Czech University of Life Sciences, Prague

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The spread and impact of non-native aquatic organisms focusing on Ponto-Caspian Region

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Doctoral Dissertation

(compilation of published scientific articles)

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I, Tatia Kuljanishvili, hereby declare that I wrote the dissertation: „The spread and impact of non-native aquatic organisms focusing on Ponto-Caspian Region“ and it was prepared under the guidance of Prof. Kalous Lukáš and Prof. Bella Japoshvili, based on my own or group projects with my colleagues, using the scientific referecnces that are properly cited. Please note that articles presented in this work that are not ‚Open Access‘ are my personal copies and are printed here only for the dissertation purposes and can not be further distributed.

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Chapter 1: Introduction

Non-native species

Human activities have led to the introduction (intentional or unintentional) of non-native species into new areas, which poses a high risk for the native biota in many terms: predation, competition, hybridization, habitat modification, and transmission of pathogens (Vitule et al. 2009; Gozlan et al. 2010). Nowadays problematic introductions of aquatic species and their potential impact on native organisms have resulted in the need to assess the impact of traded and translocated animals.

Non-native species also called "non-indigenous" "alien", or "exotic" species, are species that were not occurring in the target area naturally before and that their occurrence in the area is caused by human activities (Copp et al. 2005). In the target area, after overcoming the obstacles, non-native species are becoming invasive (Richardson et al. 2000) and negatively affecting the biodiversity (Ricciardi 2007). Some non-native species established and started their spread to the vicinity as invaders. Therefore, biological invasions are pointed to as one of the most discussed topics in the conservation management of native species and their habitats (Hulme 2009).

The detailed survey leading to the valid estimations of introductions impacts of the species is crucial for improving wildlife management. Within the framework of possible approaches to prevent new invasions, it is necessary to compile lists of introduced aquatic animals, important for aquaculture and recreational fisheries and identify those that could be dangerous in terms of potential biological invasions. Hence, the prediction models were created with specific calibration on different groups of aquatic animals (Copp et al. 2009). These models comprehensively compare biological, ecological, ethological, geographical, and historical data and exploitation of the evaluated species. In terms of climate, the models compare the native area of distribution of a non-native species with a selected/target area for which the risk assessment is computed. According to the different specific algorithms within a specific model, evaluation of potential invasiveness classifies species into one of the categories: low, medium, and high risk (Copp et al. 2009). The great advantage of predictive models is warning of potentially dangerous species before they occur. The results of the modelling of invasiveness may, therefore, serve to relevant legislative authorities and nature conservation bodies as a basis for introducing prevention of restrictive measures, which aims to minimize the possibilities of new biological invasions (Copp et al. 2009; Almeida et al. 2013; Van der Veer and Nentwig 2015). Species identified as low risk, in terms of biological invasions, should not be affected by implemented restrictions and their commercial use, therefore, should not be restricted.

Research area

We have defined thr Ponto-Caspian Region as a study area. The Ponto-Caspian region covers the Black, Azov and Caspian seas, including their river basins (Danube, Dniester, Dnieper, Don, Kuban, Kura, Aras, Terek, Volga, Ural) that are the relicts of East Paratethys (Yanina 2014). The Parathethys once (from late Eocene to late Miocene) was a shallow sea that stretched from the Western Alps through the Central Europe till the Transcaspian Basin (Rögl 1999). By the end of the Eocene, it was isolated from the Mediterranean Sea, which was formed after the Tethys vanished due to the Indian continent collision with Asia (Rögl 1999). At the end of the Miocene (5mya), the tectonic fault in the northern Caucasus has caused the separation of the Black, Azov and Caspian Seas, however, there were some temporary connections between these seas sometimes (Reid and Orlova 2002). In the Pleistocene, due to glaciations, the Black Sea was connected with the Mediterranean Sea, which has resulted in the transition of marine conditions into the previously freshwater lake (Ryan et al. 1997). In the late glacial the meltwater from the ice sheets in northern Europe was flooding the Caspian Sea, which was then, through to Kuma-Manych strait, flowing into the Black Sea basin (Bahr et al. 2005).

All these changes that have been happening for millions of years have affected the salinity and altered the connections between the Black and Caspian Sea basins, which itself had a dramatic influence on the evolution and adaptation of the Ponto-Caspian fauna (Reid and Orlova 2002). Modern Ponto-Caspian fauna includes the species, that lived in the Tethys Sea and the marine ancestry has contributed to their ability to easily adapt to different salinity fluctuations and has resulted in Ponto-Caspian species invasiveness (Reid and Orlova 2002). The spread of the Ponto-Caspian species into the Baltic Sea and the Great lakes was facilitated by human actions since the 1960s. Interconnection of the rivers via canals opened the way for Ponto-Caspian fauna to invade new environments through ballast exchange (Reid and Orlova 2002). On the other hand, the region itself is also experiencing introductions of non-native species from different source areas that are not yet assessed, in certain areas of this region.

Considering the geographical history, Naseka (2010) analysed the freshwater fish species diversity and defined the Caucasian province, from other provinces that were previously delineated by Berg, 1940 (see Naseka, 2010). Naseka (2010) suggested division of Caucasian province into six districts that bear their own and unique ichthyofauna. We focused on two divisions of the Transcaucasia (the South Caucasus), the West and East Transcaucasus districts. In political terms, we focused on three countries: Armenia, Azerbaijan, and Georgia.

Armenia, Azerbaijan, and Georgia are countries in the South Caucasus and most of their territory belongs to the Kura-Aras River drainage and the smaller watersheds of the Black and Caspian Sea (Figure 1). Located on the crossroads of Europe and Asia, the South Caucasus area is diverse with landscapes, comprising areas of high, glacial mountains to the lowlands (areas below the sea levels), which has resulted in climate variability in the area (Zazanashvili et al. 2004). The place is characterized by species diversity and high endemism level and is included in the worlds biodiversity hotspot (Mittermeier et al. 2004), meaning that species presented in this area are considered as a biogeographic unit, that is also expressed as 'ecological islands' (Myers et al. 2000). As the area of high biological diversity, conservation and monitoring of native and endemic species are very important. The area harbours relict and endangered sturgeon species.

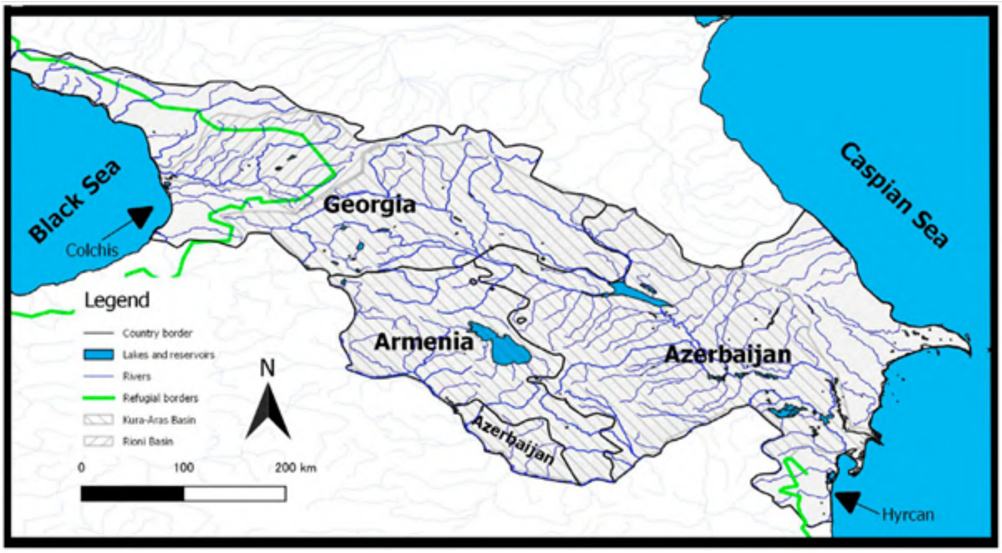

Figure 1. The map of freshwaters of Armenia, Azerbaijan and Georgia

Objectives

In order to identify the non-native freshwater fish species, first, we needed to revise all the existing freshwater species. Especially when the knowledge on freshwater biodiversity and distribution of freshwater fishes was significantly fragmented. The existing freshwater fish checklists of South Caucasian countries were outdated considering the recent taxonomic/nomenclatorial changes and distributional updates. Before there were old local publications (for instance: Abdurakhmanov, 1962; Ninua & Japoshvili, 2008; Pipoyan, 2012), sometimes on local languages limiting the species distribution only within the country borders and by creating this project and unifying these three countries we would make a big step in understanding fish distribution and diversity. Especially when this kind of work has not been done since the 1940s (see Berg, 1949).

Another important objective of this study was to create a communication, that would deal with the freshwater fish species and their local names in Georgia. This would help to avoid the mess in common names between the locals, and new species that did not have common names yet, would finally be perceivable. This work would have been important since: 1) common names are more easily adaptable among the non-scientific community; 2) the common name can play a significant role in communication efficiency among researchers, conservationists, decision-makers and local people; 3) this can affect the perception of the biodiversity and the conservation; and 4) species common names can help the biodiversity education.

In parallel to this research, we aimed to point out new species introduction records and evaluate their risks in the South Caucasian region. This would have been achieved via working on a few smaller projects to evaluate the future potential risks and their establishment that would largely contribute to the knowledge about non-native species in the area.

After revealing the freshwater biodiversity in the south Caucasian region, we aimed to discuss non-native species in detail. This would have been the first step in understanding non-native species in the region and establishing a reliable background for the risk assessment of non-native species and evaluation of their impact. The goal of this study was to comprehensively review the national legislative framework and policies to identify trends and gaps in non-native fish species management in the South Caucasus countries and to prepare an annotated list of non-native fish species with the information regarding the donor areas and pathways for fish introductions and evaluation of their establishment success.

Finally, our research would make it possible to evaluate the non-native species using Risk Assessment tools. These models comprehensively compare biological, ecological, ethological, geographical, and historical data and exploitation of the evaluated species.

Evaluation of potential invasiveness classifies species into one of the categories: low, medium, and high risk (Copp et al. 2009). This type of research would lead to discussions regarding the importance of the assessments and suggest future steps for the improvement of the management of non-native species and arguments, that should be considered by the stakeholders.

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Chapter 2: Checklist of the freshwater fishes of Armenia, Azerbaijan and Georgia

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Checklist of the freshwater fishes of Armenia, Azerbaijan and Georgia

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Abstract

We present a critical checklist of freshwater fish species found so far in the countries of Armenia, Azerbaijan and Georgia. In total 119 freshwater fishes are recorded. There are 40, 86 and 96 species currently known for Armenia, Azerbaijan and Georgia respectively. From these 119 species, seven are endemic and seven species are alien. From the alien species, only three (*Carassius gibelio*, *Gambusia holbrooki* and *Pseudorasbora parva*) can be considered as widespread and invasive. There are four species (*Gasterosteus aculeatus*, *Gobio artvinicus*, *Perca fluviatilis* and *Salmo gegarkuni*) that are translocated within the region. Seven species are confirmed or recorded for the first time including *G. artvinicus* and *Oxynoemacheilus veyselorum* for Armenia, Azerbaijan and Georgia, *Capoeta kaput* and *Rhinogobius lindbergi* for Azerbaijan and Georgia, *Capoeta razii* for Azebaijan, *Oxynoemacheilus cemali* and *Squalius agdamicus* for Georgia. In this checklist, *Acipenser colchicus* is treated as a synonym of *Acipenser persicus*. Sand smelts of the Black and Caspian Sea basin are identified as *Atherina caspia* and *Clupeonella caspia* is treated as a synonym of *Clupeonella cultriventris*. *Coregonus sevanicus* is listed as *Coregonus* sp. until the situation of Sevan whitefish is better understood. *Capoeta sevangi* and *Capoeta ekmekciae* are synonyms of *Capoeta capoeta*. The fish often identified as *Capoeta capoeta gracilis* from rivers south of the Kura most likely belong to *C. razii*. The Black and Caspian Sea *Rutilus* populations are treated as conspecific, therefore *R. kutum* is a junior synonym of *R. frisii*. *Oxynoemacheilus veyseli* is valid as *O. veyselorum*. We list the alien *Rhinogobius* species as *R. lindbergi*, however the name is provisional and needs further confirmation. All *Squalius* species from the Kura River drainage are identified as *S. agdamicus*, however in the Aras, it is replaced by *S. turcicus*. *Squalius orientalis* is treated as a valid species restricted to the eastern Black Sea basin. The four forms of Lake Sevan trout (*Salmo ischchan*, *S. gegarkuni*, *S. danilewskii* and *S. aestivalis*) are treated as valid species, two of them (*S. ischchan* and *S. danilewskii*) are extinct. *Rutilus sojuchbulagi* from Azerbaijan is also extinct.

KEYWORDS
Caucasus biodiversity hotspot, distribution, freshwater fishes, taxonomy

1 | INTRODUCTION

Nowadays, freshwater biodiversity is under heavy anthropogenic pressure (such as overexploitation, environmental pollution, habitat degradation/loss) and therefore, knowledge and monitoring of freshwater species and ecosystems under threat should be a routine for conservation planning (Dudgeon et al., 2006). To this end, reliable and accurate biodiversity data can serve as an important tool in effective conservation management (Frankham, Briscoe, & Ballou, 2002; Mace, Possingham, & Leader-Williams, 2006). However, significant challenges are related to developing countries harbouring great biodiversity. Due to a lack of biodiversity data, these areas continue to suffer inability of proper systematic conservation planning. The example of such area is the South Caucasus region, the biodiversity of which remains poorly documented (Mumladze, Japoshvili, & Anderson, 2019).

Armenia, Azerbaijan and Georgia are located mostly in the South Caucasus region, which is a part of biodiversity hotspot encompassing the Colchis and Hyrcan plio-pleistocene refugia (Figure 1) (Mittermeier et al., 2004). Though less studied, the area is known with an incredible high diversity of landscapes, habitats, species as well as elevated levels of endemism. According to Abell et al. (2008), the South Caucasus freshwater fish fauna belongs to three freshwater eco-regions (Western Transcaucasian, Kura-South Caspian and Western Caspian drainages) and harbours many species endemic to these eco-regions. Furthermore, one of the rivers of the southern Caucasus (the Rioni) is acting as one of the world's last refuge for critically endangered sturgeon species (Bacalbaşa-Dobrovici & Holčík, 2000; Guchmanidze, 2012).

Strong environmental changes started from former Soviet times and continue to occur during the last decades in the South Caucasus because of modernization and industrial development (Davtyan, 2014). Hence, the freshwater ecosystems are particularly vulnerable due to increasing land and freshwater consumption, water pollution and hydropower development. Accordingly, knowledge of freshwater biodiversity and particularly current diversity and distribution of freshwater fishes have a great potential to serve as a tool for the prioritization of conservation efforts (Skelton, Cambray, Lombard, & Benn, 1995). In spite of the recent increase of biodiversity research in the region (Mumladze et al., 2019), the knowledge on freshwater fishes still remains significantly fragmented. Along with some taxonomic uncertainties, major gaps exist in the knowledge of species distributions within the region and there is no quantitative evaluation of anthropogenic pressures freshwater fish are facing today. The inadequacy of the data was the reason why Myers, Mittermeier, Mittermeier, da Fonseca, and Kent (2000) did not include Caucasian freshwater fishes for estimating the vertebrate diversity in the original designation of the biodiversity hotspots. The existing freshwater fish checklists of South Caucasian countries (Armenia, Azerbaijan, Georgia) are outdated considering the recent taxonomic/nomenclatorial changes and distributional updates. For instance, after the collapse of the Soviet Union, a first updated checklist of Armenian fishes was compiled in 1998–2002 (Gabrielyan, 2001; Pipoyan & Tigranyan, 1998, 2002; see also Levin & Rubenyan, 2010) and the last book regarding Armenian ichthyofauna was issued in 2012 (Pipoyan, 2012). The simple list of fishes of Azerbaijan by Ibrahimov and Mustafayev (2015) was issued in Azerbaijani language, showing no significant improvement in comparison to Abdurakhmanov (1962). Based on literature review, checklist of fishes of Georgia was compiled by Ninua and Japoshvili (2008) and reissued in 2013 (Ninua, Japoshvili, & Bochorishvili, 2013). Naseka (2010) provided the checklist of freshwater fishes for wider biogeographical regions in the Caucasus and offered ecoregional delineation of the Caucasus and surrounding. This later checklist, however, is hardly applicable for country-level fish diversity analyses as well as for conservational purposes.

Thus, mostly outdated information and/or data deficiencies in terms of taxonomy, distribution and conservation status of freshwater fishes of the South Caucasian countries have resulted a necessity to assemble most up to date checklist of South Caucasian countries, which is aimed to serve as a baseline for future investigation and conservation of fish biodiversity in the region.

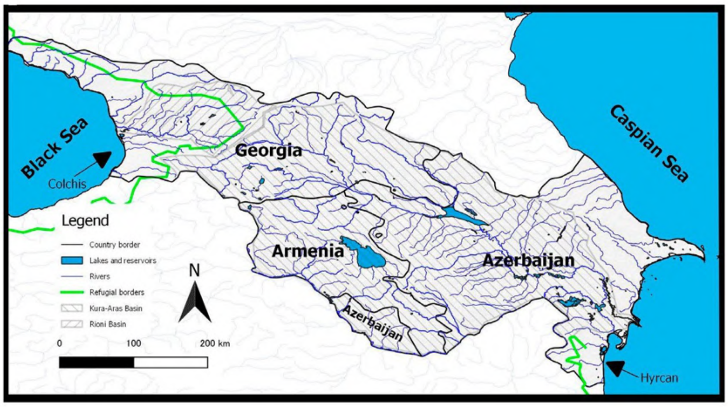

FIGURE 1 Map of freshwaters of Armenia, Azerbaijan and Georgia. Patterned areas show the main river drainages (the Kura and Rioni) encompassing almost 80% of the region. Green lines denote borders of two Caucaso-Anatolian refugia (Hyrcan and Colchis)

2 | MATERIALS AND METHODS

The study area covers the territories of Armenia, Azerbaijan and Georgia, the largest part of which is located south from the Great Caucasus Range, between the Black and Caspian Seas (Figure 1). The Eastern Black Sea basin is represented by a number of independent river systems over the territory of Georgia, one of them shared (the Coruh/Chorokhi system) with Turkey. There are other, smaller rivers including the Enguri, Supsa, Kintrishi, Coruh. The Rioni River is the largest among the eastern Black Sea tributaries in the area by means of basin extent and annual discharge (Figure 1). Almost all the South-Western Caspian Sea basin is represented by the Kura-Aras drainage discharging into the Caspian Sea from the territory of Georgia, Armenia, Azerbaijan and Turkey. Nearly 5% of the study area (north-eastern part of Azerbaijan and northern part of Georgia) is located in Northern Caucasus. This includes some headwater streams of the Terek River and other streams which originate in Georgia and discharges to the north-eastern part of Caspian Sea and some mountain streams (such as the rivers Samur, Mukhtadirchay, Gusarchay, Agchay, Garachay, Velvelechay, Gilgilchay, Atachay, Sumgaitchay, Pirsagat) of Azerbaijan.

To compile the regional checklist of fishes, we critically reviewed previously published country scale species lists (Gabrielyan, 2001; Ibrahimov & Mustafayev, 2015; Levin & Rubenyan, 2010; Ninua & Japoshvili, 2008; Pipoyan, 2012) and the literature published since then (see the references cited in the discussion section). In addition, species diversity was revised based on scattered morphological and molecular analysis done by the authors, allowing to propose changes for certain species distribution or taxonomy.

The checklist is presented in the form of a table (Table 1) which is ordered by families and genera according to the classification by Nsselson, Grande, and Wilson (2016) with the updates provided by Betancur-R et al. (2017) and the Eschmeyer's Catalogue of Fishes (Fricke, Eschmeyer & Van der Laan, 20202017). Alien and Endemic species are indicated in the table. Table also provides information about species, that were not mentioned before for Armenia, Azerbaijan and Georgia. Additional notes about distribution and taxonomy for certain species are also provided for several taxa in the discussion section.

3 | RESULTS

In total, 119 species, belonging to 26 families were recognised for the South Caucasian countries (Table 1). From these, 40 species are recorded from Armenia, 86 - from Azerbaijan, and 96 – from Georgia. This list also includes few north Caucasian species (*Barbus ciscaucasicus*, *Oxynoemacheilus merga*, *Romanogobio ciscaucasicus*, *Salmo ciscaucasicus*), mostly from Azerbaijani rivers north of Baku. Seven species (6% of the whole ichthyofauna) are endemic on a country scale level.

The region has also experienced introductions of non-native fishes. Currently there are seven (6% of all freshwater fish fauna) alien species introduced intentionally or accidentally (Table 1). From this species *Carassius gibelio*, *Pseudorasbora parva* and *Gambusia holbrooki* are considered as invasive species posing a serious threat to the native fauna and freshwater ecosystems (see also the following section). Unfortunately, there are no management strategies for any invasive species in any South Caucasian countries. There are four species which are translocated within the region: introduction of *Gasterosteus aculeatus* in the Caspian Sea basin is attributed to the opening of the Volga-Don channel in 1984 (Bogutskaya, Kijashko, Naseka, & Orlova, 2013; Musayev et al.,2004); *Gobio artvinicus* has been introduced to the Caspian Sea basin; *Perca fluviatilis* was translocated by fishermen from the Black Sea basin to the Kura River drainage; *Salmo gegarkuni* is endemic for Lake Sevan, however, it is introduced in lakes and reservoirs of Georgia and Azerbaijan.

Seven species are mentioned for the first time for the countries. These are *G. artvinicus* and *Oxynoemacheilus veyselorum* for Armenia, Azerbaijan and Georgia, *Capoeta kaput* and *Rhinogobius lindbergi* for Azerbaijan and Georgia, *Capoeta razii* for Azebaijan and *Oxynoemacheilus cemali* and *Squalius agdamicus* for Georgia.

4 | DISCUSSION

In the following, we are giving notes on several fish taxa, for which knowledge gaps are currently identified and provide additional argumentations with regards of distribution or taxonomy.

4.1 | Acheilognathidae

Based on morphological characters, *Rhodeus colchicus* was described from the River Natanebi in the Georgian Black Sea basin (Bogutskaya & Komlev, 2001) and the species is widespread from the North Caucasus to the south, not reaching Turkey. Molecular studies (Bartáková et al., 2019; Bohlen, Šlechtová, Bogutskaya, & Freyhof, 2006) revealed that *R. colchicus* is nested within *Rhodeus amarus*. In their phylogenetic analysis, Bartáková et al. (2019) found six different molecular groups of bitterlings in the western Palearctic, *R. colchicus* representing one of these. The phylogenetic structure of these six clades is poorly supported in the analysis by Bartáková et al. (2019) and it cannot be excluded, that *R. colchicus* might be the sister group of all other western Palearctic bitterling clades. Similar results have been obtained earlier by the study done by Bohlen et al. (2006). Geiger et al. (2014) suggest that lineages of European bitterlings are very closely related and might be better considered as a separate populations rather than species. However, as the consensus of species status of *R. colchicus* is not yet reached, we keep *R. colchicus* as a separate species in the presented list.

4.2 | Acipenseridae

The actual distribution of sturgeons in the study area is not well understood. *Acipenser sturio* and *A. nudiventris* have vanished from the Caucasus since no records for decades exists. Even whether other

TABLE 1 Checklist of freshwater fishes of Armenia, Azerbaijan and Georgia

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Acheilognathidae					
Rhodeus amarus (Bloch, 1782)			x	x	x
Rhodeus colchicus Bogutskaya & Komlev 2001					x
Acipenseridae					
Acipenser gueldenstaedtii Brandt & Ratzeburg 1833				x	x
Acipenser nudiventris Lovetsky, 1828				x	x
Acipenser persicus Borodin, 1897				x	x
Acipenser stellatus Pallas, 1771				x	x
Acipenser sturio Linnaeus, 1758					x
Huso huso (Linnaeus, 1758)				x	x
Anguillidae					
Anguilla anguilla (Linnaeus, 1758)				x	x
Atherinidae					
Atherina caspia Eichwald 1831				x	x
Clupeidae					
Alosa caspia (Eichwald, 1838)				x	
Alosa immaculata Bennett, 1835					x
Alosa kessleri (Grimm, 1887)				x	
Alosa maeotica (Grimm, 1901)					x
Alosa tanaica (Grimm, 1901)					x
Clupeonella cultriventris (Nordmann, 1840)				x	x
Cobitidae					
Cobitis amphilekta Vasil'eva & Vasil'ev, 2012				x	
Cobitis derzhavini Vasil'eva, Solovyeva, Levin, & Vasil'ev, 2020		x		x	
Cobitis saniae Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh & Esmaeili, 2017			x	x	x
Cobitis satunini Gladkov 1935					x
Sabanejewia aurata (De Filippi, 1863)			x	x	x
Sabanejewia caspia (Eichwald, 1838)				x	
Coregonidae					
Coregonus albula (Linnaeus, 1758)	x				x
Coregonus sp.	x		x		x
Cyprinidae					
Barbus ciscaucasicus Kessler, 1877				x	x
Barbus cyri De Filippi, 1865			x	x	x
Barbus rionicus Kamensky, 1899					x
Capoeta banarescui Turan, Kottelat, Ekmekçi & Imamoglu, s2006					x
Capoeta capoeta (Güldenstädt, 1773)			x	x	x
Capoeta kaput Levin, Prokofiev & Roubenyan 2019			x	x*	x*
Capoeta razii Jouladeh-Roudbar, Eagderi, Ghanavi & Doadrio, 2017				x*	
Capoeta sieboldii (Steindachner, 1864)					x
Carassius gibelio (Bloch, 1782)	x		x	x	x
Cyprinus carpio Linnaeus, 1758			x	x	x
Luciobarbus brachycephalus (Kessler, 1872)				x	x
Luciobarbus capito (Güldenstädt, 1773)			x	x	x
Luciobarbus mursa (Güldenstädt, 1773)			x	x	x

(Continues)

TABLE 1 (Continued)

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Esocidae					
Esox lucius Linnaeus, 1758				x	x
Gasterosteidae					
Gasterosteus aculeatus Linnaeus, 1758				(x)	x
Pungitius platygaster (Kessler, 1859)				x	
Gobiidae					
Babka gymnotrachelus (Kessler, 1857)				x	x
Mesogobius batrachocephalus (Pallas, 1814)					x
Neogobius fluviatilis (Pallas, 1814)			x	x	x
Neogobius melanostomus (Pallas, 1814)				x	x
Neogobius pallasi (Berg, 1916)				x	
Ponticola constructor (Nordmann, 1840)					x
Ponticola cyrius (Kessler, 1874)			x	x	x
Ponticola gorlap (Iljin, 1949)				x	x
Ponticola syrman (Nordmann, 1840)				x	x
Proterorhinus nasalis (De Filippi, 1863)				x	x
Gobionidae					
Gobio artvinicus Turan, Japoshvili, Aksu & Bektaş 2016			(x)*	(x)*	(x)*
Gobio caucasicus Kamensky, 1901					x
Pseudorasbora parva (Temminck & Schlegel, 1846)	x		x	x	x
Romanogobio ciscaucasicus (Berg, 1932)				x	
Romanogobio macropterus (Kamensky, 1901)			x	x	x
Leuciscidae					
Abramis brama (Linnaeus, 1758)			x	x	x
Acanthobrama microlepis (De Filippi, 1863)			x	x	x
Alburnoides eichwaldii (De Filippi, 1863)			x	x	x
Alburnoides fasciatus (Nordmann, 1840)					x
Alburnoides samiii Mousavi-Sabet, Vatandoust & Doadrio, 2016				x	
Alburnus alburnus (Linnaeus, 1758)				x	x
Alburnus chalcoides (Güldenstädt, 1772)				x	x
Alburnus derjugini Berg, 1923					x
Alburnus filippii Kessler, 1877			x	x	x
Alburnus hohenackeri Kessler, 1877			x	x	x
Ballerus sapa (Pallas, 1814)				x	x
Blicca bjoerkna (Linnaeus, 1758)			x	x	x
Chondrostoma colchicum Derjugin, 1899					x
Chondrostoma cyri Kessler, 1877			x	x	x
Chondrostoma oxyrhynchum Kessler 1877				x	
Leuciscus aspius (Linnaeus, 1758)			x	x	x
Leucaspius delineatus Heckel, 1843			x	x	x
Pelecus cultratus (Linnaeus, 1758)				x	
Petroleuciscus borysthenicus (Kessler, 1859)					x
Phoxinus colchicus Berg, 1910					x
Rutilus atropatenus Derjavin, 1937		x		x	
Rutilus frisii (Nordmann, 1840)				x	x

(Continues)

TABLE 1 (Continued)

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Rutilus lacustris (Pallas 1814)			x	x	x
Rutilus sojuchbulagi Abdurakhmanov 1950		x		x	
Scardinius erythrophthalmus (Linnaeus, 1758)				x	x
Squalius agdamicus (Kamensky 1901)				x	x*
Squalius orientalis Heckel, 1847					x
Squalius turcicus De Filippi, 1865			x	x	x
Vimba vimba (Linnaeus, 1758)				x	x
Lotidae					
Lota lota (Linnaeus, 1758)				x	
Moronidae					
Dicentrarchus labrax (Linnaeus, 1758)					x
Mugilidae					
Chelon auratus (Risso, 1810)				x	x
Chelon labrosus (Risso, 1827)					x
Chelon ramada (Risso, 1827)					x
Chelon saliens (Risso, 1810)				x	x
Mugil cephalus Linnaeus, 1758					x
Nemacheilidae					
Oxynoemacheilus bergianus (Derjavin, 1934)			x	x	x
Oxynoemacheilus brandtii (Kessler, 1877)			x	x	x
Oxynoemacheilus cemali Turan, Kaya, Kalayci, Bayçelebi & Aksu 2019					x*
Oxynoemacheilus lenkoranensis (Abdurakhmanov, 1962)				x	
Oxynoemacheilus merga (Krynicki, 1840)				x	
Oxynoemacheilus veyselorum Cicek, Eagderi & Sungur, 2018			x*	x*	x*
Oxudercidae					
Knipowitschia caucasica (Berg, 1916)			x	x	x
Knipowitschia longecaudata (Kessler, 1877)				x	x
Rhinogobius lindbergi Berg, 1933	x			x*	x*
Percidae					
Perca fluviatilis Linnaeus, 1758			(x)	x	x
Sander lucioperca (Linnaeus, 1758)			x	x	x
Petromyzontidae					
Caspiomyzon wagneri (Kessler, 1870)				x	
Lampetra ninae (Naseka, Tuniyev & Renaud 2009)					x
Poeciliidae					
Gambusia holbrooki Girard, 1859	x		x	x	x
Salmonidae					
Salmo aestivalis Kessler 1877		x	x		
Salmo caspius Kessler, 1877			x	x	x
Salmo ciscaucasicus Kessler, 1877				x	x
Salmo coruhensis Turan, Kottelat & Engin 2010					x
Salmo danilewskii Gulelmi 1888		x	x		
Salmo gegarkuni Kessler, 1877		x	x	(x)	(x)
Salmo ischchan Kessler 1877		x	x		
Salmo labrax Pallas 1814					x

(Continues)

TABLE 1 (Continued)

Taxa	Alien	Endemic	Armenia	Azerbaijan	Georgia
Salmo rizeensis Turan, Kottelat & Engin 2010					x
Siluridae					
Silurus glanis Linnaeus, 1758			x	x	x
Syngnathidae					
Syngnathus abaster Risso, 1827					x
Syngnathus caspius Eichwald 1831				x	
Tincidae					
Tinca tinca (Linnaeus, 1758)				x	x
Xenocyprididae					
Hemiculter leucisculus (Basilewsky, 1855)	x			x	
Total	7	7	40	86	96

Note: A cross – x, indicates the occurrence of the species in corresponding column; a cross in parenthesis – (x), indicates species translocated within the region, an asterisk – *, indicates the species that were not mentioned before for the countries.

sturgeon species still spawn regularly in the Kura and Rioni rivers, is doubtful. *Acipenser colchicus* was considered the Black Sea population of the Persian sturgeon (*Acipenser persicus*) by Marti (1940) or even the eastern Black Sea population of the Russian sturgeon *A. gueldenstaedti* by Berg (1949). It has been accepted as a valid and the Black Sea endemic species by Kottelat and Freyhof (2007). Following Marti (1940) we identify *A. colchicus* as the Black Sea population of *A. persicus* until the separate species status is confirmed.

Acipenser ruthenus has been recorded from the Kura River drainage in Azerbaijan and Berg (1949) mentions occasional findings from the western Caspian coast and the lower Kura River. However, there is no record or indication that there had ever been an established population of this species in the area. It is suspected, that sterlets found in the Kura mouth were migrants from the Volga population. The anadromous sterlet population in the Volga has vanished in the 20th century (Kottelat & Freyhof, 2007) and more recent records of sterlets are very likely to have come from fish farms. It should be mentioned that, apart from anadromous populations, there are resident populations within the Volga drainage, for instance the Oka and Sura Rivers populations (Ivancheva & Ivanchev, 2008; Shilin, 2001).

4.3 | Anguillidae

Anguilla anguilla is occasionally found in the Caspian Sea basin, for example in the Aras River in Armenia in 2015 (Pipoyan, 2015). As this species only spawns in the Atlantic Ocean, all records in the Caspian Sea basin must originate from stocking or from individuals which reach the Caspian Sea basin through canals.

4.4 | Atherinidae

Atherina caspia is widespread in the Black and Caspian Seas, where it is often identified as *A. boyeri* or *A. pontica* (see Vasil'eva, 2017). Naseka and Bogutskaya (2009), based on Tarasov (2001), treat *A. caspia* as a separate species. Own (unpublished) molecular and morphological studies support the Black and Caspian Sea populations as a distinct species from the Mediterranean *A. boyeri*, but fail to distinguish *A. pontica* (the Black Sea) from *A. caspia* (the Caspian Sea). *A. caspica* has been earlier treated as a valid species by Naseka and Bogutskaya (2009), while *A. pontica* was not. Here we also treat *A. pontica* as a synonym of *A. caspia*.

4.5 | Clupeidae

The diversity of shads (*Alosa*) from the Black and Caspian Seas is poorly understood and early authors follow Berg (1949) listing 15 species and forms from the Caspian Sea only. The number of species has not been reviewed comprehensively in recent years and we treat the species accepted by Kottelat and Freyhof (2007) as valid awaiting a sound taxonomic review especially of the Caspian marine species. *Alosa curensis* is sometimes mentioned as a valid species from Azerbaijan. This very poorly known shad was only found once by Suvorov (1907) and Berg (1949) mentioned that it is only known from the types collected from Kizilagach Bay (Qizilagac Bay) in Azerbaijan. *Alosa curensis* has teeth on the jaws, 31–43 gill rakers, and the gill rakers length exceed the length of the gill filaments. With this combination of characters, *A. curensis* keys out as *A. sphaerocephala* with the identification key given by Berg (1949) and we cannot exclude that it might be a synonym of this marine species. Since no records of *A. curensis* exist from freshwaters, we therefore excluded it from the current checklist.

Clupeonella cultriventris is widespread along coasts of the Black and Caspian Seas, entering lower reaches of rivers. Own (unpublished) molecular and morphological studies fail to distinguish the Caspian population (*C. caspia*) from the Black Sea population (*C. cultriventris*). Hoestlandt (1991) followed by Kottelat and Freyhof (2007) separate these two tyulkas largely based on the length of paired fins, a character we found to be very much overlapping in own materials examined. Therefore, we treat *C. caspia* as a synonym of *C. cultriventris*.

Clupeonella tscharchalensis is described from Lake Chelkar (47°50′N 59°36′E) in Kazakhstan. It is considered to be a freshwater species inhabiting rivers of the northern Caspian Sea basin (Hoestlandt, 1991) and to be invasive in the Don River. Tyulkas analysed from the Don are identified as *C. cultriventris* by own (unpublished) molecular and morphological data. Until tyulkas from Lake Chelkar are further studied, we treat *C. tscharchalensis* as a valid species having less gill-rakers than *C. cultriventris*.

4.6 | Coregonidae

Coregonus albula was introduced to Tabatskuri and Paravani Lakes (southern Georgia) from the Volkhov Hatchery at Ladoga Lake in 1930s (Barach, 1941). The local populations are still present and commercially valuable in Georgia though the abundance is decreasing due to the unavailability of local hatcheries (Kuljanishvili, Mumladze, Kalous, & Japoshvili, 2018).

The taxonomic status of the introduced Caucasian whitefish remains unsolved. Based on literature (Barach, 1940; Dadikyan, 1964; Mailyan, 1957) two species were introduced to Lake Sevan in the 1920s: *Coregonus ludoga* from Lake Ladoga and *Coregonus maraenoides* from Lake Chudskoe (both in Northern European Russia). These two species naturalised and hybridised in the lake (Mailyan, 1957) and an intermediate phenotype was subsequently described as a *Coregonus lavaretus sevanicus* by Dadikyan (1986). *Coregonus sevanicus* might represent a valid species of hybrid origin. However, due to scarcity of recent data we cannot confirm or reject any taxonomic hypothesis related to Caucasian whitefishes. Thus, we list only *Coregonus* sp. in the checklist. According to Elanidze (1983) in 1930-ies *C. ludoga* was also introduced from Volkhov hatchery to Lake Tabatskuri in Georgia through the species was not recorded for at least the last 50 years. As there are no recent records of any *Coregonus* from Lake Tabatskuri, we suspect that the population might be extirpated.

4.7 | Cyprinidae

There are three species of *Barbus* distributed in the region. *Barbus ciscaucasicus* in northern parts of Georgia and Azerbaijan, *B. cyri* in the Kura and Aras drainage and *B. rionicus* in the Black Sea basin in the west Georgia. The recent study done by Levin, Gandlin, et al. (2019) has shown that *Barbus goktschaicus* does not differ from *B. cyri* genetically and they both are distributed in the Kura-Aras system. Therefore, *B. goktschaicus* is synonymised with *B. cyri*.

Capoeta capoeta is widespread in the Kura and Aras drainages. Occurs in Lake Sevan and in Lake Urmia basin. *Capoeta sevangi* from Lake Sevan is treated as a valid species by Gabrielyan (2001), Ninua and Japoshvili (2008) and Ninua et al. (2013) without presenting any evidence. Levin, Prokofiev, and Roubenyan (2019) identify the *Capoeta* from Lake Sevan as *C. capoeta*, and we follow Levin, Gandlin, et al. (2019) treating *C. sevangi* as a synonym of *C. capoeta*, also supported by our own (unpublished) molecular data.

Capoeta ekmekciae is considered to be endemic to the Eastern Black Sea basins from the Rioni River south to the Coruh. The molecular sequences that were analysed by Bektas et al. (2017) suggest that this species is very closely related to *C. capoeta*. After checking the morphological characters given by Turan, Kottelat, Gülsün Kirankaya, and Engin (2006) to distinguish this species from *C. capoeta*, we found them largely overlapping. As we could not find other distinguishing characters between *C. capoeta* and *C. ekmekciae* in our own materials, we treat *C. ekmekciae* as a synonym of *C. capoeta*.

Capoeta gracilis was described from the area of Isfahan in Iran, this name is not available for the *Capoeta* species in the Caspian Sea basin. Despite, the fish from rivers south of the Kura was often identified as *Capoeta capoeta gracilis*. This fish most likely belong to *C. razii* that was described from the eastern Caspian Sea basin in Iran by Jouladeh-Roudbar, Eagderi, Ghanavi, and Doadrio (2017). Jouladeh-Roudbar et al. (2017) already reported this species to be very widespread in the rivers of the Iranian Caspian coast north-west to the Choobar River, close to the Azerbaijan border.

Carassius gibelio is widely introduced all over the study area. It appeared in the late 1970-ies in Armenia (Pipoyan & Rukhkyan, 1998), followed by record from Georgia in the middle 1980th by Daraselia (1985) (as *C. carassius*). Currently *C. gibelio* is the most widespread and abundant invasive species which distribution ranges from lowland waterbodies as high as mountain lakes that are up to 2,100 m above sea level (Bogutskaya et al., 2013; Gabrielyan, 2001; Japoshvili, Mumladze, & Küçük, 2013; Japoshvili, Mumladze, & Murvanidze, 2017; Kuljanishvili, Japoshvili, Mumladze, & Kalous, 2018; Oganesyan & Smolej, 1985; Pipoyan, 1993, 2012; Pipoyan & Rukhkyan, 1998). While *C. gibelio* and *C. auratus* can be distinguished by molecular characters (Rylková, Kalous, Bohlen, Lamatsch, & Petrtýl, 2013), there is no agreed set of morphological characters to separate both species and we cannot exclude that many records of *C. gibelio* actually refer to *C. auratus*. It should also be noted, that *Carassius langsdorfi* as an additional, superficially very similar species (Vetešník, Papoušek, Halačka, Lusková, & Mendel, 2007) is also expected to occur in the area.

4.8 | Gobiidae

Ponticola iranicus had been described by Vasil'eva, Mousavi-Sabet, and Vasil'ev (2015) from the Gisum and Sefid Rivers in Iran. As the fauna of the Sefid River is relatively similar to the fauna of coastal rivers of the Azerbaijani coast and the Aras, it is expected to find this species here in the future.

4.9 | Gobionidae

Gobio artvinicus was described from the lower Coruh River in Turkey and is expected to occur also in the Georgian part of the same river. The species is widespread in the Georgian Black Sea basin, where it is at least confirmed to the north to the Rioni River. Gudgeons are alien in the Kura and Aras River drainage. Gudgeons discovered in the Metsamor River (Armenia) in 1998 and identified as *Gobio gobio* by Pipoyan (1998) might also belong to *G. artvinicus*. The fish found in the Tashir and Debed rivers (The Kura river drainage) in Armenia are also morphologically similar to *G. artvinicus*. Own (unpublished) sequence data identify the *Gobio* species alien in the Kura in Georgia and Azerbaijan as *G. artvinicus* and the species might be widespread also in the Aras.

The species identity of other *Gobio* species in the region remains unclear. *Gobio caucasicus* was described as *Gobio lepidolaemus* var. *caucasica* by Kamensky 1901 based on a syntype series from three rivers: the Rioni in the Georgian Black Sea basin, the Podkumok in northern Dagestan, and the Sulak in southern Dagestan, both in the Caspian Sea basin. Furthermore, *Gobio holurus* was described from the Terek River drainage, also in the Caspian Caucasus. Kottelat and Freyhof (2007) treat *G. holurus* as a valid species and Turan, Japoshvili, Aksu, and Bektaş, (2016) treat *G. caucasicus* as a valid species. Our own (unpublished) molecular data suggest that at least two species (excluding *G. artvinicus*) might be involved in the Caucasian gudgeon complex and both seem to occur in the Black Sea as well as in the Caspian Sea basin in northern Caucasus and both are closely related, if not conspecific with adjacent species as *Gobio kubanicus*, *G. krymensis* and *G. brevicirris*. Further study is needed to unambiguously resolve the Caucasian gudgeon complex in the future.

Pseudorasbora parva was accidentally introduced together with Chinese carps fry (e.g. *Hypophthalmichthys nobilis*, *H. molitrix* or *Ctenopharyngodon idella*) from Asia followed the establishment of viable populations throughout the region (Bogutskaya et al., 2013; Gabrielyan, 2001; Naseka & Bogutskaya, 2009; Ninua & Japoshvili, 2008; Pipoyan, 2012; Pipoyan & Arakelyan, 2015). The species is still expanding its range in the study area and recently it was discovered in closed mountain lake Kartsakhi (N41.216 E43.231) in the Javakheti highland (Southern Georgia) (own unpublished data).

4.10 | Leuciscidae

Alburnoides samii was found by Levin, Simonov, Matveyev, et al. (2018) in the Lenkoran River in Azerbaijan. Future studies might show that some *Alburnoides* populations in rivers and streams south of the Kura and Aras drainages belong to this species.

Alburnus chalcoides is widespread in Azerbaijan and Georgian part of the Kura basin. It is also expected to occur in the Armenia (the Aras River) but has not yet been recorded here.

Rutilus caspicus from the Caspian Sea basin, *R. heckelii* from the Black Sea basin, and *R. shelkovnikovi* from the Metsamor River in the Ararat Valley (Armenia) were synonymised with *R. lacustris* based on their mtDNA identity (Levin et al., 2017). This opinion is followed here.

Rutilus kutum is treated as a valid species by Bogutskaya and Iliadou (2006: 294) and following authors without a detailed discussion. The gene trees presented by Kotlík et al. (2008) showed clear geographical structure between the Black and Caspian Sea populations but the two populations were not reciprocally monophyletic at any of the three loci studied and there had been considerable gene flow suggested from the Caspian population into the Black Sea population. Own (unpublished) molecular data (COI) fail to distinguish the Caspian *R. kutum* from the Black Sea *Rutilus frisii*. We were unable to find evidence why both species should be separated despite that they are frequently listed in un-commented checklists. Therefore, we treat both populations as conspecific, *R. kutum* being a junior synonym of *R. frisii*.

Rutilus sojuchbulagi was known only from the Akstafa Region in Azerbaijan where it has not been found despite of intensive fieldwork and specific research during the last ten years. Therefore, we assume that this species has gone extinct. *R. atropatenus* and *R. sojuchbulagi* from Azerbaijan were initially described as members of the genus *Rutilus* (Abdurakhmanov, 1950; Derzhavin, 1937) but later placed in *Pseudophoxinus* by Bogutskaya, Küçük, and Atalay (2006) without detailed discussion or arguments. Saç, Özuluğ, Geiger, and Freyhof (2019) place both species back to *Rutilus* based on molecular COI sequence data confirming the original generic placement (Abdurakhmanov, 1950; Derzhavin, 1937) Future research based on nuclear molecular data might again challenge this placement.

Squalius agdamicus was described from a small river called the Kuyra at Agdam town, not reaching the Kura River (Berg, 1949). Doadrio and Caramona (2006) treat this species as valid and our own (unpublished) data strongly suggest that it is widespread all over the Kura River drainage, but there is no indication, that it also occur in the Aras, where it is replaced by *Squalius turcicus*.

Squalius orientalis is treated as a valid species restricted to the eastern Black Sea basin (Khaefi, Esmaeili, Sayyadzadeh, Geiger, & Freyhof, 2016) and identifications are largely based on the COI gene of *Squalius* from the Ashe River in Russia, south to the Coruh River in Turkey. Its distribution range might be much larger and this molecular lineage is very closely related to a molecular lineage that is widely distributed in the northern Black Sea basin as well as in the Baltic Sea basin west to the Elbe River in the Czech Republic and Germany, where it is identified as *S. cephalus*.

Squalius turcicus was described from the upper Aras River in Turkey. Özulug and Freyhof (2011) suggested that this might be the species distributed in the southern Caspian Sea basin. Turan, Kottelat, and Doğan (2013) treat *S. turcicus* as a distinct species from *S. orientalis*. Also Khaefi et al. (2016) treat *S. turcicus* as a valid species. Accordingly, all previous notes on *Squalius* species from Aras river is treated as *S. turcicus*. As of now, there is no comprehensive distribution data available for *S. turcicus* and *S. agdamicus* in the Caspian Sea drainage and they might frequently live in sympatry.

Apparently, more data is needed to better understand the distribution of these two species.

4.11 | Lotidae

Only single individual of *Lota lota* was caught in 1920 in the Kura River which was considered as an accidental migrant from the northern Caspian basin species (Abdurakhmanov, 1962; Derzhavin, 1949; Musayev et al., 2004). However, *L. lota* also occurs in Iran and might have spread from this area to the north.

4.12 | Nemacheilidae

Oxynoemacheilus cemali was described from the Coruh River drainage in north-eastern Turkey by Turan, Kaya, Kalayci, Bayçelebi, and Aksu (2019). The species is expected to occur in the Georgian part of the river also.

Oxynoemacheilus merga is distributed in northern Caucasian rivers (the Kuma, Terek, Sulak) which flow to the Caspian Sea. Although it has not been recorded in Georgia yet, we assume that the fish could be distributed in the European part of the country.

Oxynoemacheilus veyseli was described from the upper Aras River in Turkey by Çiçek, Eagderi, and Sungur (2018). The species name is incorrect and must be replaced by *O. veyselorum*, since this species was named after two men, the father and son of the first author (see Çiçek et al., 2018). Loaches usually identified as *O. angorae* in the Kura and Aras Rivers (Abdurakhmanov, 1962; Barach, 1940, 1941; Berg, 1949; Dadikyan, 1986; Elanidze, 1983; Gabrielyan, 2001; Levin & Rubenyan, 2010; Ninua & Japoshvili, 2008; Pipoyan, 2012; Pipoyan & Tigranyan, 1998) might largely belong to *O. veyselorum*.

Overall, the systematics of *Oxynoemacheilus* species in the study area is still poorly understood and the taxonomic status of species such as *O. angorae alasanicus*, *O. bergi*, *O. brandti gibbusnazus*, and *O. lenkoranensis* need to be revisited.

4.13 | Oxudercidae

Betancur-R et al. (2017) recognised Gobionellidae as a junior synonym for Oxudercidae. Here we follow Betancur-R et al. (2017) and update the family name for the species which were before placed in Gobionellidae as Oxudercidae.

Knipowitschia bergi, sometimes considered as *Hyrcanogobius bergi*, has not been found in the last decades and new field data is encouraged to test, if this species might actually be extirpated in the area.

The *Rhinogobius* species, considered invasive in the Caspian Sea basin, seems to be now widespread also in the Caspian part of the study area (Japoshvili et al., 2020) and is expected to enter the Black Sea basin soon. There had been several studies aiming to identify this species, which was treated as *R. cheni* (Vasil'eva & Kuga, 2008), *R. similis* (Esmaeili et al., 2014), and *R. lindbergi* (Esmaeili, Sayyadzadeh, Eagderi, & Abbasi, 2018; Sadeghi, Esmaeili, Zarei, Esmaeili, & Abbasi, 2019). The name of this species in our list is provisional and needs further confirmation.

4.14 | Salmonidae

The diversity of Caucasian trouts is very poorly understood, what is related to the very recent evolutionary history of all species. Actually, trouts of the northern Caspian Caucasus are identified as *S. ciscaucasicus*, described from the Keyranchay River. Those of the southern Caucasus are identified as *S. caspius*, described from the Kura in Azerbaijan. Ninua, Tarkhnishvili, and Gvazava, (2018) support this view and suggest using *S. caspius* for trouts from the rivers flowing into the Caspian Sea south of the Caucasus and *S. ciscaucasicus* for that found in the Terek River and other rivers flowing into the Caspian north of the Caucasus. This system might be oversimplified and more research will for sure challenge our understanding of the species diversity of Caspian trouts. The Black Sea trouts were usually identified as *S. labrax* until Turan, Kottelat, and Engin (2009) showed that within the Coruh River drainage, two forms coexist, which they named *S. rizeensis* and *S. coruhensis*, the former purely riverine and the latter anadromous. Ninua et al. (2018) suggested that the mitochondrial haplogroup of *S. rizeensis* was never found in fish caught in the Black Sea. Ninua et al. (2018) support *S. rizeensis* and *S. coruhensis* as own species different from *S. labrax* but indicate some hybridisation between *S. coruhensis* and *S. labrax* in the Georgian Caucasus, a situation expected to happen in two anadromous, migratory species at their distribution border area.

The trouts of the *Salmo ischchan* complex are endemic to Lake Sevan (Armenia). Four "forms" of Sevan trouts are generally recognised (*S. ischchan*, *S. gegarkuni*, *S. danilewskii* and *S. aestivalis*) (Barach, 1940; Dadikyan, 1986; Gabrielyan, 2001; Levin, Simonov, Rastorguev, et al., 2018; Pipoyan, 2012). These have been interpreted as subspecies, ecological forms or life-history types. The available evidence strongly suggests that they had been species following the Evolutionary Species Concept (see Kottelat, 1997 for definitions). The four trout species were very closely related and might have been the result of sympatric speciation events happening after the last glacial times (<10,000 years ago). While Sevan trout diversity has been poorly studied, this very interesting example of intra-lacustrine trout speciation has already been lost. The abundance of all Sevan trouts drastically declined in the twentieth century due to overfishing and loss of spawning grounds due to water diversion and a critical 18.5-m drop of the lake water level finally leading to the extinction of two of the endemic species. *S. ischchan* and *S. danilewskii* are considered to be extinct since the 1980-ies (Levin & Rubenyan, 2010; Pipoyan, 2012). The two other species, *S. gegarkuni* and *S. aestivalis* are believed to still exist but they are largely or completely dependent on artificial reproduction and stocking.

4.15 | Syngnathidae

According to Berg (1949) there was *Syngnathus nigrolineatus* distributed in the Black Sea (Lake Paliastomi, the Rioni delta, Lake Nuriegel in Batumi, riverlets in Batumi region) and subspecies *S. n. caspius* in whole Caspian Sea and downstream of the Kura River. Naseka and Bogutskaya (2009) elevated *S. n. caspius* to the species level (*S. caspius*) manly based on the arguments provided in Tarasov (2001). Here we follow Naseka and Bogutskaya (2009) treating *S. caspius* as a valid species from the Caspian Sea basin.

4.16 | Xenocyprididae

Hemiculter leucisculus is fast expanding its range into the southern Caspian Sea basin in Iran (Zareian, Esmaeili, Zamanian Nejad, & Vatandoust, 2015) and was recently recorded from Azerbaijan by Mustafayev, Ibrahimov, and Levin (2015). Its invasion of the Black Sea basin is only a question of time.

5 | CONCLUSION

During the period of the Soviet Union, large-scale industrial projects have taken place in all the republics of the Southern Caucasus. This resulted in strong impacts on freshwater biodiversity of the whole region (Freyhof et al., 2015). For example, construction of the Mingachevir Dam in Azerbaijan blocked the migration path for Caspian lamprey, shads and sturgeons. As a result, a significant part of the spawning areas (which included the middle and upper part of the Kura and Alazani Rivers in Georgia) for many species has been lost. During the same period, alien species were introduced intentionally or accidentally in the region such as for instance Prussian carp (*C. gibelio*) and topmouth gudgeon (*P. parva*) (Gabrielyan, 2001; Ibrahimov & Mustafayev, 2015; Ninua & Japoshvili, 2008; Pipoyan & Arakelyan, 2015, 2018). Although the pace of large industrial developments slowed down after the collapse of the Soviet Union, the pressure on freshwater species did not weaken. Extensive poaching and the recent explosion of hydropower plant developments in the region take freshwater biodiversity to the even higher treats. Most notably, population dynamics of range-restricted species, community composition, effects of alien species and intensity of threats throughout the region remains poorly known (Freyhof et al., 2015; Katouzian et al., 2016; Mumladze et al., 2019). Recent molecular-genetic studies (as discussed in previous section) have revealed several new species as well as taxonomic rearrangement in freshwater fish fauna of South Caucasus. However, yet less than half of the taxa were studied with the aid of molecular genetics methods. Unfortunately, we could not resolve all the issues regarding the freshwater fish fauna in Armenia, Azerbaijan and Georgia within our knowledge. Therefore, we point out the need of further morphological and genetic studies to address the questions that evolved within this study. In addition, we urge enhanced investigation of freshwater fish communities in the South Caucasian countries to ensure species conservation and mitigate biodiversity loss.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests with the present study.

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Chapter 3: Fish species composition, sex ratio and growth parameters in Saghamo Lake (Southern Georgia)

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Fish species composition, sex ratio and growth parameters in Saghamo Lake (Southern Georgia)

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Abstract

We provide a first investigation of fish species composition, sex ratios, age, length-weight relationships and growth models in Saghamo Lake located in Javakheti highland (Georgia). In total 713 specimens belonging to 8 species were collected included non-native *Coregonus albula*, *Carassius gibelio* and native *Alburnoides bipunctatus, Squalius cephalus, Capoeta capoeta, Romanogobio persus, Salmo* cf. *caspius* and *Barbus lacerta,* among which later two were recorded for the first time in the lake. In overall, relative abundances of all species is low while some species may not be presented with viable populations. Deviation from expected sex ratio, growth at age and age structure indicates severe anthropogenic pressure as a potential driver of fish community degradation in the lake.

Keywords Javakheti plateau · Fish community · Population parameters

Introduction

Javakheti plateau (South Georgia) is characterized by wealth of freshwater resources (Maruashvili 1964; Apkhazava 1975) among which lake ecosystems are of specific interest (Matcharashvili et al. 2004). The lakes of Javakheti plateau are recently recognized as a globally important wetland area (Matcharashvili et al. 2004) as well as an important bird area included in IBA system (Birdlife International 2016). Georgian government in 2011 has reflected the importance by establishing of Javakheti Protected Area system covering some wetland ecosystems within the region (http://apa.gov.ge/en/).

During the last century, lakes of Javakheti plateau were extensively used for fisheries and irrigation purposes and had a great economic importance at regional scale (Savvaitova and Petr 1999). In 1930s the vendace (*Coregonus albula* Linnaeus, 1758) was introduced into the Lakes Tabatskuri and Paravani from Ladoga Lake (Northwestern Russia) (Barach 1941) for a commercial purpose and annual catches were reaching 200 tons during the time of Soviet Union (Japoshvili 2012). Beside *C. albula*, other fish species mostly introduced had also an important economic value including common carp *Cyprinus carpio* (Linnaeus, 1758), Caspian trout *Salmo* cf. *caspius* (Kessler, 1877), khramulya *Capoeta capoeta* (Güldenstädt, 1773) and chub *Squalius cephalus* (Linnaeus, 1758) (Elanidze and Demetrashvili 1973; Elanidze 1983). After Georgian independence in 1991 there was a sharp decrease in catches as a result of overfishing and interruption of management systems (Japoshvili 2012). However, in this period the invasive *Carassius gibelio* (Bloch, 1872) was quickly spread in all freshwater systems of Georgia and is currently the dominant in regular catches (Japoshvili et al. 2013; our unpublished data).

Although there are some progresses in the protection of lake ecosystems of Javakheti plateau after establishment of protected areas, effective management including sustainable fisheries is still absent. Unregulated exploitation of all non-protected lakes with very limited knowledge of their aquatic biodiversity (Gabelashvili et al. 2016) is alarming. Lack of data regarding the lake ecosystems of Javakheti highland, impedes the proper management of this important wetland area.

Saghamo Lake is the sixth largest lake of Javakheti plateau and as non-protected area it is extensively utilized by local people for fishing, fish farming and waste disposal

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(Matcharashvili et al. 2004). Although there is information available regarding the fish composition in the lake (Barach 1941; Kasymov 1972; Apkhazava 1975; Elanidze 1983), however, this data is mostly sourced from Barach (1941), who have never sampled the lake. Instead, he assumed that species that are presented in Lake Paravani would also occur in Saghamo Lake, due to their interconnectivity. Later authors simply reiterated this information without any additional data. The only known ichthyological survey of fishes in Saghamo Lake was conducted by Pipoyan et al. (2013), who reported occurrence of five species (Table 1). However, since these authors did only time limited littoral sampling, the community composition of the Saghamo Lake would be underestimated.

The aim of our study was to investigate recent composition of fish community in Saghamo Lake. We also investigate some basic population parameters (age structure and growth, sex ratio, length-weight relationship) for each species in order to evaluate fish stocks in Saghamo Lake and build foundation for future monitoring and fisheries management.

Materials and methods

Study area Saghamo Lake (N 41.305474; E 43.739367) is located in the eastern part of Javakheti plateau (Caspian Sea basin) at an elevation of 2000 m a.s.l. (Fig. 1). The lake is rather shallow (mean depth 2.6 m) with the surface area of 4.58 km^2. It is feeding mainly from the river Paravani that is an outflow of the Lake Paravani (located 10 km northward) and other numerous small rivulets. The surface of the lake is frozen during winter (from October–November until March–April) and the maximum temperature of water reaches 15 °C in summer (Apkhazava 1975). The lake is under pressure of artisanal fisheries and the lake surroundings are used for agricultural activities (mainly pasture). Saghamo village is situated on the eastern bank of the lake with approximately 153 inhabitants (National Statistics office of Georgia 2014). We interviewed local fishermen (10) focusing on species composition and usage of fishing methods.

Fish sampling We conducted fish sampling in spring (7 May) and summer (8 August) 2015 and in spring 2016 (13 and 14 May) using three mesh sized gillnets of 80 m long, 1.5 m depth with mesh size 15, 26 and 38 mm respectively in each sampling episode. Gillnets were exposed in the water for 9 h (from 8 pm to 5 am) to avoid fish stealing by gulls. Additionally, we also used two round cage nets (diameter 40 cm; mesh size 10 mm) in littorals at each occasion to supplement the gillnet sampling. All live-fished individuals were immediately anaesthetized by a lethal dose of tricaine methanesulfonate (MS–222, Sigma Aldrich Co.). Fishes were identified to the species level according to Kottelat and Freyhof (2007) and Ninua et al. (2013), were counted, measured and weighed. All measurements were done to the nearest millimeter and gram. To determine the sex, we dissected all fished individuals and evaluated the presence of male or female gonads.

We took three to five scales from each individual from the center of the body above the lateral line. An age of each individual was determined by the examination of three to five scales under the stereo-microscope according to Cailliet et al. (1996).

Statistical analyses We modeled length-weight relationship (LWR) using the logarithmic form of power function ($W = aL^b$) were W and L stands for length and weight respectively (Anderson and Neuman 1996). The differences in b coefficients between males and females were compared using chi-squared test (Soper 2017). Binomial test was applied to test the deviation of sex ratio from 1:1 for species with more than 10 individuals in pooled data.

We estimated growth parameters using von Bertalanffy equation - $L_t = L_\infty*(1\text{-exp}[-K(t\text{-}t_0)])$, where L_t – is length at age t; L_∞-is asymptotic length (or maximum length

Table 1 Fish species composition in Saghamo lake with historical records

Species	Barach (1941)	Elanidze (1983)	Pipoyan et al. (2013)	Current study
Squalius cephalus	?		+	+
Carassius gibelio			+	+
Alburnoides bipunctatus	?	?	+	+
Capoeta capoeta	?	?	+	+
Romanogiobio persus			+*	+
Coregonus albula	?			+
Barbus lacerta				+
Cyprinus carpio	?			
Salmo cf. *caspius*	?	?		+

Question mark indicates unconfirmed records. Asterisk indicates a record of *Gobio* cf. *gobio* after Pipoyan (2013) which is most probably misidentified *R. persus*. Note that all listed species except in bold face are also recorded from Paravani - a lake interconnceted with Saghamo (Elanidze 1983)

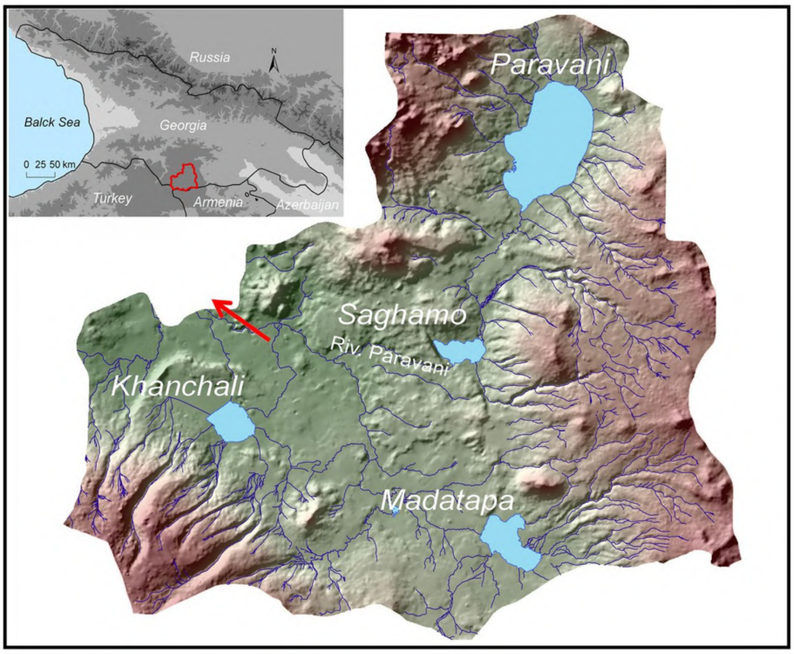

Fig. 1 A map of Javakheti highland showing the large lakes of the region and river network. All the tributaries belong to the drainage of Paravani River and the flow direction is indicated by red arrow

available from FishBase); *K*- is the growth coefficient; t_0 – is the theoretical age at zero length (In our study $t_o = 0$). Mean length for species in each age group were calculated in the statistical program R (R Development Core Team 2016), then obtained data was rearranged in spreadsheet as suggested by King (2007) to estimate best fit parameters of nonlinear least squares regressions for each species.

Estimation of catch per unit effort (CPUE) was calculated as an average number of individuals captured per single gillnet (King 2007).

Results

In total 713 individuals belonging to 8 species were collected among which 4 species were represented with more than 10 individuals (Table 2). Most of *Alburnoides bipunctatus* (Bloch, 1782) (90%) were caught by cage nets, while all others were captured by gillnets. The most abundant species (>50% of total catch) was *S. cephalus* while the *S.* cf. *caspius* and Kura barbel *Barbus lacerta* (Heckel, 1843) was represented both by one individual only.

Regarding sex of fishes, not all analyzed fish species showed balanced ratio of males and females. *S. cephalus* is strongly male biased ($p < 0.01$) ($n = 401$) while *A. bipunctatus* ($n = 79$) and *C. capoeta* ($n = 62$) are female biased ($p < 0.01$). For other species, there is no significant deviation from 1:1 sex ratio, or we did not catch a sufficient number of individuals (Table 2).

Due to low number of individuals in fishes, we analyzed age class distribution only for 6 species (Table 2). Maximum age was found to be 'V' for *A. bipunctatus, C. capoeta, Romanogobio persus* (Günther, 1899) *and S. cephalus*. The '0' age group was poorly represented in a total catch except *A. bipunctatus* presumably due to selectivity of gill nets. Only single individual of *C. albula* and 9 individuals of *C. gibelio* was represented at '0' age group.

Length- weight relationships

Data summarizing the length-weights of the species is provided in Table 3. Only *A. bipunctatus* showed positive allometric growth ($b = 3.3$) while other remaining three species had negative allometric growth ($b < 3$). There are also differences in growth parameters between males and females for some species. For instance, males of *C. capoeta* exposed lowest and females largest *b*-value among all species ($p < 0.01$). In contrast, males of *S. cephalus* showed perfect isometric growth, while females showed negative allometric growth ($p < 0.01$). For other two species, there were no significant differences in LWR models between sexes.

Table 2 Summary table of the fish species in Saghamo lake including the binomial test (p) for sex ratio and distribution of age classes

Scientific name	Native	N[a]	M[b]	F[c]	P[*]	Age classes[d]					
						0	I	II	III	IV	V
S. cephalus	yes	403	295	106	<0.01	0	2	48	72	6	1
C. gibelio	no	133	62	69	0.7	9	42	32	25	6	0
A. bipunctatus	yes	90	23	56	<0.01	19	16	19	9	3	1
C. capoeta	yes	73	18	44	<0.01	0	1	24	25	16	2
R. persus	yes	7	2	5	0.4	0	0	1	0	3	3
C. albula	no	5	3	1	0.6	1	0	1	1	0	0
B. lacerta	yes	1	–	–	–	–	–	–	–	–	–
S. cf. *caspius*	yes	1	–	–	–	–	–	–	–	–	–

* significance value for difference in sex ratio

[a] total number of specimens

[b] male

[c] female

[d] Sex and age was not determined for all the individuals

Growth

Fitting of von-Bertalanffy growth curve was only possible for four species (Fig. 2) (*A. bipunctatus* - $L_t = 18.5*(1-\exp[-0.3*t])$; *C. capoeta* - $L_t = 41*(1-\exp[-0.3*t])$; *C. gibelio* - $L_t = 46*(1-\exp[-0.1*t])$; *S. cephalus* - $L_t = 60*(1-\exp[-0.1*t])$.

Catch per unit effort (CPUE)

The CPUE was estimated as 71 individuals per gillnet including all species combined. Among captured speceis, *S. cephalus* was dominated in total catch with CPUE of 44 individuals followed *C. gibelio* and *C. capoeta* with CPUE of 14 and 8 individuals respectively. Note that 95% of *A. bipunctatus* were captured by cage nets not a gillnet and hence did not included in CPUE calculation.

Discussion

In overall, total catch was very low. For instance, the same CPUE of *C. gibelio* in nearby Madatapa Lake resulted in 70 individuals (Japoshvili et al. 2017). This indicates a very low total abundance of fishes in the lake although we are aware that gillnets may insufficiently sample the littoral parts and hence underestimate a true population density in the lake. Nevertheless, we assume no significant deviation related to species composition, relative abundance and sex ratio. The fish community in the lake is composed by six native species *S. cephalus, A. bipunctatus, C. capoeta, R. persus, S.* cf.

Table 3 Length and weight distribution of fish species in Saghamo Lake; regression coefficients; significance test statistics of Length-Weight Relationships

		Standard Length		Weight		Regression parameters			Females				Males				
									Regression parameters				Regression parameters				
Species	N[a]	Min[b]	Max[c]	Min	max	b[d]	95% CI(b)[e]	r^2[*]	N	b	95% CI(b)	r^2	N	b	95% CI(b)	r^2	p[**]
Alburnoides bipunctatus	90	55	123	2.7	39	3.3	<0.01	0.9	56	3.2	<0.01	0.9	23	3.2	<0.01	0.9	0.9
Capoeta capoeta	73	148	262	58	331	2.8	<0.01	0.9	18	2.3	<0.01	0.9	44	3.4	<0.01	0.9	0.0
Carassius gibelio	133	67	203	11	313	2.9	<0.01	0.9	69	3	<0.01	0.9	63	2.8	<0.01	0.9	0.7
Squalius cephalus	403	102	229	19	214	2.9	<0.01	0.9	106	3	<0.01	0.9	295	2.7	<0.01	0.8	0.001

* coefficient of determination

** Significance of the difference between Two Slopes

[a] sample size

[b,c] minimum and maximum standard length (cm)

[d] slope; [e] confidence interval of b

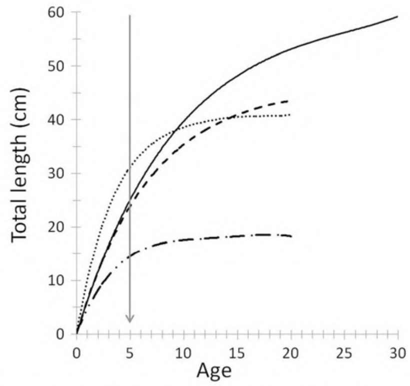

Fig. 2 Growth models for four species occurring in Saghamo Lake. Continuous line – *Squalius cephalus*; dashed line – *Carassius gibelio*; point line – *Capoeta capoeta*; dashed line with points – *Alburnoides bipunctatus*. Arrow indicates maximum age class occurring in our catch

caspius, *B. lacerta*, from which later two were recorded for the first time in the lake (also note that Pipoyan et al. (2013) described gudgeon in Saghamo Lake as *Gobio* cf. *gobio*, which presumably was misidentified with *R. persus*. In addition, two non-native species (*C. gibelio* and *C. albula*) are inhabiting the lake. The native fishes in the lake originated most likely from the river Paravani, which is the right tributary of the Kura River. Interestingly, Barach (1941, 1964) who never collected fishes in the lake, predicted the occurrence of most of them based on their occurrence in the lake Paravani (Table 1). In contrast to previous works about fishes of Saghamo Lake, the most common species nowadays is *S. cephalus*, not *C. Gibelio* which invaded the Georgian waters during last decades of twentieth century and spread to many lakes in Georgia (Japoshvili et al. 2013). Although this species is usually outnumbering all other species roughly of the same size (Lusk et al. 2010; Lusková et al. 2010; Japoshvili et al. 2013) this is not the case for Saghamo Lake, which could be related to high fishing pressure. Additionally, although *C. carpio* have been present in Paravani Lake (Japoshvili 2009), it is most likely not occurring in the lake Saghamo, which is supported by our survey and confirmed by local anglers. Nevertheless, fish communities in Saghamo and Paravani lakes are highly similar and richer in number of species than other large lakes in the Javakhety highland (Madatapa and Khanchali lakes) that are currently harboring a single species - *C. gibelio* (Fig. 1; Table 1). This could be attributed to either harsher environmental conditions in case of Madatapa Lake (Matcharashvili et al. 2004) or severe anthropogenic disturbance in case of Khanchali Lake (Gabelashvili et al. 2016).

The rarest species in our catch were: *R. persus* ($n = 7$), *C. albula* ($n = 5$), *B. lacerta* ($n = 1$) and *S.* cf. *caspius* ($n = 1$) (Table 1). The lower proportion of these species could be expected since *R. persus, B. lacerta* and *S.* cf. *caspius* are usually more common in rivers and the relative abundance of these species is not high in lakes (Elanidze 1983). The Saghamo Lake was populated by non-native *C. albula* through the river connection with nearby located Paravani Lake where it was introduced during the mid-20th centuries and since then fry was released regularly into this lake (Japoshvili 2012). According to local fishermen the last stocking of *C. albula* fry was performed in 2005. Hence, the finding of the young generation of this species indicates its naturalization in Saghamo Lake but with no commercial value due to small population size.

We found that the sex ratio was significantly male biased in *S. cephalus* population and significantly female biased in populations of *A. bipunctatus* and *C. capoeta*. In contrast, *C. gibelio* population did not show significant deviation from balanced sex ratio (1:1). The imbalanced sex ratio could be driven by different factors (Devlin and Nagahama 2002) and even the different species could have diverse response to the same factor (Ospina-Álvarez and Piferrer 2008). In this respect, *C. gibelio* is rather extensively studied. The populations of *C. gibelio* are usually female biased and sometimes the population consist of females only (Tsoumani et al. 2006). This pattern is usually attributed to the gynogenetic mode of reproduction but may exhibits great variation; however, the proportion of males seldom approaches to 50% (Vetemaa et al. 2005; Liasko et al. 2011). High proportion of males in a population of *C. gibelio* (especially in case of occurrence of other cyprinid species) indicates a dominance of sexual reproduction mode in Saghamo Lake that is hypothesized as a result of stressful environment (Smith 1978; Burt 2000; Liasko et al. 2011). Since Saghamo Lake offers milder environmental conditions than the nearby Madatapa Lake (Japoshvili et al. 2017) environmental severity cannot explain the observed sex ratio. Instead, heavy and long-term human disturbance in Saghamo Lake might be hypothesized as the structuring force of sex ratio in *C. gibelio* population. On the other hand, *C. gibelio* has very complicated genetic background (Rylková et al. 2013), and cytogenetic mechanisms, which still not have been well understood (Kalous and Knytl 2011).

A number of studies are reporting the different sex ratio in populations of *S. cephalus* (Ünver 1998; Türkmen et al. 1999; Koç et al. 2007), *C. capoeta* (Abdoli et al. 2008; Patimar et al. 2009, 2011) and *A. bipunctatus* (Patimar et al. 2012). It seems that the sex ratio in these species could vary greatly, however there is no studies explicitly addressing the potential mechanism explaining those patterns. According to Nikolsky (1963) males are predominating in fish populations in early life stages while females – at later due to probable adaptive mechanisms. Although '0' and '1' age groups are purely represented in our data most probably due to selectivity of gill nets, nevertheless the distribution trends of sex ratio for any species does not support this prediction (Fig. 3). Data limitation does not allow us to

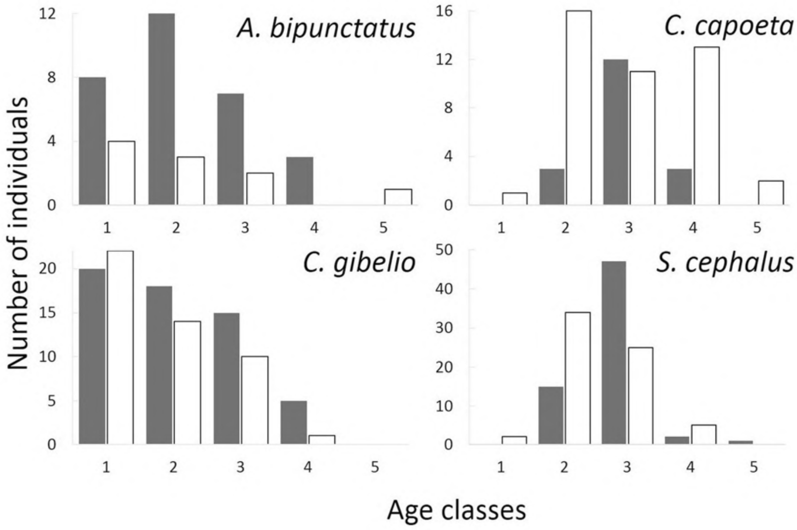

Fig. 3 Age-dependant sex distribution of four fish species in Saghamo Lake. Filled bars - males; unfilled bars – females

make any deeper analyses of the underlying mechanisms, although these inconsistencies could also be related to overfishing.

This view is supported by the low value of maximum age and sizes of species captured in the lake. In particular, *C. gibelio* attains far higher age (9 years) and large size (330 mm) in Madatapa Lake (own unpublished data), while in Saghamo Lake its maximum age does not exceed five years and the total length of 253 mm. No comparable data for all other species is available from surrounding areas although maximum sizes is far larger than ours based on data of FishBase (Froese and Pauly 2015). This clearly indicates that the species could not attain possible maximums due to fishing pressure. Furthermore, length at age distribution (Fig. 4) showed that the older individuals were not larger in any species and the maximum length is approached at 'II' – 'III' age classes. This pattern can be explained by the overfishing as the anglers are usually fishing using gill-nets with large mesh sizes (more than 30 mm). Modeled growth when asymptote length was applied from the literature showed that except *A. bipunctatus*, no other species (*C. gibelio, C. capoeta* and *S. cephalus*) can attain their maximum sizes (Riehl and Baensch 1990; Kottelat and Freyhof 2007; Verreycken et al. 2011). This in turn indicates the existence of stressful abiotic environment rather than effect of overfishing. In addition, Saghamo Lake is high mountain

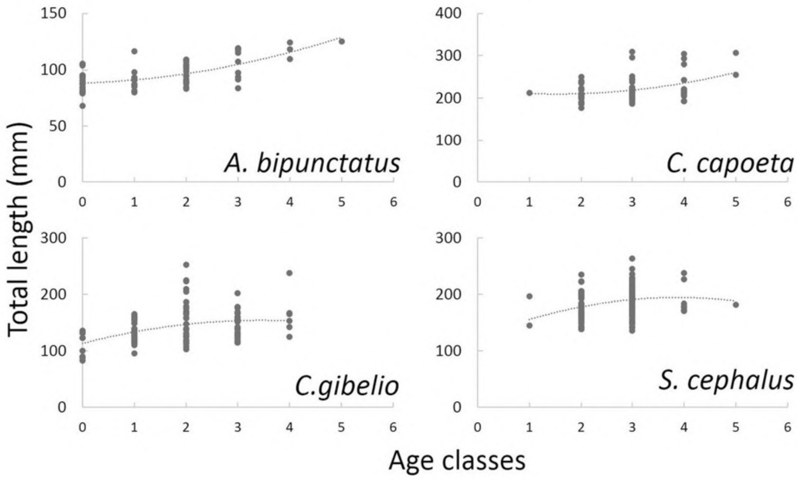

Fig. 4 Length at age relationship of four species from Saghamo Lake

oligotrophic lake that may be related to slow growth rate of fish stock.

We found all species (except *A. bipunctatus*) exhibiting very low growth rate up to asymptote (Fig. 2). In particular, *C. gibelio* for which the maximum age is thought to be 10 years (Kottelat and Freyhof 2007) needs almost 20 years to reach maximum length reported in FishBase (Froese and Pauly 2015). Similarly, the *S. cephalus* that can grow as large as 60 cm in length and live up to 22 years (Kottelat and Freyhof 2007; Froese and Pauly 2015), needs to live for 30 years to gain reported maximum size according to our growth model. No such data are available for *C. capoeta* and we cannot confidently judge the parameters of its growth model.

In summary, the fish community of Saghamo Lake consists currently of eight fishes among which *S. cephalus, C. gibelio, C. capoeta* and *A. bipunctatus* are relatively abundant, while others (*C. albula, B. lacerta, R. persus* and *S.* cf. *caspius*) are very rare. Especially the dominance of non-native *C. gibelio* is alarming since it is classified as the invasive species with potentially severe impact on ecosystem (Copp et al. 2009). However, the populations of even those relatively abundant species seems to be much diminished and probably in a critical condition. Strong overfishing is evident in Saghamo Lake that is supposedly a main contributor in fish community degradation although there are signs of poor abiotic conditions as well. Overall, fish species diversity and distribution of Georgia and South Caucasus in general is purely documented (for instance, the area was not thoroughly considered in the last compendium by Kottelat and Freyhof (2007) due to lack of information). Hence, the studies similar to ours can greatly contribute in the understanding of fish diversity and distribution of the region. In addition, provided information regarding the fish species of Saghamo Lake could be effectively used to better understand the lake ecosystem as well as for a sustainable fisheries management and monitoring purposes.

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Chapter 4: Evaluation of the potential establishment of black-striped pipefish transferred by cultural drivers

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Evaluation of the potential establishment of black-striped pipefish transferred by cultural drivers

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ABSTRACT
Introduction of non-native species is considered a grave threat for global biodiversity. It can negatively affect native biota and cause serious socioeconomic losses. In this study, we confirmed the existence of black-striped pipefish (*Syngnathus abaster*) in the freshwater Tbilisi Reservoir (the middle Kura Drainage), which had been translocated from the Black Sea Basin. The introduction was in part a result of the local name of the target freshwater reservoir called the "Tbilisi Sea," which implied a connection with the Black Sea for local hobbyists. *S. abaster* is expanding its range with or without human help, and introduction of this species is expected in other regions. To address this problem, we made a species distribution model using MaxEnt software to test the species environmental suitability around the globe. Lowland inland waterbodies and shorelines in temperate, Mediterranean, and arid climate zones were indicated as localities where this fish could survive if released. The risk of introduction currently seems low, but exploitation of *S. abaster* for ornamental purposes due to expanding local and international pet trade increases the likelihood of future releases. For this reason, it is important to verify new cases of successful establishment of this species.

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Introduction

Pipefishes in the family Syngnathidae include about 220 species that inhabit mostly tropical marine, brackish (in coastal and lagoon), and freshwater environments (Dawson 1984, Kottelat and Freyhof 2007). Syngnathid fishes are characterised by an elongated body covered by dermal plates and a tube-shaped snout. "Male pregnancy" in pipefishes is a remarkable example of sexual dimorphism (Dawson 1984, Kottelat and Freyhof 2007). Syngnathids are appreciated for their unusual appearance and are exploited for traditional medicine, aquarium displays, and curiosities (Vincent et al. 2011).

The black-striped pipefish (*Syngnathus abaster* Risso, 1827) is normally found in coastal waters of the Mediterranean, Black, and Azov seas, but has expanded its range upstream in the Danube, Dniester, Dnieper, Don, and Volga rivers (Berg 1949, Svetovidov 1964, Kottelat and Freyhof 2007). Recently, this species has also become an invader of freshwater reservoirs (Kiryukhina 2013a, 2013b, Tereshchenko et al. 2016, Didenko et al. 2018, Marenkov 2018). These introductions into new areas were mostly due to self-spreading (Kuderskii 1971) but also to human-mediated activities such as river regulation, fish stocking (Slynko et al. 2011, Didenko et al. 2018, Marenkov 2018), and ballast water transport (Lavoie et al. 1999). The creation of the Volga-Don channel in the 1960s opened the way for *S. abaster* and other organisms to spread from the Don and Dnieper deltas into Volga reservoirs (Kuderskii 1971, Kiryukhina 2013a, 2013b).

Georgia is a country located at the crossroads of western Asia and eastern Europe on the southern slope of the Caucasus (Fig. 1a). The country is divided by the Likhi mountain range into 2 main water basins: the western belonging to the Black Sea basin and the eastern to the Kura River drainage in the Caspian Sea basin. Five Sygnathid species are distributed in the Georgian part of the Black Sea: *S. abaster*, *S. schmidti*, *S. tenuirostris*, *S. typhle*, and *S. variegatus* (Japoshvili and Ninua 2008, Ninua et al. 2013); and one, *S. caspius* Eichwald 1831 in the Caspian Sea (Naseka and Bogutskaya 2009, Bogutskaya et al. 2013).

The presence of *Syngnathus* sp. in eastern Georgia in the Tbilisi Reservoir (Fig. 2b; Kura River drainage), which locals call the "Tbilisi Sea," was recorded first by Kokosadze et al. (2000) in local proceedings (grey

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literature) in the Georgian language. The authors concluded that *Syngnathus* sp. was intentionally translocated from the Black Sea basin to the Tbilisi Sea by "exotic fish lovers" in the 1980s. The reason attributed to the translocation of this fish by hobbyists was the colloquial "marine" name of the freshwater target area. As the authors reported, the main argument for the translocation event was the following: if *Syngnathus*. sp. was living in the Black Sea, why could it not have been present in the Tbilisi 'Sea'? (Kokosadze et al. 2000).

Introduction of non-native species into new areas is considered one of the main threats to global biodiversity (Rosenthal 1980, Allan and Flecker 2006). Negative effects include predation, competition, hybridisation, distribution of diseases and pathogens, disruption of food webs, eutrophication, and habitat alteration, which itself leads to homogenisation of the ecosystem and disappearance of native biota (Manchester and Bullock 2000, Savini et al. 2010). In addition, invasive species can cause socioeconomic losses (Gallardo and Aldridge 2013). The unusual nature of this translocation from "sea to sea" emphasises the importance of understanding the social and cultural drivers that can lead to introductions of alien species as well as the development of measures for successful prevention (Patoka et al. 2018).

In this study, we confirmed the existence of pipefish in the Tbilisi Reservoir 21 years after it was first reported there and identified at the species level. We also discuss the reasons for its introduction. Because *S. abaster* is known to be expanding its range with or without human help, we modelled the risk of establishment of the species worldwide, assuming it will be introduced in other regions.

Materials and methods

The Tbilisi Reservoir, locally called the Tbilisi Sea, is located in the northeastern part of Tbilisi, the capital of Georgia (Fig. 1a). Built in 1953 on the Iori River (the Kura River drainage), the reservoir covers an area of 11.6 km^2 with a maximum depth of 45 m. The reservoir supplies the city of Tbilisi with drinkable water and is an important area for recreation.

We collected fish at the northwestern edge of the reservoir (41°45′50.46″N, 44°48′44.69″E; Fig. 2b) using a 30 cm × 30 cm D-frame kick net with a mesh size of 0.5 mm. Sampling was performed in summer (2 Jul 2018) from 0700 h until 0900 h. The site was characterized by a gravel substrate covered with submerged vegetation. Fishing was conducted 1–2 m from the bank where the depth was about 50 cm by gently kicking the net on the bottom. Collected fish were transported to the laboratory at the Ilia State University (Tbilisi, Georgia) and euthanized by overdosing with a solution of methanesulfonate (MS–222, Sigma Aldrich Co., St. Louis, MO, USA). Fish were photographed, and fin clips from the right pectoral fins from the adults and caudal fins from the juveniles were stored in 96% EtOH for later DNA analysis.

Fish were identified using the Dawson (1984) morphological key by measuring the following plastic and meristic characters: standard length; head length; snout length; snout depth; caudal fin length; orbit diameter; interorbital length; trunk depth; dorsal fin base; number of fin rays in pectoral, dorsal and caudal fins; number of rings on trunk (i.e., count of the trunk rings that start at the pectoral fin base and end at the ring that bears the anus) and tail (i.e., count of the tail rings that start at the first ring behind the anus and end at penultimate ring, not including the terminal element that is bearing the caudal fin); number of subdorsal trunk rings covered by the dorsal fin base; number of tail rings covered by the dorsal fin base; and subdorsal rings in total. Fish were compared to the voucher specimens from the Berlin Natural History Museum, Germany (voucher numbers: 32482, 32485, and 32039), and the original species description was also checked.

Adults were preserved in 4% formaldehyde and deposited in A. Koenig's Natural History Museum, Bonn, Germany (voucher numbers: ZFMK-ICH 119662-119665). Juveniles were preserved in 96% EtOH and deposited in the collections of the Department of Zoology and Fisheries, Czech University of Life Sciences Prague, the Czech Republic under the numbers (5NFTK-9NFTK).

Genomic DNA from individual fish was extracted using the Blood and Tissue Kit (Qiagen, Germantown, MD, USA) according to the manufacturer's protocol. Sequences of mtDNA gene cytochrome b (Cyt *b*) were amplified by polymerase chain reaction (PCR) using the following pair of primers: forward, LCO1490 5′-GGT CAA CAA TCA TAA AGA TAT TGG-3′; and reverse, HCO2198 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′. The primer pair for the mtDNA COI gene: forward, FishF2-5′TCGACTAATCATAAAGATATCGGCAC3′; and reverse, FishR2-5′ACTTCAGGGTGACCGAAGAATCAGAA3′. The PCR reaction (50 µL total) consisted of 15.5 µL Combi PPP Master Mix (Top-bio, Vestec, Czech Republic), 5 µL of each primer, 5 µL of template DNA, and PRC water (Top-bio). The PCR was carried out on an MJ Mini thermocycler (Bio-Rad, Hercules, CA, USA). Amplification of the Cyt *b* gene started with 2 min initial

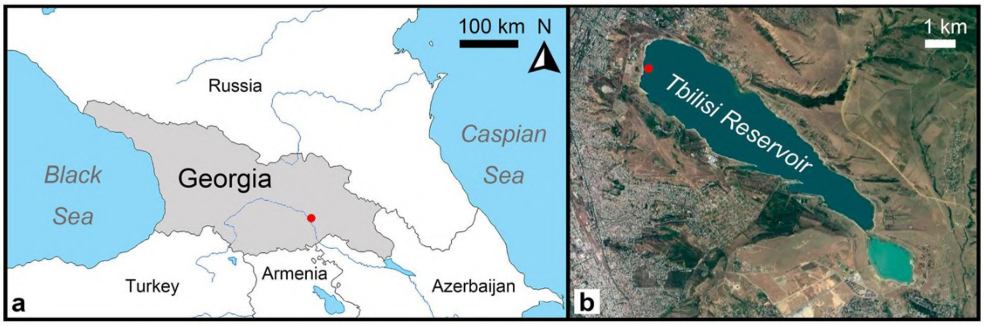

Figure 1. (a) Location of the study area; (b) sampling area on the Tbilisi Reservoir.

denaturation at 94 °C, followed by 35 cycles, each consisting of denaturation at 94 °C for 1 min, primer annealing at 50 °C for 1 min, and elongation at 72 °C for 1 min. PCR was terminated by a final elongation of 72 °C for 5 min (Sanna et al. 2013). For the COI gene, amplification started with a 2 min initial denaturation at 95 °C, followed by 35 cycles, each consisting of denaturation at 94 °C for 0.5 min, primer annealing at 54 °C for 0.5 min, and elongation at 72 °C for 1 min. Amplification was terminated by a final elongation of 10 min at 72 °C (Kolangi-Miandare et al. 2013). PCR products were purified and sequenced by Macrogen, Inc. (Seoul, Korea). Each sample was sequenced from the 3′ and 5′ ends of both fragments using the same primers as for double-strand PCR amplification. The raw chromatograms were manually assembled and checked for potential mistakes using the BioEdit 5.0.9. software. Sequences were compared with the GenBank (National Center for Biotechnology Information [NCBI]) nucleotide database using the BLASTn 2.3.1+ programme (Morgulis et al. 2008). Results of the search were used to identify isolates to the species level, and the sequences were uploaded to NCBI.

Climate matching based on temperature characteristics was modelled from a dataset of environmental layers and native ranges of *S. abaster* using the MaxEnt programme 3.4.1 (https://biodiversityinformatics.amnh.org/open_source/maxent) to determine the fish's environmental adaptability around the globe in case of potential introduction. Available GPS coordinates of the native range were obtained from FishBase (Froese and Pauly 2019) and missing georeferenced locations were manually added according to the literature (Berg 1949, Svetovidov 1964, Dawson 1984, Kottelat and Freyhof 2007). This method follows Gallardo and Aldridge (2013) to account for the missing native distribution points into the database. If the species was known from a particular stretch, 2–6 random points were selected on this stretch according to the distribution data from the literature (e.g., Berg 1949, Svetovidov 1964, Dawson 1984, Kottelat and Freyhof 2007). For example, if *S. abaseter* was known to be distributed approximately 100 km along the Dniester River from the estuary to Bender (Moldova), we selected 5 points: the estuary, Bender, and 3 randomly chosen along the length of the river between the estuary and Bender. Using this method, points were selected on the following rivers: Bug, Danube, Dniester, Dnieper, Don, Inhulets, Iput, Korilistskali, Kuban, Mejinistskali, Prutt, Rioni, and Sozh. We added single points randomly in following lakes: Bratesh, Burgas, Kahul, Katlabukh, Nuri, Paliastomi, Shablal, Varensko, and Yalpuh; and single points randomly in the Bosporus strait and in the Marmara Sea. In the Azov Sea, we knew that *S. abaster* was distributed along the coastline, and therefore we followed the mapped shoreline and randomly marked 12 points. In total, 718 points were used for the analysis.

For species distribution modelling, we obtained bioclimatic variables from the WorldClim database (v.2.0; http://www.worldclim.org; Hijmans et al. 2005) with a spatial resolution of 2.5 arcmins (~1 km^2). Bioclimatic variables are significant factors drawn from the monthly temperatures and rainfall values that show annual trends, seasonality, and extremes important for species survival. For aquatic species, a correlation has been found between species distribution pattern and climatic variables, especially temperature (Gallardo et al. 2012). These environmental layers were assembled in QGIS 3.8.2 Zanzibar (https://qgis.org/en/site/) to ASCII format for use with the MaxEnt algorithm (Phillips 2005), a maximum entropy model well suited for species distribution mapping (Phillips and Dudík 2008). The MaxEnt model describes a continuous probability surface of habitat suitability in the target area and is widely used to forecast alien species distribution (Ward 2007, Giovanelli et al. 2008, Yonvitner et al. 2020). The final set of bioclimatic predictors comprised temperature within the annual mean temperature (BIO1), temperature seasonality (BIO4), the maximum temperature of the warmest month (BIO5), and minimum temperature of the coldest month (BIO6). These variables were selected because temperature can affect growth, reproductive behaviour (Ahnesjo 1995, Monteiro et al. 2001, Silva et al. 2007), egg development, abundance, and survival of offspring (Kirby et al. 2006). As the cumulative output, a continuous map was generated and visualised in QGIS 3.8.2 Zanzibar.

Results

Nine individuals were collected: 4 adults (3 females and 1 male carrying about 40 fertilized eggs inside the pouch; Fig. 2) and 5 small juveniles. All individuals were morphologically identified as *S. abaster* (Fig. 2, Table 1). Measurements of collected specimens fit those of voucher specimens of *S. abaster* from the Black Sea (Romanian shore) deposited in the Berlin Natural History Museum (Table 1).

The total length of sequences obtained was 501 bp for Cyt *b* (access numbers MK605639, MK605641, MK605643, MK605645, MK605647, MK605649, MK605651, MK605653, and MK605655) and 626 bp for COI (access numbers MK605640, MK605642, MK605644, MK605646, MK605648, MK605650, MK605652, MK605654, and MK605656). All 9 samples shared the same haplotype in both Cyt *b* and COI region. Cyt *b* had no suitable strand length match, resulting in ambiguous pairing with several species. The COI haplotype was 100% identical to the *S. abaster* sequences (access numbers MG131907, MG131908, and MG131909) from the Black Sea (Oruç and Engin 2018). *S. abaster* from the Black Sea was originally described as *S. nigrolineatus* Eichwald and later synonymized as *S. abaster* by some authors (Eschmeyer's Catalog of Fishes; Fricke et al. 2020). We checked the identification key of *S. nigrolineatus* provided by Berg (1949), and the morphological variables fit.

We mapped environmentally suitable areas for the establishment of *S. abaser* based on global climatic variables (Fig. 3). This map output allows a fine distinction among the modelled environmental suitability of different areas for the evaluated species. MaxEnt calculated a threshold value for *S. abaster* of 12.8. A value of the climate match reaching or exceeding this threshold was interpreted as no evidence for climatic constraints to the survival of the species (shown in red on the map). The value for the area under the receiver operator curve (AUC) was 0.97, representing a 97% probability that a random selection from presence records had a model score greater than a random selection from the absence records (Ward 2007). The localities where *S. abaster* might survive after release are lowland inland waterbodies and shorelines in temperate, Mediterranean, and arid climate zones of 6 continents. In North America, these areas include the western shoreline of the Pacific Ocean from Vancouver to San Diego, the Great Salt Lake, the area west of El Paso, the northeastern area of the Mexican Plateau, shorelines of the Gulf of Mexico (except Florida) and the Atlantic Ocean from Houston to Boston, the Great Lakes, and slopes of the Appalachian Mountains. Areas in South America cover parts of Chile, Argentina, Uruguay, and the southern part of Brazil. The regions in Europe cover most of the continent, including the Baltic, North, Atlantic, Mediterranean, and Black sea basins and their shorelines. In Asia areas include the Black, Mediterranean,

Figure 2. Male individual of *Syngnathus abaster* (standard length = 117 mm) found in the Tbilisi Reservoir, with about 40 fertilized eggs inside the pouch.

Table 1. Minimum (Min), maximum (Max), and mean values of plastic and meristic measurements of *Syngnathus abaster* ($n = 4$) from the Tbilisi Reservoir and voucher specimens ($n = 3$) from the Black Sea deposited in the Berlin Natural History Museum.

		Tbilisi Reservoir				Voucher specimens from the Berlin Natural History Museum			
		Min	Max	Mean	SD	Min	Max	Mean	SD
1	SL	104.00	119.00	113.50	6.66	80.00	165.50	135.83	48.38
2	HL	11.97	13.46	12.58	0.64	10.20	17.00	13.93	3.45
3	SnL	5.24	6.35	5.85	0.51	5.40	8.80	6.93	1.72
4	SnD	1.07	1.38	1.22	0.16	1.00	1.80	1.33	0.42
5	CL	1.79	2.94	2.46	0.55	2.10	5.00	3.53	1.45
6	OD	1.49	1.90	1.69	0.17	2.00	2.80	2.53	0.46
7	IOW	0.69	0.97	0.87	0.13	1.00	1.50	1.17	0.29
8	TD	2.95	4.23	3.66	0.62	2.50	5.00	3.67	1.26
9	DB	11.47	12.59	11.97	0.51	9.00	20.00	13.93	5.59
10	PR	12	12	—	0	12	13	—	0.58
11	DR	38	42	—	1.7	36	39	—	1.53
12	CR	10	10	—	0	10	10	—	0.00
13	TR	38	38	—	0	38	38	—	0.00
14	TrR	17	17	—	0	16	16	—	0.00
15	SDR1	1	1	—	0	1	1	—	0.00
16	SDR2	8	8	—	0	7	8	—	0.58
17	SDR	9	9	—	0	8	9	—	0.58

Measurements: row 1 in mm, rows 2–9 in percentage of standard length, rows 10–17 are counts. Abbreviations are as follows: SL standard length, HL head length, SnL snout length, SnD snout depth, CL caudal fin length, OD orbit diameter, IOW interorbital length, TD trunk depth, DB dorsal fin base, PR fin rays in pectoral fin, DR fin rays in dorsal fin, CR fin rays in caudal fin, TrR rings on the trunk, TR rings on the tail, SDR1 trunk rings covered by dorsal fin base, SDR2 tail rings covered by dorsal fin base, SDR subdorsal rings.

and Caspian sea basins and shorelines, some localities in Red Sea Basin, areas around the Gulf of Oman, western Iran and Pakistan, and some small localities in southeast Uzbekistan and northeast Afghanistan. Other areas are the southern slopes of the Himalayan Mountains (Nepal, India), northern parts of Southeast Asia, and the southeastern part of China. The Korean peninsula and Japanese Islands are also included. In Africa, indicated localities are the whole northern shoreline of the continent, some areas in the Sahara, and the Cape region of South Africa. In Oceania the southwestern, central, southern, and southeastern parts of Australia and the northern part of New Zealand are also recognized as possible habitats for *S. abaster* (Fig. 3).

Discussion

We confirmed the existence and successful establishment of black-striped pipefish in the Tbilisi Reservoir. *S. abaster* has lived and been reproducing in the reservoir for at least 4 decades. The population is well established and self-sustaining, evidenced by finding both

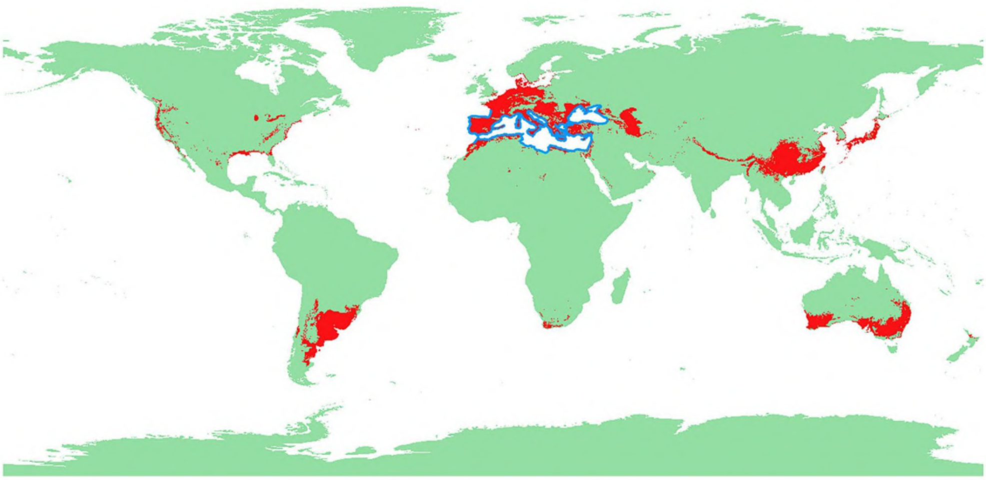

Figure 3. Native range (in blue) and environmentally suitable areas for the establishment (in red) of *Syngnathus abaster* worldwide.

adults and juveniles in the reservoir. The species was identified by morphological and genetic analyses as *S. abaster*, a euryhaline species that can tolerate a wide range of salinities: freshwater, brackish, and marine conditions (Dawson 1984, Kottelat and Freyhof 2007). Previous reports have shown that this fish has the ability to invade various freshwater environments, especially reservoirs (Semenov 2010, Khrystenko et al. 2015, Fedonenko et al. 2016, Tereshchenko et al. 2016, Marenkov 2018) like the one in Tbilisi.

Genetic analysis confirmed that pipefish from the Tbilisi Reservoir shared the same haplotype as those from the Black Sea basin (Oruç and Engin 2018), supporting the idea that the *S. abaster* in Tbilisi Sea was introduced from the Black Sea area. One of the reasons for their introduction is most likely linguistic. It is interesting to speculate that the "marine" name of the place, Tbilisi Sea, would suggest to locals that *S. abaster* belongs there and should be introduced. This phenomenon could be represented by the Latin proverb, *nomen est omen*, which means that one's name determines one's destiny, as described in many disciplines (Plangger et al. 2013, Lange et al. 2014, Brylla 2017, Smith et al. 2018). Although we cannot prove or confirm this linguistic connection in the introduction of *S. abaster* into the Tbilisi Reservoir with certainty, other reasons for its introduction are unlikely. In Georgia, the pipefish is not a target of recreational fishing, is not consumed as food, and is not grown in aquaculture. It has not been regularly stocked into the reservoir, and self-spreading from the Black Sea basin is impossible because the eastern Georgian watershed is isolated from the western one. Without human assistance, this species could not have reached the Tbilisi Reservoir, an thus this could be the first case of introduction of a species based on linguistic reasons.

The predicted potential distribution of this species in Europe and Asia—the shorelines of the Mediterranean, Black, Azov, and Caspian seas; the Iberian Peninsula; and the Atlantic coast from the Bay of Biscay to the Baltic Sea—fits the current distribution of *S. abaster* summarised by Monteiro and Vieira (2017). In the risk assessment analysis provided by Snyder et al. (2014), *S. abaster* was included with *Atherina boyeri*, *Clupeonella caspia*, and *Neogobius fluviatilis* as likely to survive transfer in ballast water and become a successful invader of the Great Lakes, North America, resulting in a negative impact on the ecosystem. Although the invasiveness of *S. abaster* was doubted by MacIsaac et al. (2015), the species is considered likely to establish in the Great Lakes, and *S. abaster* is expected to expand its range and successfully take over new areas because of its ability to osmoregulate, which allows it to quickly adapt to salinity changes (Snyder et al. 2015). In a new environment, the pipefish may negatively affect the native ecosystem by feeding on zooplanktonic communities, mainly copepods, but it can also affect native fish species by predating their larvae (Didenko et al. 2018).

No records exist of *S. abaster* distribution on the South American, African, and Australian continents, and east Asia (indicated in red in our environmental modelling; Fig. 3). The current risk of introduction of *S. abaster* in these places is low because globally this species has no significant commercial value (TRIDGE, available from https://www.tridge.com/). Note, however, that future exploitation of *S. abaster*, at least for ornamental purposes, cannot be excluded because increasingly more marine and freshwater fish species are being promoted by the local and international pet trade (Rhyne et al. 2017, Novák et al. 2020). For instance, *S. abaster* is being taken from the Tbilisi Reservoir as an aquarium fish by local hobbyists (G. Berenchikidze, pers. comm., 2018; TK, pers. observ.) because they are easy to catch, are attractive, and can survive and reproduce in freshwater. Because this tolerant species can be exploited, transported, and traded, the risk of future release or escape into new localities is great and could result in the successful establishment of new populations in suitable regions. Therefore, the verification of successful establishment cases into new invaded areas is important. Our findings call attention to this threat and should be heeded by conservationists, wildlife managers, and other decision-makers, especially in countries where ornamental fish keeping is popular and well developed.

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Chapter 5: The first unified inventory of non-native fishes of the South Caucasian countries, Armenia, Azerbaijan, and Georgia

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The first unified inventory of non-native fishes of the South Caucasian countries, Armenia, Azerbaijan, and Georgia

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Abstract – The South Caucasus (SC) region is recognized for its high biological diversity and various endemic animal taxa. The area has experienced many fish introductions over the years, but the overall information about non-native fishes in the three SC countries, Armenia, Azerbaijan, and Georgia did not exist. Although these three countries belong to the Kura River drainage, Caspian Sea basin (only the western half of Georgia drains into the Black Sea), the legislative framework for each country regarding introduction of non-native fish species and their treatment is different and poorly developed. The goal of the present study was to make an initial inventory of non-native fish species in the three SC countries, and summarize the existing knowledge as a basis for future risk assessment models and formulation of regional management policies. Here, we present a unified list of 27 non-native species recorded in the wild in Armenia, Azerbaijan, and Georgia. Among these 27 species, eight were translocated from the Black Sea basin to the Caspian Sea basin. Out of these 27 non-native fishes, 15 species have become established (three of them being considered invasive) and six fish species could not survive in the wild.

Keywords: Introduction / invasive species / translocated species / aquaculture / recreational fisheries

Résumé – Le premier inventaire commun des poissons non indigènes des pays du Caucase du Sud, Arménie, Azerbaïdjan et Géorgie. La région du Caucase du Sud (SC) est reconnue pour sa grande diversité biologique et ses divers taxons animaux endémiques. La région a connu de nombreuses introductions de poissons au fil des ans, mais il n'existait pas d'informations générales sur les poissons non indigènes dans les trois pays du SC, l'Arménie, l'Azerbaïdjan et la Géorgie. Bien que ces trois pays appartiennent au bassin versant de la rivière Kura, dans le bassin de la mer Caspienne (seule la moitié occidentale de la Géorgie se déverse dans la mer Noire), le cadre législatif de chaque pays concernant l'introduction d'espèces de poissons non indigènes et leur traitement est différent et peu développé. L'objectif de la présente étude était de dresser un premier inventaire des espèces de poissons non indigènes dans les trois pays du SC, et de résumer les connaissances existantes afin de servir de base aux futurs modèles d'évaluation des risques et à la formulation de politiques de gestion régionales. Nous présentons ici une liste unifiée de 27 espèces non indigènes enregistrées à l'état sauvage en Arménie, en Azerbaïdjan et en Géorgie. Parmi ces 27 espèces, huit ont été transférées du bassin de la mer Noire au bassin de la mer Caspienne. Sur ces 27 poissons non indigènes, 15 espèces se sont établies (trois d'entre elles étant considérées comme invasives) et six espèces de poissons n'ont pas pu survivre à l'état sauvage.

Mots clés : Introduction / espèces invasives / espèces déplacées / aquaculture / pêche récréative

1 Introduction

Non-native (alien) species are of great concern for their potential effects on biological diversity (Vitousek *et al.*, 1996;

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Jeschke *et al.*, 2014). Aquatic alien species can be introduced intentionally, through aquaculture, recreational fisheries, or ornamental trade; or unintentionally, through ballast water transport, as a contaminant of parcels, or accidental escapes from captivity. Less frequently, species can also invade new areas without human involvement (Copp *et al.*, 2005 and citations herein). Non-native invasive species can cause significant negative impacts on native fauna, and cause socio-economic losses to affected regions (Ricciardi, 2003; Gallardo and Aldridge, 2013). For this reason, it is worthwhile to invest in the prevention of introductions and if a species is introduced, develop plans for functional management of the invasive alien species. In this regard, one of the vital steps is to scrutinize the alien species lists for a particular region of interest to identify, assess, monitor, and mitigate non-native species in a timely manner. Most countries in Europe and overseas have developed such lists and most importantly, this information has subsequently been used in drafting more effective legislations (Manchester and Bullock, 2000; Copp *et al.*, 2005; Povž and Šumer, 2005; Koščo *et al.*, 2010; Mastitsky *et al.*, 2010; Musil *et al.*, 2010; Lenhardt *et al.*, 2011; Ribeiro and Leunda, 2012; Simonović *et al.*, 2013; Piria *et al.*, 2017). However, there are still some countries that do not maintain lists of invasive species introductions or have developed strategies for managing non-native species and these include the South Caucasian (SC) countries, Armenia, Azerbaijan, and Georgia.

Situated at the junction of Europe and Asia as the land between the Black and Caspian seas, the SC region encompasses a total area of 186,000 km^2 and has an incredibly variable landscape and climate. It is characterized by wide species diversity and a high level of endemism. Due to these and other reasons, the region has been recognized as one of the world's biodiversity hotspots (Myers *et al.*, 2000). According to aquatic biodiversity, the SC can be divided into the basins of the Black and Caspian Seas. However, based on its complex geological and paleo-ecological history, coupled with characteristic aquatic assemblages, finer scale biogeographical subdivisions have also been proposed (Abell *et al.*, 2008; Naseka, 2010). On a wider biogeographical scale, the SC belongs to the Ponto-Caspian region which is one of the primary sources of aquatic invaders worldwide, causing huge economic losses and biodiversity impacts (Bij de Vaate *et al.*, 2002). Unsurprisingly, the SC region is also a recipient of alien non-native aquatic species. However, the changes in diversity, the invasion trends, and the socio-economic impact of non-native aquatic species in the SC ecosystems are still poorly understood (Japoshvili *et al.*, 2020). For instance, the diversity, distribution, and the effect of non-native fish species on autochthonous biota and the local economy in the SC have never been the subject of a research study. Even a basic list of non-native fish species does not exist for the entire region or any country within it. According to a recent checklist of fishes (Kuljanishvili *et al.*, 2020), 119 freshwater fish species are currently recognized in the region with many relics and endemic species, and several introduced (established) species. However, these only included established non-native species and no other introduced non-native species such as, for instance, Chinese carps of the genera *Ctenopharyngodon* and *Hypophthalmichthys* have been discussed. Thus, in-depth understanding of the relationship between native and non-native fish fauna of the SC countries cannot be obtained.

The overarching goal of the present research was to produce a unified, mainly literature-based inventory of the non-native fish species of Armenia, Azerbaijan, and Georgia, and to summarize the existing knowledge base for creating risk assessment models and drafting region-scale management policies. Firstly, we aimed to comprehensively review the national legislative framework and policies to identify trends and gaps in non-native fish species management in SC countries. Secondly, we attempted to prepare an annotated list of non-native fish species agreed upon by fish experts for SC countries. Lastly, we sought to identify the donor areas and pathways for fish introductions and evaluate the establishment success for each non-native fish species.

2 Materials and methods

2.1 Study area

The SC and its three countries, Armenia, Azerbaijan, and Georgia (around 5% of the area of eastern Azerbaijan and northern Georgia belongs to the Northern Caucasus) is encompassed between the Black and Caspian seas. Only the western half of Georgia drains into the Black Sea and accordingly most of the SC represents the Caspian Sea basin (Fig. 1).

Among the SC countries, only Armenia is landlocked with an area of about 30,000 km^2 inhabited by approximately 3 million people. Most of Armenia is situated in the highlands of the Lesser Caucasus and belongs to either the Aras River basin, which is a tributary of the Kura River, or the closed basin of the largest lake in SC, Sevan Lake, with a surface area of 1,250 km^2 and its own river system (Central Intelligence Agency, 2020). Azerbaijan lies on the easternmost southern slope of the Greater Caucasus bordering the Caspian Sea on the east. The country covers almost 83,000 km^2 and is inhabited by approximately 10 million people. Most of the country is formed by the Kura-Aras lowland and the Karabakh upland (Central Intelligence Agency, 2020). In addition to the Kura River, there are numerous small streams directly flowing into the Caspian Sea. Georgia is located mostly along the southern slope of the Greater Caucasus bordering the Black Sea on the west and the Lesser Caucasus Mountains in the south of the country. Georgia covers a terrestrial area of almost 70,000 km^2 and is inhabited by approximately 4 million people (Central Intelligence Agency, 2020). The landscape is largely mountainous with the Colchis lowland on the west and the lowland between the Kura and Alasani Rivers in the east. The territory of Georgia is divided into the Black Sea and the Caspian Sea basins, and thus is the only SC country that includes both sea basins.

2.2 Legislative framework

To review and compare the legislative regulations for aquaculture, introduction and stocking of non-native fishes, and recreational fisheries, the laws and subordinate documents were obtained from the Ministry of Environment of the Republic of Armenia (MERA), the Ministry of Ecology and

Fig. 1. The map of freshwaters of South Caucasian countries: Armenia, Azerbaijan, and Georgia.

Natural Resources of the Azerbaijan Republic (MENRAR), the Ministry of Emergency Situations of the Azerbaijan Republic (MESAR), and the Ministry of Environmental Protection and Agriculture of Georgia (MEPAG). Particular attention was given to the following topics: (1) regulation of fish stocking in rivers, lakes and reservoirs, (2) definition and representation of non-native fish species in the legislative framework, (3) treatment of non-native fish species in aquaculture, to see if there is different treatment for native and non-native fish species in aquaculture farming, (4) regulations about the introduction of non-native fish species, (5) regulations on stocking non-native fish species in rivers, lakes and reservoirs, and (6) regulations on recreational fisheries.

2.3 The list of non-native species

Under the term non-native fish, we included species that were introduced from outside the SC, and translocated species, those native to a part of the SC region that were introduced to a new part of the region where they did not occur before (mainly species translocated from the Black to the Caspian basin). Due to ethnocultural heterogeneity in the region, the exchange of information is complicated and often delayed. Most of the information is only available in the local languages of Armenian, Azeri, or Georgian, or in Russian, which makes it impossible to access or even search for a wider international community. For that reason, we collated and synthesized information on non-native fish species within the SC countries based on all available sources, including scientific and 'grey' (both recent and old) publications, reports, and self-observations.

The non-native species list includes all the non-native and translocated species that have been recorded in the wild in the study area. In addition, the list includes the species that do not exist anymore. Each species in the list is discussed separately with the notes. These notes were arranged in five topics including (1) the origin and native range, (2) donor areas, the place the species was introduced from, (3) the chronology of species introduction events showing in which decades the most introductions happened (for species lacking a documented introduction date, the years of first introductions were recorded as decades after reviewing their first mention in the scientific literature), (4) pathways of introductions, and (5) establishment status of non-native fish species, showing whether the species have created self-sustainable populations in new areas and discussion of the potential risk of invasion and their effect on biodiversity, ecosystems and socio-economic conditions. Species invasiveness was evaluated according to a literature review of the records of the species invasiveness in countries with similar climate conditions, and self-observations. The last section provided individual summaries and discussions about the donor areas, decades of introduction, and pathways of introduction, and establishment state of non-native species.

3 Results and discussion

3.1 The legislative framework

Armenia, Azerbaijan, and Georgia are currently classified as developing countries (https://www.worldbank.org/) that started their independent destinies in 1991 after the dissolution of the Soviet Union. During the Soviet era, there was the centralized Soviet legislative body regulating the fifteen republics united within the Soviet Union. However, after dissolution, the independent states including the SC countries started developing their own legal frameworks, but with little solid experience. A constitution as the main format for the legal system was adopted in 1995 by all three SC countries, and

Table 1. The list of legislatives concerning the regulation of aquaculture/fish stocking, introduction of non-native species, stocking of non-native species, and recreational fisheries in Armenia, Azerbaijan, and Georgia.

	Aquaculture/fish stocking	Introductions of non-natives	Stocking of non-natives	Recreational fisheries
Armenia	2007[1]; 2002(a)[2]; 2009(a)[3]; 2019(a)[4]	2002(b)[5]	–	2003[6]; 2009(b)[7]; 2009(c)[8]; 2019(b)[9]; 2020[10]
Azerbaijan	2017[11]	1998[12]	2017[11]	2017[11]
Georgia	2005[13]; 2021[14]	1996[15]	2021[14]	2013[16]

[1]The Republic of Armenia Law No. 176 of 2007 "On Hunting and Maintaining Hunting Economy".
[2]The Republic of Armenia Government Decision No. 1380-N of 22.08.2002(a) "On Approving the Regulation of Concluding Contracts on the Use of Fauna Objects for Agricultural and Industrial Purposes in the Republic of Armenia".
[3]The Republic of Armenia Government Decision No. 975-N of 13.08.2009(a) "On Approving the State Registration Program of the Republic of Armenia Fauna".
[4]Order of the Republic of Armenia Minister of Nature Protection of 12.02.2019(a) "On Approving Contracts and Applications on the Use of Wild Animals (Amateur Hunting and Fishing, Animal Hunting That are not Objects of Fishing or Hunting) for Social Purposes in the Republic of Armenia".
[5]The Republic of Armenia Government Decision No. 1174-N of 18.07.2002(b) "On the Regulation of the Export of Wild Animals, Zoological Collections and Individual Samples from the Territory of the Republic of Armenia and Their Import into the Territory of the Republic of Armenia".
[6]The Republic of Armenia Government Decision No. 884-N of 10.07.2003 "On Approving the Regulation of Concluding Contracts on the Use of Fauna Objects for Social Purposes".
[7]The Republic of Armenia Government Decision No. 121-N of 22.01.2009(b) "On Approving the Regulation of Organizing and Implementing the Fauna Monitoring".
[8]Order of the Minister of Nature Protection of the Republic of Armenia No. 9-N of 15.02 2009(c) "On Approving Hunting Rules".
[9]The Republic of Armenia Government Decision No. 1667-N of 21.11.2019(b) "On Approving the Regulation of the Restoration, Preservation and Reproduction of Fish and Crayfish Stocks in the Lake Sevan, as well as Their Stocks Definition, the Quantities, Forms and Organization of Fish and Crayfish Industrial Hunting".
[10]Order of the Minister of the Nature Protection of the Republic of Armenia No. 36-N of 3.02.2020 "On Defining the Permissible Quantities for Hunting (Amateur Fishing) for Social Purposes in the Water Areas of the Republic of Armenia During 2020".
[11]Decision of the Cabinet of Ministers of the Republic of Azerbaijan No. 243 of 2.06.2017 "On the Rules for the Fishing and Extraction Other Aquatic Biological Resources".
[12]Law of the Azerbaijan Republic "On Fisheries" of 27.03.1998.
[13]Order of the Government of Georgia No. 138. of 11.09.2005 "On Approving of the Regulation on the Rules and Conditions for Issuing a Fishing Licenses".
[14]Law of Georgia on Aquaculture of 1.03.2021.
[15]Law of Georgia on Animal World of 26.12.1996.
[16]Order of the Government of Georgia No. 423 of 31.12.2013 "On Approving of the Technical Regalement on Fishing and Protection of Fish Stocks".

additional legislation has been passed over the years since then, although, not surprisingly, environmental, and biodiversity-related laws are not among the front-runners. For example, the regulation and management of the fisheries industry, as well as related environmental issues, was deregulated during the last decade of the 20th century. These regulations have been gradually reinstated over the past two decades but they are still being considered for development. For instance, the pet trade is one of the main routes for non-native species introductions (Magalhães and Vitule, 2013; Patoka *et al.*, 2018), yet it still lacks an effective legislative framework in the three SC countries.

In Table 1, we summarized the legislation concerning regulation of the introduction and stocking of non-native species for aquaculture and recreational fisheries in Armenia, Azerbaijan, and Georgia. We reviewed ten current legislative acts related to aquaculture and fisheries in the SC countries and found that Armenia had no laws regulating the stocking of non-native fish species. Currently, there are only two relevant sets of regulations in Azerbaijan and four in Georgia (Tab. 1).

3.1.1 Regulation of fish stocking in rivers, lakes, and reservoirs

Fish stocking in the wild is allowed in Armenia and is carried out on a scientific basis with the consent of the MERA. In general, however, the government does not support fish stocking in rivers, lakes, and reservoirs unless really necessary. In Azerbaijan, fish stocking in natural water bodies is possible only upon obtaining a license from MENRAR and MESAR and allowing them to conduct scheduled monitoring of all natural reservoirs. As in Azerbaijan, stocking into natural water bodies in Georgia is possible after obtaining a license from the National Environmental Agency (NEA) of the MEPAG. The license can be obtained through an auction and is usually good for 20 years. The applicant for the license must present to the NEA a plan describing what kind of fish and in what quantities will be released. The license holder is obligated to present a five-year plan about the species composition and quantity in the water body as well as report monthly catches to the NEA. Although the regulations exist, the subsequent monitoring is poorly run. It is allowed to release non-native fish for stocking into state-owned water bodies in Armenia, Azerbaijan and Georgia though a license, but local anglers frequently release the fry of various non-native fishes into these water bodies intentionally without a license (unpublished information). This may explain the rapid spread of notoriously invasive fish such as the Prussian carp (*Carassius gibelio*) and stone moroko (*Pseudorasbora parva*).

3.1.2 Definition and representation of non-native fish species in the legislative framework

Although non-native fish species are managed by some laws, the definition of 'non-native' and a list of these species are not to be found in the legislation of Armenia and Azerbaijan. There is a definition of 'Introduction' in Georgian laws, as "introduction of species of alien fauna or flora for the purpose of releasing them into nature". However, the act does not provide a list of non-native fish species. In the National Biodiversity Strategy and Action Plan (NBSAP) of Georgia, approved in 2014, there was a national target (B2) aiming by 2020 to evaluate the diversity of invasive species in Georgia, the pathways of their introduction, and their effects on ecosystems (NBSAP, 2014). Unfortunately, the target was not prioritized, and no actions have been taken towards it. Currently, there is no catalogue of alien invasive species indicating which should be placed on a black-list banning them from introduction into the SC countries. Because of the lack of official guidelines for identifying non-native species, decision-makers in SC countries are using the existing scientific literature (Pipoyan, 2012; Ninua *et al.*, 2013; Ibrahimov and Mustafayev, 2015; Pipoyan *et al.*, 2018) and their own knowledge as a rough guide.

3.1.3 Treatment of non-native fish species in aquaculture

Current Armenian legislation does not include any statements concerning non-native species in aquaculture. In Azerbaijan, some non-native fish species such as *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *H. molitrix*; *Onchorhynchus* sp. and *Salmo gegarkuni* are allowed as acclimatization objects in aquaculture and *Chelon auratus* and *C. saliens* are allowed for industrial fishing, provided that permission is obtained from MENRAR. According to the Law on the Animal World, introduction of non-native species in Georgia is forbidden. In practice, however, some exceptions are allowed for species used in aquaculture. For instance, according to the current laws on fishing and protection of fish stocks, the owner of a certain body of water can get a license for the introduction of non-native species such as Chinese carps for stocking in aquaculture since, these species have been introduced in the early 20th century (Barach, 1941; Elanidze, 1983) and thus they are considered as important acclimatized fisheries subjects and are included in books on Georgian fishes (Elanidze, 1983). To introduce a non-native species, license holders are only required to provide a relevant veterinary certificate (*pers. comm.*, Partsvania, 2018; Mdivani, 2018).

Drafting guidelines for laws dealing with non-native fish for aquaculture should be a primary goal of each country's legislation. Although there are laws at least partly dealing with non-native species introductions in all three SC countries, they are ineffective. This is largely because of flaws in the understanding of the definition of non-native fish species and the possibility that non-native species may be viewed as something else entirely.

3.1.4 Regulations about the introduction of non-native fish species

Illegal imports of animals to the Republic of Armenia, relocation to another habitat, acclimatization, and use for selective purposes had been strictly forbidden. According to the former Armenian Law on Environmental Impact Assessment and Expertise, the process of importing non-native species into the territory of the Republic of Armenia was subject to environmental impact assessment by experts. However, that law was repealed in 2014, so that currently there is no normative act restricting fish species introduction. Meanwhile, in Azerbaijan, the introduction of any non-native fish species for the purpose of production, restoration, protection, or acclimatization is legally possible by obtaining the permission of MENRAR. To stock fish in Georgia, the farmer must present a document to the NEA affirming that the introduced species will not affect native species, their habitats, or ecosystem in case it escapes into the wild. For example, this was the way the African catfish (*Clarias gariepinus* Burchell, 1822) was imported from European countries such as Poland, Netherlands, and Moldova, and was introduced and kept in specially established aquaculture facilities in Georgia. A similar situation applied to brook trout (*Salvelinus fontinalis* Mitchill, 1814) and arctic char (*Salvelinus alpinus* L., 1758), which were purchased as fertilized eggs from Canada and kept in a closed systems (*pers. comm.*, Partsvania, 2018). From these examples, it can be seen that the introduction of non-native fish species is poorly regulated in SC countries. If the introduction of non-native species is essentially unrestricted according to Armenian laws, if non-native species are considered as acclimatized in Azerbaijani laws, and if non-native species such as Chinese carp introduced for aquaculture are not considered as non-natives in Georgia, it simply means that introductions of non-native fish species are allowed for all SC countries with no further limitations.

3.1.5 Regulations on stocking non-native fish species in rivers, lakes and reservoirs

There are no laws for the regulation of non-native fish stocks in Armenia except where resettlement and reproduction in natural conditions are concerned; then, it must be carried out with the consent of the MERA. In Azerbaijan, the process of introducing non-native fish and stocking for acclimatization follows rules that require the farmer to prepare a location scheme showing where the fish will be introduced, the species of fish to be acclimatized, their biological characteristics and source of acquisition, how technologies and fishery reclamation works will be applied, the volume of fish production, the chief characteristics of the fish production facility, energy consumption, types of feed to be used, and types and amounts of waste. There are no specific laws regulating the stocking of non-native species in Georgian legislation.

3.1.6 Regulations on recreational fisheries

Recreational fishing in Armenia is allowed through an annual license, which defines the species, the number of adult individuals, and the area and time of fishing. A recreational fishery may be operated in Azerbaijan without any special permits, meaning that no license is needed. There are some limitations regarding the place and time of fishing and the size of the fish. For example, from July 1 to April 30 in the river mouths at the Caspian Sea, an angler can catch up to 5 kg of the Black Sea roach, *Rutilus frisii* (Nordmann, 1840), with a total body length no less than 35 cm. Recreational fishing is free in Georgia, however, the allowed fishing gear and methods are defined in the Technical Agreement on Fishing and Protection of Fish Stocks. The recreational fisheries are monitored at random or based on anonymous reports by the Department of Environmental Supervision (DES) of MEPAG.

What all SC countries have in common with regard to recreational fishing is that anglers are not obligated to report their catches and no statistical data is collected on the number of anglers, fished species and amounts, or even the types of licenses. Accordingly, there is no way to evaluate the contribution of recreational fisheries or to judge the effectiveness of the existing poorly developed regulations. Local fishers often have little knowledge of the risks and intentionally introduce non-native species or bring in native fish from other populations to 'support local stocks' (authors' observations).

3.2 List of non-native fish species

In total, 27 records of non-native species were surveyed in the SC countries of Armenia, Azerbaijan, and Georgia (Tab. 2). Among these taxa, eight species were translocated from the Black to the Caspian Sea basin and six species were unable to survive in the wild (Tab. 2). In Armenia, there were 16 non-native fish species among which, three (*Gobio artvinicus*, *Perca fluviatilis* and *Sander lucioperca*) were translocated and three (*Anguilla anguilla*, *Ictalurus punctatus* and *Mylopharyngodon piceus*) could not survive in natural water bodies. In Azerbaijan, there were 17 non-native fish species, from which five (*Gasterosteus aculeatus*, *Chelon auratus*, *C. saliens*, *G. artvinicus* and *Salmo gegarkuni*) were translocated and two (*Mugil cephalus* and *Oncorhynchus kisutch*) could not survive in the wild. In the western part of Georgia, eight non-native species were recorded while there were 16 in the eastern part of the country. Among these 16 species, four (*G. artvinicus*, *P. fluviatilis*, *Salmo gegarkuni* and *Syngnathus abaster*) were translocated and one species (*Oreochromis niloticus*) could not survive in wild. There were eight non-native species commonly occurring in all three countries: *Carassius gibelio*, *Ctenopharyngodon idella*, *Gambusia holbrooki*, *Gobio artvinicus*, *Hypophthalmichthys molitrix* and *H. nobilis*, *Oncorhynchus mykiss*, and *Pseudorasbora parva* (Tab. 2). There were 15 species that have become established in SC countries (Tab. 2).

3.2.1 Notes on non-native species currently living in the wild

Coregonidae

Coregonus albula is native to the Baltic Sea basin, lakes of the upper Volga River drainage, and also in some lakes of the White Sea basin and the North Sea basin (Kottelat and Freyhof, 2007). It was introduced from the Volkhov hatchery at Lake Ladoga (Russia) to southern Georgia, in Lakes Paravani and Tabatskuri during the 1930s (Barach, 1941; Elanidze, 1983; Japoshvili, 2004; 2012). In the beginning, this species was commercially very important and local hatcheries were involved in artificial reproduction of *C. albula* and release of fry into the lake (Japoshvili, 2012). As of 2005, these hatcheries ceased operation and it was expected that *C. albula* populations would become extinct over time. However, twelve years later young individuals were found in Saghamo Lake, which is connected to Paravani Lake by the Paravani River. This meant that they had become naturalized in the area, although the population density was extremely low (Kuljanishvili *et al.*, 2018).

Commercially valuable species such as *Coregonus* sp. and *C. ludoga* from Ladoga Lake, and *C. maraenoides* from Chudskoe Lake were introduced to Sevan Lake in Armenia from 1924–1927, to support fish production (Barach, 1940; Dadikyan, 1964). During these three years, these coregonids were transported in the form of fertilized eggs from the Volkhov Hatchery in Russia, and already in 1927, fish farms around the lake reproduced whitefishes and released them into the lake (Dadikyan, 1964). Later on, these species were interbred (Mailyan, 1957) and a hybrid form arose as a new subspecies *C. lavaretus sevanicus* (Dadikyan, 1986). Because of insufficient taxonomic evidence, Kuljanishvili *et al.* (2020) reported the Sevan Lake whitefish as *Coregonus* sp. Sevan Lake coregonids are still commercially valuable and their populations are self-sustainable as no artificial propagation occurs in the lake. In addition, it is forbidden to fish in Sevan Lake during the breeding period. According to Elanidze (1983) in 1930, *C. ludoga* was introduced from the Volkhov hatchery at Ladoga Lake to Tabatskuri Lake in Georgia. For some reason, however, the species was not recorded for the past several decades.

Cyprinidae

Although *Carassius gibelio* is considered to be native to an area from central Europe to Siberia and eastern Asia (Rylková *et al.*, 2013), its origin still remains unknown because of the incomplete introduction history and confusion with *Carassius* spp. (Kottelat and Freyhof, 2007). The species introduced into Armenia came from the Odessa region of the Ukraine in the 1960s or 1970s together with the Chinese carps (Pipoyan, 1993, 2012; Pipoyan and Rukhkyan, 1998). Later, during the acclimatization of the herbivorous Chinese carps, *Carassius gibelio* was also accidentally introduced and spread to most of the water bodies in Azerbaijan and was spotted for the first time in the small Kyzylagach Bay in 1986 (Musayev *et al.*, 2004). During the same period, *C. gibelio* was also recorded in Paliastomi Lake (western Georgia) (Daraselia, 1985). Its introduction into Georgia was most likely unintentional. As discussed by Japoshvili *et al.* (2013), this species was

Table 2. List of non-native and translocated fish species, date of the first record, origin, donor areas, pathways of introduction, introduction purpose, and establishment status in Armenian, Georgian, and Azerbaijani freshwaters.

Species	ARM	AZR	W. GEO	E. GEO	Origin	Donor area	Pathway of introduction	Introduction purpose	Establish-ment
Anguillidae									
Anguilla anguilla (Linnaeus, 1758)+	2015	1964			Atlantic Ocean, Black Sea	Intra-regional	Unknown	Accidental	no
Cichlidae									
Oreochromis niloticus (Linnaeus, 1758)+				2019	Sub-Saharan Africa	unknown	A	Intentional	no
Coregonidae									
Coregonus albula (Linnaeus 1758)				1930s	Europe, Russia	Russia	A	Intentional	yes
Coregonus sp.	1920s			1930s	Chudskoe Lake, Russia	Russia	A	Intentional	yes
Cyprinidae									
Carassius gibelio (Bloch 1782)#	1960s	1980s	1980s	1980s	Central Europe to Siberia	Ukraine; Russia	A	Accidental	yes
Gasterosteidae									
Gasterosteus aculeatus Linnaeus 1758*		1984			Black Sea	Intra-regional	SS	Independent invader	yes
Gobionidae									
Gobio artvinicus Turan, Japoshvili, Aksu and Bektas 2016*	x	x		x	Black Sea basin, the lower Choruh River	Intra-regional	A	Accidental	yes
Pseudorasbora parva (Temminck and Schlegel 1846)#	1960s	x	1960s	1960s	Asia: Amur to Zhujiang drainages	China; intra-regional	A	Accidental or Independent invader	yes
Ictaluridae									
Ictalurus punctatus Rafinesque, 1818+	1980s				North America	Russia	A	Intentional	no
Mugilidae									
Chelon auratus (Risso 1810)*		1930			Black Sea	Intra-regional	A	Intentional	yes
Chelon saliens (Risso 1810)*		1930			Black Sea	Intra-regional	A	Intentional	yes
Mugil cephalus Linnaeus, 1758+		1930s			Black Sea	Intra-regional	A	Intentional	no
Oxudercidae									
Rhinogobius lindbergi Berg 1933		2010s		2010s	Asia: Amur River basin	Unknown	A	Accidental or Independent invader	yes
Percidae									
Gymnocephalus cernua (Linnaeus, 1758)			2010s		Northern Black And Azov Sea basins	Unknown	Unknown	Unknown	yes
Perca fluviatilis Linnaeus 1758*	x			1950s	Black Sea	Intra-regional	R	Intentional or accidental	yes
Sander lucioperca (Linnaeus 1758)*	2000s				Black Sea	unknown	A	Intentional	no

Table 2. (continued).

Species	ARM	AZR	W. GEO	E. GEO	Origin	Donor area	Pathway of introduction	Introduction purpose	Establish-ment
Poeciliidae									
Gambusia holbrooki Girard 1859#	1934	1934	1925	1940	North America	Italy; intra-regional, Kazakhstan	BC	Intentional	yes
Salmonidae									
Oncorhynchus kisutch (Walbaum, 1792)+		1970s			North Pacific	Russia	A	Intentional	no
Oncorhynchus mykiss (Walbaum, 1792)	1960s	1970	1930s	1940s	North America	Russia; intra-regional; Latvia	A, R	Intentional	no
Salmo gegarkuni Kessler 1877*		1970s		1930s	Armenia	Intra-regional	A, R	Intentional	yes
Salmo trutta Linnaeus 1758	2000s			1926	Europe and Asia	Russia	A, R	Intentional	no
Syngnathidae									
Syngnathus abaster Risso 1827*				1980s	Black Sea	Intra-regional	R	Intentional	yes
Xenocyprididae									
Ctenopharyngodon idella (Valenciennes 1844)	1960s	1960s	1960s	1960s	Asia: China to eastern Siberia	China	A	Intentional	no
Hemiculter leucisculus (Basilewsky 1855)	2010s	2012			Asia	unknown	A	Accidental or Independent invader	yes
Hypophthalmichthys molitrix (Valenciennes 1844)	1960s	1960s	1960s	1960s	Asia: China to eastern Siberia	China	A	Intentional	no
Hypophthalmichthys nobilis (Richardson 1845)	1960s	1960s	1960s	1960s	Asia: China to eastern Siberia	China	A	Intentional	no
Mylopharyngodon piceus (Richardson 1846)+	1990s				Asia	Russia	A	Intentional	no
Total number of species	**16**	**17**	**8**	**16**					

Abbreviations are as follows: +, species that were spotted but do not exist in wild currently; *, translocated species; #, invasive species; ARM, Armenia; AZR, Azerbaijan, W.GEO, west Georgia; E.GEO, east Georgia; x, introduction time unknown; SS, self-spread; A, aquaculture; R, recreation; BC, biological control.

mistakenly introduced as, or with *Cyprinus carpio,* and it was spread to different water bodies by local people later (Japoshvili *et al.*, 2013). According to local anglers, in the 1980s, tons of *C. gibelio* fry were released in the Alazani River from local hatcheries that were raising *C. carpio* previously introduced from Russia. However, this action was not documented and there is no other evidence except the statements of the fishers. Today, *C. gibelio* is distributed in almost every water body and is usually the dominant species, especially in stagnant water (Japoshvili *et al.*, 2013; Japoshvili *et al.*, 2017; Kuljanishvili *et al.*, 2017; 2020). Also, it is worth noting that in this region, *C. gibelio* can flourish in very harsh conditions such as Madatapa Lake, which at 2,100 meters above sea level is frozen almost six months out of the year (Japoshvili *et al.*, 2017). *Carassius gibelio* is considered as one of the most aggressive and invasive species worldwide (Paulovits *et al.*, 1998; Vetemaa *et al.*, 2005; Mahmoud *et al.*, 2009; Ribeiro and Leunda, 2012; Wouters *et al.*, 2012; Simonović *et al.*, 2013; Copp *et al.*, 2016; Ruppert *et al.*, 2017), although its effect on native fishes and ecosystems in the SC area is unknown.

Gasterosteidae

Gasterosteus aculeatus is a translocated species. It is native to the Black Sea basin and has been dispersed to the Caspian Sea basin and into Azerbaijan. The species appeared there for the first time in 1984 (Guliyev, 2006; Bogutskaya *et al.*, 2013) and was an independent invader. The building of the Volga-Don canal opened a way for this species to disperse into the Caspian Sea basin (Bogutskaya *et al.*, 2013) and it is now widely distributed throughout the Azerbaijani coast of the Caspian Sea and into the river mouths (Ibrahimov and Mustafayev, 2015). The fish enters the rivers during reproduction (Yusifov *et al.*, 2017).

Gobionidae

Gobio artvinicus is a recently described species in the Black Sea basin from the lower Coruh River in Turkey (Turan *et al.*, 2016). Kuljanishvili *et al.* (2020) proposed that *G. artvinicus* in the Kura-Aras system is alien species translocated from the Black Sea basin. However, there is no detailed information available when or for what reason this species was translocated, but its introduction was likely accidental and related to aquaculture activities. Currently, this species is widely distributed in the Kura-Aras system. It should also be mentioned that the species reported from the Metsamor River as *Gobio gobio* by Pipoyan (1998) and found also by Levin and Rubenyan (2010) is most probably *G. artvinicus* (Kuljanishvili *et al.*, 2020).

Pseudorasbora parva is native to the Amur to Zhujiang River drainages in Asia (Kottelat and Freyhof, 2007). It was introduced into Armenian and Georgian water bodies from East Asian countries in the 1960s (Elanidze, 1983; Shonia *et al.*, 2010; Pipoyan, 2012; Ninua *et al.*, 2013; Pipoyan and Arakelyan, 2015). However, this species was discovered in Azerbaijan as late as 2008 (Karabanov *et al.*, 2013), although this does not mean that the species was introduced in that year. In Armenia and Georgia, this species was accidentally introduced together with *Hypophthalmichthys nobilis* or *Ctenopharyngodon idella* fry that were being imported from China at this time (Pipoyan, 2012; Ninua *et al.*, 2013). However, in Azerbaijan, it was suggested that this species penetrated from Armenian water bodies through the Akstafa River. This species has established viable populations in the area and is now very common in almost every water body in the region, even at the relatively high altitude of Kartsakhi Lake at 2000 meters above sea level (Kuljanishvili *et al.*, 2020). Since this species is known to have a huge negative impact on the native environment where it is introduced (Gozlan *et al.*, 2005; Pinder *et al.*, 2005), some adverse consequences of its introduction to the SC are expected.

Mugilidae

Chelon auratus and *C. saliens* are native to the Black Sea basin, and both species were translocated to the Caspian Sea basin in 1902. The first attempt to introduce them by Russian fish farmers was not successful, but another attempt in the 1930s resulted in the acclimatization of these two species in the Caspian Sea basin (Bogutskaya *et al.*, 2013). Both species formed self-sustaining populations (Ibrahimov and Mustafayev, 2015) with current distributions over the entire Caspian sea basin (Bogutskaya *et al.*, 2013; Yusifov *et al.*, 2017). Moreover, these fish are also found in the brackish and fresh waters of the Caspian Sea coast (authors' observations).

Oxudercidae

Rhinogobius lindbergi is a newly recorded non-native fish species in the SC part of the Caspian Sea basin. Its native range is in the Amur River drainage and it is widely introduced in western Asia (Sadeghi *et al.*, 2019), but the exact source from which the species was introduced has not been confirmed. Most probably it entered the SC rivers no earlier than the 2010s and today the species is distributed only in the eastern part of Georgia and Azerbaijan (Epitashvili *et al.*, 2020; Japoshvili *et al.*, 2020; Kuljanishvili *et al.*, 2020). It is assumed that this species is likely to be already present in the Black Sea basin (Kuljanishvili *et al.*, 2020). The vector for this species introduction in the region was either aquaculture or accidental, as it was introduced to Central Asia and Kazakhstan together with Chinese carps (Vasil'eva, 2007). Natural spread from neighbouring countries is also possible. This species is well established and seems to be abundant in eastern Georgian rivers (Epitashvili *et al.*, 2020).

Percidae

Gymnocephalus cernua is distributed in the North, Baltic, Black, Azov and Caspian Sea basins (Kottelat and Freyhof, 2007). This species has not been recorded as native in the area of the SC. This species was recorded in the wild for the first time by Epitashvili *et al.* (2020) in the Rioni River of western Georgia. It's introduction is assumed to have been in the late 2010s, although it is unclear how it could have overcome the natural barriers and appeared in west Georgia. *Gymnocephalus cernua* has become established, and is even invasive, in France, northern Italy, northern Great Britain, and the Great Lakes in North America (Kottelat and Freyhof, 2007), Germany, Austria, Switzerland and Norway (Gutsch and Hoffman, 2016). If established, this species might create competitive pressure for the native *Perca fluviatilis* (Lorenzoni *et al.*, 2009).

Perca fluviatilis is native to the Black Sea basin and was not recorded in eastern Georgia until the early 1960s (Barach, 1964). In the 1950s it appeared in the Alazani River after Mingachevir Reservoir construction (Elanidze, 1983). This species also appeared in the Ararat region in Armenia (Kuljanishvili *et al.*, 2020). Currently, this species is being translocated from western Georgian water bodies (where it is native) to the eastern ones by local fishers for angling purposes. It is commonly distributed in many eastern Georgian water bodies such as the Algeti and Tbilisi Reservoirs, Bareti, Bazaleti, Lisi, Turtle, and other lakes. Accidental occurrences of *P. fluviatilis* in the Ararat Region in Armenia might be connected to fishing activities (Pipoyan, 2020). Its establishment has not been reported in Armenia.

Sander lucioperca is native to the Black and Caspian Sea basins. This species was not recorded in Armenia before 2000. It has been caught in the Aras River at the Agarak-Megri district (Pipoyan and Tigranyan, 2002; Pipoyan, 2012) and was also recorded by Levin and Rubenyan (2010) in the Aras River. Though not officially reported, the repeated findings indicate the existence of established populations in Armenia, but the vector of introduction of this species is unknown.

Poeciliidae

Gambusia holbrooki is native of North America and was introduced into Georgia from Italy in 1925 by Dr. Rukhadze as a biological pest control agent against mosquitoes transmitting malaria, and since 1940, it has been introduced into the whole Georgian territory (Barach, 1941; Elanidze, 1983; Ninua *et al.*, 2013). In Armenia, it was introduced for the same purpose from Georgia in 1934 (Dadikyan, 1986; Pipoyan, 2012). In Azerbaijan, it was also introduced in 1933–1934 (Abdurakhmanov, 1966) from Kazakhstan (Dengina, 1946) for mosquito control (Dengina, 1946). *Gambusia holbrooki* is acclimatized and has spread throughout the water bodies of Azerbaijan. Currently, this species is distributed widely and is considered invasive in the SC (Kuljanishvili *et al.*, 2020). In addition, the appearance of the closely related *G. affinis* is also expected in SC since these species were and still are frequently misidentified (Vidal *et al.*, 2010). Thus, more data is needed to better understand the distribution and diversity of *Gambusia* species in the SC.

Salmonidae

Oncorhynchus mykiss is native to North America with a range from the Pacific basin to northern Mexico. It is also native from Kamchatka to the lower Amur drainage in Asia (Kottelat and Freyhof, 2007). It was introduced into the USSR in Leningrad and the Kursk regions, and from the Kursk region, it was introduced into the Black Sea basin in 1936–1940 (Ninua and Japoshvili, 2008). Subsequently, it was introduced into the Tbilisi, Tkibuli, Shaori, Kumisi and other reservoirs in the region (Ninua *et al.*, 2013). In Armenia, it was introduced from Georgia (Abkhazia region) in the 1960s–70s (Pipoyan, 2012). In Azerbaijan, it was introduced from Latvia, from the hatcheries in Russia and Abkhazia during the 1970–80s (Guliyev, 2006; Ibrahimov and Mustafayev, 2015) for aquaculture. This species is also very popular in recreational fisheries and anglers frequently release fry in reservoirs. Therefore, this species accidentally occurs in the wild throughout the SC (authors' observation, Levin and Roubenyan, 2010; Pipoyan, 2012). Despite this fact, self-sustaining populations of this species have not yet been reported in any of the SC countries.

Salmo gegarkuni is endemic to Sevan Lake, Armenia. This species was introduced into Georgia in the 1930s, first to Tabatskuri Lake in 1930–35, then in Paravani Lake in 1970, and in Tbilisi Reservoir around the 1980s. It was released into the Azerbaijani reservoirs and lakes in the 1970s (*e.g.*, Maralgel Lake in 1977) for aquaculture (Elanidze, 1983; Musayev *et al.*, 2004; Kuljanishvili *et al.*, 2020). It was established in natural water bodies and still occurs in Georgia in the Tbilisi Reservoir and possibly also in mountain lakes of the Kalbajar region of Azerbaijan. Yusifov *et al.*, (2017) reported that after the introduction of *S. gegarkuni* (named as *S. ischchan*) in the Kalbajar region, the native trout species populations decreased in abundance to the point where they were included on the Red List of Endangered Species in Azerbaijan.

Salmo trutta is native to the Atlantic, North and White Sea basins in Europe from Spain to the Chosha Bay in Russia (Kottelat and Freyhof, 2007). It was introduced from the Baltic Sea basin to a fish farm in Tbilisi, Georgia, from which fish were transported to a specifically constructed fish farm in the Natakhtari village (the Kura River drainage) in 1926 and this procedure was instigated by F. F. Kavrajsky (Dzerzhavin, 1941). In Armenia, it was introduced from Europe since the 2000s by farmers under the commercial name 'trout' together with *O. mykiss*. Some were released or accidentally appeared in open waters in Armenia (authors' observation). In Georgia, *S. trutta* has not been recorded after its introduction. However, in Armenia, this fish continues to be valued for use in recreational fisheries (authors' observation). To the best of our knowledge, *S. trutta* should not form sustainable viable populations in Armenia (authors' observation). It should be noted that both older and some recent references erroneously mentioned the taxon, *S. trutta*. This was the result of an obsolete taxonomic approach in which many forms or subspecies of *S. trutta* were used, *e.g. S. t. fario* and *S. t. caspius* for the fauna of Armenia, Azerbaijan and Georgia (Barach, 1940; 1941; Berg, 1949; Elanidze, 1983; Gabrielyan, 2001; Pipoyan and Tigranyan, 2002; Ninua and Japoshvili, 2008; Pipoyan, 2012; Ninua *et al.*, 2013). In this case, the taxon *Salmo trutta* was used for native *Salmo caspius*, not introduced *S. trutta*.

Syngnathidae

Syngnathus abaster is a marine and brackish water species native to the Black Sea, but is also able to survive in fresh water (Kiryukhina, 2013a; b; Tereshchenko *et al.*, 2016; Didenko *et al.*, 2018; Marenkov 2018). It was translocated from the Black Sea basin in the western part of Georgia to the eastern part of the country in the 1980s. As reported by Kuljanishvili *et al.* (2021b), the species was translocated by local hobbyists to the Tbilisi Reservoir, which is locally referred to as 'Tbilisi

Sea'. This translocation was intentional because of the marine name of the freshwater reservoir. Currently, this species has established self-sustainable populations in the reservoir and could be further distributed into nearby water bodies.

Xenocyprididae

Ctenopharyngodon idella, *Hypophthalmichthys molitrix* and *H. nobilis* all have a similar introduction history in the SC. These species were introduced from China in the 1960s for aquaculture to all three countries almost simultaneously (Abbasov, 1972) and have continued to be released into the wild up till now (Elanidze, 1983; Dadikyan, 1986; Gabrielyan, 2001; Ninua and Japoshvili, 2008; Naseka and Bogutskaya, 2009; Pipoyan, 2012; Bogutskaya *et al.*, 2013). These species were unable to sustain populations there and for that reason, their regular stocking has continued.

Hemiculter leucisculus is native to southeastern Asia and the Amur River basin. The species appeared on the Iranian shores of the Caspian Sea in the 1990s as an accidental contaminant of Chinese carp parcels (Zareian *et al.*, 2015). It was recorded in Azerbaijan for the first time in 2012 (Mustafayev *et al.*, 2015), but the exact time of its introduction and the pathways are still unknown. Most probably it entered from the neighbouring areas, where it was already introduced (Mustafayev *et al.*, 2015). *H. leuciscus* was also recently discovered in Armenia, in the Arpa River by Pipoyan and Arakelyan (2021). The first introduction date and pathway of this species introduction in Armenia is unknown, but it probably penetrated from neighbouring areas. The species is currently well established in the Caspian Sea basin and Kuljanishvili *et al.* (2020) and it is assumed that it will soon penetrate the Black Sea basin.

3.2.2 Notes on non-native species that no longer occur in the wild.

Anguillidae

Anguilla anguilla is native to the Atlantic Ocean and is distributed in all European rivers that flow into the Mediterranean, North and Baltic Seas, with some individuals entering the Black Sea (Kottelat and Freyhof, 2007). Passing through the Volga-Baltic waterway, *A. anguilla* accidentally appeared in the Volga River, and from there made its way into the Caspian Sea. Since 1964, individuals of this species have been found at the mouths of rivers in Azerbaijan (Abdurakhmanov, 1966; Ibrahimov, 2012). In 2015, it was also observed in the middle Aras River (Pipoyan, 2015), which is classified as an accidental occurrence rather than as an introduction record (Kuljanishvili *et al.*, 2020).

Cichlidae

Oreochromis niloticus is native to Sub-Saharan Africa and it is one of the most cultured fishes worldwide (Trewavas, 1983). Because of this reason, this species has been widely introduced for aquacultural purposes to different countries, which had led to its successful establishment in some areas of USA (Zambrano *et al.*, 2006) and Brazil (Britton and Orsi, 2012). In summer of 2019, *Oreochromis* sp. has appeared to the local angler's catch in the small River Baisubniskhevi, eastern Georgia (Lagodekhi Region) (Kuljanishvili *et al.*, 2021a). Probably it appeared in the river from the nearby fish farms, where the owners might have been stocking tilapias for aquaculture. Local angler collected around 10 individuals and provided photographs to the authors. According to our observation, the fish was identified as *O. niloticus*. And after repeated sampling in the same year, later in the season, the fish did not appear in our catch. However, this case needs attention, since it was not believed that *O. niloticus* could establish in temperate US, however, it was later shown that they have established successful populations (Grammer *et al.*, 2012). Since the climatic conditions in the eastern Georgia fluctuates from cold mountainous to humid subtropical type it is necessary to monitor the success of this species establishment in the area.

Ictaluridae

Ictalurus punctatus, the venomous catfish native to North America, was brought to Armenia from Krasnodar, Russia in 1981, 1983, 1991. It is currently absent in the wild in the region (Pipoyan and Tigranyan, 1998, 2002; Pipoyan 2012).

Mugilidae

Mugil cephalus is native to the Black Sea basin. It was brought together with *Chelon auratus* and *C. saliens,* from the Black Sea and released into the Caspian Sea for acclimatization in 1930–34. Unlike *Chelon auratus* and *C. saluens*, *M. cephalus* could not adapt to local environmental conditions in the Caspian Sea and disappeared (Bogutskaya *et al.*, 2013).

Salmonidae

Oncorhynchus kisutch is originally from the northern Pacific Ocean. This species was transported from Kamchatka in Russia to Azerbaijan in 1977–83, to the Chaykend fish hatchery in the form of fertilized eggs. After incubation, the hatched fry were released into the Caspian Sea (Musayev *et al.*, 2004). However, there is no data on the results of the acclimatization of this fish and no records from the wild.

Xenocyprididae

Mylopharyngodon piceus is native to the Amur River basin to southern China and was introduced in Armenia from Krasnodar, Russia, in 1990. It was reported as an acclimatized species by (Pipoyan and Tigranyan, 1998; 2002), but without giving any details of the species distribution in the wild (Levin and Rubenyan, 2010). Later, Pipoyan (2012) reported that this species was distributed in the Metsamor River drainage; however, due to discontinuation of artificial reproduction, it disappeared from the inland waters of Armenia (Pipoyan, 2012). Unless there is new evidence of this species being caught in the wild in Armenia, we believe that this species should be taken off the list of non-native species of Armenia.

3.3 Summary of the list of non-native fish species

3.3.1 Donor areas

To summarize the previous section, the most important donor area for fish introduction was intra-regional spread (Tab. 2). In total, 12 species were spread within the

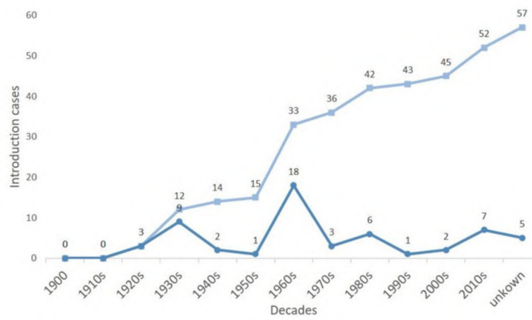

Fig. 2. Number of introduction cases by the decades in Armenia, Azerbaijan and Georgia together expressed by dots; and total number of introduction cases by the decades expressed by squares. Note that, the total number of cases is 57 since there are species that were introduced in different times in different countries.

SC countries, mostly intentionally (Tab. 2). These species were *Anguilla anguilla*, *Gasterosteus aculeatus*, *Gobio artvinicus*, *Pseudorasbora parva*, *Chelon auratus*, *C. saliens, Mugil cephalus*, *Perca fluviatilis, Gambusia holbrooki, Oncorhynchus mykiss*, *Salmo gegarkuni,* and *Syngnathus abaster.*

The largest number of non-native species was introduced from Russia (Tab. 2). This is a consequence of the fact that all three countries were part of the Soviet Union in the past, because most introductions happened during that period. Eight species were introduced intentionally. These are *Coregonus albula, Coregonus* sp., *Carassius gibelio*, *Ictalurus punctatus*, *Oncorhynchus kisutch*, *O. mykiss*, *Salmo trutta*, and *Mylopharyngodon piceus* (Tab. 2). Four species widely used in aquaculture were introduced intentionally in all three SC countries from China mainly because this country is the main provider of the species that are easy to keep and culture in ponds, and pond aquaculture is currently very popular in this region (Tab. 2). These species are *Pseudorasbora parva*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*, and *H. nobilis* (Tab. 2). Two non-native introductions came from Europe (Tab. 2): *Gambusia holbrooki* was introduced from Italy into Georgia, and rainbow trout *Oncorhynchus mykiss* came from Latvia to Azerbaijan (Tab. 2). One species, *Carassius gibelio*, came from Ukraine into Armenia (Tab. 2), and *Gambusia holbrooki* was introduced to Azerbaijan from Kazakhstan. The donor area is unknown for five cases (Tab. 2). From where *Sander lucioperca* got to Armenia, *Hemiculter leucisculus* to Azerbaijan, *Rhinogobius lindbergi* to Azerbaijan and Georgia, *Gymnocephalus cernua* and *Oreochromis niloticus* to Georgia is unknown (Tab. 2).

3.3.2 Decades of introductions

There were four peaks of non-native species introductions in the 1930s, the 1960s, 1980s, and the 2010s with 9, 18, 6 and 7 new introduction events (Fig. 2). The remaining decades were characterized by fewer introductions (Fig. 2). While most of the introductions and translocations of local species, both intentional and unintentional, happened during the Soviet Era before the 1990s, the species introductions also continued during the last 30 years of the later period and tended to increase in 2010s.

It is not surprising that the largest number of introductions happened during the Soviet era as this period was characterized in general by a significant increase in new introductions and translocations worldwide (Copp *et al.*, 2005). The 1920s–30s is also an interesting period when intensive introductions for aquaculture purpose were pioneered by the scientist, Dr. Rukhadze, and Prof. Derzhavin in the SC. It is also known that during the Soviet regime, fish introductions for acclimatization and stocking for aquaculture were encouraged by the central government (Copp *et al.*, 2005). It should be also mentioned that the recent decade was characterised with high number of non-native species, meaning that developing of monitoring strategies is crucial.

3.3.3 Pathways of introductions

The basic pathway for fish introductions in the SC with 18 cases out of 27 introductions, was aquaculture followed by aquaculture and recreational fisheries (with three cases), and lastly, recreational fisheries (with two cases) (Tab. 2). *Gambusia holbrooki* was only species, that was introduced for biocontrol purposes and only species such as *G. aculeatus* are thought to be independent (self-spreading) intruders to new areas in the SC (Tab. 2). Except for a few cases, all introductions were intentional with direct human assistance (Tab. 2). This includes target species and accompanied non-target species introductions.

3.3.4 State of non-native fish species establishment

Out of 27 non-native fish species recorded, 15 are currently established. The remaining 12 species are dependent on human support (Tab. 2). Among these 15 established species, *Carassius gibelio*, *Pseudorasbora parva* and *Gambusia holbrooki* are of primary importance because of their aggressive, invasive behavior. Before reliable risk/impact assessments of these species will be done, we have to rely on existing literature to assess their invasive potential. For instance, *C. gibelio* is considered one of the most successful invasive fish worldwide (Copp *et al.*, 2005; Gozlan *et al.*, 2010) with a negative impact on the environment because of its foraging behaviour and unusually high abundances (Vetemaa *et al.*, 2005; Lusková *et al.*, 2010). The presence of *C. gibelio* is known to increase water turbidity (Crivelli, 1995) and lead to alterations in the nutrient cycle (Paulovits *et al.*, 1998). It negatively affects native species including plants and animals in terms of grazing pressure and by direct competition with natives (Gaygusuz *et al.*, 2007; Ribeiro and Leunda, 2012; Tarkan *et al.*, 2012; Ruppert *et al.*, 2017). *C. gibelo* may also cause the introduction of non-indigenous parasites and pathogens into newly invaded areas (Žitnan, 1974; Mahmoud *et al.*, 2009). Like *C. gibelio*, *Pseudorasbora parva* is also a widely distributed invasive species. Once established, it creates dense populations that become dominant and compete aggressively with native species for resources (Britton *et al.*, 2007). It is also known to feed on the eggs and larvae of native species (Pinder *et al.*, 2005). Moreover, it is a host of *Sphaerothecum destruens*, the novel fish pathogen in Europe (Gozlan *et al.*, 2010). *Gambusia holbrooki* is known to have a negative impact on native fish and amphibian fauna

(Hamer *et al.*, 2002; Alcaraz *et al.*, 2008). Because of its rapid reproduction, it can quickly reach high abundance, which makes this species more invasive (Benejam *et al.*, 2009). Its feeding habit plays an important role in shaping the community of zooplankton (Margaritora *et al.*, 2001) and it successfully competes for food with native species (Scalici *et al.*, 2007). These well-known invasive species are currently widespread and frequently dominant in the whole SC area, and have already had a significant impact on the native fauna and ecosystems. There has been no attempt to quantify the effects of invasive fish species in the SC countries, or to extrapolate their future impact if left unchecked. Thus, it is not surprising that no strategy exists for mitigating the impact of invasive species in Armenia, Azerbaijan, and Georgia.

4 Conclusions

For many decades, there has been little attention paid to the history of introduction and the status of non-native fish species in the SC countries of Armenia, Azerbaijan, and Georgia. To the best of our knowledge, this study is the first attempt to establish a reliable background for risk assessment of non-native species and evaluation of their impact on the area. This paper should help the national decision-makers to categorize native and non-native species and to improve legislation for regulating introduction and stocking of non-native species, aquaculture production and the pet trade, which our study revealed are poorly designed and not consistent across the three SC countries. These countries share the same river basin, the Kura-Aras, and up to now, there has been no initiative among them addressing the problem of invasive freshwater fishes (or other invasive taxa such as crustaceans and molluscs), either at the governmental or the academic level. Thus, transboundary monitoring schemes and data-sharing systems do not exist. In general, the lack of rules or enforcement of existing standards regarding introductions and translocations have contributed to an increase in the number of non-native species, which endangers native organisms (Copp *et al.*, 2005) and threatens to cause serious socio-economic losses in the region. Therefore, we strongly encourage substantive discussions and planning to deploy systems designed to protect the highly vulnerable freshwater ecosystems in the Caucasus biodiversity hotspot.

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Chapter 6: Risk assessment of non-native freshwater fishes for the Caucasus biodiversity hotspot [in preparation]

Risk assessment of non-native freshwater fishes for the Caucasus biodiversity hotspot

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Abstract

The invasion of non-native fish species is the most threatening process for freshwater ecosystems. Yet the knowledge of fish species invasions, affecting mechanisms or possible future scenarios after introductions is rather limited in most of the parts of the world. These challenges are at least partially solved by the recently developed AS-ISK tool kit that enables researchers to screen the species for their invasiveness (i.e. horizon scanning) given the current and predicted climate change scenarios and make conclusions for future actions. Here, based on AS-ISK methodology, we screened 32 to introduced non-native established/not established species, not yet introduced and locally translocated species for their invasiveness in the South Caucasus (including Armenia, Azerbaijan and Georgia). The screening was conducted three local fish biologists independently to minimize human related errors. Among the screened species, we found that 21 species are pose high risks of invasiveness for the risk assessment area. The results were highly similar irrespective to involved expert. Eight of those high risk species are already widespread within the region and hence having yet unmeasured impact on the local environment/economy. The other species while others are expected to worsen the situation if become established. We draw special attention to the AS-ISK tool as a new open source and easy to use opportunity for local stakeholders to run horizon screening of potentially invasive species. Also identify the gaps in knowledge of non-native fish species in the South Caucasus region and provide recommendations for future directions towards the comprehensive understanding and enhanced management of non-native fish taxa.

Keywords
AS-ISK, South Caucasus, Invasive fishes, Risk Screening

Introduction

Biological invasions are considered a topping threat to global biodiversity and pose significant human well-being problems (Mazza et al. 2014; Shackleton et al., 2018). Protecting the biodiversity and maintaining ecosystem functioning under the pressure of invasion of non-native species (NNS) (also referred to as "alien") require huge expenditure annually. The growing trend of invasion events and extents (Seebens et al., 2017) are proportionally getting more and more costly (Diagne et al. 2020). Undoubtedly, eradication and later management of established invasive taxa are much demanding in terms of costs and challenges in mitigation than the prevention of initial penetration (Simberlof et al. 2013). The latter one can be achieved by country-based regulations to control species translocation within/among countries and immediate eradicative response once the invasive species first appear locally (according to the priorities for the management of invasive species adopted by "Convention of Biological Diversity" in 2002; Simberlof et al. 2013).

However, concerted work to slow down the invasion process and management of invasive taxa seem to be challenging at a global scale (Genovesi et al. 2013; Tittensor et al., 2014; CBD, 2018). One of the main reasons hindering the effective cross-national strategic planning against the invasion process is the absence of quality data for most of the countries (Latombe et al. 2017). For example, most developing countries lack an effective list of invasive species, do not have monitoring capacities or have not adopted strategies of how to deal with invasive taxa, and are usually characterized by delayed reporting of new arrivals (Early et al. 2016).

A good example is the South Caucasus comprising territories of Armenia, Azerbaijan and Georgia, which forms the major part of the Caucasus biodiversity hotspot (Mittermeier et al. 2004). Despite declared willingness to do so (e.g. signing parties on CBD), there are no yet working nationwide incentives to inventory the invasive species, nor monitoring programs for SC countries exist. Only marginal activities run by academic researchers provide the authoritative but still limited inventory of non-native plants (Kikodze et al. 2010; Fayvush and Tamanyan 2014; Sharabidze et al. 2018; Abdiyeva 2019), freshwater molluscs (Mumladze et al. 2019), insects (Aleksidze et al. 2021) and fishes (Kuljanishvili et al. 2021). Unfortunately, none of those mentioned above inventory (except fishes) is complete enough or up-to-date to be useful at a national or regional level. The effects of the NNS on biodiversity and ecosystems in SC have never been evaluated for even a single species. Undoubtedly, the NNS have already caused some environmental and economic loss in SC countries. However, the cost amount or expenditure trends are unknown. There is no information on the economic costs of the NNS to the South Caucasian countries in the most up-to-date InvaCost database of Diagne et al. (2020). Located south of the Great Caucasus mountain chain and stretched between the Black and Caspian Seas, the 80% of the SC territory belongs to the Kura-Aras (the Caspian Sea basin) drainage shared with Turkey and Iran. Only 20% of the area (western SC) belongs to the Black Sea basin. The SC is widely recognized as a biodiversity hotspot having a great diversity of landscapes, climate zones, and respectively sheltering incredibly diverse plants and animal taxa. Freshwater biodiversity is perhaps the most understudied ecosystem type in the SC region (Mumladze et al., 2020). While the SC region is diverse with aquatic resources, the rivers are mainly considered as hydropower production potential (Freyhof et al., 2015; Mumladze et al., 2019). Other services such as up-taking drinking water, fisheries, irrigation, recreation etc., while extensively used, is less acknowledged locally. Out of few recent studies, no threats facing the freshwater biodiversity and ecosystems in the SC (and Caucasus biodiversity hotspot in general) have been evaluated and hence very poorly understood. This is particularly evident when considering freshwater NNS's current and potential impact. Indeed, the knowledge about the threats that freshwater ecosystems face to in the SC area is virtually non-existent. Only in the recent study, Japoshvili et al. (2021) showed the effect of existing and planned hydropower plants on the connectivity of fish communities within Georgia. Another study by Kuljanishvili et al. (2021), based on the literature review, recent collections and social-media data about the diversity, distribution and introduction history, developed the most up-to-date inventory for all known non-native introduced/locally translocated species for the SC countries. This later work aimed to help future risk assessment models using the expert-based Aquatic Species Invasion Screening Kit – AS-ISK (Copp et al. 2016, 2021) and inform regional management policies. AS-ISK is one of the most promising tools to help decision-makers prioritize NNS according to the invasiveness potential and plan resource allocation for their management. Based on species biology, invasion history and future climate scenarios, AS-ISK is able to rank species according to the risks they pose if penetrated to a geographic area of interest (Risk Assessment or RA area hereafter). AS-ISK can provide a practical evaluation of the invasiveness of NNS that are either present and established in the area or have not yet arrived in a RA area. To understand the potential risks of the NNS in RA, we aimed to screen: (1) the locally translocated species that are *a priori* not usually perceived as invasive or non-native; (2) species that are (or were) a subject of introductions (established/not yet established) in RA area; (3) several freshwater fish species not yet introduced to RA area; (4) and the ones, which were recorded but have not been found in natural waters in the RA. This is the first analysis of its kind for the region. It is anticipated to step forward to understand better the impact and risks of potential environmental/economic loss caused by invasive fish species in the region. Since the whole SC shares river basins, it is essential to evaluate the invasion risks at the level of the entire river basins. Thus such results are expected to be of high value for policy and environmental decision-makers at the country and international levels to plan invasive species management and mitigation.

Methods

Risk assessment (RA) area

The South Caucasus is politically subdivided into three independent states Armenia, Azerbaijan and Georgia. However, the SC is also pronounced geographic unit. Indeed, the region is bordered by the insurmountable Great Caucasus mountain range from north and the Black and Caspian Seas, respectively, from west to east (fig. 1). The southern border is depicted less precisely, but it reflects the watercourses of two large rivers – the Kura/Aras and Chorokh, which springs in the Anatolian plateau. The longest in the region, the Kura-Aras river flows into the Caspian Sea. Almost all the eastern South Caucasian rivers are draining to this water basin except a few short rivers in extreme north-east Azerbaijan.

On the other hand, western Georgia is draining into the Black Sea through numerous small to medium-sized rivers. Above this, there are several isolated mountainous lakes with their independent basin. Among those, the Sevan Lake in Armenia is the largest, with a surface area of 1239 km^2 and the basin with 5000 km^2. This lake harbours several endemic freshwater taxa. According to Kuljanishivili et al. (2021), some species were translocated from the Black Sea rivers of Western Georgia to the Kura-Aras system intentionally or unintentionally. In addition, a few species, such as Sevan trout was also introduced from Sevan Lake to similar mountainous lakes within the Kura-Aras basin. Other species were introduced accidentally or intentionally through the SC area. Thus, as the RA area, we consider either whole 'South Caucasus' for species already (or can be) introduced in the region or 'eastern SC' (Caspian Sea Drainage) for translocated species.

Risk screening

In total, 32 freshwater fish species were selected for risk assessment of their invasiveness in the South Caucasus region – hereafter, also referred to as the 'RA area'. RA (for each of 32 species) were carried out independently by BJ, GE and TK under the supervision of LM and LV, who were respectively responsible for quality control of the biological/ecological data and of the methodological (i.e. database-related) aspects of the study. The criteria for species selection were as follows (Table 1):

1. Translocated species (*n* = 8);
2. Non-native species already present in the RA area (*n* = 14);
3. Non-native species established in neighbouring countries or countries of similar climate to the RA area (*n* = 5);
4. Non-native species were recorded in the RA area but not in the wild (*n* = 5).

The species for the first three criteria were based on the most recent non-native species list published by Kuljanishvili et al. (2021), and the later one were selected based on the literature search.

Identifying potentially high-risk species was undertaken using the AS-ISK (available for free download at www.cefas.co.uk/nns/tools/). This decision-support tool complies with the 'minimum standards' for screening NNS under EC Regulation No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (European Union, 2014). The AS-ISK consists of 55 questions: the first 49 questions comprise the Basic Risk Assessment (BRA) and address the biogeography/invasion history and biology/ecology of the species; the last six questions comprise the Climate Change Assessment (CCA) and require the assessor to predict how future predicted climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. For the purposes of the CCA component of the screening protocol, an increase in temperature on average by 2ºC relative to current conditions is predicted (Hansen et al., 2010; Beck et al. 2018).

To achieve a proper screening, the assessor must provide a response for each question, a level of confidence (see below), and a justification based on literature sources. The outcome BRA score, ranges from −20 to 68, and a composite BRA+CCA score, ranges from −32 to 80. It means after adding or subtracting up to 12 points to the BRA score or leaving it unchanged in case of a CCA score equal to 0. Scores < 1 suggest that the species is unlikely to become invasive in the RA area and poses a low risk, whereas scores > 1 indicate a medium or high risk of being invasive species in the RA area. The threshold values for the BRA and BRA+CCA that distinguish between medium-risk and high-risk levels are obtained through a procedure of RA area-specific 'calibration', which is achieved by Receiver Operating Characteristic (ROC) curve analysis (Bewick et al., 2004). Additionally, for the species classified as high risk, a distinction was made in this study of the 'very high risk' species based on an *ad hoc* threshold weighted according to the range of high-risk score values obtained for the BRA and BRA+CCA (e.g. Clarke et al., 2020; Interesova et al., 2020; Killi et al., 2020; Moghaddas et al., 2021; Uyan et al., 2020). Importantly, identifying the very high-risk species is helpful to prioritize the allocation of resources because of a full RA (Copp et al., 2016a). This examines in detail the risks of: (i) introduction (entry); (ii) establishment (of one or more self-sustaining populations); (iii) dispersal (more widely within the RA area, i.e. so-called secondary spread or introductions); and (iv) impacts (to native biodiversity, ecosystem function and services, and the introduction and transmission of diseases).

For ROC curve analysis to be implemented, the species selected for screening must be categorized *a priori* as 'non-invasive' or 'invasive' using literature sources. The *a priori* categorization was implemented as follows (see also Clarke et al. 2020): (i) the first search was made in the FishBase (www.fishbase.org), where, the species that were regarded as 'harmless' were categorized as non-invasive in our study and the species that were regarded as 'potential pest', were categorized as invasive. Species, not evaluated or not listed in the above database were scored as absent; (ii) a second search was made of the Global Invasive Species Database (www.iucngisd.org) and the Centre for Agriculture and Bioscience International Invasive Species Compendium (www.cabi.org/ISC), with the species categorized as invasive if it appeared in any of such lists or scored as absent if not listed; (iii) a third search was made of the Invasive and Exotic Species of North America list (www.invasive.org), with the species categorized as invasive if it appeared in any of such lists or scored as absent if not listed; (iv) except for those species categorized as invasive in any (or all) of the previous three steps, a Google Scholar (literature) search was performed to check whether at least one peer-reviewed reference is found that 'demonstrates' (hence, not 'assumes') invasiveness/impact. The latter was then taken as 'sufficient evidence' for categorizing the species as invasive; whereas, if no evidence was found, then the species was categorized as non-invasive.

The Confidence level (CL) in the responses to questions in the AS-ISK is ranked using a 1–4 scale (1 = low; 2 = medium; 3 = high; 4 = very high) as per the Intergovernmental Panel on Climate Change (IPCC 2005; see also Copp et al., 2016b). Based on the CL allocated to each response, a confidence factor (CF) is obtained as:

$CF = \sum(CL_{Qi})/(4 \times 55)$ $(i = 1, \ldots, 55)$

where CL_{Qi} is the CL for Qi, 4 is the maximum achievable value for confidence (i.e. very high: see above) and 55 is the total number of questions comprising the AS-ISK questionnaire. The CF ranges from a minimum of 0.25 (i.e. all 55 questions with confidence level equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Based on all 55 Qs of the AS-ISK questionnaire, the 49 Qs comprising the BRA and the six Qs comprising the CCA: for the CL, the CL_{Total}, CL_{BRA} and CL_{CCA} are computed, respectively; and for the CF, the CF_{Total}, CF_{BRA} and CF_{CCA}.

Statistical analysis

A ROC curve is a graph of sensitivity *vs* 1 – specificity for each threshold value, wherein the present context, sensitivity and specificity will be the proportion of *a priori* invasive and non-invasive fish species, respectively, that are correctly identified by the AS-ISK as such. A measure of the accuracy of the calibration analysis is the area under the curve (AUC). Given that AUC is equal to 1, the test is 100% accurate because both sensitivity and specificity are 1, and there are neither 'false positives' (*a priori* non-invasive species classified as high risk, hence invasive) nor 'false negatives' (*a priori* invasive species classified as low risk, hence non-invasive). If the AUC is equal to 0.5, then the test is 0% accurate as it cannot discriminate between 'true positives' (*a priori* invasive species classified as high risk, hence invasive) and 'true negatives' (*a priori* non-invasive species classified as low risk, hence non-invasive).

Following ROC analysis, the best threshold value that maximizes the true positive rate and minimizes the false positive rate was determined using Youden's *J* statistic; whereas the 'default' threshold of 1 was set to distinguish between low-risk and medium-risk species. Notably, the true/false positive/negative outcome distinction was not applied to the medium-risk species, as they can be either included or not into a full (comprehensive) RA (see 2.1 *Risk Screening*) depending on priority and/or availability of financial resources. Fitting of ROC curves was performed by the package pROC (Robin et al., 2011) for R x64 v3.2.0 (R Core Team, 2019) using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along with the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals).

Differences in CF between components (i.e. BRA and BRA+CCA) were tested with permutational ANOVA based on a one-factor design. Analysis was implemented in PERMANOVA+ for PRIMER v6, with normalization of the data and using a Bray-Curtis dissimilarity measure, 9999 unrestricted permutations of the raw data, and with statistical effects evaluated at $\alpha = 0.05$.

Results

For both the BRA and the BRA+CCA, there were no statistically significant differences between AUCs from the three assessor-specific ROC curves (BJ *vs* GE: $P = 0.679$ for the BRA and 0.546 for the BRA+CCA; BJ *vs* TK: $P = 0.869$ and 0.811; GE *vs* TK: $P = 0.607$ and 0.404). These results justified the computation of two ROC curves based on the mean BRA and

BRA+CCA outcomes scores for the screened species, respectively. Accordingly, the ROC curve for the BRA resulted in an AUC of 0.7316 (0.5274–0.9358 95% CI) and that for the BRA+CCA in an AUC of 0.6926 (0.4895–0.8958 95% CI). These AUCs indicated that AS-ISK was able to reliably distinguish between non-invasive and invasive freshwater fish species for the RA area. Youden's *J* provided the BRA and BRA+CCA thresholds of 18.0 and 20.3, respectively, which were used to calibrate the risk outcomes for the distinction between medium-risk and high-risk species. The AS-ISK report for the 32 screened species is provided as Supplementary Material 1.

Based on the BRA threshold (Table 2):

- 21 (65.6%) species were classified as high risk and 11 (34.4%) as a medium risk;
- Amongst the 21 species categorized *a priori* as invasive, 18 were true positives;
- Out of the 11 medium-risk species, eight were *a priori* non-invasive and three invasive.

Based on the BRA+CCA threshold (Table 2):

- 21 (65.6%) species were classified as high risk, 10 (31.3%) as medium risk, and one (3.1%) as low risk;
- Amongst the *a priori* invasive species, 17 were true positives; amongst the *a priori* non-invasive species, one was a true negative (*Coregonus* sp.);
- Out of the 10 medium-risk species, six were *a priori* non-invasive and four invasive.

The highest-scoring ('top invasive') species (BRA score ≥ 40 and BRA+CCA score ≥ 45, both taken as *ad hoc* 'very high risk' thresholds) were gibel carp (*Carassius gibelio*), Zander (*Sander lucioperca*), North African catfish (*Clarias gariepinus*) and topmouth gudgeon (*Pseudorasbora parva*) for both the BRA and BRA+CCA, and ruffe (*Gymnocephalus cernua*) for the BRA+CCA only. The CCA resulted in an increase in the BRA score (cf. BRA+CCA score) for 26 species and in a decrease for the remaining six species (Table 3). Differences in BRA scores between the three assessors ranged from 1 to 34, with a mean of 13.0 and median of 11.5, and 5% and 95% CIs of 6.0 and 23.0, respectively (Figure 2a). Differences in BRA+CCA scores ranged from 4 to 35, with a mean of 16.3 and a median of 13.5 and 5%, and 95% CIs of 5.8 and 32.0, respectively (Figure 2b).

In terms of confidence in the responses, the mean CL_{Total} was 2.70 ± 0.03 SE, the mean CL_{BRA} 2.77 ± 0.03 SE, and the mean CL_{CCA} 2.11 ± 0.07 SE (hence, indicating medium confidence). The mean CF_{Total} was 0.674 ± 0.008 SE, the mean CF_{BRA} 0.692 ± 0.008 SE, and the mean CF_{CCA} 0.527 ± 0.017 SE. Statistically, the CL_{BRA} was significantly higher than the CL_{CCA} ($F^{\#}_{1,62} = 76.72$, $P < 0.001$).

Discussion

NNS in SC region

Presented work is the first attempt to evaluate risks of freshwater NNS invasiveness in the Caucasus biodiversity hotspot. Employed AS-ISK tool-kit was able to successfully identify and classify freshwater associated NN fish species according to the risks they pose in the SC region. The obtained BRA and BRA+CCA thresholds for the SC can be then used for further risk screenings of any single fish species in the region. Worse to note that our calibration resulted relatively higher thresholds for both BRA/BRA+CCA then reported by a Vilizzi et al (2021) indicating the peculiarity of particular RA area under question. Furthermore, the either threshold values can be adjusted after obtaining new biological data on separate species or by improving climate prediction scenarios. Thus re-screening of the species after some time can be worthwhile. Due to inherent ambiguity related to expert-based evaluations, we replicated screening procedures independently for each species and found no statistically significant differences between assessors. This further justifies the reliability of the obtained results.

Among the screened species (32 in total), 21 species were classified among the high-risk bearing for both BRA and BRA+CCA (Table 2). From those high-risk species, there are three false positives (not *a priory* invasive), including three-spined stickleback (*Gasterosteus acculeatus*), ruffe (*Gymnocephalus cernua*), and pumpkinseed (*Lepomis gibbosus*). However, the high-risk status of those species can easily be justified for the RA area. As an example, *G. aculeatus* is widespread circum-arctic/temperate species that naturally occurs in the Black Sea basin. However, species expanded its range to the Caspian Sea using Volga-Don channel and now is widely established along the Caspian Sea coast (in particular eastern SC region) and actively inters to a lower reaches of rivers (Ibrahimov and Mustafayev, 2015). Due to its high tolerance to salinity and temperature and its reproductive/foraging characteristics (e.g. Roch et al. 2018; Candolin, 2019), the species can be considered as high risk-bearing within the RA area. Similarly, *G. cernua* is also a widespread species around the RA area (natively), but was naturally absent in SC. This species is widely introduced throughout European countries and USA. Due to its biology and trend of range expansion, species is well suited to invasive status; however, still, there is no consensus whether the species possess high risks of biodiversity and local economies within invaded areas (Gunderson 2021). *G. cernua* was only recently recorded from RA area and have established a population in at least a single (Rioni) river (Epitashvili et al. 2020). Thus, with wide distribution potential, species can significantly alter the invaded ecosystems through competition to native fauna and alter endemic/range restricted invertebrates. The last species from this category is *L. gibosus*, a widely established species in

Europe that originated from North America. Though species have not yet been recorded from RA area, it is established in western neighbour countries, and there are no known obstacles preventing its introduction. Pumpkinseed is known to adjust its life-history traits to new environments (Copp et al., 2007) and can even be an aggressive competitor for native fauna (Almeida et al., 2014; Copp et al., 2017). Although the species is not declared as highly invasive globally, it can be a serious concern in the RA area. Thus, all three species screened as false positive could be considered a high risk bearing for the RA irrespective of their global status.

The rest of the *a priory* invasive 18 species with a high risk of invasiveness (Table 1,2,) are supposed to bring a significant treat to a native species and ecosystems. Of those species, 14 are already established in the RA area (Table 1, category 2), and some of these species (such as *Carassius gibelio* or *Pseudorasbora parva*) are already recognized as a serious concern at a regional scale (Kuljanishvili et al., 2021). Unfortunately, quantitative data on the threats or effects to native species and ecosystems as well as the economic loss related to those established species, is non-existent for RA area.

Among the 8 translocated species within RA area, only two (*Sander lucioperca, perca fluviatilis*) are considered as *a priory* invasive (Table 1,2). These two species with *Salmo ischchan* were intentionally and repeatedly introduced to non-native waterbodies while the introduction pathways of the rest of species are not well understood (Kuljanishivili et al., 2021). Whatever the reasons or ways of introduction, all of the locally translocated species turned out to pose medium to high risk of being invasive.

Other species that are not known as established in RA area in spite of their previous introductions, the highly invasive *Mylopharyngodon piceus* and *Ictalurus punctatus* seem to benefit from climate change significantly (Table 2). The same goes to a sample of fish species that are not yet spotted within a RA area bat are known from proximity (3rd category in Table 1). These species have a high risk of being invasive within a RA area. Thus their future introductions should be avoided.

Conservation issues in SC on the light of NNS

Most of the freshwater NNS in the South Caucasus region were introduced intentionally, including some of the most invasive species (e.g. *Gambusia holbrooki*). Among the NN fish species, seven species (*Anguilla anguilla, Carassius gibelio, Gobio artvinicus, Pseudorasbora parva, Rhinogobius lindbergi, Perca fluviatilis, and Hemiculter leucisculus*) are unintentional introductions (or hitchhikers) or are naturally increasing their range to RA area from neighbour countries (Kuljanishvili et al. 2021). Without exceptions, once the species is established in SC, it continues further colonization of the RA area. The natural geographic barrier dividing the Caspian and Black Sea basins is only temporarily effective to slow down the NNS range expansion. The alien species introduced to one of those basins appear later in another basin. For instance, it is believed that *C. gibelio* was accidentally introduced in the eastern Georgian fish farms erroneously as a *Cyprinus carpio*. After realizing the farmer discarded an unwanted fish batch in the nearest river. Ever since, this species has been intentionally introduced in different water bodies throughout the whole Georgia, in different altitudes (Kuljanishvili et al 2021). Another example is *R. lindbergi* (species with no aquaculture/recreational value), which first invaded the Kura river drainage. It is also detected in the Black Sea Basin (our yet unpublished data). Two other recently established NNS (*G. cernua*, *H. lucisculus*) are still awaiting the between basin jumping opportunities.

NNS, if invasive, pose a significant threat to native fish populations through predation, food and shelter competition, habitat modifications, etc. (Gozlan et al., 2010). However, the effects of invasive NNS are difficult to evaluate without dedicated research and monitoring. Unfortunately, studies assessing such changes, threats to local ecosystems, or expected environmental/economic effects are completely lacking. Considering that the Prussian carp is not only established invasive NNS and many others have the potential to become invasive in the region, it is clear that urgent actions are needed to plan a working strategy regarding freshwater NNS.

Geopolitical context

In the presented assessment, we considered the basins rather than political boundaries in the SC region for the risk assessments. The species native to RA area that were translocated within the area are primarily translocation cases from Black to Caspian Sea basins. The only exception is *S. ischchan*, which is translocated from Lake Sevan, forming independent basins to lakes belonging to the Kura drainage in the Caspian Sea basin. At the current knowledge, the reason behind this directional bias is not apparent and deserves more research. Nevertheless, species translocations either within Georgia or between countries do not face any difficulty until now. Recent and almost simultaneous detection of *Rhinogbius lindbergi* in the Kura and Rioni rivers (Epitashvili et al., 2020; Japoshvili et al., 2020; own unpublished data) indicate that the untraced species introductions still take place.

On the other hand, since the Kura-Aras basin is shared among all SC countries, establishing any invasive species in any of them means the future potential impact for all three countries. In addition, the Kura and Aras rivers are also shared between Turkey and SC and the Chorokhi river between Turkey and Western Georgia. Thus evaluation of the impact of potentially invasive species (i.e. risk screening) as well as the regulations of NNS introductions must be agreed upon and implemented SC-wide rather than independently for each country.

Unfortunately, SC is also a geopolitical hotspot with permanent military tensions in its different parts. Solely on this reason, it is hardly possible to communicate the environmental issues between SC countries (e.g. between Armenia and Azerbaijan) or even within the country (e.g. 20% of Georgia is inaccessible due to Russian occupation). These long-unsolved political oscillations are aggravating the nature conservation problems.

Concluding remarks and way forward

The crucial consequence of species invasions is so-called 'biotic homogenization. It drives the earth's biodiversity to global homogenization and impoverishment (McKinney and Lockwood, 1999). The freshwater environment of the SC region is particularly vulnerable due to an existing (and intensive) anthropogenic pressure, including water intake, legal or illegal fishing, gravel mining, pollution, hydropower plant development, irrigation, invasion. None of the listed threats have been studied or evaluated adequately (the only exception is Japoshvili et al. (2021) providing evidences of effects of hydropower dams on fish population connectivity). No agreed working strategy (either within countries or between countries) exists in SC to reduce or mitigate the threats on freshwater ecosystems. With this respect, we strongly advocate the idea that the countrywide and nationwide strategies related to freshwater NNS must be urgently developed and implemented. Such a strategy must adopt several overarching conceptual goals, including:

1. Gap analyses and further improvement of the legal basis for species introduction related to aquaculture/game fisheries and pet trade. This legislation should be jointly agreed upon among SC countries to be effective.
2. The early detection and communication of freshwater NNS is the process that is happening on its own (e.g. researchers are regularly publishing results about new introductions or invasive species, and citizen science platforms also regularly receive data from the general public on new species). However, the data and knowledge developing over time must be standardized, rapidly communicated and reflected among stakeholders. In addition, measures should be taken to enhance the data collection and generation from all potential sources. For instance, currently, there is no information about the exotic species available on the local markets.
3. The risks assessment for new introductions of potential invasive taxa should be directed to species that are generally considered invasive, especially if the species are already found close to a region. Whenever available an existing analysis (such as presented

here), an in-depth study of potentially high invasive fish species (e.g. full risk assessments of potentially high invasive species (Copp et al., 2016)) must be conducted and the results adequately used by decision makers.

4. Continuously developing an in-depth monitoring scheme (including infrastructure for filed data collection based on traditional field works, barcoding/metabarcoding approaches, data management and representation). This is a critical step to understand the history of NNS colonization and accompanying processes related to NNS effects, local community perceptions, damage/mitigation associated costs etc.
5. Prevention of introductions (the black-list of species). Since there is a huge amount of data on freshwater NNS worldwide, it is just a matter of relatively limited resources to develop a list of potentially invasive species for SC. The control of the introduction of most threatening species (if not all), could be implemented then by a government. At least such practice exists (Battisti et. al. 2019; Gederaas et. al. 2012; Essl et. al. 2011; Poeta et. al. 2017) and should be discussed/adopted within SC too.
6. Impact assessments research, including related to already established invasive taxa. Currently, there is no evaluation of economic/environmental costs related to freshwater NNS (nor for terrestrial). Therefore it is difficult to judge the negative (or any positive) effect of NNS on local communities and optimally manage the available resources to prevent/mitigate the NNS introductions.

Thus, in this manuscript, we showed a number of established and not yet established highly invasive freshwater fish species in the SC region that deserves further attention from the stakeholders (including all the governmental institutions, scientists, and local people). A lot is still to be done in the SC region to handle freshwater NNS-related challenges and maintain the highly vulnerable biodiversity hotspot stability. We hope that the provided risk screening exercise could help build a solid basis for a better understanding of NN fish species in the SC region and open a window for future risk evaluations and NNS management planning.

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Tables

Table 1 Freshwater fish species evaluated with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for their potential risk of invasiveness in the South Caucasus region the risk assessment (RA) area. For each species, the following information is provided: criterion (Crit.) for selection (1 = translocated species; 2 = non-native species already present in the RA area; 3 = non-native species established in neighbouring countries or countries of similar climate to the RA area; 4 = non-native species recorded in the RA area but currently not found in the wild); *a priori* categorization outcome into Non-invasive or Invasive. For the *a priori* categorization, the results of the four steps of the protocol (see text for details) are indicated: (i) FishBase (www.fishbase.org); (ii) Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI: www.cabi.org/ISC) and Global Invasive Species Database (GISD: www.iucngisd.org); (iii) Invasive and Exotic Species of North America list (IESNA: www.invasive.org); (iv) Google Scholar literature search. N = no impact/threat; Y = impact/threat; '–' = absent; n.e. = not evaluated (but present in database); n.a. = not applicable.

			***A priori* categorization**					
Species name	**Common name**	**Crit.**	**FishBase**	**CABI**	**GISD**	**IESNA**	**Google Scholar**	**Outcome**
Ameiurus melas	black bullhead	3	Y	–	Y	–	n.a.	Invasive
Anguilla anguilla	European eel	4	N	–	Y	–	n.a.	Invasive
Carassius gibelio	gibel carp	2	Y	–	Y	–	n.a.	Invasive
Chelon auratus	golden grey mullet	1	N	–	–	–	N	Non-invasive
Chelon saliens	leaping mullet	1	N	–	–	–	N	Non-invasive
Clarias gariepinus	North African catfish	3	Y	Y	Y	–	n.a.	Invasive
Coregonus albula	vendace	2	N	–	Y	–	n.a.	Invasive
Coregonus sp.*	–	2	N	–	–	–	N	Non-invasive
Ctenopharyngodon idella	grass carp	2	Y	Y	Y	Y	n.a.	Invasive
Gambusia holbrooki	eastern mosquitofish	2	Y	Y	Y	–	n.a.	Invasive

Species name	Common name	Crit.	*A priori* categorization					
			FishBase	CABI	GISD	IESNA	Google Scholar	Outcome
Gasterosteus aculeatus	three-spined stickleback	1	N	–	–	–	N	Non-invasive
Gobio artvinicus	[Artvin gudgeon]	1	N	–	–	–	N	Non-invasive
Gymnocephalus cernua	ruffe	2	-	–	N	–	N	Non-invasive
Hemiculter leucisculus	sharpbelly	2	Y	–	N	–	n.a.	Invasive
Hypophthalmichthys molitrix	silver carp	2	Y	Y	Y	Y	n.a.	Invasive
Hypophthalmichthys nobilis	bighead carp	2	Y	Y	Y	Y	n.a.	Invasive
Ictalurus punctatus	channel catfish	4	Y	–	Y	–	n.a.	Invasive
Lepomis gibbosus	pumpkinseed	3	-	–	N	–	N	Non-invasive
Micropterus salmoides	largemouth bass	3	Y	Y	Y	–	n.a.	Invasive
Mugil cephalus	flathead grey mullet	4	N	–	–	–	n.a.	Non-invasive
Mylopharyngodon piceus	black carp	4	Y	–	Y	Y	n.a.	Invasive
Oncorhynchus kisutch	coho salmon	4	N	–	–	Y	n.a.	Invasive
Oncorhynchus mykiss	rainbow trout	2	Y	Y	Y	Y	n.a.	Invasive
Oreochromis niloticus	Nile tilapia	2	Y	Y	Y	Y	n.a.	Invasive
Perca fluviatilis	Eurasian perch	1	Y	Y	Y	–	n.a.	Invasive
Pseudorasbora parva	topmouth gudgeon	2	Y	–	Y	–	n.a.	Invasive

			A priori categorization					
Species name	**Common name**	**Crit.**	**FishBase**	**CABI**	**GISD**	**IESNA**	**Google Scholar**	**Outcome**
Rhinogobius lindbergi	[Lin's goby]	2	N	–	–	–	n.a.	Non-invasive
Salmo ischchan	Sevan trout	1	-	–	–	–	n.a.	Non-invasive
Salmo trutta	brown trout	2	Y	Y	Y	Y	n.a.	Invasive
Salvelinus fontinalis	brook trout	3	Y	Y	Y	–	n.a.	Invasive
Sander lucioperca	pikeperch	1	Y	–	Y	–	n.a.	Invasive
Syngnathus abaster	black-striped pipefish	1	N	–	N	–	N	Non-invasive

* Reference species for the *a priori* categorization: *Coregonus lavaretus*.

Table 2 Risk outcomes for the freshwater fish specis screened with AS-ISK for the South Caucasus region. For each species, the following information is provided: *a priori* categorization for invasiveness (N = non-invasive; Y = invasive: see Table 1), BRA and BRA+CCA scores with corresponding risk outcomes (Out: L = Low; M = Medium; H = High; H* = Very high based on *ad hoc* thresholds: see text for details) and classification (Class: FN = false negative; FP = false positive; TN = true negative; TP = true positive; '–' = not applicable as medium-risk: see text for details), difference (Delta) between BRA+CCA and BRA scores. Risk outcomes for the BRA and BRA+CCA are based on the following thresholds: BRA = 18.0, BRA+CCA = 20.3. Risk outcomes for the BRA are computed as: Low, with score within interval [−20, 1[; Medium, [1, 18.0[; and High,]18.0, 68]; and for the BRA+CCA as: Low, with score within interval [−32, 1[; Medium, [1, 20.3[; High,]20.3, 80] (note the reverse bracket notation indicating in all cases an open interval).

		BRA			**BRA+CCA**			
Species name	***A priori***	**Score**	**Out**	**Class**	**Score**	**Out**	**Class**	**Delta**
Ameiurus melas	Y	30.5	H	TP	35.8	H	TP	5.3
Anguilla anguilla	Y	9.3	M	–	8.0	M	–	−1.3
Carassius gibelio	Y	44.0	H	TP	55.3	H	TP	11.3
Chelon auratus	N	17.0	M	–	17.7	M	–	0.7
Chelon saliens	N	16.0	M	–	16.7	M	–	0.7
Clarias gariepinus	Y	40.3	H	TP	49.7	H	TP	9.3
Coregonus albula	Y	11.2	M	–	1.2	M	–	−10.0
Coregonus sp.	N	8.7	M	–	−0.7	L	TN	−9.3
Ctenopharyngodon idella	Y	20.7	H	TP	24.7	H	TP	4.0
Gambusia holbrooki	Y	34.2	H	TP	42.8	H	TP	8.7
Gasterosteus aculeatus	N	38.0	H	FP	41.3	H	FP	3.3
Gobio artvinicus	N	8.3	M	–	11.7	M	–	3.3
Gymnocephalus cernua	N	37.0	H	FP	48.3	H	FP	11.3
Hemiculter leucisculus	Y	33.5	H	TP	43.5	H	TP	10.0
Hypophthalmichthys molitrix	Y	22.8	H	TP	28.8	H	TP	6.0
Hypophthalmichthys nobilis	Y	27.2	H	TP	31.2	H	TP	4.0
Ictalurus punctatus	Y	25.7	H	TP	35.7	H	TP	10.0
Lepomis gibbosus	N	29.5	H	FP	40.2	H	FP	10.7
Micropterus salmoides	Y	30.2	H	TP	40.2	H	TP	10.0
Mugil cephalus	N	11.3	M	–	14.7	M	–	3.3
Mylopharyngodon piceus	Y	21.3	H	TP	31.3	H	TP	10.0
Oncorhynchus kisutch	Y	10.8	M	–	11.5	M	–	0.7
Oncorhynchus mykiss	Y	18.8	H	TP	15.5	M	–	−3.3

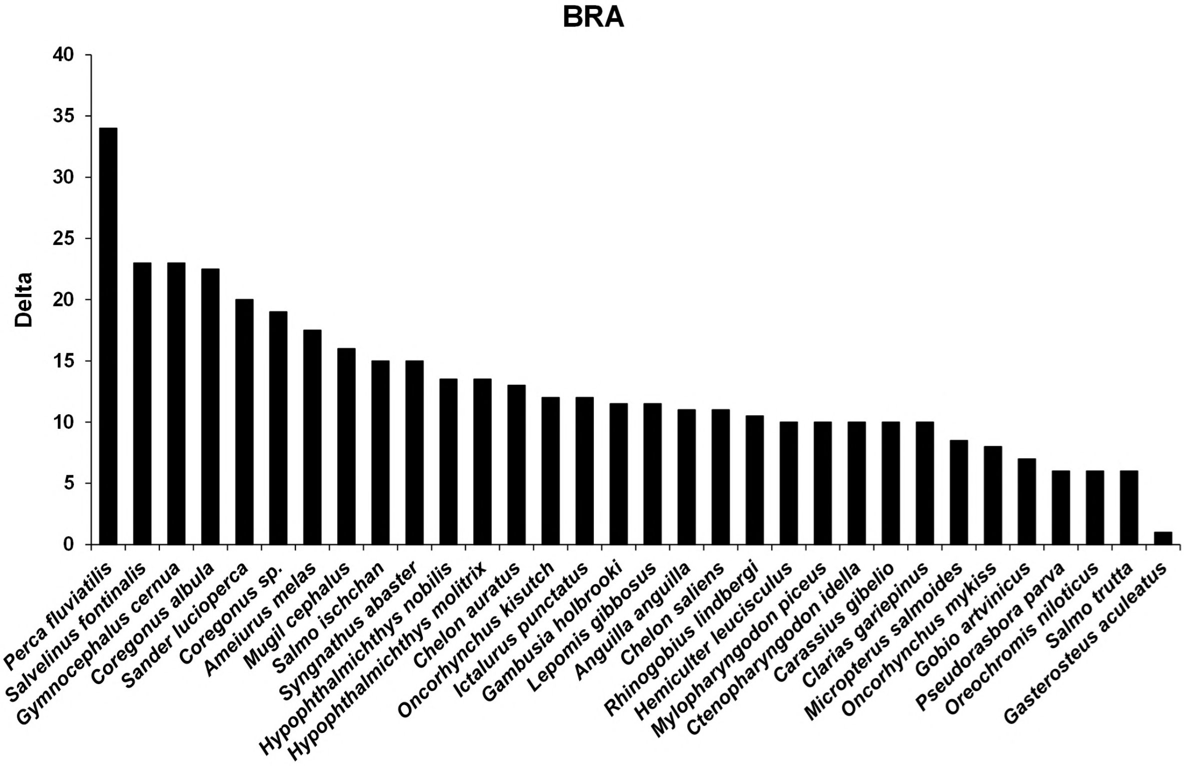
Species name	*A priori*	BRA			BRA+CCA			
		Score	Out	Class	Score	Out	Class	Delta
Oreochromis niloticus	Y	31.7	H	TP	41.0	H	TP	9.3
Perca fluviatilis	Y	32.0	H	TP	41.3	H	TP	9.3
Pseudorasbora parva	Y	40.0	H	TP	49.3	H	TP	9.3
Rhinogobius lindbergi	N	17.2	M	–	27.8	H	FP	10.7
Salmo ischchan	N	16.7	M	–	10.0	M	–	−6.7
Salmo trutta	Y	36.0	H	TP	36.7	H	TP	0.7
Salvelinus fontinalis	Y	21.3	H	TP	20.7	H	TP	−0.7
Sander lucioperca	Y	43.0	H	TP	46.3	H	TP	3.3
Syngnathus abaster	N	16.0	M	–	20.0	M	–	4.0

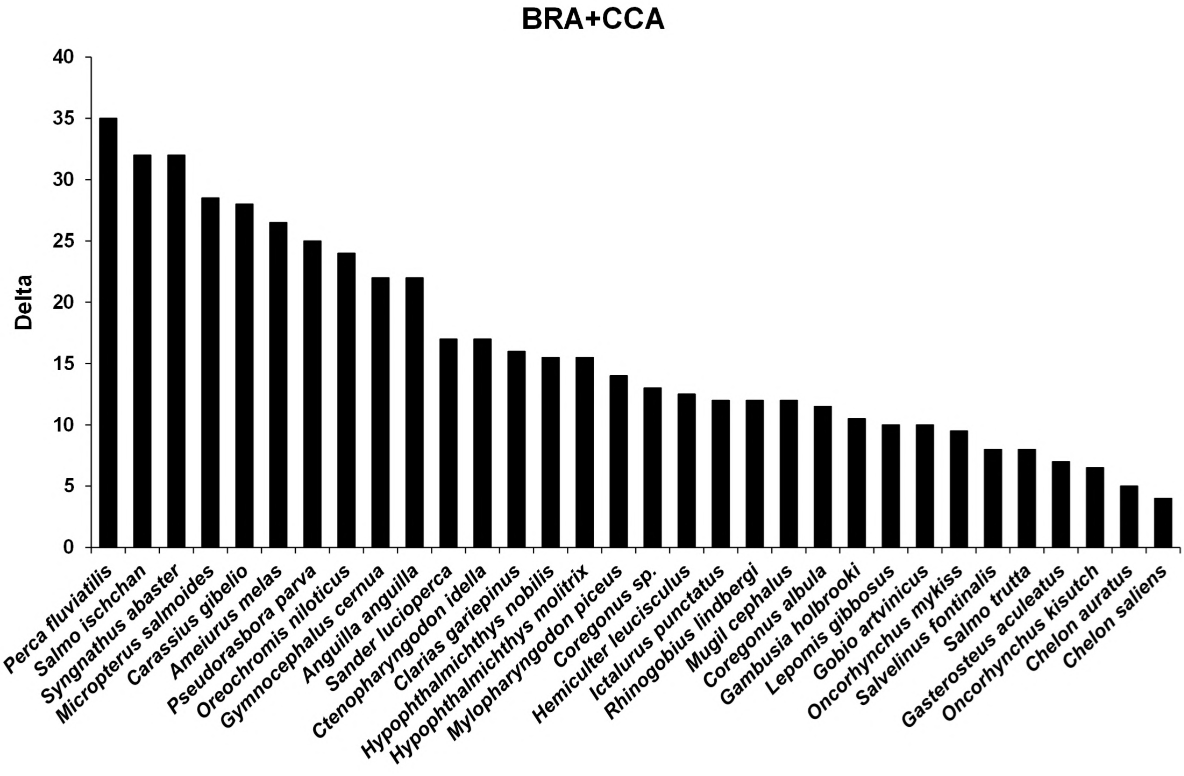
Figure legends

Figure 1 Map of the risk assessment area (South Caucasus region) and neighbouring countries.

Figure 2 (a) Between-assessor differences in the BRA (Basic Risk Assessment) score for the species screened with the Aquatic Species Invasiveness Screening Kit for the Anzali Wetland Complex; (b) same for the BRA+CCA (Climate Change Assessment) score. See also Table 2.


Russia
Black Sea
Caspian Sea
Georgia
South Caucasus
Armenia
Azerbaijan
Turkey
Iran
Caspian Sea Basin
Black Sea Basin
Country Borders
Rivers
Lakes
0
10
20 km


BRA
Delta
40
35
30
25
20
15
10
5
0
Perca fluviatilis
Salvelinus fontinalis
Gymnocephalus cernua
Coregonus albula
Sander lucioperca
Coregonus sp.
Ameiurus melas
Mugil cephalus
Salmo ischchan
Syngnathus abaster
Hypophthalmichthys nobilis
Hypophthalmichthys molitrix
Chelon auratus
Oncorhynchus kisutch
Ictalurus punctatus
Gambusia holbrooki
Lepomis gibbosus
Anguilla anguilla
Chelon saliens
Rhinogobius lindbergi
Hemiculter leucisculus
Mylopharyngodon piceus
Ctenopharyngodon idella
Carassius gibelio
Clarias gariepinus
Micropterus salmoides
Oncorhynchus mykiss
Gobio artvinicus
Pseudorasbora parva
Oreochromis niloticus
Salmo trutta
Gasterosteus aculeatus


BRA+CCA
Delta
40
35
30
25
20
15
10
5
0
Perca fluviatilis
Salmo ischchan
Syngnathus abaster
Micropterus salmoides
Carassius gibelio
Ameiurus melas
Pseudorasbora parva
Oreochromis niloticus
Gymnocephalus cernua
Anguilla anguilla
Sander lucioperca
Ctenopharyngodon idella
Clarias gariepinus
Hypophthalmichthys nobilis
Hypophthalmichthys molitrix
Mylopharyngodon piceus
Coregonus sp.
Hemiculter leucisculus
Ictalurus punctatus
Rhinogobius lindbergi
Mugil cephalus
Coregonus albula
Gambusia holbrooki
Lepomis gibbosus
Gobio artvinicus
Oncorhynchus mykiss
Salvelinus fontinalis
Salmo trutta
Gasterosteus aculeatus
Oncorhynchus kisutch
Chelon auratus
Chelon saliens

Chapter 7: General discussion

The Caucasus area is considered as one of the biodiversity hot spots (Mittermeier et al., 2004; Zazanashvili et al., 2004). The place is characterized by species diversity and high endemism level, meaning that species presented in this area are considered as a biogeographic unit. As the area of high biological diversity, the detailed survey leading to the valid estimations of the impacts of the introductions and translocation of alien species is crucial for improving wildlife management. Even though the three countries, Armenia, Azerbaijan, and Georgia belong to the same river system, the knowledge about the freshwater species has always been only reviewed within the countries territories (Ibrahimov & Mustafayev, 2015; Ninua & Japoshvili, 2008; Pipoyan, 2012). The political situation between these countries was a challenge (see Welt, 2021) for the study, however, we managed to collaborate and create the research, that had led to a huge contribution towards understanding the impact of non-native species in the area on a global scale.

The results of this dissertation revealed a few important aspects. The unified checklist of freshwater fish species in the area has not been published since the 1940s. Due to outdated information and/or data gaps in terms of taxonomy, distribution, and conservation status of freshwater fishes in the South Caucasus, it has become necessary to compile the most up-to-date checklist of the region, which would serve as a baseline for future research and conservation of fish biodiversity in the region. The result of our primary project was the unified checklist of freshwater fishes of South Caucasian countries (Kuljanishvili et al. 2020a) (Chapter 2). In this work, we have recorded 119 freshwater fish species distributed in Armenia, Azerbaijan and Georgia. In the freshwater fish fauna of the South Caucasus, the research done by us and our colleagues has revealed several new species and there has been some taxonomic rearrangement as well that were discussed in our paper (Kuljanishvili et al., 2020a). We emphasized the necessity for more morphological and genetic research to address the issues raised in the study.

Measurement of biodiversity and species conservation is an important challenge and has a worldwide biological importance (Williams, 2014). Recent taxonomic research done by Kuljanishvili et al (2020a) discovered 96 freshwater fish species in Georgia, several of which lacked native Georgian names. These include newly described species, first country records, and species that have previously been incorrectly identified. We created the list of freshwater fishes and their Georgian local names found in the country and explained the significance of local names (Kuljanishvili et al. 2020b) (Appendix 1).

Our first step to evaluate risks of non-native species in the South Caucasian region was in 2018, when we were working on high altitude, glacial lakes ichthyofauna dynamics. We revealed two new non-native species and shortly discussed their potential impact on native ichthyofauna (Kuljanishvili et al., 2018) (Chapter 3). In addition to these findings, we managed to discover non-native species in unusual areas, such as finding Prussian carp in the northern Caucasian mountainous lakes (Kuljanishvili et al. 2017) (Appendix 2). This was the first record of this fish in the Northern Caucasus on the territory of Georgia, and we considered it as a potential invader in the nearby water ecosystems. Appearing of non-native species is always worth attention. Therefore, after the Nile tilapia's first appearance in eastern Georgian rivers, we sampled the place and studied this species biological characteristics and climate tolerance to identify it as a future threat (Kuljanishvili et al. 2021a) (Appendix 3). Another unusual new appearance did not go unnoticed. We obtained some translocated black-striped pipefish in the freshwater reservoir (Kuljanishvili et al. 2021c) (Chapter 4). We reviewed the species identity using genetic and morphologic methods, we assessed the species invasiveness and modelled its potential establishment throughout the world.

Kuljanishvili et al (2020) lead to an important result: The Unified checklist of the non-native freshwater fishes in the area (Kuljanishvili et al. 2021b) (Chapter 5). The possible effects of non-native (alien) species on biological diversity are a major topic of concern (Vitousek et al. 1996; Jeschke et al. 2014). Non-native invasive species have the potential to have a considerable detrimental impact on native fauna as well as socioeconomic losses in afflicted areas (Ricciardi 2003; Gallardo and Aldridge 2013). As a result, it is worthwhile to spend in preventing introductions and developing plans for the functional management of invasive alien species once one is imported. In this regard, one of the most important stages is to review the alien species lists for a specific region of interest to quickly identify, analyze, monitor, and mitigate non-native species. Most European and international governments have created such lists, and this information has been utilized to construct more effective legislation as a result (Manchester and Bullock 2000; Copp et al. 2005; Povž and Šumer 2005; Koščo et al. 2010; Mastitsky et al. 2010; Lenhardt et al. 2011; Ribeiro and Leunda 2012; Simonović et al. 2013; Piria et al. 2017; Musil et al., 2010). The review of the legislative framework revealed that even though these laws have been gradually reestablished during the last two decades, further development is still needed, and we suggest that they should reflect the situation.

Last but not least, the research led to the first attempt to assess the risks of freshwater non-native species invasiveness in the area (Mumladze et al. 2022) (Chapter 6). The AS-ISK toolkit was effectively used to detect and classify freshwater non-native fish species according to the threats they pose in the area. We evaluated the risks of 32 non-native species in the South Caucasian countries. From these 32 species, 14 species are introduced; eight were translocated; five were recorded but did not occur at the time of writing, and five were established in neighbouring countries or countries of similar climate to the South Caucasus. The study emphasizes the importance of monitoring the introductions. It also suggests that prompt action is required to develop a working strategy for managing the non-native species introductions on a wider scale (not individually within each country). This study also reveals the overarching conceptual goals that must be adopted in such approach: 1) Conduction of gap studies and improvements to the legal basis for species introduction in aquaculture, game fisheries, and the pet trade are all underway. To be successful, such law should be at least mutually agreed upon by SC countries; 2) Detection of freshwater non-native species and fast communication (rapid action). Data and information that accumulates overtime must be standardized, distributed quickly, and mirrored across stakeholders; 3) Risk assessment and forecasting for future invasive species introductions; 4) Creating a comprehensive monitoring plan; 5) Prevention of introductions (black list of species). A government could perhaps set controls on the introduction of the most dangerous species; 6) Assessment of socio-economic and environmental costs caused by the non-native species.

It should be mentioned that our research has also contributed to global collaboration which was dedicated to developing the risk screening tool AS-ISK that helped the calibration and generalized thresholds for different groups of animals under current and future climatic conditions (Vilizzi et al. 2021) (Appendix 4).

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Appendix 1: Freshwater fish species diversity in Georgia (South Caucasus Region) and their local names

Freshwater fish species diversity in Georgia (South Caucasus Region) and their local names

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Abstract

Georgia is a country of great diversity of freshwater fish species that is facilitated due to the landscape diversity and richness of water resources. As an area of global biological importance, measuring biodiversity and the conservation of species is a significant issue. Recent taxonomic research found 96 freshwater fish species, from which several species did not have local Georgian names. These includes recently described species, or first country records, and species that were named wrongly in the past. In this paper, we provide the Georgian local names of all the freshwater fish species distributed in the country and discussed the importance of local names.

Key words: Biodiversity, Etymology, common names.

1. Introduction

The republic of Georgia is located in the south of the Great Caucasus Mountainous Range. This territory is a part of two world's biodiversity hotspots, meaning that the existing unique and endemic biodiversity is at the same time vulnerable for various types of pressure, caused by human activities (Mumladze *et al.* 2019; Zazanashvili *et al.* 2004). Therefore, the conservation of the species and ecosystems in this area is a top priority.

Georgia is rich with water resources. There are more than 26 000 rivers and streams, and around 860 lakes in the territory of Georgia belonging to two different sea basins (the Black and Caspian Seas) (Apkhazava 1975; Maruashvili 1964; Ninua *et al.* 2013). Diversity of landscapes and richness of water resources coupled with complex geological history, has resulted in indigenous freshwater fish fauna, which is represented with 119 freshwater species in the South Caucasian region (territories of Armenia, Azerbaijan and Georgia) (Kuljanishvili *et al.* 2020).

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In an updated checklist, Kuljanishvili *et al.* (2020) listed 96 freshwater species that are currently recorded for Georgia. The list includes well known species as well as species, that were either recently described as new, or were first time reported from Georgia. Accordingly, there are no vernacular names available for those species in Georgian language. Furthermore, even few species known for Georgia already long ago, still are without common names.

Having the common local names of the fish species is important for several reasons. It is believed that the common names are more easily adaptable among non-scientific community compared to Latin binomens and the same time might also be more stable in a long run (Bailey *at al.* 1960). With this respect, the common name can play a significant role in communication efficiency among researchers, conservationists, decision makers and local people (including the country scale legal terminology, trade names etc). On the other hand, it is also important to standardize already established common names and give them proper definition. This can affect the perception of the biodiversity and the conservation thereof. For instance, there are some species with different common names in different regions of the country, or vice versa, different species have the same name (for instance, three species - *Rutilus lacustris*, *Alburnoides fasciatus*, and *A. eichwaldii* are all called as "roach" (as of genus *Rutilus*)). This situation makes it difficult to perceive the diversity properly and consequently help to species conservation. Furthermore, the availability of the fish species common names can help the biodiversity education (at the primary school level, in the museum exhibitions etc.) and provide better means for effective and precise communication of natural heritage to a local people.

In this communication, we list the common names in Georgina language for all the freshwater fish species reported so far by Kuljanishvili *et al.* (2020) and suggest some clarifications to step towards standardization of the vernacular terminology.

2. Materials and methods

The literature search was done to identify the freshwater fish species that did not have local names in the Georgian language. We named the species according to their English name, available from FishBase (https://www.fishbase.de/) and IUCN Red List database (https://www.iucnredlist.org/). When not available, from these databases, the genus name was taken from the already known species genus name and the species name was translated from Latin to Georgian language, if the meaning was relevant or characteristic for a particular taxon. If the species Latin names were linked to the authority names or did not provide meaningful translation, we suggested synonyms of common Georgian names according to their distribution.

The table consists of the Latin binomens of the species, their English and Georgian names, and the Georgian name transcriptions in Latin letters. Etymological notes for each species are also provided.

3. Results

From the 96 species, distributed in Georgia, we created, or updated names for 24 species (indicated with an asterisk in Table 1). The names of the rest of the species are retained from Ninua *et al.* 2013.

The common Georgian names of the freshwater fish species are updated in the Georgian biodiversity database (http://biodiversity-georgia.net/).

Etymology notes

Cobitis saniae is a species recently described by Eagderi *et al.* (2017) and it is included neither in FishBase, nor in IUCN red list. The English name was not given in scientific literature. The genus name was taken from other known species of this genus “spined loach” and the species name was given according to the distribution “South Caucasian”, making it “South Caucasian spined loach - სამხრეთკავკასიური გველანა/Samkhretkavkasiuri gvelana”.

Barbus ciscaucasicus has English and Russian names in the scientific literature, however the name of this species was not mentioned in Georgian language scientific literature. The genus and species names were translated from the English names, “Terek barbel” that was available on FishBase, making it “თერგის წვერა/Tergis tsvera” in Georgian.

Capoeta banarescui was misunderstood with *C. tinca* (Anatolian khramulya) in the past, which is not distributed in the area. Therefore, *C. banarescui* did not have a common name. The genus name was taken from other known species of this genus “barb” and the species name was translated from its Latin “Banarescu’s”, making it “Banarescu’s barb - ბანარესკუს ხრამული/Banareskus khramuli”.

Capoeta kaput is also a recently described species by Levin *et al.* (2019). They named this species according to bluish colour. Translated from the Latin, the English common name of this species should be “Blue barb” making it “ლურჯა ხრამული/Lurja khramuli” in Georgian.

Luciobarbus brachycephalus was mentioned as subspecies - Caspian barbel (Каспийский усач - *Barbus brachycephalus caspius*) by Berg (1949), which is now valid as *Luciobarbus caspius*: (Fricke *et al.* 2020). The common name of *Luciobarbus brachycephalus*, should be “Aral barbel” because of the type locality, the Syr-Darya River, that belongs to the Aral Sea basin, making it “არალის წვერა/Aralis tsvera” in Georgian.

Ponticola cyrius and *Ponticola gorlap* were mentioned for Georgia by Freyhof (2011), however these species were never mentioned in Georgian language literature. The genus and the species names were translated from their English names, “Kura goby” for *P. cyrius* and “Caspian bighead goby” for *P. gorlap*, that were available on FishBase, making them “მტკვრის ღორჯო/Mtkvris ghorjo" and “კასპიური დიდთავა/Kaspiuri didtava ghorjo” in Georgian, respectively.

Proterorhinus nasalis was mentioned for Georgia in Russian language literature (Barach 1941; Berg 1949) and was not mentioned in Georgian language literature. The genus and the species names were translated from the English name, “Eastern tubenose goby” that was available on FishBase, making it “აღმოსავლური მილცხვირა ღორჯო/Aghmosavluri miltskhvira ghorjo” in Georgian.

Rhinogobius lindbergi did not have Georgian name. The genus and the species names were translated from the English name, “Amur goby” available on FishBase, making it “ამურის ღორჯო/Amuris ghorjo” in Georgian.

Gobio artvinicus is also a recently described species (Turan *et al.* 2016) named after the type locality (Artvin city). The species did not have the English name. The genus and the species names were translated from the Latin, “Artvin gudgeon - ართვინული ციმორი/Artvinuli tsimori”.

Romanogobio macropterus did not have Georgian name. The genus and the species names were translated from the English name, “South Caucasian gudgeon” available on FishBase, making it “სამხრეთკავკასიური ციმორი/Samkhretkavkasiuri tsimori” in Georgian.

Leucaspius delineates did not have a Georgian name. The genus and the species names were translated from the Austrian name (since the species was described by the Austrian scientist, Heckel, and this term describes it’s appearance well), that was available on FishBase “Sunbleak”, making it “მზისებრი თაღლითა/Mzisebri taghlita” in Georgian.

Squalius agdamicus was named after its type locality (Agdam village). The species did not have English name. Since, it is known that the species is distributed in all over the Kura River and it is endemic for this area, the English name was given after the River Kura: “Kura chub”, making it “მტკვრის ქაშაპი/Mtkvris kashapi” in Georgian.

Squalius orientalis was mentioned as *S. cephalus* for Georgia by Ninua *et al.* (2013) as “Caucasian chub”. *S. cephalus* is distributed in Europe and cannot be named as “Caucasian chub”. On the other hand, this species is not at all distributed in Georgia. This was most possible species *S. orientalis*, which did not have an English name. The common name for *Squalius orientalis* was translated from Latin “Oriental chub - აღმოსავლური ქაშაპი/Aghmosavluri kashapi” in Georgian.

Squalius turcicus was not mentioned in Georgian language literature. For Georgian naming, the genus and the species names were translated from the English name, “Transcaucasian chub” that was available on FishBase, making it “სამხრეთკავკასიური ქაშაპი/Samkhretkavkasiuri kashapi” in Georgian.

Chelon labrosus and *Chelon ramada* were not mentioned in Georgian language literature. The genus and the species names were translated from the English names, “Thicklip grey mullet” for *C. labrosus* and “Thinlip grey mullet” for *C. ramada*, that were available on FishBase, making them “სქელტუჩა კეფალი/Skeltucha kephali” and “თხელტუჩა კეფალი/Tkheltucha kephali” in Georgian, respectively.

Oxynoemacheilus bergianus was not mentioned in Georgian language literature. The genus and the species names were translated from the English name, “Kura sportive loach” that was available on FishBase, making it “მტკვრის სპორტული გოჭალა/Mtkvris sportuli gotchala”. We also suggest the synonyms of these species as “Berg’s loach - ბერგის გოჭალა/Bergis gotchala” in Georgian.

Oxynoemacheilus cemali and *Oxynoemacheilus veyselorum* are recently described species, named after “Cemal Turan” (Turan *et al.* 2019) and “Veysel Cicek” (Çiçek *et al.* 2018). These two species did not have English names. The genus name was taken from other known species of this genus “loach” and the species name was translated from latin “Cemali” for *O. cemali* and “Veyseli” for *O. veyselorum*, making them “ცემალის გოჭალა/Tsemalis gotchala” and “ვეისელის გოჭალა/Veiselis gotchala” in Georgian, respectively. Since local people perceive species better with their names linked to their distribution, we suggest the synonyms of these species as “Choruh loach-ჭოროხის გოჭალა/Tchorokhis gotchala” and Araks loach- არაქსის გოჭალა/Araksis gotcha” for *O. cemali* and *O. veyselorum* loach respectively.

Lampetra ninae was mostly confused with Ukrainian lamprey *Eudontomyzon mariae*. This species was not mentioned in Georgian language literature. The genus and the species names were translated from the English name, “Western Transcaucasian lamprey” that was available on FishBase, making it “დასავლეთ ამიერკავკასიური სალამურა/Dasavlet amierkavkasiuri salamura” in Georgian.

Salmo ciscaucasicus, Salmo coruhensis and *Salmo rizeensis* did not have names in Georgian language literature. The genus and the species names were translated from their English names, that was available on FishBase: Caspian salmon for *S. ciscaucasicus*, Coruh trout for *S. coruhensis*, and Rize trout for *S. rizeensis*, making them “თერგის კალმახი/Tergis kalmakhi,”

"ჭოროხის კალმახი/Tchorokhis kalmakhi." and "რიზეს კალმახი/Rizes kalmakhi" in Georgian respectively.

4. Discussion

Our personal experience shows that valid scientific names (e.g. Latin binomial names) of fishes is not a primary (or even wanted) way of communication for the local anglers, students and other interested non-scientific parties in Georgia. Having species with no local name, or sometimes incorrect or multiple common names, creates significant obstacles during the spreading of biodiversity information and species conservation activities. It should be mentioned that most of the time, even the most experienced anglers treat different species as the same. For instance, they treat three different species of *Rutilus lacustris*, *Alburnoides fasciatus*, and *A. eichwaldii* under the same name "roach" as of genus Rutilus (Napota-ნაფოტა). Even more, *Romanogobio macropterus* and *Luciobarbus mursa* both are called as "gudgeons" as of genus *Romanogobio* (Tsimori-ციმორი), meaning that these two species are one. Sometimes, one specie is treated as different species in different regions. For instance, in western Georgia, species of *Oxynoemacheilus* are called as "loaches" as of genus *Cobitis* (Gvelana-გველანა) or as "goby" as of genus Gobiidae (Ghorjo-ღორჯო). Even more complicated cases exist. In eastern Georgia, locals call *Barbus cyri* (Tsvera -წვერა) as "mursa" as *Luciobarbus mursa* (Murtsa-მურწა) and the mursa (*L. mursa*), itself is called as "gudgeon" as of genus *Romanogobio* (Tsimori-ციმორი, as mentioned above). Having the list of all the freshwater fish species with their local Georgian names is important, as locals now, can refer to each species individually.

This work is a first step towards creating a new field guide of freshwater fish species in Georgia, with information about their biological characteristics, distribution, and identification keys. This will make the identification of freshwater fish species easier for the local researchers, anglers, students or any interested parties during field trips or recreation.

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Table 1. List of freshwater fish species in Georgia and their English and Georgian common names. Georgian names are followed by Latin transcriptions. *- indicates species that did not have the Georgian common names before.

Taxa	English common Name	Georgian common name
Acheilognathidae		
Rhodeus amarus (Bloch, 1782)	European bitterling	ტაფელა/Taphela
Rhodeus colchicus Bogutskaya & Komlev 2001	Colchic bitterling	კოლხური ტაფელა/kolkhuri taphela
Acipenseridae		
Acipenser gueldenstaedtii Brandt & Ratzeburg 1833	Russian sturgeon	რუსული ზუთხი/rusuli zutkhi
Acipenser nudiventris Lovetsky, 1828	Fringebarbel sturgeon	ჯარღალა/jarghala
Acipenser persicus Borodin, 1897	Persian sturgeon	სპარსული ზუთხი/sparsuli zutkhi
Acipenser stellatus Pallas, 1771	Starry sturgeon	ტარაღანა/taraghana
Acipenser sturio Linnaeus, 1758	Atlantic sturgeon	ატლანტური ზუთხი/atlanturi zuthkhi
Huso huso (Linnaeus, 1758)	Beluga	სვია/svia
Anguillidae		
Anguilla anguilla (Linnaeus, 1758)	European eel	მდინარის გველთევზა/mdinaris gveltevza
Atherinidae		

Atherina caspia Eichwald 1831	Big-scale sand smelt	შავი ზღვის ათერინა/shavi zghvis aterina
Clupeidae		
Alosa immaculata Bennett, 1835	Pontic shad	პონტოური ქაშაყი
Alosa maeotica (Grimm, 1901)	Black sea shad	შავი ზღვის ქაშაყი/shavi zghvis kashaki
Alosa tanaica (Grimm, 1901)	Azov shad	აზოვის ზღვის ქაშაყი/azovis zghvis kashaki
Clupeonella cultriventris (Nordmann, 1840)	Black and Caspian Sea sprat	შავი ზღვის სარდელი, ქარსალა/shavi zghvis sardeli, karsala
Cobitidae		
Cobitis saniae Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh & Esmaeili, 2017*	South Caucasian spined loach	სამხრეთკავკასიური გველანა/samkhretkavkasiuri gvelana
Cobitis satunini Gladkov 1935	Colchic spined loach	კოლხური გველანა/kolkuri gvelana
Sabanejewia aurata (De Filippi, 1863)	Golden spined loach	ოქროსფერი გველანა/okrospheri gvelana
Coregonidae		
Coregonus albula (Linnaeus, 1758)	Vendace	ევროპული ჭაფალა/evropuli tchaphala
Coregonus sp.		სიგი/sigi
Cyprinidae		
Barbus ciscaucasicus Kessler, 1877*	Terek barbel	თერგის წვერა/tergis tsvera

Barbus cyri De Filippi, 1865	Kura barbel	მტკვრის წვერა/mtkvris tsvera
Barbus rionicus Kamensky, 1899	Colchic barbel	კოლხური წვერა/kolkhuri tsvera
Capoeta banarescui Turan, Kottelat, Ekmekçi & Imamoglu, 2006*	Banarescu's barb	ბანარესკუს ხრამული/banareskus khramuli
Capoeta capoeta (Güldenstädt, 1773)	Khramulya	მტკვრის ხრამული/mtkvris khramuli
Capoeta kaput Levin, Prokofiev & Roubenyan 2019*	Blue barb	ლურჯა ხრამული/lurja khramuli
Capoeta sieboldii (Steindachner, 1864)	Colchic khramulya	კოლხური ხრამული/kolkhuri khramuli
Carassius gibelio (Bloch, 1782)	Prussian carp	ჩვეულებრივი კარჩხანა/chveulebrivi karchkhana
Cyprinus carpio Linnaeus, 1758	Common carp	ჩვეულებრივი/სარკისებრი კობრი, გოჭა/Chveulebrivi/sarkisebri kobri, gocha.
Luciobarbus brachycephalus (Kessler, 1872)*	Aral barbel	არალის წვერა/aralis tsvera
Luciobarbus capito (Güldenstädt, 1773)	Bulatmai barbel	ჭანარი/tchanari
Luciobarbus mursa (Güldenstädt, 1773)	Mursa	მურწა/murtsa
Esocidae		
Esox lucius Linnaeus, 1758	Northern pike	ქარიყლაპია/წერი/kariklapia/tseri
Gasterosteidae		
Gasterosteus aculeatus Linnaeus, 1758	Three-spined stickleback	სამნემსა მახათა/samnemsa makhata

Gobiidae		
Babka gymnotrachelus (Kessler, 1857)	Racer goby	მდევარა ღორჯო/mdevara ghorjo
Mesogobius batrachocephalus (Pallas, 1814)	Knout goby	შოლტა ღორჯო/sholta ghorjo
Neogobius fluviatilis (Pallas, 1814)	Monkey goby	მექვიშია ღორჯო/mekvishia ghorjo
Neogobius melanostomus (Pallas, 1814)	Round goby	შავპირა ღორჯო/shavpira ghorjo
Ponticola constructor (Nordmann, 1840)	Caucasian goby	კავკასიური მდინარის ღორჯო/kavkasiuri mdinaris ghorjo
Ponticola cyrius (Kessler, 1874)*	Kura goby	მტკვრის ღორჯო/mtkvris ghorjo
Ponticola gorlap (Iljin, 1949)*	Caspian bighead goby	კასპიური დიდთავა ღორჯო/kaspiuri didtava ghorjo
Ponticola syrman (Nordmann, 1840)	Syrman goby	ღორჯო შირმანი/ghorjo shamani
Proterorhinus nasalis (De Filippi, 1863)*	Eastern tubenose goby	აღმოსავლური მილცხვირა ღორჯო/aghmosavluri miltskhvira ghorjo
Oxudercidae		
Knipowitschia caucasica (Berg, 1916)	Caucasian dwarf goby	კავკასიური ჯუჯა ღორჯო/kavkasiuri juja ghorjo
Knipowitschia longecaudata (Kessler, 1877)	Longtail dwarf goby	გრძელკუდა ღორჯო/grdzelkuda ghorjo
Rhinogobius lindbergi Berg, 1933*	Amur goby	ამურის ღორჯო/amuris ghorjo
Gobionidae		

Gobio artvinicus Turan, Japoshvili, Aksu & Bektaş 2016*	Artvin gudgeon	ართვინული ციმორი/artvinuli tsimori
Gobio caucasicus Kamensky, 1901	Colchic gudgeon	კავკასიური ციმორი/kavkasiuri tsimori
Pseudorasbora parva (Temminck & Schlegel, 1846)	Stone moroko	ფსევდორაზბორა/phsevdorazbora
Romanogobio macropterus (Kamensky, 1901)*	South Caucasian gudgeon	სამხრეთკავკასიური ციმორი/samkhretkavkasiuri tsimori
Leuciscidae		
Abramis brama (Linnaeus, 1758)	Freshwater bream	კაპარჭინა/kaparchina
Acanthobrama microlepis (De Filippi, 1863)	Blackbrow bleak	შავწარბა/shavtsarba
Alburnoides eichwaldii (De Filippi, 1863)	Kura chub	მტკვრის მარდულა, სწრაფულა/mtkvris mardula, stsraphula
Alburnoides fasciatus (Nordmann, 1840)	Transcaucasian spirlin	სამხრეთული მარდულა, ფრიტა/samkhretuli mardula, phrita
Alburnus alburnus (Linnaeus, 1758)	Bleak	თაღლითა, თეთრულა/ taghlita, tetrula
Alburnus chalcoides (Güldenstädt, 1772)	Danube bleak	შამაია/shamaia
Alburnus derjugini Berg, 1923	Georgian shemaya	ბათუმის შამაია/batumis shamaia
Alburnus filippii Kessler, 1877	Kura bleak	მტკვრის თაღლითა/mtkvris taghlita
Alburnus hohenackeri Kessler, 1877	North Caucasian bleak	ამიერკავკასიური თაღლითა/amierkavkasiuri taghlita

Ballerus sapa (Pallas, 1814)	White-eye bream	თეთრთვალა/tetrtvala
Blicca bjoerkna (Linnaeus, 1758)	White bream	ჩვეულებრივი ბლიკა/chveulebrivi blika
Chondrostoma colchicum Derjugin, 1899	Colchic nase	კოლხური ტობი/kolkhuri tobi
Chondrostoma cyri Kessler, 1877	Kura nase	მტკვრის ტობი/mtkvris tobi
Leuciscus aspius (Linnaeus, 1758)	Asp	ჩვეულებრივი ჭერეხი/chveulebrivi tcherekhi
Leucaspius delineatus Heckel, 1843*	Sunbleak	მზისებრი თაღლითა/mziseburi taghlita
Petroleuciscus borysthenicus (Kessler, 1859)	Dnieper chub	ჯუჯა ქაშაპი/juja kashapi
Phoxinus colchicus Berg, 1910	Colchic minnow	კოლხური კვირჩხლა/kolkhuri kvirchkhla
Rutilus frisii (Nordmann, 1840)	Kutum	მორევის ნაფოტა, კუტუმი/morevis naphota, kutumi
Rutilus lacustris (Pallas 1814)	Roach	ნაფოტა/naphota
Scardinius erythrophthalmus (Linnaeus, 1758)	Rudd	ფარფლწითელა/pharphltsitela
Squalius agdamicus (Kamensky 1901)*	Kura chub	მტკვრის ქაშაპი/mtkvris kashapi
Squalius orientalis Heckel, 1847*	Oriental chub	აღმოსავლური ქაშაპი/aghmosavluri kashapi
Squalius turcicus De Filippi, 1865*	Transcaucasian chub	სამცრეთკავკასიური ქაშაპი/samkhretkavkasiuri kashapi
Vimba vimba (Linnaeus, 1758)	Vimba bream	ვიმბა/vimba

Moronidae		
Dicentrarchus labrax (Linnaeus, 1758)	European seabass	ლავრაკი/lavraki
Mugilidae		
Chelon auratus (Risso, 1810)	Golden grey mullet	სინგილი/singili
Chelon labrosus (Risso, 1810)*	Thicklip grey mullet	სქელტუჩა კეფალი/skeltucha kephali
Chelon ramada (Risso, 1810)*	Thinlip grey mullet	თხელტუჩა კეფალი/thkeltucha kephali
Chelon saliens (Risso, 1810)	Leaping mullet	მახვილცხვირა კეფალი/mskhviltskhvira kephali
Mugil cephalus Linnaeus, 1758	Flathead grey mullet	ლობანი/lobani
Nemacheilidae		
Oxynoemacheilus bergianus (Derjavin, 1934)*	Kura sportive loach; Berg's loach	მტკვრის სპორტული გოჭალა/mtkvris sportuli gotchala; ბერგის გოჭალა/bergis gotchala
Oxynoemacheilus brandtii (Kessler, 1877)	Kura loach	მტკვრის გოჭალა/mtkvris gotchala
Oxynoemacheilus cemali Turan, Kaya, Kalayci, Bayçelebi & Aksu 2019*	Cemali loach; Coruh loach	ცემალის გოჭალა/tsemalis gotchala; ჭოროხის გოჭალა/tchorokhis gotchala
Oxynoemacheilus veyselorum(Cicek, Eagderi & Sungur, 2018)*	Veyseli loach; Araks loach	ვეისელის გოჭალა/veislelis gotchala; არაქსის გოჭალა/araksis gotchala
Percidae		

Perca fluviatilis Linnaeus, 1758	European perch	მდინარის ქორჭილა/mdinaris kortchila
Sander lucioperca (Linnaeus, 1758)	Pike-perch	ფარგა/pharga
Petromyzontidae		
Lampetra ninae (Naseka, Tuniyev & Renaud 2009)*	Western Transcaucasian lamprey	დასავლეთ ამიერკავკასიური სალამურა/dasavlet amierkavkasiuri salamura
Poeciliidae		
Gambusia holbrooki Girard, 1859	Mosquitofish	გამბუზია/gambuzia
Salmonidae		
Salmo caspius Kessler, 1877	Caspian trout	კასპიური მდინარის კალმახი/kaspiuri mdinaris kalmakhi
Salmo ciscaucasicus Kessler, 1877*	Caspian salmon	თერგის კალმახი/tergis kalmakhi
*Salmo coruhensis*Turan, Kottelat & Engin 2010*	Coruh trout	ჭოროხის კალმახი/tchorokhis kalmakhi
Salmo gegarkuni Kessler, 1877	Sevan trout	იშხანი/სევანის კალმახი/ischkhani/sevanis kalmakhi
Salmo labrax Pallas 1814	Black Sea salmon	შავი ზღვის ორაგული.shavi zghvis oraguli
Salmo rizeensis Turan, Kottelat & Engin 2010*	Rize trout	რიზეს კალმახი/rizes kalmakhi
Siluridae		
Silurus glanis Linnaeus, 1758	Wels catfish	ლოქო/loko

Syngnathidae		
Syngnathus abaster Risso, 1827	Black-striped pipefish	შავი ზღვის ლოყაფუნთუშა ნემსთევზა/shavi zghvis lokaphuntusha nemstevza
Tincidae		
Tinca tinca (Linnaeus, 1758)	Tench	გუწუ/gutsu

Appendix 2: Preliminary information about the occurrence of Prussian carp *Carassius gibelio* (Bloch 1782) in mountainous Lake Devdoraki (Caucasus, Georgia).

Preliminary information about the occurrence of Prussian carp *Carassius gibelio* (Bloch 1782) in mountainous Lake Devdoraki (Caucasus, Georgia)

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Abstract

In this conference communication, we briefly inform about the finding of non-native fish species morphologically identified as Prussian carp (*Carassius gibelio*) in Devdoraki Lake in the world biodiversity hotspot of the Caucasus Mountains. Additional analyses of the material are in process.

Key words: *Carassius gibelio,* lake Devdoraki, Caucasus, Georgia

1. Introduction

Spreading of non-native species is considered as one of the main drivers of biodiversity loss (Keller et al., 2011). One of the most successful fish invaders are from the species complex of the genus *Carassius* (Jarocki 1822) usually sorted into the species Prussian carp *Carassius gibelio* (Bloch 1782) (Kottelat & Freyhof, 2007) but the situation seems to be much more complicated (Liu et al., 2017; Rylková et al., 2013).

This species complex is invasive in many places in Europe (Ribeiro et al., 2015; Rylková et al., 2013) but recently also in North America, where it is spreading fast and affecting the native biota in the lake ecosystems (Ruppert et al., 2017).

Prussian carp was found in Georgia in various water bodies (Japoshvili et al., 2013; Japoshvili et al., 2017), but the distribution did not include Caucasian Mountains because until now, the occurrence of the Carassius in the area was not known and even not expected.

In this paper, we present the finding of fish from *Carassius* complex morphologically identified as Prussian carp (*Carassius gibelio*) in Devdoraki Lake (Caucasus, Georgia).

2. Materials and methods

During the touristic trip to the lake Devdoraki on 27th April 2017, afternoon, we found more than 100 fish with the size from 7 cm to 20 cm, fish appeared as they are freshly out of the water (some still alive) Figure 1. Devdoraki Lake is situated 42.722616, 44.623018, 1505 m a.s.l. with well-developed vegetation (Fig. 2).

Figure 1. Fish in the grass nearby Devdoraki Lake

Figure 2. Lake Devdoraki (Caucasus, Georgia)

We collected six freshly dead individuals and transported them to the laboratory of the Department of Hydrobiology and Ichthyology of the Institute of Zoology of Ilia State University in Tbilisi and we stored them in 70% alcohol.

Fish identification followed characters given in Berg (1949), Kottelat and Freyhof (2007). We measured total length and analyzed the following characters: number of spiny and branched fin rays in dorsal fin (DF), with attention to the serration of the last spiny fin ray in DF and number of spiny and branched fin rays in anal fin (AF); number of gill rakers; number of scales in lateral line and the color of the peritoneum. We identified sex according to the presence of testes or ovaries

3. Results and Discussion

All six individuals were morphologically identified as *Carassius gibelio*, details of the morphological analyses are given in the table. 1

We identified fish found on the bank of the lake Devdoraki morphologically as Prussian carp (*Carassius gibelio*), which was reported from Georgia for the first time by Japoshvili et al. (2013). Analyzed individuals showed following characteristics: 3 spiny and 17 branched rays in dorsal fin; 2 spiny and 6 branched rays in anal fin; 44-49 gill rakers; 30-31 scales in lateral line; peritoneum - black; dorsal fin with strongly serrated last spiny ray. All analyzed specimens were females. We are aware that more detailed study is necessary for identification of the species and this short contribution should be considered as preliminary information.

Devdoraki Lake is a small, isolated lake, which is very close to the confluence of the Amali and the Terek Rivers. The presence of C. gibelio in the lake is most likely connected to human activities such as recreational fishing as it was reported in other literature (e.g. Corn et al., 2001).

Table 1. Morphological characters of *Carassius gibelio* from Devdoraki Lake

Specimen	**Berg, 1949**	**1**	**2**	**3**	**4**	**5**	**6**
TL	-	124	117	93	102	99	94
Number of spiny fin rays in DF	III-IV	III	III	III	III	III	III
Number of branched fin rays in DF	15-19	17	17	17	17	17	17
Number of spiny fin rays in AF	II-III	II	II	II	II	II	II
Number of branched fin rays in AF	5-6	6	6	6	6	6	6
Gill rakers	39-50	49	48	44	45	45	46
Lateral line scales	28-33	30	31	30	30	30	30
Peritoneum black	YES	YES	YES	YES	YES	YES	YES
Last spiny ray of DF is strongly serrated	YES	YES	YES	YES	YES	YES	YES
Sex	-	F	F	F	F	F	F

We do not know why the fish were in the grass outside the lake. However, we speculate that it could be the result of flash flood since we observed outflow from the lake. It is possible that fish were flushed out by the flood wave caused by the melting snow from the mountain over the lake. During our visit, water runoff from the lake was low and fish could not swim and remained in the grass. However, in case of more intensive flooding, fish could be easily flushed into the Terek River and spread further, in the water bodies of the Northern Caucasus. This incidental finding brings the interesting documentation of the appearance of Prussian carp in the Caucasus Mountains. This fish is highly adaptable, which has made this specie highly invasive (Copp et al., 2009). We assume that it may become a successful invader in sensitive mountainous ecosystems with negative effects on aquatic environment especially benthic fauna in the Caucasian biodiversity hotspot.

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Appendix 3: Finding of nile tilapia *Oreochromis niloticus* (Cichliformes: Cichlidae) in Georgia, the South Caucasus.

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Finding of nile tilapia *Oreochromis niloticus* (Cichliformes: Cichlidae) in Georgia, the South Caucasus

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Abstract. Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is a fish from the family Cichlidae, native to Sub-Saharan Africa. Nile tilapia is one of the most cultured freshwater fish species worldwide and because of its commercial value and well-developed aquaculture technologies, it has been introduced to many countries. Nile tilapia also has become invasive in areas of suitable climate when escaped from aquaculture facilities to the wild. Georgia is the country in the Ponto-Caspian region situated on the southern slopes of the Great Caucasus eastwards from the Black Sea coast. Due to its very variable landscape, the climate of Georgia fluctuates from cold mountainous to humid subtropical type. Here we present the finding of *Oreochromis niloticus* in freshwaters of eastern Georgia for the first time, with the discussion of risk assessment with the climate suitability for potential establishment.

Keywords: caucasus; climate; non-native species; risk assessment

1. Introduction

The introduction of non-native species is a global problem. Some introduced species cause negative effects on native organisms and cause their decline [1, 2]. Aquaculture is one of the pathways of new fish species introductions [3, 4].

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is a cichlid fish, native to tropical and subtropical regions of the African continent and the Nile River [5]. Nile tilapia is the most cultured species worldwide from all tilapiine fishes [6]. Because of its commercial value and well-developed aquaculture technologies, it has been introduced in many countries. Due to the species ability of fast growth, paternal care, age, and size at maturity, this species has become successful invaders outside its native range and have become invasive in several countries such as Brazil [7] and the USA [8].

Nile tilapia was found in Turkey by Mert & Cicek [9], which was supposed to be its maximum northern distribution in the Mediterranean area. Authors, however, emphasized that the confirmation of this species establishment was needed [9]. Later, Nile tilapia was included in the checklist of the freshwater fishes of Turkey [10]. A few years later, Nile tilapia appeared in local anglers caught in a small village in neighboring Georgia.

Georgia is a country located in the South of the Great Caucasus Mountains eastwards from the Black Sea coast, and this area is included in global biodiversity hotspots [11]. The region is characterized by different altitude landscapes that facilitated the climate variability from cold mountainous to humid subtropical type [12]. In total, 119 freshwater fish species can be found in the South Caucasian region (Georgia, Armenia, and Azerbaijan), with seven endemic species [13].

Here we present the finding of Nile tilapia in freshwaters of Eastern Georgia for the first time, with the discussion of climate suitability for the species establishment and associated risks in the area.

2. Material and methods

In the summer of 2019, a tilapia (*Oreochromis sp.*) fish appeared in local anglers caught in a small village Mshvidobani (Lagodekhi region) in Eastern Georgia. The territory of the Lagodekhi municipality is characterized by a mild humid climate with hot summers and mildly cold winters. The average annual humidity of the air is 72%, the annual sum of precipitation is 1004 mm. The annual summer temperature is 12.6°C. The coldest month (January) has an average temperature of 0.9°C. The hottest period of the year is July-August with an average temperature of 24.1°C (http://www.lagodekhi.gov.ge).

In a small river, Baisubniskhevi (GPS coordinates N41.7634466 E46.1304388, the Kura River drainage) the local angler caught 10 individuals of the tilapia species with the fishing rod and put the photograph on the forum for identification on 29 September 2019. The record of the angler's catch was found on the fishermen forum (https://bit.ly/3lKSasu). The river, where the tilapia fish was found is used to supply the fish pond and fish farms in the area. From the bridge, down the river, south-west, in around 100 m, there are two ponds where a small aquaculture facility is located. Outflow from the ponds flows to the Baisubniskhevi River. The study area is shown in figure 1.

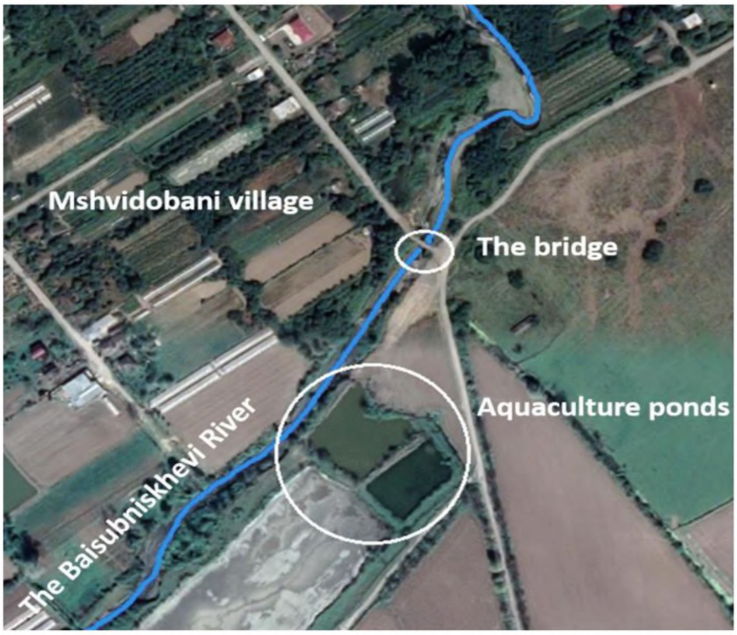

Figure 1. The south-east of the Mshvidobani Village: the Baisubniskhevi River, the bridge, and the aquaculture ponds.

Identification of caught individuals was done using the available picture (figure 2). We took morphological characters, which we were able to measure from the photograph and compared to the identification key [5]. We used the following characters: the number of scales in the lateral line and the presence of vertical spines on the caudal fin.

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Figure 2. *Oreochromis niloticus* caught in the Baisubniskevi River near village Mshvidobiani, Lagodekhi region (Georgia) on 23.09.2019. (c) Sportfishing.ge/forum.

After two months, on 29 November 2019, we visited the locality where the angler's cough the tilapia fish. We interviewed local inhabitants and applied electro-fishing with device EFGI 650. We sampled in shallow waters, at a maximum 70 cm depth, with an effective radius of 1m and 100-volt strength at the Baisubniskhevi River close to the Mshvidobiani village. The starting point was at the bridge (N41.763548 E46.130234) upstream northeast in the transect of 100 m length, and we also sampled the channels that are the outflow of aquaculture facilities.

We used the program Climatch v1.0 (Bureau of Rural Sciences) to test the climate suitability of the tilapia's native range, Source Area (SFA) versus Georgia, Target Area (TA). Five variables including annual mean temperature, the temperature of coldest and warmest months, and temperature of coldest and warmest quarters were used for the test. The test gives a final score from 1-10, meaning that the SA and TA climate are matching if the result scores are more than 7. Any less score indicates no suitability of climate between SA and TA [14].

3. Results

We were not able to catch fish during electrofishing. Nevertheless, the unavailability of the specimen we could still distinguish the species from the photograph (figure 2) as Nile tilapia (*Oreochromis niloticus*) according to the number of scales from lateral lines which were 33 and the most distinguishable character for *O. niloticus*, presence of vertical stripes on the caudal fin.

We interviewed the son of the owner of the aquaculture facility and fishponds (that is 100 meters far away down from the bridge) because the owner himself was unavailable. His son considered the picture of tilapia as a Prussian carp (*Carassius gibelio*) and he said that they have many Prussian carps in the pond. In the end, it was quite unclear if they have this fish there. However, after interviewing locals, they admit that they have caught tilapia fish several times from the bridge and from the channel which supplies the river with water.

We compared Nile tilapias native area (SA) tropical and subtropical regions of the African continent and the Nile River to its introduced area (TA) Georgia. The result of Climate analysis identified the TA as a not suitable climate for the Nile tilapias distribution (figure 3). The maximum similarity value between SA and TA within the whole of Georgia was 5 in the Abkhazia Region (northwest of Georgia, the Black Sea Basin), where minimum temperatures in winter vary between 4-6 °C.

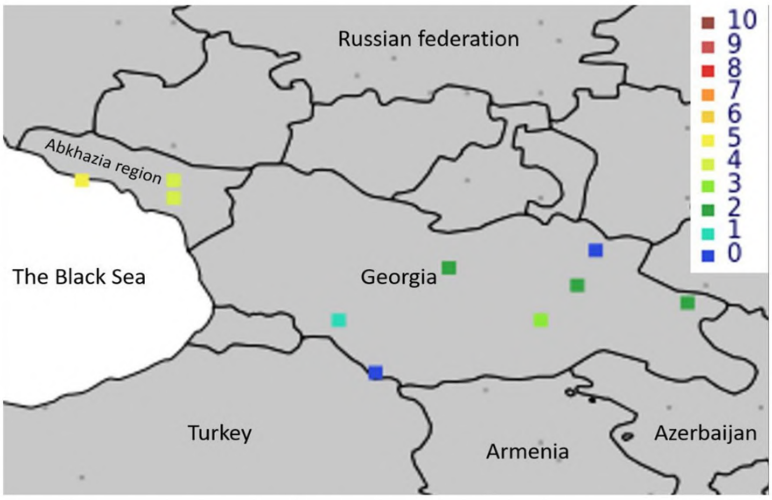

Figure 3. Climatch results for Nile tilapias Native Area versus Georgia. The scale indicates matching of the climatic variables from 0-10.

4. Discussion

The fish found in the Lagodekhi region was identified as Nile tilapia, however without having the voucher specimen. The obtained morphological characteristics observed in a photograph perfectly matched those presented by Trewavas [5]. Nile tilapia is the most commonly distributed cichlid fish outside of its native range due to its trade value [6]. Aquaculture is also most likely the reason for this species' appearance in the Lagodekhi region. This fish likely escaped from nearby fish farms that are just 100 meters far away from where the fish was spotted. To track the exact place and time of Nile tilapias introduction in the area was impossible since the owner of the facility was not able to communicate with us and it was not possible to confirm the information that we got from locals.

Even if the Climate analysis did not confirm the environmental suitability for the Nile tilapia in Georgia, the risk of this species establishment still exists. For example, Nile tilapia was not considered to be established in temperate environments in the US, since it was believed that the species could not survive the winter. However, the study done by Grammer *et al.* [15] proved its successful establishment in temperate Mississippi (Southeastern Mississippi, the Pascagoula River). Although the extended temperatures range for Nile tilapia is 8-42 °C [16], it was found to be well adapting to the outflows of the aquaculture farms, where water is warmer (so-called thermal refugia), which then could have led to survival and establishment of Nile tilapia in temperate regions in the USA [17].

Global climate change is believed to be the factor of some non-native species to become invasive, it was hypothesized that climate change might alert the mechanisms of transportation and introduction of non-native species as commercial and recreational activities will increase the propagule pressure of non-native species [18, 19]. In the view of global climate change the probability of species establishment and spreading is increasing as the fish enter the open waters. We suggest observing the locality if the fish is likely to overcome the temperature barriers over the winter period and establish a viable population.

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Appendix 4: A global-scale screening of non-native aquatic organisms to identify potentially invasive species under current and future climate conditions.

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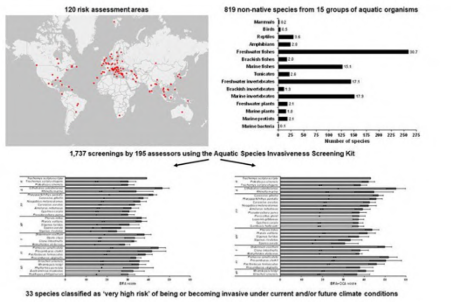
H I G H L I G H T S

- A global approach is required to identify invasive species posing high risk impact.
- 195 assessors screened 819 non-native species from 15 groups of aquatic organisms.
- Risk thresholds were identified for 14 aquatic organism groups.
- The resulting risk thresholds and rankings will help management and conservation.

G R A P H I C A L   A B S T R A C T



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A B S T R A C T

The threat posed by invasive non-native species worldwide requires a global approach to identify which introduced species are likely to pose an elevated risk of impact to native species and ecosystems. To inform policy, stakeholders and management decisions on global threats to aquatic ecosystems, 195 assessors representing 120 risk assessment areas across all six inhabited continents screened 819 non-native species from 15 groups of aquatic organisms (freshwater, brackish, marine plants and animals) using the Aquatic Species Invasiveness Screening Kit. This multi-lingual decision-support tool for the risk screening of aquatic organisms provides assessors with risk scores for a species under current and future climate change conditions that, following a statistically based calibration, permits the accurate classification of species into high-, medium- and low-risk categories under current and predicted climate conditions. The 1730 screenings undertaken encompassed wide geographical areas (regions, political entities, parts thereof, water bodies, river basins, lake drainage basins, and marine regions), which permitted thresholds to be identified for almost all aquatic organismal groups screened as well as for tropical, temperate and continental climate classes, and for tropical and temperate marine ecoregions. In total, 33 species were identified as posing a 'very high risk' of being or becoming invasive, and the scores of several of these species under current climate increased under future climate conditions, primarily due to their wide thermal tolerances. The risk thresholds determined for taxonomic groups and climate zones provide a basis against which area-specific or climate-based calibrated thresholds may be interpreted. In turn, the risk rankings help decision-makers identify which species require an immediate 'rapid' management action (e.g. eradication, control) to avoid or mitigate adverse impacts, which require a full risk assessment, and which are to be restricted or banned with regard to importation and/or sale as ornamental or aquarium/fishery enhancement.

1. Introduction

The threat posed by invasive non-native species (NNS) worldwide to native species and ecosystems requires a global approach to identify species that pose a high risk of becoming invasive across varied geographies and climates (Gordon et al., 2008). Use of the same risk screening protocols worldwide can provide the larger-scale information with which to inform the decisions of policy makers and environmental managers in dealing with species invasions (Early et al., 2016; Shackleton et al., 2019). Indeed, reliable, evidence-based risk assessment (RA) methods are vital to decision-making in biosecurity management at national and international levels in order to prevent or mitigate unwanted biological invasions (Kaiser and Burnett, 2010). Ideally, the RA outcomes should compare and prioritise NNS according to their potential invasiveness under current and future climate conditions (Barney and DiTomaso, 2010), which aids in identifying the underlying factors associated with invasion hotspots (O'Donnell et al., 2012; Chapman et al., 2020).

The first step in the NNS risk analysis process is risk screening (i.e. hazard identification), which aims to identify the NNS that are likely to pose an elevated threat to native species and ecosystems and, therefore, warrant more comprehensive (i.e. full) RA (Copp et al., 2005b). Aquatic species that are likely to carry a high risk of becoming invasive, hence posing a threat to native species, usually possess life-history traits including frequent reproduction with a high incidence of ovoviviparity, large body size and a long life span, the ability to exploit opportunistically available food resources, a history of invasion success, and a close climate matching with the RA area (Statzner et al., 2008; Chan et al., 2021). In addition, these species often tolerate higher salinity, broader environmental temperatures and higher levels of organic pollution than native species (Leuven et al., 2009). Importantly, the identification of species posing a high risk of being (or becoming) invasive in a certain RA area provides a basis for advice to policy, decision-makers and other stakeholders regarding management options for existing and potential future invasive NNS (Copp et al., 2005b, 2016b; Mumford et al., 2010; David et al., 2013; David and Gollasch, 2018, 2019).

The objectives of the present study were to: (i) construct a global database of risk screenings that span the broadest range of aquatic organisms possible, given available resources, across the widest possible geographical spread; (ii) subject the global database of screenings to calibration and accuracy analysis; and (iii) generate global-scale risk thresholds at the organism group and climate class/marine ecoregion levels under both current and future climate conditions. The global-scale thresholds identified will provide a basis against which thresholds calibrated for specific RA areas may be interpreted and will also allow the 'rapid risk screening' of individual species for a certain RA area whenever specific calibration is not possible. In addition, these global-scale thresholds will place RA area-specific risk screening calibrations within a broader, global context, also accounting for climate change predictions. In turn, this will enhance the value and scope of more localised calibrations to inform environmental policy and decision-makers of the relative risk rankings of aquatic NNS so as to facilitate the cost-effective allocation of management resources.

2. Materials and methods

2.1. Risk screening procedure

For the purposes of this study, 'invasive species' are defined, as per Copp et al. (2005c, p. 244), as those NNS "that spread, with or without the aid of humans, in natural or semi-natural habitats, producing a significant change in composition, structure, or ecosystem processes, or cause severe economic losses to human activities". Species were evaluated for their potential to become invasive in the assessor(s)-defined RA area using the Aquatic Species Invasiveness Screening Kit (AS-ISK: free download at www.cefas.co.uk/nns/tools/). This is a decision-support tool (Copp et al., 2016b, 2021) adapted from the Fish Invasiveness Screening Kit (Copp et al., 2009, 2005a), which itself was derived from the globally-applied Weed Risk Assessment of Pheloung et al. (1999). The AS-ISK comprises questions from the generic screening module of the European Non-native Species in Aquaculture Risk Analysis Scheme (Copp et al., 2016a) and incorporates the 'minimum requirements' (Roy et al., 2018) for the assessment of invasive NNS with regard to the 2014 EU Regulation 1143/2014 (European Union, 2014). As a taxon-generic toolkit, the AS-ISK is applicable to any aquatic species (other than parasites and pathogens) in virtually any climatic/marine ecoregion zone (Copp et al., 2016b; Table 1), and allows the screening of 27 groups of aquatic organisms in total (taxonomy after Ruggiero et al., 2015): mammals, birds, reptiles, amphibians, fishes (freshwater, brackish, marine), tunicates, lancelets, invertebrates (freshwater, brackish, marine), 'other' animals (freshwater, brackish, marine), plants (freshwater, brackish, marine), protists (freshwater, brackish, marine), fungi (freshwater, brackish, marine), and bacteria (freshwater, brackish, marine).

The screening protocol consists of 55 questions (Copp et al., 2016b). The first 49 questions comprise the Basic Risk Assessment (BRA), which are concerned with the biogeographical and biological aspects of the species being screened. The last six questions address the Climate Change Assessment (CCA), which require the assessor to evaluate how future predicted climate conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. To achieve a valid screening, for each question the assessor must provide a response, a level of confidence in the response, and a justification. In all cases, the assessor is a specialist in the biology/ecology of the aquatic organism under screening for the RA area under study. Upon completion of the screening, the species receives both a BRA score and a BRA + CCA (composite) score (ranging from −20 to 68 and from −32 to 80, respectively). Scores < 1 suggest that the species is unlikely to become invasive and is therefore classified as 'low risk' (Pheloung et al., 1999). Higher scores classify the species as posing either a 'medium risk' or a 'high risk' of becoming invasive. Distinction between medium-risk and high-risk levels depends upon setting a 'threshold' value (see Section 2.2 *Data processing and analysis*).

The ranked levels of confidence (1 = low; 2 = medium; 3 = high; 4 = very high) associated with each question-related response mirror the confidence rankings recommended by the International Programme on Climate Change (IPCC, 2005; see also Copp et al., 2016b). Based on the confidence level (CL) allocated to each response, a confidence factor (CF) is computed as:

$$CF = \sum (CL_{Qi})/(4 \times 55)\ (i = 1, \ldots, 55)$$

where CL_{Qi} is the confidence level for the *i*th Question (*Qi*), 4 is the maximum achievable value for confidence (i.e. very high: see above) and 55 is the total number of questions. Based on the 49 Qs comprising the BRA and the six Qs comprising the CCA, the CL_{BRA} and CL_{CCA} are also computed (out of the CL_{Total} for all 55 Qs).

Table 1
Published initial applications of the Aquatic Species Invasiveness Screening Kit (AS-ISK) by aquatic organismal group.

Aquatic organismal group	Reference(s)
Reptiles	Ruykys et al. (2021)
Amphibians	Ruykys et al. (2021)
Freshwater fishes	Glamuzina et al. (2017), Li et al. (2017), Tarkan et al. (2017a), Tarkan et al. (2017b), Dodd et al. (2019), Suresh et al. (2019), Interesova et al. (2020), Moghaddas et al. (2020), Zięba et al. (2020), Glamuzina et al. (2021), Haubrock et al. (2021), Kumar et al. (2021), Moghaddas et al. (2021), Radočaj et al. (2021), Ruykys et al. (2021), Wei et al. (2021)
Brackish fishes	Castellanos-Galindo et al. (2018), Clarke et al. (2020)
Marine fishes	Filiz et al. (2017a), Filiz et al. (2017b), Bilge et al. (2019), Clarke et al. (2020), Lyons et al. (2020), Uyan et al. (2020)
Tunicates	Clarke et al. (2020)
Freshwater invertebrates	Paganelli et al. (2018), Ruykys et al. (2021), Semenchenko et al. (2018)
Brackish invertebrates	Clarke et al. (2020), Ruykys et al. (2021)
Marine invertebrates	Clarke et al. (2020), Killi et al. (2020), Stasolla et al. (2020), Ruykys et al. (2021)
Freshwater plants	Ruykys et al. (2021)
Marine plants	Clarke et al. (2020), Ruykys et al. (2021)
Marine protists	Clarke et al. (2020)

2.2. Data processing and analysis

Data consisted of: (i) individual contributions to the present study by assessors invited to screen one (or more) NNS belonging to one or more aquatic organismal groups of choice (i.e. falling within their expertise) for a certain RA area; and (ii) datasets from more comprehensive screening studies of NNS for a certain RA area, both published (see Table 1) and unpublished. For each species screened, the scientific name used in the original contribution or study was updated to the most recent taxonomy after the World Register of Marine Species (www.marinespecies.org), else after the Integrated Taxonomic Information System (www.itis.gov/) or FishBase (www.fishbase.org). This was followed by 'cross-checking' for the existence of at least one peer-reviewed publication that used the updated scientific name in case of a change in taxonomy. A notable exception was the retention of the original name *Crassostrea gigas* instead of the recently proposed *Magallana gigas* for the Pacific oyster (see Bayne et al., 2017). Taxonomic details of the corresponding Order and Family were also retrieved for each species screened.

Except for marine regions, for each RA area the corresponding Köppen-Geiger climate class (i.e. Tropical, Dry, Temperate, Continental, Polar: Peel et al., 2007) was identified, noting that in several cases more than one climate class applied to the same RA area. For marine regions, the classification by Spalding et al. (2007) was used including: (i) Arctic, (ii) Temperate Northern Atlantic and Temperate Northern Pacific (grouped in the present study into 'Temperate marine'), and (iii) Central Indo-Pacific, Tropical Atlantic, Tropical Eastern Pacific and Western Indo-Pacific (grouped in the present study into 'Tropical marine').

The shape of the global distribution of the BRA and BRA + CCA scores was tested in R x64 v3.6.3 (R Development Core Team, 2020) using the package 'moments' v0.14 (Komsta and Novomestky, 2015), with normality, skewness and kurtosis evaluated by the Jarque-Bera (*JB*), D'Agostino and Anscombe tests, respectively. Computation of risk outcomes was based on receiver operating characteristic (ROC) curve analysis (Bewick et al., 2004). An ROC curve is a graph of sensitivity vs

1 – specificity for each threshold value, where in the present context sensitivity and specificity will be the proportion of a priori invasive and non-invasive species, respectively, correctly identified as such. For ROC curve analysis to be implemented, the species selected for screening must be categorised a priori as non-invasive or invasive using independent literature sources.

The a priori categorisation was as follows (see also Clarke et al., 2020): (i) a first search was made of FishBase for any reference to the species' threat, with the species categorised as non-invasive if listed as 'harmless', categorised as invasive if listed as 'potential pest', or scored as absent if either not evaluated or not listed in the above database; (ii) a second search was made of the Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI ISC: www.cabi.org/ISC) and the Global Invasive Species Database (GISD: www.iucngisd.org), with the species categorised as invasive if it appeared in any of such lists or scored as absent if not listed; (iii) a third search was made of the Invasive and Exotic Species of North America list (www.invasive.org), with the species categorised as invasive if it appeared in any of such lists or scored as absent if not listed; (iv) except for those species categorised as invasive in any (or all) of the previous three steps, a Google Scholar (literature) search was performed to check whether at least one peer-reviewed reference is found that 'demonstrates' (hence, not 'assumes') invasiveness/impact. The latter was then taken as 'sufficient evidence' for categorising the species as invasive; whereas, if no evidence was found, then the species was categorised as non-invasive. Overall, the advantage of this method is that, by virtue of its meta-analytical foundation, it draws upon and combines previous approaches into a multi-tiered protocol. This maximises the amount of information collectable about the NNS under screening, thereby increasing the accuracy of the screening outcomes (Vilizzi, Copp and Hill, unpublished).

A measure of the accuracy of the calibration analysis is the Area Under the Curve (AUC), which ranges from 0 to 1: a model whose predictions are 100% correct has an AUC of 1, one whose predictions are 100% wrong has an AUC of 0. In the former case there are neither 'false positives' (a priori non-invasive species classified as high risk, hence false invasive) nor 'false negatives' (a priori invasive species classified as low or medium risk, hence false non-invasive); in the latter case, the test cannot discriminate between 'true positives' (a priori invasive species classified as high risk, hence true invasive) and 'true negatives' (a priori non-invasive species classified as low or medium risk, hence true non-invasive). Following ROC curve analysis, the best threshold value that maximises the true positive rate and minimises the false positive rate was determined using Youden's J statistic.

Because of sample size constraints (see Vilizzi et al., 2019), group-specific thresholds for both the BRA and BRA + CCA were fitted to those groups of aquatic organisms for which >10 species were screened. Consequently, for mammals and birds for which there were low numbers of taxa a combined threshold was computed by pooling together the screened species for these groups with those screened for reptiles and amphibians. This rendered the respective thresholds statistically significant, permitting their use for distinguishing between high-risk and low-to-medium risk species until such time that RA-area-specific calibrations can be undertaken for those taxonomic groups. To highlight 'very high risk' species for the aquatic organismal groups with large enough sample sizes, ad hoc thresholds for the BRA and BRA + CCA were set weighted according to the range of scores for the high-risk species (see Clarke et al., 2020) and with the constraint that the species was screened for a 'representative' number of RA areas (i.e. weighted according to the corresponding organismal group). Additionally, climate class-specific thresholds were computed for freshwater fishes, and marine ecoregion-specific thresholds for marine fishes and invertebrates – the aquatic organismal groups with large enough sample size for successful computation of such thresholds. In all cases, ROC curve analysis was carried out with the package 'pROC' (Robin et al., 2011) for R x64 v3.6.3 using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals).

Following Smith et al. (1999), three measures of accuracy were defined:

1) for a priori invasive species: $A_i = (I_r/I_t) \times 100$, where I_r is the number of a priori invasive species rejected, and I_t the total number of a priori invasive species screened;
2) for a priori non-invasive species: $A_n = (N_a/N_t) \times 100$, where N_a is the number of a priori non-invasive species accepted and N_t the total number of a priori non-invasive species screened;
3) overall: $A_o = (N_a + I_r)/(N_t + I_t)$.

In all cases, values above 50% are indicators of the accuracy of the screening tool.

3. Results

In total, 1730 screenings were conducted by 195 assessors (the co-authors of this study) on 819 taxa comprising 798 species, nine subspecies, three hybrids, and nine genera (Supplementary data Table S1). Of these taxa (hereafter, loosely termed 'species'), 562 (68.6%) were categorised a priori as non-invasive and 257 (31.4%) as invasive (Supplementary data Table S1) and were screened relative to 120 RA areas (Supplementary data Tables S2 and S3) across all six inhabited continents (Fig. 1). The RA areas consisted of extensive geographical areas, regions, countries, parts of countries, states, other political entities, water bodies, river basins, lake drainage basins, and marine regions (Supplementary data Table S2). Screenings encompassed 15 groups of aquatic organisms (Fig. 2) in 104 Orders (Supplementary data Table S2), with 24 species assigned to two different groups depending on the RA area's aquatic habitat (Supplementary data Table S4).

The BRA scores ranged from −15.0 to 55.0, with a mean = 18.6, a median = 18.0, and 5th and 95th percentiles = −4.0 and 42.5. Their distribution was not normal ($JB = 36.664$, $P < 0.001$), not skewed (skewness = 0.076, $z = 1.230$, $P = 0.195$), but platykurtic (kurtosis = 2.305, $z = -9.359$, $P < 0.001$) (Fig. 3a). The BRA + CCA scores ranged from −27.0 to 67.0, with a mean = 22.3, a median = 22.0, and 5th and 95th percentiles = −5.6 and 51.1. Their distribution was not normal ($JB = 16.378$, $P < 0.001$), not skewed (skewness = −0.039, $z = -0.675$, $P = 0.499$), but platykurtic (kurtosis = 2.531, $z = -5.210$, $P < 0.001$) (Fig. 3b). The majority of delta values (i.e. differences between BRA + CCA and BRA scores, hence accounting for climate change predictions) were equal to 0, 4, 6 and 10 (>10% of the total in all cases), and overall the proportion of the positive differences was much larger than that of the negative differences (68.8% vs 16.7%) (Fig. 3c). Across all species, the mean CL values were: $CL_{Total} = 2.73 \pm 0.01$, $CL_{BRA} = 2.78 \pm 0.01$, and $CL_{CCA} = 2.25 \pm 0.02$, indicating in all cases medium to high confidence (Supplementary data Table S5).

Thresholds were computed for all screened groups of aquatic organisms except those represented by ≤10 species (Table 2). For reptiles, amphibians, freshwater and marine fishes, tunicates, freshwater and brackish invertebrates and marine protists, the BRA threshold was lower than the BRA + CCA one, whereas the opposite was true for brackish fishes, marine invertebrates, and freshwater and marine plants. Except for marine protists (BRA), the mean AUC values (in Table 2) were in all cases > 0.5 – this confirmed the ability of the toolkit to differentiate between a priori invasive and non-invasive species. After pooling, BRA and BRA + CCA thresholds could be computed for mammals and birds, and in both cases the BRA threshold was lower than the BRA + CCA one (Table 2).

Based on the aquatic organismal group-specific thresholds (excluding the pooled ones), all three measures of accuracy had a mean value ≥50% for all groups except tunicates (BRA + CCA only), marine plants and marine protists (both BRA and BRA + CCA) – a result of the relatively small sample sizes (Table 3). The number (and proportion) of true positives

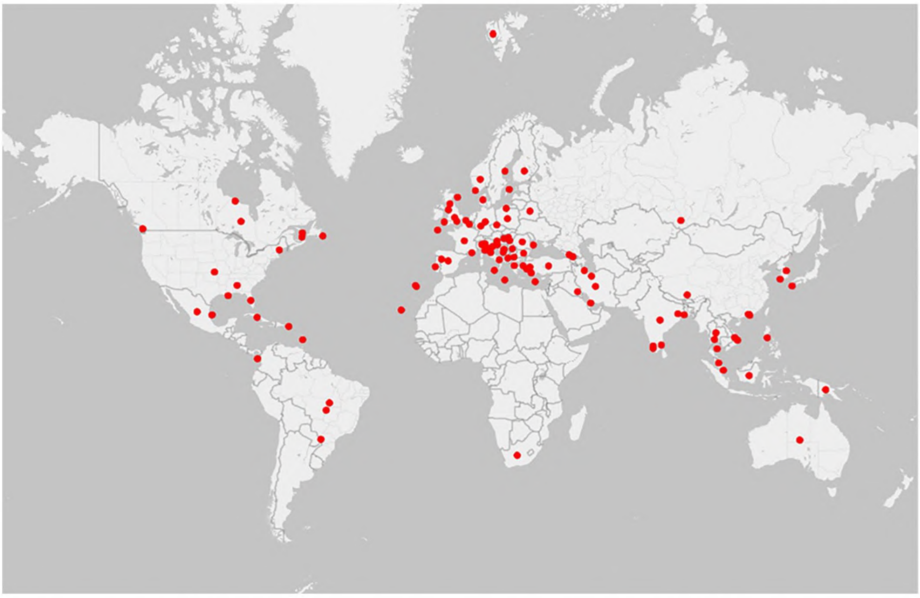

Fig. 1. Map of the risk assessment areas for which species were screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK; see also Supplementary data Table S2).

was consistently larger than that of the false negatives, which in all cases accounted for only 0–5.6% of the screened species for each group (Table 4). Similarly, the proportion of false positives was in most cases smaller than that of the true positives, and the proportion of the medium-risk species was always relatively high. In total, 33 species were identified as carrying a very high risk of invasiveness: 26 species based on both the BRA and BRA + CCA, four on the BRA only, and three on the BRA + CCA only (Fig. 4a, b).

Of the 82 non-marine region RA areas, 56 included one climate class. To these RA areas, an additional four were added for which the second climate class (namely, Continental) was only marginally represented (Supplementary data Table S2), whereas the only RA area represented by the Dry climate was removed from further analysis (noting that the Polar climate was found only in combination with the Temperate and/or Continental climates, hence could not be analysed separately regardless of sample size). In total, 59 non-marine region RA areas were therefore considered. For freshwater fishes in tropical, temperate and continental climates, both the BRA and BRA + CCA thresholds were higher for the tropical climate, lower for the temperate and even lower for the continental climate, whereas the BRA + CCA was similar to the BRA in all cases (Table 5). Of the 38 marine ecoregion RA areas, four fell within the Arctic ecoregion, 24 in the Temperate grouping (including 23 RA areas in the Temperate Northern Atlantic and one in the Temperate Northern Pacific ecoregions), and ten in the Tropical

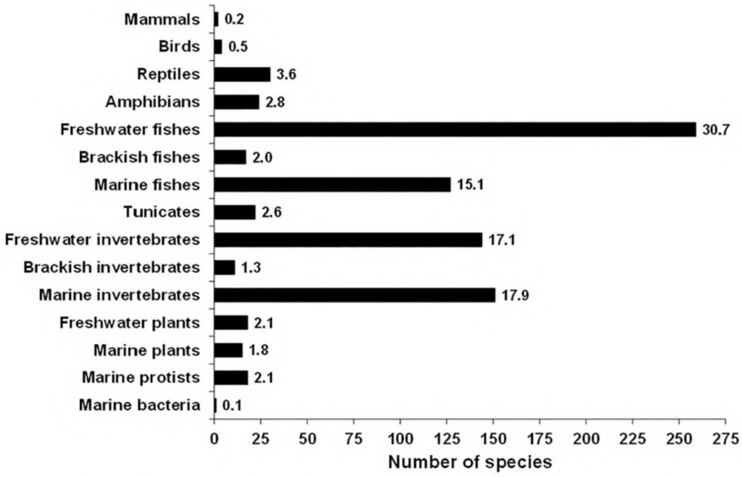

Fig. 2. Number (and corresponding percentage) of species screened according to aquatic organismal group.

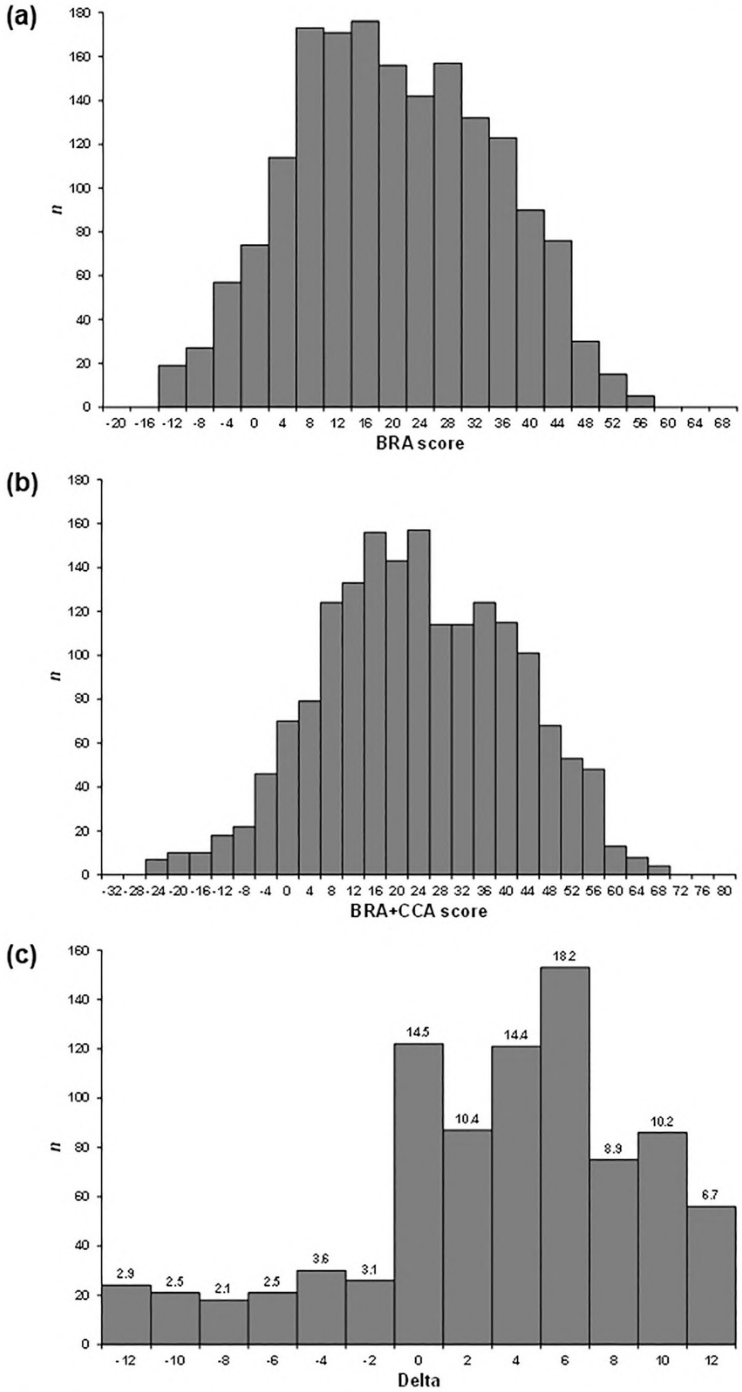

Fig. 3. (a) Frequency distribution of the Basic Risk Assessment (BRA) scores for the species screened with AS-ISK; (b) same for the BRA + CCA (Climate Change Component) scores; (c) same for delta CCA values (i.e. differences between BRA + CCA and BRA scores for each of the species screened) with corresponding percentage.

Table 2
Basic Risk Assessment (BRA) and BRA + Climate Change Assessment (CCA) AS-ISK thresholds from receiver operating characteristic curve analysis for the groups of aquatic organisms screened in the present study (Marine bacteria added for completeness). For each aquatic organismal group, the following is provided: number of screened species (n), threshold (Thr) values for the BRA and BRA + CCA [the Area Under the Curve (AUC) values are >0.5, and therefore are statistically valid, including for Mammals, Birds, Reptiles and Amphibians – with the latter two groups pooled together with Mammals and Birds for computation of the thresholds], with corresponding mean, lower confidence interval (LCI) and upper confidence interval (UCI) for the AUC, very high risk threshold (VH Thr) and minimum number of risk assessment areas (RAAs) for selection of the very high risk species. Note that threshold values are given in increments of 0.05 and rounded to the first or second decimal (as applicable, following AS-ISK threshold notation).

Aquatic organismal group	n	BRA						BRA + CCA					
		Thr	Mean	LCI	UCI	VH Thr	RAAs	Thr	Mean	LCI	UCI	VH Thr	RAAs
Mammals	2	25.5	0.7180	0.5834	0.8525	–	–	31.6	0.6639	0.5223	0.8056	–	–
Birds	4	25.5	0.7180	0.5834	0.8525	–	–	31.6	0.6639	0.5223	0.8056	–	–
Reptiles	30	28.5	0.6550	0.4176	0.8924	–	2	36.5	0.5975	0.3489	0.8461	–	2
Amphibians	24	15.5	0.7983	0.6144	0.9822	–	3	19.5	0.7815	0.5934	0.9696	–	3
Freshwater fishes	259	14.7	0.8446	0.7957	0.8936	30	10	17.7	0.8213	0.7691	0.8735	36	10
Brackish fishes	17	38	0.7917	0.5542	1.0000	–	–	29.5	0.6875	0.3969	0.9781	–	–
Marine fishes	127	12.75	0.8254	0.7089	0.9420	–	3	19	0.7819	0.6541	0.9096	–	3
Tunicates	22	22.5	0.6417	0.3943	0.8890	–	2	36.75	0.5792	0.3119	0.8464	–	2
Freshwater invertebrates	144	13.25	0.8243	0.7185	0.9301	30	4	25.75	0.8199	0.7076	0.9322	36	4
Brackish invertebrates	11	15	0.6111	0.0247	1.0000	–	–	26.9	0.7222	0.4142	1.0000	–	–
Marine invertebrates	151	15.1	0.8842	0.8333	0.9351	30	3	14.25	0.8483	0.7859	0.9107	36	3
Freshwater plants	15	24.5	0.8611	0.6697	1.0000	–	–	12.5	0.9028	0.7522	1.0000	–	–
Marine plants	15	32	0.6161	0.3098	0.9224	–	–	27.25	0.6250	0.3197	0.9303	–	–
Marine protists	18	34	0.4545	0.1639	0.7452	–	–	42.75	0.5779	0.2899	0.8659	–	–
Marine bacteria	1	–	–	–	–	–	–	–	–	–	–	–	–

grouping (including three RA areas in the Central Indo-Pacific, four in the Tropical Atlantic, one in the Tropical Eastern Pacific, and two in the Western Indo-Pacific ecoregions) (Supplementary data Table S2). However, owing to low sample sizes, thresholds could not be computed for any aquatic organismal group in the Arctic ecoregion. For marine fishes, the BRA and BRA + CCA thresholds were higher for the temperate relative to the tropical ecoregion grouping, and the BRA + CCA threshold was higher than the BRA threshold in both cases (Table 5). For marine invertebrates, the BRA and BRA + CCA thresholds were higher for the tropical relative to the temperate ecoregion grouping, and the BRA threshold was similar to the BRA + CCA for the temperate ecoregion grouping but lower for the tropical ecoregion grouping (Table 5). In all cases, the mean AUC values were well above 0.5.

Based on the climate/marine ecoregion-specific thresholds, all three measures of accuracy had a mean value well above 50% in all cases and for both the BRA and BRA + CCA (Table 6). The number (and proportion) of true positives was consistently larger than that of the false negatives, which always accounted for only 0–1.6% of the screened species for each combination (Table 7). Similarly, the proportion of false positives was in most cases smaller than that of the true positives, and the proportion of medium-risk species was always relatively high.

Table 3
Accuracy measures for screenings on the groups of aquatic organisms for which BRA and BRA + CCA thresholds were directly computed (cf. Table 2). A_i = accuracy for a priori invasive species; A_n = accuracy for a priori non-invasive species; A_o = overall accuracy (see text for details). In italics, values < 50%.

Aquatic organismal group	BRA			BRA + CCA		
	A_i	A_n	A_O	A_i	A_n	A_O
Reptiles	70.0	75.0	73.3	70.0	75.0	73.3
Amphibians	100.0	70.6	79.2	50.0	50.0	50.0
Freshwater fishes	83.9	74.7	78.0	77.4	71.7	73.7
Brackish fishes	62.5	100.0	82.4	62.5	88.9	76.5
Marine fishes	88.9	75.2	77.2	72.2	79.8	78.7
Tunicates	80.0	58.3	68.2	*40.0*	91.7	68.2
Freshwater invertebrates	76.9	83.9	82.6	65.4	95.8	90.3
Brackish invertebrates	88.9	50.0	81.8	66.7	100.0	72.7
Marine invertebrates	86.4	78.3	81.5	83.1	75.0	78.1
Freshwater plants	83.3	100.0	88.9	58.3	100.0	72.2
Marine plants	*42.9*	87.5	66.7	*28.6*	75.0	53.3
Marine protists	*42.9*	36.4	38.9	71.4	*27.3*	*44.4*

4. Discussion

4.1. Risk screening extent

In this study, fishes and invertebrates represented the largest proportion of screened aquatic species, thus reflecting the composition of introduced animal species recorded for e.g. European waters (Alcaraz et al., 2005; Gherardi et al., 2009; Katsanevakis et al., 2013) but also the relative number of experts (cf. assessors) in the various aquatic organismal groups. After freshwater fishes, freshwater and marine invertebrates comprised the second most-widely screened group of aquatic organisms, with the marine invertebrates including a large proportion of Decapoda – an Order that comprises several of the world's worst invasive species (Lowe et al., 2000; Souty-Grosset et al., 2006). The large number of screenings for freshwater fishes in this study can be attributed to the importance of inland waters as providers of ecosystem services for human societies (e.g. Wilson and Carpenter, 1999) and to the fact that these habitats are under high human-induced pressure, including NNS introductions (e.g. Hughes et al., 1998; Rahel, 2000). The broad geographical spread of most of the screened freshwater fish species reflects the increasing homogenisation of aquatic fauna and flora as a result of worldwide introductions (e.g. McKinney, 1998; Rahel, 2000). Further, the taxonomic Orders that were more frequently screened are those usually ecologically flexible, able to withstand adverse ecological conditions, generally widespread over large spatial scales, and often of economic importance (e.g. Hulme, 2009).

Despite the large number of aquatic species screened in this study, bacteria were represented by only one species and no screenings for fungi were contributed. Risk screenings of these groups of aquatic organisms would require the participation of experts in the fields of microbiology and mycology, respectively. This points to the need for greater multi-disciplinarity in future risk identification/assessment studies, which is particularly important as both aquatic bacteria and fungi are known to exert in some cases severe ecological impacts once established and spread in their invasive range (Litchman, 2010), similar to their terrestrial counterparts (Alderman, 1996; Loo, 2008). Regardless, it must be noted that pathogenic and parasitic organisms are normally evaluated separately from other NNS using risk assessment protocols specific to infectious agents (e.g. Peeler et al., 2007; D'hondt et al., 2015; Copp et al., 2016a). In addition, the diminutive size of these taxa could cause their presence to go un-noticed, thereby limiting knowledge of their spread and extent of invasiveness.

Table 4
Risk outcomes (given as number of screened species and corresponding percentage) for the BRA and BRA + CCA for the groups of aquatic organisms for which risk thresholds were directly computed. Species are categorised a priori as either Non-invasive or Invasive (see Supplementary data Table S2). Medium-risk and high-risk outcomes for each group are based on the thresholds given in Table 2, whereas low-risk outcomes are based on a 'default' threshold of 1.

Aquatic organismal group	BRA				BRA + CCA			
	Non-invasive		Invasive		Non-invasive		Invasive	
	n	%	*n*	%	*n*	%	*n*	%
Reptiles								
Low	0	0.0	1	3.3	0	0.0	1	3.3
Medium	15	50.0	2	6.7	15	50.0	2	6.7
High	5	16.7	7	23.3	5	16.7	7	23.3
Amphibians								
Low	1	4.2	0	0.0	12	50.0	0	0.0
Medium	11	45.8	0	0.0	5	20.8	7	29.2
High	5	20.8	7	29.2	17	70.8	7	29.2
Freshwater fishes								
Low	43	16.6	2	0.8	50	19.3	3	1.2
Medium	81	31.3	13	5.0	69	26.6	18	6.9
High	42	16.2	78	30.1	47	18.1	72	27.8
Brackish fishes								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	9	52.9	3	17.6	8	47.1	3	17.6
High	0	0.0	5	29.4	1	5.9	5	29.4
Marine fishes								
Low	36	28.3	1	0.8	33	26.0	1	0.8
Medium	46	36.2	1	0.8	54	42.5	4	3.1
High	27	21.3	16	12.6	22	17.3	13	10.2
Tunicates								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	7	31.8	2	9.1	11	50.0	6	27.3
High	5	22.7	8	36.4	1	4.5	4	18.2
Freshwater invertebrates								
Low	14	9.7	0	0.0	1	0.7	0	0.0
Medium	85	59.0	6	4.2	112	77.8	9	6.3
High	19	13.2	20	13.9	5	3.5	17	11.8
Brackish invertebrates								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	1	9.1	1	9.1	2	18.2	3	27.3
High	1	9.1	8	72.7	0	0.0	6	54.5
Marine invertebrates								
Low	15	9.9	0	0.0	16	10.6	0	0.0
Medium	57	37.7	8	5.3	53	35.1	10	6.6
High	20	13.2	51	33.8	23	15.2	49	32.5
Freshwater plants								
Low	1	5.6%	0	0.0%	4	22.2%	1	5.6%
Medium	5	27.8%	2	11.1%	2	11.1%	4	22.2%
High	0	0.0%	10	55.6%	0	0.0%	7	38.9%
Marine plants								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	7	46.7	4	26.7	6	40.0	5	33.3
High	1	6.7	3	20.0	2	13.3	2	13.3
Marine protists								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	4	22.2	4	22.2	3	16.7	2	11.1
High	7	38.9	3	16.7	8	44.4	5	27.8

4.2. Risk outcomes under current and future climate conditions

The high proportion of positive 'delta' values (i.e. after accounting for climate change predictions: Fig. 3c) is in line with findings that climate change is likely to exacerbate the risk of introduction, establishment, dispersal and impact of several NNS, though some species might respond negatively to changes in climate conditions (Kernan, 2015). With predicted warmer temperatures, reduced lake ice cover, altered flow regimes, increased salinity due to changes in precipitation and saltwater intrusion, and increased environmental disturbances, climate change is likely to favour the spread of NNS along their invasion pathways as these conditions present opportunities for enhanced survival and lower invasion resistance of the invaded habitats (Rahel and Olden, 2008). Further, climate change may result in altered transport and/or introduction mechanisms or reduced effectiveness of control strategies (Hellmann et al., 2008).

Several of the top (i.e. 'very high risk') species under current climate conditions achieved an even higher score under conditions of climate change (Fig. 4). Of these species, most are either primarily warm-water/tropical or have wide thermal tolerances. For example, the red-eared slider *Trachemys scripta scripta* is a very common semi-aquatic turtle native to the south-eastern USA (Florida to south-eastern Virginia: Powel et al., 1991) and in its introduced range occurs in a wide variety of habitats, including slow-flowing rivers, floodplain swamps, marshes, seasonal wetlands and permanent ponds (Scriber et al., 1986). Both goldfish *Carassius auratus* and gibel carp *Carassius gibelio* are known to establish across a wide geographical and climatic range. These species are widespread and locally invasive both in Europe's more northerly parts, such as Finland and Poland (e.g. Grabowska et al., 2010; Puntila et al., 2013), across the Mediterranean region (e.g. Crivelli, 1995; Tarkan et al., 2012), and further afield in Australia (e.g. Beatty et al., 2017) and the Americas (Magalhães and Jacobi, 2013; Halas et al., 2018). The common lionfish *Pterois miles* is another highly invasive species, especially since its invasion of the western Atlantic and Mediterranean Sea, which has been unprecedentedly rapid (Bariche et al., 2017; Schofield, 2010). The channelled applesnail *Pomacea canaliculata* is native to South America and has been introduced as an ornamental species in Europe and the Mediterranean area, but also elsewhere in the world through aquaculture (https://www.cabi.org/isc/datasheet/68490). *Phyllorhiza punctata* is native to the tropical Western Pacific, i.e. from Australia to Japan (Rippingale and Kelly, 1995) and has been reported across the Mediterranean Region (Abed-Navandi and Kikinger, 2007; Boero et al., 2009; Çevik et al., 2011; Gueroun et al., 2014; Deidun et al., 2017). An in-depth, species-specific description of all the 'very high risk' species identified in this study (Fig. 4) is provided in the Supplementary data, Appendix A1.

Overall, the responses to climate-change questions tended to increase scores as well as thresholds for most taxonomic groups in most climatic regions. Species with broad distributions tended to possess broad thermal tolerances, suggesting that those species are likely to be able to expand their ranges, and thus impacts, poleward under future climate conditions. For example, six freshwater fish species not native to Great Britain were predicted to benefit from the forecasted future climate conditions, thus offering the potential to expand their ranges (Britton et al., 2010), which in Great Britain would be northward. Such poleward shifts in marine species distributions were initially deemed likely (VanDerWal et al., 2013), based in part on shifts observed in previous warm periods (Drinkwater, 2006), and they have been predicted for some freshwater fishes, e.g. channel catfish *Ictalurus punctatus* (McCauley and Beitinger, 1992). Indeed, poleward shifts have been documented in a recent meta-analysis of marine species distributions (Chaudhary et al., 2021). As such, tropical species are likely to expand poleward into temperate regions (e.g. Quero, 1998; Scavia et al., 2002), and temperate species to expand poleward into continental regions (Root et al., 2003; Hickling et al., 2006). Whereas, species with more limited thermal tolerances are likely to undergo shifts in their distributions (and thus impacts), which are characterised by range recessions and range expansions in a poleward direction (Roessig et al., 2004; Rahel and Olden, 2008; Eissa and Zaki, 2011; Renaud et al., 2012). Wide thermal tolerances may be enhanced by local adaptation, such as is apparent in the cold-adapted population of eastern mosquitofish *Gambusia holbrooki*, which has established a self-sustaining population in Normandy, France (Beaudouin et al., 2008). This reflects the wide geographical distribution of *Gambusia holbrooki* in its native range, which

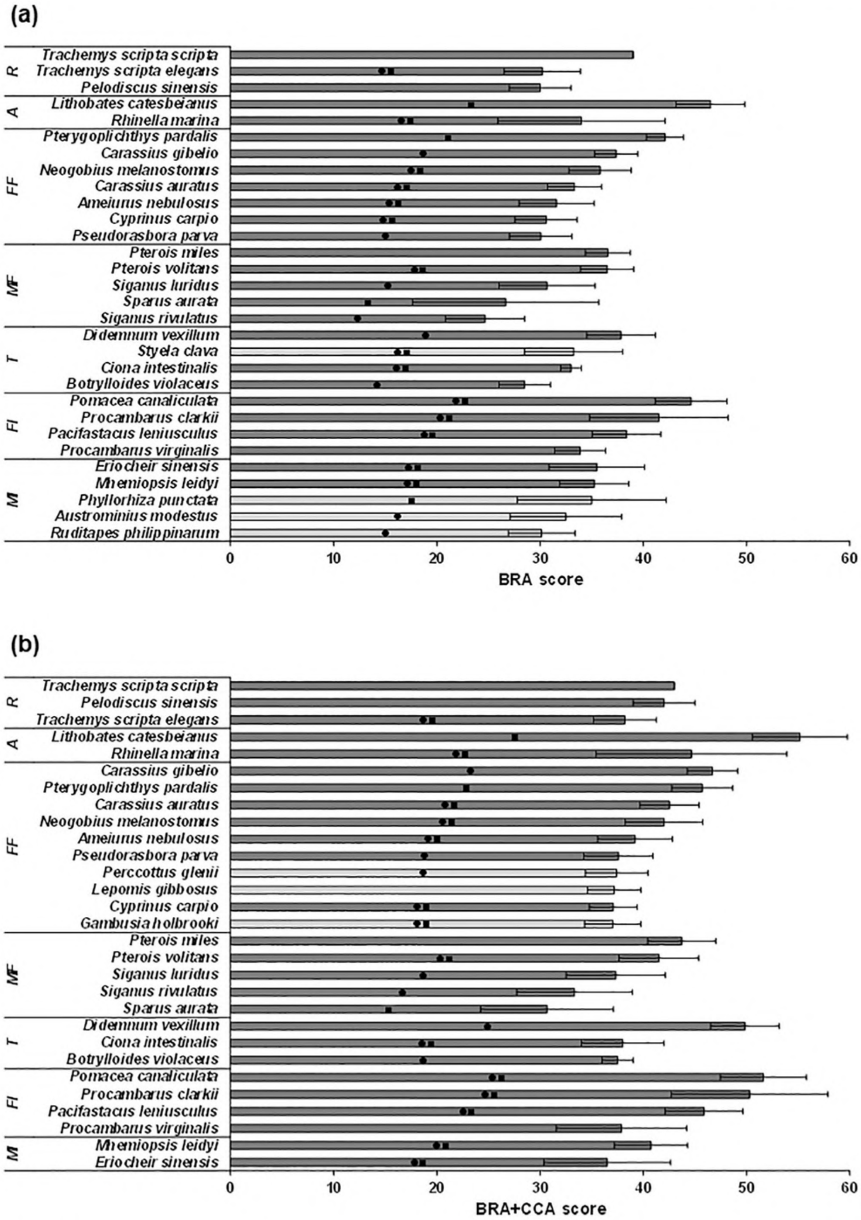

Fig. 4. (a) Very high risk species based on the BRA score (±SE) and screened for a representative number of risk assessment areas; (b) Same for the BRA + CCA. In dark gray, very high risk species for both the BRA and BRA + CCA. Black circle (•): listing in the Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI ISC); black square (▪): listing in the Global Invasive Species Database (GISD). R = Reptiles; A = Amphibians; FF = Freshwater fishes; MF = Marine fishes; T = Tunicates; FI = Freshwater invertebrates; MI = Marine invertebrates. Within each aquatic organismal group, species are ordered according to decreasing score. See Table 2 for very high risk thresholds and number of risk assessment areas criteria.

extends along the Mississippi River basin from the Gulf of Mexico northward to midway up the states of Indiana and Illinois (Aislabie et al., 2019).

Thus, in many cases, increased scores for BRA + CCA for freshwater species are warranted. Further, this phenomenon, which is based on the interaction of climate and physiology, should pertain also to species in different aquatic environments such as brackish and marine systems. For example, red lionfish *Pterois volitans* scores in Florida (USA) increased slightly from the BRA to the BRA + CCA, with modest increases mainly due to a greater potential for the species to survive inshore in the northern Gulf of Mexico during winter, thereby extending annual impacts (Lyons et al., 2020). Conversely, within tropical climate zones, warmer conditions under climate-change scenarios are not likely to

Table 5
BRA and BRA + CCA thresholds from receiver characteristic curve analysis for freshwater fishes relative to three climate classes (after Peel et al., 2007) and for marine fishes and invertebrates relative to two marine ecoregion groupings (modified after Spalding et al., 2007). Abbreviations and notation as in Table 2.

Climate/marine ecoregion	*n*	BRA				BRA + CCA			
		Thr	Mean	LCI	UCI	Thr	Mean	LCI	UCI
Freshwater fishes									
Tropical	63	18.4	0.9153	0.8370	0.9936	19.6	0.8986	0.8088	0.9885
Temperate	200	15.9	0.8685	0.8197	0.9174	16.0	0.8493	0.7974	0.9012
Continental	58	12.9	0.7844	0.6579	0.9110	13	0.7387	0.5992	0.8782
Marine fishes									
Temperate	46	19.5	0.8083	0.6422	0.9745	31.5	0.8208	0.6589	0.9828
Tropical	83	12.5	0.8521	0.6994	1.0000	23.4	0.8016	0.6390	0.9643
Marine invertebrates									
Temperate	97	15.1	0.9236	0.8643	0.9829	15.6	0.8871	0.8153	0.9588
Tropical	63	35.75	0.7386	0.6085	0.8688	23.25	0.7279	0.5895	0.8662

incite large alterations to the spread, abundance, or impacts of tropical species. The same can be said for continental climate zones with regard to cold-water species due to their existing adaptations to that climate types. Whereas range recession of a species' distribution could occur should a future temperature regime exceed the species' thermal tolerances (Scavia et al., 2002).

Despite the general emphasis on range expansion and greater impacts of invasive species due to warmer conditions (Rahel and Olden, 2008; Bradley et al., 2010), climate change is a complex issue for NNS risk assessment. When providing responses to CCA questions and ranking their confidence in those responses, the assessor must consider a great breadth of information, including climate-match model predictions (e.g. Britton et al., 2010), if available for their RA area, as well as emissions scenarios, climate-model outputs, and time frames (Kennedy, 1990). The IPCC (2014) presents a variety of scenarios based on future emissions levels, with the extremes represented by RCP8.5 and RCP2.6. Further, there are numerous climate models that may be used for guidance, though these may differ in profound ways from one another in terms of predicted future temperature and precipitation regimes (e.g. Kirtman et al., 2017). Other anomalies such as Artic warming, which are expected to lead to harsh, cold winters in mid-latitude areas of North America and Asia (Cohen et al., 2014; Kug et al., 2015), or non-analogue climates (Fitzpatrick and Hargrove, 2009) occur. Extreme events can set back or cancel species range expansion (Canning-Clode et al., 2011; Rehage et al., 2016) and thus may influence future risk estimates. Multi-directional range shifts are not only possible, but likely (VanDerWal et al., 2013). The time frame for such predictions is an important variable as well, given that the potential outcomes of range expansion, contraction, or oscillation in size are relative to current NNS ranges. In view of the complexity of climate change interactions with biologically important factors such as physiology, dispersal, demography, species interactions, and evolution, not all changes in climate may result in greater spread or heighten NNS impacts (Urban et al., 2016).

4.3. Management implications

The very low proportion and, in most cases, near or total absence of false negatives across the representative groups of aquatic organisms screened in the present study is an indicator of the accuracy of the risk screenings (cf. Kumschick and Richardson, 2013). The management consequences of this elevated accuracy could be that of a large number

Table 6
Accuracy measures for screenings on freshwater fishes relative to three climate classes and for marine fishes and invertebrates relative to two marine ecoregion groupings (see Table 5). Abbreviations and notation as in Table 3.

Climate/marine ecoregion	BRA			BRA + CCA		
	A_i	A_n	A_0	A_i	A_n	A_0
Freshwater fishes						
Tropical	84.6	94.6	90.5	84.6	89.2	87.3
Temperate	82.5	75.0	78.0	87.5	64.2	73.5
Continental	81.1	66.7	75.9	83.8	57.1	74.1
Marine fishes						
Temperate	80.0	77.8	78.3	80.0	83.3	82.6
Tropical	91.7	81.7	83.1	66.7	88.7	85.5
Marine invertebrates						
Temperate	88.5	90.1	89.7	84.6	85.9	85.6
Tropical	38.6	100.0	57.1	65.9	78.9	69.8

Table 7
Risk outcomes (given as number of screened species and corresponding percentage) for the BRA and BRA + CCA for freshwater fishes relative to three climate classes and for marine fishes and invertebrates relative to two marine ecoregion groupings (see Table 5). See Table 4 for details.

Climate/marine ecoregion	BRA				BRA + CCA			
	Non-invasive		Invasive		Non-invasive		Invasive	
	n	%	*n*	%	*n*	%	*n*	%
Freshwater fishes								
Tropical								
Low	10	15.9	1	1.6	8	12.7	1	1.6
Medium	25	39.7	3	4.8	25	39.7	3	4.8
High	2	3.2	22	34.9	4	6.3	22	34.9
Temperate								
Low	31	15.5	0	0.0	38	19.0	1	0.5
Medium	59	29.5	14	7.0	39	19.5	9	4.5
High	30	15.0	66	33.0	43	21.5	70	35.0
Continental								
Low	8	13.8	0	0.0	8	13.8	0	0.0
Medium	6	10.3	7	12.1	4	6.9	6	10.3
High	7	12.1	30	51.7	9	15.5	31	53.4
Marine fishes								
Temperate								
Low	1	2.2	0	0.0	1	2.2	0	0.0
Medium	27	58.7	2	4.3	29	63.0	2	4.3
High	8	17.4	8	17.4	6	13.0	8	17.4
Tropical								
Low	33	39.8	1	1.2	31	37.3	1	1.2
Medium	25	30.1	0	0.0	32	38.6	3	3.6
High	13	15.7	11	13.3	8	9.6	8	9.6
Marine invertebrates								
Temperate								
Low	15	15.5	0	0.0	12	12.4	0	0.0
Medium	49	50.5	3	3.1	49	50.5	4	4.1
High	7	7.2	23	23.7	10	10.3	22	22.7
Tropical								
Low	0	0.0	0	0.0	4	6.3	1	1.6
Medium	19	30.2	27	42.9	11	17.5	14	22.2
High	0	0.0	17	27.0	4	6.3	29	46.0

of species ultimately warranting comprehensive (full) RA. In this study, accuracy was measured explicitly and represented the 'pragmatic' approach, given that a full (comprehensive) RA, which might follow for species identified as potentially posing a high risk of invasiveness, would normally involve a major economic commitment. This is contrary to the 'idealistic' approach, which would involve assessing both medium- and high-risk species, given the even higher economic commitment of accounting for both. An even more pragmatic approach could be therefore to base management decisions (or species' rankings) on the risk screening outcomes until such time that a full RA can be undertaken. This approach has been employed by the UK's Alien Species Group in its 'impact' ranking of aquatic NNS with regard to waterbody classification under the EU Water Framework Directive (https://ec.europa.eu/environment/water/water-framework/index_en.html). Within this context, aquatic NNS are listed provisionally as being of low, medium or high impact (UK-TAG ASG, 2021) pending the outcome of a full, and in some cases rapid, RA commissioned by the Great Britain Non-native Species Secretariat (www.nonnativespecies.org). The categorisation of the species is then subsequently confirmed or changed according to the outcome of the full or rapid RA.

As shown in the present study, invasive and non-invasive species could be distinguished accurately across aquatic organisms to a greater degree than would be expected by chance alone. Calibrated thresholds could be computed for several taxonomic groups, and for freshwater and marine fishes and for marine invertebrates also based on climate/ecoregion. In RA areas for which no calibration is possible, e.g. due to a statistically insufficient number of screenings and/or to the requirement of screening only one target species (or small group thereof), these generalised thresholds (i.e. at the organism group or climate class level) can be reliably used in future risk screening applications for distinguishing between species that pose a high risk of being invasive from those posing a low-to-medium risk. Use of these thresholds may be therefore of particular relevance in cases of individual species being risk screened (e.g. Castellanos-Galindo et al., 2018; Zięba et al., 2020), including 'rapid risk assessment' studies, or for RA areas where the number of NNS is too limited for a valid calibration to be undertaken (e.g. Filiz et al., 2017b; Paganelli et al., 2018; Semenchenko et al., 2018; Dodd et al., 2019; Lyons et al., 2020).

As is common in NNS risk analysis (e.g. Caley et al., 2006; Barry et al., 2008), the available scientific information (both peer-reviewed and gray literature) about the species being screened was reflected in the confidence rankings assessors attributed to their responses. As such, given the robust confidence levels, the present study provides a means for existing risk rankings to be adjusted and a stronger evidence base to identify: (i) which species require an immediate 'rapid' management action (e.g. eradication, control) to avoid or mitigate adverse impacts; (ii) which to subject to a full RA; and (iii) which to restrict or ban with regard to importation and/or sale as ornamental or aquarium/fishery enhancement. To this end, biological monitoring programmes may explicitly search for high-scoring species because the sooner these species are found in new areas the more likely are eradication programmes to be successful. Such monitoring could be supported by developing techniques such as DNA metabarcoding (Brown et al., 2016) and eDNA surveys (Holman et al., 2019). Risk identification therefore plays an important role in the provision of advice to policy makers, for the development of appropriate legislation, and associated regulation and management pertaining to NNS. In this perspective, the present study has also provided a means of fine-tuning NNS risk analysis procedures in countries that encompass more than one climatic class by the computation of generalised thresholds. In conclusion, the present study provides a comprehensive baseline to help identify (through risk screening using AS-ISK) for management priority high risk species across a range of taxonomic groups and geographical/climatic regions, even where existing information on such species invasiveness/impact is limited.

CRediT authorship contribution statement

LV and GHC designed the concept, co-wrote and edited the manuscript; LV analysed the data, and all other authors contributed data to and participated in the composition and editing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147868.

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