Morphological and Trophic Features of the Invasive Babka gymnotrachelus (Gobiidae) in the Plain and Mountainous Ecosystems of the Dniester Basin: Spatiotemporal Expansion and Possible Threats to Native Fishes

Sergey Afanasyev 1,*, Olena Hupalo 1, Nataliia Tymoshenko 1, Olena Lietytska 1, Anatolii Roman 1, Oksana Manturova 1 and Doru Bănăduc 2,*

1 Institute of Hydrobiology, National Academy of Sciences of Ukraine, 04210 Kyiv, Ukraine; cloudy@ukr.net (O.H.); natali_tim@i.ua (N.T.); lietitska@ukr.net (O.L.); aroman.fish@gmail.com (A.R.); omaniturova@ukr.net (O.M.)
2 Applied Ecology Research Center, Lucian Blaga University of Sibiu, 550024 Sibiu, Romania
* Correspondence: safanasyev@ukr.net (S.A.); ad.banaduc@yahoo.com (D.B.)

Abstract: Over the last few years, the racer goby B. gymnotrachelus quickly expanded in the mountainous rivers of the Dniester basin at a height of 300 m a.s.l. and above. The occurrence frequency of the racer goby in fish communities in the mountainous rivers of the Carpathian ecoregion remains low (up to 20%), as compared with the plain rivers, where the species occurs in 70–100% of cases. The major prey groups in its diet in both plain and mountainous were Chironomidae, Diptera, and Crustacea. Chironomidae formed the maximal portion (35% at an occurrence frequency of 72.5%), and Trichoptera formed 18% at an occurrence frequency of 41%, whereas fish larvae were absent in their diet in mountainous rivers. The peculiar environmental conditions of mountainous rivers caused the adaptive modifications of the morphological features of the racer goby at the subpopulation level, which comprised a decrease in the specimens’ size in rivers with a flow velocity of above 1.5 m/s and rivers with pebble content of above 50% in the bottom sediments. The modifications showed an increase inbody streamlining as an adaptation to flow velocity and turbulence and the stony substrate of the river’s bottom. Thus, the morphological adaptation of B. gymnotrachelus to the conditions of the mountainous rivers is in progress, and the formation of anastable population in these rivers can be expected. In contrast, the native fish species’ resilience in the face of newcomers is still high, and this is because their alevins are not food for invaders.

Keywords: racer goby Babka gymnotrachelus; Gobiidae; Dniester River basin; Carpathians; Eastern plains; highland and lowland streams; morphology; diet; alien fish; adaptations; native fish species resilience characteristics

Key Contribution: The relatively widespread racer gobyfish in the basins of the Marmara, Black, and Caspian Seas exhibits continual expansion. Both its biological and ecological characteristics help this species conquer new areas, but the resilience capacity of the native fish species, especially in the mountainous ecosystems, exhibits ongoing spatiotemporal dynamics. For this species, by carrying out spatiotemporal trend assessments and monitoring and management, this study highlighted the importance of morphological and trophic characteristic dynamics both for the racer goby, which is expanding, and for the existent native fish species, which still exhibit their resilience capacity to fight against newcomers. All of this “fight for territory” phenomenon currently occurs in the Dniester River basin, a well-known biodiversity hot spot. This study’s results offer not only a new perspective on the local fish fauna but also a better understanding of the associated risks for decision making in management.
1. Introduction

The rapid development of civilization negatively affected water and aquatic ecosystems [1,2], and specifically, over the last century, it significantly accelerated the natural processes of the spread species and enabled the exchange of species between previously isolated regions [3]. Aquatic ecosystems, especially those that are already disturbed by various human activities, appear to be particularly vulnerable to these invasions [4,5]. The spread of alien fish species is caused not only by purposeful or accidental introductions but also by the species’ migration beyond their natural range due to various anthropogenic stressors, which induce natural habitat degradation: the construction of dams, the modification of river flow, climate change, sea level rise, water pollution, etc. [6–9].

The native freshwater fish species are among those that are most affected by the alien species’ introduction [10–13].

Biological invasions, which occur when species are transferred to new geographical regions and spread and form new ranges, are of special concern because of potentially negative ecological and economic effects [14].

Racer goby \textit{Babka gymnotrachelus} (Kessler, 1857) (syn. \textit{Neogobius gymnotrachelus}) [11,15] is a relict Ponto–Caspian species of the family Gobiidae: It is a freshwater and brackish-water benthic non-migratory fish that is widely spread in Ukraine and in the desalinized sections of the Black Sea and the Sea of Azov, and they are specifically observed in inlins and coastal lakes at a salinity of up to \(50/00\). In rivers, this species inhabits the bottom, preferring habitats with silted sandy or fine stony substrates, zones with slow flow, and depths of up to 2–5 m [11,12,16]. RG is one of the most successive invaders in European continental waters, and it actively expands its range, especially in the Danube and Vistula basins [3,17–20].

The recent rapid natural expansion of the range of the racer goby \textit{Babka gymnotrachelus} (Kessler, 1857) is one of the mechanisms of natural diversification, and it is a threat to the native biodiversity of the mountainous tributaries of the Dniester River. Due to various degradation types of the natural habitats in the Carpathian mountain basin [21–28], the high adaptive potential and ecological flexibility of the species catalyzed its expansion in many European water courses, particularly in the Dniester basin [10,14,29].

Information on RG spread from websites such as fishbase.org (accessed on 27 February 2023), http://www.nobanis.org (accessed on 1 March 2023), and https://www.iucnredlist.org (accessed on 2 March 2023) is not complete, especially regarding the Dniester basin. The map, given in the monograph from [30], is based only on the records of Polish specialists Kukula and Bylak [31], who wrongly stated the practical absence of data regarding RG spread in the Dniester River basin, relying only on the modern reprint of the popular book [32].

At the same time, the natural range of this Ponto–Caspian species covers the entire lower section of the Dniester River within the ecoregion Pontic province. In fact, just the natural spread of the Ponto–Caspian species is one of the reasons and markers for the delineation of the ecoregions of the Pontic province and Eastern plains. We have not determined the time of RG penetration beyond the limits of its natural range; however, it can be argued that since the XIX cent., it has been reliably recorded in the upper Dniester section within the limits of Eastern plains [33–35], but it has not been recorded upstream of the Zbruch River’s mouth. Up until the mid-XX cent., RG spread upstream the Dniester to the town of Sambir [36–40]. Over the next years, the population of RG increased, and it became quite widely spread and started to invade left-bank tributaries, which was confirmed by numerous records over the 1970–1990s in the rivers Zhvanchyk, Smotrych, Zbruch, and Strypa [41,42] (Figure 1).

In right-bank tributaries, the first RG specimens were found in 1976 in the lower section of the Lukva and Strałyzh rivers and the upper section of Bystrytsia Tysmenytska [42] (see Figure 1).
The aim of this study is to reveal the invasion’s scope and directions and the morphological peculiarities of *B. gymnotrachelus* in the plain and mountainous rivers of the Dniester basin within the Carpathian and Eastern Plainecoregions.

In right-bank tributaries, the first RG specimens were found in 1976 in the lower section of the Lukva and Strviazh rivers and the upper section of Bystrytsia Tysmenytska [42] (see Figure 1).

**Figure 1.** Expansion of *B. gymnotrachelus* in the middle and upper sections of the Dniester River over the last decades (the map was plotted using internet resource [43]): 1—Dniester; 2—Strviazh; 3—Bystrytsia Tysmenytska; 4—Trudnytsia; 5—Repchanka; 6—Stryi; 7—Opir; 8—Svicha; 9—Syvka; 10—Limnytsia; 11—Lukva; 12—Bystrytsia; 13—Bystrytsia Nadvirnianska; 14—Vereshchytsia; 15—Shchyrets; 16—Zubra; 17—Lug; 18—Svirzh; 19—Gnyla Lypa; 20—Zolota Lypa; 21—Strypa; 22—Seret; 23—Zbruch; 24—Zhvanchyk; 25—Smotrych; 26—Yagorlyk. Blue line means the Dniester basin limits, red line means the state boundaries.

**2. Material and Methods**

The invasion vectors and spread dynamics of the racer goby *B. gymnotrachelus* (hereinafter referred to as RG) in the Dniester basin were studied using the original records and archive materials of complex surveys carried out by the Institute of Hydrobiology NAS of Ukraine starting from 1983 and literature data. In addition, in 2019, special surveys were carried out in the main Dniester channel (upstream the Smotrych River inflow) and its tributaries: Strviazh, Bystrytsia Tysmenytska, Trudnytsia, Repchanka, Lug, Svirzh, Gnyla Lypa, Syvka, Limnytsia, Bystrytsia, Zolota Lypa, Seret, and Smotrych; the surveys aimed to search for localization sites and the spread of RG. In 2021–2022, complementary surveys were carried out in the Svicha and Yagorlyk rivers (see Figure 1).

Until 2018, samplings were carried out according to the State Fishery Agency licenses. In 2019–2022, catches were carried out using the gear permitted by the rules of amateur and sport fishing [44]: an ichthyological sac used for 1 h at each site (in the sections, it was 100–200 m long within the determined sites, and the sampling area was equal to...
500 m$^2$) and fishing rods at the potential occurrence sites of the species. The species was identified in situ according to the key provided in [11].

At each site, field protocols were filled in with records of water and air temperatures, flow velocity, water transparency, and bottom substrate and sediment type and composition [45].

Samples were preserved using 4% formaldehyde solution for further analysis in the laboratory. In total, 118 RG specimens were processed using I.F. Pravdin’s method [46,47]. Age was determined using scales and gill covers. Measurements were carried out using the standard methodology for fam. Percidae, with complementary measurements for the fam. Gobiidae (Figure 2).

![Figure 2. Scheme of racer goby measurements (modified from [48]).](image)

Morphometric analysis was carried out on 67 specimens. The morphological variability of RG was analyzed using a total of 5 meristic traits: scale number in the lateral line (Squ) and ray number in the first ($D_1$) and second ($D_2$) dorsal fins and in anal ($A$) and pectoral ($P$) fins. Twenty-two morphometric traits were examined: standard length ($l$); body length ($l_{cor}$); snout length ($lr$); horizontal diameter of eye ($do$); inter-orbital width ($io$); maximal head width ($ic$); maximal ($H$) and minimal ($h$) body height; maximal ($IH$) and minimal ($ih$) body depth; peduncle length ($pl$); anterodorsal ($AD$); antennal ($aA$); post-orbital ($po$) distance; length of the second dorsal fin base ($D_2$); length of anal fin base ($L_2$); length of pectoral fin base ($IP$); length of suction cup ($IV$); length of caudal fin ($IC$); height of first ($hD_1$) and second ($hD_2$) dorsal fins; head height ($hu$) (see Figure 1).

Data were statistically processed in Past v 2.17 [49].

The morphological features of RG in terms of plastic traits in different ecoregions were examined in three samples from mountainous rivers (Strviazh, Limnytsia, and Svicha) and four samples from plain rivers (main Dnieper channel, Seret, Smotrych, and Yagorlyk). Sample similarity was assessed within the principal component space using a total of 22 morphometric traits with an account of several environmental factors (flow velocity, projective cover of higher aquatic vegetation, and granulometric composition of bottom substrate). Specimens with incomplete datasets were not considered in the analysis.

In order to assess differences in the morphology of RG samples from mountainous and plain rivers, PCA was carried out using a set of morphometric traits, with an account
of some environmental factors (flow velocity using the OTTMFproWaterFlowMeter device, projective cover of higher aquatic vegetation, and substrate composition).

The significance of ecological factors in RG sample differentiation with respect to morphological traits was assessed using non-metric multidimensional scaling (NMDS), and it was based on a matrix of distances between the individual specimens calculated using Euclidean distances in 3D space. Morphological analysis was carried out in the combined mountainous and plain riversamples, and RG specimens were 3–7 cm long and 1–3 years old. Normality was tested using the Shapiro–Wilk test. Morphological analysis was carried out using the two-sample t-test. The correlation between flow velocity and specimens’ body length and between pebble portion in substrates and body length was established via acorrelation coefficient. The qualitative analysis of stomach content was carried out in 45 RG specimens. Each food category was identified individually. The material was weighed at 0.01 g.

3. Results

The analysis of the entiredataset revealed the occurrence of RG in 46 sites in the Dniester basin. According to catches of the Institute of Hydrobiology and catches by the authors since 1983, RG inhabited and still inhabits all areas of the ecoregion Pontic province (where it should not be considered invasive) and beyond its native range—in the Eastern plainecoregion and in the main channel of the Dniester River in the Carpathianecoregion. Outside the main channel in the Carpathian ecoregion, it was first recorded in 2000 when five specimens were caught in the Strviazh River near Zarichchia vil. at a height of 352 m a.s.l. In 2001, two specimens were caught in the Stryi River upstream of Rozgirche vil. at a height of 320 m a.s.l., and during 2002–2005, we recorded this species in the Svicha, Syvka, and Limnytsiarivers. On 23 July 2003, it was found by Polish specialists in the Strviazh River near the Ukrainian–Polish state boundary. Since 2009, the first RG specimens have been found at a height of above 400 m a.s.l. in the Opir and BystrytsiaNadvirnianskarivers (Table 1).

Table 1. Chronology of the first records of *B. gymnotrachelus* in the Dniester tributaries above 300 m a.s.l.

<table>
<thead>
<tr>
<th>Site</th>
<th>River</th>
<th>n of Specimens</th>
<th>Date</th>
<th>Height m a.s.l.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zarichchia village</td>
<td>Strviazh</td>
<td>5</td>
<td>12 June 2000</td>
<td>352</td>
</tr>
<tr>
<td>Rozgirche village</td>
<td>Stryi</td>
<td>2</td>
<td>12 July 2001</td>
<td>320</td>
</tr>
<tr>
<td>Koroscenko village (Poland)</td>
<td>Strviazh</td>
<td>2</td>
<td>23 July 2003</td>
<td>395</td>
</tr>
<tr>
<td>Broshniv-Ocada village</td>
<td>Syvka</td>
<td>3</td>
<td>2004</td>
<td>330</td>
</tr>
<tr>
<td>Vygodivka village</td>
<td>Svicha</td>
<td>2</td>
<td>2004</td>
<td>393</td>
</tr>
<tr>
<td>Berlogy village</td>
<td>Limnytsia</td>
<td>4</td>
<td>2005</td>
<td>329</td>
</tr>
<tr>
<td>Mezhybrody village</td>
<td>Stryi</td>
<td>2</td>
<td>2006</td>
<td>374</td>
</tr>
<tr>
<td>Dubyna village</td>
<td>Opir</td>
<td>2</td>
<td>2009</td>
<td>417</td>
</tr>
<tr>
<td>Nadvirna town</td>
<td>BystrytsiaNadvirnianska</td>
<td>1</td>
<td>2009</td>
<td>406</td>
</tr>
</tbody>
</table>

We have analyzed the records, biological characteristics, and feeding spectra of the species in some typical rivers beyond their native range in two samples from the Carpathian
and Eastern Plainecoregions. The maximal portion of both samples comprised two-year-old specimens with body lengths of 4–5 cm (Figure 3). On the contrast to the mountainous rivers, in the plain rivers portion of older specimens of the body length 6–8 cm also was big (see Figure 3).

![Figure 3](image-url)  
**Figure 3.** Size and age distribution of *B. gymnotrachelus* in the mountainous rivers of the Carpathian region (a) and the plain rivers of the Eastern plains (b).

In both samples, the mean ratio of males and females was close to 1:2. In mountainous rivers, most females were 4–5 cm long, and in the plain rivers, they were 5–6 cm long (see Figure 4). In both samples, males were somewhat larger than females, although this is not a characteristic of this fish species.

![Figure 4](image-url)  
**Figure 4.** Sexual distribution of *B. gymnotrachelus* in the mountainous rivers of the Carpathian region (a) and the plain rivers of the Eastern plains (b).

The abundance of RG in mountainous and plain rivers notably differed (Table 2), and this was likely dependent on a set of abiotic conditions (Table 3).

In mountainous rivers (Stryvazh, Svicha, and Limnytsia), the density and habitat characteristics were as follows.

In the Strviazh River near Zasadky village, RG’s density was 0.8 ind/100 m². The mean flow velocity amounted to 1.7 m/s, the depth measured up to 1 m, and the bottom was covered with pebbles. In the Svicha River near Liutynka village, the mean flow velocity was equal to 0.8 m/s, the depth measured up to 1.2 m, and the bottom was covered with gravel and pebbles and was partly silted. RG’s density was equal to 1.4 ind/100 m². In the Limnytsia River near the town of Kalush, RG’s density was equal to 1.2 ind/100 m², the mean flow velocity was 1.5 m/s, the depth measured up to 0.7 m, and bottom sediments consisted of pebbles and was covered in silt and filamentous algae.
Table 2. Density of *B. gymnotrachelus* (per 100 m²) and its biological characteristics in the mountainous and plain rivers of the Dniester basin (2019–2022).

<table>
<thead>
<tr>
<th></th>
<th>Density, ind/100 m²</th>
<th>Body Length, cm, M ± m</th>
<th>Body Weight, g, M ± m</th>
<th>Sexes Ratio, ♂ ♀</th>
<th>n of Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mountainous rivers—Carpathian ecoregion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strviazh</td>
<td>0.8</td>
<td>4.4 ± 0.35</td>
<td>1.7 ± 0.43</td>
<td>1:3</td>
<td>4</td>
</tr>
<tr>
<td>Svicha</td>
<td>1.4</td>
<td>4.5 ± 0.11</td>
<td>1.7 ± 0.14</td>
<td>6:1</td>
<td>7</td>
</tr>
<tr>
<td>Limnytsia</td>
<td>1.2</td>
<td>4.1 ± 0.49</td>
<td>1.5 ± 0.59</td>
<td>only ♀</td>
<td>6</td>
</tr>
<tr>
<td><strong>Plain rivers—Eastern plain ecoregion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dniester</td>
<td>3.8</td>
<td>5.6 ± 0.34</td>
<td>4.0 ± 0.80</td>
<td>1:1</td>
<td>19</td>
</tr>
<tr>
<td>Seret</td>
<td>4.6</td>
<td>5.6 ± 0.20</td>
<td>3.9 ± 0.04</td>
<td>1:2</td>
<td>23</td>
</tr>
<tr>
<td>Smotrych</td>
<td>3.0</td>
<td>4.1 ± 0.33</td>
<td>1.5 ± 0.38</td>
<td>1:2</td>
<td>6</td>
</tr>
<tr>
<td>Yagorlyk</td>
<td>1.8</td>
<td>5.9 ± 0.19</td>
<td>4.1 ± 0.46</td>
<td>only ♀</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 3. The main hydromorphological characteristics of *B. gymnotrachelus* habitats.

<table>
<thead>
<tr>
<th>River</th>
<th>River Type</th>
<th>Mean Flow Velocity, m/s</th>
<th>Projective Cover of Higher Vegetation, %</th>
<th>Sediments Composition, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Silt</td>
</tr>
<tr>
<td>Strviazh</td>
<td>mountainous</td>
<td>1.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Svicha</td>
<td>mountainous</td>
<td>0.8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Limnytsia</td>
<td>mountainous</td>
<td>1.5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Dniester</td>
<td>plain</td>
<td>0.7</td>
<td>7</td>
<td>30</td>
</tr>
<tr>
<td>Seret</td>
<td>plain</td>
<td>0.6</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Smotrych</td>
<td>plain</td>
<td>0.6</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Yagorlyk</td>
<td>plain</td>
<td>0.4</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

The density of RG in the plain rivers was notably higher. In the main channel of Dniester (near the towns of Staryi Martyniv and Galych), it amounted to 3.8 ind/100 m², the mean flow velocity in this section was 0.7 m/s, the depth is up to 1.5 m, and the bottom was covered with pebbles and silted in places. In the Seret River (near the villages of Ostrivets, Ugryn, and Lysivtsi), RG’s density was recorded at the maximum in this study at 4.6 ind/100 m². The mean flow velocity in this section amounted to 0.6 m/s, the depth measured up to 2 m, and the bottom sediments consisted of pebbles and silted sand. In some places, the silt layer reached 0.1 m. In the Smotrych River (near the town of Smotrych), the RG density was equal to 3.0 ind/100 m², flow velocity was 0.6 m/s, the depth measured 1.5–2 m, and bottom sediments consisted of gravel and pebbles, and the bottom was covered silt and sand layers up to 0.2 m thick. In the Yagorlyk River, the RG density value was the lowest of the considered plain rivers—1.8 ind/100 m²; the mean flow velocity was 0.3 m/s, and depth was measured at 0.4–0.6 m. The bottom sediments consisted of gravel and sand, and it was covered with silt layers up to 0.2 m thick.

Analyses of the feeding spectra of RG showed that Chironomidae larvae were the main diet component, which formed 35% of the food volume. Their occurrence frequency in the stomach content in mountainous and plain rivers amounted, respectively, to 72.5 and 95.7% (Figures 5 and 6). The only notable differences comprised the presence of Trichoptera larvae and the absence of fish larvae in the stomachs of RG from mountainous rivers. At the same time, Trichoptera were absent in the stomachs of specimens from plain rivers, whereas fish larvae formed up to 25% of the stomach content with an occurrence frequency of 91.3%. Other feeding categories were almost similar in the stomachs of specimens from both river types (see Figures 5 and 6).
Analyses of the feeding spectra of RG showed that Chironomidae larvae were the main diet component, which formed 35% of the food volume. Their occurrence frequency in the stomach content in mountainous and plain rivers amounted, respectively, to 72.5 and 95.7% (Figures 5 and 6). The only notable differences comprised the presence of Trichoptera larvae and the absence of fish larvae in the stomachs of RG from mountainous rivers. At the same time, Trichoptera were absent in the stomachs of specimens from plain rivers, whereas fish larvae formed up to 25% of the stomach content with an occurrence frequency of 91.3%. Other feeding categories were almost similar in the stomachs of specimens from both river types (see Figures 5 and 6).

Figure 5. Portion (%) of food items in the total stomach content of *B. gymnotrachelus* in mountainous rivers (Strviazh, Syvka, and Limnytsia) and plain rivers (Seret).

Figure 6. Occurrence frequency (%) of food items in *B. gymnotrachelus* feeding in the mountainous rivers (Strvyazh, Syvka, and Limnytsia) and plain rivers (Seret).

Morphological differences between RG samples from the rivers of Carpathian and Eastern plain ecoregions were examined using the two-sample Student’s *t*-test, and samples of specimens aged 1–3 years with body lengths of 3–6 cm were used to avoid the effects of size and age variability. Both samples were found to be normally distributed: in mountainous rivers, *n* = 14, *W* = 0.95, and *p* = 0.59; in plain rivers, *n* = 34, *W* = 0.97, and *p* = 0.60.

Notable confident differences with respect to the morphological characteristics of RG from mountainous and plain rivers were revealed in 9 (40.9%) of 22 considered morphometric traits (Table 4).

Specimens from mountainous rivers were characterized by smaller body dimensions with respect to length (*l*), depth (*h*), and maximal (*H*) and minimal (*h*) height; shorter antennal distance (*aA*), snout length (*lr*), and head height (*hc*); and maximal head width (*ic*), whereas the eye’s diameter (*do*) was larger.
Table 4. Morphological characteristics of *B. gymnotrachelus* samples from mountainous and plain rivers, *M* ± *m*. Confident values are given in **bold**.

<table>
<thead>
<tr>
<th>Traits</th>
<th>Mountainous Rivers, <em>n</em> = 14</th>
<th>Plain Rivers, <em>n</em> = 34</th>
<th>Student’s <em>t</em>-Test</th>
<th>Confidence Level, <em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>l</em>, mm</td>
<td>42.79 ± 1.32</td>
<td>51.59 ± 1.12</td>
<td>−4.52</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td><em>l</em> <em>cor</em></td>
<td>71.04 ± 0.37</td>
<td>71.95 ± 0.23</td>
<td>−2.12</td>
<td>0.0393</td>
</tr>
<tr>
<td><em>pl</em></td>
<td>16.33 ± 0.50</td>
<td>17.36 ± 0.30</td>
<td>−1.83</td>
<td>0.0738</td>
</tr>
<tr>
<td><em>IC</em></td>
<td>21.42 ± 0.29</td>
<td>20.83 ± 0.20</td>
<td>1.60</td>
<td>0.1154</td>
</tr>
<tr>
<td><em>H</em></td>
<td>15.58 ± 0.30</td>
<td>17.31 ± 0.22</td>
<td>−4.46</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td><em>dH</em></td>
<td>13.06 ± 0.34</td>
<td>14.90 ± 0.20</td>
<td>−4.93</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td><em>h</em></td>
<td>7.62 ± 0.16</td>
<td>8.47 ± 0.14</td>
<td>−3.52</td>
<td>0.0010</td>
</tr>
<tr>
<td><em>ih</em></td>
<td>3.14 ± 0.20</td>
<td>3.53 ± 0.12</td>
<td>−1.72</td>
<td>0.0915</td>
</tr>
<tr>
<td><em>aD</em></td>
<td>33.59 ± 0.23</td>
<td>33.37 ± 0.20</td>
<td>0.63</td>
<td>0.5329</td>
</tr>
<tr>
<td><em>aA</em></td>
<td>53.98 ± 0.40</td>
<td>57.82 ± 0.31</td>
<td>−6.94</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td><em>hD</em></td>
<td>12.79 ± 0.43</td>
<td>13.40 ± 0.28</td>
<td>−1.18</td>
<td>0.2434</td>
</tr>
<tr>
<td><em>ID</em></td>
<td>35.53 ± 0.54</td>
<td>34.91 ± 0.29</td>
<td>1.09</td>
<td>0.2814</td>
</tr>
<tr>
<td><em>H1</em></td>
<td>13.69 ± 0.49</td>
<td>13.75 ± 0.21</td>
<td>−0.14</td>
<td>0.8899</td>
</tr>
<tr>
<td><em>dH</em></td>
<td>13.69 ± 0.49</td>
<td>13.75 ± 0.21</td>
<td>−0.14</td>
<td>0.8899</td>
</tr>
<tr>
<td><em>IP</em></td>
<td>21.95 ± 0.63</td>
<td>22.09 ± 0.26</td>
<td>−0.25</td>
<td>0.8038</td>
</tr>
<tr>
<td><em>IV</em></td>
<td>20.07 ± 0.37</td>
<td>20.48 ± 0.21</td>
<td>−1.01</td>
<td>0.32</td>
</tr>
<tr>
<td><em>IA</em></td>
<td>28.78 ± 0.49</td>
<td>28.00 ± 0.39</td>
<td>1.12</td>
<td>0.2666</td>
</tr>
<tr>
<td><em>Ir</em></td>
<td>26.52 ± 0.81</td>
<td>31.86 ± 0.50</td>
<td>−5.68</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td><em>po</em></td>
<td>49.48 ± 0.96</td>
<td>48.69 ± 0.59</td>
<td>0.72</td>
<td>0.4757</td>
</tr>
<tr>
<td><em>do</em></td>
<td>29.01 ± 0.62</td>
<td>27.13 ± 0.39</td>
<td>2.58</td>
<td>0.0130</td>
</tr>
<tr>
<td><em>oi</em></td>
<td>9.14 ± 0.76</td>
<td>10.11 ± 0.41</td>
<td>−1.21</td>
<td>0.2309</td>
</tr>
<tr>
<td><em>hc</em></td>
<td>58.31 ± 0.73</td>
<td>69.50 ± 0.66</td>
<td>−9.89</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td><em>ic</em></td>
<td>71.91 ± 1.79</td>
<td>81.14 ± 0.88</td>
<td>−5.18</td>
<td>&gt;0.0001</td>
</tr>
</tbody>
</table>

Flow velocity and pebble portions in bottom substrates exhibited negative linear regression with respect to the body size of same-age specimens (Figure 7).

Figure 7. Linear regression applied on *B. gymnotrachelus* body size relative to flow velocity (a) and pebble portion (%) in bottom substrates (b).

PCA results (Figure 8) demonstrated a distribution of all samples into two clusters. The first combined samples were from mountainous rivers (Strviazh, Limnytsia, and Svicha), and the second combined samples were from plain rivers (Dniester, Seret, Smotrych, and Yagorlyk). The first principal component accounted for 27.6% of the values, and a strong
positive correlation was revealed in terms of body length ($r = 0.69$), body depth ($r = 0.63$), antennaldistance ($r = 0.67$), snout length ($r = 0.55$), head height ($r = 0.79$), maximal head width ($r = 0.66$), and negative correlation in terms of eye diameter ($r = -0.60$). A strong correlation was also revealed in the samples’ differentiation via the bottom substrate type (silt $r = 0.92$; sand $r = 0.83$) and projective cover of higher vegetation ($r = 0.71$), and a negative correlation was observed with respect to pebble portions in substrates ($r = -0.94$) and flow velocity ($r = -0.80$).

The second principal component accounted for 13.0% of values, and a strong positive correlation was revealed by the pectoral fin’s length ($r = 0.68$), caudal fin’s length ($r = 0.54$), and maximal body depth ($r = 0.54$) and by pebble portions in bottom substrates ($r = 0.62$), whereas a negative correlation was revealed due to the projective cover of higher vegetation ($r = -0.53$).

On the whole, it is evident that RG samples from the plain rivers of the Eastern plain ecoregion are close to one another in terms of morphological traits, which enabled their combination into one sample in order to facilitate further analyses of the morphological differences between samples from different ecoregions. Samples from the mountainous rivers of the Carpathian ecoregion were combined as well.

The results of the NMDS analysis demonstrated the samples’ separation by morphological traits and environmental factors (Figure 9). The Shepard plot parameters were equal to the following: stress = 0.0962; Axis 1 = 0.823; Axis 2 = 0.0786.

Pebble substrates and flow velocity were found to be the most determinative factors in mountainous rivers, and the silt layer was the most determinative in plain rivers.
Competition for feeding objects and spawning areas, as a rule, results in further expansion when the population increases and smaller specimens are forced to change habitats [10,50]. RG was also considered invasive in the transboundary of the Evros River basin [51].

For the first time, in the Carpathian ecoregion, we recorded this species in 2001 in the Stryi River, and in 2003, two specimens were caught in the Polish section of the basin in the Carpathian ecoregion [31]. In 2009, for the first time, in the Dniester basin, RG crossed the height mark of 400 m a.s.l., and in the upper section of the Strviazh River, RG crossed the height of 398 m a.s.l.; its population was so numerous that it dominated the local ichthyofauna [16,29,31,35]. The DNA analysis of the specimens demonstrated that RG in the Strviazh River exhibited Black Sea origins [16,35,52].

General analyses showed that the invasion of RG in the Dniester basin occurred in “splashes”: Until the early XX century, RG practically did not inhabit the upstream area of the middle Dniester section (upstream the Zbruch River’s mouth). In the first part of the XX century, it actively spread along the main Dniester channel. Starting from the 1980s to 1990s, RG actively expanded into left- and right-bank tributaries within the limits of the Eastern Plain ecoregion, and starting from the early 2000s, the species penetrated the Carpathian ecoregion and invaded the piedmont and mountainous sections of the rivers (see Figure 1).

The species actively expanded into the left- and right-bank tributaries of the Dniester River within the Eastern plain ecoregion (Bystrytsia, Tysmenytska and its tributaries Tysmenytsia, Ripchanka, Trudnytsia, and Seret). Surveys of the upper and middle sections of the Dniester basin carried out in 2018–2020 revealed many new localities of RG [40,53].

Moving upstream, RG occupies free ecological niches in these sections, and they are different due to the set of hydrological conditions, the availability of certain feeding objects, the occurrence of predators, etc. Due to adaptation to various habitats, RG developed

Figure 9. Effect of environmental factors on the differentiation of *B. gymnotrachelus* by morphological parameters: 1—combined sample of mountainous rivers (Strviazh, Svicha, and Limnytsia); 2—combined sample of plain rivers (Dniester middle section, Seret, Smotrych, and Yagorlyk); V—velocity; Veg—vegetation; S—sand; Si—silt; G—gravel; P—pebbles.

4. Discussion

RG is considered “potentially invasive” in the “blacklists” of Austria and Germany. Moving upstream, RG actively expanded into left- and right-bank tributaries within the limits of the Eastern Plain ecoregion, and starting from the early 2000s, the species penetrated the Carpathian ecoregion and invaded the piedmont and mountainous sections of the rivers (see Figure 1).
complex combinations of ecomorphological features, which are associated with movement, feeding, and environmental conditions [54]. The set of environmental factors affect body shape and manifests themselves as changes in meristic and morphological traits. Phenotypic variability and the flexibility of life processes facilitate successive invasions, enabling easy adaptation to different environmental conditions overall invasion stages [54]. In our opinion, the changes in morphological traits in the *B. gymnotrachelus* sample from the rivers of the Carpathian ecoregion were not related to size and age variability and sexual dimorphism. Examining the set of morphological traits, the RG sample from mountainous rivers confidently differed with respect to smaller body dimensions: body length (*l*), height and depth (*H*, *iH*, and *ih*), antennal distance (*Aa*), snout length (*lr*), head height (*hc*), and maximal head width (*ic*); in contrast, the eye’s diameter (*do*) was larger. These morphological modifications of RG in mountainous rivers indicated its adaptation to high flow velocity and pebble substrates (these two factors were the most significant).

Regarding the potential effects of RG on the local ichthyofauna, we should stress that food competition with aboriginal species is a key element of its negative effect. Insect larvae and imago and crustaceans, on which RG feeds, in the mountainous sections of the rivers are also the main feeding objects of rare species *Salmo trutta* Linnaeus, 1758; *Hucho hucho* (Linnaeus) 1758; and *Thymallus thymallus* (Linnaeus, 1758) [11,12], species which experience significant negative human impact in the Carpathian basin [55–60]; they exhibit decreasing population frequency, abundance, and ecological status. Thus, even the partial overlapping of the feeding spectra along with the low biomass of food organisms can cause tension in the trophic relations of fish. Depending on food availability, RG is able to modify its feeding strategy, and it has a wide spectrum of potential prey that facilitate its invasion into new habitats [3,61].

Most specialists tend to think that RG spreads due to intensive navigation. However, this is not true for the Dniester basin, as the river is not navigable. It was supposed that *B. gymnotrachelus* invasion into the Piedmont rivers of the Dniester basin was connected to the Stebnyk chemical enterprise catastrophe in 1983, which caused mass fish death within the river section from the Brystrytsia Tysmenytska’s inflow to the Dniester’s mouth; this section was 500 km long and had highly mineralized waters. Thus, it released many ecological niches in the rivers of the Dniester basin [41]. It is probable that this ecological catastrophe induced a series of morphological adaptations in RG, which enabled its active spread along the mountainous tributaries of the Dniester River.

We also suppose that the intensification of anthropogenic load on the rivers and climate change facilitated *B. gymnotrachelus* expansion upstream into the mountainous tributaries, and the species successfully occupies available ecological niches and competes with native fish species.

5. Conclusions

Thus, recently *B. gymnotrachelus* rapidly spread in the mountainous rivers of the Dniester basin in the Carpathian ecoregion at a height of above 300 m a.s.l. The occurrence frequency of *B. gymnotrachelus* in fish communities in the mountainous rivers of the Carpathian ecoregion remains relatively low at about 10–20%, whereas it ranges between 70 and 100% in plain rivers.

The main components of the *B. gymnotrachelus* diet were observed to be Chironomidae, Diptera, and Crustacea. Chironomidae larvae formed the maximal portion of diets in both mountainous and plain rivers (35%). In mountainous rivers, the feeding spectra of *B. gymnotrachelus* partially overlapped with the feeding spectra of rare native fish, including salmonids. The food competition with native fish is probably the key element of the negative impact of the racer goby on the Carpathian ichthyofauna. Fortunately, in mountainous areas, in the trophic context, native fish species larvae are not under direct threat as potential food for *B. gymnotrachelus*.

Specific environmental conditions of the mountainous rivers caused adaptive modifications with respect to the morphological traits at the subpopulation level, and these modifications...
manifested as a decrease in body size in same-agespecimens in rivers with a flow velocity above 1.5 m/s and pebble portions of above 50% in bottom sediments. Modifications showed an increase in body streamlining due to adaptation to flow velocity and turbulence in the mountainous rivers and stony substrate of the river bottom. Thus, the morphological adaptation of the species to the conditions of mountainous rivers is in progress, and the formation of stable populations in these rivers can be expected.

The Ponto–Caspian fauna to which the RG fish species belong has evolved in an unusually rich and peculiar way over the millennia due to its dynamic geology; this was observed in a series of lakes and seas with widely varying salinity and water levels and during the intermittent periods of separation and the establishment of connections between the basins of the Caspian and Black seas; connections between these seas and the Mediterranean Sea; and connections between the world’s oceans and seas [62,63]. This evolutionary history backgroung makes RG a skilled and invasive alien fish species in the Dnieper basin, and its expansion range is expected to extend in the future.

The relatively widespread racer goby fish species in the Marmara, Black, and Caspian Sea basins continue their expansion. Both the species’ biological and ecological characteristics that orient them toward conquering new areas and the resilience capacity of the native fish species, especially in mountainous ecosystems, exhibit ongoing spatiotemporal dynamics. By carrying out spatiotemporal trend assessments, monitoring, and management, this study highlighted the importance of morphological and trophic characteristic dynamics both for the racer goby, which is expanding, and for the existing native fish species, which still exhibit their resilience capacity to fight against the newcomers. All of this “fight for territory and resources” phenomenon is currently occurring in the well-known diversity basin of the Dniester River. This study’s results offer not only a new perspective on local fish fauna but also a better understanding of the associated risks of the studied aquatic ecosystems for management decision makers.


**Funding:** This research is based on the work supported by the State program of Ukraine “Support of priority fields of scientific researches” N 6541230 and received no external funding. The APS was kindly funded by Ecotur Sibiu.

**Institutional Review Board Statement:** The study was conducted under the permits of the Ministry of Ecology and Natural Resources: No. 2019/10 of 26 April 2019 for the Dniester (with tributaries) within the Lviv region; No. 2019/11 of 1 May 2019 for the Dniester (with tributaries) within the Ternopil region; No. 2019/12 of 14 May 2019 for the Dniester (with tributaries) within the Ivano-Frankivsk region; and No. 2020/4 of 25 September 2020 for the Dniester (with tributaries) within the Ivano-Frankivsk region. Fishing was carried out in accordance with the rules of the Order of the State Committee of Fisheries of Ukraine No. 19 of 15 February 1999. The work was performed in accordance with the bioethical requirements for animals in accordance with the Law of Ukraine “On Protection of Animals from Cruelty” of 21 February 2006. All work was performed in compliance with the international standards of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Strasbourg, 1986) and in accordance with the “General Ethical Principles for Animal Experiments”, and the study was approved by the First National Congress on Bioethics (Kyiv, 2001). Before formalin fixation, all fish were killed by overdosing using anesthetics (1.5–2.0 mL/L solution of 2-phenoxyethanol for 5 min). The method of “killing fish by overdosing on anesthetics” meets European bioethical requirements under Directive 2010/63/EU.

**Data Availability Statement:** All this study data can be obtained at request from the first correspondence author.
References


27. Burcea, A.; Boerag, I.; Mihiu, C.-M.; Bânăduc, D.; Matei, C.; Curtean-Bânăduc, A. Adding the Mureș River Basin (Transylvania, Romania) to the list of hotspots with high contamination with pharmaceuticals. *Sustainability* 2020, 12, 10197. [CrossRef]


31. Kukuła, K.; Bylak, A. Adding the Mureș River Basin (Transylvania, Romania) to the list of hotspots with high contamination with pharmaceuticals. *Sustainability* 2020, 12, 10197. [CrossRef]


34. Kessler, K. Description of fish belonging to families common to the Black and Caspian Seas.


42. Sabaniejew, L.P. Ryby Rosji. *Zhitni i lavlja (uzhieni) nasyshkh prsnovodnykh ryh*; Fizkultura i Sport: Moskwa, Russia, 1982; Volume 1.


46. Pravdin, I.F. *Guide to the Study of Fish; Pishhevaja promyslennost*; Moscow, Russia, 1966; 875p.

47. Pulypenko, Y.V.; Shevchenko, P.G.; Tsedyk, V.V.; Korniyenko, V.O. *Methods of Ichthyological Research (Tutorial); OLDI-PLUS:* Kherson, Ukraine, 2017; 432p.


52. Ohayon, J.L.; Stepien, C.A. Genetic and biogeographic relationships of the racer goby *Neogobius gymnotrachelus* (Gobiidae: Teleostei) from introduced and native Eurasian locations. *J. Fish Biol.* 2007, 71, 360–370. [CrossRef]


Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.