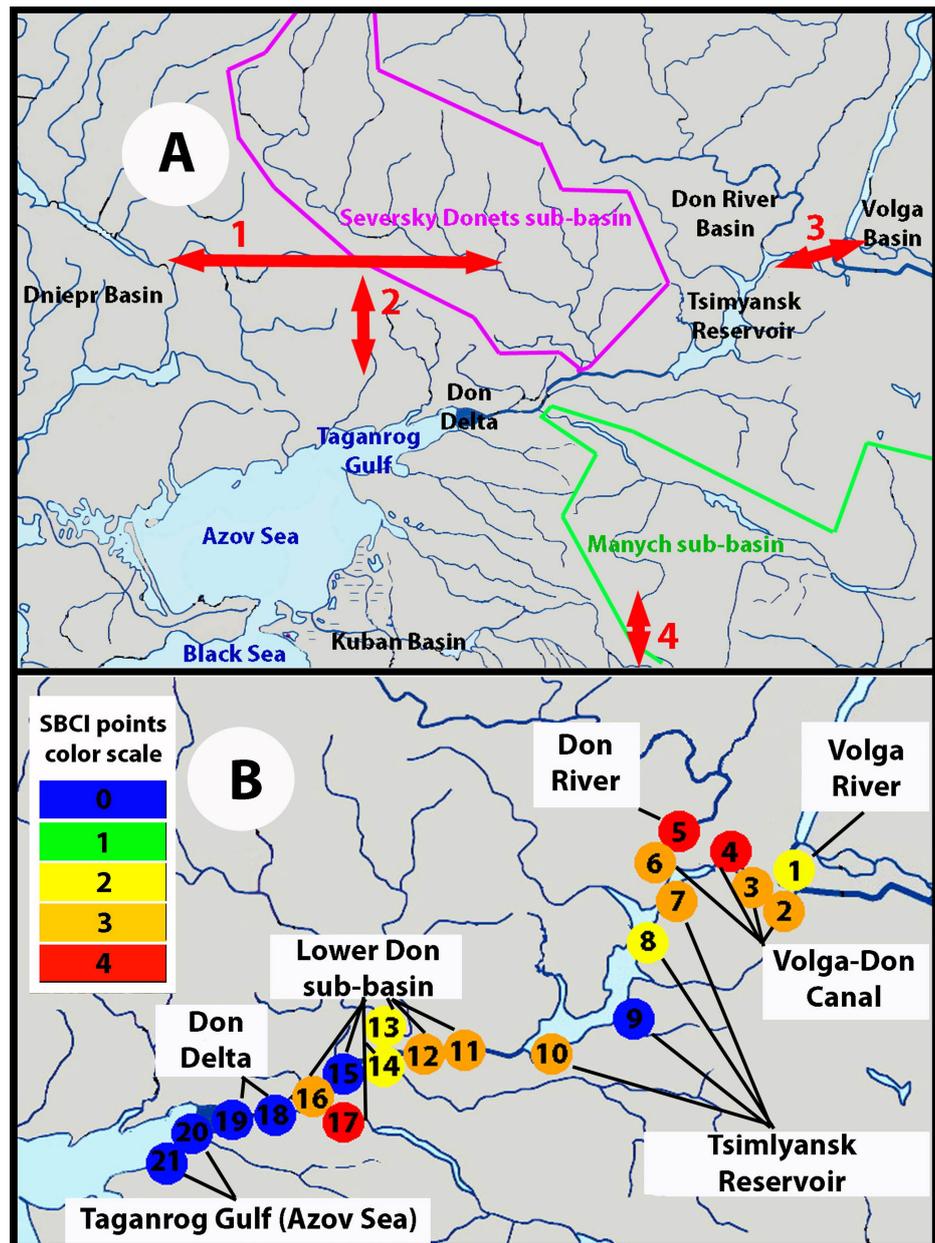


Figure 1. Study area. A: River basins and inter-basin connections (1 – Seversky Donets-Donbass Canal; 2 – Dnieper-Donbass Canal; 3 – Volga-Don Canal; 4 – Nevinnomysky Canal). B. Sample stations. The color scale of the Water Framework Directive for ecological indicators marks Site-specific Biocontamination Index (SBCI) points (blue – 0; yellow – 2; orange – 3; red – 4).

Reservoir down to the Taganrog Gulf (Don Delta), the Severskyi Donets River sub-basin, and finally the Manych River sub-basin.

Lower and estuarine parts of the Don Basin are key areas of the European invasion network and form both an “invasion gateway” for transcontinental shipping (Panov et al. 2009) and the location of several important inter-basin connections (Figure 1). Currently, the Don Basin is connected directly with the Volga basin through the Volga-Don Canal. The sub-basins of the Don River tributaries are also connected with other rivers; the Manych sub-basin is connected with Kuban by the Nevinnomysky Canal, the Seversky Donets sub-basin is connected with the Dnieper by the

Dnieper-Donbass Canal and the Kalmius sub-basin is joined to the Azov Sea by the Seversky Donets-Donbass Canal.

The current study is based predominantly on a hydrobiological survey conducted from 16–21/08/2011 at 21 sampling stations (Figure 1). Sampling sites were sub-divided into the following seven assessment units: 1) Volga River, 2) Don River upstream from the Tsimlyansk Reservoir, 3) Volga-Don Canal (VDC) including associated small reservoirs, 4) Tsimlyansk Reservoir including the mouth of the Aksay Kurmoyarsky River, 5) Lower Don River basin including Severskyi Donets and Manych mouths, 6) Don Delta, and 7) Taganrog Gulf of the Azov Sea.

Sampling methods

The Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates (AQEM) protocol was used for the description of the sampling stations (AQEM Consortium 2002). At each station, benthic samples were taken in all microhabitats that covered more than 5%. A kick-net (ISO 7828) was dragged along the shore on 5 m distance. The collector moved in zigzags to cover depths from the water edge to one meter during 15 minutes, with 4–5 replicates. Stones, flooded wood, and other large objects were inspected separately. Also, bottom sediments were washed through laboratory sieves for sediment granulometry with a metal wire mesh (mesh diameter = 2 and 1 mm).

Collected macroinvertebrates were immediately preserved in 70% ethanol in the field, and later in the laboratory, samples were sorted and macroinvertebrate taxa counted and identified to either species or family level (Supplementary material Table S1 and Table S2). The abundance of each taxon at a station was calculated as an average from all replicates. To assess the structure of macroinvertebrate communities, we calculated the relative abundances of each taxon. This comparison of relative abundances, instead of absolute abundances could potentially reduce operator error. Supplementary to our standard sampling protocol described above, we also used additional macroinvertebrate benthos sampling techniques where appropriate/necessary, such as snorkeling with hand sampling.

Indicators

The biological contamination of each station was assessed with a quantitative estimate of alien species presence (Panov et al. 2009). We used the Site-specific Biocontamination Index (SBCI), based on both taxonomic composition and relative abundance; namely the proportion of different invasive taxa as opposed to native taxa present (Richness Contamination Index) and the numerical relative abundance of invaders compared to natives (Abundance Contamination Index) in the sample (Arbačiauskas et al. 2008). The values of SBCI (ranging from 0 to 4 points) are aligned to the five-point scale of the EEC Water Framework Directive with absence of

aliens (0) regarded as the highest ecological status (Arbačiauskas et al. 2008). We used two standard biological water quality indices, the Trent Biotic Index (TBI) (Woodiwiss 1964) and the Biological Monitoring Working Party index (BMWP) (Armitage et al. 1983), to assess the relationship between biological contamination and biological water quality. Although the TBI has been superseded by more modern indices in Western Europe, it remains a main official index of water quality monitoring in Post-Soviet countries, so that it may be useful in the future for comparison with other regional studies.

The Species-specific Biological Pollution Risk (SBPR) index and the Integrated Biopollution Risk (IBPR) index are two closely-related indices of biological pollution that were used to assess invasion species risk. SBPR is based on the general assessment of invasiveness of a specific alien species according to the estimates of high risk of dispersal, establishment in a new environment, and ecological and negative socio-economic impacts. The SBPR index uses criteria of high risk of dispersal (HRD), establishment (HRE), and impact (HRI) (Panov et al. 2009).

HRD and HRE assessments are based on the invasive history of species (including other regions) and have already been published for the species mentioned in this study with the exception of invaders of Caspian origin (Panov et al. 2009). Required data for the Caspian species are given in results (new data) and subsection “Distribution of alien species”. HRI assessment, which has a narrower regional character, is based on data from subsection “Impact of alien species.” New assessments in this part of the study (local impact of individual alien species) are provided by the expert consensus of Mikhail Son and Alexander Prokin as regional researchers. The SBPR assessment is based both on the invasive history of species in other regions and on the new data provided in this study. In particular, species with SBPR = 3 are high-risk aliens and form a “black list” of aliens (1 and 2 show low and moderate levels of invasiveness, respectively).

IBPR is based on semi-quantitative data on the dispersal ability and distribution of alien species with a high level of SBPR within the “assessment unit”, with 7 units in the current study (Panov et al. 2009). The values of IBPR (in a range of 0–4 points) are adapted to the five-point scale of the EEC Water Framework Directive, with absence of aliens regarded as the zero point (from Good to Bad ecological status respectively).

As biotic index data defied normalization, we used the non-parametric Spearman rank correlation coefficient to assess the relationship between the three indices (SBCI, TBI, and BMWP). All the calculations were performed in R 3.3 statistical analysis environment.

Results

During our 2011 survey, seven alien macroinvertebrate species were recorded in the Don River Basin. The North American freshwater limpet

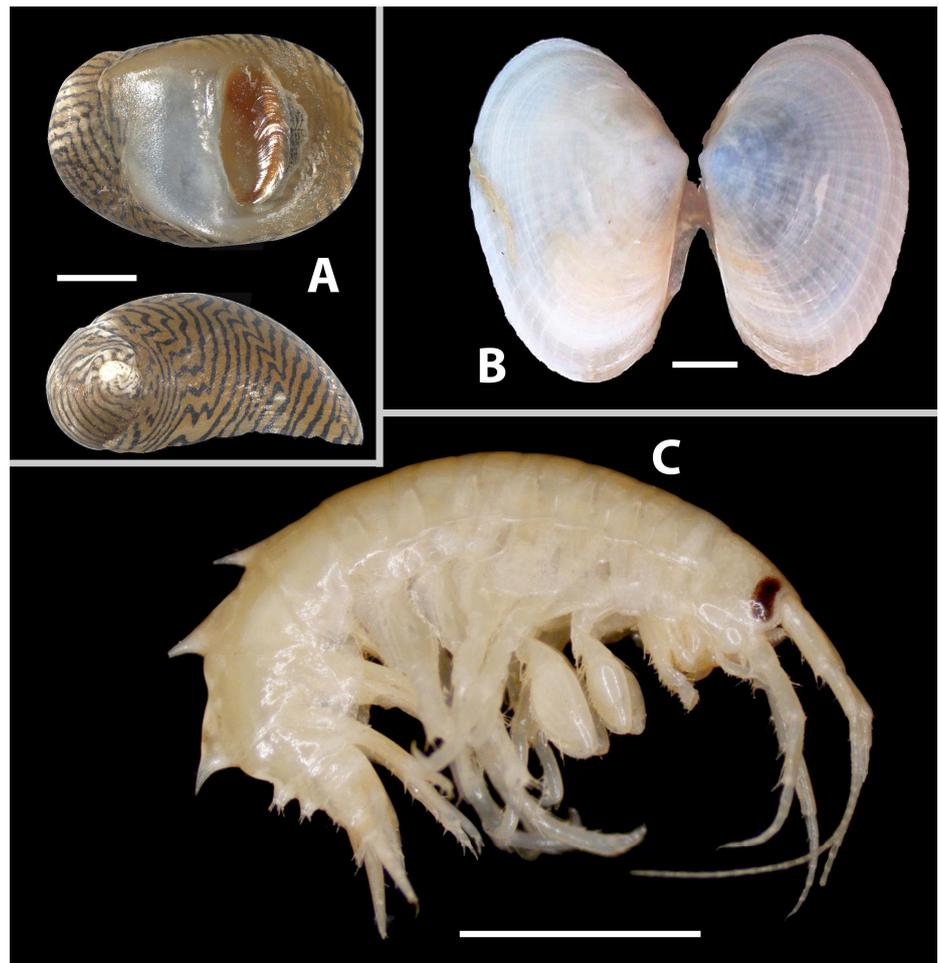


Figure 2. Species invaded the Don River from the Caspian Sea (2 mm scale): A – *Theodoxus major* (photo of A.A. Prokin); B – *Adacna glabra* (photo of M.O. Son); C – *Dikerogammarus caspius* (photo of A.A. Prokin).

Ferrissia californica (Rowell, 1863) was sampled in the VDC in the city of Volgograd (station 2). The New Zealand mud snail *Potamopyrgus antipodarum* (J.E. Gray, 1853) was sampled in thickets of *Vallisneria spiralis* L. in warm shallows of the Karpovsk Reservoir. The popular ornamental bladder snail *Physella acuta* (Draparnaud, 1805) was sampled in a thermally polluted area of a canal linked to the cooling pond of the Rostov Nuclear Power Plant (station 10). The quagga mussel *Dreissena bugensis* (Andrusov, 1897) was found during the expedition in the Volga River (although only empty shells) and in several localities downstream of the Tsimlyansk reservoir (stations 14, 15, 16, 18). Three species (Figure 2) that invaded from the Caspian Sea basin through the Volga-Don Canal are alien for the Don River. The Caspian endemic *Adacna glabra* (Ostroumoff, 1905) was recorded in the Tsimlyansk Reservoir. During the expedition, *Theodoxus major* (Issel, 1865) was sampled in the Volga River, VDC and its three associated reservoirs (Breslavsk, Karpovsk, and Varvarovsk), the Tsimlyansk Reservoir, the Lower Don, and mouth of the Manych River.

The Caspian amphipod, *Dikerogammarus caspius* (Pallas, 1771) was found in the VDC, Tsimlyansk Reservoir, and in the Don River upstream and

downstream of the Tsimlyansk Reservoir (in 12 of the 21 stations in our study). *D. caspius* was most dominant (> 50% of all the individual macroinvertebrates recovered) in the small and medium-diverse benthic communities of the Don River upstream and downstream of the Tsimlyansk Reservoir (stations 5 and 16) and in the Manych River (station 17). The species was less dominant (17.5%) in the Volga River (station 1). The most common other amphipod species in these four stations were *P. robustoides* and *O. crassus*, but *D. caspius* was by far the most dominant amphipod species present, ranging from 56.6% (station 16) to 96.8% (station 5) of all amphipods present. At the other stations where the species was present, its relative proportion of all macroinvertebrates in the benthic communities was much lower, ranging from 0.7% (station 6) to 8.1% (station 14) and, in terms of amphipod species only, ranged from 1.5% (station 13) to 18.5% (station 4). Two species, *D. bugensis* and *Lithoglyphus naticoides* (C. Pfeiffer, 1828), are alien for the Volga River (in the Don Basin, the latter species is native).

In addition, our study shows there has been a number of incursions of species native to the Pontic region into the region (i.e., alien species originating from nearby areas). For example, the bivalve species *Monodacna colorata* (Eichwald, 1829) is alien to the Tsimlyansk Reservoir, and three amphipod species are reported for the first time in the Manych River sub-basin (*Dikerogammarus villosus* Sowinsky, 1894; *Pontogammarus robustoides* Sars, 1894; and *Obesogammarus crassus* Sars, 1894). These amphipod incursions are recent, as none of the three species had been recorded during previous investigations of the same area (Lubina and Sayapin 2008).

The distribution of SBCI values is shown on the Figure 1, and more detailed results of the expedition are provided in the Table S1. The high values of the SBC Index were noted in the communities of the Breslavsk Reservoir of Volga-Don Canal, the port in Kalach-on-Don, the Manych River (4 assessment units), and at some localities of VDC, Tsimlyansk Reservoir, and Lower Don (3 assessment units). It was only in the Karpovsk Reservoir (station 6), that the high value of SBCI (4 points) was caused by a change of taxonomic composition, while in other cases, such SBCI values resulted from a high abundance of individual alien species of the Caspian origin (Figure 3).

The zones of greatest biological contamination were found under relatively favorable conditions of water quality, evaluated with biological indicators TBI and BWMP (Table 1, Figure 4). In contrast, uncontaminated stations tended to be those under the most severe pressures: proximity to urban areas (stations 16, 18 and 19), drying up and low flows (station 9), or unstable estuarine conditions of the Taganrog Gulf (stations 20 and 21). Strong positive correlations of the SBCI with both the TBI and BWMP biological water quality indices ($\rho = 0.7$, $Z = 3.17$, p -value = 0.002 for the TBI and $\rho = 0.69$, $Z = 3.10$, p -value = 0.002 for the BWMP) were shown.

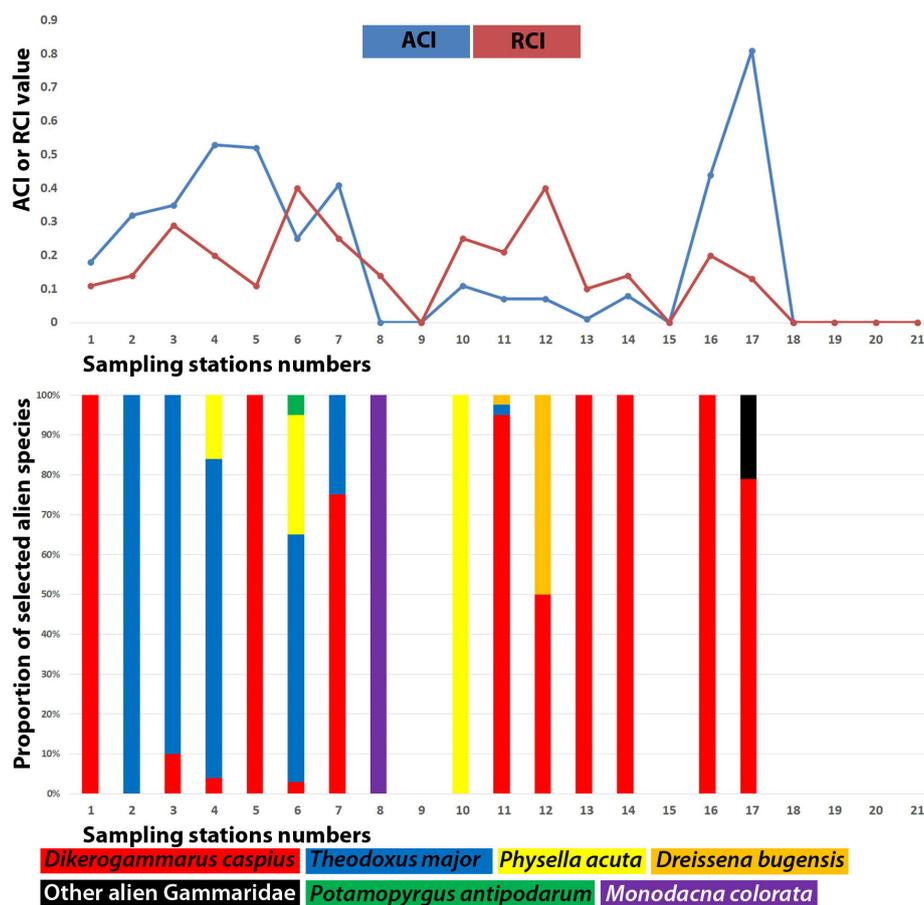


Figure 3. Biocontamination parameters (ACI: Abundance Contamination Index, RCI: Richness Contamination Index) and relative proportion of alien macroinvertebrates at all 21 sites.

Table 1. The point values of the indices: the Site-specific Biocontamination Index (SBCI), the Biological Monitoring Working Party index (BMWP), the Average Score Per Taxon (ASPT), and the Trent Biotic Index (TBI).

No	Location	SBCI	BWMP	ASPT	TBI
1	Volga River	2	20	4	5
2	VDC, Volgograd	3	18	3.6	5
3	VDC, Varvarovsk Reservoir	3	14	3.5	5
4	VDC, Breslavsk Reservoir	4	49	5.4	8
5	Kalach-on-Don, Don River, port	4	31	3.4	5
6	VDC, Karpovsk Reservoir	3	30	4.3	6
7	Tsimlyansk Reservoir near dam of VDC	3	22	4.4	5
8	Tsimlyansk Reservoir	2	9	3	5
9	Aksay Kurmoyarsky River mouth	0	7	1.8	4
10	Rostov Nuclear Power Plant	3	26	5.2	6
11	Don River	3	50	5.6	9
12	Don River	3	13	4.3	5
13	Severskyi Donets River	2	39	4.3	7
14	Don river	2	21	4.2	4
15	Don River	0	13	4.3	4
16	Don River	3	15	3.8	4
17	Manych River mouth	4	34	5.7	6
18	Don Delta	0	8	4	4
19	Don Delta	0	8	4	4
20	Taganrog Gulf	0	11	5.5	4
21	Taganrog Gulf	0	19	4.8	4

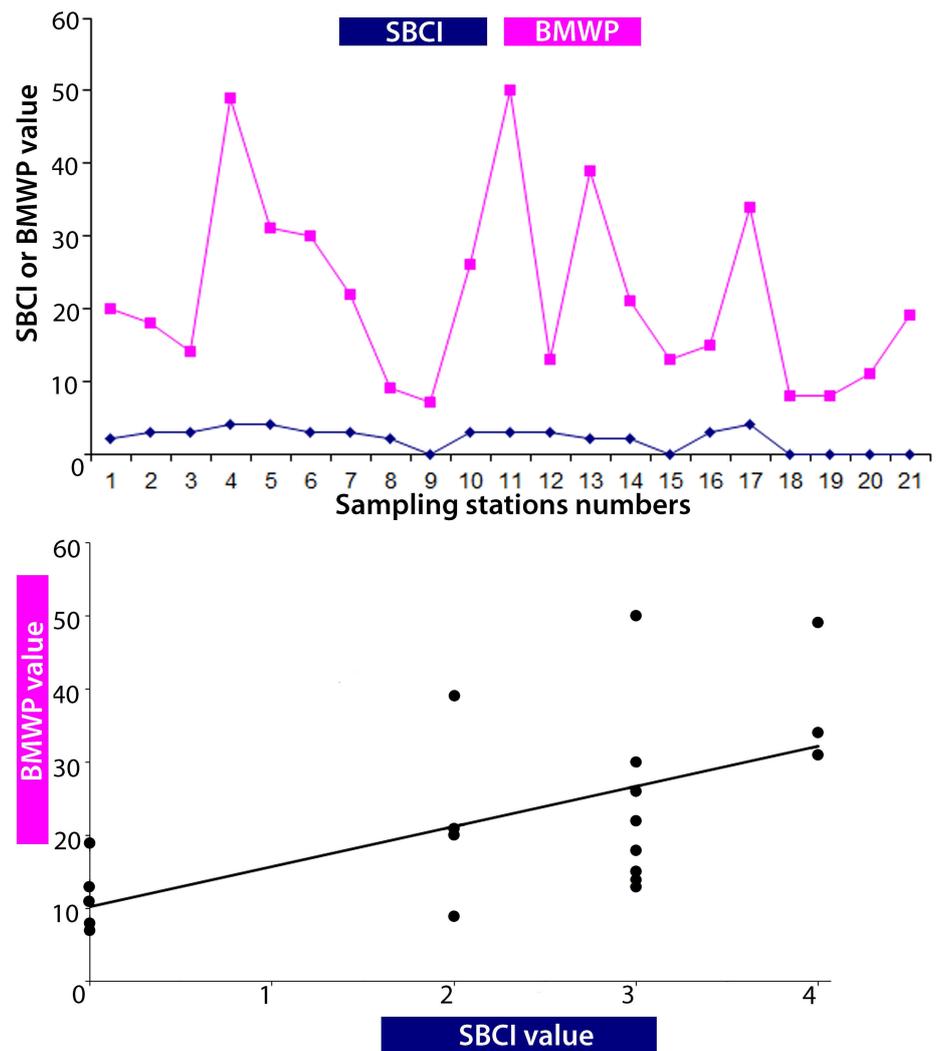


Figure 4. Relationships between Site-specific Biocontamination Index (SBCI) and the Biological Monitoring Working Party index (BMWP). Black line indicate linear fit.

Table 2. Assessments of risk level for alien species in the region (HRD – high risk of dispersal; HRE – high risk of establishing; HRI – high risk of impacts, SBPR – Species-specific Biological Pollution Risk index).

Species	HRD	HRE	HRI	SBPR	Risk classification
<i>Lithoglyphus naticoides</i> (for the Volga River Basin)	+	+	change of parasite cycles	3	high
<i>Ferrissia californica</i>	+	+		2	moderate
<i>Dreissena bugensis</i>	+	+	change of communities	3	high
<i>Theodoxus major</i>	+	+	displacement of native species	3	high
<i>Adacna glabra</i>	+		competition	3	high
<i>Dikerogammarus caspius</i>	+	+	competition	3	high
<i>Physella acuta</i>	+	+		2	moderate
<i>Potamopyrgus antipodarum</i>	+		new communities	3	high

We estimated the invasiveness risk of species alien for the Don Basin with the SBPR index (Table 2). With the exception of the Caspian invaders who have not been previously assessed, these estimates coincide with those previously made in the studies of the Dnieper, Danube, and other major European rivers (Panov et al. 2009; Semenchenko et al. 2015).

The values of IBPR for assessment units are provided in the Table 3. We did not compare it with biological water quality indices, since this index is

Table 3. Values of Integrated Biopollution Risk index (IBPR), high-risk alien species, and range of Site-specific Biocontamination Index (SBCI) at all assessment units.

Unit, stations	Alien species with high range of Species-specific Biological Pollution Risk	IBPR	SBCI
Volga River (1)	<i>Theodoxus major</i> , <i>Lithoglyphus naticoides</i> , <i>Dreissena bugensis</i>	N/A	3
VDC (2, 3, 4, 6)	<i>Dikerogammarus caspius</i> , <i>Th. major</i> , <i>Potamopygus antipodarum</i>	4	4-5
Don upstream Tsimlyansk (5)	<i>D. caspius</i>	N/A	5
Tsimlyansk Reservoir (7–10)	<i>D. caspius</i> , <i>Th. major</i> , <i>Adacna glabra</i>	4	1-4
Lower Don (11–17)	<i>D. bugensis</i> , <i>D. caspius</i> , <i>Th. major</i>	4	1-5
Don Delta (18–19)	<i>P. antipodarum</i> (according to previous expeditions)	3	1
Taganrog Gulf (20–21)	–	N/A	1

not tied to specific stations but represents an assessment for the whole assessment unit. Together with indicators of biological contamination (SBCI) and the distribution of the high-risk species, the IBPR values indicate increased risk of biological invasions for the Volgo-Don Canal (VDC), Tsimlyansk Reservoir with adjacent sites of the Don River, and certain sections of the Lower Don, including the mouth of the Manych tributary.

It should be noted that the Volga, the Taganrog Gulf and Don River upstream from the Tsimlyansk Reservoir were not studied in detail, and we only carried out a risk assessment in areas immediately adjacent to our main sampling station areas.

Discussion

Distribution of alien species

Among the reported alien macroinvertebrate species, three are transcontinental invaders (*F. californica*, *P. antipodarum* and *Ph. acuta*), one species originating from the Black Sea region (*D. bugensis*) and three originating from the Caspian Basin (*D. caspius*, *A. glabra* and *Th. major*). The invasion of *D. bugensis* is well described for the region (Zhulidov et al. 2004, 2005, 2006; Son 2007a; Nabozhenko and Son 2012). Currently, this species is distributed up the Don River to the mouth of the Potudan' River in the Voronezh Oblast (Krylov et al. 2010). In recent years, it has reached to the Voronezh Reservoir (*unpublished data*). The Caspian endemic *A. glabra* was recorded in the Tsimlyansk Reservoir. Earlier reports document it from the Don River at the dam of the Tsimlyansk Reservoir and in the Taganrog Gulf (Nabozhenko 2008; Son 2010). The gastropod, *L. naticoides*, native to the Don River, reached the Volga Basin after construction of the VDC (Pirogov 1972).

Last molecular revisions claim the synonymy of all four Caspian *Theodoxus* with the name *Theodoxus major* Issel, 1865 having priority (Sands et al. 2019a, b). This conclusion seems somewhat surprising, taking into account not only morphological differences (for example *Theodoxus schultzei* is very different from all other representatives of the genus), but also environmental differences. Separate morphospecies belonging to this group live in different salinity conditions and do not cross the critical salinity threshold between fresh water and mesohaline zone making

reproductive success dubious. This issue can probably be resolved using experimental crossbreeding. However, since this article is not taxonomic, we use the results of these revisions with some important comments.

In the Azov Sea basin, two populations of this group with different origin and ecological features were noted. Marine populations in the most saline sites of Azov Sea and its estuaries are traditionally identified as “*Theodoxus pallasi*” (Mordukhay-Boltovskoy 1960; Son and Prokin 2012). Origin of this population linked to Caspian Sea overflow events into the Sea of Azov (Sands et al. 2019a). This morphospecies is considered as mesohaline and in Azov Sea is found in salinity 11–14 PSU (Mordukhay-Boltovskoy 1960). Under experimental conditions, “*Th. pallasi*” (from Aral Sea population) gradually acclimatized in desalinated water up to 4 PSU (Bekmurzaev 1970), but under natural conditions it is absent in the transitional waters of the Azov Sea. In general, adaptation to the mesohaline zone is not characteristic for most Ponto-Caspian relicts (Mordukhay-Boltovskoy 1960) and requires complex evolutionary changes (Pauli et al. 2018). “*Theodoxus astrachanicus*” was known from the Lower Volga as freshwater species closely related to the marine “*Th. pallasi*”. An upstream expansion of this morphospecies was reported for the Volga Basin (Zinchenko and Kurina 2011). In our expedition, expansion of these snails was noted in the Don River Basin, but according to a personal message from Dr. Maxim Nabozhenko, it started at least a few years earlier. Both avoid the transitional regions of the Sea of Azov, whose salinity is probably lethal to them. Thus, now they are isolated from each other.

The Caspian amphipod, *D. caspius*, is the most widespread macroinvertebrate invader in the Lower Don basin. This species is reported from the Volga Delta and from the Damchik “ilmen” (a lagoon in the Caspian Sea basin) (Benning 1924). This species was initially reported in the Don River basin at the beginning of the 2000s, being found to be common in the delta and riverbed of the Don, while being scarce in the Taganrog Gulf of Asov Sea and in the Veselovskoye Reservoir. In an upstream section of the Don, it was registered near Romanovskaya village, 9 km below the dam of the Tsimlyansk HPP (Lubina and Sayapin 2008). In the Middle Don, the species was recorded in 2009 from the mouth of the Tolucheevka River in the Voronezh Oblast (Krylov et al. 2010), although it had previously reached this point in 2003, but those specimens (from a Don River tributary, the Kolodyezhny stream) were erroneously identified as *Gmelinopsis tuberculata* G.O. Sars, 1986 (Silina 2005). In the Volga Basin, decades after it had been first reported in the Damchik “ilmen” and the river delta (Benning 1924), its range expansion had progressed to the Volgograd Reservoir and the Saratov Reservoir (Sonina and Filinova 2011).

As we see, transcontinental exotic species had a small point distribution. The range of freshwater limpet *F. californica* in the studied region is restricted by VDC. This alien snail is widespread in the Ponto-Caspian

Basin and was already recorded from the Volga Basin (Son 2007b; Tokinova 2012; Semenchenko et al. 2016). The New Zealand mud snail, *P. antipodarum*, is widespread along the Black Sea coast, but in the Azov Sea Basin besides of the Karpovsk Reservoir was found earlier only in the springs on the banks of the Lower Don River near Rostov-on-Don (Son 2008; Son et al. 2008). The popular ornamental snail, *Ph. acuta*, is well-known from Southern Russia (Vinarsky 2017), but in the Lower Don it had not been previously reported. However, it was reported from other Don River sub-basins, i.e. from the Seversky Donets River Basin (Zatravkin 1980) and from the Middle Don River Basin in the cooling pond of the Novovoronezh Power Plant and from the nearby channel of the Don River (Silina et al. 2005). The main pathway of their introduction into this area is the secondary natural dispersion from an already established invasive area, while the introduction of Caspian relics is associated with the construction of inter-basin canals.

Our study records the situation in 2011, when new Caspian invaders were already widely distributed in the study area, but only a small number of exotic species present and whose presence was spatially restricted and episodic. Since then, many exotic species have been reported from the Don Delta or Taganrog Gulf, including the bivalves *Corbicula fluminea* (O.F. Müller, 1774) (Zhivoglyadova and Revkov 2018) and *M. leucophaeata* (Zhulidov et al. 2018), the decapod *Macrobrachium cf. rosenbergii* (De Man, 1879) (Shokhin 2018), and the polychaetes *Streblospio cf. gynobranchiata* Rice et Levin, 1998 (Syomin et al. 2017b) and *Marenzelleria neglecta* Sikorski et Bick, 2004 (Syomin et al. 2016, 2017a). In addition, there are also alien polychaetes, which have been misidentified as “*Aracia* sp.” (Syomin et al. 2015) or “*Laonome calida*” (Boltachova et al. 2017), now described as the new species *Laonome xeprovala* Bick et Bastrop in Bick, Bastrop, Kotta, Meißner, Meyer et Syomin, 2018 (Bick et al. 2018). Most of these new invaders are probably spread by shipping. The estuary system of the Don River (Taganrog Gulf and Don Delta) serves as a “gateway” that, because of its salinity regime and high level of shipping, may serve as an “acclimatization chamber” for potentially euryhaline species, enhancing their ability to colonize inland waters (Panov et al. 2009).

Impact of alien species

The ecological impacts of two of the alien species featured in our survey have been relatively well studied. *L. naticoides* may have influenced parasite-host interactions in the Volga Basin, not least via the introduction of new trematodes associated with this snail (Tyutin and Slynko 2010). Second, the impact of *D. bugensis* as an “ecosystem engineer” and its relationship with the closely related *D. polymorpha* (Pallas, 1771) has been well-studied (Zhulidov et al. 2004, 2006, 2010; Son 2007a; Nabozhenko and

Son 2012; Karatayev et al. 2015; D'Hont et al. 2018; Kemp and Aldridge 2018). A notable finding of the current study area was that *D. bugensis* did not appear to have replaced or out-competed *D. polymorpha*, and its abundance appears to have been gradually decreasing after the initial expansion associated with its arrival in the system (Zhulidov et al. 2010). Habitat partitioning is evident in the Lower Don and Lower Volga, with the two species occupying different niches; *D. polymorpha* dominates the main bottom habitats, and *D. bugensis* is apparently restricted to a narrow stretch in the splash zone (Nabozhenko and Son 2012). Another dreissenid species, *Mytilopsis leucophaeata* (Conrad, 1831), was found in the Taganrog Gulf near Don Delta, but its relationships with *Dreissena* spp. have thus far not been investigated (Zhulidov et al. 2018). The New Zealand mud snail, *P. antipodarum*, forms numerous mass populations in small streams along the Black Sea coast, often forming a new community with extreme dominance, but this was observed in the studied region only in the springs on the banks of the Lower Don River near Rostov-on-Don (Son 2008; Son et al. 2008).

Expansion of *A. glabra* led to the formation of new stable communities in the Taganrog Gulf, including the associated Yeisk Estuary. In addition, the species was found in one location in the Don River, downstream of the Tsimlyansk Reservoir dam. Here, it was part of a community dominated by dreissenids and Limnocaridiidae (*M. colorata* and *A. glabra*) on mixed sediments of mud, shells, and gravel (Nabozhenko 2008). In contrast, in sandy mud in the upper part of the Tsimlyansk Reservoir, we found a community dominated by *A. glabra* and *M. colorata*, accompanied by Ponto-Caspian mysids and with *Dreissena* absent. In the Taganrog Gulf, *A. glabra* competes with *M. colorata* (Shokhin et al. 2006), which is a key species of the historical native community. However, in the Tsimlyansk Reservoir and in the adjacent region of the Don River, both species were present and abundant, a novel situation for this part of the Don Basin. Before construction of the Tsimlyansk Reservoir, there were more rheophilic communities evident, with colonies of massively abundant *D. polymorpha*, associated with Ponto-Caspian amphipods, molluscs, and hydroids (Mordukhay-Boltovskoy 1960).

Another species that clearly impacted the native community is *Th. major*. The native *Theodoxus fluviatilis* (Linnaeus, 1758) was absent from all the localities invaded by *Th. major* and was only present in the Don River channel, upstream from the Tsimlyansk Reservoir where the invader was absent. This may indicate that the closely-related invader outcompeted and displaced the native species, which had previously dominated the rocky substrates (Mordukhay-Boltovskoy 1960).

The amphipod *D. caspius* is little studied compared to other species in the genus, several of which have been identified as aggressive invaders (Rewicz et al. 2015). Despite this, some evidence of its impact on benthic

communities have been reported: especially displacement of resident amphipod species in littoral habitats of the Voronezh Reservoir (Kurina et al. 2018) and alteration of the structure of malacostracan assemblages in the littoral vegetation of the Volgograd Reservoir (Sonina and Filinova 2011). As the results described above show, this species has become a key species in the amphipod assemblages of the studied region.

It should be noted that samples from our expedition were included in a study of the molecular phylogeography of the amphipod *P. robustoides* in its native and invaded range (Rewicz et al. 2018). Preliminary results suggest that the lower Don River is inhabited predominantly by a population of Caspian origin (Rewicz *personal communication*), which may indicate a displacement of local amphipod populations even at the intraspecific level.

In summary, we can conclude that at the time of our study, species originating from the Caspian caused the greatest impact on “local” species and communities in the Don Basin.

Indicators of biological pollution

In common with other European biotic indices based on the presence of pollution sensitive indicator groups, the reliability of TBI and BMWP to accurately reflect changes in water quality may be compromised in regions with Ponto-Caspian macroinvertebrates. First, such indices do not include a number of sensitive relict groups of marine origin (Mysida, Cumacea, Cardiidae, etc.), and such absences in index generation could potentially underestimate water quality in stations with some of the most diverse and stable communities. Second, the sensitivity/tolerance of some Ponto-Caspian species to organic water quality may be significantly different from confamiliar species widely distributed in Europe and which feature in indices such as the TBI and BMWP. A clear example of this can be found with amphipod taxa in central Europe, as all amphipods, such as invasive and native *Gammarus* spp. and invasive *Crangonyx pseudogracilis* Bousfield, 1958, may be collectively scored as a single indicator amphipod taxa in many biotic indices (MacNeil et al. 2012). This lack of resolution/accuracy may be exacerbated by the presence of Ponto-Caspian species such as *D. villosus* (MacNeil et al. 2012) among others (Grabowski et al. 2009; Guareschi and Wood 2019). For this reason, indices assessing the average sensitivity of indicator groups, such as the BMWP and its derived index, the Average Score Per Taxon (ASPT), are problematic to use in the Lower Don area. In particular, at a number of stations with low species diversity (12, 15, 18–21), the presence of eurybiont amphipods sharply overestimated the quality of water when calculating ASPT (Table 1), which made us reject the use this indicator.

We noted a positive correlation between biocontamination and common biological water quality indices, which may be caused by Ponto-Caspian invasive fauna forming rich and diverse benthic communities sensitive to

existing physico-chemical regimes, particularly dissolved oxygen level (Mordukhay-Boltovskoy 1960). Meltdown of the two complexes of relict species originating from Caspian and Azov-Black Sea basins can lead to intra-guild competition but probably does not change the overall picture of the role of the Ponto-Caspian fauna in this ecosystem. Among both the Caspian invaders and the exotic macroinvertebrates found during our research, there were no opportunistic species that could take advantage of the deterioration of organic water quality. This is different from many other scenarios where invasive alien species tend to be more tolerant of organic pollution than the natives they replace (MacNeil et al. 2010; Čuk et al. 2019). However, this situation may change if the study area is invaded by more opportunistic, pollution tolerant fauna. Indeed, as shown above, during recent years, many exotic worms and other macroinvertebrate taxa were reported from the Don Delta or Taganrog Gulf, and this requires new research and environmental assessments for this region.

Estimation of invasiveness risk of species alien for the Don Basin with SBPR index (Table 2) coincide with those previously made in the study of the Dnieper, Danube, and other major European rivers with the exception of the Caspian invaders who have not been previously assessed (Panov et al. 2009; Semenchenko et al. 2015).

Invasion corridors

Our study clearly shows that that existing assumptions about the main invasive corridors of Europe are not consistent with the processes we observed in this region. The original concept of the Northern invasion corridor was based on the upstream expansion of Ponto-Caspian relict species from estuaries to upper parts of rivers and to new basins (Bij de Vaate et al. 2002). In this context, the “latitudinal” exchange of Ponto-Caspian fauna between different estuaries of the Azov, Black, and Caspian seas or downstream expansion of exotic species was considered of secondary importance. The Azov-Caspian inter-basin connections were considered in the sense that several species that expand upstream the Volga Basin were of Azov-Black Sea origin. Later, different schemes of this invasion network system used names of invasion corridors without this context but already as “two-way roads” for all aliens (Galil et al. 2007; Leuven et al. 2009; Panov et al. 2009). In this framework, we now have to clearly separate two distinct processes; firstly, the Azov-Caspian exchange, and, secondly, the invasion of the Caspian-Volgo-Baltic waterway. It should also be borne in mind that many species spread mainly horizontally without a significant upstream expansion and indeed, the horizontal expansion along the Azov-Black Sea coast was noted as the Southern Meridian Corridor (Panov et al. 2009). Our study of the Lower Don region involving both the Azov-Caspian exchange via VDC and eastward invasion of exotic species, allows identification of a single Ponto-Caspian Invasion Corridor that ultimately connects all three marine basins of the Ponto-Caspian region.

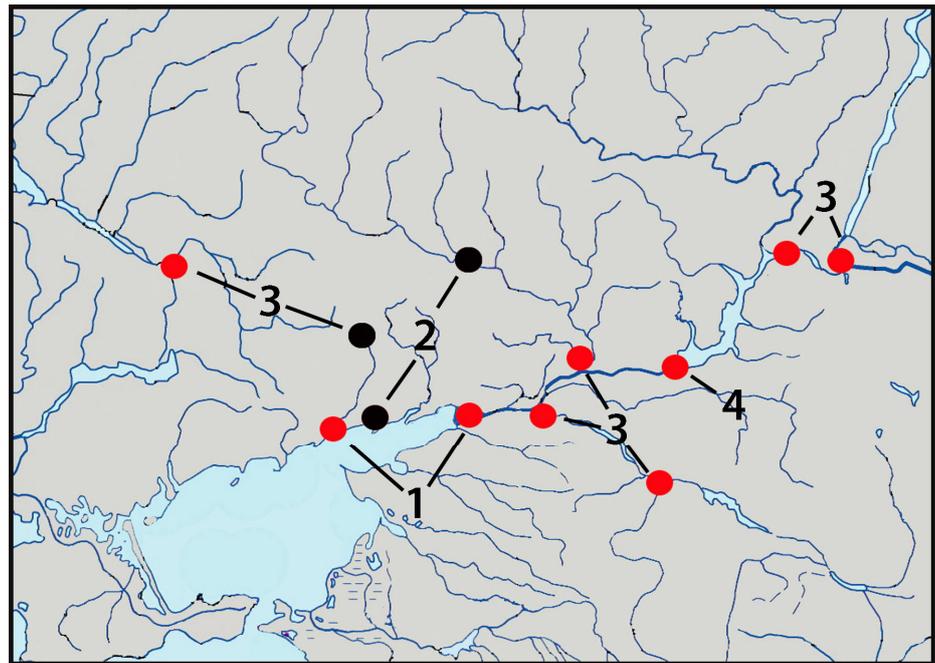


Figure 5. The key points for monitoring of aquatic alien species distribution in the study area and neighboring river basins: red circles – available monitoring zones; black circles – points in a zone of military conflict: 1 – invasion gateways; 2 – cross-border areas; 3 – the ends of the canals and the mouths of the tributaries connected by canals to other basins; 4 – warm-water zone near Rostov Nuclear Power Plant.

Regional problems of alien species monitoring and management in the context of basin approach

The above results show the critical importance of the studied region as a point of exchange of species between the neighboring river basins of Volga, Don, and Dnieper. These two basins are cross-border between Russia and Ukraine. The focal points for monitoring of aquatic invasions here should be “invasion gateways” presented by estuarine zones with large ports; cross-border areas in the basin of the Seversky Donets and the coast of the of Azov Sea; the end-points of canals and the mouths of tributaries connected by canals to other basins; and, finally, the warm-water zone near Rostov Nuclear Power Plant (Figure 5).

In both Ukraine and Russia, water use and management of inland waters are regulated by Water Codes, which in recent times have been based on a river basin approach to river management and monitoring, similar to the catchment approach in Western Europe (MacNeil *pers. obs.*). In Russia, 21 river basin districts (RBD) have been created, and nine have been created in Ukraine. This study covers three Russian RBD (Don, Kuban, and Lower Volga) and three Ukrainian RBD (Dnieper, Don, and Azov Sea rivers). Biological invasions are among the key anthropogenic factors driving aquatic ecosystem change, and therefore their management should be a core component of river basin management plans, and their indicators should be routinely included in assessments of the ecological status of water bodies. Traditionally for Post-Soviet countries, biological parameters (TBI,

species composition, etc.) are auxiliary elements in the monitoring system for chemical and organic pollution of water bodies. Thus, more focused monitoring methods and environmental assessments related to biological invasions need to be developed and legally implemented.

A large-scale reorganization of Ukrainian legislation was driven by implementation of the EU Water Framework Directive. The most fundamental changes in the management of water resources was the adoption of the river basin approach and an increase in the importance of biological indicators. A number of elements of monitoring and management that require “biological” information (assessment of ecological status, assessment of environmental potential, allocation of valuable sites, etc.) were introduced, which set a range of new protocols and sampling regimes which were in marked contrast to traditional Post-Soviet approaches (Son 2019). New procedures for state water quality monitoring include identification of alien species and information about their establishment for further implementation of RBD monitoring and management programs, environmental assessments and future planning.

The development of monitoring networks in Ukraine in the context of these changes is at the initial stage of development. In this case, a major problem is that several important monitoring locations are in a zone of military conflict. These are non-controlled areas in the Donetsk urban agglomeration (Seversky Donets – Donbass Canal), the Lugansk City surroundings (transboundary area of Seversky Donets River) and the transboundary area of Azov Sea coast (Figure 5). Management and monitoring of these areas requires a lot of focused activity and a pragmatic approach, which differ from the conventional management of water bodies in other jurisdictions, and sometimes must be additionally coordinated with different ministries and departments.

In Russia, RBD monitoring and management programs (state monitoring of water bodies) are separated from the system of state monitoring of quality and pollution of environment (carried out by the Russian Federal Service for Hydrometeorology and Environmental Monitoring) and do not include biological elements. The system of state monitoring of quality and pollution of environment use the parameters of phytoplankton, zooplankton and zoobenthos in analysis of surface water pollution, but primary data on alien species are not separately considered decision-making information. In principle, this monitoring system can be easily adapted for the purpose of obtaining information on biological invasions. However, an important underlying methodological problem that remains is an existing monitoring network that reflects the previously purely auxiliary nature of biological information collection, rather than has such biological monitoring at its core.

Although, initially, the monitoring network covered economically important, slightly polluted, and heavily polluted areas, it has been a frequent practice that in more recent monitoring of biological parameters

in good water quality areas to cease this once water quality has remained consistently good over an extended time period (unlike monitoring of chemical indicators) (Buyvolov et al. 2016). As we have previously shown, the fact of a positive correlation of biological contamination with TBI, which is an official part of the monitoring system, is contradictory to the underlying assumptions and usefulness of the existing monitoring network for alien species risk assessment. There is an obvious need for conceptual modernization of the current monitoring network.

Conclusion

Our study clearly identifies the Caspian Sea basin as a new important source of invaders. The Don River could be considered as the “first line of defense” in the path of such Caspian invaders, as they head towards Central and Western Europe. We can observe the processes of competition/predation between complexes of relict species similar in origin and ecology, that may have been isolated from one another for millennia. Such scenarios may be being currently repeated in many Azov-Black Sea rivers, and in the future, may be witnessed in other regions, which are currently occupied by Ponto-Caspian species.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Details of sample stations.

Table S2. Species records.

This material is available as part of online article from:

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