Delayed expansion of Ponto-Caspian gobies (Pisces, Gobiidae, Benthophilinae) in the Nemunas River drainage basin, the northern branch of the central European invasion corridor

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Received: 20 October 2017 / Accepted: 13 April 2018 / Published online: 26 April 2018
Handling editor: Charles W. Martin

Abstract
During the past few decades, rapid expansion of Ponto-Caspian gobies has been observed in the rivers connected to the central European invasion corridor. The Nemunas River catchment has been connected to this corridor for more than two hundred years, yet there is no evidence of Ponto-Caspian gobies in the Nemunas River. This study aimed to assess the presence of Ponto-Caspian gobies within the Nemunas River and its main tributaries, specifically in the Lithuanian part of the Nemunas and Neris Rivers, which may serve as possible routes for these fishes’ expansion. Of all the presumptive Ponto-Caspian goby species only Neogobius fluviatilis (Pallas, 1814) and Neogobius melanostomus (Pallas, 1814) were caught. The relative abundance and biomass of both species was very low. The pilot analysis of piscivorous fish diet revealed that N. fluviatilis is already selectively consumed by Esox lucius. Although both species can reproduce and sustain viable populations in the Nemunas River, different scenarios for their future establishment within the Nemunas River drainage basin are predicted. The hypothesized distribution of N. melanostomus will remain restricted only to the outlets of the Nemunas River; while the distribution of N. fluviatilis in the future will be wider. We presume that the area colonised by N. fluviatilis will expand until the species establishes in the entire Nemunas River drainage system.

Key words: aquatic invasion, alien species, fish introduction, Neogobius fluviatilis, Neogobius melanostomus

Introduction
One of the main causes of biodiversity loss is the spread of invasive species. These species reduce local biodiversity through both indirect competitive and direct predatory impacts on resident native populations (Goudswaard et al. 2002; Reshetnikov 2003). The spread of invasive species is an international problem, and local governments are working to prevent their spread and reduce their impacts (EU Regulation No 1143/2014). However, over the past few decades, rapid expansion of Ponto-Caspian gobies has been observed in the rivers connected to the central European invasion corridor. Four Ponto-Caspian gobies Babka gymnotrachelus (Kessler, 1857), Neogobius fluviatilis (Pallas, 1814), Neogobius melanostomus (Pallas, 1814), and Proterorhinus marmoratus (Pallas, 1814) have successfully established in the Dnieper, Vistula, Pripyat and Oder Rivers (Wolter and Röhr 2010; Semenchenko et al. 2011). Rapid colonisation of inland European waters by these gobies could be explained by their effective reproductive strategies (multiple spawning, egg laying in nests, paternal care), extreme resistance to adverse habitat conditions (stagnant, flowing, euryhaline waters) and human assisted introduction (Kornis et al. 2012). At present, these species are considered globally invasive and are found in waters of most countries of Central and Eastern Europe posing serious ecological threats to fauna of invaded waters (Wolter and Röhr 2010; Semenchenko et al. 2011). However, their presence in the Nemunas River has not yet been reported (Semenchenko et al. 2009; Rakauskas et al. 2016), although N. melanostomus is
known to have established a viable population in the Curonian lagoon (Rakauskas et al. 2013).

The Nemunas River catchment, which is connected to the Pripyat and Vistula rivers by the Oginski (opened in 1783; Figure 1A) and Augustow (opened in 1839; Figure 1B) canals, forms the northern branch of the central European invasion corridor (Bij de Vaate et al. 2002; Panov et al. 2009). Connections between the watersheds of the Nemunas and Dnieper Rivers form the most probable pathway for new invasions of this region, primarily those of Ponto-Caspian origin (Arbačiauskas et al. 2011; Rakauskas et al. 2016). Many non-indigenous freshwater invertebrate species have dispersed into the Nemunas River drainage area via this invasion route (Arbačiauskas et al. 2011). Yet, no non-indigenous fish species (hereafter NIFS) have been shown to have spread into the Nemunas River via this invasion corridor (Rakauskas et al. 2016), despite dense populations of the four invasive gobies in the connected Vistula and Pripyat Rivers (Semenchenko et al. 2011).

The purpose of this study was to confirm the presence of Ponto-Caspian goby species in the Nemunas River and, in case of positive results, to identify their possible invasion routes within the drainage area. Furthermore, to evaluate the integration of Ponto-Caspian gobies into higher trophic levels of the invaded rivers’ ecosystems, we analyzed the diet of one local piscivorous fish.

Material and methods

Study area

The Nemunas River catchment area (Figure 1) stretches along the south-eastern shore of the Baltic Sea and drains an area of approximately 97,863 km². It is the fourth largest river catchment within the Baltic
Neogobius fluviatilis invades Nemunas River drainage

Sea drainage system covering 72% of Lithuanian territory (Kažys 2013). However, only the lower reaches and part of the mid-reaches (47.7% of the Nemunas River basin) falls into the territory of Lithuania. Other parts belong to the Republic of Belarus (46.4%), Poland (2.5%), Latvia (0.1%) and the Russian Federation (3.3%) (Kažys 2013). The Neris River is the biggest tributary of the Nemunas River. Its catchment basin covers approximately 24,942 km² (Kažys 2013), almost half of which (13,981 km²) is in Lithuanian territory. The upper reaches of the Neris River (44% of its drainage basin) flow through the territory of Belarus. The ichthyological research into the occurrence of Ponto-Caspian gobies in the Nemunas River drainage area was carried out in Lithuania.

**Screening for Ponto-Caspian gobies**

The presence of Ponto-Caspian gobies was assessed at 34 study sites in the Nemunas and Neris Rivers once a year from May until October in 2015, 2016 and 2017. Geo-referenced information on sampled locations is presented in the Supplementary material to this paper (Table S1) and visualized in Figure 2. Sampling was conducted using battery-powered electric fishing gear, by wading for 15 min in water depths of 0.3–1.0 m at each study site (~150 m distance along selected habitats). Gobies were collected from habitats with mixed-type bottom (sand and mud with some gravel and aquatic macrophytes) and slower water current. Such habitats are known to be typical for different species of invasive Ponto-Caspian gobies such as B. gymnotrachelus, N. fluviatilis, N. melanostomus, and P. marmoratus (see Froese and Pauly 2017).

All fish individuals were identified to species level and counted. Pursuant to state fishing laws and sampling licensing, after sampling all native fish individuals except Esox lucius Linnaeus, 1758, which were sacrificed for diet analyses, were released back into the river. The collected non-indigenous gobies
were taken for further examination. All examination procedures were carried out in strict accordance with regulations of the Republic of Lithuania. The total body length (TL) of all the collected goby specimens was measured from the tip of the snout to the end of caudal fin rays to the nearest mm. The fish taxonomy used in the present study follows the taxonomy provided in FishBase (Froese and Pauly 2017).

Analysis of operating invasion routes for natural Ponto-Caspian gobids dispersion

To assess the potential invasion route(s) of Ponto-Caspian gobies in the Nemunas River drainage, the studied drainage area was divided into four sections to cover all possible natural routes of Ponto-Caspian gobies’ invasion. Study sites were assigned to river sections based on the distance to the Nemunas River mouth and the presence of artificial barriers to natural fish spread (Figure 2). The occurrence of invasive fish species in the lower reaches of the Nemunas River (study sites 1–5, the river section stretching from the river mouth up to 100 km upstream) would indicate the marine vector of invasion. The presence of gobies in the middle reaches of the river (study sites 6–9, from approximately 100 km upstream of the river mouth up to the dam of the Kaunas Hydroelectric Power Plant) would point to the Venta and Lielupe Rivers as invasion pathways. Occurrence of gobies in the upper reaches of the Nemunas River (study sites 10–18, from the Kaunas Hydroelectric Power Plant dam upstream to the Lithuania-Belarus border) would indicate goby dispersion via the Oginski and/or Augustow canals. Finally, catches of gobies in the Neris River (study sites 19–34) would suggest that gobies invaded the river via the connection of the Neris and Berezina Rivers.

Analysis of piscivorous fish diet

For the analysis of piscivorous fish diet, we used fish specimens sampled in 2017 by electric fishing from those study sites where invasive gobies were present. From all local piscivorous fish species only E. lucius spatially overlapped with Ponto-Caspian gobies and were therefore selected for diet analyses (n = 59). Only specimens with non-empty guts were included (N = 42), TL of which varied from 21.5 to 69.2 cm.

After fish had been euthanized (immersed into 1.5–2.0 ml L⁻¹ solution of 2-phenoxethanol for 5 min), their digestive tracts were immediately removed and preserved in 10% formaldehyde solution until necropsy in the laboratory. Gut contents were dissected out and food items were identified to the lowest possible taxa under a stereomicroscope. The undigested prey items were grouped by taxonomic rank and wet-weighted, their proportions in the total gut content weight being assessed. Digested prey fish species were identified from undigested species-specific bones and other hard remains such as otoliths (sagittae), pharyngeal teeth, jaws, and chewing pads. Fish lengths and weights were reconstructed using allometric relationships, estimated from the reference collection supplemented with published relationships for some species (Piltys and Zarankaitė 2010).

Fish feeding selectivity for Ponto-Caspian gobies was calculated as the Ivlev selectivity index (Eᵢ) (Ivlev 1955):

\[ Eᵢ = \frac{rᵢ - pᵢ}{rᵢ + pᵢ}, \]

where \( rᵢ \) and \( pᵢ \) are proportions of Ponto-Caspian gobies biomass in fish diet and environment, correspondingly. \( Eᵢ \) selectivity is expressed on a scale from −1 to 1. When \( Eᵢ = 0 \), there is no selective feeding on Ponto-Caspian gobies, whereas \( Eᵢ > 0 \) or \( Eᵢ < 0 \) indicates positive or negative selectivity, respectively.

Results

In total, 23 995 specimens representing 36 fish species were sampled. Of all presumptive Ponto-Caspian gobies only N. fluviatilis and N. melanostomus were recorded, together comprising only 0.2% (n = 37) of all specimens. Alburnus alburnus (Linnaeus, 1758), Gobio gobio (Linnaeus, 1758), Squalius cephalus (Linnaeus, 1758), and Rutilus rutilus (Linnaeus, 1758) were the most abundant native fish species, accounting for 68% of all individuals.

Distribution

Neogobius fluviatilis was recorded at 10 study sites in the Neris River (Table S2, Figure 2). It was present in very low numbers and never exceeded more than 6 individuals per sampling occasion. Its relative abundance and biomass were also very low and never exceeded 1% of the total fish catch per study site.

Only three Neogobius melanostomus individuals were recorded all at one study site in the Nemunas River delta (Table S3, Figure 2). Its relative abundance and biomass were lower than 1% of the total fish catch at this study site per sampling occasion.

The white-finned gudgeon, Romanogobio albipinnatus (Lukasch, 1933), another recent invader of the Nemunas River drainage area, was also recorded during this study. However, the distribution of R. albipinnatus was restricted to one site (Table S4, Figure 2) in the Neris River. It was present in very low numbers, never exceeding five individuals per
Neogobius fluviatilis invades Nemunas River drainage

Table 1. Prevalence of Neogobius melanostomus and Neogobius fluviatilis in the diet of Esox lucius from different study sites in the Nemunas and Neris Rivers and its load per fish specimen: study site number (Site No); number of investigated E. lucius specimens (n); total body length of E. lucius (TL); percentage of E. lucius individuals containing N. fluviatilis (P); number of N. fluviatilis individuals per fish (I); percentage share of N. fluviatilis biomass in the total diet of E. lucius (S); feeding selectivity for Ponto-Caspian gobies (Ei).

<table>
<thead>
<tr>
<th>Site No (Map Ref.)</th>
<th>n</th>
<th>TL (cm)</th>
<th>P (%)</th>
<th>I (ind.)</th>
<th>S (%)</th>
<th>Ei</th>
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<tbody>
<tr>
<td>N. melanostomus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>3</td>
<td>52.9 ± 13.9</td>
<td>66.7</td>
<td>1 ± 0</td>
<td>66.7</td>
<td>0.99</td>
</tr>
<tr>
<td>23</td>
<td>3</td>
<td>41.7 ± 9.7</td>
<td>33.3</td>
<td>1</td>
<td>33.3</td>
<td>0.99</td>
</tr>
<tr>
<td>24</td>
<td>7</td>
<td>36.1 ± 14.9</td>
<td>28.6</td>
<td>1 ± 0</td>
<td>16.7</td>
<td>0.95</td>
</tr>
<tr>
<td>25</td>
<td>4</td>
<td>47.3 ± 4.9</td>
<td>25.0</td>
<td>1</td>
<td>25.0</td>
<td>0.98</td>
</tr>
<tr>
<td>26</td>
<td>3</td>
<td>37.2 ± 7.8</td>
<td>33.3</td>
<td>1</td>
<td>33.3</td>
<td>0.99</td>
</tr>
<tr>
<td>27</td>
<td>2</td>
<td>36.8 ± 11.0</td>
<td>50.0</td>
<td>1</td>
<td>50.0</td>
<td>0.99</td>
</tr>
<tr>
<td>28</td>
<td>5</td>
<td>39.8 ± 7.2</td>
<td>20.0</td>
<td>1</td>
<td>20.0</td>
<td>0.99</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>38.3 ± 3.9</td>
<td>33.3</td>
<td>1</td>
<td>33.3</td>
<td>0.99</td>
</tr>
<tr>
<td>33</td>
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<td>39.9 ± 8.0</td>
<td>25.0</td>
<td>1</td>
<td>25.0</td>
<td>0.99</td>
</tr>
<tr>
<td>34</td>
<td>6</td>
<td>44.1 ± 5.4</td>
<td>33.3</td>
<td>1 ± 0</td>
<td>33.3</td>
<td>0.97</td>
</tr>
</tbody>
</table>

sampling occasion with relative abundance and biomass always below 1% of the total fish catch.

**Spreading rate**

The annual investigations revealed the spreading rate of *N. fluviatilis* individuals, though relatively limited attempts (one-time 15 minutes long electric fishing samplings) were made to track this species in studied river sites. In 2015, the species was found only at one study site near the border with Belarus (study site 34, Figure 2). In 2016, it was recorded 54 km downstream from site 34 (study site 27, Figure 2) while in 2017, it was recorded 56 km further downstream again (study site 22, Figure 2). Overall, in two years *N. fluviatilis* had moved roughly 110 km downstream indicating the continued spread of the species via the Neris River at the average rate of 55 km per year.

The spreading rate of *N. melanostomus* was indeterminable as it was recorded only at one study site throughout the study period.

**Accompanying fish species**

During sampling in the Neris River, 18 other fish species besides *N. fluviatilis* were recorded. Eight of these occurred sympatrically with *N. fluviatilis*: i.e. *Barbatula barbatula* (Linnaeus, 1758), *Cobitis taenia* Linnaeus, 1758, *Cottus gobio* Linnaeus, 1758, *Gobio gobio*, *E. lucius*, *Perca fluviatilis* (Linnaeus, 1758), *R. rutilus*, and *S. cephalus*.

Fish species accompanying *N. melanostomus* were: *A. alburnus*, *C. taenia*, *E. lucius*, *Gasterosteus aculeatus* Linnaeus, 1758, *P. fluviatilis*, *R. rutilus*, as well as single individuals of *Blicca bjoerkna* (Linnaeus, 1758), and *S. cephalus*.

*Esox lucius* was the only recorded piscivorous fish species spatially overlapped with either *N. fluviatilis* or *N. melanostomus* throughout the study period. Other accompanying and potentially piscivorous species such *P. fluviatilis*, *S. cephalus* or *Leuciscus aspius* (Linnaeus, 1758), were too small at the time of capture to prey on fish.

**Piscivorous fish diet**

The analysis of *E. lucius* diet showed that at the studied river sites cyprinids (*R. rutilus*, *A. alburnus*, *G. gobio*, *S. cephalus*) were the dominant prey item, constituting more than 58.5% of *E. lucius* diet. Neogobius fluviatilis made up a substantial part of *E. lucius* diet in the Neris River, varying from 16.7 to 66.7% of the total diet content (Table 1). No individuals of *N. melanostomus* were found in any dissected *E. lucius* specimen from either studied river. Other prey fish species (*P. fluviatilis*, *B. barbatulus*, *G. aculeatus*) were scarce and together did not exceed 7.9% of *E. lucius* diet.

Surprisingly, 32.5% of all the analysed *E. lucius* specimens from the Neris River consumed *N. fluviatilis*. Furthermore, *N. fluviatilis* proved to be a constituent, yet small part of *E. lucius* diet at all the studied sites in this river. Overall, the prevalence of *N. fluviatilis* as prey varied from 20.0 to 66.7% (Table 1). As most of *E. lucius* individuals consumed only one individual of prey fish, *N. fluviatilis* contribution to the total predators’ diet was very similar to its prevalence, varying from 16.7 to 66.7% at different study sites (Table 1).
Comparison of *N. melanostomus* and *N. fluviatilis* biomass in the total fish catch and as prey showed *E. lucius* to be highly selective in its food choice of *N. fluviatilis*: the relative biomass of *N. fluviatilis* were very low and never exceeded 1% of the total fish catch per study site in the Neris River. However, none of the studied *E. lucius* specimen had positive selectivity for *N. melanostomus* in the Nemunas River (Table 1).

**Discussion**

**Current status of invasive gobies**

This study revealed the current range of *N. fluviatilis* and *N. melanostomus* in the Nemunas River drainage basin. Furthermore, it indicates further rapid range expansion of *N. fluviatilis* in the Neris River, the right tributary of the Nemunas River. Although the abundance of *N. fluviatilis* in sampled fish communities in the Neris River was very low, there is no doubt that the species has established a viable population. *Neogobius fluviatilis* has been annually observed in the upper reaches of the Neris River (Belarus) since 2013 (Rizevsky et al. 2015) and in the lower reaches of the Neris River (Lithuania) since 2015. Furthermore, during each sampling occasion, we detected individuals of different ages, which suggest local breeding. Overall, the spread of *N. fluviatilis* within the Nemunas River drainage basin is continuing.

The presence of *N. melanostomus* in the Nemunas River was first recorded in 2010. At that time, *N. melanostomus* was found at several study sites at the outlet of the Nemunas River (Rakauskas et al. 2016). This study again confirmed that the recent distribution of *N. melanostomus* has been restricted only to the lowest part of the Nemunas River. To date, only adult specimens of *N. melanostomus* were found. Similarly, seldom have *N. melanostomus* individuals been reported entering small rivers in Sweden and rivers situated at the northern coast of Estonia (Verliin et al. 2017; Puntila et al. 2018). However, no data about viable riverine *N. melanostomus* populations are known from other relatively large northern Baltic Sea tributaries such as Daugava, Neva, and Narva. Overall, these findings contradict the previous assumption about the further rapid spread of *N. melanostomus* upstream in the Nemunas River (Rakauskas et al. 2013). Such a pattern was observed in Poland, when the first record of *N. melanostomus* was reported from the Puck Bay and afterwards this species was recorded as far as 130 km upstream of the Vistula River’s mouth (Kostrzewa et al. 2004; Grabowska et al. 2008).

Explanations for such delayed and slow colonisation of the Nemunas River waters by *N. melanostomus* include native species predation and/or climatic conditions.

There are some indications that native fish species feeding on NIFS could contribute considerably to ecosystem resistance against their invasion (Robinson and Wellborn 1988; Baltz and Moyle 1993). The broad range and relatively dense populations of piscivorous fish species (*E. lucius*, *L. aspius*, *S. glanis* (Linnaeus, 1758), *L. lota* (Linnaeus, 1758), large *Perca fluviatilis* (Linnaeus, 1758), or *Sander lucioperca* (Linnaeus, 1758)) in the lower reaches of the Nemunas River (Kesminas and Repėka 2005; Žiliukas and Žiliukienė 2006) may provide extensive and selective predation on initial *N. melanostomus* populations. Previous studies from Europe (Plačhocki et al. 2012; Mikl et al. 2017) or North America (Dietrich et al. 2006; Madenjian et al. 2011) have similarly highlighted the importance of piscivorous fish species in controlling *N. melanostomus* populations. However, more comprehensive research on predator-prey interactions between native piscivores and invaders is needed to understand predator role in ecosystem resistance against the invasion of Ponto-Caspian gobies in newly invaded areas of large European rivers.

The northern climatic conditions of Lithuania could be another reason for lack of *N. melanostomus* expansion in the Nemunas River. *Neogobius melanostomus* natural range covers coastal areas of the Black and Caspian Seas, the Azov Sea and the Marmara Sea (Froese and Pauly 2017). This species can tolerate a temperature range of 0 to 30 °C, but mainly thrives in warm waters (Froese and Pauly 2017) suggesting physiological performance may be suppressed in more severe northern climatic conditions, especially in winter. To date, viable riverine populations of *N. melanostomus* in the whole Baltic Sea catchment are known only from southern tributaries, such as the Vistula and Oder Rivers (Grabowska et al. 2008; Schomaker and Wolter 2014). The Nemunas River catchment is relatively further north from these rivers, and possesses more severe thermic conditions. Features such as a relatively short warm season, frequent fluctuations of water temperature in early summer, and severe winters may be lethal for *N. melanostomus* larvae and/or juveniles, and may also make it more susceptible to predators. A similar pattern was observed in the Great Lakes, where *N. melanostomus* is most widespread and at its greatest densities in the warmest Lake Erie and has the smallest range and lowest densities in the coldest Lake Superior. Water temperature was also significantly higher at river
sites invaded by *N. melanostomus* compared to sites where the species was absent (Kornis and Vander Zanden 2010). Overall, further studies are needed to explain such delayed and slow colonisation of the Nemunas River waters by *N. melanostomus*.

**Piscivorous fish diet**

The integration of invasive gobies in higher trophic levels occurs mostly through *E. lucius* predation. Although the conclusions drawn from our predatory fish diet analysis are limited due to the low number of piscivorous fish species and specimens used for gut content analyses (especially in the Nemunas River), some predictions about their ability to feed on invasive gobies (at least in Neris River) can be made. The percentage of *N. fluviatilis* in the diet of *E. lucius* from the Neris River was significant, although the amount of consumed gobies was negligible. Moreover, the share of *N. fluviatilis* in *E. lucius* diet was rather high compared to its relative abundance in the fish community. In the newly invaded river sites, *E. lucius* seems to prey on *N. fluviatilis* selectively. A similar pattern was observed in the Vistula River, where Ponto-Caspian gobids within 3–4 years became an important dietary component of common predatory fish, such as *E. lucius*, *S. lucioperca* and large *P. fluviatilis* (Plčhocki et al. 2012). Overall, such data contradict the general opinion that potential local predators need some time to adapt feeding on new exotic prey species effectively.

All studied *E. lucius* specimen showed negative selectivity for *N. melanostomus* in the Nemunas River, though the small sample size indicates these results should be treated with caution. However, our findings are coincident with earlier results from piscivorous *S. lucioperca* and large *P. fluviatilis* (Plčhocki et al. 2012). Overall, such data contradict the general opinion that potential local predators need some time to adapt feeding on new exotic prey species effectively.

**Operating invasion vectors for Ponto-Caspian gobies dispersion**

Six vectors for the introduction of NIFS into the inland waters of Lithuania were determined: aquaculture, development of fisheries, bio-control of aquatic plants, ornamental fish (release of unwanted fish or escapees from ornamental ponds or aquaria), unintentional as a contaminant of imported stocks, and natural spread of the species introduced into neighbouring countries (Rakauskas et al. 2016). However, legal deliberate introduction of NIFS for the development of fisheries should be rejected as currently it is regulated by local (Anonymous 2002a; 2002b) and European laws (EU Regulation No. 1143/2014). Intentional, though illegal, introduction of Ponto-Caspian gobies is also unlikely as these species have never been treated as target species by anglers. Therefore, the recent appearance of Ponto-Caspian gobies in the Nemunas River drainage area is likely associated with its natural or semi-natural (human accelerated “jump dispersal”) spread from neighbouring watersheds via connecting canal systems (Figure 1).

**Neogobius fluviatilis invasion route**

*Neogobius fluviatilis* had never been recorded in the Lithuanian part of the Nemunas River drainage area before 2015 (Rakauskas et al. 2016). However, for many years *N. fluviatilis* was already present in rivers of neighbouring countries, in the Vistula and Pripyat Rivers which are connected to the Nemunas River via the Oginski and Augustow canal systems (Figure 1A, B). The first record of *N. fluviatilis* in the Pripyat River dates back to 1957 (Semenchenko et al. 2009) and it was first recorded in the Vistula River in 1997 (Danilkiewicz 1998). *Neogobius fluviatilis* was found in the upper reaches of the Neris River (Belarus) in the Vilejskaje water reservoir in 2007 (Burko 2008) and by 2013, it was a common species both in the reservoir and downstream of the dam (Rizevsky et al. 2015).

The distribution of *N. fluviatilis* is still restricted to the upper and middle reaches of the Neris River and it has never been recorded in the Nemunas River, implying only one invasion route for the spread of this species into the Nemunas River drainage area. This invasion route connects the Dnieper and Nemunas Rivers (the Dnieper River → the Berezina River → the Svislach River → the Zaslavskaye water reservoir → the channel → the Vilejskaje water reservoir → the Neris River → the Nemunas River) (Figure 1D). We suggest that the upward spread of *N. fluviatilis* to the upper reaches of the Neris River was semi-natural. Its occurrence at the Zaslavskaye and Vilejskaje water reservoirs probably resulted from human-mediated transfer (i.e., “hitch-hiking” as a bait species) from the Svislach River as several relatively large artificial dams are present upstream. However, further spread of
N. fluviatilis downstream from Vilejskaje water reservoir to the upper and middle reaches of the Neris River occurred naturally.

Neogobius melanostomus invasion route

The distribution of N. melanostomus is still restricted to the outlets of the Nemunas River. It has never been reported from other parts of the Nemunas River drainage area (Semenchenko et al. 2009; Rakauskas et al. 2016), suggesting that N. melanostomus has naturally penetrated into the Nemunas River drainage area from the Baltic Sea via the Curonian Lagoon (the Baltic Sea → the Curonian lagoon → the Nemunas River). Neogobius melanostomus was reported from the Lithuanian coastal waters of the Baltic Sea by 2002 (Zolubas 2003). The appearance of N. melanostomus in the Baltic Sea waters in 1990 has been associated with international shipping (Grabowska et al. 2008).

Future distribution

Although expansion of both Ponto-Caspian gobies N. fluviatilis and N. melanostomus into the Nemunas River drainage area is still ongoing, some predictions about long-term consequences of this invasion could be made. Both species can reproduce and sustain viable populations in temperate zone rivers without human support (Semenchenko et al. 2011). However, quite different scenarios can be argued for their future establishment within the Nemunas River drainage basin.

Neogobius melanostomus was first recorded in the Nemunas River drainage area (the Curonian Lagoon) in 2002 (Zolubas 2003). In 2010 it was directly found in the Nemunas River waters (Rakauskas et al. 2016), and has been present there for almost a decade. Although N. melanostomus was shown to spread upstream via the Vistula River (Grabowska et al. 2008), we doubt if this scenario will occur within the Nemunas River drainage area. First, the abundance of N. melanostomus in the Curonian Lagoon seems to have stabilized at relatively low densities (Rakauskas et al. 2013). Second, it has been present at the outlets of the Nemunas River for almost a decade (Rakauskas et al. 2016), though has still not spread any farther upstream and is found at very low densities. Therefore, we believe the distribution of N. melanostomus will remain restricted only to waters of the Curonian Lagoon and the outlets of the Nemunas River. Overall, it is unlikely that in the future this intruder could dramatically change the composition of fish assemblage in the Nemunas River and cause shifts in fishery yields.

Meanwhile, N. fluviatilis is a very recent invader of the Nemunas River drainage area. It was first recorded in the Neris River waters in 2013 (Rizevsky et al. 2015). However, based on a spreading rate of 55 km per year, a wider distribution of N. fluviatilis is expected in the future. At this dispersion rate, the whole Neris River and the lower reaches of the Nemunas River are likely to be colonised by N. fluviatilis within a few years and is expected to reach the Curonian Lagoon (~ 400 km away from the recent species location) by 2024. Furthermore, N. fluviatilis may reach Latvian waters. The Venta and Lielupe Rivers flowing from Lithuania to Latvia may serve as donors of this species to the Latvian ichthyofauna as they are connected with the Nemunas River drainage area by canals. Two such invasion pathways may be operating: (1) The Nemunas River → the Nevēžs River → the Sanžilē canal → Lēvuo River → the Mūša River → the Lielupe River and (2) the Nemunas River → the Dubysa River → the Windawski canal → the Venta River (Figure 1E, F). Overall, the area colonised by N. fluviatilis will expand until the species has established in the entire Nemunas River drainage area. However, further studies are needed to answer the question of whether the expansion and increase of N. fluviatilis population will significantly affect fish community structure and cause shifts in the diet of top predators in the Nemunas River drainage.

Acknowledgements

Dr Audrius Steponėnas and Dr Egidijus Leliūnas are greatly appreciated for their assistance in the field. A draft of this manuscript has considerably benefited from the comments and suggestions of anonymous reviewers.

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Neogobius fluviatilis invades Nemunas River drainage


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Supplementary material

The following supplementary material is available for this article:

**Table S1.** Geo-referenced information on all sampled locations.

**Table S2.** Records of *Neogobius fluviatilis* in the Nemunas River drainage.

**Table S3.** Records of *Neogobius melanostomus* in the Nemunas River drainage.

**Table S4.** Records of *Ramonogobio albipinnatus* in the Nemunas River drainage.

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