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Risk assessment of non-native fishes in the Balkans Region using FISK, the invasiveness screening tool for non-native freshwater fishes

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Abstract

A high level of freshwater fish endemism in the Balkans Region emphasizes the need for non-native species risk assessments to inform management and control measures, with pre-screening tools, such as the Fish Invasiveness Screening Kit (FISK) providing a useful first step. Applied to 43 non-native and translocated freshwater fishes in four Balkan countries, FISK reliably discriminated between invasive and non-invasive species, with a calibration threshold value of 9.5 distinguishing between species of medium and high risk *sensu lato* of becoming invasive. Twelve of the 43 species were assessed by scientists from two or more Balkan countries, and the remaining 31 species by a single assessor. Using the 9.5 threshold, three species were classed as low risk, 10 as medium risk, and 30 as high risk, with the latter category comprised of 26 moderately high risk, three high risk, and one very high risk species. Confidence levels in the assessments were relatively constant for all species, indicating concordance amongst assessors.

Keywords: Non-native fish, Balkans inland waters, identification of invasiveness, Fish Invasiveness Screening Kit.

Introduction

The Balkans possesses a highly unique native freshwater fish fauna, including several endemic genera, e.g. *Delminichthys*, *Economidichthys* and *Pelagius* (*sensu* Kottelat, 1997), *Aulopyge*, *Phoxinellus* and *Romanichthys*, as well as many endemic species of otherwise widespread genera (e.g. *Barbus*, *Cobitis*, *Eudontomyzon*, *Gobio*, *Knipowitschia*, *Rutilus* and *Zingel*) (Bănărescu, 1990). In Bulgaria, 15% (26 of 173 species) of fishes are non-native (Uzunova & Zlatanova, 2007) with similar proportions of non-natives in the freshwater fish faunas of Serbia (23%, 22 of 96 species; Lenhardt *et al.*, 2011), Montenegro (19.5%, 15 of 77 species; Marić & Milošević, 2011) and the Former Yugoslav Republic of Macedonia (FYROM) (16.7%, 14 of 84 species; Kostov *et al.*, 2010, 2011ab; Ristovska *et al.*, 2011; Kostov & Ristovska, 2012). In the specific case of the River Dan-

ube, 11.4% (5 of 44) of fishes are non-native (Polačik *et al.*, 2008), although in the Serbian section, which partially overlaps with the Croatian and Romanian sections, 51.5% (17 of 31) of fishes are introduced (Simonović *et al.*, 2010a).

Risk (or hazard) identification is an important first step in evaluating the risk of non-native species to native species and ecosystem biodiversity (Kolar & Lodge, 2002; Copp *et al.*, 2005a), especially for regions characterised by a high level of endemism such as the Mediterranean and the Balkans. Perhaps the most popular tool for the pre-screening of non-native freshwater fishes is FISK, the Fish Invasiveness Screening Kit (Copp *et al.*, 2005a, 2009), which has been applied in a number of risk assessment areas world-wide (Mastitsky *et al.*, 2010; Onikura *et al.*, 2011), encompassing sub-tropical and warm temperate regions such as Brazil and Iberia (Troca & Vieira, 2012; Almeida *et al.*, 2013). Similar to

Iberia, the Balkans Region (henceforth, 'the Balkans') represents a remarkable biodiversity hotspot within the wider Mediterranean Region (Blondel & Aronson, 1999; Médail & Quézel, 1999), which comprises the north-Mediterranean, mid-European and Ponto-Caspian sub-regions (Bănărescu, 1990).

The main sources of non-native fish introductions to the Balkans have been ascribed to recreational fisheries (i.e. stocking: Uzunova & Zlatanova, 2007), aquaculture (i.e. escapees: Simonović *et al.*, 2010a; Lenhardt *et al.* 2011) and ballast water transfers (Jude *et al.*, 1992; Skora & Stolarski, 1993; Simonović *et al.*, 2001; Grigorovich *et al.*, 2003). European inland waterways, encompassing 28 000 navigable km and 37 countries, have facilitated the natural and assisted dispersal of aquatic non-native species, some of which have become highly invasive (Copp *et al.*, 2005a). The River Danube, which drains a large part of the Balkans, is a component of the 3500 km Southern Invasion Corridor, one of four main invasion pathways in Europe that links the Black and Northern Seas via the River Danube, the Rhine–Main canal and the River Rhine (Bij de Vaate *et al.*, 2002), comprising more than 125 harbours and 67 locks (Panov *et al.*, 2008).

The state of inland waterways described above renders the Balkans particularly vulnerable to the potential impacts of invasive non-native species (Cirruna *et al.*, 2004), thus requiring adequate assessment of potential risks as well as implementation of appropriate management and control measures in order to ensure compliance with the European Union's Water Framework Directive (WFD, 2000). Although non-native species are not identified in the main text of the WFD, they are mentioned in the Directive's annexes as an important environmental pressure and as such require appropriate risk analysis (i.e. identification, assessment, management and communication). The zoogeographic uniqueness of the Balkans, along with the strong pressure it sustains from the introduction of non-native fishes, deserves appropriate attention, but so far this has been lacking despite an increased awareness of the risks and potential adverse effects posed by non-native species introductions on the native fishes. To address this problem, the aim of the present study was to expand an initial, preliminary trial application of FISK to non-native fishes in Serbia (Simonović, 2009) and apply this risk identification tool to non-native fish species in drainage basins of the Black, Aegean and Adriatic seas that surround the Balkan Peninsula, encompassing four Balkan countries (Bulgaria, FYROM, Montenegro, Serbia). The specific objectives of the present study were to: 1) undertake the calibration of FISK for the Balkans, i.e. determine the threshold value for distinguishing between fishes of medium and high risk of being (or becoming) invasive; 2) evaluate the confidence levels (i.e. the certainty) of the assessors in their species assessments; and 3) interpret the FISK scores relative to independent cat-

egorizations of the species with regard to their invasiveness and conservation (i.e. threatened) status.

Materials and Methods

FISK v2 (Lawson *et al.*, 2013) assessments were carried out on 43 fish species (Table 1), which were selected based on the criterion of introduction applied to check lists and other kind of publications related to fish fauna of inland waters occurring so far in Bulgaria (Vassilev & Pehlivanov, 2005; Uzunova & Zlatanova, 2007; Polačik *et al.* 2008; Economidis *et al.*, 2007), FYROM (Kostov *et al.*, 1998, 2010, 2011 a, b; Kostov, 2007, 2008a, b; Kostov & Van der Knaap, 2009; Ristovska *et al.*, 2011; Kostov & Ristovska, 2012), Montenegro (Marić & Milošević, 2011) and Serbia (Simonović & Nikolić, 1997; Simonović, 2001; Simonović *et al.*, 2010b; Lenhardt *et al.*, 2011). Fish species introduced or translocated were regarded as non-native following the definitions and terminology given in Copp *et al.* (2005b), e.g. Lake Ohrid trout *Salmo letnica*, Lake Skadar rudd *Scardinius knezevici* and Lake Ohrid bleak *Alburnus scoranza* from Lake Ohrid (FYROM) to Lake Vlasina Reservoir (Simonović & Nikolić, 1997; Simonović, 2001; Simić *et al.*, 2012); the translocation of Macedonian trout *Salmo cf. macedonicus* from the River Struma drainage basin (Bulgaria and Serbia) to the River Nišava catchment (Black Sea Basin, Serbia) (Marić *et al.*, 2006); the introduction of grayling *Thymallus thymallus* from the Slovenian part of the River Danube catchment to the River Morača (Adriatic Sea catchment, Montenegro) (Marić & Milošević, 2011); and the introduction of Eurasian perch *Perca fluviatilis* from the River Danube catchment across the entire Balkans into Lake Skadar, Montenegro (Knežević & Marić, 1979).

FISK evaluations were carried out independently by assessors from each country (initials: AA for Bulgaria, VK for FYROM, DM for Montenegro, PS for Serbia) on a different number of species, resulting from one to four replicate scores for each species (Table 1). Receiver operating characteristic (ROC) analysis (Bewick *et al.*, 2004) was then used to assess the predictive ability of FISK to discriminate between invasive and non-invasive species. To this end, species were classified *a priori* as either invasive or non-invasive based on information available from the Invasive Species Specialist Group database (<http://www.issg.org>) and from FishBase (www.fishbase.org).

Statistically, a ROC curve is a graph of sensitivity vs 1 – specificity (or, alternatively, sensitivity vs specificity), where in the present context sensitivity and specificity will be the proportion of invasive and non-invasive fish species, respectively, that are correctly identified by the FISK tool as such. A measure of the accuracy of the calibration analysis is the area under the ROC curve (AUC). If the AUC is equal to 1.0 (i.e. the ROC 'curve' consists

of two straight lines, one vertical from 0,0 to 0,1 and the other horizontal from 0,1 to 1,1), then the test is 100% accurate because both sensitivity and specificity are 1.0 and there are neither false positives (i.e. non-invasive species categorized as invasive) nor false negatives (i.e. invasive species categorized as non-invasive). Conversely, if the AUC is equal to 0.5 (i.e. the ROC 'curve' is a diagonal line from 0,0 to 1,1), then the test is 0% accurate as it cannot discriminate between true positives (i.e. actual invasive species) and true negatives (i.e. actual non-invasive species). Typically, the AUC will range between 0.5 and 1.0, and the closer the AUC to 1.0 the better the ability of FISK to differentiate between invasive and non-invasive species.

The best FISK threshold, i.e. the cut-off value that maximizes the probability of correct classification of a species as invasive whilst minimizing that of incorrect classification as non-invasive, was determined using both Youden's *J* statistic (Youden, 1950), and the point closest to the top-left part of the plot with perfect sensitivity or specificity. Bootstrapped confidence intervals were computed for the AUC (DeLong *et al.*, 1988) and a smoothed mean ROC curve was also generated along with bootstrapped confidence intervals of specificities along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). ROC analyses were done with package pROC for R (R Development Core Team, 2008) using the *n* = 2000 default bootstrap replicates.

As each response in FISK for a given species is allocated a certainty score (1 = very uncertain; 2 = mostly uncertain; 3 = mostly certain; 4 = very certain), a 'certainty factor' (CF) was computed as:

$$\sum(CQ_i)/(4 \times 49) \quad (i = 1, \dots, 49),$$

where CQ_i is the certainty for question *i*, 4 is the maximum achievable value for certainty (i.e. 'very certain') and 49 is the total number of questions comprising the FISK tool. The CF therefore ranges from a minimum of 0.25 (i.e. all 49 questions with certainty score equal to 1) to a maximum of 1 (i.e. all 49 questions with certainty score equal to 4).

Results

Of the 43 species in total, twelve were evaluated by assessors from two or more Balkan countries, and the remaining 31 by a single assessor from one Balkan country only, yielding a range of FISK scores (Table 1). The calibration threshold of 9.5 of FISK risk outcomes for the Balkans was set after that same best threshold value that both Youden's and closest point statistics provided. The AUC for the ROC curve equal to 0.67 (0.50–0.83, 95% C.I.) (Fig. 1) indicated that FISK was able to discriminate reliably between invasive and non-invasive species. Accordingly, the 9.5 threshold was used to dis-

tinguish between 'medium risk' species (i.e. species with FISK scores within the interval [1, 9.5]) and 'high risk *sensu lato*' species (i.e. species with FISK scores within the interval [9.5, 57]), with the latter further categorized as per Britton *et al.* (2010), into 'moderately high risk' (interval [9.5, 25]), 'high risk' (interval [25, 30]), and 'very high risk' (interval [30, 57]), and with 'low risk' species having a FISK score within the interval [–15, 1]. Based on the above threshold, three (7.0%) species were categorized as low risk, 10 (23.3%) as medium risk, and the remaining 30 as high risk *sensu lato*, of which 26 (86.7%; 60.5% of total) were categorized as moderately high risk, three (10.0%; 7.0%) as high risk, and one (3.3%; 2.3%) as very high risk. The highest scoring (i.e. very high risk) species was gibel carp *Carassius gibelio*, followed by the three high risk species, brown bullhead *Ameiurus nebulosus*, Amazon sailfin catfish *Pterygoplichthys pardalis* and western mosquitofish *Gambusia affinis*; whereas, the lowest scoring (i.e. low risk) species were the European whitefish *Coregonus lavaretus*, the Mississippi paddlefish *Polyodon spatula* and the Arctic char *Salvelinus alpinus* (Table 1). Finally, the mean score for the 'Non-invasive/Not evaluated' species group (19.5 ± 5.7 SE) was higher than all other *a priori* categories for invasive species (Fig. 2).

Mean certainty in response for all species was 3.5 ± 0.2 SE and mean certainty factor (CF) was 0.87 ± 0.04 SE, ranging from a minimum of 2.6 (CF: 0.65) for Lake Skadar rudd *Scardinius knezevici* to a maximum of 4.0 (CF: 0.99) for European perch *Perca*

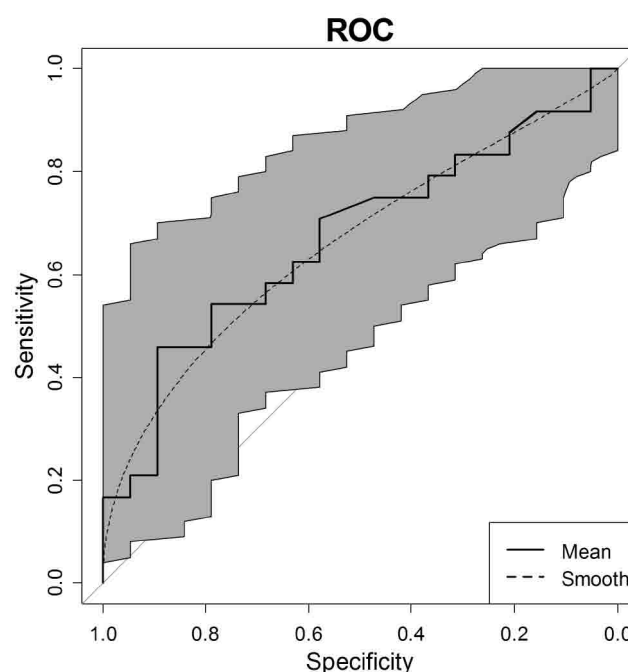


Fig. 1: Receiver operating characteristic (ROC) curve for 43 fish species assessed with the FISK v2 tool for four countries in the Balkans, with smoothing line and confidence intervals of specificities. See also Table 1.

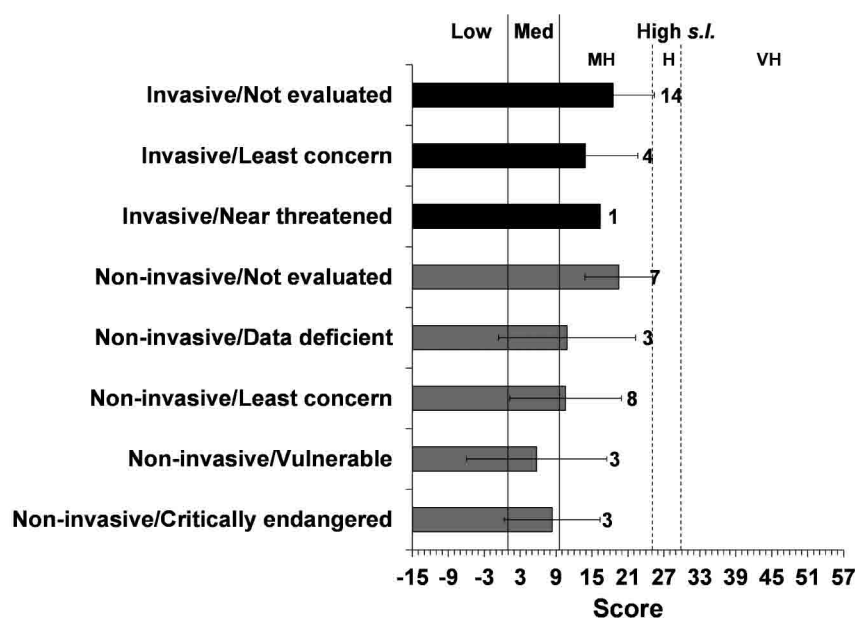


Fig. 2: Mean scores (\pm SE and n) for 43 fish species assessed by FISK for four countries in the Balkans and categorised according to their invasiveness and protection status (cf. Table 1). Thresholds are: < 1 ('low risk') and ≥ 9.5 ('high risk *sensu lato*'), with 'medium risk' species in between. Risk categories are: L = 'low risk': $[-15, 1[$; M = 'medium risk': $[1, 9.5[$; MH = 'moderately high risk': $[9.5, 25[$; H = 'high risk': $[25, 30[$; VH = 'very high risk': $[30, 57]$.

fluviatilis and the Atlantic strain of brown trout *Salmo trutta trutta*.

The threshold value that distinguishes between medium and high risk species achieved for the Balkans is approximately half that reported for other countries where FISK calibrations have been undertaken, i.e. the U.K.

Discussion

Table 1. Fish species assessed with FISK v2 for four countries of the Balkans. For each species, a priori invasiveness (as per <http://www.issg.org> and www.fishbase.org) and protection status (as per www.iucnredlist.org), the assessment country, and summary statistics (SE = standard error) for corresponding FISK score, (risk) outcome and certainty factor (CF: see text) are reported. Outcome is based on a threshold of 9.5 between medium risk and high *sensu lato* risk species and classified as: Medium (M) = $[1, 9.5[$; Moderately high (MH) = $[9.5, 25[$; High (H) = $[25, 30[$; Very high (VH) = $[30, 57]$. bg = Bulgaria; mk = FYROM; me = Montenegro; rs = Serbia

Species name	Common name	Invasiveness/Protection status	Country	Score				Outcome	CF			
				Mean	Min	Max	SE		Mean	Min	Max	SE
<i>Acipenser gueldenstaedtii</i>	Danube sturgeon	Non-invasive/Critically endangered	mk	16.0	–	–	–	MH	0.75	–	–	–
<i>Acipenser ruthenus</i>	sterlet	Invasive/Not evaluated	mk	18.0	–	–	–	MH	0.75	–	–	–
<i>Alburnus scoranza</i>	Lake Ohrid bleak	Non-invasive/Vulnerable	rs	2.5	–	–	–	M	0.89	–	–	–
<i>Ameiurus melas</i>	black bullhead	Invasive/Not evaluated	rs	24.5	–	–	–	MH	0.92	–	–	–
<i>Ameiurus nebulosus</i>	brown bullhead	Non-invasive/Least concern	mk ,me, rs	29.7	29.0	31.0	0.7	H	0.90	0.83	0.95	0.04
<i>Babka gymnotrachelus</i>	racer goby	Invasive/Not evaluated	rs	24.0	–	–	–	MH	0.96	–	–	–
<i>Carassius gibelio</i>	gibel carp	Invasive/Not evaluated	mk, me, rs	30.5	26.5	34.0	2.2	VH	0.94	0.89	1.00	0.03
<i>Coregonus lavaretus</i>	European whitefish	Non-invasive/Vulnerable	bg	– 4.0	–	–	–	L	0.90	–	–	–
<i>Coregonus peled</i>	peled	Invasive/Least concern	rs	3.0	–	–	–	M	0.76	–	–	–
<i>Ctenopharyngodon idella</i>	grass carp	Non-invasive/Not evaluated	bg, mk, me, rs	17.5	15.0	21.0	1.5	MH	0.89	0.82	0.93	0.03
<i>Gambusia affinis</i>	western mosquitofish	Invasive/Not evaluated	mk	27.0	–	–	–	H	0.84	–	–	–
<i>Gambusia holbrooki</i>	eastern mosquitofish	Invasive/Not evaluated	bg, me	19.0	12.0	26.0	5.7	MH	0.89	0.89	0.90	0.01
<i>Gymnocephalus cernua</i>	ruffe	Invasive/Least concern	mk	18.5	–	–	–	MH	0.85	–	–	–
<i>Hypophthalmichthys molitrix</i>	silver carp	Invasive/Near threatened	bg, mk, me, rs	16.4	12.0	20.5	2.1	MH	0.88	0.81	0.93	0.03
<i>Hypophthalmichthys nobilis</i>	bighead carp	Invasive/Not evaluated	bg, mk, me, rs	13.9	7.0	20.5	3.3	MH	0.88	0.81	0.93	0.03

(continued)

Table 1 (continued)

Species name	Common name	Invasiveness/Protection status	Country	Score				Outcome	CF			
				Mean	Min	Max	SE		Mean	Min	Max	SE
<i>Ictalurus punctatus</i>	channel catfish	Invasive/Not evaluated	bg	10.0	–	–	–	MH	0.79	–	–	–
<i>Lepomis gibbosus</i>	pumpkinseed	Non-invasive/Not evaluated	bg, mk, rs	21.3	18.0	24.0	1.8	MH	0.87	0.86	0.88	0.01
<i>Megalobrama terminalis</i>	black Amur bream	Non-invasive/Not evaluated	me	23.5	–	–	–	MH	0.81	–	–	–
<i>Micropterus salmoides</i>	largemouth (black) bass	Invasive/Not evaluated	rs	18.0	–	–	–	MH	0.87	–	–	–
<i>Mugil soiyu</i>	so-iuy mullet	Non-invasive/Not evaluated	bg	12.0	–	–	–	MH	0.85	–	–	–
<i>Mylopharyngodon piceus</i>	black carp	Invasive/Least concern	bg	11.0	–	–	–	MH	0.84	–	–	–
<i>Neogobius fluviatilis</i>	monkey moby	Non-invasive/Not evaluated	rs	18.0	–	–	–	MH	0.91	–	–	–
<i>Neogobius melanostomus</i>	round goby	Non-invasive/Not evaluated	rs	15.0	–	–	–	MH	0.94	–	–	–
<i>Oncorhynchus mykiss</i>	rainbow trout	Invasive/Not evaluated	bg, mk, me, rs	15.3	12.0	18.0	1.6	MH	0.93	0.89	0.99	0.02
<i>Oxynoemacheilus bureschi</i>	Bureschi loach	Non-invasive/Least concern	mk	8.0	–	–	–	M	0.83	–	–	–
<i>Pachychilon macedonicum</i>	Macedonian roach	Non-invasive/Data deficient	rs	3.5	–	–	–	M	0.78	–	–	–
<i>Perca fluviatilis</i>	Eurasian perch	Invasive/Least concern	me	23.0	–	–	–	MH	0.99	–	–	–
<i>Perccottus glenii</i>	Amur (Chinese) sleeper	Non-invasive/Vulnerable	bg, rs	18.8	18.5	19.0	0.2	MH	0.80	0.73	0.88	0.07
<i>Polyodon spathula</i>	Mississippi paddlefish	Non-invasive/Critically endangered	bg, rs	0.0	–3.0	3.0	2.4	L	0.86	0.83	0.88	0.03
<i>Ponticola kessleri</i>	bighead goby	Non-invasive/Least concern	rs	17.0	–	–	–	MH	0.91	–	–	–
<i>Proterorhinus semilunaris</i>	western tubenose goby	Non-invasive/Least concern	rs	13.0	–	–	–	MH	0.89	–	–	–
<i>Pseudorasbora parva</i>	topmouth gudgeon	Invasive/Not evaluated	bg, mk, me, rs	18.3	12.0	26.0	3.7	MH	0.85	0.81	0.89	0.02
<i>Pterygoplichthys pardalis</i>	Amazon sailfin catfish	Non-invasive/Not evaluated	rs	29.0	–	–	–	H	0.81	–	–	–
<i>Rutilus sp.</i>	Adriatic roach	Non-invasive/Least concern	rs	7.0	–	–	–	M	0.73	–	–	–
<i>Salmo letnica</i>	Ohrid trout	Non-invasive/Data deficient	rs	5.0	–	–	–	M	0.88	–	–	–
<i>Salmo macedonicus</i>	Macedonian trout	Non-invasive/Data deficient	rs	24.0	–	–	–	MH	0.90	–	–	–
<i>Salmo trutta trutta</i>	brown trout	Invasive/Not evaluated	me	22.0	–	–	–	MH	0.99	–	–	–
<i>Salvelinus alpinus</i>	Arctic char	Non-invasive/Least concern	rs	0.0	–	–	–	L	0.90	–	–	–
<i>Salvelinus fontinalis</i>	brook trout	Invasive/Not evaluated	bg, mk, rs	4.3	0.0	12.0	3.8	M	0.89	0.87	0.90	0.01
<i>Sander lucioperca</i>	pikeperch	Invasive/Not evaluated	mk	14.5	–	–	–	MH	0.91	–	–	–
<i>Scardinius knezevici</i>	Lake Skadar rudd	Non-invasive/Critically endangered	rs	9.0	–	–	–	M	0.65	–	–	–
<i>Syngnathus abaster</i>	black-striped pipefish	Non-invasive/Least concern	rs	5.0	–	–	–	M	0.68	–	–	–
<i>Thymallus thymallus</i>	grayling	Non-invasive/Least concern	me	5.0	–	–	–	M	0.94	–	–	–

(Copp *et al.*, 2009), Japan (Onikura *et al.*, 2011), Australia (Vilizzi & Copp, 2013), and most notably the western extent of the Mediterranean Region, Iberia (Almeida *et al.*, 2013), which has a similar high level of endemism to the Balkans. This lower threshold for the Balkans region is probably due to the elevated number of translocations within countries of this region, in particular to closed, often artificial waters (e.g. newly-constructed reservoirs like Lake Vlasina), which limit their further dispersal. As such, many species were evaluated for invasiveness even though they are not normally classed as particularly invasive (i.e. elevated FISK scores) across a broader geographical scale.

The current assessment of inland fish species invasiveness in the Balkans revealed a very high risk outcome for gibel carp, herewith denoting the complex of mtDNA molecular lineages assigned to ginbuna *Caras-*

sius langsdorfii, goldfish *Carassius auratus* and gibel carp, which were recently recorded using the *cytochrome b* gene as a molecular marker, whose reliable identification in field or laboratory using only morphological characters is not possible (Kalous *et al.*, 2013). Despite the erstwhile unintentional introduction of gibel carp into the Balkans (Plančić, 1967) and subsequent dispersal after 1975 (Maletin & Budakov, 1982), the species continues to spread very rapidly in all four of the Balkan countries and has been blamed for declines of native crucian carp *Carassius carassius*, common carp *Cyprinus carpio* and tench *Tinca tinca* (Maletin *et al.*, 1997). The main factors responsible for gibel carp invasiveness are its ability to reproduce gynogenetically (e.g. Peñáz & Dulmaa, 1987), its adaptability to various, including harsh, environmental conditions (e.g. Vetemaa *et al.*, 2005; Tarkan *et al.*, 2012), and its strong competitiveness for feeding re-

sources (e.g. Demeny *et al.*, 2009). Gynogenesis appears to enhance significantly the invasiveness of gibel carp in the Balkans, where the species makes use of males from closely-related carp species to activate its eggs. This is particularly acute in the large, Mediterranean-zone lakes of Montenegro (e.g. Lake Skadar) and FYROM (e.g. Lake Ohrid), where the local high levels of endemism are at risk from the adverse effects of the gibel carp (see also Leonardos *et al.*, 2008). Additionally, the naturalization process in gibel carp appears to be complete in the Balkans, as males have begun to appear in populations that were hitherto composed exclusively of female clones (Simonović & Jovanović, 1991).

The second most invasive species in the region, brown bullhead, was also introduced during 1885 for rearing in aquaculture (Holčik, 1991) and reached the greatest abundance in the 1950s, declining thereafter. The spread of brown bullhead has been through human action (unintentional stocking) as well as natural dispersal (via inland waterways), with establishment facilitated by its life-history strategy (e.g. high fecundity and parental care), resistance to harsh conditions, adaptability to various environments (rivers, lakes, ponds, reservoirs) and great dietary plasticity (Pujin & Sotirov, 1966). Although small-bodied species, mosquitofishes share similar biological traits with brown bullhead and the climate in the species' native range is similar to that of FYROM, where they outcompete native fishes and exert impacts on both aquaculture and natural ecosystems (Kostov, 2008a).

The higher mean score achieved by the 'Non-invasive/Not evaluated' species group, relative to all other *a priori* categories of invasive species, suggests that the introduction of any non-native freshwater fish species poses a risk to native species and ecosystems, especially when the climatic and environmental conditions in the recipient area match those in the donor area. This result also suggests that *a priori* invasiveness assigned for particular species in other recipient areas, especially when it is arbitrary (i.e. not supported by published evidence), should be avoided. This is because a number of alien species have been established in certain inland waters of the Balkans for a long time (e.g. black bullhead, Cvijanović *et al.*, 2005; grass carp, Janković, 1998; pumpkinseed, Pehlivanov & Leonardakis, 2009; monkey goby and round goby, Simonović *et al.*, 2001) and some are invasive in some water bodies and not in others. This appears to be in accordance with their medium-high risk of being invasive (Table 1).

The high risk of invasiveness revealed by the Amazon sailfin catfish *Pterygoplichthys pardalis* was a result of its previous history of introductions, impacts posed to the recipient ecosystems, lack of natural predators, environmental versatility and reproductive features. However, the low CF value achieved for this species' assessment comes mainly from a lack of information for answering questions related to this tropical species' reproductive traits, toler-

ances to environmental factors and its ecosystem impacts in a temperate river such as the Danube (Simonović *et al.*, 2010b). Regardless, the awareness that such a high potential risk of being invasive in the Balkans gives a good reason for future environmental surveillance.

The FISK score achieved for certain species, which occur in more than one of the Balkan countries and were evaluated by separate assessors (e.g. brown bullhead, grass carp, pumpkinseed, rainbow trout and Amur sleeper *Perccottus glenii*), were nonetheless rather similar regardless of their *a priori* assigned invasiveness risk (Table 1). This may be attributed to their similar introduction history and degree of establishment in the Balkans. However, for certain species such as gibel carp, both western and eastern mosquitofishes, Mississippi paddlefish and topmouth gudgeon, FISK scores were more variable at the country level, and the variability of risk assessment was very high, ranging from medium to high. The uniformity and low variability in CF values in particular countries indicates a similar level of familiarity amongst assessors concerning these species. Finally, no clear relationship was found between the interval of the time since the introduction of a species and the level of certainty amongst experts regarding their traits in the recipient ecosystems.

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References

- Almeida, D., Ribeiro, F., Leunda, P.A., Vilizzi, L., Copp, G.H., 2013. Effectiveness of an invasiveness screening tool for non-native freshwater fishes (FISK) to perform risk identification assessments in the Iberian Peninsula. *Risk Analysis*, (<http://dx.doi.org/10.1111/risa.12050>)
- Bănărescu, P., 1990. *Zoogeography of Fresh Waters*. Vol. 1. *General Distribution and Dispersal of Freshwater Animals*. AULA-Verlag, Wiesbaden, 511 pp.
- Bewick, V., Cheek, L., Ball, J., 2004. Statistics review 13: Receiver operating characteristic curves. *Critical Care*, 8, 508-512.
- Bij de Vaate, A., Jazdzewski, K., Ketelaars, H.A.M., Gollasch, S., Van der Velde, G., 2002. Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, 1159-1174.
- Blondel, J., Aronson, J., 1999. *Biology and Wildlife of the Mediterranean Region*. Oxford University Press, Oxford, 352 pp.
- Britton, J.R., Cucherousset, J., Davies, G.D., Godard, M., Copp, G.H., 2010. Non-native fishes and climate change: predicting species responses to warming temperatures in a temperate region. *Freshwater Biology*, 55, 1130-1141.
- Cirruna, K.A., Meyerson, L.A., Gutierrez, A., 2004. *The ecological and socio-economic impacts of invasive alien species in*

- inland water ecosystems. Report to the Conservation on Biological Diversity on behalf of the Global Invasive Species Programme. Washington, D.C., 34 pp.
- Copp, G.H., Garthwaite, R., Gozlan, R.E., 2005a. *Risk identification and assessment of non-native freshwater fishes: concepts and perspectives on protocols for the UK*. Cefas Science Technical Report. Lowestoft, UK, 36 pp. Available at: <http://www.cefas.co.uk/publications/techrep/tech129.pdf> (Accessed 13 May 2013).
- Copp, G.H., Bianco, P.G., Bogutskaya, N., Erős, T., Farka, I., et al., 2005b. To be, or not to be, a non-native freshwater fish? *Journal of Applied Ichthyology*, 21, 242-262.
- Copp, G.H., Vilizzi, L., Mumford, J., Fenwick, G.V., Godard, M.J. et al., 2009. Calibration of FISK, an invasive-ness screening tool for non-native freshwater fishes. *Risk Analysis*, 29, 457-467.
- Cvijanović, G., Lenhardt, M., Hegediš, A., 2005. The first record of black bullhead *Ameiurus melas* (Pisces, Ictaluridae) in Serbian waters. *Archives of Biological Sciences*, 57 (4), 21.
- DeLong, E.R., DeLong, D.M., Clarke-Pearson, D.L., 1988. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*, 44, 837-845.
- Demeny, F., Šipoš, S., Ittzes, I., Szabo, Z., Levai, P., et al., 2009. Observations of the crucian carp (*Carassius carassius*) pond culture. p. 138-144. In: *Proceedings of the IV International Conference "Fishery", Belgrade, 27-29 May 2009*. Faculty of Agriculture, University of Belgrade, Zemun-Belgrade.
- Economidis, P.S., Koutrakis, M., Apostolou, A., Vassilev, M., Pehlivanov, L., 2007. *Atlas of River Nestos fish fauna*. Prefectural authority of Drama-Kavala-Xanthi, Kavala, Greece: NA-GREF-Fisheries Research Institute & Bulgarian Academy of Sciences, 181 pp.
- Grigorovich, I.A., Colautti, R.I., Mills, E.L., Holeck, K., Ballert, A.G., et al., 2003. Ballast-mediated animal introduction in the Laurentian Great Lakes: retrospective and prospective analyses. *Canadian Journal of Fisheries and Aquatic Sciences*, 60, 740-756.
- Holčík, J., 1991. Fish introductions in Europe with particular reference to its Central and Eastern part. *Canadian Journal of Fisheries and Aquatic Sciences*, 48, 13-23.
- Janković, D., 1998. Natural reproduction by Asiatic herbivorous fishes in the Yugoslav section of the River Danube. *Italian Journal of Zoology*, 65 (Suppl. 1), 227-228.
- Jude, D.J., Reider, R.H., Smith, G.R., 1992. Establishment of Gobiidae in the Great Lakes Basin. *Canadian Journal of Fisheries and Aquatic Sciences*, 49, 416-421.
- Kalous, L., Rylková, K., Bohlen, J., Šanda, R., Petrýl, M., 2013. New mtDNA data reveal a wide distribution of the Japanese ginbuna *Carassius langsdorfii* in Europe. *Journal of Fish Biology*, 82, 703-707.
- Knežević, B., Marić, D., 1979. *Perca fluviatilis* Linnaeus, 1758 (Percidae, Pisces), nova vrsta za jugoslovenski dio Skadarskog jezera. *Glasnik Republičkog zavoda za zaštitu prirode – Prirodnjačkog muzeja*, 12, 177-180.
- Kolar, C.S., Lodge, D.M., 2002. Ecological predictions and risk assessment for alien fishes in North America. *Science*, 298, 1233-1236.
- Kostov, V., 2007. Nutrition and growth of *Hypophthalmichthys molitrix* (Valenciennes, 1844) and *Hypophthalmichthys nobilis* (Richardson, 1845) from reservoir Streževo – Two fish species used like biomanipulative tool. p. 149-158. In: *3rd International Conference "Fishery", Belgrade, 1-3 February 2007*. University of Belgrade, Faculty of Agriculture, Zemun-Belgrade.
- Kostov, V., 2008a. Results of ichthyofauna investigation in Macedonian part of Lake Dojran. p. 189-201. In: *1st Symposium for Protection of Natural Lakes in Republic of Macedonia, Ohrid, 31 May-3 June 2007*. Ministry of Education and Sciences of Republic of Macedonia, Ohrid.
- Kostov, V., 2008b. First record of species *Acipenser ruthenus* Linnaeus 1758 into the waters of R. Macedonia. p. 210-216. In: *1st Symposium for Protection of Natural Lakes in Republic of Macedonia, Ohrid, 31 May-3 June 2007*. Ministry of Education and Sciences of Republic of Macedonia, Ohrid.
- Kostov, V., Georgiev, S., Nastova, R., Naumovski, M., 1998. First report about common ruffe *Gymnocephalus cernua* Linnaeus, 1758, in the waters of the Republic of Macedonia. p. 167-172. *Proceedings papers devoted to Cyril Apostolski*. Institute of Animal Science, Skopje.
- Kostov, V., Rebok, K., Slavevska-Stamenković, V., Ristovska, M., 2010. Fish fauna of Bregalnica River (R. Macedonia). p. 1-8. In: *BALWOIS, Conference on water observation and information system for decision support, Ohrid, 25-29 May 2010*. Ministry of Education and Sciences of Republic of Macedonia, Ohrid. (available online <http://www.balwois>).
- Kostov, V., Ristovska, M., 2012. Checklist of fish fauna in Republic of Macedonia. *Macedonian Journal of Animal Science*, (in press).
- Kostov, V., Ristovska, M., Prelić, D., Slavevska-Stamenković, V., 2011b. Assessment of the ecological status of the Crna River based on the fish fauna – contribution to the establishment of the monitoring system of rivers in R. Macedonia. *Macedonian Journal of Animal Science*, 1 (1), 261-270.
- Kostov, V., Ristovska, M., Slavevska-Stamenković, V., Miljanović, B., Paunović, M., 2011a. Setting up a system for ecological status assessment based on Fish Fauna – the Pčinja River – case study. *Macedonian Journal of Animal Science*, 1 (2), 369-376.
- Kostov, V., Van der Knaap, M., 2009. The collapse of Fisheries of Lake Dojran – Reasons, Actual situation and Perspectives. p. 239-246. In: *Proceedings of the IV International Conference "Fishery", Belgrade, 27-29 May 2009*. Faculty of Agriculture, University of Belgrade, Zemun-Belgrade.
- Kottelat, M., 1997. European freshwater fishes. *Biologia*, 52 (Suppl. 5), 1-271.
- Lawson, L.L., Vilizzi, L., Hill, J.E., Hardin, S., Copp, G.H., 2013. Revisions of the Fish Invasiveness Scoring Kit (FISK) for its application in warmer climatic zones, with particular reference to peninsular Florida. *Risk Analysis*, (doi: 10.1111/j.1539-6924.2012.01896.x).
- Lenhardt, M., Marković, G., Hegediš, A., Maletin, S., Ćirković, M., et al., 2011. Non-native and translocated fish species in Serbia and their impact on the native ichthyofauna. *Reviews in Fish Biology and Fisheries*, 21, 407-421.
- Leonardos, I.D., Tsikliras, A.C., Eleftheriou, V., Cladas, Y., Kagalou, I., et al., 2008. Life history characteristics of an invasive cyprinid fish (*Carassius gibelio*) in Chimaditis Lake (northern Greece). *Journal of Applied Ichthyology*, 24, 213-217.
- Maletin, S., Budakov, L.J., 1982. The incidence of *Carassius auratus gibelio* in the Danube through Vojvodina (In Serbian with English summary). *Vodoprivreda*, 14 (1), 75-76.
- Maletin, S., Djukić, N., Miljanović, B., Ivanc, B., 1997. Status of allochthonous ichthyofauna of Pannonian basin in Yugoslavia

- via. *Acta Biologica Iugoslavica – Ekologija*, 32 (1), 87-98.
- Marić, D., Milošević, D., 2011. *Katalog slatkovodnih riba (Os-teichthyes) Crne Gore [Catalogue of freshwater fishes (Os-teichthyes) of Montenegro]*. Crnogorska akademija nauka i umjetnosti, Podgorica. 114 pp.
- Marić, S., Sušnik, S., Simonović, P., Snoj, A., 2006. Phylogeographic study of brown trout from Serbia, based on mitochondrial DNA control region analysis. *Genetique, Selection, Evolution*, 38, 411-430.
- Mastitsky, S.E., Karatayev, A.Y., Burlakova, L.E., Adamovich, B.V., 2010. Non-native fishes of Belarus: diversity, distribution, and risk classification using the Fish Invasiveness Screening Kit (FISK). *Aquatic Invasions*, 5, 103-114.
- Médail, F., Quézel, P., 1999. Biodiversity hotspots in the Mediterranean Basin: Setting global conservation priorities. *Conservation Biology*, 13, 1510-1513.
- Onikura, N., Nakajima, J., Inui, R., Mizutani, H., Kobayakawa, M., et al., 2011. Evaluating the potential of invasion by non-native freshwater fishes in northern Kyushu Island, Japan, using the Fish Invasiveness Scoring Kit. *Ichthyological Research*, 58, 382-387.
- Panov, V., Alexandrov, B., Arbaciauskas, K., Binimelis, R., Copp, G.H., et al., 2008. *Interim protocols for risk assessment of aquatic invasive species introductions via European inland waterways; ALARM Project, Project Website*. www.alarm-project.net. (December 2012).
- Pehlivanov, L., Leontarakis, P., 2009. *Lepomis gibbosus* (Linnaeus, 1758). p. 106-107 In: *Atlas of River Nestos Fish Fauna*. P.S. Economidis, Koutrakis M., A. Apostolou, M. Vassilev & Pehlivanov L. (Eds). Prefectural authority of Drama-Kavala-Xanthi, NAGREF-Fisheries Research Institute and Bulgarian Academy of Sciences, Kavala and Sofia.
- Peňáz, M., Dulmaa, A., 1987. Morphology, population structure, reproduction and growth in Mongolian populations of *Carassius auratus gibelio* (Pisces: Cyprinidae). *Folia Zoologica* 36, 161-173.
- Plančić, J., 1967. Prussian carp (*Carassius auratus gibelio*) a new member of our ichthyofauna. *Ribarstvo Jugoslavije*, 22, 6.
- Polačik, M., Trichkova, T., Janáč, M., Vassilev, M., Jurajda, P., 2008. The ichthyofauna of the shoreline zone in the longitudinal profile of the Danube River, Bulgaria. *Acta Zoologica Bulgarica*, 60 (1), 77-88.
- R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org> (January 2013).
- Pujin, V., Sotirov, S., 1966. A study of the nutrition of the dwarf catfish (*Ictalurus nebulosus* Le Sueur) (In Serbian). *Annales of Scientific Work at Faculty of Agriculture in Novi Sad*, 10 (1), 147-156.
- Ristovska, M., Kostov, V., Prelic, D., Slavevska-Stamenković, V., Arsovska, J., 2011. Fish community structure and water quality assessment of Babuna River, *Journal of International Environmental Application & Science*, 6, 508-517.
- Simić, V., Simić, S., Paunović, M., Simonović, P., Radojković, N., et al., 2012. *Scardinius knezevici* Bianco & Kottelat, 2005 and *Alburnus scoranza* Bonaparte, 1845: new species of ichthyofauna of Serbia and the Danube basin. *Arch. Biol. Sci. Belgrade*, 64 (3), 981-990.
- Simonović, P., 2001. *Ribe Srbije (Fishes of Serbia)*. NNK International, Zavod za zaštitu prirode Srbije & Biološki fakultet, Belgrade. 247 pp.
- Simonović, P., 2009. Invazija riba [Fish Invasion]. *Phlogiston*, 17(1), 43-64.
- Simonović, P., Jovanović, V., 1991. Sexual dimorphism in the Prussian carp (*Carassius auratus gibelio* Bloch, 1783). *Acta biologica Iugoslavica – Ichthyologia Belgrade*, 23 (1), 59-72.
- Simonović, P., Nikolić, V., 1997. Freshwater fish of Serbia: an annotated check list with some faunistic and zoogeographic considerations. *Bios Thessaloniki*, 4 (1), 137-156.
- Simonović, P., Nikolić, V., Stefanović, K., Tomović, J., 2010a. Influence of invasive alien fish species to the ecological status of the Danube River and its main tributaries in Serbia after terms of the EU Water Framework Directive. p. 281-302 In: *The Danube in Serbia – The Results of National Program of the Second Joint Danube Survey*. Simonović P., Simić V., Simić S., Paunović M., (Eds.). Ministry of Agriculture, Forestry and Water Management, University of Belgrade, Institute for Biological Research “Siniša Stanković” and University of Kragujevac, Faculty of Science, Belgrade and Kragujevac.
- Simonović, P., Nikolić, V., Grujić, S., 2010b. Amazon sailfin catfish *Pterygoplichthys pardalis* (Castelnau, 1855) (Loricariidae, Siluriformes), a new fish species recorded in the Serbian section of the Danube River. *Biotechnology and Biotechnological Equipment*, 24 (Special Edition), 655-660.
- Simonović, P., Paunović, M., Popović, S., 2001. Morphology, feeding and reproduction of the round goby, *Neogobius melanostomus* (Pallas), in the Danube River basin, Yugoslavia. *Journal of Great Lakes Research*, 27, 281-289.
- Skora, K.E., Stolarski, J., 1993. New fish species in the Gulf of Gdansk *Neogobius* sp. [cf. *Neogobius melanostomus* (Pallas, 1911)]. *Bulletin of the Sea Fisheries Institute*, 1, 83.
- Tarkan, A.S., Copp, G.H., Top, N., Özdemir, N., Önsöy, B., et al., 2012. Are introduced gibel carp *Carassius gibelio* in Turkey more invasive in artificial than in natural waters? *Fisheries Management & Ecology*, 19, 178-187.
- Troca, D.F.A., Vieira, J.P., 2012. Poencial invasor dos peixes não nativos cultivados na região cisteura do Rio Grande do Sul, Brasil. [Potential invasive non-native fish farmed in the coastal region of Rio Grande do Sul, Brazil] *Boletim do Instituto de Pesca, São Paulo*, 38 (2), 109-120.
- Uzunova, E., Zlatanova, S., 2007. A review of fish introductions in Bulgarian freshwaters. *Acta Ichthyologica et Piscatoria*, 37, 55-61.
- Vassilev, M., Pehlivanov, L., 2005. Checklist of Bulgarian freshwater fishes. *Acta Zoologica Bulgarica*, 57, 161-190.
- Vetemaa, M., Eschbaum, R., Albert, A., Saat, T., 2005. Distribution, sex ratio and growth of *Carassius gibelio* (Bloch) in coastal waters of Estonia (eastern Baltic Sea). *Journal of Applied Ichthyology*, 21, 287-291.
- Vilizzi, L., Copp, G.H., 2013. Application of FISK, an invasiveness screening tool for non-native freshwater fishes, in the Murray-Darling Basin (south-eastern Australia). *Risk Analysis* (<http://dx.doi.org/10.1111/j.1539-6924.2012.01860.x>).
- WFD, 2000. *Directive of the European Parliament and of the Council 2000/60/EC establishing a framework for community action in the field of water policy*. European Union, the European Parliament and Council, Luxembourg.
- Youden, W.J., 1950. Index for rating diagnostic tests. *Cancer*, 3, 32-35.