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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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 Pelasgus sensu Kottelat, 1997, Aulopyge, Phoxinellus and Romanichthys, as well as many endemic species of otherwise widespread genera (e.g. Barbus, Cobitis, Eudontomyzon, Gobio, Knipowitschia, Rutillus 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 Danube, 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 (Troc & 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, 1982). The Balkan Peninsula, encompassing four countries across the entire Balkans into Lake Skadar, 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 scoaranza 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 graying 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 values (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:

\[ \sum(CQ_i)(4 \times 49) \]  

where CQ, 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 4 (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 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 spathula* 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, 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
fluviatilis and the Atlantic strain of brown trout Salmo trutta trutta.

Discussion

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.

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

<table>
<thead>
<tr>
<th>Species name</th>
<th>Common name</th>
<th>Invasiveness/Protection status</th>
<th>Country</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SE</th>
<th>Outcome</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SE</th>
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<td>Acipenser gueldenstaedtii</td>
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<td>mk</td>
<td>16.0</td>
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<td>–</td>
<td>–</td>
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<td>sterlet</td>
<td>Invasive/Not evaluated</td>
<td>mk</td>
<td>18.0</td>
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<td>–</td>
<td>–</td>
<td>MH</td>
<td>0.75</td>
<td>–</td>
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<td>–</td>
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<td>Lake Ohrid bleak</td>
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<td>rs</td>
<td>2.5</td>
<td>–</td>
<td>–</td>
<td>M</td>
<td>0.89</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>rs</td>
<td>24.5</td>
<td>–</td>
<td>–</td>
<td>MH</td>
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<td>mk, me, rs</td>
<td>29.7</td>
<td>29.0</td>
<td>31.0</td>
<td>0.7</td>
<td>H</td>
<td>0.90</td>
<td>0.83</td>
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<td>–</td>
<td>MH</td>
<td>0.96</td>
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<td>26.5</td>
<td>34.0</td>
<td>2.2</td>
<td>VH</td>
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<td>0.89</td>
<td>1.00</td>
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<td>bg</td>
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<td>–</td>
<td>–</td>
<td>L</td>
<td>0.90</td>
<td>–</td>
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<tr>
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<td>peled</td>
<td>Invasive/Least concern</td>
<td>rs</td>
<td>3.0</td>
<td>–</td>
<td>–</td>
<td>M</td>
<td>0.76</td>
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<tr>
<td>Ctenopharyngodon idella</td>
<td>grass carp</td>
<td>Non-invasive/Not evaluated</td>
<td>bg, mk, me, rs</td>
<td>17.5</td>
<td>15.0</td>
<td>21.0</td>
<td>1.5</td>
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<td>0.82</td>
<td>0.93</td>
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<td>mk</td>
<td>27.0</td>
<td>–</td>
<td>–</td>
<td>H</td>
<td>0.84</td>
<td>–</td>
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<tr>
<td>Gambusia holbrooki</td>
<td>eastern mosquitofish</td>
<td>Invasive/Not evaluated</td>
<td>bg, me</td>
<td>19.0</td>
<td>12.0</td>
<td>26.0</td>
<td>5.7</td>
<td>MH</td>
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<td>–</td>
<td>–</td>
<td>M</td>
<td>0.85</td>
<td>–</td>
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<tr>
<td>Hypophthalmichthys molitrix</td>
<td>silver carp</td>
<td>Invasive/Near threatened</td>
<td>bg, mk, me, rs</td>
<td>16.4</td>
<td>12.0</td>
<td>20.5</td>
<td>2.1</td>
<td>MH</td>
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<td>0.81</td>
<td>0.93</td>
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<tr>
<td>Hypophthalmichthys nobilis</td>
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<td>bg, mk, me, rs</td>
<td>13.9</td>
<td>7.0</td>
<td>20.5</td>
<td>3.3</td>
<td>MH</td>
<td>0.88</td>
<td>0.81</td>
<td>0.93</td>
<td>0.03</td>
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</table>

(continued)
Table 1 (continued)

<table>
<thead>
<tr>
<th>Species name</th>
<th>Common name</th>
<th>Invasiveness/Protection status</th>
<th>Country</th>
<th>Score</th>
<th>CF</th>
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</thead>
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<td>Ictalurus punctatus</td>
<td>channel catfish</td>
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<td>bg</td>
<td>10.0</td>
<td>–</td>
</tr>
<tr>
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<td>pumpkinseed</td>
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<td>bg, mk, rs</td>
<td>21.3</td>
<td>18.0</td>
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<tr>
<td>Megalobrama terminalis</td>
<td>black Amur bream</td>
<td>Non-invasive/Not evaluated</td>
<td>me</td>
<td>23.5</td>
<td>–</td>
</tr>
<tr>
<td>Micropterus salmoides</td>
<td>largemouth (black) bass</td>
<td>Invasive/Not evaluated</td>
<td>rs</td>
<td>18.0</td>
<td>–</td>
</tr>
<tr>
<td>Mugil cephalus</td>
<td>so-iuy mullet</td>
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<td>bg</td>
<td>12.0</td>
<td>–</td>
</tr>
<tr>
<td>Mylopharyngodon piceus</td>
<td>black carp</td>
<td>Invasive/Least concern</td>
<td>bg</td>
<td>11.0</td>
<td>–</td>
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<tr>
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<td>monkey moby</td>
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<td>rs</td>
<td>18.0</td>
<td>–</td>
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<tr>
<td>Neogobius melanostomus</td>
<td>round goby</td>
<td>Non-invasive/Not evaluated</td>
<td>rs</td>
<td>15.0</td>
<td>–</td>
</tr>
<tr>
<td>Oncorhynchus mykiss</td>
<td>rainbow trout</td>
<td>Invasive/Not evaluated</td>
<td>bg, mk, me, rs</td>
<td>15.3</td>
<td>12.0</td>
</tr>
<tr>
<td>Oxyoemachelus bureschi</td>
<td>Bureschi loach</td>
<td>Non-invasive/Least concern</td>
<td>mk</td>
<td>8.0</td>
<td>–</td>
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<tr>
<td>Pachychilus macedonicum</td>
<td>Macedonian roach</td>
<td>Non-invasive/Data deficient</td>
<td>rs</td>
<td>3.5</td>
<td>–</td>
</tr>
<tr>
<td>Perca flavissilis</td>
<td>Eurasian perch</td>
<td>Invasive/Least concern</td>
<td>me</td>
<td>23.0</td>
<td>–</td>
</tr>
<tr>
<td>Percottus glenii</td>
<td>Amur (Chinese) sleeper</td>
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<td>bg, rs</td>
<td>18.8</td>
<td>19.0</td>
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<tr>
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<td>Mississippi paddlefish</td>
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<td>bg, rs</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Poncilia kessleri</td>
<td>bighead goby</td>
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<td>rs</td>
<td>17.0</td>
<td>–</td>
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<tr>
<td>Proterorhinus semilunaris</td>
<td>western tubenose goby</td>
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<td>rs</td>
<td>13.0</td>
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</tr>
<tr>
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<td>topmouth gudgeon</td>
<td>Invasive/Not evaluated</td>
<td>bg, mk, me, rs</td>
<td>18.3</td>
<td>12.0</td>
</tr>
<tr>
<td>Pterygoplichthys pardalis</td>
<td>Amazon salinl catfish</td>
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<td>rs</td>
<td>29.0</td>
<td>–</td>
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<tr>
<td>Rutilus sp.</td>
<td>Adriatic roach</td>
<td>Non-invasive/Least concern</td>
<td>rs</td>
<td>7.0</td>
<td>–</td>
</tr>
<tr>
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<td>Ohrid trout</td>
<td>Non-invasive/Data deficient</td>
<td>rs</td>
<td>5.0</td>
<td>–</td>
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<td>Macedonian trout</td>
<td>Non-invasive/Data deficient</td>
<td>rs</td>
<td>24.0</td>
<td>–</td>
</tr>
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<td>–</td>
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<td>4.3</td>
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<tr>
<td>Sander lucioperca</td>
<td>pikeperch</td>
<td>Invasive/Not evaluated</td>
<td>mk</td>
<td>14.5</td>
<td>–</td>
</tr>
<tr>
<td>Scardinius kzeveizi</td>
<td>Lake Skadar rudd</td>
<td>Non-invasive/Critically</td>
<td>rs</td>
<td>9.0</td>
<td>–</td>
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<tr>
<td>Synagrus abaster</td>
<td>black-striped pipefish</td>
<td>Non-invasive/Least concern</td>
<td>rs</td>
<td>5.0</td>
<td>–</td>
</tr>
<tr>
<td>Thymallus thymallus</td>
<td>grylling</td>
<td>Non-invasive/Least concern</td>
<td>me</td>
<td>5.0</td>
<td>–</td>
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</tbody>
</table>

(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 endemicism 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 gibeluna Carassius langsfordii, 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. Peňáz & 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 & Leontarakis, 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, tolerances 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 Percottus 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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