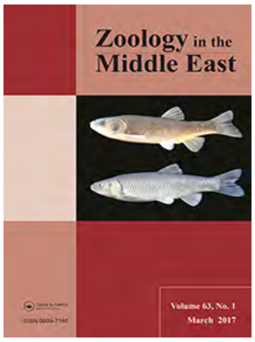

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Risk screening of non-native and translocated freshwater fish species in a Mediterranean-type shallow lake: Lake Marmara (West Anatolia)

Ali Serhan Tarkan[a*], Hasan M. Sarı[b], Ali İlhan[b], Irmak Kurtul[b] and Lorenzo Vilizzi[c]

[a]*Muğla Sıtkı Koçman University, Faculty of Fisheries, Kötekli, Muğla, Turkey;* [b]*Ege University, Faculty of Fisheries, Bornova, İzmir, Turkey;* [c]*Istanbul University, Faculty of Fisheries, Laleli, İstanbul, Turkey*

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Risk screening tools to identify species with a high or low risk of invasiveness are being increasingly used for effective management purposes. Amongst the available tools, the Fish Invasiveness Screening Kit (FISK) has been used extensively and successfully in large risk assessment (RA) areas, and was recently upgraded to the new generic tool Aquatic Species Invasiveness Screening Kit (AS-ISK). The aim of the present study was to assess with AS-ISK the invasive potential of introduced non-native and translocated fishes in a Mediterranean-type shallow lake (Lake Marmara) located in west Anatolia (Turkey). Based on independent evaluations of 35 species by two assessors, calibration of AS-ISK resulted in a threshold score of -3.65, which reliably distinguished between potentially invasive (high risk) and potentially non-invasive (medium to low risk) fishes. Of the 35 species assessed, 17 were categorised as ‘low risk’ and included native/endemic and translocated natives, and the remaining 18 as ‘high risk’ and comprised non-natives and translocated natives. *Carassius gibelio* had the highest score in the Climate Change Assessment section, suggesting that it might potentially impact on the native fish fauna under likely climate change scenarios for the RA area. Some cool water non-native (*Oncorhynchus mykiss*, *Salvelinus fontinalis*), translocated native (*Luciobarbus lydianus*) and endemic species (*Ladigesocypris mermere*) in the catchment will likely be affected negatively by predicted climate change conditions.

Keywords: AS-ISK; endemic species; biological invasions; shallow lakes

Introduction

With some 320 fish species of which 215 endemic (Freyhof et al., 2014), Turkey is an important biodiversity hotspot for inlands waters and a natural corridor for the distribution of species at both the endemic and global scale (Tarkan, Marr, & Ekmekçi, 2015). However, biological invasions are becoming an increasingly major threat to biodiversity worldwide (Copp et al., 2005), and this is true also for Anatolia (Freyhof et al., 2014), which is subject to introductions of non-native freshwater fishes (Tarkan et al., 2015). This problem is exacerbated by the translocation of native fish species either accidentally or intentionally (i.e. through government-led initiatives) for fisheries improvement, aquaculture and biological control (Gaygusuz et al., 2015).

Currently, risk screening tools are being increasingly used to predict the invasiveness of species introduced into new ecosystems (Copp et al., 2016). Amongst the available tools, one that has been used extensively and successfully for freshwater fishes is

*Corresponding author. Email: serhantarkan@gmail.com

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the Fish Invasiveness Screening Kit (FISK: Copp et al., 2009). FISK consists of 49 questions within two main sections (i.e. Biogeography/History and Biology/Ecology) and provides an outcome score with which to categorise the potential risk of a species being invasive as ‘low’, ‘medium’, or ‘high’. Recently, FISK has been upgraded to the Aquatic Species Invasiveness Screening Kit (AS-ISK), which incorporates the ‘minimum requirements’ for the assessment of species with regard to the recent EU Regulation on the prevention and management of the introduction and spread of invasive alien species. AS-ISK is a major improvement over FISK due to its applicability to all aquatic organisms (i.e. marine, brackish and freshwater) and the possibility to incorporate assessments based on climate change scenarios (Copp et al., 2016).

In Turkey, both FISK and AS-ISK have been used successfully for the screening of non-native and translocated fish species at the broader (regional) scale of Thrace and Anatolia (Tarkan, Ekmekçi, Vilizzi, & Copp, 2014; A. S. Tarkan, L. Vilizzi, N. Top, F. G. Ekmekçi, P. D. Stebbing, G. H. Copp, unpubl.). However, there are approximately 200 natural lakes in Turkey with different ecological (e.g. rich endemic fish faunas) and geomorphological features, and many of them are located in densely-populated areas. It is therefore expected that the native/endemic fish faunas of these lakes can be affected to a different extent by the introduction of non-native fish species. Clearly, there is a need to assess the risks posed by non-native fishes at local geographical scales, particularly in those natural lakes with relatively rich endemic fish faunas (e.g. Ferincz et al., 2016).

One of these water bodies is Lake Marmara, which is a medium-size shallow Mediterranean lake inhabited by five endemic and three non-native and translocated fish species. Given that further introductions into the lake’s catchment are highly likely and its endemic fish diversity is currently under threat by the non-native and translocated fish species already present, there is a pressing need to identify which other species are likely to pose a risk to the lake’s ecosystem. The aim of the present study therefore was to assess the invasive potential of introduced non-native and translocated fishes in the Lake Marmara Basin (LMB) using the recently developed AS-ISK tool, and to evaluate the applicability of AS-ISK to a smaller risk assessment (RA) area compared to the larger country or regional-scale levels at which it has been recently applied (Copp et al., 2016). Notably, the outcomes of the present study are expected to assist local environmental managers and stakeholders in the implementation of suitable policies for the prevention, management and containment of potential, existing and future undesired translocations of freshwater fishes.

Material and Methods

Study area. Lake Marmara is a Mediterranean-type shallow lake located in Manisa Province in north-west Anatolia (38°37'N, 28°04'E). The lake is 12 km long and 6 km wide, is at 75 m a.s.l, and it has a surface area of 4500 ha and an average depth of approximately 5 m (Ustaoğlu, 1989). Lake Marmara is an alluvial-set lake that belongs to the alluvial valley of the Gediz River; it is shared between Gölmarmara county to the northwest and Salihli county to the southeast, and represents an important location for fishery and irrigation activities, and also an Important Bird Area with 144 bird species (Gül, 2008). The Gediz River Basin, in which Lake Marmara is located, has a predominantly continental climate with some areas characterised by a Mediterranean climate (Peel, Finlayson, & McMahon, 2007). The freshwater fish fauna of Lake Marmara consists of 20 species, of which several are endemic, non-native and translocated, and with the Cyprinidae representing the most abundant family (İlhan & Sarı, 2011).

The İzmir Minnow *Ladigesocypris mermere* (Ladiges, 1960) lives exclusively in this lake, whereas Bergama Barb *Capoeta bergamae* Karaman, 1969, İzmir Nase *Chondrostoma* *holmwoodii* (Boulenger, 1869), and Küçük Menderes Spined Loach *Cobitis fahirae* Erk'akan, Atalay-Ekmekçi & Nalbant, 1998, and Marmara Goby *Knipowitschia mermere* Ahnelt, 1995 belong to the Gediz River System and West Anatolia (İlhan & Sarı, 2011). The lake is also inhabited by three non-native fish species of which Gibel Carp *Carassius gibelio* (Bloch, 1782) and Topmouth Gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846) have been introduced unintentionally, whereas Eastern Mosquitofish *Gambusia holbrooki* Girard, 1859 has been stocked for mosquito control. The three translocated fish species found in the lake are Big-scale Sand Smelt *Atherina boyeri* Risso, 1810, Common Carp *Cyprinus carpio* Linnaeus, 1758, and Pikeperch *Sander lucioperca* (Linnaeus, 1758).

Data analysis. The fish species included for risk screening were selected based on five criteria: 1- Non-native species already present in LMB; 2- Non-native species currently not present in LMB but established in nearby areas of Turkey or neighbouring countries; 3- Translocated species already present in LMB; 4- Translocated native species not present in LMB but established in nearby areas of Turkey; 5- Native species already occurring in LMB. All of the 35 species eventually selected based on the above criteria were assessed by two of the authors (H.M.S. and A.İ.), who are knowledgeable in the freshwater fish fauna of LMB. Of the selected species, three (8.6%) corresponded to criterion 1, six (17.1%) to criterion 2, three (8.6%) to criterion 3, twelve (34.3%) to criterion 4, and eleven (31.4%) to criterion 5.

Assessments were made with the AS-ISK software application (available at www.cefas.co.uk/nns/tools) and involved collection of species-specific literature information on biogeographical/historical traits (AS-ISK Section 1) and biological/ecological characteristics (AS-ISK Section 2). In retrieving this information, priority was given to peer-viewed publications, with Internet databases, thesis dissertations and, occasionally, reports used whenever necessary. For the questions on climate change (AS-ISK Section 3), a relevant study on likely future climate scenarios for Turkey was used (Demir, Kılıç, & Coşkun, 2008). Notably, most scenarios predict an increase of 0.5–1°C in air temperature in the next 50 years, from which water temperature was calculated based on the relationship between water temperature (T_w) and air temperature (T_a) (after Erickson & Stefan, 1996): $T_w = 3.47 + 0.898 x T_a$. For each AS-ISK assessment two separate scores are eventually produced, namely a Basic Risk Assessment (BRA) score and a Climate Change Assessment (CCA) score (Copp et al., 2016).

Receiver Operating Characteristic (ROC) analysis (Bewick, Cheek, & Ball, 2004) was used to assess the predictive ability of AS-ISK to discriminate between species posing a 'high risk' (hence, categorised as invasive) and those posing a 'low risk' of being invasive (hence, categorised as non-invasive). Species were categorised *a priori* in terms of their perceived invasiveness (i.e. invasive or non-invasive) and protection status (i.e. conservation concern category: Least Concern, Near Threatened, Vulnerable, Not Evaluated) based on information retrieved from the Invasive Species Specialist Group database (www.issg.org) and FishBase (Froese & Pauly, 2016). 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 AS-ISK tool as such. A measure of the accuracy of the calibration analysis is the area Area Under the 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 AS-ISK to differentiate between invasive and non-invasive species.

The two assessors carried out independently their assessments on all 35 species. As a result, separate ROC curves were initially generated for the two assessors and differences between the corresponding AUCs were statistically tested (Mann-Whitney U-statistic: $\alpha = 0.05$; applet StAR available at http://protein.bio.puc.cl/star/home.php: Vergara, Norambuena, Ferrada, Slater, & Melo, 2008). Following ROC analysis, the best AS-ISK threshold (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. Additionally, a smoothed mean ROC curve was generated and boot-strapped confidence intervals of specificities were computed along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). Analyses were carried out with package pROC for R x64 v3.0.3 (R Development Core Team, 2015) using 2000 bootstrap replicates. Also, as each response of AS-ISK for a given species is allocated a confidence level (1 = low; 2 = medium; 3 = high; 4 = very high), a confidence factor (CF) was computed as: $\sum(CQ_i)/(4 \times 55)$ ($i = 1, \ldots, 55$), where CQ_i is the certainty for question *i*, 4 is the maximum achievable value for certainty (i.e. 'very certain') and 55 is the total number of questions comprising the AS-ISK tool. The CF therefore ranges from a minimum of 0.25 (i.e. all 55 questions with certainty score equal to 1) to a maximum of 1 (i.e. all 55 questions with certainty score equal to 4).

For further 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, very high). The CFs for the 35 species were also compared between assessors by a one-way permutational univariate analysis of variance (PERANOVA), using a Euclidean dissimilarity measure following normalisation and $n = 9999$ permutations of the raw data (PERMANOVA+ for PRIMER v6: Anderson, Gorley, & Clarke, 2008). All statistical tests were at $\alpha = 0.05$.

Results

As the AUCs from the two assessors did not differ significantly ($P = 0.759$) (Figure 1, left) a combined ROC curve was computed resulting in an AUC of 0.947 (0.864–1.000 95% CI), hence well above 0.5 (Figure 1, right). This indicated that AS-ISK was able to discriminate reliably between invasive and non-invasive species for the LMB. Youden's *J* and the closest point statistics provided the same best threshold of -3.65, which was chosen as the calibration threshold of the AS-ISK risk outcomes for the RA area under study (see Annex). This threshold allowed to distinguish between 'low risk' species, with BRA scores within the interval [-20, -3.65[, and 'high risk' species, with scores within [-3.65, 68] (note the reverse bracket notation indicating an open interval).

Based on the above threshold and corresponding intervals, 17 (48.6%) of the 35 species assessed were categorised as 'low risk' and the remaining 18 (52.4%) as 'high risk'. The highest-scoring species (BRA score ≥ 20) were non-native *C. gibelio*, Western Mosquitofish *Gambusia affinis* (Baird & Girard, 1853), *G. holbrooki* and *P. parva*, and translocated *A. boyeri*, North African Catfish *Clarias gariepinus* (Burchell, 1822), *C. carpio* and *S. lucioperca*; the lowest-scoring species were Kuru's spined loach *Cobitis kurui* Erk'akan, Atalay-Ekmekçi & Nalbant, 1998 and 'Lesbos Stone Loach' *Oxynoemacheilus theophilii* Stoumboudi, Kottelat & Barbieri, 2006 (see Annex).

Climate Change Assessment resulted in a decrease in the CCA relative to the BRA score for 22 species, in an increase for nine species and in no change for the reaming four (Annex). The maximum achievable score of 12 for this Section was achieved for non-native *C. gibelio* (which also scored highest in the BRA), and other score increases resulted for the warm-water species *C. gariepinus*, *G. affinis*, *G. holbrooki*, Silver Carp *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Lepomis gibbosus* (Linnaeus, 1758), and *P. parva*. The highest decrease in CCA relative to the BRA score was observed for species that thrive in cool waters (i.e. rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and brook trout *Salvelinus fontinalis* (Mitchill, 1814)), for endemic *C. kurui* and *L. mermere*, and for some translocated/non-native species (i.e. Bleak *Alburnus alburnus* (Linnaeus, 1758), Grass Carp *Ctenopharyngodon idella* (Valenciennes, 1844) and Monkey Goby *Neogobius fluviatilis* (Pallas, 1814)) (see Annex).

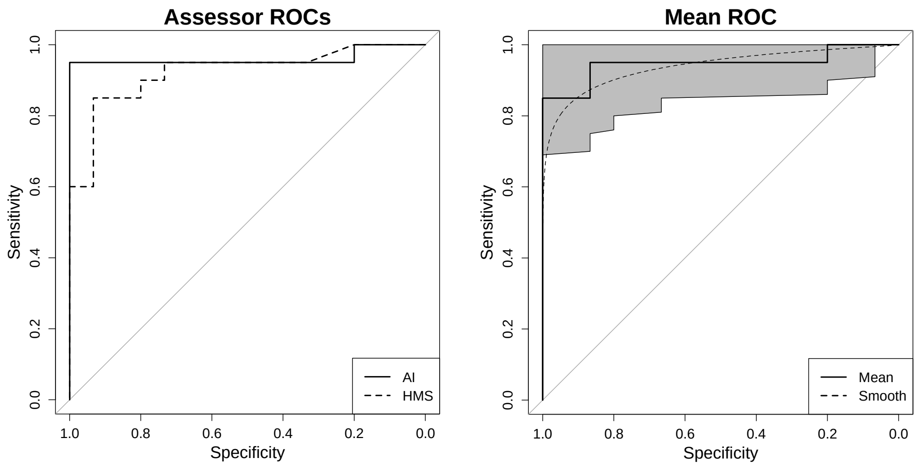

Figure 1. Left: Receiver operating characteristic (ROC) curves for two assessors (initials of the first two authors) for 35 fish species evaluated with the Aquatic Species Invasiveness Scoring Kit (AS-ISK) for the Lake Marmara Basin. Right: Mean ROC curve based on mean scores from the two assessors, with smoothing line and confidence intervals of specificities (see Annex).

All invasive species classified *a priori* as Least concern, Near threatened, Vulnerable or Not evaluated attracted scores within the 'high risk' category (Figure 2). On the contrary, all non-invasive species classified *a priori* as Near threatened, Critically endangered, Vulnerable or Not evaluated were ranked as 'low risk', except for the Least concern group that fell in the 'high risk' category (Figure 2). Mean confidence in assessments was 2.11±0.03 (hence, above the category 'mostly uncertain'), and the CF was 0.528±0.01, ranging from a minimum of 1.96±0.01 (CF: 0.49 ±0.01) for *S. fontinalis* to a maximum of 2.38±0.01 (CF = 0.60 ±0.02) for European Eel *Anguilla anguilla* (Linnaeus, 1758). Finally, coincidence rate between the two assessors was 74.2%, and there was a significant difference in mean CF values between the first one (A.İ.) (0.54±0.03) and the second one (H.M.S.) (0.52±0.02) (permutational $F_{1,68}$ = 8.34, P=0.0045).

Discussion

Similar to a recent, broad-scale risk assessment study for Turkey (A. S. Tarkan et al., unpubl.), the present results indicate that AS-ISK could reliably discriminate between invasive and non-invasive species at the smaller scale of the LMB. The mean threshold value of -3.65 was considerably lower than the previous FISK and AS-ISK thresholds for Turkey of 23 and 28, respectively (Tarkan et al., 2014; A. S. Tarkan et al., unpubl.), but also relative to other FISK thresholds elsewhere (Piria et al., 2016). The same was true for the only other smaller-scale FISK assessment currently available (Lake Balaton catchment, Hungary), which yielded a threshold of 11.4 (Ferincz et al., 2016). The lower threshold values for the LMB could be attributed to the climate of the area, but also to species-specific features, namely the higher number of local and endemic species screened in the current assessment. These species are either native to Turkey or to neighbouring regions of the RA area, but have been introduced (i.e. translocated) outside their native range within the RA area, and this might render them less invasive compared to non-native species introduced from other continents. Similarly, a low proportion of translocated species has been considered one main reason for threshold differences amongst RA areas (Almeida, Ribeiro, Leunda, Vilizzi, & Copp, 2013; Tarkan et al., 2014). And, another reason for the lower threshold detected for the LMB could be related to the smaller area under investigation compared to the much larger scale of other risk assessments (see Copp, 2013).

In the present study, there was an overall balance in the proportion of low and high risk species. Cyprinids, North African clariid catfishes and poeciliids were the highest scoring – an outcome similar to previous studies elsewhere (Almeida et al., 2013; Tarkan et al., 2014; Piria et al., 2016). The highest-scoring species in the present study was *C. gibelio*, similar to previous FISK assessments for Asia and Europe. This species is possibly the most abundant invasive freshwater fish in Turkey and possesses a very high risk to its freshwater biodiversity (Tarkan et al., 2012, 2014). After its first report from the Thrace region in the 1980s, *C. gibelio* has become widespread across Anatolian lakes including Lake Marmara (Tarkan et al., 2012). Also, similar to other lakes of the region, millions of eggs, larvae and juveniles of *C. carpio* are stocked into Lake Marmara on an annual basis to support fishing through government-led initiatives. However, this practice carries the risk of accidental, concomitant introductions of non-native species (Gaygusuz et al., 2015). In the case of Lake Marmara, and similar to other lakes of the region, this is believed to have been one pathway of introduction for *C. gibelio* and *P. parva*.

Lepomis gibbosus, another high-risk scoring, non-native species, has not yet been found in the LMB. However, its presence in the adjacent Gediz Basin (which is part of the RA) makes it highly likely to be introduced into the LMB in the near future. Conversely, *C. carpio*, despite its yielding a very high score of being invasive, remains a highly-demanded species, and is not only the main target of fishermen but also the most commonly-reared fish species in hatcheries across the country (Gaygusuz et al., 2015). Yet, *C. carpio* is one of the least caught fish species in Turkish lakes, including artificial reservoirs where it is continuously stocked (Gaygusuz et al., 2015). For this reason, the risk status of this species should be further investigated at the country and/or regional level, especially as regards its nativeness in some areas (Vilizzi, 2012). This fuzziness surrounding the role of *C. carpio* is also evidenced by its current *a priori* classification as both ‘vulnerable’ (in its original wild form) and ‘invasive’ (in its feral/domesticated form) (see Annex).

The other two high-scoring species, namely the mosquitofishes *G. affinis* and *G. holbrooki*, were probably first introduced in Turkey in the 1920s into Lake Amik for the control of malaria and they probably represent the first deliberate non-native freshwater fish introductions in the country (Tarkan et al., 2015). However, recent studies carried out in similar geographic areas (cf. Iberian Peninsula) suggest that these species may cause negative impacts on endemic fish faunas (Carmona-Catot, Magellan, & García-Berthou, 2013), whilst exerting almost no pressure on mosquito larvae (García-Berthou, 1999). The real status of these species in Turkey is still unknown, so further studies are needed on their distribution and possible impacts to native freshwater faunas.

The clariid Catfish *C. gariepinus*, which was identified in the present study as another high-risk species, occurs in several ecoregions and hot springs of Turkey, where water temperatures are always high enough for its survival (Emiroğlu, 2011). However, similar catfish species have expanded their distributional range in Europe, and these include Black Bullhead *Ameiurus melas* (Rafinesque, 1820). This species is known be tolerant of pollution and low dissolved oxygen levels (Novomenská & Kováč, 2009), and its omnivorous and highly aggressive behaviour make it a high risk species for Turkey. Arguably, catfishes may also pose a considerable risk to the LMB.

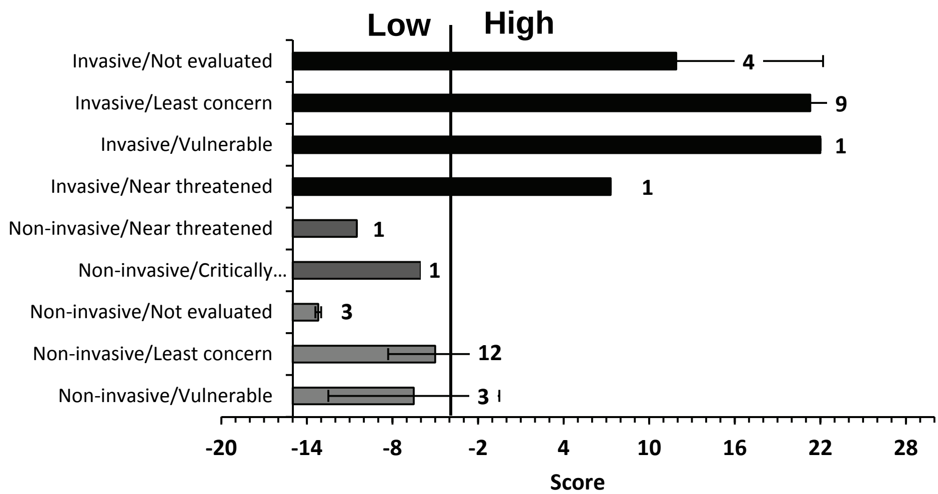

Figure 2. Mean scores (±SE and *n*) for 35 freshwater fish species assessed by AS-SK for Lake Marmara Basin and ranked according to their *a priori* invasiveness and protection status. See Annex for risk levels.

Although generally ignored, nationally translocated species (*sensu* Copp et al., 2005) have been shown to pose risks of comparable extent to those from non-native or invasive species (Copp et al., 2005; Tarkan et al., 2014, 2015; Piria et al., 2016). This was suggested in the present study by the high risk scores for a number of translocated species such as *A. boyeri*, Northern Pike *Esox lucius* Linnaeus, 1758, Eurasian Perch *Perca fluviatilis* Linnaeus, 1758, *S. lucioperca* and Tench *Tinca tinca* (Linnaeus, 1758). *S. lucioperca* is a widely-distributed native species in Turkey, but occurs in the LMB as a translocated species. It is known to be an active predator, which could make it very threatening to the rich endemic fauna of Lake Marmara. On the other hand, some other high-scoring, translocated species such as *A. boyeri* and *T. tinca* would require further investigation as to their likely impacts on native faunas and ecosystem functioning.

Based on Climate Change Assessments (CCA), the highest BRA-scoring species *C. gibelio* also achieved the highest CCA score, and was followed by other high risk species such as *G. affinis*, *G. holbrooki*, *L. gibbosus*, *P. parva* and tropical North African Catfish *C. gariepinus*. The potential impact of these species is expected to increase under the conditions of climate change predicted for Turkey (Demir et al., 2008), especially in relation to their risks of entry, establishment, dispersal and competition with native/endemic species. Conversely, a decrease in CCA scores suggested that projected increases in water temperature might have a negative effect, and in the present study this applied mainly to local and endemic species (with the notable exception of Gediz Shemaya *Alburnus battalgilae* Özuluğ & Freyhof, 2007).

Coincidence rate between the two assessors in the present study was higher (74%) than that obtained in previous FISK assessments for Turkey (71%: Tarkan et al., 2014) and Iberia (69%: Almeida et al., 2013). Regardless, in all of these studies (including the present one) there were significant differences amongst assessors in certainty of responses. This finding supports the recommendation by Almeida et al. (2013) and Tarkan et al. (2014) that each species should be assessed by several independent assessors to get the most accurate and representative assessments for a particular RA area.

In conclusion, this AS-ISK based assessment for a relatively small and isolated catchment has proved useful for evaluating the potential invasiveness of some freshwater fish species and has also emphasised the importance of the potential risks associated with lesser-known translocated species. Undoubtedly, the outcomes of this study will become more valuable if tighter collaboration is established between fish biologists, fisheries scientists and environmental managers/stakeholders, in a joint effort to ensure better conservation and management actions with regard to the role of non-native fishes in inland waters of Turkey. The results obtained from the employment of risk identification tools such as AS-ISK could be used to prioritise actions and implementation of measures to prevent the further spread and/or introduction of species identified as 'high risk'. This could be very important for a country such as Turkey, where the impacts of non-native species are usually underestimated in the absence of necessary basic biological/ecological information. Further, the development and adoption of proper risk assessment schemes is strongly encouraged given not only the economic relevance of fisheries across the country (Turkish Statistical Institute, 2014) but also current efforts to align with EU Regulation for the management of non-native species entry and control (e.g. European Union Regulation, 2014).

Supplementary Material

The Annex is given as a Supplementary Material, which is available via the "Supplementary" tab on the article's online page (http://dx.doi.org/10.1080/09397140.2017.1269398).

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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