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ARTICLE *in* FISHERIES MANAGEMENT AND ECOLOGY · JULY 2015

Impact Factor: 1.76

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F M E	12147	Wiley	Dispatch: 17.8.15	CE: Sangeetha
Journal Code	Manuscript No.		No. of pages: 11	PE: Bhuvaneswari C

Fisheries Management and Ecology, 2015

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Abstract The Fish Invasiveness Screening Kit (FISK) version 2 was used to assess the invasiveness potential of 40 introduced and translocated freshwater fish species to Croatia and Slovenia. Based on *a priori* classification of invasiveness, receiver operating characteristic analysis of FISK scores from two independent assessors resulted in a statistically significant calibration threshold of 11.75. This indicated that FISK was able to discriminate reliably between non-native species likely to pose a high risk of being invasive and those likely to pose a medium or low risk of invasiveness. Seven species were categorised as 'medium risk' and the other 33 as 'high risk', whereas no species 4 was categorised as 'low risk'. The two highest scoring species were European catfish *Silurus glanis* and North African catfish *Clarias gariepinus*. Mean scores for all species classified *a priori* as invasive were ranked as 'high risk' *sensu lato* and fell into the 'moderately high risk' subcategory. FISK proved to be a valid tool for assessing the risks posed by non-native fishes in Croatia and Slovenia. For this reason, it can be adopted as a reliable tool for the prevention of new translocations or introductions of potentially invasive species in the risk assessment area, as well as to assist in decisions regarding future management (i.e. monitoring, control and eradication) and conservation strategies.

KEYWORDS: Balkan Peninsula, hazard identification, inland waters, invasive potential, non-native fish species, risk analysis.

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Introduction

An essential first step in risk analysis is the identification of potential hazards. In the case of non-native species, several risk screening tools have been developed to identify the potentially invasive plants and animals in both 5 terrestrial (e.g. Pheolung *et al.* 1999) and aquatic ecosystems (e.g. Kolar & Lodge 2002; Copp *et al.* 2005a,b). Risk screening is important in all geographical regions, but especially in areas with high endemism and/or biodiversity such as the Iberian (e.g. Almeida *et al.* 2013) and the Balkans Peninsulas (Simonović *et al.* 2013), the latter being one of the world's 35 biodiversity hotspots (Hewitt 2011). Croatia and Slovenia are situated in the northern part of the Balkan's Peninsula, encompassing three zoogeographical areas: the continental-Pannonian Valley, the continental Hills and Mountains (Alps and Dinarides) and the Mediterranean. The continental region, which includes the first two zoogeographical areas, is part of the River Danube Basin, with the rivers Sava and Drava being the two largest tributaries in this section of the Danube. By contrast, the coastal rivers are part of the Adriatic drainage (Mediterranean zoogeographical area) and are generally relatively short, unnavigable and isolated, and characterised by a high level of endemism.

The freshwater ichthyofauna of Croatia is diverse and includes 150 riverine species, of which 21 are found in brackish and salt waters (e.g. Mediterranean toothcarp *Aphanius fasciatus*, twaite shad *Alosa fallax* and three-spine stickleback *Gasterosteus aculeatus*). Croatia's ichthyofauna is also rich in endemic species, with 38 present in the Adriatic Basin and 12 in the Danube Basin (Ćaleta *et al.* 2015). Of the non-native species introduced during the 19th century, 10 currently inhabit the Adriatic and 20 inhabit the Danube Basin (Mrakovčić *et al.* 2006), with 13 of the introduced species translocated from the Danube to the Adriatic basin (Ćaleta *et al.* 2015). The ichthyofauna of Slovenian inland waters is somewhat less rich than that of Croatia, represented by 70 native and 19 introduced species (Povž & Ocvirk 1988; Povž *et al.* 2003; Povž & Gregori 2014). Eleven of the introduced species are translocations between the Danube and the Adriatic basins (Povž & Šumer 2005). Slovenia and Croatia share both the Adriatic and Danube basins, and therefore, eight of the endemic species are present in both countries in either of the two basins (Ćaleta *et al.* 2015): Adriatic, with Padanian barbel *Barbus plebejus*, Istrian chub *Squalius janae*, Adriatic sturgeon *Acipenser naccarii*, Padanian goby *Padogobius bonelli*, black spot goby *Pomatoschistus canestrinii*, marble trout *Salmo marmoratus*, triotto *Rutilus aula* and Adriatic dwarf goby *Knipowitschia panizzae*; Danube, with cactus roach *Rutilus virgo*, whitefin gudgeon *Romanogobio vladykovi*, stone gudgeon *Romanogobio uranoscopus*, huchen *Hucho hucho*, gudgeon *Gobio obtusirostris*, schraetzer *Gymnocephalus schraetser*, Danubian brook lamprey *Eudontomyzon vladykovi* and Balkan loach *Cobitis elongata*.

The first accounts of fish introductions to the region were of rainbow trout *Oncorhynchus mykiss* in 1883 (Franke 1913; Bojčić 1997). Subsequent non-native fish introductions were made mainly by anglers, either intentionally for sport fishing and aquaculture or accidentally whilst restocking rivers with native species. Recently, seven new non-native species have been recorded in the Croatian part of the Danube Basin (Ćaleta 2007, 2010; 6 Piria *et al.* 2011a,b; Jelkić & Opačak 2013; Safner *et al.* 2013; Šanda *et al.* 2013), of which monkey goby *Neogobius fluviatilis*, round goby *Neogobius melanostomus* and bighead goby *Ponticola kessleri* have established self-sustaining populations (Piria *et al.* 2015). Although many of these introductions have occurred on a regular basis (Povž & Ocvirk 1988), their impacts are completely unknown due to a scarcity of studies on non-native fishes (Povž *et al.* 2003), and risk analysis of non-native freshwater fishes is virtually non-existent.

The objective of this study was to carry out a screening of non-native freshwater fishes for Croatia and Slovenia to determine which species are likely to pose a risk of becoming (or becoming) invasive in the northern (Croatian and Slovenian) parts of the Balkans region. This information can be used by decision makers to develop guidelines for the management of non-native fishes (including future introductions) and conservation strategies for native fish species.

Materials and methods

The screening of non-native and translocated fish species was undertaken using the Fish Invasiveness Scoring Kit (FISK), which was adapted by Copp *et al.* (2005a,b) from the Australian weed risk assessment (RA) tool (Pheolung *et al.* 1999). Fish Invasiveness Screening Kit (FISK) was recently revised to produce version 2 (Lawson *et al.* 2013) and has since been applied to several RA areas across a wide range of climatic regions in Europe (Almeida *et al.* 2013; Puntila *et al.* 2013; Simonović *et al.* 2013), Australasia (Vilizzi & Copp 2013; Tarkan *et al.* 2014) and North America (Lawson 2014), indicating that FISK is a useful and viable tool for identifying potentially invasive non-native fishes for management and conservation purposes (review in Copp 2013). Fish Invasiveness Screening Kit consists of 49 questions in two main sections (Biogeography/History and Biology/Ecology) and eight categories (domestication/cultivation; climate and distribution; invasive elsewhere; undesirable traits; feeding guild; reproduction; dispersal mechanisms; and persistence attributes), with outcome scores ranging from −11 to 57 (L. Vilizzi, unpublished data). Based on the resulting score, three levels of potential risk of a species being invasive are identified: 'low', 'medium' and 'high' (Britton *et al.* 2010).

Species selection was based on four criteria: (1) native species translocated from the Danube to the Adriatic Basin; (2) native species translocated outside their native range but within the same drainage basin; (3) non-native species already present and established (in one or both drainage basins); (4) non-native species recently found but without knowledge of their establishment history. With Croatia and Slovenia as the RA area, assessments were carried out by the first two authors, who are freshwater fish experts for the RA area. Receiver operating characteristic (ROC) analysis (Bewick *et al.* 2004) was used to assess the predictive ability of FISK to discriminate between invasive and non-invasive species. Species were categorised *a priori* in terms of their perceived invasiveness (i.e. invasive or non-invasive) and protection status (i.e. conservation concern) based on information available from the Invasive Species Specialist
7 Group database (http://www.issg.org/) and from FishBase (Froese & Pauly 2014). Statistically, a ROC curve is a graph of sensitivity vs specificity, where in the present context sensitivity and specificity are 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 categorised as invasive) nor false negatives (i.e. invasive species categorised 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 two assessors carried out separate and independent assessments on 40 species in total. Of these, 37 species were assessed for Croatia and 26 for Slovenia, with 23 species assessed for both countries (Table 1). Separate ROC curves were initially generated for the two assessors based on the 23 species in common and differences between corresponding AUCs were statistically tested (Mann–Whitney U-statistic, $\alpha = 0.05$) (online applet StAR: http://protein.bio.puc.cl/star/home.php) (Vergara *et al.* 2008). Following between-curve comparison, a global ROC curve was computed on the mean scores from all 40 species evaluated by the two assessors. Based on the global ROC curve, the best FISK threshold (or cut-off) value that maximises the true positive rate (i.e. true invasive classified as invasive) and minimises the false-positive rate (i.e. true non-invasive classified as invasive) was determined using a combination of Youden's J statistic (Youden 1950) and the point closest to the top-left part of the plot with perfect sensitivity or specificity. A smoothed mean ROC curve was also generated and boot-strapped confidence intervals (2000 replicates) of specificities were computed along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). Package pROC (Robin *et al.* 2011) for R x64 v3.03 (R Development Core Team, 2014) was used for 8
analysis.

As each response of 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 follows:

$$\sum \frac{(CQ_i)}{(4 \times 49)}, i = 1, \ldots, 49$$

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).

Finally, for assessment of the consistency between assessors, an error (or confusion) matrix (Renken & Mumby 2009) was computed and the corresponding coincidence rate determined for species categorisation according to risk extent (i.e. 'medium', 'moderately high', 'high' and 'very high').

Results

For the 40 freshwater fish species assessed for their invasiveness potential in the RA area (Table 1), there were no statistically significant differences ($P = 0.289$) between AUCs from the two assessor-specific ROC curves based on the 23 species evaluated for both countries that comprise the RA area (Fig. 1a). With a caveat

Table 1. Fish species assessed with FISK v2 for Croatia (hr) and Slovenia (si)

Species name	Common name	Invasiveness/Protection status	Country	FISK score				Outcome	Criterion	CF			
				Mean	Min	Max	SE			Mean	Min	Max	SE
Silurus glanis	European catfish	Invasive/Least concern	hr, si	33.0	30.0	36.0	2.4	VH	1	0.90	0.89	0.91	0.01
Clarias gariepinus	North African catfish	Invasive/Not evaluated	si	32.0	32.0	32.0	–	VH	4	0.88	0.88	0.88	–
Sander lucioperca	Pikeperch	Invasive/Not evaluated	hr	29.0	–	–	–	H	1	0.90	0.90	0.90	–
Carassius gibelio	Gibel carp	Invasive/Not evaluated	hr, si	28.5	22.0	35.0	5.3	H	3	0.93	0.92	0.94	0.01
Ameiurus nebulosus	Brown bullhead	Non-invasive/Least concern	hr, si	28.0	22.0	34.0	4.9	H	3	0.88	0.86	0.89	0.01
Neogobius melanostomus	Round goby	Non-invasive/Not evaluated	hr	28.0	28.0	28.0	–	H	3	0.92	0.92	0.92	–
Perccottus glenii	Chinese (Amur) sleeper	Non-invasive/Vulnerable	hr	27.0	–	–	–	H	4	0.84	0.84	0.84	–
Ameiurus melas	Black bullhead	Invasive/Not evaluated	hr, si	26.5	21.0	32.0	4.5	H	3	0.86	0.84	0.88	0.02
Micropterus salmoides	Largemouth (black) bass	Invasive/Not evaluated	hr, si	26.3	26.0	26.5	0.2	H	3	0.89	0.87	0.91	0.02
Salmo trutta	Brown trout Atlantic lineage	Invasive/Not evaluated	hr, si	25.5	23.0	28.0	2.0	H	1, 2	0.93	0.90	0.95	0.02
Oncorhynchus mykiss	Rainbow trout	Invasive/Not evaluated	hr, si	24.5	22.0	27.0	2.0	MH	3	0.94	0.93	0.94	0.01
Cyprinus carpio	Common carp	Invasive/Vulnerable	hr, si	22.5	19.0	26.0	2.9	MH	1	0.94	0.93	0.94	0.00
Mylopharyngodon piceus	Black carp	Invasive/Least concern	si	22.0	–	–	–	MH	4	0.87	0.87	0.87	–
Neogobius fluviatilis	Monkey goby	Non-invasive/Not evaluated	hr	22.0	–	–	–	MH	3	0.90	0.90	0.90	–
Pseudorasbora parva	Topmouth gudgeon	Invasive/Not evaluated	hr, si	21.5	17.0	26.0	3.7	MH	3	0.91	0.90	0.91	0.00
Ctenopharyngodon idella	Grass carp	Non-invasive/Not evaluated	hr, si	21.0	21.0	21.0	0.0	MH	3	0.90	0.89	0.90	0.00
Abramis brama	Common bream	Non-invasive/Least concern	hr	20.0	–	–	–	MH	1	0.86	0.86	0.86	–
Scardinius erythrophthalmus	Rudd	Invasive/Least concern	hr	20.0	–	–	–	MH	1	0.86	0.86	0.86	–
Esox lucius	Northern pike	Invasive/Least concern	hr, si	18.5	14.0	23.0	3.7	MH	1	0.89	0.88	0.90	0.01
Ponticola kessleri	Bighead goby	Non-invasive/Least concern	hr	18.0	–	–	–	MH	3	0.87	0.87	0.87	–
Gambusia holbrooki	Eastern mosquitofish	Invasive/Not evaluated	hr, si	17.5	12.0	23.0	4.5	MH	3	0.88	0.88	0.88	0.00
Lepomis gibbosus	Pumpkinseed	Invasive/Not evaluated	hr, si	17.5	9.0	26.0	6.9	MH	3	0.92	0.91	0.93	0.01
Carassius auratus	Goldfish	Invasive/Not evaluated	hr, si	17.0	12.0	22.0	4.1	MH	3	0.92	0.92	0.93	0.00
Salvelinus alpinus	Arctic char	Non-invasive/Least concern	hr, si	16.5	11.0	22.0	4.5	MH	3	0.86	0.81	0.92	0.05
Hypophthalmichthys nobilis	Bighead carp	Invasive/Not evaluated	hr, si	16.0	15.0	17.0	0.8	MH	3	0.92	0.91	0.93	0.01
Thymallus thymallus	Grayling	Non-invasive/Least concern	hr, si	16.0	15.0	17.0	0.8	MH	1	0.91	0.91	0.91	0.00
Hypophthalmichthys molitrix	Silver carp	Invasive/Near threatened	hr, si	15.8	13.5	18.0	1.8	MH	3	0.92	0.90	0.94	0.02
Oreochromis niloticus	Nile tilapia	Invasive/Not evaluated	hr, si	15.0	10.0	20.0	4.1	MH	3	0.81	0.78	0.85	0.03
Chondrostoma nasus	Common nase	Non-invasive/Least concern	si	14.0	–	–	–	MH	1	0.96	0.96	0.96	–
Salvelinus fontinalis	Brook trout	Invasive/Not evaluated	hr, si	13.5	9.0	18.0	3.7	MH	3	0.91	0.89	0.92	0.01
Piaractus brachypomus	Pirapatinga	Non-invasive/Not evaluated	hr	13.0	–	–	–	MH	4	0.78	0.78	0.78	–
Babka gymnotrachelus	Racer goby	Invasive/Not evaluated	hr	12.0	–	–	–	MH	4	0.83	0.83	0.83	–
Coregonus peled	Peled	Invasive/Least concern	hr	12.0	–	–	–	MH	3	0.82	0.82	0.82	–
Coregonus lavaretus	European whitefish	Non-invasive/Vulnerable	hr, si	11.5	9.0	14.0	2.0	M	3	0.85	0.83	0.88	0.02
Anguilla anguilla	European eel	Non-invasive/Critically endangered	hr, si	9.8	6.5	13.0	2.7	M	4	0.89	0.88	0.89	0.00
Rutilus rutilus	Roach	Invasive/Least concern	hr	9.0	–	–	–	M	1	0.87	0.87	0.87	–
Squalius cephalus	Chub	Non-invasive/Least concern	hr	9.0			–	M	1	0.85	0.85	0.85	–

(continued)

Table 1. (continued)

Species name	Common name	Invasiveness/Protection status	Country	FISK score				Outcome	Criterion	CF			
				Mean	Min	Max	SE			Mean	Min	Max	SE
Morone chrysops × *Morone saxatilis*	Wiper/sunshine bass hybrid	Non-invasive/Least concern	hr	7.5	–	–	–	M	4	0.67	0.67	0.67	–
Polyodon spathula	Mississippi paddlefish	Non-invasive/Critically endangered	hr, si	4.5	3.0	6.0	1.2	M	4	0.84	0.84	0.84	0.00
Rutilus basak	Neretvan roach	Non-invasive/Least concern	hr	3.0	–	–	–	M	2	0.92	0.92	0.92	–

CF, certainty factor; FISK, Fish Invasiveness Screening Kit. For each species, *a priori* invasiveness (as per http://www.issg.org/ and http://www.fishbase.org) and protection status, along with corresponding FISK score and CF (see text for computation), are reported. Outcome is based on a calibration threshold of 11.75 between medium and high risk species *sensu lato* and classified by [lower, upper]/[scores as: medium risk (M) = [1, 11.75[; moderately high risk (MH) = [11.75, 25[; high risk (H) = [25, 30[; very high risk (VH) = [30, 57] (NB: Open square brackets indicate an open interval). Criterion: 1 = native species translocated from the Danube to the Adriatic Basin; 2 = native species translocated outside their native range but within the same drainage basin; 3 = non-native species already present and established (in one or both drainage basins); 4 = non-native species recently found but without knowledge of their establishment history.

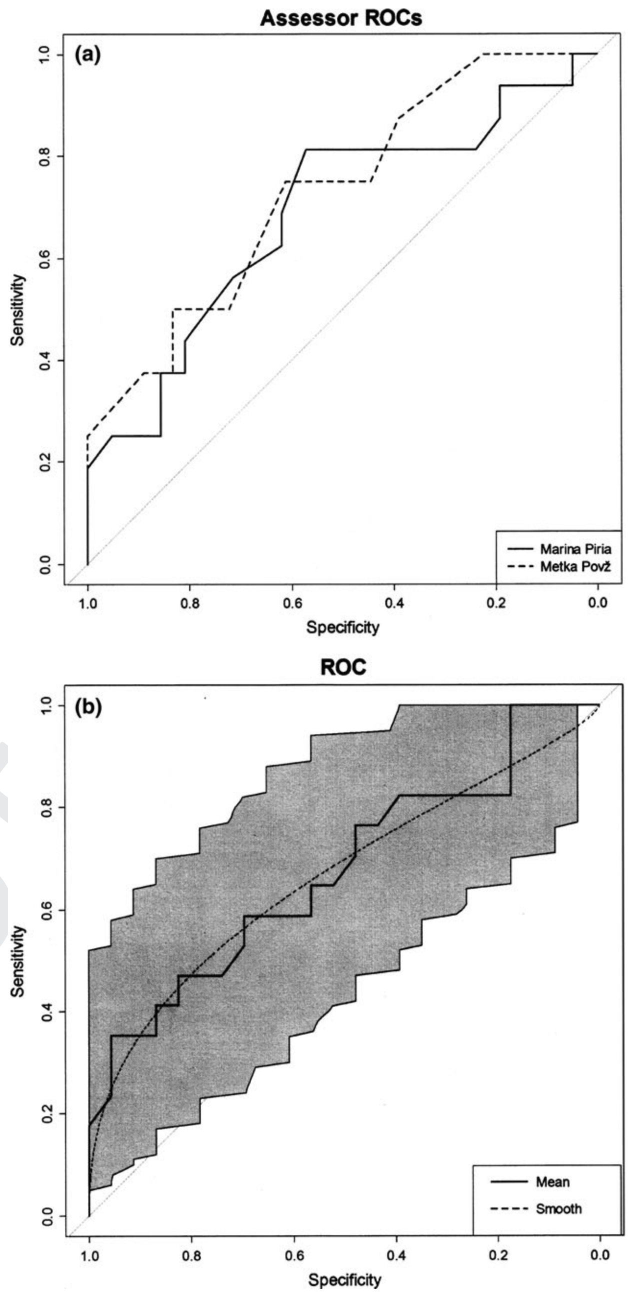

Figure 1. Receiver operating characteristic (ROC) curve for 40 non-native freshwater fish species assessed with FISK v2 for Croatia and Slovenia (Table 1). Smoothing line and confidence intervals of specificities are provided. 20

for this being a representative subsample of the entire set of 40 species, computation of a global ROC curve based on the mean scores for the latter was justified. This resulted in an AUC equal to 0.6752 (0.5000–0.8504 95% CI), hence above 0.5 (Fig. 1b), indicating that FISK was able to discriminate reliably between invasive and non-invasive species for the RA area. Youden's *J* and closest point statistics provided the same best threshold value of 11.75, which was therefore chosen as the calibration threshold of FISK risk outcomes for Croatia and Slovenia (Table 1). Accordingly, the threshold was used to distinguish between ‘medium risk’ species (that is, species with scores within the interval [1, 11.75[) and ‘high risk *sensu lato*’ species (that is, species with scores within the interval [11.75, 57]). The latter interval was further categorised, as per Britton *et al.* (2010), into ‘moderately high risk’ (interval [19, 25[), ‘high risk’ (interval [25, 30[) and ‘very high risk’ (interval [30, 57]) and with ‘low risk’ species having a score within the interval [−15, 1[. (NB: open square brackets indicate an open interval).

Based on the above threshold and corresponding intervals, none of the 40 species assessed was categorised as ‘low risk’. Conversely, 7 (17.5%) species were categorised as ‘medium risk’ and the remaining 33 (82.5%) as ‘high risk’ *sensu lato*, of which 23 (69.7%; 57.5% of total) as ‘moderately high risk’, 8 (24.2%; 20.0% of total) as ‘high risk’ and 2 (6.1%; 5.0% of total) as ‘very high risk’. The latter species were European catfish *Silurus glanis* and North African catfish *Clarias gariepinus*; whereas, the lowest scoring species (‘medium risk’) were Neretvan roach *Rutilus basak*, Mississippi paddlefish *Polyodon spatula*, wipe/sunshine bass hydrid *Morone chrysops* × *Morone saxatilis*, chub *Squalius cephalus*, roach *Rutilus rutilus*, European eel *Anguilla anguilla* and European whitefish *Coregonus lavaretus* (Table 1).

Mean scores for all species classified *a priori* as invasive were ranked as ‘high risk *sensu lato*’ and fell into the ‘moderately high risk’ subcategory (Fig. 2). However, the mean scores for non-invasive species both of least concern and vulnerable threat status also were ranked as ‘moderately high risk’, with only non-invasive critically endangered Mississippi paddlefish and European eel categorised as ‘medium risk’ (Fig. 2). Mean certainty in assessor responses for all species was 3.5 ± 0.1 SE (i.e. above the category ‘mostly uncertain’), and the mean CF was 0.89 ± 0.05 SE (Table 1). Finally, the coincidence rate between the two assessors was 57%.

Discussion

Unlike two previous calibrations for other RA areas (Onikura *et al.* 2012; Almeida *et al.* 2013), which had FISK threshold values (i.e. for distinguishing between medium and high risk species) similar to that of the UK (≥19; Copp *et al.* 2009a), the calibrated threshold value for Croatia and Slovenia was 11.75. This is close to the 9.5 value obtained for the Balkan countries of Serbia, Montenegro, FYROM and Bulgaria (Simonović *et al.* 2013), which are situated in the southern part of the Balkan Peninsula. The Serbian RA area partially overlaps with the Croatian and Slovenian RA area in terms of the number of introduced fish species. As suggested by Simonović *et al.* (2010), a low threshold value for 9 the Balkans Peninsula is probably due to the elevated number of translocations (44%, 19 of 43 species in total) across the countries of this region. Indeed, translocations from the Danube to the Adriatic Basin, as well as within

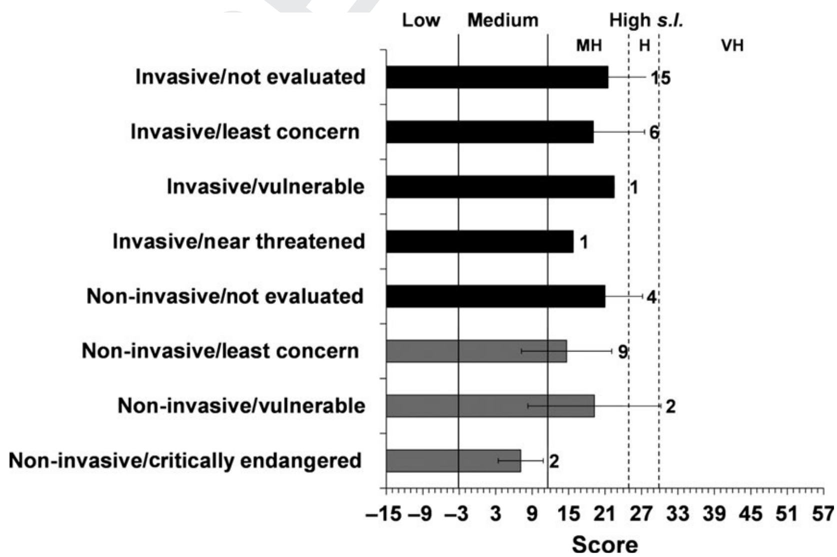

Figure 2. Mean scores (±SE and *n*) for the 40 non-native freshwater fish species assessed according to their *a priori* invasiveness and protection status (Table 1). Thresholds are: <1 (low risk) and ≥11.75 (high risk *sensu lato*), with medium risk species in between. Risk categories and [lower, upper]/[scores are as follows: L = low risk [−15, 1[; M = medium risk [1, 11.75[; MH = moderately high risk [11.75, 25[; H = high risk [25, 30[; VH = very high risk [30, 57]. (NB: open square brackets indicate an open interval).

the same drainage basin, constitute 30% (12 of 40) of species introductions in Croatia and Slovenia. In other Mediterranean regions, where translocated species represent a lower proportion of the introduced fishes (Iberia = 18%; Turkey = 14.29%), the threshold values were 20.25 (Almeida *et al.* 2013) and 23 (Tarkan *et al.* 2014), respectively.

Of the 40 species assessed in the present study, no species was categorised as ‘low risk’. A similar result was obtained for Iberia and Turkey (Almeida *et al.* 2013; Tarkan *et al.* 2014), both countries being characterised by a high level of endemism and a heightened threat to native species diversity. The highest score (i.e. ‘very high risk’) for Croatia and Slovenia was obtained for two top predators, namely the European catfish and the North African catfish. European catfish possesses the attributes of a species well adapted to introductions outside its native range and can establish self-sustaining populations relatively easily in warmer climates (Copp *et al.* 2009b). Native to the River Danube basin, which drains parts of Croatia and Slovenia, European catfish was translocated several decades ago into the karstic Lake Vransko (Adriatic basin, Croatia) for aquaculture (Treer 1989) as well as into the karstic River Vipava (Slovenia) for angling purposes (Povž & Ocvirk 1988). The Mediterranean climate in those areas is thought to provide appropriate environmental conditions for introduced species such as the European catfish, despite the oligotrophic status of some waters (e.g. Lake Vransko; Treer *et al.* 2011). The North African catfish was first reported in two gravel pits of the River Mura basin (Slovenia) in 1997, having been introduced for angling purposes without legal consent, and is currently found in fish farms nearby (Povž 2007a). Although this species is widely tolerant of extreme environmental conditions (Froese & Pauly 2014), it is unable to survive winter conditions in the continental part of the RA area when water temperatures fall to <10 °C. Nevertheless, a threat still exists due to potential translocations to thermal spring habitats – as demonstrated by the adaptation of Nile tilapia *Oreochromis niloticus* in Slovenia (Povž 2009) and to the Mediterranean part of the RA area.

Amongst the eight species evaluated as ‘high risk’, round goby *Neogobius melanostomus* is known to be a successful Ponto-Caspian invader (Kornis *et al.* 2012; Manné *et al.* 2013), with self-sustaining populations recently established in the Croatian part of the Danube basin and further spread upstream also reported (Jakovlić *et al.* 2015). Furthermore, recent research of newly invaded parts of Croatia demonstrates the round goby’s potential to invade and exert impacts on native Balkan golden loach *Sabanejewia balcanica* (Piria *et al.* 2015). This contrasts Slovenia, where there have so far been no records of any Ponto-Caspian gobies. The high scores achieved for brown bullhead *Ameiurus nebulosus* and black bullhead *Ameiurus melas*, two very robust and adaptable species characterised by high plasticity, were also obtained in most of the other RA areas where these species have been evaluated (i.e. Copp *et al.* 2009a; Almeida *et al.* 2013; Simonović *et al.* 2013; Vilizzi & Copp 2013; Tarkan *et al.* 2014) – the exception here is Finland, where the colder climate relative to the UK and southern Europe may limit the species’ invasiveness (Puntila *et al.* 2013). A high FISK score also was achieved for the Amur sleeper *Perccottus glenii*, which is regarded as one of the most invasive fish species in Eastern and Central Europe (Grabowska *et al.* 2011). In Croatia, the Amur sleeper was recorded in 2008 in a channel that is part of a drainage system from nearby common carp *Cyprinus carpio* rearing ponds (Ćaleta *et al.* 2010). As no additional records are available for the Amur sleeper, it is difficult to predict its acclimatisation to Croatian inland waters. However, based on reports from eastern Europe (Koščo *et al.* 2003; Nowak *et al.* 2008) and neighbouring countries to the RA area (Hegediš *et al.* 2007), future spread of this species into Croatian waters cannot be ruled out (Ćaleta *et al.* 2010).

The gibel carp (*Carassius gibelio*) has already been evaluated as invasive in other countries (Mastitsky *et al.* 2010; Almeida *et al.* 2013; Simonović *et al.* 2013; Tarkan *et al.* 2014). The factors responsible for gibel carp invasiveness are its ability to reproduce gynogenetically; its adaptability to various environmental conditions; its effect on the reproduction of native fishes (Tarkan *et al.* 2012); and its strong competitiveness for feeding resources (Simonović *et al.* 2013). This species can, therefore, pose a serious threat to Mediterranean inland waters with high levels of endemism (Crivelli 1995). Moreover, the naturalisation 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 *et al.* 2013), and the same is true for both drainage basins in Croatia and Slovenia (M. Piria and M. Povž, personal observation).

In the Adriatic catchment of Slovenia, brown trout *Salmo trutta* of Atlantic lineage is one of the most attractive recreational salmonid species. It was introduced for the first time into the River Soča in 1906 (Ocvirk 1989; Povz & Ocvirk 1990), where it hybridised 10
successfully with endemic salmonids (Crivelli 1995). In the late 1980s, only eight genetically pure (i.e. not introgressed) marble trout populations were identified in the upper part of the Soča basin (Fumagalli *et al.* 2002), whereas all other populations were found to be introgressed with non-native trout of Danubian and Atlantic

origin (Berrebi *et al.* 2000; Snoj *et al.* 2000). Stocking of brown trout of Atlantic lineage has also occurred in the 5-km-long stretch of the River Jadro in Croatia, which provides suitable habitat for a highly endangered endemic population of softmouth trout *Salmo obtusirostris* (Snoj *et al.* 2007). Population-specific microsatellite allele profiles indicate hybridisation between brown trout of Atlantic lineage and Jadro softmouth trout, pointing to a likely ancient origin (Sušnik *et al.* 2007). This has raised concerns that introductions of brown trout of Atlantic and Danube linage into the river catchment may be leading to the extinction of trout species endemic to the River Jadro but also to the Adriatic basin (Snoj *et al.* 2008; Mrdak *et al.* 2012). Supportive evidence for this can be found in the River Danube basin, that is introgression of alien At1 haplotype of sea trout (Marić *et al.* 2006, 2010, 2012), which caused the loss of intraspecific variability after the introduction of non-native strains and a change in genetic composition of native brown trout stock of Danube lineage (Horváth *et al.* 2014; Simonović *et al.* 2014). Considering the impact of introduced foreign-sourced brown trout on native brown trout stocks in Serbia, brown trout of the Atlantic lineage was found to be the most invasive alien brown trout strain in Serbia (Simonović *et al.* 2015).

Pikeperch *Sander lucioperca* is a widespread species in the River Neretva in neighbouring Bosnia and Herzegovina, where it has been introduced for angling purposes (Trožić-Borovac & Škrijelj 2007; Škrijelj *et al.* 2011). A top predator, the pikeperch, can occupy a higher trophic level relative to other native and non-native predatory species (Kopp *et al.* 2009). In this respect, stomach content analysis of pikeperch in the River Neretva indicated the lack of food competitors, and the aggressive behaviour of the larger-sized individuals exerted negative ecological changes across the river system and represented a threat for autochthonous salmonids and cyprinids (Trožić-Borovac & Škrijelj 2007).

With regard to rainbow trout *Oncorhynchus mykiss*, which achieved a 'moderately high risk' score in the present evaluation, it is widely farmed and stocked for angling purposes across the RA area. Although, in the past, this species was not known to breed in open waters, breeding behaviour was recently reported along with the successful establishment of self-sustaining populations (Povž & Šumer 2005). Also, rainbow trout can have a severe negative impact on other salmonid species through redd superimposition and competition for space and food (see Stanković *et al.* 2015). This may represent another potential threat for native and, especially, endemic species such as trout in the RA area.

The lowest scoring of the assessed species, Neretvan roach, is endemic in Croatian lakes and small rivers near Imotski and in the River Neretva drainage area, which belongs to the Adriatic basin (Mrakovčić *et al.* 2006; Froese & Pauly 2014). Neretvan roach was translocated to Lake Vransko ≈60 years ago (Treer 1989), where it currently represents <0.1% of the total fish biomass (Mrakovčić 2004). Populations in its native area of dis- 11
tribution are seriously threatened (Matić-Skoko *et al.* 2011), and the low score recorded in the present study suggests low establishment potential in new areas (Puntila *et al.* 2013; but see Onikura *et al.* 2012).

The mean scores for all species classified *a priori* as invasive were ranked as 'high risk' *sensu lato* and fell into the 'moderately high risk' subcategory. Mean certainty in response for all species was above the category 'mostly uncertain' and the corresponding CF was comparatively high except for wiper/sunshine bass hybrid, which reflected the paucity of published data for this taxon.

In conclusion, the present results indicate that FISK v2 applied to the RA area, which comprises Croatia and Slovenia, was successful in distinguishing species of high invasiveness risk from those of low-to-medium risk. As such, it can be used as a reliable tool for categorising non-native freshwater fishes and thus informing policy and management decisions with regard to further translocations or introductions of potentially of invasive species. This is of particular relevance to authorities in the formulation of future conservation strategies for the protection of native species in Croatian and Slovenian inland waters.

Acknowledgments

This study was supported by University of Zagreb, Faculty of Agriculture and Slovenian Institute of Natural Sciences. Participation by GHC funded by the UK Department of Environment, Food and Rural Affairs. We thank N. Šprem and A. Gregori for assistance in the initial stages of this investigation. Contribution by LV was through a 2221 Fellowship provided by The Scientific & Technological Research Council of Turkey (TÜBİTAK) and The Department of Science Fellowships & Grant Programs (BİDEB). PS was funded by Grant 173025 of the Ministry of Education, Science and Technological Development of Serbia.

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