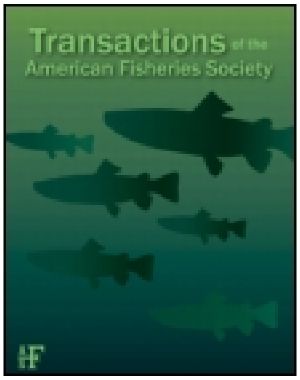

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Assessment of the Risks of Transgenic Fluorescent Ornamental Fishes to the United States Using the Fish Invasiveness Screening Kit (FISK)

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ARTICLE

Assessment of the Risks of Transgenic Fluorescent Ornamental Fishes to the United States Using the Fish Invasiveness Screening Kit (FISK)

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Abstract
Three species of transgenic fluorescent ornamental fish are commercially available to the public in the United States—Zebra Danio *Danio rerio*, Black Tetra *Gymnocorymbus ternetzi*, and Tiger Barb *Systomus tetrazona*. Despite qualitative assessments of the risks of these transgenic fishes by the U.S. Food and Drug Administration and two state agencies, critics argue that the risk assessment and approval processes were not transparent and that the results were never published or otherwise open to scientific scrutiny. We used an internationally recognized risk screening tool, the Fish Invasiveness Screening Kit (FISK), to provide a transparent, peer-reviewed assessment for the conterminous United States. We found that the three transgenic fluorescent ornamental fishes in question represent a low risk of invasiveness. Any risk is limited to the warmer regions of the country. No potential for hybridization with native species, little history of invasiveness elsewhere, a lack of traits associated with persistence, and small body size coupled with predation-enhancing fluorescence all indicate that the ability of these species to become established and have impacts is limited even in warm regions. Our finding of low risk is consistent with the results of unpublished, qualitative agency assessments using expert panels or in-house expertise. The risk screens identified few data gaps, areas of important uncertainty, or potentially elevated risk levels, thus suggesting that there would be limited gain to committing resources to a full risk assessment. A low-risk result further indicates little need for risk management actions in addition to those already being taken. Risk screens such as FISK can have high value for managers because they capture important elements of risk, providing vital information for assessment and management decisions with relatively small investments in time and funding.

Three species of transgenic ornamental fish are commercially available to the public in the United States. They are marketed under the trade name GloFish (Yorktown Technologies, Austin, Texas). A trait shared by all of these species is the expression of fluorescent proteins designed to heighten the color of the fish and make them more attractive to aquarium hobbyists (Gong et al. 2003; Stewart 2006; Nagare et al. 2009; see also Pan et al. 2008). A transgenic red-fluorescent-protein (RFP) Zebra Danio *Danio rerio* (Gong et al. 2003) was the first marketed variety, in 2003; an updated RFP fish (Hill et al. 2011) and four additional color varieties of Zebra Danio followed. A green-fluorescent-protein (GFP) Black Tetra *Gymnocorymbus ternetzi* was marketed in 2012, followed by two additional color varieties. A GFP Tiger Barb *Systomus tetrazona* (also known as *Puntius tetrazona*) was commercialized recently.

Commercialization of transgenic fluorescent ornamental fish has met with controversy (Nagare et al. 2009), with opponents expressing concern over their escape and establishment

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outside of captivity and subsequent environmental impacts, such as competition with native species and interbreeding with wild fish (Rao 2005; Peddie 2008). Critics also point out that the U.S. Food and Drug Administration (FDA), the lead regulatory agency in the United States for transgenic animals, did not require a full New Animal Drug Approval process prior to allowing the sale of RFP Zebra Danios (FDA 2003; The one that got away, 2004; Bratspies 2005; Otts, in press). Recent marketing of new species and varieties has led to numerous media reports, with advocacy groups and some scientists questioning the environmental safety of transgenic fluorescent ornamentals and the regulatory process that allowed them to be sold. For example, the *Washington Post* published an article indicating that environmentalists were concerned about the GFP Black Tetra, a story that was picked up and commented on by many major print and television news outlets (Appel 2012).

Various agencies have evaluated the risks of transgenic fluorescent Zebra Danios, including the FDA (FDA 2003), the California Department of Fish and Game (CDFG 2003), and the Florida Department of Agriculture and Consumer Services (FDACS 2004a, 2004b). The two state efforts included qualitative risk assessments based on expert panels. In all cases, the agencies concluded that fluorescent Zebra Danios present little or no increased risk over the wild type, which has no history of invasiveness despite its widespread presence in the aquarium industry. These assessments led to the legalization of sales of Zebra Danios in the USA (FDA 2003; see also FDA–CVM 2011) and of commercial production in Florida (FDACS 2004b); both sales and production remain illegal in California, however, due to a general ban on the public possession and sale of all transgenic aquatic animals in that state (Van Eenennaam and Olin 2006; CNRA 2013). The other two fluorescent ornamentals have undergone in-house evaluations by the FDA and FDACS. Critics of these assessments argue that the risk assessment and approval processes were not transparent and that the results were never published or otherwise opened to scientific scrutiny.

Risk assessment is used to evaluate the potential for the establishment by and major impacts of nonnative species and to inform management (Orr 2003; Hill and Zajicek 2007). Early on, risk assessment was recognized as a necessary management tool for transgenic and other genetically modified aquatic organisms in aquaculture (Kapuscinski and Hallerman 1990, 1991; Muir and Howard 1999, 2002; Maclean and Laight 2000). Despite the growing literature recommending risk assessments for transgenic fish (e.g., Kapuscinski et al. 2007b; Dana et al., in press), no published risk screens or full risk assessments are available in the literature. Public access to the agency reviews of RFP Zebra Danio variants is limited, with only brief summaries or conclusions being reported on agency Web sites (CDFG 2003; FDA 2003; FDACS 2004a, 2004b); no specific information is available for in-house assessments of the remaining species or color varieties.

Despite the paucity of information about the previous risk assessments, enough data are available to perform risk screens on the commercialized transgenic fluorescent ornamentals. A dissertation by Khee (2006) provides data for a limited qualitative assessment of GFP Zebra Danios. A growing literature of experimental studies of reproductive fitness (Gong et al. 2003; Amanuma et al. 2008), physiological tolerances (Cortemeglia and Beitinger 2005, 2006a; Amanuma et al. 2008), behavior (Snekser et al. 2006; Jiang et al. 2011), and vulnerability to predators (Cortemeglia and Beitinger 2006b; Hill et al. 2011) provide additional information. We therefore evaluated the three commercial transgenic fluorescent ornamental fishes using an internationally recognized risk screening tool, the Fish Invasiveness Screening Kit (FISK; Copp et al. 2005, 2009; Lawson et al. 2013), to provide a transparent, peer-reviewed assessment for the conterminous United States. These assessments are the first use of FISK with transgenic organisms. This risk screening is timely due to recent improvements to FISK (Lawson et al. 2013), a growing body of experimental data on transgenic fluorescent ornamentals, the probable marketing of new transgenic ornamentals, and the potential for release of these fishes outside of the captive environment by the public.

METHODS

Study species.—Fact sheets available in Fishbase (Froese and Pauly 2013) and the U.S. Geological Survey's Nonindigenous Aquatic Species (NAS) Database (USGS 2013) summarize existing information on Zebra Danios (family Cyprinidae), Black Tetras (Characidae), and Tiger Barbs (Cyprinidae). The basic biology, ecology, and performance of these species are well known because they are common in the international aquarium trade. The wild-type and multiple nontransgenic varieties of each species are cultured in the USA, primarily in Florida (authors' personal observations). The Zebra Danio is also a model species in several scientific disciplines (Spence et al. 2008 and references therein). All three species are small bodied ($\leq$75 mm total length), feeding mainly on small invertebrates such as crustaceans and insects. Egg fertilization is external, with eggs being scattered onto aquatic plants or the substrate with no parental care.

The Zebra Danio is native to India, Bangladesh, and Nepal, where it occurs mostly in sluggish streams, floodplains, and flooded rice fields (Spence et al. 2008). The Black Tetra is native to the Rio Guapore in Brazil and Bolivia and the Rio Paraguay in Argentina, where it is found in floodplain wetlands and seasonal marshes along rivers and streams. The native range of the Tiger Barb is Borneo and Sumatra, where it primarily occurs in the floodplains of rivers and streams.

FISK assessments.—Screening tools such as FISK are used to identify potentially invasive fishes in a rapid and cost-effective manner (e.g., Copp et al. 2005). These tools thus help to inform managers concerning the need to expend resources for full risk assessments (e.g., see Hardin and Hill 2012). We used FISK 2.03 (Lawson et al. 2013) as our risk screening tool to estimate the invasive potential of transgenic Zebra Danios, Black Tetras, and Tiger Barbs. The original version of FISK has primarily been used to provide preliminary estimates of the risk of nonnative fishes in temperate regions (Copp 2013). Subsequent versions were used to assess fishes from numerous regions of the world, including Asia, Australia, and Europe (e.g., Onikura et al. 2012; Almeida et al. 2013; Vilizzi and Copp 2013; see also Copp 2013). The modifications included in FISK 2.03 enhanced the clarity, ecological applicability, and geographic coverage of the widely used FISK 1 (Copp et al. 2005, 2009), making it more suitable for use in the conterminous United States, a region containing a wide range of climate types. The only published application to North America is an assessment of the risks of the Barcoo Grunter *Scortum barcoo*, an Australian species of interest for food fish aquaculture in Florida (Lawson et al. 2013).

FISK is a straightforward adaptation of the Australian Weed Risk Assessment (WRA; Pheloung et al. 1999) and follows the same premise that species that are invasive in one part of the world are more likely to be invasive in other regions with similar climate and other habitat characteristics (Copp et al. 2005; Copp 2013). Important elements of potential invasiveness are grouped into two major sections within FISK. The Biogeography/History section includes factors related to climate, human use, and invasion history. The Biology/Ecology section considers the potentially undesirable traits of invasive species (e.g., that they are venomous or attain large body sizes), their physiological tolerances (e.g., to cold), and characteristics that might enhance their dispersal (e.g., life stages especially adapted for dispersal).

FISK 2.03 consists of 49 questions related to the characteristics of the assessed species and region. Each question comes with guidance to assist the assessor and to reduce subjectivity. A numerical score is assigned to each response, and the sum of the scores for all questions results in a risk score ranging from -15 to $+57$ (L. Vilizzi, Murray–Darling Freshwater Research Center, personal communication). Species with scores <1 are considered to have low risk of invasiveness, those with scores of 1–6 to have medium risk, and those with scores >6 to have high risk; note, though, that the calibrated score thresholds may vary by region (e.g., medium risk = 1–19 in the UK; Copp et al. 2009). Each response is assigned a certainty rating, and most questions allow the answer "Don't Know" to account for gaps in information. As with the WRA, individual questions do not determine a species' risk category and a small number of missing responses still allows for the effective use of the program (Pheloung et al. 1999; Gordon et al. 2012).

Two assessors from the University of Florida (J.E.H. and L.L.L.) independently scored each species, as suggested by Hayes et al. (2007), resulting in mean, minimum, and maximum FISK scores. Information sources included online databases such as FishBase (Froese and Pauly 2013), the Food and Agriculture Organization of the United Nations' Database of Introductions of Aquatic Species (FAO 2013), and the USGS' NAS Database (USGS 2013); the primary literature; Köppen-Geiger climate maps (Kottek et al. 2006); and personal experience with the species, including the transgenic variants specifically addressed in the FISK assessments. Assessors took a conservative approach and responded to most questions by considering the wild type unless the fluorescent trait itself specifically required a different response. For example, questions concerning invasion history were answered for the entire species because the fluorescent strains represent only a recent subset of the varieties used in trade and have no history of introduction outside the captive environment. Conversely, question 4.01 ("Is the species poisonous/venomous, or poses other risks to human health?") was answered for the transgenic varieties specifically to capture any potential additional risks of the fluorescent strains associated with the genetic modifications. Not all fluorescent strains and species have been studied to the same extent, and more information is available for GFP and RFP Zebra Danios. The assessors assumed that the genetic modifications to the two less-studied species were not fundamentally different from those to the well-studied Zebra Danio. In the absence of specific studies, the assessors used personal experience and studies of other fluorescent varieties to determine their FISK responses. A coauthor with a management and regulatory background and knowledge of ecological risk analysis (S.H.) reviewed the FISK assessments. This process is consistent with many management scenarios, in which risk assessments would be peer-reviewed by agency staff (Hardin and Hill 2012).

RESULTS

All three of the transgenic fluorescent ornamental species were scored as low risk (Table 1), with relatively high certainty in responses (Figure 1). Zebra Danios had slightly negative scores for the Biogeography/History section, whereas the scores of Black Tetras and Tiger Barbs were slightly above 0. All species averaged <1 for the Biology/Ecology section. Specific answers and degrees of certainty are presented in Table A.1 in the appendix.

Domestication/Cultivation (questions 1.01–1.03).— Nontransgenic varieties of all three species are common in the aquarium trade in the United States and are cultured commercially in Florida. Nevertheless, the data show reduced reproductive fitness for transgenic fluorescent Zebra Danios compared with wild types (Khee 2006; Nagare et al. 2009) or no advantage (Gong et al. 2003). The adult viability of GFP Zebra Danios has been shown to be less than that of wild types in laboratory trials (Gong et al. 2003). Fluorescence increases the vulnerability of small fish to predators and thus decreases their fitness relative to that of wild types (Hill et al. 2011).

Transgenic fluorescent ornamentals have not been reported outside of captivity. The wild types of the three species have been introduced into the noncaptive environment, but their history of establishment is mixed. Both assessors responded "No" to question 1.02 ("Has the species established self-sustaining populations where introduced?") with relatively high uncertainty for Zebra Danios and "No" or "Don't Know" for Black Tetras because of the sparse data and uncertainty about their actual establishment in the environment. There is more evidence of establishment for Tiger Barbs (USGS 2013), though the degree of certainty is relatively low because of the sparse data for nearly

TABLE 1. FISK scores for three species of transgenic fluorescent ornamental fish. The mean score is the average of the overall scores of the two assessors (J.E.H., L.L.L.; the individual scores are given in parentheses). The assessors' overall scores are the sums of the scores for the two sections, Biogeography/History (questions 1.01 through 3.05) and Biology/Ecology (questions 4.01 through 8.05). Scores are also shown for subsections. Scores <1 indicate low risk (Copp et al. 2005, 2009).

Variable	Zebra Danio	Black Tetra	Tiger Barb
Mean score	−0.5 (−1, 0)	−3.5 (−6, −1)	−2.5 (−3, −2)
Risk category	Low	Low	Low
Biogeography/Historical	0, –1	0, 2	2, 1
1. Domestication/cultivation	0, 0	0, 0	0, 0
2. Climate and distribution	2, 1	2, 2	1, 0
3. Invasive elsewhere	−2, −2	−2, 0	1, 1
Biology/Ecology	−1, 1	−6, −3	−5, −3
4. Undesirable traits	1, 2	0, 2	1, 2
5. Feeding guild	0, 0	0, 0	0, 0
6. Reproduction	2, 2	0, 0	0, 0
7. Dispersal mechanisms	−1, −1	−1, −1	−1, −1
8. Tolerance attributes	−3, −2	−5, −4	−5, −4

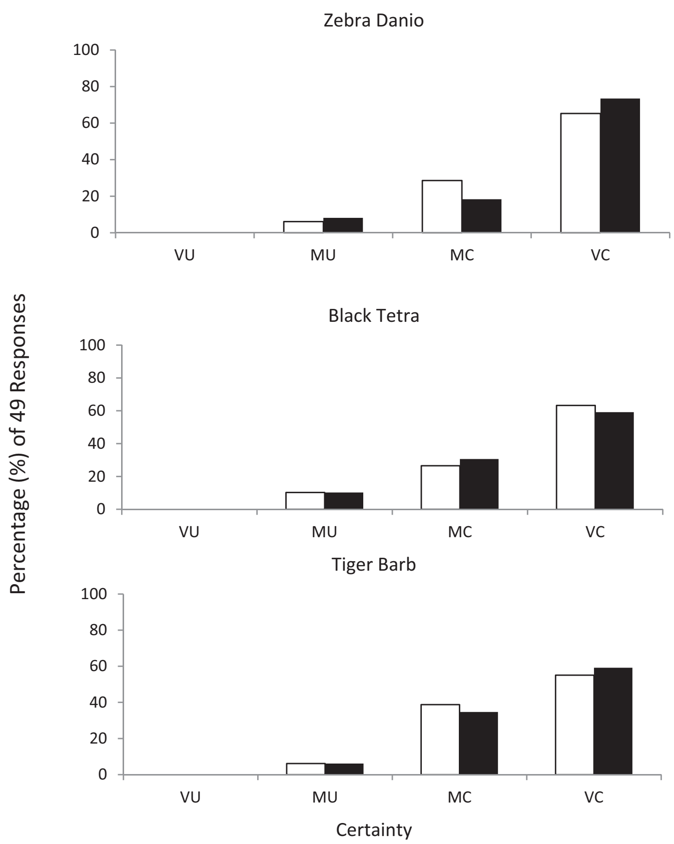

FIGURE 1. Distributions of certainty for the answers to 49 FISK questions by two assessors (J.E.H. [light bars] and L.L.L. [dark bars]) for transgenic fluorescent varieties of Zebra Danio, Black Tetra, and Tiger Barb. Abbreviations are as follows: VU = very uncertain, MU = mostly uncertain, MC = mostly certain, and VC = very certain.

all locations and the highly localized nature of the remaining examples.

Climate and distribution (questions 2.01–2.05).—Data from the native range, reported temperature ranges, and the authors' experience with these species show that all three species require warm temperatures (>18°C). Most of the conterminous United States has unsuitable climate for them outside of thermal refuges (e.g., warm springs). Both assessors gave Black Tetra a high climate score based on the climate of its native range and the fact that warm temperate and subtropical regions occur in the USA (Kottek et al. 2006). One assessor gave Zebra Danio a high climate score because of a climate match with Florida, a narrow strip along the Gulf of Mexico, and southern California, whereas the other assessor gave it a low score because the match only occurred over a limited portion of the risk assessment area (Cortemeglia and Beitinger 2005, 2006a; Cortemeglia et al. 2008). A medium or low score was given to Tiger Barbs based on the fact that the only suitable climate occurs in southern Florida (Kottek et al. 2006). All three species have been introduced into the risk assessment area from outside of their native ranges (USGS 2013).

Invasive elsewhere (questions 3.01–3.05).—Zebra Danios and Black Tetras have a questionable history of establishment when introduced outside their native ranges. There is no evidence of negative impacts by introduced populations of the three species. Zebra Danios and Tiger Barbs have limited histories of establishment, and no congeners have become invasive. No members of the genus *Danio* are established in the USA (USGS 2013). *Gymnocorymbus* is a monotypic genus and thus lacks invasive congeners. The recent revision of the genus *Puntius* (Pethiyagoda et al. 2012) placed the Tiger Barb in the genus *Systomus*, a group with little history of invasiveness. Within the genus *Puntius* (sensu lato), the Rosy Barb *Pethia conchonius* (also known as *Puntius conchonius*) is established in Puerto Rico (USGS 2013) and locally in Australia (Koehn and MacKenzie 2004; Corfield et al. 2007), but no impacts are known.

Undesirable (or persistence) traits (questions 4.01–4.12).—There is no evidence that either the nontransgenic or transgenic versions of any of the three species are toxic or otherwise pose risks to human health (e.g., FDA 2003; Nagare et al. 2009; Froese and Pauly 2013). The fluorescent proteins used in ornamental fishes naturally occur in some marine invertebrates and are considered nontoxic and of low allergenic risk (e.g., Richards et al. 2003; Shaner et al. 2005). None of these fish parasitize other species or transfer novel pathogens listed by the Office International des Epizooties (OIE 2012).

All three species are unlikely to outcompete native species or to significantly impact native species previously subject to low predation in the conterminous United States. Small insectivorous fishes have little documented history of impact within the regions of suitable climate in the United States (see USGS 2013 species accounts). None of the three species alter habitat through their feeding or other activities.

These transgenic ornamentals are limited by cold temperatures and elevated salinity. These species naturally occur in water bodies with a range of water velocities, though all seem most common in shallow, slowly moving or still habitats (e.g., Spence et al. 2006, 2008). Zebra Danios are relatively tolerant of low dissolved oxygen (Hill, unpublished data). None of the three species are capable of surviving desiccation.

Feeding guild (questions 5.01–5.04).—All three species are small-bodied insectivores. Although each feeds on benthos to a limited extent, these species are unlikely to have significant impacts on native species based on the information on established small-bodied fishes in the United States (USGS 2013).

Reproduction (questions 6.01–6.07).—All three species are egg-scatterers and provide no parental care. All have short generation times, but only the Zebra Danio has a lengthy spawning season outside of captivity (Spence et al. 2008). Hybridization is not a concern for the conterminous United States due to the lack of close relatives for any of the three transgenic ornamentals.

Dispersal mechanisms (questions 7.01–7.08).—The three species possess few or no biological attributes to enhance dispersal. The main mechanism of introduction would be release by aquarists.

Tolerance attributes (questions 8.01–8.05).—As with undesirable (persistence) traits (above), the three fluorescent species have few tolerance attributes that increase their FISK scores. All three are susceptible to rotenone at doses legally permitted by the U.S. Environmental Protection Agency (Hill, personal observations; see also Finlayson et al. 2010). All are highly vulnerable to native predators and aggressive competitors in the United States (e.g., Cortemeglia and Beitinger 2006b; Hill et al. 2011; Hill, personal observations; confer Jha 2010 for India).

Agreement and certainty.—Assessor agreement was high (88%), with only 3 cases of their giving opposite responses to the same question. Most disagreements were the result of one assessor assigning a "Yes" or "No" and the other assigning a "Don't Know" (11 cases). One assessor was more conservative when answering questions about climate match (see Discussion) and Köppen-Geiger climate zones (4 cases).

Both assessors rated certainty as "mostly certain" or "very certain" for 88–94% of their responses (Figure 1). Uncertainty was highest with respect to the ability of species to persist at low population density (question 4.12); the capacity of larvae, juveniles, and adults to disperse (questions 7.05, 7.06, and 7.08); and their adaptability to environmental disturbance (question 8.04). In addition, there was uncertainty about Black Tetras establishing populations outside their native range (questions 1.02 and 3.01).

FISK reviewer's comments.—Overall, the reviewer was in agreement with the assessors' responses and justifications. There were a total of 21 comments on the combined 294 assessor responses (7.1%), ranging from 1 to 5 comments per assessment. Only a single change in response occurred due to the review, a case in which the reviewer discovered an obvious error in the response based on the justification. Three comments were notes of clarification that the assessor's justification was based on the question guidance.

Comments regarding climate matching were the most common (6 comments) and were related to the issue of the strength of the climate match, climate match data quality, and the interpretation of Köppen-Geiger climate maps when a match was found for only a small portion of the risk assessment area. The reviewer also noted discrepancies in the invasion history data and the resulting disagreement in responses for some species. Examples include the responses to questions 1.02 and 3.01 (established, introduced populations), where one assessor answered "No" and the other answered "Don't Know" due to sparse and anecdotal information on the status of Black Tetra introductions.

The reviewer also noted differences in the interpretation of question 8.04 ("Does the species tolerate or benefit from environmental disturbance?") on the issue whether a species spawning during seasonal flooding qualifies for a "Yes" response. A discrepancy was noted between assessors on the ability of Zebra Danios to tolerate a wide range of water velocity (question 4.01). The remaining comment was on the assessors' differences concerning the ability of Zebra Danios to tolerate a wide range of environmental conditions, noting that there was disagreement on the tolerance of a low oxygen level and that the species is not able to tolerate cool temperatures.

DISCUSSION

We conclude that the three transgenic fluorescent ornamental fishes evaluated represent a low risk of invasion in the conterminous United States. There is no potential for hybridization or the passing of genes to native species, a major risk factor with transgenics (Kapuscinski et al. 2007a). In addition, a questionable history of invasiveness elsewhere, the lack of traits associated with persistence, and small body size coupled with predation-enhancing fluorescence indicate that the ability of these species to become established and have impacts is limited even in warm regions. Our conclusion of low risk using FISK is consistent with the results of unpublished, qualitative agency assessments using expert panels or in-house expertise done by the FDA, CDGF, and FDACS.

Potential pleiotropic and other unintended effects of the transgene and the novelty of genetically modified organisms suggest that the risks posed by transgenic fish differ from those of nontransgenic, nonnative species in fundamental ways (Muir and Howard 2002; Kapuscinski 2005; Gong et al. 2007). Nevertheless, our results suggest that transgenic fluorescent fish are not fundamentally different from their nontransgenic conspecifics in terms of invasiveness, being of similar or less risk than nontransgenics. We assumed that the genetic modifications of the two less-studied species did not impart unknown, hypothetical advantages. This assumption is supported by considerable experience with transgenic and nontransgenic varieties of these species and research demonstrating that fluorescent individuals behave similarly to nonfluorescent fish and lack advantages in reproduction, growth, or survival (e.g., Gong et al. 2003; Jiang et al. 2011). Transgenic fish are usually at a disadvantage when a difference is apparent. For example, transgenic RFP Zebra Danios are slightly less thermally tolerant for both minimum and maximum temperatures than the wild type (Cortemeglia and Beitinger 2005), though this difference did not reduce the climate match in FISK for this species. Large differences in predation vulnerability are apparent under nearly natural experimental conditions, with RFP Zebra Danios being about twice as vulnerable to predators as wild types (Hill et al. 2011). The RFP Zebra Danios were less able to tolerate harassment by Eastern Mosquitofish *Gambusia holbrooki*, an aggressive competitor and predator of small-bodied fishes, in a laboratory experiment (Hill et al. 2011). Conversely, heightened vulnerability to predators was not captured in the FISK scores because the nontransgenic varieties of all three species are already vulnerable to a wide range of predators (questions 4.04 and 8.05). Fluorescence simply enhances the already high predation vulnerability of many small-bodied fishes. A considerable literature suggests that predators strongly influence the establishment and persistence of susceptible fishes (Tonn and Magnuson 1982; Harvey et al. 2004; Aiken et al. 2012; Thompson et al. 2012), implying that transgenic fish entail lower risk of establishment than wild types (Hill et al. 2011).

Although FISK was not specifically designed to screen for the risks of transgenic fish, it is flexible and inclusive enough to readily accommodate fluorescence and potentially other genetically modified, ecologically meaningful traits (e.g., growth enhancement and cold tolerance). A strength of FISK is the ability it gives risk managers to view each question's answer and certainty ratings individually and to make informed decisions based on scenario-specific factors if desired (Lawson et al. 2013). Thus, risk managers can use FISK to screen transgenic fishes prior to or following development to determine data gaps or factors of particular concern. Moreover, FISK may be used to establish a baseline estimate of risk for the wild type prior to an evaluation of transgenic variants. Incorporation of FISK into a more complex, module-based risk assessment scheme (such as that for nonnatives in the European Nonnative Species in Aquaculture Risk Assessment Scheme; Copp et al. 2008) could enable it to be used in the early stages of assessment of transgenics. All of these potential uses for FISK in evaluating transgenic fishes can provide low-cost, timely information to assist in determining the need for additional research, full risk assessment, or preliminary risk management actions.

History of invasiveness elsewhere is an important predictive factor for invasiveness across a variety of taxa (Hayes and Barry 2008; Kulhanek et al. 2011). Invasion history and the occurrence of impacts following introduction strongly influence the outcome of many risk screening and assessment methods, including FISK; thus, there is a critical need for better data on the status and impacts of introduced fish populations worldwide. Assessor judgment was necessary to sort out the sparse and often confusing literature on the invasion histories of the nontransgenic versions of the ornamental species in our study. Information for the United States was readily available through the USGS' NAS Database (USGS 2013). Information for other regions of the world was of relatively low quality, and some databases contained information on introduction status and impacts that elaborated far beyond that provided by the source references. In particular, it is difficult to distinguish introductions into open waters from transfers of species into a country for aquaculture or ornamental purposes. For example, most databases rely heavily on Welcomme's (1988) report on international transfers of fishes. The introduction to that report states that a species is introduced to a country once it has crossed national boundaries. This means that species are included in the listing if they are transported into a country as part of current commercial practice for food or ornament and not for stocking into natural environments.

Welcomme and Vidthayanon (2003) stated in their review of nonnative fishes in the Mekong River that the contemporary persistence of populations of some species listed in the report may be "based on outdated and superseded information." In our collective experience, population status and impacts are the types of data that are most difficult for database managers to obtain and keep up to date and the most difficult for risk assessors to evaluate, even for the better databases. The FISK question guidance indicates that there should be documentation of establishment and impact for positive responses. The occurrence of single specimens, highly isolated and localized reproduction, and speculation that there are impacts are not sufficient to justify positive responses. We therefore recommend that assessors carefully evaluate the quality of the data they are using, provide detailed justifications for their answers, and use certainty ratings to indicate questionable data. In particular, we discourage the uncritical citation of questionable distribution and impact data so as to prevent their reification (Slobodkin 2001) and thus their receiving unwarranted weight in future risk assessments or other analyses of nonnative species.

The insights of the FISK reviewer, who had the ability to research and compare the assessors' responses, not only provided a critical evaluation of the assessments but also pointed out questions and topics about which there was disagreement or possible confusion. In addition to the previously noted differences with respect to climate match and invasion history, differences were found between the information in some databases and that in the primary literature. Moreover, the assessors interpreted some questions, question guidance, and species information differently, adding variation in assessor scores, as seen in other FISK applications (e.g., Copp et al. 2009; Vilizzi and Copp 2013). Assessor and reviewer knowledge of the species in question and the relevant literature is important to account for information discrepancies and to increase certainty in the FISK assessment.

The FISK screens pointed to few data gaps, important areas of uncertainty, or potentially elevated risk levels for the three transgenic species, thus suggesting that there is little need to commit resources to a full risk assessment, such as has been done nationally with Black Carp *Mylopharyngodon piceus* (Nico et al. 2005) or regionally with Barramundi Perch *Lates calcarifer* (Hardin and Hill 2012). Critics of transgenic fluorescent ornamental fish also perceived a lack of clear risk management actions by agencies to reduce risks to acceptable levels (see Hill and Zajicek 2007). The results from the FISK screening of the three commercial fluorescent ornamentals that we studied indicate little need for additional risk management actions. We suggest that outcomes similar to those presented here would result from FISK assessments of many other hypothetical fluorescent ornamental fish unless there are important, risk-enhancing differences in novel transgenic species such as cold-hardiness or the ability to hybridize with native fishes. Nevertheless, our current level of knowledge warrants continuing to evaluate specific combinations of species, traits, and transgene constructs individually while building a track record of assessments of transgenic aquatic species (Kapuscinski 2005; Gong et al. 2007). Risk screens such as FISK can have high value to managers for such evaluations because they capture important elements of risk, providing vital information for assessment and management decisions with relatively small investments in time and funds.

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APPENDIX: DETAILED RESPONSES TO QUESTIONS

Table A.1. FISK assessment for two assessors (J.E.H. and L.L.L.) evaluating three species of transgenic fluorescent ornamental fish; RA = risk assessment. The answer to each question (Y = yes, N = no, and ? = don't know) is followed by a certainty rating (VU = very uncertain, MU = moderately uncertain, MC = moderately certain, and VC = very certain) (Copp et al. 2005, 2009; Lawson et al. 2013).

		Zebra Danio		Black Tetra		Tiger Barb	
	Question	J.E.H.	L.L.L.	J.E.H.	L.L.L.	J.E.H.	L.L.L.
1.01	Is the species highly domesticated or widely cultivated for commercial, angling, or ornamental purposes?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
1.02	Has the species established self-sustaining populations where introduced?	N—MU	N—VC	N—MU	?—MU	Y—MU	Y—MC
1.03	Does the species have invasive races/varieties/subspecies?	N—VC	N—VC	N—VC	N—MC	N—VC	N—VC
2.01	What is the level of matching between the species' reproductive tolerances and the climate of the RA area?	3—VC	2—MC	3—VC	3—MC	2—MC	1—MC
2.02	What is the quality of the climate match data (1–low; 2–medium; 3–high)?	3—VC	3—VC	3—VC	2—MC	3—MC	2—MC
2.03	Does the species have self-sustaining populations in three or more (Köppen-Geiger) climate zones?	Y—VC	Y—VC	Y—MU	Y—MC	N—VC	N—MC
2.04	Is the species native to, or have established self-sustaining populations in, regions with similar climates to the RA area?	Y—VC	N—MC	Y—VC	Y—MC	Y—VC	N—MC
2.05	Does the species have a history of being introduced outside its natural range?	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC
3.01	Has the species established one or more self-sustaining populations beyond its native range?	N—MU	N—MU	N—MU	?—MU	Y—MU	Y—MC
3.02	In the species' introduced range, are there impacts to wild stocks of angling or commercial species?	N—MC	N—VC	N—MC	N—MC	N—MC	N—VC
3.03	In the species' introduced range, are there impacts to aquacultural, aquarium, or ornamental species?	N—MC	N—VC	N—MC	N—VC	N—MC	N—VC
3.04	In the species' introduced range, are there impacts to rivers, lakes, or amenity values?	N—MC	N—VC	N—MC	N—VC	N—MC	N—MC
3.05	Does the species have invasive congeners?	N—VC	N—VC	N—VC	N—VC	N—MC	N—VC
4.01	Is the species poisonous/venomous, or poses other risks to human health?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
4.02	Does the species outcompete with native species?	N—MC	N—MC	N—MC	N—MC	N—MC	N—MC
4.03	Is the species parasitic of other species?	N—VC	N—VC	N—VC	N—VC	N—VC	N—MC
4.04	Is the species unpalatable to, or lacking, natural predators?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC

(Continued on next page)

TABLE A.1. Continued.

	Question	Zebra Danio		Black Tetra		Tiger Barb	
		J.E.H.	L.L.L.	J.E.H.	L.L.L.	J.E.H.	L.L.L.
4.05	Does species prey on a native species previously subjected to low (or no) predation?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
4.06	Does the species host, and/or is it a vector for, one or more recognized nonnative infectious agents?	N—MC	N—MC	N—MC	N—MC	N—MC	N—MC
4.07	Does the species achieve a large ultimate body size (i.e., >15 cm total length) (more likely to be abandoned)?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
4.08	Does the species have a wide salinity tolerance or is euryhaline at some stage of its life cycle?	N—MC	N—VC	N—VC	N—MC	N—MC	N—MC
4.09	Is the species able to withstand being out of water for extended periods (e.g., minimum of one or more hours)?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
4.10	Is the species tolerant of a range of water velocity conditions (e.g., versatile in habitat use)	Y—VC	Y—MC	N—MU	Y—MC	Y—MC	Y—MC
4.11	Does feeding or other behaviors of the species reduce habitat quality for native species?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
4.12	Does the species require minimum population size to maintain a viable population?	?—MU	N—MC	?—MU	N—MU	?—MU	N—MU
5.01	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?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
5.02	If the species is an omnivore (or a generalist predator), then is its foraging likely to have an adverse impact in the RA area?	N—VC	N—MC	N—VC	N—MC	N—VC	N—MC
5.03	If the species is mainly planktivorous or detritivorous or algivorous, then is its foraging likely to have an adverse impact in the RA area?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
5.04	If the species is mainly benthivorous, then is its foraging likely to have an adverse impact in the RA area?	N—MC	N—VC	N—MC	N—VC	N—MC	N—VC
6.01	Does it exhibit parental care and/or is it known to reduce age at maturity in response to environment?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC

(Continued on next page)

TABLE A.1. Continued.

	Question	Zebra Danio J.E.H.	Zebra Danio L.L.L.	Black Tetra J.E.H.	Black Tetra L.L.L.	Tiger Barb J.E.H.	Tiger Barb L.L.L.
6.02	Does the species produce viable gametes?	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC
6.03	Is the species likely to hybridize with native species (or use males of native species to activate eggs) in the RA area?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
6.04	Is the species hermaphroditic?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
6.05	Is the species dependent on the presence of another species (or specific habitat features) to complete its life cycle?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
6.06	Is the species highly fecund (>10,000 eggs/kg), iteropatric, or has an extended spawning season relative to native species?	Y—MC	Y—MC	N—MC	N—MC	N—MC	N—MC
6.07	What is the species' known minimum generation time (in years)?	1—VC	1—VC	1—VC	1—VC	1—VC	1—VC
7.01	Are life stages likely to be dispersed unintentionally?	N—MC	N—MC	N—MC	N—VC	N—MC	N—VC
7.02	Are life stages likely to be dispersed intentionally by humans (and suitable habitats abundant nearby)?	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC
7.03	Are life stages likely to be dispersed as a contaminant of commodities?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
7.04	Does natural dispersal occur as a function of egg dispersal?	N—VC	N—VC	N—VC	N—VC	N—VC	N—MC
7.05	Does natural dispersal occur as a function of dispersal of larvae (along linear and/or "stepping stone" habitats)?	N—MC	?—MU	N—MC	N—MU	N—MC	N—MC
7.06	Are juveniles or adults of the species known to migrate (spawning, smolting, feeding)?	N—MC	N—VC	?—MC	N—MC	?—MC	N—VC
7.07	Are eggs of the species known to be dispersed by other animals (externally)?	N—MC	N—VC	N—MC	N—VC	N—MC	N—VC
7.08	Is dispersal of the species density dependent?	N—MC	?—MU	N—MC	N—MU	?—MC	?—MU
8.01	Any life stages likely to survive out-of-water transport?	N—VC	N—VC	N—VC	N—VC	N—VC	N—VC
8.02	Does the species tolerate a wide range of water quality conditions, especially oxygen depletion and temperature extremes?	Y—VC	Y—VC	N—VC	Y—MC	N—MC	N—MC
8.03	Is the species readily susceptible to piscicides at the doses legally permitted for use in the risk assessment area?	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC
8.04	Does the species tolerate or benefit from environmental disturbance?	N—MC	?—MU	N—MC	Y—MC	N—MC	?—MU
8.05	Are there effective natural enemies of the species present in the risk assessment area?	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC	Y—VC

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