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Applicability of Fish Risk Assessment (FISK) to ornamental species

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Dissertação
Mestrado em Biologia da Conservação

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Orientadores: Professora Doutora Filomena Magalhães

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Abstract

A third of the world's worst aquatic invasive species are ornamental species, with about 90% being freshwater fishes. It is thus important to identify undesirable ornamental species before they can spread and become established in natural environments. This study analyses the applicability of the Fish Invasiveness Screening Kit (FISK) in assessing the invasion risk of the 40 freshwater ornamental fishes most commonly sold in Lisbon and explores whether FISK can be used in conservation management. The response rate to FISK questions and the certainty of answers were high, evaluations performed by independent assessors were largely consistent, and there was little variation between FISK and IRI. The certainty level was positively correlated with FISK scores, indicating that FISK may perform better when there is more information available to support the assessment. Also FISK can be superior to IRI as it produces an immediate score which makes it easy to read by managers. FISK scores ranged between 0 and 38, covering all risk categories. Nine species were considered to have a high risk of invasiveness in Iberian Peninsula, namely *C. auratus, C. carpio, H. plecostomus, P. reticulata, X. helleri, X. maculatus, T. trichopterus, P. sphenops* and *D. rerio*. Moreover there was a positive correlation between FISK scores and the Frequency of Occurrence (FO%) and the Numeric Frequency (FN%) in the aquarium stores in Lisbon, indicating that species most popular in stores tended to have the highest FISK scores. FISK assessments should be repeated through time for strengthening species assessment, with multiple evaluations allowing the identification and filling of current gaps. This tool should be integrated in environmental programs for non-native species because is very easy to apply, has a low cost and is also very effective even when low amount of species information is available.

KEY-WORDS: aquatic invasion; conservation management; Iberian Peninsula; non-native freshwater fish; ornamental trade;

RESUMO

Os peixes estão entre os vertebrados mais introduzidos no mundo. A aquariofilia é actualmente um dos principais vectores para a introdução de peixes não-nativos na Europa. Um terço das espécies aquáticas invasoras são espécies de aquariofilia sendo 90 a 96% dos indivíduos desta indústria peixes dulçaquícolas. Os impactos potenciais causados por estas espécies não-nativas são enormes, quer em termos ecológicos e quer em termos económicos. A Bacia Mediterrânica é um hotspot de biodiversidade, e em particular a Península Ibérica, estando a fauna piscícola desta região entre as mais ameaçadas globalmente, com mais de 70% das espécies listadas com algum nível de ameaça, devido aos impactos causados pelas espécies não-nativas. Actualmente, cerca de 19% das introduções de peixes dulçaquícolas na Península Ibérica estão associadas à aquariofilia, por exemplo, espécies como *Poecilia reticulata, Astronotus ocellatus* e *Xiphophorus helleri* foram introduzidas na Península Ibérica devido a este vector humano. A introdução de espécies não-nativas pode ser reduzida através de três ações essenciais: erradicação, controlo ou prevenção. A prevenção parece ser a mais efectiva pois apresenta um menor custo comparativamente à erradicação ou ao controlo de espécies já existentes. Uma medida de prevenção passa por usar ferramentas de avaliação de risco como o *Fish Invasiveness Screening Kit* (FISK), de forma a identificar espécies não-desejáveis (ex. “listas negras”) antes que estas sejam importadas e se estabeleçam na área recipiente. Apesar do FISK ter sido usado para avaliar o risco de invasão de espécies de peixes não-nativas em várias regiões geográficas (ex. Austrália, Florida, Japão, Belarússia, UK) nunca foi aplicado em espécies de um vector tão importante como a aquariofilia. Avaliações anteriores do risco de invasão de espécies não-nativas na Penínusa Ibérica focaram-se também em espécies já introduzidas, negligenciando espécies actualmente vendidas em aquariofilia. Este estudo analisa a aplicabilidade do FISK como ferramenta para avaliar o risco de invasão das 40 espécies de peixes dulçaquícolas de aquariofilia mais vendidas nas lojas de aquariofilia em Lisboa (Portugal) considerados num trabalho anterior e explica de que forma o FISK pode ser usado na gestão da conservação. A taxa de resposta às questões do FISK e a certeza das respostas foram elevadas, as avaliações efectuadas por avaliadores independentes foram em grande parte consistentes e houve pouca variação entre o FISK e o *Iberian Risk Index* (IRI). O nível de certeza apresentou-se positivamente correlacionado com os valores de FISK, indicando que quanto maior o valor de FISK maior o nível de certeza associado. Isto pode ser atribuído ao facto das espécies com baixos valores de FISK (valores entre -15 e 18) terem menos estudos e bibliografia disponíveis, o que significa menor confiança na resposta dada, contrariamente a espécies com valores mais elevados de FISK que apresentam um maior leque

de informação, o que resulta em respostas mais completas e com maior certeza. Desta forma, o FISK pode ser mais eficazmente executado se houver mais informação disponível para suportar a avaliação das espécies. O FISK parece ser também superior ao IRI pois produz um valor imediato de risco de invasão, o que faz com que seja de fácil interpretação por gestores. Os valores de FISK variaram entre 0 e 38 (dentro de um universo entre -15 e 57) e abrangeram todas as categorias de risco de invasão desde a categoria de baixo risco (LR) até à categoria de risco muito elevado (VHR). Apesar da maioria das espécies avaliadas (77.5%) pertencer às categoria de risco mais baixas, nove espécies foram consideradas como tendo um elevado risco de invasão na Península Ibérica, nomeadamente *C. auratus, C. carpio, H. plecostomus, P. reticulata, X. helleri, X. maculatus, T. trichopterus, P. sphenops* e *D. rerio*. As espécies para as quais se obtiveram os maiores valores de FISK para a categoria de risco mais elevada (*C. auratus* e *C. carpio)* são também já consideradas espécies invasoras na Península Ibérica, causando perturbações elevadas no ambiente recipiente. Apesar de algumas espécies com risco elevado de invasão não estarem estabelecidas com sucesso na Península Ibérica (ex. *H. plecostomus, X. maculatus*, *T. trichopterus*, *P. sphenops* e *D. rerio)* o seu controlo deve ser tido em conta de forma a evitar o seu estabelecimento pois apresentaram algumas características biológicas propícias à sua invasão, por exemplo são capazes de se reproduzir em climas semelhantes aos encontrados na Penísula Ibérica. Além disso, houve uma correlação positiva entre os valores de FISK e a Frequência de Ocorrência (FO%) e a Frequência Numérica (FN%) nas lojas de aquariofilia em Lisboa, indicando que as espécies mais populares nas lojas tendem a ter os valores mais elevados de FISK. As relações foram particularmente evidentes para as espécies mais frequentes nas lojas (FO>80%) classificadas dentro das categorias de risco mais elevadas no FISK, como *C. auratus, P. reticulata, X. helleri, X. maculatus, P. sphenops* e *D. rerio.* Desta forma, as espécies que têm uma elevada probabilidade de se estabelecer com sucesso na Península Ibérica são também as espécies que são mais frequentemente vendidas em grandes quantidades nas lojas, o que pode resultar numa elevada pressão de propágulo e colocar maiores riscos de impacto no ecosistema recipiente. Uma vez que o risco de introdução destas espécies através do descarte humano é elevado nestas espécies, é essencial o controlo do seu comércio para evitar futuras introduções não-desejáveis na Península Ibérica. Este controlo deve também ser extendido a *C. carpio, H. plecostomus* e *T. trichopterus* pois pois apesar de apresentarem um baixo risco de libertação nos ecossistemas (FO% <0.80%), tiveram um elevado risco de invasão de FISK. As avaliações do FISK são essenciais na avaliação de risco de invasão das espécies e devem ser repetidas ao longo tempo de forma a fortalecer estas avaliações, com avaliações múltiplas (pelo menos dois avaliadores) permitindo a identificação e o preenchimento de lacunas existentes. Um elevado nível de questões não-

respondidas e um aumento da incerteza para uma espécie deverá motivar decisores ambientais a adoptarem uma abordagem preventiva devido ao baixo nível de confiança na avaliação, devendo esta ser uma prioridade. Avaliações futuras devem também ter em atenção a avaliação de espécies que toleram temperaturas mais quentes de forma a identificar possíveis espécies com um elevado risco de invasão, tendo em conta os cenários futuros actuamente previstos para o clima na Península Ibérica (ex. *H. plecostomus, T. trichopterus, P. sphenops* e *D. rerio*). A alteração de habitats ribeirinhos através da construção de barragens poderá contribuir para um aumento do risco de invasão uma vez que os regimes térmicos da água são alterados. Apenas espécies com um baixo risco de invasão deverão ser incluídas numa “lista-branca” de espécies, desta forma autorizando a comercialização em lojas de aquariofilia. Estratégias para a prevenção da introdução de novas espécies não-nativas, como a preparação de listas de espécies com elevado risco de invasão utilizando o FISK, a implementação de métodos alternativos de descarte de espécies não-desejáveis e a educação das pessoas envolvidas na aquariofilia são as ações potencialmente mais eficazes. O FISK deverá ser integrado em programas de avaliação de risco para espécies não-nativas pois a sua aplicação é bastante fácil, tem um custo baixo, sendo muito eficiente mesmo quando está disponível pouca informação sobre as espécies ornamentais.

PALAVRAS-CHAVE: invasão aquática; gestão da conservação; Península Ibérica; peixes dulçaquícolas não-nativos; aquariofilia;

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1 – INTRODUCTION

Non-native species are recognized as one of the leading threats to native biodiversity and ecosystem function (1). The ornamental species industry is among the most important vectors for the introduction of non-native species and has significantly increased the risk of intentional and unintentional introductions worldwide (2, 3, 4). The ecological and economic costs pose by the introduction of non-native species are very high (5, 6, 7) and can be reduced through three main actions: eradication, control and prevention (6). Among these three actions, prevention appears to be the most cost effective because the costs of both eradication and control largely exceed those of prevention (2). Prevention measures include environmental education, increase stakeholder awareness and risk assessment. The risk-assessments are key to identify new invaders before they can spread and become established (8). Indeed risk assessments may identify undesirable species to be included in black lists being highly recommended to the aquarium trade since many non-native ornamental species pose risks of invasion (being an invasive species a species that can establish successfully in the new environment and cause severe ecological damages to it) if introduced in natural aquatic systems (9).

Fishes are among the most introduced vertebrates in the world (10) and the aquarium industry is currently one of the main vectors for introductions of non-native fishes (11). Despite research on fish invasions is increasing and the urgency to identify new potential invaders brought by main vectors is clear, risk assessment of non-native fishes remains limited (12).

The identification of which species pose the highest invasiveness risk is often difficult (13) and the available screening tools must be adapted to provide reliable information for particular species groups (14). Screening of invasion risk for ornamental fishes is challenging, given ecological knowledge is often limited and highly efficient screening tools are thus required to evaluate these species. Nevertheless, this would greatly aid managers in determining the potential risks presented by ornamental species (15) and developing pro-active measures to avoid and control their introduction in the wild (16).

1.1 – Global trade of ornamental freshwater fishes

The number of species introduced worldwide has more than doubled in the last three decades with fishes listing among the most introduced aquatic animals in the world (10, 11). The global pathways of introduction of fishes are from aquaculture (51%), ornamental industry (21%), sport fishing (12%) and fisheries (7%) (11).

Aquarium keeping is amongst the most popular of hobbies with millions of enthusiasts (5, 7) supporting a $25 billion-per-year worldwide industry (3). This industry is growing by 14% annually worldwide and pet industry surveys have estimated the aquarium industry to worth over $1,000 million (3). A third of the world's worst aquatic invasive species are ornamental species with 90-96% of the individuals in the trade being freshwater fishes (12). European Union is considered to be the largest market for ornamental fish species (17, 18). Despite invasion by ornamental fishes is a major threat to native fishes it has received less attention than other vectors such as angling (2).

Ornamental fishes are introduced into recipient waters mainly as a disposal method apparently perceived as more humane than various euthanasia options with the most popular species probably being the most frequently introduced (18). In this way the popularity of a species can be a good indicator of propagule pressure (2) which is a measure of the number of individuals introduced and the frequency of their introduction (19).

For an effective prevention of the introduction of ornamental fishes, managers need to have some knowledge about which species are being introduced and which can be successfully established, so that they can target their efforts towards the ornamental species that pose a higher risk of introduction (16, 20).

1.2 – Fish introductions in the Mediterranean Basin

The Mediterranean Basin is a biodiversity hotspot (21, 22) including about 250 endemic freshwater fishes (23), representing about 1% of the world species (10). The fish fauna of the region is also among the most threatened worldwide, with more than 70% of the species listed as endangered or already extinct (24). Concurrently, the Mediterranean Basin is one of the six global invasion hotspots regions (22, 25). Indeed, non-native species are currently the major threat to Mediterranean fish fauna, followed by water pollution, extraction, damming, agriculture and fishing (26, 27).

In particular, the freshwater fish fauna of the Iberian Peninsula (IP) has a high rate of endemics and includes 59 species with some threat level (28, 29). Since the XXth century freshwater fish introductions increased substantially in IP (30, 31) and this has been associated with the loss of native biodiversity (25, 26). About 19% of freshwater fish introductions in IP are associated with aquarium trade (2). Moreover, the trade of ornamental freshwater fish species is growing in IP, with several species sold in the market becoming potential new invaders in rivers (2). For example, species like *Poecilia reticulata* Peters 1859, *Astronotus ocellatus* (Agassiz, 1831) and

Xiphophorus helleri Heckel, 1848 were introduced in IP through aquarium trade which emphasizes the need for prevention tools like risk assessments in this region (26, 32).
Previous evaluations of the invasion risk of non-native freshwater fish in IP focused on species already introduced (13, 33), largely neglecting species currently sold in aquarium trade. Risk assessment tools should thus urgently be used to evaluate species in the aquarium industry of the region, so that more effective measures to avoid their introduction in the wild can be taken.

1.3 – Risk Assessment of non-native fish species

Three approaches have been developed for assessing potential invasiveness in fish and were already used in IP: the invasive fish biological trait profiling (hereafter Fish Profiling), the Iberian Risk Index (IRI, 13) and the Fish Invasiveness Screening Kit (FISK) (33, 34) adapted from the Weed Risk Assessment (35).
Fish Profiling is a quantitative approach that was first developed to predict potential fish invaders to the Great Lakes based mainly on biological characteristics of successful and failed invaders (8). This is a quantitative approach that requires a large knowledge of the species biological traits (1, 14) and strong statistical analysis. However it does not assigns a final score to each species and focus more on the arrival and establishment stages rather than the spread and impact of non-native species. Fish Profiling was previously used to assess non-native fishes more common in Iberian watersheds (36).
The Iberian Risk Index (IRI) is also a quantitative approach that was specifically developed to identify likely future introductions in the IP (13, 33). This region-specific procedure covers all sequential stages of introduction, including spread and impact and three biological traits, namely relative growth, number of taxa in diet and minimum temperature. The procedure is dependent on detailed knowledge of species biological traits and the factors that determine its invasion success, requires strong statistical analyses but, unlike the fish profiling, assigns a final score to each species facilitating interpretation by stakeholders and managers (33).
Conversely, FISK is a semi-quantitative method that primarily requires expert judgment. The tool is easy to apply (37), accounts for all introduction stages (34) and species can still be evaluated even in the absence of detailed ecological information (38). Moreover, this tool produces an immediate score of the invasiveness risk for each species which makes it easy to read by managers (35). A new version of FISK (i.e. FISK v2) was recently developed for subtropical climatic zones, such as peninsular Florida, United States (39). This version is also

applicable in Mediterranean climates, and has recently been used to evaluate the invasiveness risk of freshwater fish already introduced with success in the IP (33).

Although FISK has been used to assess the invasiveness risk of non-native freshwater fish species in numerous geographical regions (39, 40, 41), it was never been used to evaluate invasiveness risk of ornamental freshwater fish species commonly trade in one particular region. Also, the applicability of FISK has never been evaluated with species whose ecological traits are poorly known, as is the case ornamental freshwater fish species.

1.4 – Objectives

This study addresses the applicability of FISK in assessing the invasion risk of freshwater ornamental fishes currently sold in Lisbon. Specific objectives were (i) to analyze the relationships between FISK scores and the quantity and quality of available information (ii) to compare FISK scores between independent assessors and with IRI, thus evaluating the performance of this assessment tool and iii) to analyze relationships between FISK scores and the popularity of the species.

Results were then used to explore whether FISK can discriminate ornamental freshwater fish species most likely to succeed if introduced in the wild and may thus be used as a preventive tool in conservation management and to advance future perspectives for research. This is particularly important for ornamental freshwater fish species sold in Portugal, for which lack of ecological information severely limits the use of alternative risk assessment approaches.

2 – MATERIAL AND METHODS

2.1 – Study area and selection of species

Analysis focused on the most popular species in ornamental trade in the region of Lisbon (Table 1), identified in a previous work conducted between October of 2010 and January of 2011 in 37 stores (32).

Species popularity in stores can be used as a surrogate to the propagule pressure, which is a measure of the number of individuals introduced and the frequency of their introduction (19, 32). Given the numbers of individuals and introduction events in rivers are often unknown, species popularity can be a good indicator of propagule pressure (2, 33). It is expected that the popular species are the ones which have the highest probability of being released, increasing the probability of establishment (2, 19, 33).

Species popularity was thus assessed from its Frequency of Occurrence (FO%; number of stores where the species occurs/total stores visited) and Numeric Frequency (FN%; number of individuals of the species/total individuals found) in stores. Focus was on 40 out of the 259 species which had a Frequency of Occurrence above 40% or a Numeric Frequency above 1% (Table 1).

Table 1 – List of the 40 freshwater fish species in the ornamental trade in Lisbon (32) selected for this study. For each species are indicated the scientific name, the authority and the species code.

Species	Author	Species Code
Carassius auratus	(Linnaeus, 1758)	Caur
Poecilia reticulata	Peters, 1859	Pret
Paracheirodon innesi	(Myers, 1936)	Pinn
Poecilia sphenops	Valenciennes, 1846	Psph
Hemigrammus erythrozonus	Durbin, 1909	Hery
Danio rerio	(Hamilton, 1822)	Drer
Xiphophorus maculatus	(Gunther, 1866)	Xmac
Paracheirodon axelrodi	(Schultz, 1956)	Paxe
Corydoras aeneus	(Gill, 1858)	Caen
Hemigrammus rhodostomus	(Ahl, 1924)	Hrho
Trigonostigma heteromorpha	(Duncker, 1904)	Thet
Xiphophorus helleri	Heckel, 1848	Xhel
Hyphessobrycon pulchripinnis	(Ahl, 1937)	Hpul
Pterophyllum scalare	(Schultze, 1823)	Psca
Puntius tetrazona	(Bleeker, 1855)	Ptet
Hyphessobrycon herbertaxelrodi	(Géry, 1961)	Hher
Pristella maxillaris	(Ulrey, 1894)	Pmax
Puntius titteya	(Deraniyagala, 1929)	Ptit
Tanichthys albonubes	(Lin, 1932)	Talb
Gyrinocheilus aymonieri	(Tirant, 1883)	Gaym
Hypostomus plecostomus	(Linnaeus, 1758)	Hple
Betta splendens	(Regan, 1910)	Bspl
Moenkhausia sanctaefilomenae	(Steindachner, 1907)	Msan
Thayeria boehlkei	(Weitzman, 1957)	Tboe
Symphysodon aequifasciatus	(Pellegrin, 1904)	Saeq
Hyphessobrycon eques	(Steindachner, 1882)	Hequ
Aulonocara sp.		Asp.
Corydoras paleatus	(Jenyns, 1842)	Cpal
Hyphessobrycon rosaceus	(Durbin, 1909)	Hros
Gymnocorymbus ternetzi	(Boulenger, 1895)	Gter
Pelvicachromis pulcher	(Boulenger, 1901)	Ppul
Mikrogeophagus ramirezi	(Myers & Harry, 1948)	Mram
Chromobotia macracanthus	(Bleeker, 1852)	Cmac
Cyprinus carpio	Linnaeus, 1758	Ccar
Balantiocheilos melanopterus	(Bleeker, 1850)	Bmel
Rasbora trilineata	(Steindachner, 1870)	Rtri
Trichopodus trichopterus	(Pallas, 1770)	Ttri
Labidochromis caeruleus	(Fryer, 1956)	Lcae
Trichogaster lalius	(Hamilton, 1822)	Tlal
Astronotus ocellatus	(Agassiz, 1831)	Aoce

2.2 – Fish Invasiveness Screening Kit (FISK)

FISK v2 includes 49 questions divided into two main sections (34): Biogeography/History (Section A) and Biology/Ecology (Section B) (Table 2). The Biogeography/History section has a total of 13 questions and includes three categories, namely Domestication/Cultivation, Climate and Distribution, and Invasive Elsewhere. The Biology/Ecology section has a total of 36 questions divided in five categories, namely Undesirable Traits, Feeding Guild, Reproduction, Dispersal Mechanisms and Tolerance Attributes. All questions have three possible answers (yes/no/don't know), to which a certainty level must be attributable (see Annex I), thus reflecting the quality of the answer given (see Figure 1 for an example). Total FISK scores may range from -15 to 57 (34, 35, 37).

Table 2 – FISK sections, categories and number of questions (n) (adapted from (35)).

Sections	Categories	N
A.Biogeography/Historical	1. Domestication/Cultivation	3
	2. Climate and Distribution	5
	3. Invasive Elsewhere	5
B. Biology/Ecology	4. Undesirable (or persistence) Traits	12
	5. Feeding Guild	4
	6. Reproduction	7
	7. Dispersal Mechanisms	8
	8. Tolerance Attributes	5

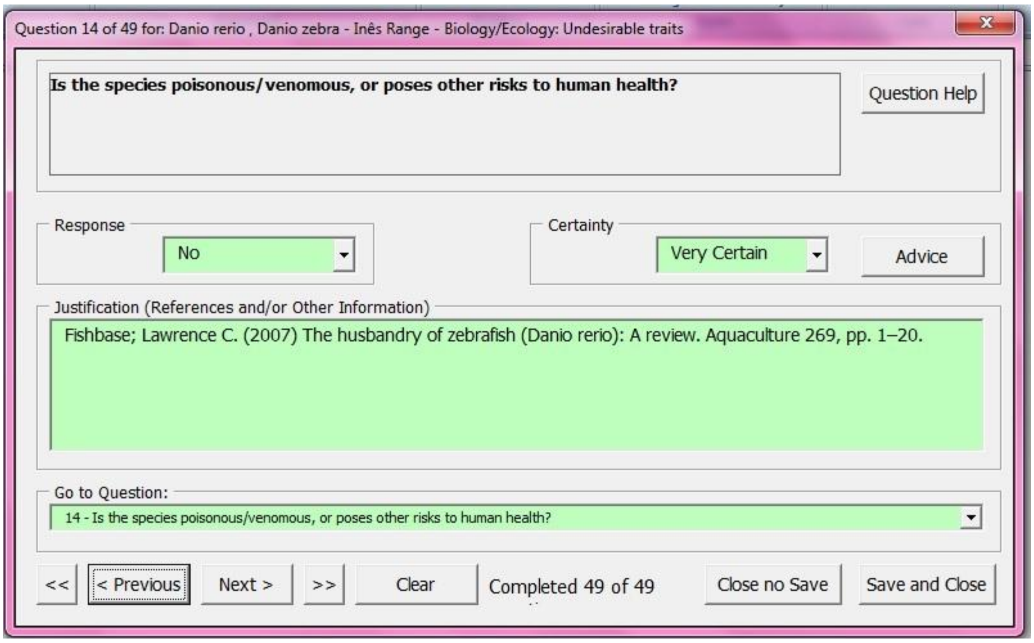

Figure 1 – Example of a Q&A dialog (Question 14) in FISK v2 in section B (Undesirable Traits) for *Danio rerio,* from assessor Inês Range.

2.2.1 – Interpretation of questions

Questions were answered following the guidelines in the help function for each question in FISK v2 (39), as well as information on previous papers addressing FISK applicability (34, 35, 39). Because some personal judgment is always required in each question, a reference guide with the interpretation of each question was produced (Annex II), to assure that answers were consistent among species and to avoid potential biases on the total scores.

An extensive literature search on biogeographical, historical, biological and ecological traits of each species was carried out in scientific papers, books, internet platforms and online forums about ornamental fishes (see Annex III). Key words were introduced in the search engine of the internet (42) to obtain information to questions for each species (e.g. "species name", "temperature tolerance" and "area of dispersion" for the questions related to climate distribution) and information was selected prioritizing the most recent publications available. Primary references were scientific papers and books, with those that were more cited and published in the last five years being considered the most reliable and further used in the analysis. When no scientific papers or books where available, FishBase (43) was the alternative source for information. Ultimately, a search was conducted in forums and general websites for ornamental fishes. The DIAS FAO (Database on Introductions of Aquatic Species of Food and Agriculture Organization of the United Nations, 44) was used to confirm the introduction status for all the species for questions 2 to 6.

2.2.2 – Level of certainty

The level of certainty of each question was assigned as very certain—high confidence (4); mostly certain—medium high confidence (3); mostly uncertain—medium low confidence (2) and very uncertain—low confidence (1). Answers based on information in papers or books were classified as very certain (4) and those based in FishBase as mostly certain (3). Answers that were only supported by information from online forums and general websites were classified as mostly uncertain (2). Classification very uncertain (1) was only attributable when no information was available, and answers were based in personal judgment only.

The average level of certainty of answers for each species was determined, ranging from 1 to 4, with 1 indicating that no previous information was available and 4 indicating that all answers were strongly supported.

2.2.3 – Total Scores

Total scores were assigned into three general categories: low risk ([-15,1[), medium risk ([1,20[) and high risk ([20,57]) based on previous work (33, 39). The high risk category was further sub-divided in: moderately high risk ([20,25[), high risk ([25,30[) and very high risk ([30, 57]) according to 33 and 39.

2.3 – Comparison of FISK scores between assessors

In order to assess author dependent variability in FISK scores, several comparisons were made. First, evaluations by Inês Range (IR) were compared with evaluations conducted independently by David Almeida (DA) and Pedro Leunda (PL), in a previous work (see 33). Given this included only 6 of the species under study, the evaluation was further extended to another independent evaluator, Carlos Mourão (CM) and to a larger set of species. Specifically fourteen among the 40 study species were randomly selected for the analysis by CM and IRI. Carlos Mourão was selected as assessor based on his experience and previous work with aquarium fish trade (see 32).

To guarantee independence between IR and CM, assessments were done separately by each assessor, based on their own guidelines. Although both assessors had access to the reference list and guidelines given in FISK (34) they did not share any other information about the interpretation of questions.

2.4 – Comparison between FISK and the Iberian Risk Index (IRI)

FISK scores were compared with Iberian Risk Index (IRI, 13) to assess consistency in species evaluation between assessment approaches. The IRI ranges from 0 (minimal risk of invasion) to 25 (high risk of invasion) (13). This analysis included only the seven species common to this study and the one by (13).

2.5 – Data analysis

Analysis focused on: (i) relationships between FISK scores and the percentage of questions answered (Qa) and the mean certainty value of answers (Ce), ii) comparisons of FISK scores between independent assessors (IR, DA, PL and CM), iii) comparisons of FISK and IRI scores, and (iv) relationships between FISK scores and species popularity in aquarium shops, as assessed from its Frequency of Occurrence (FO%) and Numeric Frequency (FN%).

All relationships were quantified using the correlation coefficient of Pearson (45). Analysis was conducted using R Development Core team (2008) and significance level was set at 0,05 for all tests (45).

3 – RESULTS

3.1 – FISK scores

The response rate to FISK questions was very high, with 36 questions (73.5%) answered for all species and only four questions (8.2%) having a response rate below 50% (Figure 2). The questions with the lowest response rates belonged to section Biology/Ecology (section B), namely to the Undesirable (or persistence) Traits (Q25), Dispersal Mechanisms (Q41 and Q44) and Tolerance Attributes (Q49) (see Annex II).

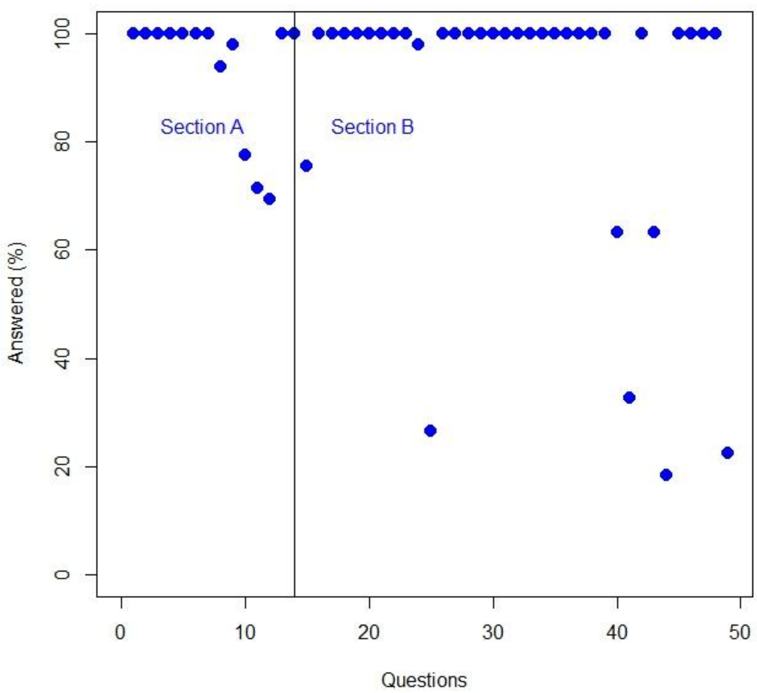

Figure 2 – Percentage (%) of answers to each of the 49 questions in FISK v2 (34) for the 40 evaluated freshwater fish species. Section A: Biogeography/Historical with 13 questions covering three categories; Section B: Biology/Ecology with 36 questions covering five categories.

Mean value of certainty for each question ranged between 2 and 4, with 23 questions (46.9%) being always classified as very certain (4) and only two (4.1%) being classified as uncertain (2) (Figure 3). No questions were classified as very uncertain (1).

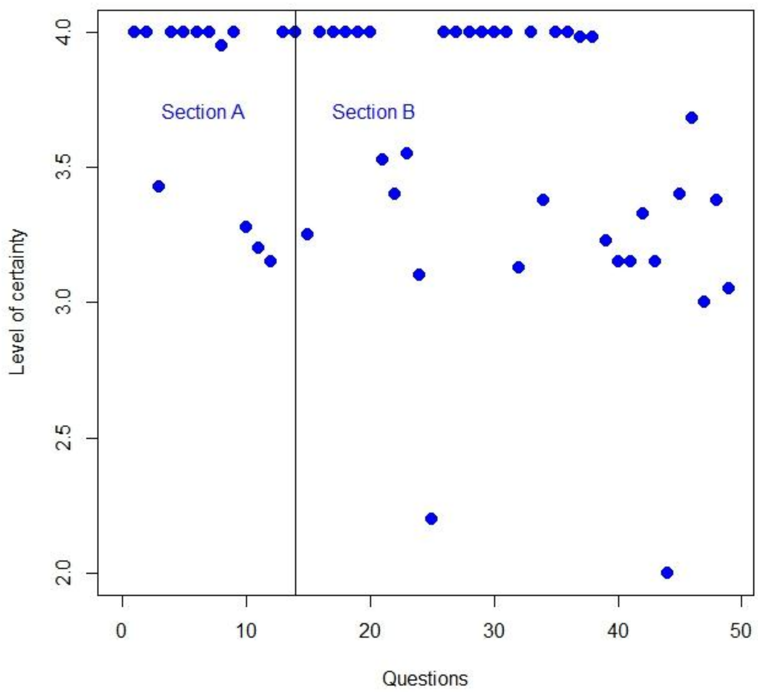

Figure 3 – Mean value of certainty of each question in FISK v2 (34) based on the 40 evaluated freshwater fish species. Section A: Biogeography/Historical with 13 questions covering three categories; Section B: Biology/Ecology with 36 questions covering five categories. Categories of certainty level: very certain—high confidence (4); mostly certain—medium high confidence (3); mostly uncertain—medium low confidence (2) and very uncertain—low confidence (1).

FISK scores for the 40 species analyzed ranged between 0 and 38, covering all categories from low risk (LR) to very high risk (VHR) (Table 3).

Table 3 – Fisk results for the 40 freshwater fish species. For each species are indicated the Frequency of Occurrence (FO%) and the Numeric Frequency (FN%) in stores determined by Carlos Mourão (32), the FISK score, the risk category (33, 39), the percentage of questions answered (Qa) and the mean certainty value of answers (Ce).

Species	FO(%)	FN(%)	Score	Category	Qa (%)	Ce
Caur	100	10.6	38	VHR	93.9	3.9
Pret	100	8.6	27	HR	95.9	3.9
Pinn	89	5.7	3	MR	79.6	3.6
Psph	92	3.4	20	MHR	91.8	3.8
Hery	62	3	0	LR	87.8	3.5
Drer	95	2.6	20	MHR	85.7	3.7
Xmac	84	2.5	21	MHR	93.9	3.8
Paxe	19	2.5	4	MR	93.9	3.6
Caen	86	2.4	10	MR	83.7	3.6
Hrho	70	2.3	2	MR	85.7	3.5
Thet	76	2.2	0	LR	89.8	3.5
Xhel	84	2.2	27	HR	93.9	3.9
Hpul	49	2	3	MR	91.8	3.6
Psca	95	1.9	0	LR	91.8	3.5
Ptet	76	1.8	5	MR	79.6	3.5
Hher	65	1.7	0	LR	91.8	3.5
Pmax	51	1.6	2	MR	89.8	3.5
Ptit	43	1.6	7	MR	83.7	3.6
Talb	62	1.5	7	MR	79.6	3.6
Gaym	57	1.4	8	MR	89.8	3.6
Hple	68	1.4	29	HR	87.8	3.8
Bspl	70	1.2	10	MR	81.6	3.7
Msan	54	1.2	1	MR	91.8	3.5
Tboe	49	1.1	2	MR	91.8	3.5
Saeq	51	1	6	MR	87.8	3.6
Hequ	57	1	4	MR	83.7	3.6
Asp.	46	1	2	MR	83.7	3.6
Cpal	57	1	5	MR	87.8	3.6
Hros	41	1	1	MR	91.8	3.5
Gter	68	1	2	MR	83.7	3.5
Ppul	49	0.8	6	MR	79.6	3.7
Mram	62	0.8	4	MR	87.8	3.5
Cmac	46	0.7	0	LR	87.8	3.6
Ccar	51	0.7	36	VHR	93.9	3.8
Bmel	65	0.6	5	MR	89.8	3.5
Rtri	41	0.6	8	MR	83.7	3.5
Ttri	49	0.5	21	MHR	85.7	3.7
Lcae	51	0.5	1	MR	93.9	3.6
Tlal	46	0.5	9	MR	79.6	3.6
Aoce	41	0.2	18	MR	83.7	3.7

Note: FISK scores may range between -15 and 57.

The majority of species (77.5%) were below the 20 threshold (Figure 4). The sub-group low risk (LR: [-15,1[) had the lowest frequency of species (12.5% of total species) and the sub-group medium risk (MR: [1,20[) had the highest frequency of species (65% of total species).

Among species with scores above the 20 threshold, species with very high risk (VHR) were *C. auratus* (score: 38) and *C. carpio* (score: 36). Species with high risk (HR) were *H. plecostomus* (score: 29), *P. reticulata* (score: 27) and *X. helleri* (score: 27) and species with moderately high risk (MHR) were *X. maculatus* (score: 21), *T. trichopterus* (score: 21), *P. sphenops* (score: 20) and *D. rerio* (score: 20).

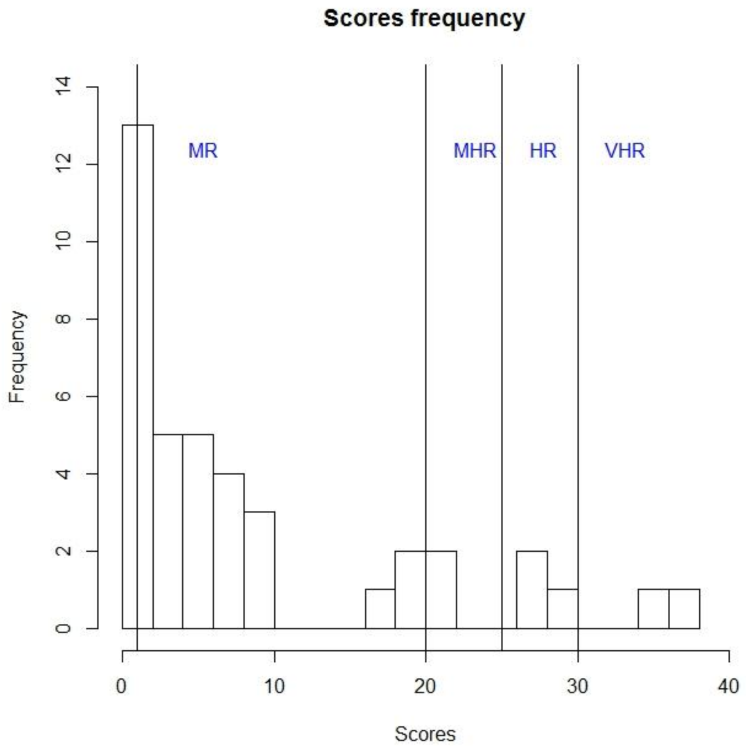

Figure 4 – Histogram of FISK scores ([0,38) for the 40 freshwater fish species. FISK scores were categorized according to (33, 39) as LR (low risk), MR (medium risk), MHR (medium high risk) HR (high risk) and VHR (very high risk). High risk species are above the 20 threshold.

3.2 – Relations between FISK scores and answers to questions

FISK scores were independent of the percentage of questions answered (R=0.27, p=0.093), but showed a positive correlation with the mean certainty value of answers (Figure 5, R=0.92, p<0.001).

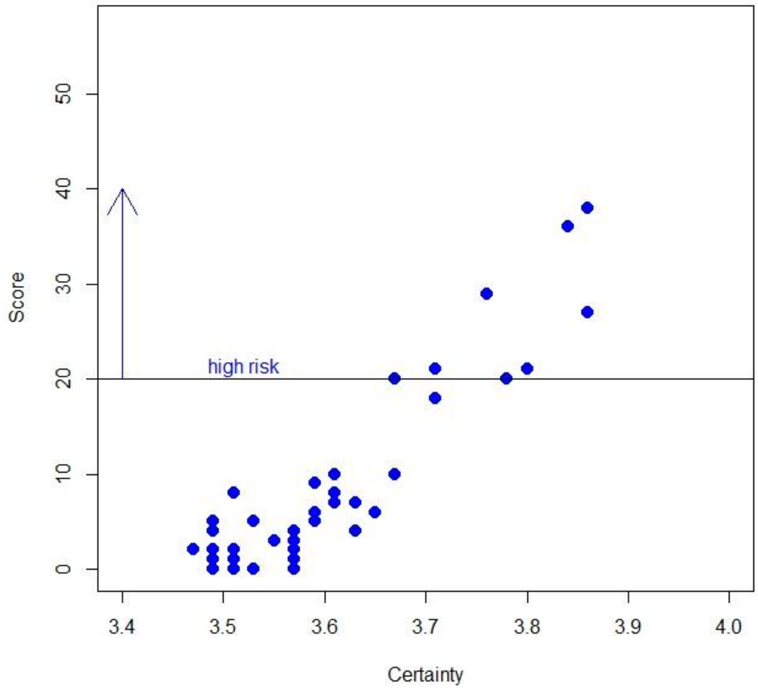

Figure 5 - Relation between FISK scores and mean certainty value of answers for the 40 freshwater fish species. High risk species are above the 20 threshold.

3.3 – Comparison of FISK scores between assessors

FISK scores obtained for a set of six species by Inês Range (IR), David Almeida (DA) and Pedro Leunda (PL) were similar, ranging between 18 and 38 for IR, between 18 and 44 for DA, and between 14 and 39 for PL (Table 4).

Table 4– FISK scores for the six freshwater fish species assessed by Inês Range (IR), David Almeida (DA, 33) and Pedro Leunda (PL, 33). Risk categories for FISK scores were low risk (LR), medium risk (MR), moderately high risk (MHR), high risk (HR) and very high risk (VHR) guided by (33, 39).

Species	**IR**	**DA**	**PL**
Carassius auratus	38 (VHR)	44 (VHR)	39 (VHR)
Cyprinus carpio	36 (VHR)	44 (VHR)	39 (VHR)
Poecilia reticulata	27 (HR)	28 (HR)	23 (MHR)
Xiphophorus helleri	27 (HR)	20 (MHR)	14 (MR)
Xiphophorus maculatus	21 (MHR)	18 (MR)	14 (MR)
Astronotus ocellatus	18 (MR)	19 (MR)	19.5 (MR)

Likewise, there was considerable similarity between evaluations conducted by Carlos Mourão (CM) and Inês Range (IR) for a set of 14 species (Table 5). FISK scores ranged between 0 and 38 for IR and between -5 and 35 for CM. There was a difference of 4.64 between the mean FISK scores for IR and those for CM. Inês Range classified a total of seven species in the highest categories, including two species within the VHR category, two species within the HR category and three species in the MHR category. Carlos Mourão (CM) only classified three species in the highest categories, including one species within the VHR category and two species within the MHR category. The categories of seven species matched between the two assessors, including in the MR category *C. aeneus, P. axelrodi, P. innesi* and *H. rhodostomus*, in the LR category *T. heteromorpha* and *H. erythrozonus* and in the VHR category *C. auratus*. Differences in categories were found for *C. carpio* (VHR for IR and MHR for CM)*, P. reticulata* and *X. helleri* (HR for IR and MR for CM for both)*, X. maculatus, P. sphenops* and *D. rerio* (MHR for IR and MR for CM for both) and *A. ocellatus* (MR for IR and MHR for CM), but never exceeded more than 2 differences in risk categories (Table 5). Nevertheless, there was a positive correlation between FISK scores for IR and FISK scores for CM (Figure 6, R=0.90, p=0.0).

The percentage of questions answered (Qa) for IR was 89.51% and for CM was 84.69% and the mean certainty value was 3.71 for IR and 2.99 for CM.

Table 5 – FISK results for the fourteen freshwater fish species assessed by Inês Range (IR) and Carlos Mourão (CM). For each species is referred the FISK score for IR and CM, the percentage of questions answered (Qa) for IR and CM and the mean certainty value of answers (Ce) for IR and CM. Risk categories for FISK scores were low risk (LR), medium risk (MR), moderately high risk (MHR), high risk (HR) and very high risk (VHR) guided by (33, 39).

Species	Score (IR)	Category (IR)	Score (CM)	Category (CM)	Mean FISK scores	Qa(%) (IR)	Qa(%) (CM)	Ce (IR)	Ce (CM)
Caur	38	VHR	35	VHR	36.5	93.9	91.8	3.9	3.6
Ccar	36	VHR	22	MHR	29	93.9	87.8	3.9	2.7
Pret	27	HR	18	MR	22.5	95.9	71.4	3.9	3.1
Xhel	27	HR	17	MR	22	93.9	91.8	3.9	2.5
Xmac	21	MHR	7	MR	14	93.9	93.9	3.8	3
Psph	20	MHR	16	MR	18	91.8	65.3	3.8	3
Drer	20	MHR	9	MR	14.5	85.7	87.8	3.7	3.2
Aoce	18	MR	20	MHR	19	83.7	89.8	3.7	2.5
Caen	10	MR	11	MR	10.5	83.7	85.7	3.6	2.8
Paxe	4	MR	6	MR	5	93.9	93.9	3.6	3.2
Pinn	3	MR	2	MR	2.5	79.6	67.4	3.6	3.2
Hrho	2	MR	4	MR	3	85.7	85.7	3.5	3
Thet	0	LR	-1	LR	-0.5	89.8	91.8	3.5	2.9
Hery	0	LR	-5	LR	-2.5	87.8	81.6	3.5	3.2
Mean	16.14		11.5		13.82	89.5(±5.11)	95.6(±9.73)	3.7(±0.16)	3(±0.30)

Note: See Table 1 for species codes.

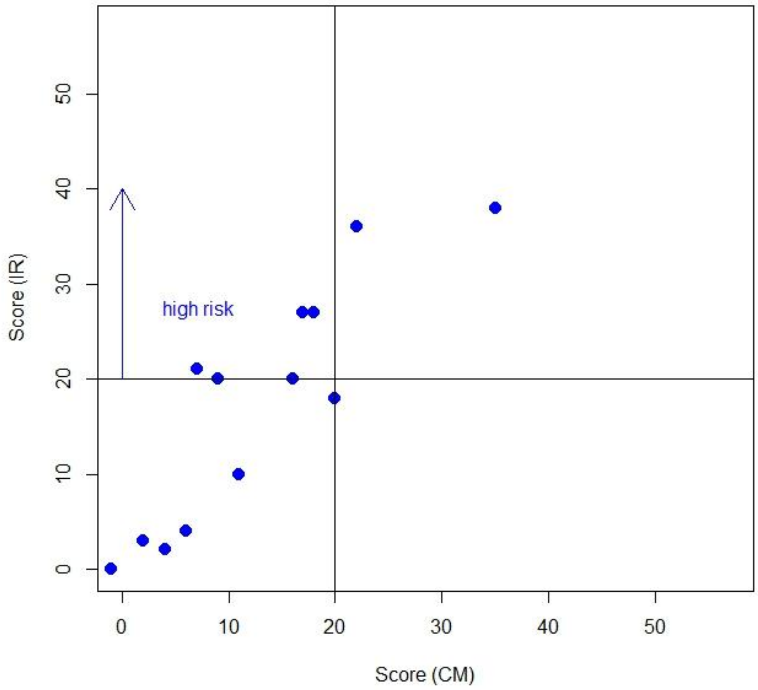

Figure 6 – Relation between FISK scores determined by IR (Inês Range) and by CM (Carlos Mourão) for fourteen freshwater fish species. High risk species are above the 20 threshold.

FISK scores were correlated with the percentage of questions answered (Qa) by IR (Figure 7, R=0.57, p=0.035) but not by CM (Figure 7, R=0.1, p=0.75).

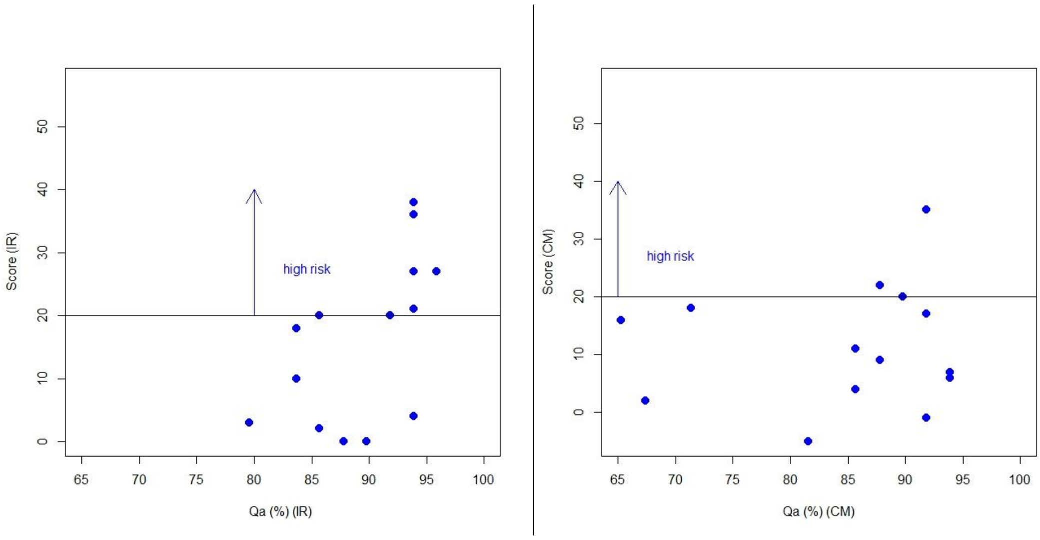

Figure 7 – Relation between FISK scores ([-5,38]) and the percentage of questions answered (Qa) for the fourteen freshwater fish species evaluated by IR and CM. High risk species are above the 20 threshold.

FISK scores were also positively correlated with the mean certainty value of answers (Ce) determined by IR (Figure 8, R=0.94, p=0) but not by CM (Figure 8, R=-0.04, p=0.90).

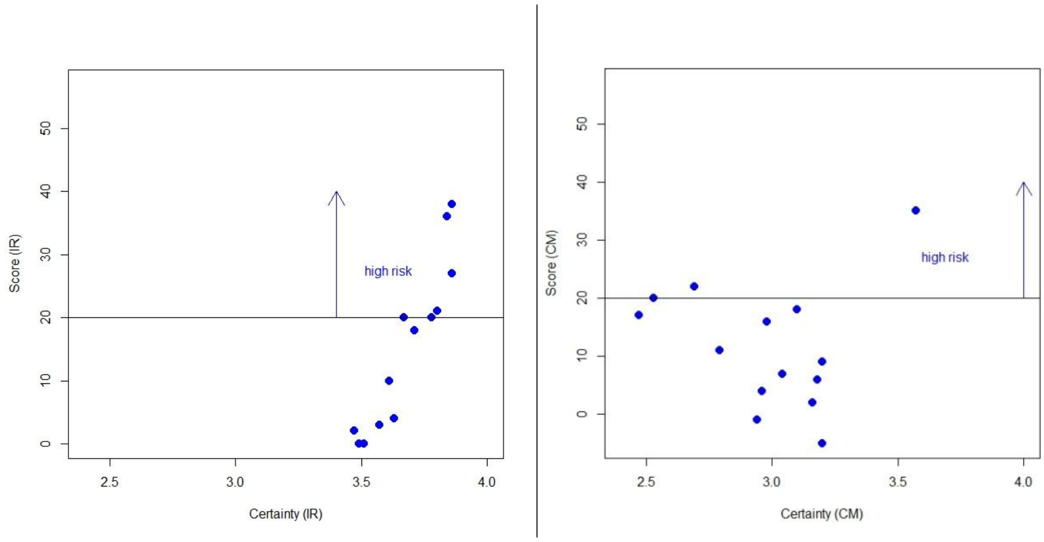

Figure 8 – Relation between FISK scores ([-5,38]) and the mean certainty value of answers (Ce) for the fourteen freshwater fish species evaluated by IR and CM. High risk species are above the 20 threshold.

3.4 – Comparison between FISK and IRI

The IRI ranged from 10 to 22 for the seven species analyzed by Miguel Clavero (Table 6, 13). Evaluations of the risk of invasion were highly consistent between indices, with the only exception being *P. sphenops* that was above the FISK threshold (20) but below the IRI threshold (14) for high invasiveness risk. *Carassius auratus* and *C. carpi*o presented both the highest IRI and the highest FISK scores.

Table 6 – Scores determined by Inês Range using FISK v2 (39) and by Miguel Clavero using Iberian Risk Index (IRI) (13) for seven freshwater fish species. For each species is indicated the FISK score ([-15,57]), the IRI score ([0,22]), the risk category for FISK (very high risk, high risk, moderately high risk, medium risk) and the IRI level (high risk and medium risk).

Species	**Score (FISK)**	**Score (IRI)**	**FISK category**	**IRI level**
Carassius auratus	38	22	very high risk	high risk
Cyprinus carpio	36	22	very high risk	high risk
Poecilia reticulata	27	18	high risk	high risk
Xiphophorus helleri	27	18	high risk	high risk
Xiphophorus maculatus	21	16	medium high risk	high risk
Poecilia sphenops	20	12	medium high risk	medium risk
Astronotus ocellatus	18	10	medium risk	medium risk

3.5 – Relations between FISK scores and species popularity

FISK scores were positively correlated with both the Frequency of Occurrence (FO%) (Figure 9, R=0.39; p=0.013) and the Numeric Frequency (FN%) of species (Figure 10, R= 0.45; p=0.004).

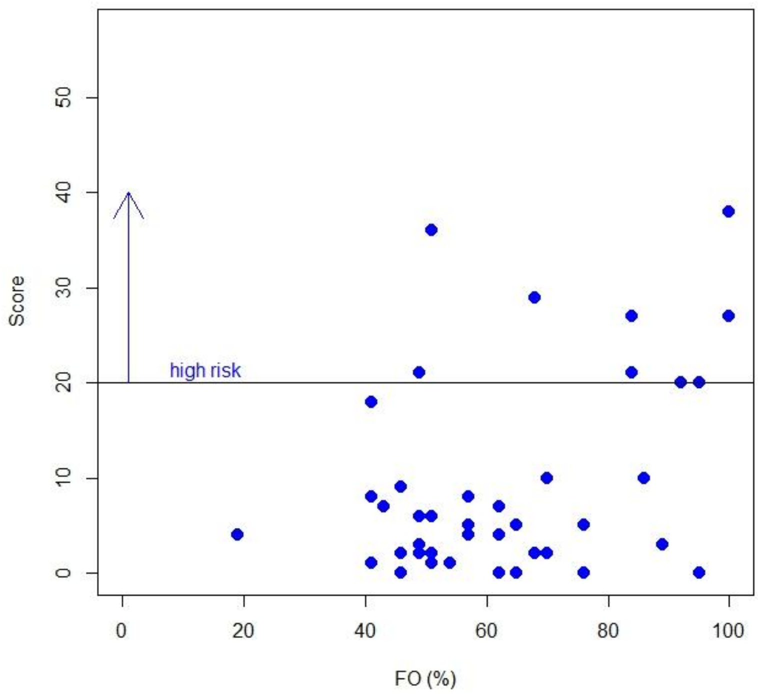

Figure 9 – Relation between FISK scores and Frequency of Occurrence (FO%) for the 40 freshwater fish species. High risk species are above the 20 threshold.

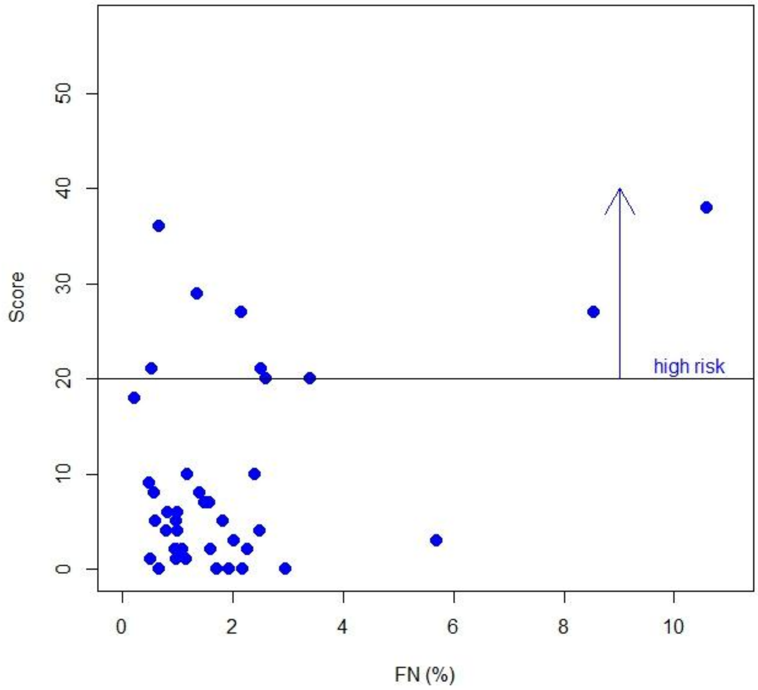

Figure 10 – Relation between FISK scores and Numeric Frequency (FN%) for the 40 freshwater fish species. High risk species are above the 20 threshold.

Among the most popular species in the region of Lisbon (FO>80%) six belonged to the three high risk categories of FISK (Figure 11), namely C. *auratus* (FO=100%; FN= 10.6%, score: 38) for the very high risk category (VHR), *P. reticulata* (FO=100%; FN=8.6%, score: 27) and *X. helleri* (FO=84%; FN=2.2%, score: 27) for the high risk category (HR) and *X. maculatus* (FO=84%; FN=2.5%, score: 21), *P. sphenops* (FO=92%, FN=3.4%, score: 20) and *D. rerio* (FO=95%; FN=2.6%, score: 20) for the medium high risk category (MHR) (Figure 11).

Among other species that had a high popularity (FO>80%), *C. aeneus* (FO=86%; FN=2.4%, score: 10) and *P. innesi* (FO=89%; FN=5.7%, score: 3) were classified as medium risk (MR) and *P. scalare* (FO=95%; FN=1.9%, score: 0) as low risk (LR) (Figure 11). *Cyprinus carpio* (FO=51%; FN=0.7%, score: 36), *H. plecostomus* (FO=68%; FN=1.4%, score: 29) and *T. trichopterus* (FO=49%; FN=0.5%, score: 21) had both FISK scores above the 20 threshold, but did not had a high popularity (FO>80%) (Figure 11).

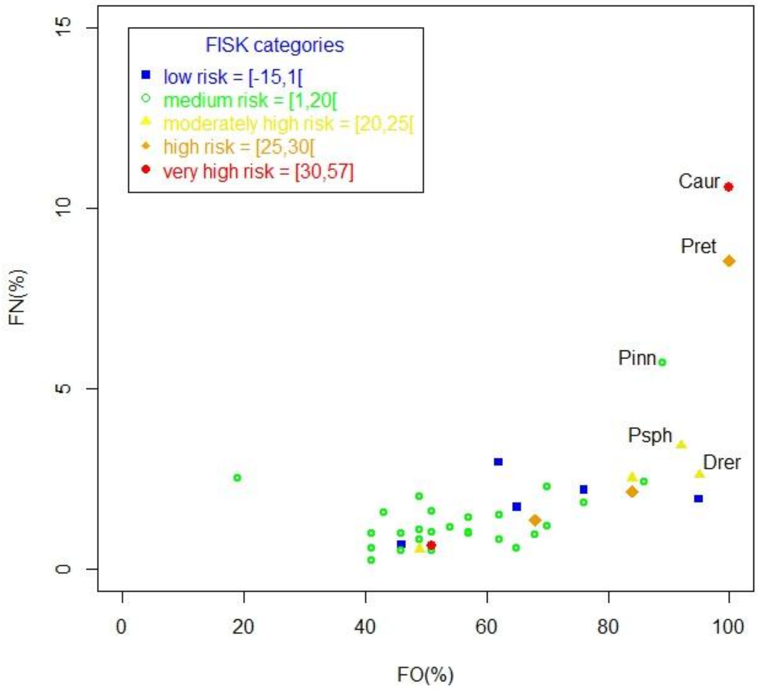

Figure 11 – Variation in categories of FISK scores among the 40 freshwater fish species in relation to its Frequency of Occurrence (FO%) and Numeric Frequency (FN%). Risk categories for FISK scores were low risk (LR-blue), medium risk (MR-green), moderately high risk (MHR-yellow), high risk (HR-orange) and very high risk (VHR-red) guided by (33, 39). **Note**: Caur (*C. auratus*), Pret (*P. reticulata*), Pinn (*P. innesi*), Psph (*P. sphenops*) and Drer (*D. rerio*).

4 – DISCUSSION

This is the first study analyzing the applicability of FISK in assessing the invasion risk of freshwater ornamental fishes, based on the performance of FISK in assessing the 40 species most popular in stores in Lisbon (32).

The response rate to FISK questions and the certainty of answers were high, evaluations performed by independent assessors were largely consistent, and there was little variation between FISK and IRI scoring. Taken together, these results indicate FISK may be a reliable tool in assessing ornamental fishes, though the scarcity of information may pose some challenges in assessing some species.

FISK scores for the 40 most common species in Lisbon stores ranged between 0 and 38, covering all categories from low risk (LR) to very high risk (VHR). Moreover, there was a positive correlation between FISK scores and the Frequency of Occurrence (FO%) and between FISK scores and the Numeric Frequency (FN%) of species.

Although the majority of species (77.5%) were below the 20 threshold, nine species were considered to have a high risk of invasiveness in IP, namely *C. auratus, C. carpio, H. plecostomus, P. reticulata, X. helleri, X. maculatus, T. trichopterus, P. sphenops* and *D. rerio*. Moreover, some of these species have already been successfully introduced in IP or in countries with similar climates, namely *C. auratus, C. carpio, P. reticulata* and *X. helleri*. Also, species with a moderately high risk or high risk of invasiveness such as *H. plecostomus, X. maculatus*, *T. trichopterus*, *P. sphenops* and *D. rerio* possess some biological characteristics (e.g. were able to reproduce in climates similar to IP) that may give them some invasiveness advantages in invading streams in IP and control should thus be undertaken to avoid their introduction and spread in this region.

4.1 – Limitations of the study

FISK application to ornamental species was influenced by the amount and quality of information available for ornamental species. Indeed it was difficult to answer questions related to the Biology/Ecology of all species, namely in what concerns their Undesirable Traits (Q25), Dispersal Mechanisms (Q41 and Q44) and Tolerance Attributes (Q49).

In these circumstances FISK assessments should not be static (37) and must be repeated through time for strengthening the identification and validation of the species scoring and risk classification since the information of species traits is expected to increase over time. Indeed, a fundamental step to improve assessments will involve the fill of the gaps of information about the species identified here, and the review and reformulation of some questions (if necessary) and scores given to alternatives responses (Yes, No or Don't know).

4.2 – FISK applicability

In general, FISK is more applicable when there is more information to support the assessment of species and when certainty is high (34). This was also evident here, as the certainty value was positively correlated with FISK scores. This can be attributed to the fact that species with lowest FISK scores (values between -15 and 18) have been less studied and there is little bibliography available, which means less confidence in the response given. This contrast with high risk species which are often invasive species widely distributed, for which there is more information available which resulted in complete answers, with high certainty. Overall, when information about the species was scarce the assessment was more difficult.

FISK scores were generally similar among independent assessors, despite some disparities between IR and CM in risk classification and the percentage and certainty of questions answered. Differences between independent assessors in risk category classification are not uncommon in FISK (38), which highlights the importance of conducting independent assessments for validation of species invasiveness risk. The evaluation by different assessors will allow identifying gaps in the evaluation and in the information available for the species, with species being analyzed and discussed together for a better assessment (39). For example, variation in the classification of *A. ocelatus* as medium risk (MR) by IR and as moderate high risk (MHR) by CM highlights the importance of this particular species being handled once again, or by a third assessor to ascertain its probability of invasiveness in the region. Likewise, *X. maculatus, P. sphenops* and *D. rerio* were classified as moderately high risk (MHR) by IR and as medium risk (MR) by CM and should be evaluated again or by a third assessor.

FISK and the Iberian Risk Index (IRI) provided similar risk categorizations, classifying *C. auratus, C. carpio, P. reticulata, X. helleri* and *X. maculatus* as having a high invasiveness risk and *A. ocellatus* as medium invasiveness risk. *Poecilia shenops* was the only species diverging in classification between the two approaches, being classified as having a medium high invasiveness risk (MHR) using FISK and as having a medium invasiveness risk using IRI (13). Nevertheless, the comparison between FISK and IRI should be extended to a larger sample of species, given shortcomings in both approaches may be more evident for species with medium to low risk because scarcity of information may enhance differences in scores.

4.3 – Invasiveness risk of ornamental freshwater fish species

Species most popular in stores tended to have the highest FISK scores, namely *C. auratus, P. reticulata, X. helleri, X. maculatus, P. sphenops* and *D. rerio.* These species may pose a severe invasion risk in IP streams, as the large quantities in stores may result in a high propagule pressure of species having a high likelihood of becoming established. Because the risk of introduction through human disposal is high for these species, it is very important to control the trade of these species to avoid their introduction in IP. This is particularly important, given *C. auratus* and *C. carpio* are already considered to be invasive in IP (30) as well as in other countries (7), and they were found to exert a high level of disturbance on the recipient ecosystems (7).

The control of trade should also be extended to *C. carpio, T. trichopterus* and *H. plecostomus* because although presenting a lower popularity (FO% <0.80%) they had a high risk of invasiveness. *Cyprinus carpio* is already established in IP (26, 30) but there are no records of introductions of *H. plecostomus* and *T. trichopterus* in the IP (26, 44). However there are records of *H. plecostomus* from Florida, USA (44).

Among the species with a moderately high risk of invasiveness (MHR) *X. maculatus*, *T. trichopterus*, *P. sphenops* and *D. rerio* have no records in IP (26), but seemed to tolerate colder water temperatures and were already introduced in regions with similar climate to IP, such as Australia, California and Florida (7, 44). The species with the highest score within the medium risk FISK category (MR) was *A. ocellatus* that was already introduced in Argentina and Florida and can also tolerate colder water temperatures (7). *Astronotus ocellatus* is not in the high risk category considered by (33, 39) but its biological and invasive attributes makes the species suitable for the IP (e.g. can reproduce and tolerate the environment conditions) and there are records of their introduction in the region (26). Because a score of 18 is very close to the threshold 20, the species is also considered to have a high probability of establishment in IP although it had not been included in the highest risk categories of FISK.

5 – CONCLUDING REMARKS

The availability of information about biology, ecology and invasive attributes is often very limited for ornamental species and this can restrain the utility of FISK. The current recommendation should thus be contingent on the number of questions answered and its certainty as an indication of its reliability (34). Because my percentage of questions answered was high (90%) and I had an high mean certainty value per question (3.61), I considered that in overall the final FISK classification produced here for ornamental fish was reliable. Moreover, because FISK covered a wide range of questions it appeared more promising than the Iberian Risk Index (IRI), given species with less information may have a higher bias in evaluation with the later tool.

A high level of questions unanswered or an increased uncertainty for a species should also motivate environmental decision makers to take a precautionary approach for that same species because of the low confidence in the assessment. This is essential because after establishment, few invading species are successfully eradicated, and this eradication is economically costly. In this way, adopting a precautionary approach using FISK results should be a priority in prevention management of species introduction, given this assessment tool is very simple, has a low cost and can be applied and integrated in the current environmental programs for non-native species. This should also be complemented with monitoring of sales in shops, taking into account that species most sold in the aquarium trade are those that need more prevention.

Only species with a low invasiveness risk should be excluded in further management planning and may be included in a "white-list" species (16). In this way, by scaling the invasiveness risk of the species we can we can prioritize the level of need for prevention. The higher the species invasiveness risk the stronger will be the management measures needed to prevent their introduction in the future.

The aquarium trade poses an increasing threat for the introduction of new species worldwide and in particular in IP, where it is the second leading cause of introduction (36). Not only the industry is in expansion with constant searching for new species to include in the market but most aquarium species are of tropical and of subtropical origin (7, 46) and the probability of their establishment in IP rises with climatic warming rise (47) as well with increasing river damming (48) which create suitable conditions for the tropical species in the recipient ecosystems.

Future assessments should thus evaluate ornamental species that tolerate warmer temperatures to identify possible high risk species under the currently predicted scenarios for future climates. For example, *H. plecostomus, T. trichopterus, P. sphenops* and *D. rerio* although not adapted to the water temperatures conditions of IP may have a high risk of invasion under altered climates. Indeed warmer climates may translate into new opportunities for these species in the future, especially if they have other biological and ecological attributes that may facilitate a successful establishment (47, 48).

A primary strategy for the prevention of introduction of new species by the aquarium trade should also be the education of people involved in the industry (e.g. sellers from aquarium stores, aquarium hobbyists) about the effects that the releases of these species could have in the environment. Particularly important will be the preparation and release of lists of species with high invasiveness risk (Annex IV) and the implementation of alternative methods of disposal for the unwanted species.

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ANNEX I – Fish Invasiveness Screening Kit (FISK) protocol for hazard identification in non-native freshwater fishes adapted from (34). An unknown response (?), a negative response (N) or a positive response (Y) can be given to each question.

Section/ Categories		Response		
A. Biogeography/Historical		**Score ?**	**N**	**Y**
1 - Domestication/Cultivation				
	1.01	2	0	2
	1.02	1	-1	1
	1.03	1	0	1
2 - Climate and Distribution				
	2.01	0 – 2		
	2.02	0 – 2		
	2.03	1	0	1
	2.04	1	0	1
	2.05		-1	2
3 - Invasive Elsewhere				
	3.01	2	-1	1
	3.02	2	0	1
	3.03	0	0	2
	3.04	4	0	2
	3.05	0	0	1
B. Biology/Ecology				
4 - Undesirable (or persistence) Traits				
	4.01	0	0	1
	4.02		0	1
	4.03	1	0	1
	4.04	0	0	1
	4.05	0	0	1
	4.06	1	0	1
	4.07	0	0	1
	4.08		0	1
	4.09		0	1
	4.1	1	0	1
	4.11	1	0	1
	4.12		1	0
5 - Feeding Guild				
	5.01	0	0	2
	5.02	1	0	1
	5.03	1	0	1
	5.04	2	0	2
6 - Reproduction				
	6.01	1	0	1
	6.02	1	-1	1
	6.03	1	-1	1
	6.04		0	1

	6.05	0	0	-1
	6.06	1	-1	1
	6.07	1 to 2		
7 - Dispersal Mechanisms				
	7.01	1	-1	1
	7.02	1	-1	1
	7.03	1	-1	1
	7.04	1	0	1
	7.05	1	0	1
	7.06	1	0	1
	7.07		0	1
	7.08	1	0	1
8 - Tolerance Attributes				
	8.01	1	-1	1
	8.02	1	-1	1
	8.03		1	-1
	8.04	1	-1	1
	8.05	-1	1	-1

ANNEX II – Guidelines to help answer FISK v2 questions, including the guidance from the programme itself and personal comments for all questions.

1 – Is this species highly domesticated or widely cultivated for commercial, angling or ornamental purposes?

- ✓ Guidance for FISK: *In order to respond "Yes," the taxon must have been grown deliberately for at least 20 generations, or is known to be easily reared in captivity (e.g., fish farms, aquaria, or garden ponds). Whereas, if the taxon has been subjected to substantial human selection that has led to reduced fitness and/or adaptability, then the response should be "No" despite the species being widely domesticated/cultivated. Commercially produced ornamental fish are often small-bodied and selected for bright coloration or increased fin length and this has been shown to increase their vulnerability to predation and decrease their likelihood of establishment in nonnative environments.*
- ✓ Personal comments: In accordance with guidance for FISK. Fishes less than 15 cm in standard length are considered small-bodied.

2 – Has the species established self-sustaining populations where introduced?

- ✓ Guidance for FISK: *The taxon must be known to have successfully established self-sustaining populations in at least one location outside its native range for an extended period of time – this "extended period" is likely to be shorter for short-lived species and longer for longer lived species.*
- ✓ Personal comments: In order to respond Yes, the taxon must be listed as "established" and "probably established" in the DIAS FAO *(Database on Introductions of Aquatic Species of Food and Agriculture Organization of the United Nations)*. If the taxon is listed as "unknown", "probably not established" and "not established" the answer must be "No".

3 – Does the species have invasive races/varieties/sub-species?

- ✓ Guidance for FISK: *This question emphasizes the invasiveness of domesticated species.*
- ✓ Personal comments: In order to answer yes, the taxon must be known to have races/varieties/subspecies (likely in ornamental species) and there must be documented evidence of their invasiveness". If the taxon doesn't have self-sustaining populations in the introduction area and isn't considered invasive and to have invasive races/varieties/subspecies the answer should be "No".

4 – Is the species reproductive tolerance suited to climates in the risk assessment area (1-low, 2-medium, 3-high)?

- ✓ Guidance for FISK: *The intention of this question is to assess the likelihood of a taxon establishing self-sustaining populations in the risk assessment area. If readily available, then a climate matching approach (e.g., Climex, GARP (*Genetic Algorithm for Rule Set Production)*, Climatch) may be used (see summary in Venette et al. 2010; BioScience 60: 349–362). If a climate matching model is not available, then make a "best estimate" through consultation of the Koppen-Geiger climate region system (see: www.hydrol-earth-syst-sci discuss.net/4/439/2007/hessd-4-439-2007.pdf) and/or local expertise.*
- ✓ Personal comments: **3-high -** taxon fulfils both the requirements of toleration of temperatures below 20ºC and has populations (natural or introduced) that occur in

similar areas to the RA area.
2-medium: taxon tolerates temperatures below 20ºC or has populations (natural or introduced) that occur in similar areas to the RA area;
1-low: taxon cannot support water temperatures below 20ºC and/or doesn't have populations (natural or introduced) that occur in similar areas to the RA area;

5 – What is the quality of the climate match area (1-low, 2-medium, 3-high)?

- ✓ Guidance for FISK: *Quality' refers to the assessor's evaluation of the information used to determine the climate match. If there are doubts about the quality of the information available, then attribute the minimum score (i.e., low).*
- ✓ Personal comments:
  3-high: both introduction area and natural dispersion area matched and the taxon can tolerate a broad scale of temperatures;
  2-medium: both introduction area and natural dispersion area don't match but the taxon can tolerate a broad scale of temperatures;
  1-low: it is verified a discrepancy between the introduction area and the natural dispersion area;

6 – Does the species demonstrate broad climate suitability?

- ✓ Guidance for FISK: *Output from climate matching can help answer this, combined with the known versatility of the taxon as regards climate region distribution. Otherwise, the response should be based on natural occurrence in three or more distinct climate categories, as defined by Koppen-Geiger (see: www.hydrol-earth-syst-sci discuss.net/4/439/2007/hessd-4-439-2007.pdf), or based on knowledge of existing presence in areas of similar climate.*
- ✓ Personal comments: In order to respond Yes, the taxon must have a toleration to lower water temperatures (<20ºC) and a broad distribution range (according with guidance for FISK).

7 – Is the species native to or has established self-sustaining populations in, regions with similar climates to the RA area?

- ✓ Guidance for FISK: *This issue raised by this question is whether or not the species actually is established in (or originates from) an area where the climate is similar to the risk assessment area.*
- ✓ Personal comments: In order to respond Yes, at least one region in the taxon's distribution area (including native and introduced areas) needs to have a Mediterranean climate (e.g. Spain, Florida, California, some areas in Australia, South Africa and Argentina) or water temperatures below 20ºC.

8 – Does the species have a history of being introduced outside its natural range?

- ✓ Guidance for FISK: *Should be relatively well documented, with evidence of translocation and introduction. A response of "Don't know" should be given where positive evidence is not available. A "No" response should be given if the taxon is a novel introduction of a single specimen.*
- ✓ Personal comments: For this question is considered all the introductions of the taxon even if they didn't result in established self-sustaining populations.

9 – Has the species established one or more self-sustaining populations beyond its native range?

- Guidance for FISK: *If uncertainty exists regarding the established, self-sustaining population(s), i.e., whether they constitute a true introduction/translocation or simply a "range expansion by natural means", then the answer is "Don't know".*
- Personal comments: In order to respond Yes, the introduced populations must be considered self-sustaining and "established" in the DIAS FAO.

10 – In the species' introduced range, are there impacts to wild stocks of angling or commercial species?

- Guidance for FISK: *There should be documented evidence of real impacts (i.e., decline of native species, disease introduction or transmission), not just circumstantial or opinion-based judgments.*
- Personal comments: The answer should be "Don't know" if the taxon has established self-sustaining populations in the introduction area but there isn't documented evidence of the dimension of their impacts. When the taxon doesn't have established self-sustaining populations in the introduction area the answer should be "No".

11 – In the species' introduced range, are there impacts to aquaculture, aquarium or ornamental species?

- Guidance for FISK: *There should be documented evidence of real impacts (e.g., increased control costs, reduced yields), not just circumstantial or opinion-based judgements.*
- Personal comments: The answer should be "Don't know" if the taxon has established self-sustaining populations in the introduction area but there isn't documented evidence of the dimension of their impacts. When the taxon doesn't have established self-sustaining populations in the introduction area the answer should be "No".

12 – In the species' introduced range, are there impacts to rivers, lakes or amenity values?

- Guidance for FISK: *Documented evidence that the species has altered the structure or function of a natural ecosystem.*
- Personal comments: The answer should be "Don't know" if the taxon has established self-sustaining populations in the introduction area but there isn't documented evidence of the dimension of their impacts. When the taxon doesn't have established self-sustaining populations in the introduction area the answer should be "No".

13 – Does the species have invasive congeners?

- Guidance for FISK: *One or more species within the genus are known to exert moderate to severe impacts.*
- Personal comments: In order to be considered "invasive", the congener needs to have records of established self-sustaining populations in the introduction area that are considered invasive. In order to respond Yes, at least one congener of the taxon has to be considered as "invasive".

14 – Is the species poisonous/venomous, or poses other risks to human health?

- ✓ Guidance for FISK: *Applicable if the taxon's presence is known, for any reason, to cause discomfort or pain to animals.*
- ✓ Personal comments: If there is no documented evidence and the taxon is unlikely to be poisonous/venomous, then the answer should be "No".

15 – Does the species out-compete with native species?

- ✓ Guidance for FISK: *There should be documented evidence that the taxon is responsible for suppression of growth or survival, and/or displacement from microhabitat, of native species.*
- ✓ Personal comments: In order to respond Yes, the taxon needs to have established self-sustaining populations in the introduction area plus evidence about their competitive behaviour towards native taxa. If the taxon doesn't have established self-sustaining populations in the introduction area the answer should be "No".

16 – Is the species parasitic of other species?

- ✓ Guidance for FISK: *Needs at least some documentation of being a parasite of other species (e.g., scale or fin nipping such as known for Pseudorasbora parva, blood-sucking such as by some lampreys).*
- ✓ Personal comments: If there is no documented evidence and the taxon is unlikely to be parasitic of other species the answer should be "No".

17 – Is the species unpalatable to, or lacking, natural predators?

- ✓ Guidance for FISK: *This should be considered with respect to the likely level of ambient natural or human predation, if any.*
- ✓ Personal comments: It is assumed that the taxon has natural predators and in order to respond Yes, there mustn't be evidence to the contrary (e.g. the taxon has characteristics that makes it little attractive to predators). Predators are assumed to be all living beings capable of exercising some level of predation against the species (including humans).

18 – Does the species prey on a native species previously subjected to low (or no) predation?

- ✓ Guidance for FISK: *There should be some evidence that the taxon is likely to establish in a hydrosystem in which predatory fish have never been present, or that is normally devoid of predatory fish (e.g., amphibian ponds), or of a fish species that possesses a predation-facilitating biological attribute (e.g., behavior, large body size, appearance).*
- ✓ Personal comments: In order to respond Yes, the taxon must have established self-sustaining populations in the introduction area and prey on fish or have a body size > 15 cm or displays aggressive behaviour towards native species in the introduction area. If the taxon doesn't have established self-sustaining populations in the introduction area and/or the species isn't a fish predator the answer should be "No".

19 – Does the species host, and/or is it a vector, for one or more recognised non-native infectious agents?

- ✓ Guidance for FISK: *The main concerns are nonnative pathogens and parasites, with the host either being the original introduction vector of the disease or as a host of the disease brought in by another taxon.*

- ✓ Personal comments: It is assumed that the non-native infectious agents can be transmitted if the taxon would be introduced in the RA area and in order to respond Yes, the taxon must have one or more of these infectious agents.

20 – Does the species achieve a large ultimate body size (i.e. > 15 cm total length) (more likely to be abandoned)?

- ✓ Guidance for FISK: *Although small-bodied fishes may be abandoned, large-bodied fishes are the major concern, as they soon outgrow their aquarium or garden pond.*
- ✓ Personal comments: For this question is considered the maximum length reported for the taxon.

21 – Does the species have a wide salinity tolerance or is euryhaline at some stages of its life cycle?

- ✓ Guidance for FISK: *Presence in low salinity water bodies (e.g., Baltic Sea, Tampa Bay) does not constitute euryhaline, so minimum salinity level should be about 15%.*
- ✓ Personal comments: For species with fewer references available records in artificial habitats are evaluated together with records in natural habitats.

22 – Is the species able to withstand being out of water for extended periods (e.g. minimum of one or more hours)?

- ✓ Guidance for FISK: *Examples are lungfishes, walking catfishes, and species with desiccation tolerant eggs.*
- ✓ Personal comments: In order to respond Yes, the taxon must to tolerate extreme hypoxia conditions. For species with fewer references available are counted records in artificial habitats of the taxon being out of water for extended periods.

23 – Is the species tolerant of a range of water velocity conditions (e.g. versatile in habitat use)?

- ✓ Guidance for FISK: *Species that are known to persist in both standing and flowing waters over a wide range of velocities (0–0.7 m/sec).*
- ✓ Personal comments: In order to respond Yes, the taxon must have a body type that enables it to swim in different environments and/or occur areas with different soil types.

24 – Does feeding or other behaviours of the species reduce habitat quality for native species?

- ✓ Guidance for FISK: *There should be evidence of bioengineering behavior, such as foraging that leads to the destruction of macrophytes or an increase in suspended solids, reducing water clarity (e.g., as demonstrated for common carp), or burrow construction, which undermines bank character and stability (e.g., armored sailfin catfishes).*
- ✓ Personal comments: In order to respond Yes, the taxon needs to have established self-sustaining populations in the introduction area and have documented evidence about their impacts.

25 – Does the species require minimum population size to maintain a viable population?

- Guidance for FISK: *If evidence of population crash or extirpation because of low numbers (e.g., over exploitation or pollution), then response should be: "yes."*
- Personal comments: Species with high fecundities are considered to not require minimum population size to maintain a viable population and the answer should be "No". If no documented evidence is available the answer should be "Don't know".

26 – If the species is mainly herbivorous or piscivorous/carnivorous (e.g. amphibia), then is its foraging likely to have an adverse impact in the RA area?

- Guidance for FISK: *Obligate herbivores and piscivores (as adults) are most likely to score here, except where there is sufficient documented evidence form the RA area (or an area considered very similar) that the species has not exerted adverse impacts and therefore the appropriate response is "No." For a herbivorous species to score here, it must feed primarily on aquatic macrophytes. In the case of some facultative piscivores, they may become more piscivorous when confronted with native prey.*
- Personal comments: In accordance with guidance for FISK.

27 – If the species is an omnivore (or a generalist predator), then is its foraging likely to have an adverse impact in the RA area?

- Guidance for FISK: *There must be evidence of foraging on a wide range of food types, including incidental piscivory. For obligate piscivores (as adults) that go through ontogenetic dietary changes (e.g., from zooplankton to macrobenthos to fish), respond"'Yes" here, but then respond "No" to the next two questions.*
- Personal comments: In accordance with guidance for FISK.

28 – If the species is mainly planktivorous or detritivorous or algivorous, then is its foraging likely to have an adverse impact in the RA area?

- Guidance for FISK: Should be primarily planktivorous*, detritivorous, or algivorous* to score here*. For obligate piscivores (as adults) that go through ontogenetic dietary changes that include these food types (e.g., from zooplankton, to macrobenthos to fish), respond "No" here. Similarly, if there is sufficient documented evidence from the RA area (or an area considered very similar) that the species has not exerted adverse impacts, then the appropriate response is "No."*
- Personal comments: In accordance with guidance for FISK.

29 – If the species is mainly benthivorous, then is its foraging likely to have an adverse impact in the RA area?

- Guidance for FISK: *Should be primarily benthivorous to score here. For obligate piscivores (as adults) that go through ontogenetic dietary changes that include these food types (e.g., from zooplankton to macrobenthos to fish), respond "No" here.*
- Personal comments: In accordance with guidance for FISK.

30 – Does the species exhibit parental care and/or is it known to reduce age- at – maturity in response to environment?

- Guidance for FISK: *Needs at least some documentation of expressing parental care, including nest guarding, mouth brooding, live bearing, etc.*
- Personal comments: In accordance with guidance for FISK.

31 – Does the species produce viable gametes?

- Guidance for FISK: *A "Yes" response requires evidence that the taxon produces viable gametes in the wild (native or introduced range). Functionally sterile hybrids, subspecies, or varieties receive a "No" response*
- Personal comments: In order to respond No, the taxon mustn't have capacity to reproduce in its natural habitat.

32 – Is the species likely to hybridize with native species (or use males of native species to activate eggs) in the RA area?

- Guidance for FISK: *Consider evidence of hybrids, occurring in the RA area or elsewhere, with related taxa under natural conditions and without human assistance.*
- Personal comments: In accordance with guidance for FISK.

33 – Is the species hermaphroditic?

- Guidance for FISK: *Needs at least some documentation of hermaphroditism.*
- Personal comments: In accordance with guidance for FISK.

34 – Is the species dependent on the presence of another species (or specific habit features) to complete its life cycle?

- Guidance for FISK: *Some species may require specialist incubators (e.g., unionid mussels used by Rhodeus amarus) or specific habitat features (e.g., fast-flowing water, particular species of plant or types of substrata) to reproduce successfully.*
- Personal comments: In accordance with guidance for FISK.

35 – Is the species highly fecund (>10,000 eggs /kg), iteropatric or has an extended spawning season relative to native species?

- Guidance for FISK: *Normally observed in medium-to-longer lived species.*
- Personal comments: In accordance with guidance for FISK.

36 – What is the species' known minimum generation time (in years)?

- Guidance for FISK: *Time from hatching to full maturity (i.e., active reproduction, not just presence of gonads). Please specify the number of years.*
- Personal comments: In accordance with guidance for FISK.

37 – Are life stages likely to be dispersed unintentionally?

- Guidance for FISK: *Unintentional dispersal resulting from human activity (e.g., bait buckets, live eggs on anglers' gear).*
- Personal comments: In order to respond Yes, the taxon must have economic value and evidence of reports of unintentional dispersal.

38 – Are life stages likely to be dispersed intentionally by humans (and suitable habitats abundant nearby)?

- Guidance for FISK: *Taxon has properties that make it attractive or desirable (e.g., as a food fish or an angling amenity, for ornament or unusual appearance, for cultural reasons).*
- Personal comments: In order to respond Yes**,** the taxon must have economic value and evidence of reports of intentional dispersal.

39 – Are life stages likely to be dispersed as a contaminant of commodities?

- Guidance for FISK: *Taxon is associated with organisms likely to be sold commercially.*
- Personal comments: In order to respond Yes, the taxon must be frequently mistaken with other species (example species within the same genera sold commercially) or has documented information that it tends to be sold together with other ornamental species.

40 – Does natural dispersal occur as a function of egg dispersal?

- Guidance for FISK: *There should be documented evidence that eggs are taken by water currents.*
- Personal comments: Live bearing species and/or species which have sticky or adhesive eggs have less chance that their natural dispersal occurs as a function of egg dispersal and the answer should be “No”. If there is no documented evidence the answer should be “Don’t know”.

41 – Does natural dispersal occur as a function of dispersal of larvae (along linear and/or ‘stepping stone’ habitats)?

- Guidance for FISK: *There should be documented evidence that larvae enter, or are taken by, water currents, or can move between water bodies via connections.*
- Personal comments: If the species is live bearing then the answer should be “No”. If there is no documented evidence the answer should be “Don’t know”.

42 – Are juvenile or adults of the species known to migrate (spawning, smolting, feeding)?

- Guidance for FISK: Th*ere should be documented evidence of migratory behavior, even at a small scale (hundreds or thousands of meters).*
- Personal comments: In accordance with guidance for FISK.

43 – Are eggs of the species known to be dispersed by other animals (externally)?

- Guidance for FISK: There should be documented evidence of such movement events, e.g., accidentally by waterfowl when they move from water body to water body.
- Personal comments: Live bearing species and/or species which have sticky or adhesive eggs score a “No” answer here. If there is no documented evidence the answer should be “Don’t know”.

44 – Is dispersal of the species density dependent?

- Guidance for FISK: *There should be documented evidence of the taxon spreading out or dispersing when its population density increases.*
- Personal comments: If there is no documented evidence the answer should be “Don’t know”.

45 – Are any life stages likely to survive out of water transport?

- ✓ Guidance for FISK: *There should be documented evidence of the taxon being able to survive for an extended period (e.g., an hour or more) out of water.*
- ✓ Personal comments: In order to respond Yes, the taxon must tolerate extreme hypoxia conditions (e.g. lungfishes, walking catfishes, and species with desiccation tolerant eggs). For species with fewer references available, are counted records in artificial habitats of the taxon being out of water for extended periods.

46 – Does the species tolerate a wide range of water quality conditions, specially oxygen depletion and temperature extremes?

- ✓ Guidance for FISK: *This is to identify taxa that can persist even in cases of low oxygen and/or elevated toxic levels of normal chemicals (e.g., ammonia) and/or temperature extremes.*
- ✓ Personal comments: In order to respond Yes, the taxon needs to tolerate at least one of these conditions: hypoxia or temperature extremes.

47 – Is the species readily susceptible to piscicides at the doses legally permitted for use in the RA area?

- ✓ Guidance for FISK: *To score a "no" response, there must be documented evidence of the taxon's resistance to chemical control agents at the doses legally permitted for use in the risk assessment area.*
- ✓ Personal comments: In order to respond Yes, there must have documented evidence that the species isn't susceptible to piscicides at the doses legally permitted for use in the RA area.

48 – Does the species tolerate or benefit from environmental disturbance?

- ✓ Guidance for FISK: *Growth and spread of taxon may be enhanced by disruptions or unusual events (floods, spates, desiccation), including both short- and long-term human impacts.*
- ✓ Personal comments: In accordance with guidance for FISK.

49 – Are there effective natural enemies of the species in the RA area?

- ✓ Guidance for FISK: *A known, effective, natural enemy of the taxon may or may not be present in the risk assessment area (this includes infectious agents that would impede establishment). Unless a specific enemy (or enemies) is known, answer "Don't know."*
- ✓ Personal comments: In accordance with guidance for FISK.

ANNEX III – List of references for the 40 freshwater fish species selected for analysis, coded by number and cited above.

Species	References codes
Carassius auratus	9); 31); 70); 71); 93); 98);
Poecilia reticulata	2); 8); 16); 23); 24); 25); 31); 34); 36); 37); 55); 60); 62); 68); 76); 90); 92); 93);
Paracheirodon innesi	57); 63); 83); 93);
Poecilia sphenops	9); 31); 47); 93); 101); 103);
Hemigrammus erythrozonus	16); 85); 93);
Danio rerio	23); 36); 74); 93); 97); 107);
Xiphophorus maculatus	1); 8); 9); 14); 31); 36); 78); 90); 93); 100); 101);
Paracheirodon axelrodi	3); 15); 30); 83); 101); 105);
Corydoras aeneus	5); 21); 51); 52); 53); 56); 86);
Hemigrammus rhodostomus	3); 72);
Trigonostigma heteromorpha	58);
Xiphophorus helleri	8); 9); 31); 36); 79); 93); 100); 101);
Hyphessobrycon pulchripinnis	18); 29); 61); 93);
Pterophyllum scalare	19); 44); 54); 84); 93);
Puntius tetrazona	59); 93); 99);
Hyphessobrycon herbertaxelrodi	93);
Pristella maxillaris	3);
Puntius titteya	10); 13); 35); 87); 93);
Tanichthys albonubes	31); 66); 93);
Gyrinocheilus aymonieri	4); 81); 93);
Hypostomus plecostomus	6); 41); 88); 93); 95);
Betta splendens	40); 93); 101); 102);
Moenkhausia sanctaefilomenae	33); 93); 106);
Thayeria boehlkei	
Symphysodon aequifasciatus	9); 26); 32); 94);
Hyphessobrycon eques	20); 21); 27); 33); 77);
Aulonocara sp.	
Corydoras paleatus	39); 86); 89);
Hyphessobrycon rosaceus	3);
Gymnocorymbus ternetzi	77); 93); 101); 104);
Pelvicachromis pulcher	73); 82);
Mikrogeophagus ramirezi	46);
Chromobotia macracanthus	11); 64); 93); 96);
Cyprinus carpio	7); 9); 31); 43); 70); 75); 79); 93); 108);
Balantiocheilos melanopterus	12); 65); 91);
Rasbora trilineata	
Trichopodus trichopterus	28); 31); 69); 93); 101); 109);
Labidochromis caeruleus	38);
Trichogaster lalius	49); 50);
Astronotus ocellatus	26); 31); 42); 45); 67); 80); 93);

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ANNEX IV – List of freshwater ornamental fish species with high invasiveness risk according to FISK results by Inês Range. For each species is given the FISK score and the risk category of FISK. FISK categories represented are very high risk (VHR), high risk (HR), medium high risk (MHR) and medium risk (MR).

Species	Score (IR)	Category
Carassius auratus	38	VHR
Cyprinus carpio	36	VHR
Hypostomus plecostomus	29	HR
Poecilia reticulata	27	HR
Xiphophorus helleri	27	HR
Trichopodus trichopterus	21	HR
Xiphophorus maculatus	21	HR
Poecilia sphenops	20	MHR
Danio rerio	20	MHR
Astronotus ocellatus	18	MR