Fisheries and Oceans Canada | Pêches et Océans Canada
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Canadian Science Advisory Secretariat (CSAS)

Research Document 2026/043

National Capital Region

Environmental Risk Assessment of the GloFish® Electric Green®, Sunburst Orange® and Moonrise Pink® Corydoras (*Corydoras aeneus*): Three Lines of Transgenic Ornamental Fish

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June 2026

Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
DFO.CSAS-SCAS.MPO@dfo-mpo.gc.ca

ISSN 1919-5044
ISBN 978-0-660-99885-5 Cat. No. Fs70-5/2026-043E-PDF

Correct citation for this publication:

McGowan, C., Walker, S., Baillie, S., and Leggatt, R. 2026. Environmental Risk Assessment of the GloFish® Electric Green®, Sunburst Orange® and Moonrise Pink® Corydoras (*Corydoras aeneus*): Three Lines of Transgenic Ornamental Fish. DFO Can. Sci. Advis. Sec. Res. Doc. 2026/043. v + 27 p.

Aussi disponible en français :

*McGowan, C., Walker, S., Baillie, S., et Leggatt, R. 2026. Évaluation des risques pour l'environnement posés par les corydoras (*Corydoras aeneus*) GloFish^MD Electric Green^MD, Sunburst Orange^MD et Moonrise Pink^MD : trois lignées de poissons ornementaux transgéniques. Secr. can. des avis sci. du MPO. Doc. de rech. 2026/043. v + 32 p.*

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ABSTRACT

Pursuant to the *Canadian Environmental Protection Act* (CEPA), a notification under the *New Substances Notification Regulations (Organisms)* (NSNR(O)) was submitted by Spectrum Brands to Environment and Climate Change Canada (ECCC) for the import of three genetically engineered *Corydoras aeneus* called the GloFish® Electric Green® Cory (GCA2021), Sunburst Orange® Cory (OCA2020) and Moonrise Pink® Cory (PiCA2020) for commercial sales in Canada. The environmental risk assessment analyzed potential hazards, likelihood of exposure and associated uncertainties, to reach a conclusion on risk. The environmental exposure assessment concluded that the occurrence of GCA2021, OCA2020 and PiCA2020 in the Canadian environment, outside of aquaria, is expected to be rare, isolated, and ephemeral due to its inability to survive typical low winter temperatures in Canada's freshwater environments. Consequently, the likelihood of exposure to the Canadian environment is ranked low. Uncertainty associated with the exposure assessment is low, given the available data for temperature tolerance of the notified lines and relevant comparators, and lack of establishment of non-transgenic *C. aeneus* in North America despite a long history of use. The environmental hazard assessment concluded that potential hazards linked with environmental toxicity, trophic interactions, hybridization, disease, biodiversity, biogeochemical cycling, and habitat are negligible. There is low hazard (i.e., no anticipated harmful effects) related with horizontal gene transfer. Uncertainty associated with the environmental hazard ratings range from negligible to moderate due to data limitations for the notified and surrogate organisms, and some reliance on expert opinion and anecdotal evidence. The use of guide RNA and Cas9 during line creation may have resulted in off-target mutations in the GloFish® Corydoras populations, adding to uncertainty in the hazard assessment, but without altering the overall conclusions on risk. Overall, there is low risk of adverse environmental effects at the exposure levels predicted for the Canadian environment from the use of GCA2021, OCA2020 and PiCA2020 as ornamental aquarium fish, or other potential uses.

1. INTRODUCTION

On January 22, 2025, GloFish LLC with Spectrum Brands submitted three regulatory packages (notifications) to Environment and Climate Change Canada (ECCC) under the *New Substances Notification Regulations (Organisms)* [NSNR(O)] of the Canadian Environmental Protection Act, 1999 (CEPA) for the GloFish® Electric Green®, Sunburst Orange®, and Moonlight Pink® Corydoras. These ornamental fish are domesticated *Corydoras aeneus* that have been genetically engineered to fluoresce different colours in home aquaria. Notification of the GloFish® Corydoras under CEPA follows previous similar GloFish® notifications for six lines of GloFish® Tetra (DFO 2018, 2019), three lines of GloFish® Danio (DFO 2020a, 2020b), three lines of GloFish® Betta (DFO 2021a), four lines of GloFish® Barb (DFO 2023), four lines of GloFish® Pristella (DFO 2024), and four lines of GloFish® Rainbow Shark (DFO 2026).

The biotechnology provisions of CEPA take a preventative approach to pollution by requiring all new living products of biotechnology, including genetically engineered fish, to be notified and assessed prior to import or manufacture in Canada, to ultimately determine whether they are "toxic" or capable of becoming "toxic". Under CEPA (Section 64), an organism is considered "toxic" if it can enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health. Anyone proposing to import or manufacture a living animal product of biotechnology in Canada, including genetically engineered fish, is required to provide ECCC with the information prescribed in the NSNR(O) at least 120 days prior to the commencement of import or manufacture of the organism. This information is used to conduct an environmental risk assessment and an assessment of indirect human health (risk to human health from environmental exposure to the living organism), which are then used to determine whether the organism is CEPA-toxic or capable of becoming CEPA-toxic. Further information on CEPA and the NSNR(O), including guidance on the regulations, detailed guidance for information requirements, use of waivers, significant new activities, risk assessment outcomes, and risk management, can be found on the Biotechnology page of the Environment and Climate Change Canada (ECCC) website.

Under a memorandum of understanding with ECCC and Health Canada (HC), Fisheries and Oceans Canada (DFO) provide science advice in the form of an environmental risk assessment for fish products of biotechnology under the NSNR(O). This advice is used to inform the CEPA risk assessment conducted by ECCC and HC. Under this arrangement, the Minister of Environment and Climate Change receives scientific advice from DFO and retains ultimate responsibility for regulatory decision-making on the use of notified fish.

It is in this context that DFO conducted this environmental risk assessment of the notified organisms under the proposed use. Here, Risk is defined as a function of the potential for Canadian environments to be exposed to the notified organisms and the potential for the notified organisms to pose hazards to the Canadian environment. Exposure and Hazard assessments are conducted separately and then integrated into an assessment of Risk. Uncertainty in Exposure and Hazard assessments are determined, and are discussed in the context of the final risk assessment. A detailed overview of the legal context for the risk assessment process, the risk assessment framework, and regulatory decision making process under CEPA is provided in Leggatt et al. (2018b).

2. CHARACTERIZATION OF THE ORGANISMS

In the current notifications, Spectrum Brands is seeking to import three new transgenic strains of Corydoras (*Corydoras aeneus*) to Canada from the United States, for sale in the ornamental aquarium industry. Trade names for the transgenic organisms are: GloFish® Electric Green® Cory (GCA2021), GloFish® Sunburst Orange® Cory (OCA2020), and GloFish® Moonrise Pink® Cory (PiCA2020); herein referred to collectively as the GloFish® Corydoras. The GloFish® Corydoras are genetically engineered variants of the domesticated albino *C. aeneus* and differ from one another in the expression of distinct fluorescent proteins that result in novel colour phenotypes. Figure 1 (B-D) demonstrates the physical appearance of the three notified GloFish® Corydoras strains, as well as a non-transgenic, albino, domesticated *C. aeneus* (A).

Though greater detail regarding the structure, development, and function of the transgene constructs used to create the GloFish® Sharks has been provided by the company for review, it is considered confidential business information and is not included in this report.

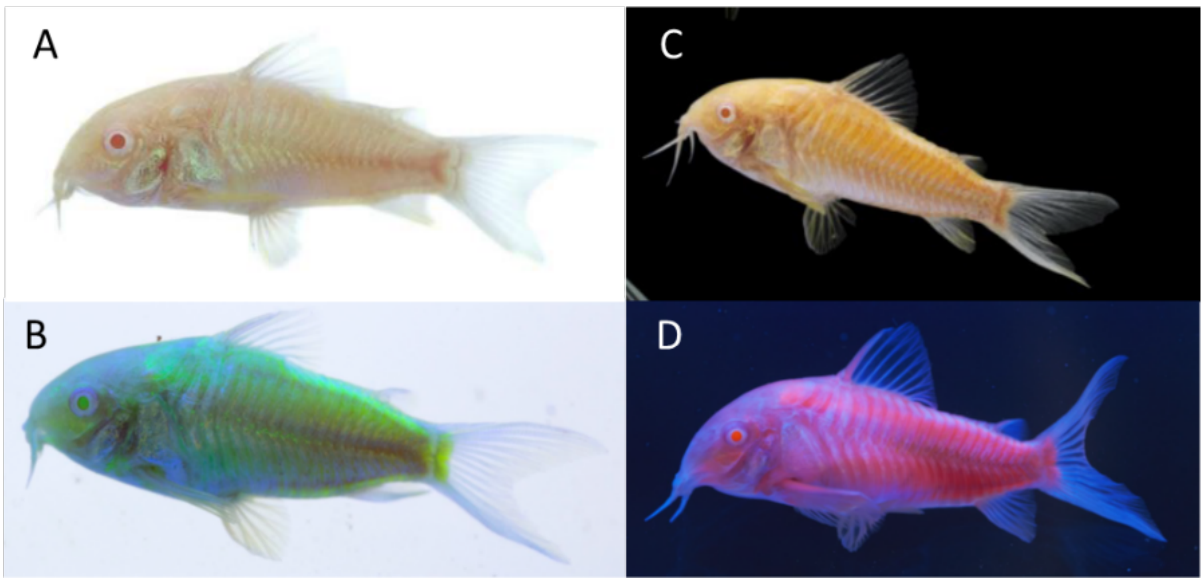

Figure 1. The current notification's transgenic fluorescent GloFish® C. aeneus variants. Non-transgenic albino C. aeneus (A), GloFish® Electric Green® Cory (B), GloFish® Sunburst Orange® Cory (C), and GloFish® Moonrise Pink® Cory (D). All images are provided by Spectrum Brands.

2.1. GLOFISH® ELECTRIC GREEN® CORYDORAS (GCA2021)

The GloFish® Electric Green® Cory is a genetically engineered albino *C. aeneus*. The genetic modification results in ubiquitous green colouration of the organism under ambient light, including sunlight and blue light (Figure 1B). The purpose of the modification is to create a new colour phenotype of *C. aeneus* for the ornamental aquarium trade.

2.1.1. Production of GCA2021

The purified transgene expression cassette was injected into newly fertilized eggs of *C. aeneus*. Further details provided by the company that describe line development and analysis to confirm that GCA2021 constitutes a single homogeneous line and that the vector backbone was not incorporated along with the transgenes are considered confidential business information and are not reported here.

2.1.2. Characterization of the transgene integrant

The sequence of the transgene construct as it is inserted into GCA2021 has not been determined, and the specific location of the insert within the organism's genome is unknown. Details regarding the analysis to confirm that multiple copies of the transgene cassette were incorporated at a single insert location are considered confidential business information and are not reported here.

2.1.3. Inheritance and stability of the transgene

The specific insert location of the transgene has not been determined, and consequently, it cannot be determined whether the transgene is inserted into a stable genome location or in an area prone to silencing. Should transgene expression be silenced in an individual, it would not display the green colouration and would, therefore, be culled from the breeding population.

Spectrum Brands has maintained this breeding line for over eight generations and have produced GCA2021 commercially since September 2021. In this time they have observed the green fluorescent phenotype to be durable and stable across generations.

2.1.4. Method to detect GCA2021

GCA2021 individuals are easily distinguished from non-transgenic domesticated *C. aeneus* by their phenotypic uniform green colouration under natural light and fluorescence under blue or UV light. GCA2021 can be distinguished genetically from other transgenic fluorescent green Corydoras carrying a different cassette using PCR.

2.1.5. Phenotypic characterization

2.1.5.1. Targeted phenotypic effects of the modification

The targeted phenotypic effect of the genetic modification is that GCA2021 appears green under ambient light and fluorescent under UV or blue light, to create a new, bright colour variant for the ornamental aquarium trade. The novel colour phenotype is present in the muscle as well as skin and eyes.

The company reports that GCA2021 individuals that are hemizygous and homozygous for the transgene insert are visually indistinguishable from each other and are both part of the commercially available population.

2.1.5.2. Additional phenotypic effects of the modification

The company provided information and data from a low-temperature tolerance experiment and a reproductive fitness experiment, to identify potential differences between GCA2021 and non-transgenic domesticated *C. aeneus* that may impact their ability to survive and reproduce in the wild. Results indicate that the transgenic Electric Green® Cory is more sensitive to cold than their non-transgenic siblings, and that the reproductive fitness of GCA2021 is low relative to domestic albino *C. aeneus*.

2.2. GLOFISH® SUNBURST ORANGE® CORYDORAS (OCA2020)

The GloFish® Sunburst Orange® Cory is a genetically engineered albino *C. aeneus*. The genetic modification results in ubiquitous orange colouration of the organism under ambient light, including sunlight and blue light (Figure 1C). The purpose of the modification is to create a new colour phenotype of *C. aeneus* for the ornamental aquarium trade.

2.2.1. Production of OCA2020

The purified transgene expression cassette was injected into newly fertilized eggs of *C. aeneus.* Further details provided by the company that describe line development and analysis to confirm that GCA2021 constitutes a single homogeneous line and that the vector backbone was not incorporated along with the transgenes are considered confidential business information and are not reported here.

2.2.2. Characterization of the transgene integrant

The sequence of the transgene expression cassette as it is inserted into OCA2020 has not been determined, and the specific location of the insert within the organism's genome is unknown. Details regarding the analysis to confirm that multiple copies of the transgene cassette were incorporated at a single insert location are considered confidential business information and are not reported here.

2.2.3. Inheritance and stability of the transgene

The specific insert location of the transgene has not been determined, and consequently, it cannot be determined whether the expression cassette is inserted into a stable genome location or in an area prone to silencing. Should transgene expression be silenced in an individual, it would not display the orange colouration and would, therefore, be culled from the breeding population.

Spectrum Brands has maintained this breeding line for over eight generations and have produced OCA2020 commercially since September 2022. In this time they have observed the orange fluorescent phenotype to be durable and stable across generations.

2.2.4. Method to detect OCA2020

OCA2020 individuals are easily distinguished from non-transgenic domesticated *C. aeneus* by their phenotypic uniform orange colouration under natural light and fluorescence under blue or UV light. OCA2020 can be distinguished genetically from other fluorescent orange Corydoras carrying a different cassette using PCR.

2.2.5. Phenotypic characterization

2.2.5.1. Targeted phenotypic effects of the modification

The targeted phenotypic effect of the genetic modification is that OCA2020 appears orange under ambient light and fluorescent under UV or blue light, to create a new, bright colour variant for the ornamental aquarium trade. The novel colour phenotype is present in the muscle, as well as skin and eyes.

The company reports that OCA2020 individuals that are hemizygous and homozygous for the transgene insert are visually indistinguishable from each other and are both part of the commercially available population.

2.2.5.2. Additional phenotypic effects of the modification

The company provided information and data from a low-temperature tolerance experiment and a reproductive fitness experiment, used to identify potential differences between OCA2020 and non-transgenic domesticated *C. aeneus* that may impact their ability to survive and reproduce in the wild. Results indicate that the transgenic Sunburst Orange® Cory is more sensitive to cold than their non-transgenic siblings, and that the reproductive fitness of GCA2021 is similar to domestic albino *C. aeneus*.

2.3. GLOFISH® MOONRISE PINK® CORY (PICA2020)

The GloFish® Moonrise Pink® Cory is a genetically engineered albino *C. aeneus.* The genetic modification results in ubiquitous pink colouration of the organism under ambient light, including sunlight and blue light (Figure 1D). The purpose of the modification is to create a new colour phenotype of *C. aeneus* for the ornamental aquarium trade.

2.3.1. Production of PiCA2020

The purified transgene expression cassette was injected into newly fertilized eggs of *C. aeneus*. Further details provided by the company that describe line development and analysis to confirm that PiCA2021 constitutes a single homogeneous line and that the vector backbone was not incorporated along with the transgenes are considered confidential business information and are not reported here.

2.3.2. Characterization of the transgene integrant

The sequence of the transgene construct as it is inserted into PiCA2020 has not been determined, and the specific location of the insert within the organism’s genome is unknown. Details regarding the analysis to confirm that multiple copies of the transgene cassette were incorporated at a single insert location are considered confidential business information and are not reported here.

2.3.3. Inheritance and stability of the transgene

The specific insert location of the transgene has not been determined, and consequently, it cannot be determined whether the transgene is inserted into a stable genome location or in an area prone to silencing. Should transgene expression be silenced in an individual, it would not display the pink colouration and would, therefore, be culled from the breeding population.

Spectrum Brands has maintained the PiCA2020 line for over eight generations in preparation for commercial production. During this time, they have observed the pink fluorescent phenotype to be durable and stable.

2.3.4. Method to detect PiCA2020

PiCA2020 individuals are easily distinguished from non-transgenic domesticated *C. aeneus* by their phenotypic uniform pink colouration under natural light and fluorescence under blue or UV light. PiCA2020 can be distinguished genetically from other transgenic fluorescent pink Corydoras carrying a different cassette using PCR.

2.3.5. Phenotypic characterization

2.3.5.1. Targeted phenotypic effects of the modification

The targeted phenotypic effect of the genetic modification is that PiCA2020 appears pink under ambient light and fluorescent under UV or blue light, to create a new, bright colour variant for the ornamental aquarium trade. The novel colour phenotype is present in the muscle as well as skin and eyes.

The company reports that PiCA2020 individuals that are hemizygous and homozygous for the transgene insert are visually indistinguishable from each other and are both part of the commercial population that will be available for sale to the public.

2.3.5.2. Additional phenotypic effects of the modification

The company provided information and data from a low-temperature tolerance experiment and a reproductive fitness experiment, used to identify potential differences between PiCA2020 and non-transgenic domesticated C*. aeneus* that may impact their ability to survive and reproduce in the wild. Results indicate that the transgenic Pink Moonrise® Cory has similar cold sensitivity as their non-transgenic siblings, and that the reproductive fitness of GCA2021 is low relative to domestic albino *C. aeneus*.

2.4. PLEIOTROPIC EFFECTS OF FLUORESCENT TRANSGENES IN OTHER FISH

Many fluorescent proteins, most commonly enhanced green fluorescent protein (eGFP), have widespread use for research in a variety of organisms, and some risk assessment relevant information is available on Zebrafish (*Danio rerio*) transgenic for red fluorescent protein (RFP) and other fluorescent proteins.

Zebrafish containing a RFP transgene were observed to be less cold tolerant than unrelated non-transgenic Zebrafish, when examined under different acclimation temperatures (Cortemeglia and Beitinger, 2005, 2006a), though differences in strain background and rearing conditions (Schaefer and Ryan, 2006) prior to experimentation may have impacted relative extreme temperature tolerance. Similarly, Leggatt et al. (2018a) reported that Zebrafish transgenic for eGFP, driven by the Fli-1 protein promoter, were less cold tolerant than the source non-transgenic strain. However, Leggatt et al. (2018a) also reported on two other eGFP lines, driven by different promoters, that did not exhibit diminished cold tolerance. This indicates that different transgenic lines may have different responses to extreme environmental stressors.

No effect of fluorescence protein transgenesis was observed on survival of RFP Zebrafish relative to related non-transgenic fish under laboratory conditions (Howard et al., 2015). In a population of eGFP, RFP, eGFP-RFP, and non-transgenic Zebrafish, eGFP fish had lower survival, but there was no effect of RFP or the double transgene on survival (Gong et al., 2003), indicating different transgenes or transgenic lines may also have different influences on survival.

Reports describing the effects of RFP transgenesis on vulnerability to predation have shown varied outcomes. Cortemeglia and Beitinger (2006b) found that RFP and unrelated non-transgenic Zebrafish were equally preyed upon. Hill et al. (2011) found that GloFish® RFP Zebrafish were two times more vulnerable to predation than unrelated non-transgenic Zebrafish. In contrast, Jha (2010) found a domesticated RFP Zebrafish strain in India was less preyed upon by wild-caught Snakeheads than were wild-caught non-transgenic Zebrafish. Factors influencing the difference in relative vulnerability of RFP Zebrafish to predation are not known, but could include differences in genetic background or rearing history of transgenic and non-transgenic Zebrafish, innate preference or life history of predators, and/or experimental conditions (e.g., presence of shelter for prey species). Jha (2010) found RFP Zebrafish were more aggressive than wild-caught unrelated Zebrafish, although this may have been due to differences in domestication and/or rearing. GloFish® Electric Green® Tetras did not differ from non-transgenic Tetras in foraging success or aggression levels in paired foraging competition trials (Leggatt and Devlin, 2020).

The reported influences of RFP and other fluorescent transgenes on reproductive success or preferences in Zebrafish are likewise inconsistent. RFP and non-transgenic Zebrafish had similar age at maturity for related females, as well as similar male and female fecundity (Howard et al., 2015). In a population containing equal numbers of eGFP and non-transgenic Zebrafish eGFP offspring had no reproductive advantage or disadvantage (Gong et al., 2003). In contrast, Owen et al. (2012) found both non-transgenic and RFP Zebrafish females (related) preferred to associate with RFP rather than non-transgenic males, regardless of the proportion of non-transgenic to RFP fish with which they were raised. In another study, (Howard et al., 2015) reported lower mating success in RFP males and less aggression towards both male and female fish compared to related non-transgenic males.

Snekser et al. (2006) found the RFP transgene did not influence social partner preferences for either shoaling or in a potential reproductive context in presumably unrelated populations of RFP and non-transgenic Zebrafish. Jiang et al. (2011) reported sex-specific differences in non-transgenic Cloud Mountain Minnow (*Tanichthys albonubes*) preference for non-transgenic or RFP transgenic conspecifics, where non-transgenic males tended to prefer transgenic females, while non-transgenic females preferred non-transgenic males and had no preference for shoal type. Howard et al. (2015) examined the fate of the RFP transgene over 15 generations in a serial competitive breeding experiment in 18 populations of GloFish® Zebrafish. In all populations, the frequency of the RFP transgene declined rapidly, and was eliminated in all populations except one, indicating a strong bias against the RFP transgene in reproduction. In previously notified GloFish®, three of six lines of Tetras, all three GloFish® Danio lines, two of three GloFish® Betta lines, all four GloFish® Tiger Barb and Pristella lines were reported to have decreased reproductive success in competition.

Overall, there are inconsistent reports of pleiotropic effects in other fluorescent protein transgenic models, and, for the most part, these effects would be considered detrimental to the organism (e.g., diminished cold tolerance, diminished reproductive success).

2.5. CHARACTERIZATION RELATIVE TO PREVIOUSLY ASSESSED GLOFISH

The GloFish® Corydoras were produced using similar methodologies and testing protocols as for previously notified and assessed GloFish® lines. All previously notified GloFish® lines have used similar transgene expression cassette production and elements (promoters, terminator sequences), though the pigment genes vary between colours of fish.

3. CHARACTERIZATION OF THE COMPARATOR SPECIES

For the purpose of this risk assessment, the domesticated *Corydoras aeneus* (Gill 1858), commonly known as Corydoras, Bronze Corydoras, Green Corydoras, Bronze Catfish, Light-spot Corydoras or Wavy Catfish (FishBase), was selected as a comparator. *C. aeneus* is a popular ornamental species that has been bred and traded in North America since about 1915 (Nico and Fuller, 2025). It is a tropical species, naturally distributed throughout South America on the eastern side of the Andes, from Columbia and Trinidad south to the Rio de la Plata basin in Brazil and Argentina (FishBase), though it is unclear if a single species occupies this entire area (Tencatt et al., 2023). In captivity, *C. aeneus* grows to a size of approximately 7 cm, with females slightly larger than males, and has a lifespan of about 10 years (Seriously Fish). The pet-trade describes *C. aeneus* as a schooling, non-aggressive, bottom-dwelling, community fish.

3.1. TAXONOMIC STATUS

C. aeneus is a freshwater tropical fish of the family Callichthyidae (order Siluriform), which are also known as armored catfish due to the two rows of dermal plates along the length of their bodies. There are currently 220 valid species within the family, of which 180 fall within the genus *Corydoras* (Tencatt et al., 2023). In a review of the sub-family Corydoradinae, Britto (2003) examined 56 *Corydoras* species and, using a cladistic analysis, found the genus is non-monophyletic, with species falling into different clades.

While there are no scientific papers describing natural hybridization between *Corydoras* species in the wild, there are scattered reports on internet forums of hybrids observed in captivity. Teletchea (2016) listed pet-trade bred *C. aeneus* as very domesticated.

3.2. PHYSICAL, CHEMICAL AND BIOLOGICAL REQUIREMENTS

In nature, *C. aeneus* typically inhabits slow moving rivers and streams, preferably with shallow, muddy waters, though they have also been found in fast flowing rivers and nearly stagnant pond and marshes (aquariumtidings.com). Their capacity to succeed in stagnant water stems from their ability to breath by taking surface air into their gut, where it can be absorbed into the blood, though predation risk may increase as they swim to the surface to gulp a breath of air (Pineda et al., 2020). They are omnivores, eating worms, crustaceans, insects and plant matter (Mills and Ververs 1982). In the aquarium, they can be voracious eaters and can significantly impact the density of benthic nematode populations (Majdi et al., 2018).

Corydoras prefer a temperature range of 22-29°C, a pH between 6 and 8, and a water hardness between 36 and 268 ppm (aquariumtidings.com). In experiments at DFO, when temperature was dropped daily by 1°C, from a starting temperature of 20.5°C, non-transgenic albino-strain pet-trade sourced Corydoras decreased activity at approximately 14.5°C, decreased feeding at 13.5°C, and lost equilibrium at a mean temperature of 12.7 ± 1.1°C (range 11-15.2°C) (Leggatt 2025 in prep).

3.3. LIFE-HISTORY

The reproductive behavior of C. aeneus is complex. As described by Sakurai et al. (1992), "*when spawning season arrives, males begin a courtship dance and touch the female's dorsal. Once the female accepts these advances, a male will vibrate his body inducing her to initiate spawning activities. The female will suck sperm out into her mouth from the male's genital pore. At this point, the female ejects a clutch of eggs which she then uniquely holds between her ventral fins. Finding a suitable place, she spits the sperm out onto this site and then attaches the eggs, at which point fertilization occurs. During the course of this repeated series of activities, over 200 eggs may be laid*." In the wild, C. aeneus reproduction coincides with the rainy season, though maturation is believed to be continuous and females may be able to breed more than once in a season (Alkins-Koo et al. 2000). In the aquarium, or under commercial production, the reproductive condition of adult fish is triggered through the manipulation of water temperatures and water replacement regimes (water chemistry). Courtship can take place over several days before fish are ready to copulate, which can occur multiple times over a period of several hours.

At 25°C, fertilized eggs will hatch after three days, and fish are ready to feed at three to five days after hatching. Female Corydoras take approximately 12 months to reach sexual maturity at a slightly larger size than males, who take about 24 months. Adult males are about 6.5 cm in length, while adult females are slightly larger at 7 cm.

3.4. BACKGROUND GENETICS

Within the genus *Corydoras* there is extensive variability in chromosome number and nuclear DNA content, indicating an evolutionary history characterized by genome duplications and extensive chromosomal rearrangements (Carvaho et al. 1998, Bell et al. 2020). Indeed, within the species *C. aeneus*, chromosome number is variable, and both diploids and natural tetraploids are observed.

Sun et al. (2022) sequenced the entire mitochondrial genome for *C. aeneus* as part of a phylogenetic analysis of 44 fish species from the order Siluriform. They found that species of the genus *Corydoras* grouped together as a single monophyletic group within the family Callichthyidae and that the mitochondrial genome size, gene content, and gene arrangement in *Corydoras* are highly conserved.

There are no published reports that consider the genetics of *C. aeneus* or other *Corydoras* species at a population level, however, Bell et al. (2020) observed low variability at the immune-related toll-like receptor gene in both *Corydoras maculifer* (n=20) and *Corydoras araguaiaensis* (n=41), two coexisting species that are respectively diploid and putative tetraploid.

3.5. HISTORY OF INVASIVENESS

Domesticated *C. aeneus* is a popular species and has been traded and sold in Canada and the United States for over 100 years, with no evidence of invasiveness. The only examples of established *C. aeneus* populations outside their native range are those reported in 1984 for freshwater streams on the islands of Oahu and Kauai, Hawaii (USFWS 2017), however, negative impacts to local ecosystems are undocumented and the current status of these populations is unknown. According to Hill et al. (2017) the invasion potential of *C. aeneus* in conterminous United States is ranked slightly above the low threshold when using the Fish Invasiveness Screening Kit (FISK).

Tuckett et al. (2017) found evidence of an established Callichthyidae species, the Brown Hoplo (*Hoplosternum littorale*) within 500 meters from the effluent of tropical ornamental fish farms near Tampa Bay, Florida, but never detected *C. aeneus*. Other introductions of unidentified *Corydoras* species have been observed in Colorado and Florida, but were last observed in 1986 and 1984 respectively, with no report of environmental impacts.

4. CHARACTERIZATION OF POTENTIAL RECEIVING ENVIRONMENT

Though the many lakes and rivers of Canada vary in their annual temperature profiles, as well as their average maximum and minimum temperatures, most reach 4°C or below at some point annually, and only a few isolated lakes in Southern Coastal British Columbia have minimum recorded temperatures above this. Of these latter lakes, all but one has a minimum temperature recorded below 6°C, and the one lake with minimum temperature recording above 6°C (Cowichan Lake) has, on occasion, minimum temperatures recorded below 6°C (see Leggatt et al., 2018a). It is worth noting that temperature recordings of these warmer lakes are often restricted to a single measurement per winter, and recorded temperatures may not represent the coldest or warmest temperature obtained during winter months. Given the above, if an introduced fish cannot survive at 4°C or below, its occurrence in the Canadian environment will be seasonal at best, with possible localized overwintering pockets (e.g., industrial effluent, hot springs etc., isolated lakes, if species can survive between 4-6°C).

It should be noted that many freshwater systems may have heterogeneity in temperature profiles. For example, groundwater contributions may increase or decrease temperatures in localized areas of a water body, and shorelines are expected to have more extreme temperatures than deep waters. Also, mean freshwater surface temperatures in Canada are rising as a result of global climate change and are projected to increase by 1.5 to 4.0°C over the next 50 years (DFO, 2013) and therefore, could increase the number of possible lakes in which organisms with moderate cold tolerance could survive.

A more detailed description of potential receiving environments in Canada relevant to the introduction of tropical freshwater fish is presented in Leggatt et al. (2018a).

5. ENVIRONMENTAL RISK ASSESSMENT

This section of the document comprises the environmental risk assessment conducted under the *Canadian Environmental Protection Act* (CEPA) with respect to the three GloFish® Corydoras lines that are described in the first part of this document and have been notified by Spectrum Brands under the *New Substances Notification Regulations (Organisms)*. Given the common comparator species, and the physiological and ecological similarities between the three lines, the following section will consider all lines at the same time. The environmental risk assessment format follows that used for previously notified GloFish® Tetras (DFO, 2018, 2019), Danios (DFO, 2020a, 2020b), Bettas (DFO 2021a), Tiger Barbs (DFO 2023), Pristellas (DFO 2024), and Rainbow Sharks (DFO 2026). Results of the current assessment are equivalent to those from previous GloFish® assessments unless otherwise stated.

5.1. EXPOSURE ASSESSMENT

The exposure assessment for the three living organisms addresses both their potential to enter the environment (release) and fate once in the environment. The likelihood and magnitude of environmental exposure is determined through an extensive, cradle-to-grave assessment that details the potential for release, survival, persistence, reproduction, proliferation, and spread in the Canadian environment. Rankings for the likelihood of exposure to the Canadian environment are provided in Table 1.

Table 1. Rankings for likelihood of exposure of genetically engineered fish to the Canadian environment.

Likelihood of Exposure	Assessment
Negligible	No occurrence; Not observed in Canadian Environment[1]
Low	Rare, isolated occurrence; Ephemeral presence
Moderate	Often occurs, but only at certain times of the year or in isolated areas
High	Often occurs at all times of the year and/or in diffuse areas

[1]extremely unlikely or unforeseeable

Given the regulatory status of any GE fish undergoing environmental risk assessment under CEPA, a lack of empirical data regarding the survival, fitness and ability of GloFish® Corydoras to reproduce in the natural environment will contribute uncertainty to the exposure assessment. Uncertainty associated with the environmental fate of an organism or the failure of biological and geographical containment may depend on the availability and robustness of the scientific information related to the biological and ecological parameters of the organism, valid surrogates, and the receiving environment. Table 2 ranks uncertainty associated with the likelihood of occurrence and fate of the organism in the Canadian environment.

All previous assessments of notified and assessed GloFish® species concluded low rating for environmental exposure with low uncertainty. There are no known molecular or phenotypic characteristics of GloFish® Corydoras that suggest a different rating than previously assessed lines, and no new scientific literature has been published that would alter the previous ratings. Consequently, the environmental exposure assessments for GloFish® Corydoras are **low**, with **low** uncertainty that is consistent with previously notified lines. Details supporting this conclusion follow.

Table 2. Ranking of uncertainty associated with the likelihood of occurrence and fate of the organism in the Canadian environment (environmental exposure).

Uncertainty	Available Information
Negligible	High-quality data on the organism (e.g., sterility, temperature tolerance, fitness). Data on environmental parameters of the receiving environment and at the point of entry. Demonstration of absence of Genotype by Environment Interaction (GxE) effects or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Low	High-quality data on relatives of the organism or valid surrogate. Data on environmental parameters of the receiving environment. Understanding of potential GxE effects across relevant environmental conditions. Evidence of variability.
Moderate	Limited data on the organism, relatives of the organism or valid surrogate. Limited data on environmental parameters in the receiving environment. Knowledge gaps. Reliance on history of use or experience with populations in other geographical areas with similar or better environmental conditions than in Canada.
High	Significant knowledge gaps. Significant reliance on expert opinion.

5.1.1. Likelihood of release

Though the stated purpose of the organism is for sale in the ornamental market, and hobbyists who purchase the product do, for the most part, follow the instructions for disposal that are recommended by the retailer or the company itself, there is still a high likelihood that GloFish® Corydoras will be introduced into the Canadian environment. Once the organism has been sold into the retail market, it is no longer under the direct control of the importer, and there can be no guarantee of appropriate containment and disposal. Numerous aquarium fish have established themselves in natural waters in North America, and reoccurring, though isolated, reports of aquarium fish in Canadian water suggest the practice of releasing aquarium fish into the environment is common and ongoing (Kerr et al., 2005; Rixon et al., 2005; Marson et al., 2009; Strecker et al., 2011, DFO 2021b). The extent to which GloFish® Corydoras may be further exposed to the environment will therefore depend heavily on their ability to survive and reproduce in Canadian lakes and rivers.

5.1.2. Likelihood of survival

As a tropical species, *C. aeneus* is not expected to survive in a temperate region, where water temperatures are below optimal for survival. Indeed, water temperature is a key abiotic factor that affects both the survival and production of most freshwater fish populations, and is a pervasive determinant of habitat suitability (Magnuson et al., 1979; Jobling, 1981).

In the aquarium, *C. aeneus* do best at temperatures between 22 and 29°C (aquariumtidings.com, see Section 3.2).

In experiments at DFO (see Section 3.2), when temperature was dropped daily by 1°C, from a starting temperature of 20.5°C, non-transgenic albino-strain pet-trade sourced Corydoras decreased activity at approximately 14.5°C, decreased feeding at 13.5°C, and lost equilibrium at a mean temperature of 12.7 ± 1.1°C (range 11-15.2°C) (Leggatt, unpublished data).

As discussed in Section 4, there are no known lakes in Canada that consistently remain above 7°C throughout the entire course of a year, or above 6°C across multiple years, and almost all do not remain above 4°C throughout the year (with the exception of hot springs and industrial effluent). Consequently, the temperatures needed for GloFish® Corydoras to survive may be possible for several Canadian lakes during the summer, but are extremely unlikely during the Canadian winter.

It should be noted that mean freshwater surface temperatures in Canada are rising as a result of global climate change. DFO (2013) has projected an increase of 1.5 to 4.0°C over the next 50 years, and the Intergovernmental Panel on Climate Change report projects increases of 2 to 7°C in lake surface temperatures globally (IPCC, 2021). Though the majority of freshwater systems experiencing significant ice coverage in the winter are expected to see a decrease in the number of ice-days (DFO, 2013), the continuation of any winter ice coverage would result in temperatures at or below 4°C at some point during the winter, preventing year-round survival of GloFish® Corydoras. Cold-tolerance data suggest negligible potential for survival in Canadian waters, even with the increased water temperatures associated with climate change. At best, its occurrence in the environment would be seasonal or ephemeral.

5.1.3. Likelihood of reproduction

Though water temperatures in Canada will limit the persistence of any GloFish® Corydoras that are introduced into the environment (see Section 5.1.2), there may still be time to reproduce, if introduced at the start of a warm season. For example, Osoyoos Lake in the BC interior is one of Canada's warmest lakes in the summer, with an average temperature between 20 and 25°C for about 2 months of the year (mid-July to mid-September), with higher temperatures (e.g., 25°C) restricted to an even shorter window (e.g., end of July – beginning of August, (BCLSS, 2013). Corydoras could also potentially reproduce in isolated areas of warm water (e.g., hot springs, industrial effluent).

In the wild, *C. aeneus* reproduction coincides with the rainy season, though maturation is believed to be continuous and females may be able to breed more than once in a season (Alkins-Koo et al. 2000). Courtship can take place over several days before fish are ready to copulate, which can occur multiple times over a period of several hours. At 25°C, fertilized eggs will hatch after three days and fish are ready to feed at three to five days after hatching (see Section 3.3).

While some lakes in Canada may reach 25°C for short periods during the summer (see Section 4), it is highly unlikely that all other seasonal environmental conditions required for *C. aeneus* to mature sexually and reproduce will occur in a temperate environment. Consequently, reproduction of *C. aeneus* during this short window of time and in a temperate environment, is highly unlikely.

5.1.4. Likelihood of proliferation and spread

The capacity for GloFish® Corydoras to proliferate and spread in the Canadian environment is prevented by the fact that *C. aeneus* cannot survive the winter. It should be noted that any released GloFish® Corydoras are expected to occupy slow moving rivers and streams, preferably with shallow, muddy waters, based on what is known of wild-type habitat preferences (see Section 3.2). These areas are expected to have more extreme temperature ranges than deep water or mid-lake areas that are often the source of water temperature measurements (Trumpikas et al., 2015). Consequently, periodic winter temperatures may be colder than indicated by recorded data, which may further reduce the potential for overwintering of GloFish® Corydoras.

5.1.5. Conclusions of the exposure assessment

Given the above analysis, the occurrence of GloFish® Corydoras in the Canadian environment is expected to be rare, isolated and ephemeral. Consequently, the likelihood of exposure of GloFish® Corydoras to the Canadian environment is ranked **low** according to Table 1. The uncertainty associated with this estimate is **low** (Table 2), given the quality of data (temperature tolerance) available for GloFish® Corydoras and valid surrogate organisms, evidence of variability (temperature tolerance and reproductive success, low variability in colour trait), and data available on the environmental parameters of the receiving environment in Canada. Though G x E interactions may contribute to the observed variability in temperature sensitivity, the trait is not expected to change beyond the normal range for low temperature tolerance and is not expected to improve the organism's ability to survive at low temperatures in the wild. This rating is consistent with the low exposure rating with low uncertainty concluded on for all other GloFish® species.

The notifying company identifies the sole intended use for the notified organism as an ornamental fish for interior, static home aquaria. However, once purchased by consumers, other unintended uses cannot be discounted (e.g., rearing in outdoor ponds, as bait fish, etc.). While some unintended uses may lead to the release of GloFish® Corydoras, they would not be expected to alter the organism's ability to overwinter in Canadian environments, or otherwise alter the low environmental exposure ranking for the organism.

Though changing water temperature patterns associated with global climate change have the potential to increase uncertainty when determining the ability of the notified organism to survive, reproduce and proliferate in Canadian freshwater ecosystems, it does not affect the final ranking of low for environmental exposure.

5.2. HAZARD ASSESSMENT

The hazard assessment examines potential impacts to the environment that could result from exposure to GloFish® Corydoras. The hazard identification process considers potential pathways to harm including through environmental toxicity (i.e., potential to be poisonous), gene transfer, trophic interactions, and as a vector for pathogens, as well as capacity to impact ecosystem components (e.g., habitat, nutrient cycling, biodiversity). Table 3 categorizes the severity of the biological consequences based on the severity and reversibility of effects to the structure and function of the ecosystem. Any difference in measurement endpoint is evaluated relative to 'normal' variation, based on published studies and expert opinion.

Table 3. Ranking of hazard to the environment resulting from exposure to the organism.

Hazard Ranking	Assessment
Negligible	No effects[1]
Low	No harmful effects[2]
Moderate	Reversible harmful effects
High	Irreversible harmful effects

[1]No biological response expected beyond natural fluctuations. [2]Harmful effect: an immediate or long-term detrimental impact on the structure or function of the ecosystem including biological diversity beyond natural fluctuations.

Uncertainty around the hazard assessment may be significant due to clear knowledge gaps and lack of empirical data around the behaviour and effects of GloFish® Corydoras in the natural environment. Criteria for the assessment of uncertainty address potential effects to the environment, which may rely heavily on information and data found in published and peer-reviewed scientific literature. A description of rankings for uncertainty regarding the potential hazards of the organism in the environment is provided in Table 4.

Table 4. Ranking of uncertainty associated with the environmental hazard.

Uncertainty Ranking	Available Information
Negligible	High quality data on the organisms. Demonstration of absence of GxE effects or complete understanding of GxE effects across relevant environmental conditions. Evidence of low variability.
Low	High quality data on relatives of the organisms or valid surrogate. Understanding of GxE effects across relevant environmental conditions. Some variability.
Moderate	Limited data on the organisms, relatives of the organisms or valid surrogate. Limited understanding of GxE effects across relevant environmental conditions. Knowledge gaps. Reliance on expert opinion.
High	Significant knowledge gaps. Significant reliance on expert opinion.

5For uncertainty, the quality of data refers to the data or information available for each parameter being examined, the integration of this information and breadth of experimental conditions examined, sample size, appropriateness of controls, statistical analysis, as well as the experimental design and interpretations of the results. Variability refers to both the range of phenotypic differences among individuals or strains within the same environment as well as the range of physical, chemical, and biological conditions that may be experienced by a GE fish in the receiving environment. Broad principles influencing uncertainty in hazard assessments of GE fish (e.g., GxE, effects of background genetics, off-target/pleiotropic effects) are detailed in Leggatt et al. (2018b) and Devlin et al. (2015).

The proposed use of GloFish® Corydoras in Canada (i.e., importation and transport in static containers, holding in static tanks in commercial wholesalers and retailers, rearing in static tanks in home aquaria) provide minimal pathways of effects to Canadian environments. The majority of potential hazards posed by GloFish® Corydoras (e.g., through interactions with other organisms, impacts to biogeochemical cycling, habitat and biodiversity) would be through direct release into natural aquatic ecosystems, although some potential hazards could act indirectly through the release of waste water and carcasses into the environment (e.g., environmental toxicity, horizontal gene transfer, as a vector for disease).

In assessments of previously notified and assessed GloFish® lines, all concluded with negligible rating for most environmental hazard pathways and low hazard rating through horizontal gene transfer (HGT), with uncertainty ranging from negligible to moderate. While *C. aeneus* differ from previously notified species in some phenotypes (i.e., reproductive behaviour and physiology), there are no known molecular or phenotypic characteristics of GloFish® Corydoras derived from the genetic modifications that suggest a different rating than previously assessed lines, and no new scientific literature has been published that would alter the previous ratings. Consequently, the environmental hazard assessments for GloFish® Corydoras follow those of the previously notified GloFish® species. Details supporting these conclusions follow, and greater description of each hazard assessment can be found in Leggatt et al. (2018b).

5.2.1. Potential hazards through environmental toxicity

Potential routes of environmental toxicity include exposure of aquatic ecosystems to the whole animal and its waste, as well as ingestion by predators. Exposure of the fluorescent proteins to the environment is expected to be lower than exposure of the proteins to GloFish® Corydoras lines; though different routes of exposure are not necessarily comparable. Fluorescent proteins are commonly used as neutral markers in research in a wide range of organisms with almost no reports of toxicity (Stewart, 2006). The few reports of negative effects are generally specific to transgenic organisms with especially high expression of fluorescent transgenes (Huang et al., 2000; Devgan et al., 2004; Guo et al., 2007). Any toxic effects to host organisms are likely due to production of the protein within the host cell, and are not expected to have equal effects from contact or ingestion exposure.

The notifications report screening the amino acid sequence of the fluorescent proteins for allergenicity on Allermatch™ and AllergenOnline that found no functional matches to known human allergen amino acid sequences. After several years of commercial production in the US, there have been no reported toxic effects resulting from exposure to other species of GloFish® containing transgenes coding the same proteins as those in the GloFish® Corydoras lines in both Canada and the United States. Consequently, the potential hazard to the environment due to environmental toxicity of GloFish® Corydoras is ranked **negligible**. The uncertainty associated with this ranking is **moderate** due to limited direct data from the notified organisms or surrogate organisms, and reliance on anecdotal evidence and indirect evidence from other organisms. This concurs with assessment rankings for previously notified GloFish® species and no new relevant data has become available since the analyses of previously notified GloFish® lines.

5.2.2. Potential hazards through horizontal gene transfer

Horizontal gene transfer (HGT) is the non-sexual exchange of genetic material between organisms of the same or different species (DFO, 2006). Pathways of exposure of free transgenic DNA to novel organisms (most likely prokaryotes) include exposure within the gut, or through feces, mucus, and other waste sloughed off by the fish into the water. The transgene constructs do not contain transposable elements that may increase the potential for DNA uptake/mobility to a new organism. In order for the transgene to be expressed and result in phenotypic change, it requires co-transfer of regulatory elements. The close proximity of the promoters to the pigment transgenes could increase the likelihood of them being co-transferred and expressed, though vertebrate promoters generally have poor activity in prokaryotes. One recent study examined the potential for HGT of fluorescent protein transgenes using genetically engineered fruit flies (transgenic for DsRed) and its parasitoid (Ramirez-Santos et al. 2018). The authors did not find any evidence of HGT of the fluorescent protein transgene over 16 generations of experimental rearing, although cautioned their experimental design may not have detected rare events of HGT or transfer of mutated transgenes.

Genes encoding fluorescence have been introduced to a wide range of organisms with few reports of harmful effects from the introduced transgenes. This suggests that the introduction of the transgene through HGT to a novel host is not expected to result in harmful effects, should it occur. Graham and Davis (2021) demonstrated HGT of an environmentally advantageous gene (antifreeze protein) between two fish species at an evolutionary scale. While this demonstrates

HGT can occur very infrequently with advantageous genes between higher organisms, the lack of fitness advantage (e.g., cold tolerance) conferred by the present fluorescent protein transgenes suggests that if HGT transfer occurred it would likely be on an individual organism level. Though the introduction of a fluorescent transgene to a novel organism in Canadian environments through HGT cannot be excluded, the absence of expected harmful effects from such an introduction result in a hazard ranking of **low**. While the transgenes are well defined, the limited knowledge of the location of the transgenes within the *C. aeneus* genome, and lack of studies examining HGT of the transgenes and resulting consequences, results in **moderate** uncertainty. This concurs with the previous assessments for the GloFish® species, though in some groups (Tetras) uncertainty was assessed as low (Table 12). Here, as with the GloFish® Danios, Bettas, Barbs, Pristellas, and Rainbow Shark, the uncertainty rating was increased to moderate to better reflect the lack of or limited number of relevant studies of HGT and resulting consequences.

5.2.3. Potential hazards through interactions with other organisms

Should GloFish® Corydoras be released to the environment, they have the potential to interact with other organisms in Canadian freshwater aquatic ecosystems, including potential prey, competitors, and predators. The trophic interactions of wild *C. aeneus* in its native range are not well documented, nor is there documentation of trophic interactions for escaped domesticated non-transgenic *C. aeneus* in other areas. Limited data described below indicate non-transgenic *C. aeneus* may have limited potential to impact Canadian species through trophic interactions, and GloFish® Corydoras would likely have similar potential to impact through trophic interactions.

Wild Corydoras are omnivores, eating worms, crustaceans, insects and plant matter (Mills and Vevers 1982). In the aquarium, they can be voracious eaters and can significantly impact the density of benthic nematode populations (Majdi et al. 2018). As such, they have the potential to impact localized populations of small prey organisms or competitors occupying similar niches at the location of release.

The pet trade describes *C. aeneus* as a schooling, non-aggressive, bottom-dwelling, community fish. Anecdotal information from the company indicates there are no detected differences in behaviour between GloFish® Corydoras and non-transgenic Corydoras sold in the pet industry. In experiments with surrogate organisms, the GloFish® Electric Green® Tetras had similar aggressive behaviour and foraging success as non-transgenic siblings in paired feeding trials (Leggatt and Devlin 2020). However, Howard et al. (2015) found that RFP Zebrafish had lower male mating aggression and success than non-transgenic siblings. These studies, though conducted on surrogate species, suggest that fluorescent protein transgenesis may decrease or not affect competitive success in tropical fish.

Given the low temperatures expected for Canadian freshwater systems for most of the year, and lack of evidence of altered behaviour from genetic modifications, potential for the anticipated release of GloFish® Corydoras to impact native aquatic species through prey acquisition and competition is expected to be negligible through most of the year, and is expected to be no greater than for non-transgenic *C. aeneus*.

Released GloFish® Corydoras also have the potential to impact native predator populations as a new prey source. This could have a positive effect on predator populations by providing a new food source, or a negative effect on predator populations if consuming GloFish® Corydoras causes deleterious effects to the predator populations. The latter is not expected as GloFish® Corydoras are not expected to be environmentally toxic (see Section 5.2.1).

Predation pressure on GloFish® Corydoras relative to non-transgenic *C. aeneus* has not been reported. It is possible that brightly coloured Corydoras may be more subject to selective pressures such as predation, an effect observed in other ornamental tropical fish species bred for colour (Tuckett et al. 2021). However, the effect of fluorescent transgenesis in other transgenic models (RFP Zebrafish) is conflicting, with RFP-expressing Zebrafish having higher (Hill et al., 2011), equal (Cortemeglia and Beitinger, 2006b), or lower (Jha, 2010) predation susceptibility relative to non-related, non-transgenic fish. These variable findings may be due to differences in rearing history, genetic background, or the experimental conditions among studies. Whether any of the above studies could be applied to the GloFish® Corydoras predation vulnerability in Canadian environments is not known and, consequently, the predation vulnerability of GloFish® Corydoras relative to non-transgenic counterparts cannot be estimated with reasonable certainty. However, due to the lack of expected toxicity from ingesting GloFish® Corydoras, and the anticipated scale of introduction, GloFish® Corydoras are not expected to pose a hazard as prey to native predators, regardless of potential predation sensitivity.

Based on the expected low activity of *C. aeneus* in cooler waters, and lack of noted alterations in trophic-related behaviour of the notified lines, GloFish® Corydoras are not expected to influence trophic interactions of native organisms beyond natural fluctuations, with associated **negligible** hazard relative to non-transgenic counterparts. The lack of studies directly examining the hazards of GloFish® Corydoras, limited available data on a surrogate (RFP Zebrafish), and poor understanding of GxE interactions in aggression and predation susceptibility in surrogate fluorescent transgenic models, result in a **moderate** level of uncertainty. This concurs with assessment rankings for previously notified GloFish® species.

5.2.4. Potential hazards through hybridization with native species

Corydoras aeneus is a freshwater fish of the family Callichthyidae (also known as Armored Catfish) that are neotropical, occurring only in South America, as far north as Panama. Corydoras do not occur in North America, or anywhere else, with the exception of an introduced population of *C. aeneus* in Hawaii that was last confirmed in 1984, and other introductions in Colorado and Florida during the mid-1980s that have not been confirmed recently. Though *C. aeneus* has many congeneric species within its natural range (Tencatt et al., 2023), none are established in Canada and there are no other genera of the Callichthyidae family native to Canada. Consequently, there is **negligible** potential for GloFish® Corydoras to cause hazard through natural hybridization with native fish in Canada. High quality data on the reproductive requirements and distribution of Callichthyidae species and related genera result in **low** uncertainty.

5.2.5. Potential to act as vector for disease agents

Commercial ornamental aquarium fish are commonly reported to carry numerous disease agents including viruses, bacteria, fungi, and parasites (e.g., Evans and Lester, 2001; Řehulka et al., 2006; Whittington and Chong, 2007; Hongslo and Jansson, 2009; Rose et al., 2013).

While disease agents are common in tropical-origin freshwater ornamental aquarium fish, any disease agents GloFish® Corydoras would be harbouring are expected to be tropical in origin, and/or persist in warm waters normally found in home aquarium (e.g., 25-28°C), and, therefore, may have limited ability to persist within or outside GloFish® Corydoras, if released into cooler Canadian freshwater environments.

C. aeneus is not listed among the few tropical species susceptible to diseases of significant importance to aquatic animal health and the Canadian economy by the Canadian Food Inspection Agency (CFIA) (Susceptible Species of Aquatic Animals). Since the principal mode of entry into Canada for GloFish® Corydoras will be through importation from the US, the CFIA will play a critical role in regulating disease agents of *C. aeneus* that are imported into Canada. Anyone importing aquatic animals on the Susceptible Species list require an Aquatic Animal Health Import Permit, while all other imports require a 'declaration' at the border.

Whether GloFish® Corydoras have altered ability to act as a vector of disease agents has not been directly examined. Some studies of fluorescent cultured cell models used in research have reported potential change in disease susceptibility. For example, GFP expression has been shown to decrease T-cell activation (Koelsch et al., 2013), induce cytokine IL-6 secretion (Mak et al., 2007), inhibit immune-related signalling pathways (Baens et al., 2006), and alter expression of genes involved in immune function (Coumans et al., 2014) and response to stress (Badrian and Bogoyevitch, 2007). As well, Chou et al. (2015) reported mice transgenic for DsRed had alterations in some white blood cell numbers (lymphocytes and monocytes) but not others. However, numerous other transgenic fluorescent aquarium species and lines have been grown on a commercial scale in the US starting in 2003, with no observed change in disease susceptibility or capacity to act as a vector. Consequently, there is **negligible** potential for GloFish® Corydoras to have altered capacity as a vector for disease relative to non-transgenic *C. aeneus*. As this has not been directly examined in GloFish® Corydoras, there are limited data on a surrogate, and there is a reliance on expert opinion, the uncertainty level for this ranking is **moderate.** This concurs with assessment rankings for previously notified GloFish® species.

5.2.6. Potential to impact biogeochemical cycling

GloFish® Corydoras are expected to contribute to nutrient cycles within habitats through ingestion of food items and release of waste (ammonia and feces). The potential effects of fluorescent protein in GloFish® Corydoras on metabolism, and hence nutrient cycling, have not been examined. In a different model organism, eGFP transgenic mice were found to have alterations in the urea cycle, nucleic acid and amino acid metabolism, and energy utilization (Li et al., 2013). What impacts these changes may have on biogeochemical cycling should GloFish® Corydoras have similar influences from fluorescent transgenic gene expression are not known. However, the small size of *C. aeneus* and potential low numbers of individuals anticipated to enter an ecosystem indicates a **negligible** potential for GloFish® Corydoras to impact biogeochemical cycling in natural environments, even with altered metabolic pathways. Uncertainty is **moderate** due to a lack of studies directly examining this hazard. This concurs with assessment rankings for previously notified lines of GloFish® species (Table 5).

5.2.7. Potential to affect habitat

Corydoras are a small species and are not known to build structures that are expected to impact habitats of other species. There have been no reports, anecdotal or otherwise, of GloFish® Corydoras having altered behaviour, relative to domesticated *C. aeneus*, that may influence effects on habitat structure. Consequently, GloFish® Corydoras are expected to have **negligible** effects on habitat with **low** uncertainty due to a lack of information regarding this hazard for GloFish® Corydoras or the comparator species. This concurs with assessment rankings for previously notified GloFish® species (Table 5).

5.2.8. Potential to affect biodiversity

Biodiversity can be negatively impacted by numerous drivers, including invasive species and the introduction of disease. Despite their long-standing use in the ornamental aquarium trade, there have been no reports of *C. aeneus* becoming invasive anywhere outside of their natural range. As well, there is no evidence that GloFish® Corydoras lines have increased fitness that may increase their invasiveness potential relative to non-transgenic Corydoras.

As elaborated above, GloFish® Corydoras are not expected to negatively impact native species through trophic or hybrid interactions, act as a vector for disease agents of concern in Canada, impact biogeochemical cycling, or impact habitat. Addition of the transgenic construct and fluorescent protein in GloFish® Corydoras is not expected to result in environmental toxicity, or cause hazards through HGT of the transgene. Taken together, there is a **negligible** hazard of GloFish® Corydoras affecting biodiversity of Canadian ecosystems. Data regarding the comparator species for invasiveness and biodiversity effects results in a **low** degree of uncertainty, in line with most other GloFish species (Table 5).

5.2.9. Conclusions of the hazard assessment

GloFish® Corydoras are not expected to be hazardous to Canadian environments. Non-transgenic Corydoras have no history of invasiveness anywhere, despite widespread use. There is no evidence of environmental toxicity associated with the constructs, and the majority of other fluorescent models do not report toxicity associated with fluorescent transgenes. There is also no indication of potential effects to the environment via transfer of the transgene to native Canadian species through hybridization, or HGT. GloFish® Corydoras and other fluorescent fish models have no reported differences in survival, disease susceptibility, behaviour, or husbandry care, and are not expected to have an altered ability to act as a vector for disease or impact biogeochemical cycling.

The examined hazards have negligible to low rankings (Table 5), while uncertainty ranged from negligible to moderate, due to limited data specific to GloFish® Corydoras, limited direct data on comparator species, variable data from surrogate models (e.g., RFP Zebrafish), and the reliance on expert opinion for the assessment of some hazards. Outside of its intended use as an ornamental fish in static aquaria, GloFish® Corydoras are not expected to pose unique hazards beyond those of the intended use. Hazard ranking concurred with that previously assessed for GloFish® species, though uncertainty differed from that assessed in GloFish® Tetras, Danios, Bettas, Pristellas and Rainbow Sharks in several hazard categories due to increased acknowledgement of data limitations (through HGT), or differences in family distributions (through hybridization).

Table 6. Summary of all ranks and uncertainty ratings for environmental risk assessments of currently notified GloFish® Corydoras lines, as well as previously notified GloFish® Tetras, Danios, Bettas, Barbs, Pristellas, and Rainbow Sharks. Underlines indicate where previous assessments differ from the current assessment. Neg=Negligible, Mod=Moderate, Low=Low.

Assessment	**Rank/Uncertainty**						
	GloFish® Corydoras	**GloFish® Sharks**	**GloFish® Pristella**	**GloFish® Barbs**	**GloFish® Bettas**	**GloFish® Danios**	**GloFish® Tetras**
Exposure	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**	**Low/Low**
Hazards:							
1. Environmental toxicity	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod
2. HGT	Low/Mod	Low/Mod	Low/Mod	Low/Mod	Low/Mod	Low/Mod	Low/<u>Low</u>
3. Trophic interactions	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod
4. Hybridization	Neg/Neg	Neg/<u>Mod</u>	Neg/Neg	Neg/<u>Low</u>	Neg/Neg	Neg/<u>Mod</u>	Neg/Neg
5. Vector for disease	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod
6. Biogeochemical	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod	Neg/Mod
7. Habitat	Neg/Low	Neg/<u>Mod</u>	Neg/Low	Neg/Low	Neg/Low	Neg/Low	Neg/Low
8. Biodiversity	Neg/Low	Neg/<u>Mod</u>	Neg/Low	Neg/Low	Neg/Low	Neg/Low	Neg/Low
Environmental Risk	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**	**Low**

5.3. ASSESSMENT OF RISK

Risk is the likelihood that a harmful effect is realized as a result of exposure to a hazard. The risk assessment incorporates the nature and severity of the harmful effect, the likelihood that the harmful effect is realized, and the uncertainty associated with each conclusion. DFO's science advice to ECCC and HC for a regulatory decision is based on the overall risk of the organism, carried out in the context of the applicant's proposed use scenario, and all other potential use scenarios. An overall conclusion on Risk is based on the classic paradigm where Risk is proportional to Hazard and Exposure:

Risk $\propto$ Exposure $\times$ Hazard

For each endpoint, hazard and exposure are ranked as: negligible, low, moderate, or high, and include an analysis of uncertainty for each. Overall Risk is estimated by plotting Hazard against Exposure, using a matrix or heat map, as illustrated in Figure 2. Though the matrix cannot be used as a tool for establishing a discreet conclusion or decision on risk, it can be used to facilitate communication and discussion. The uncertainty associated with the overall Risk is not estimated, rather, uncertainty in the hazard and exposure assessments are discussed in the context of a final conclusion on risk.

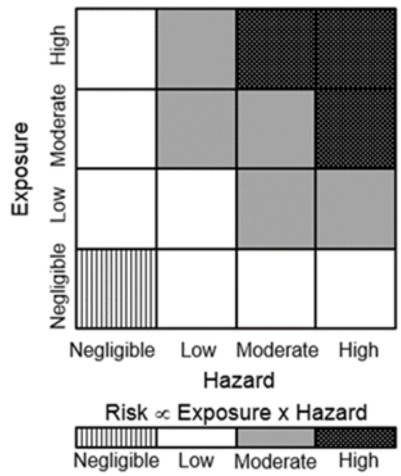

Figure 2. Risk matrix and pattern scale to illustrate how exposure and hazard are integrated to establish a level of risk in the environmental risk assessment.

5.3.1. Risk assessment of the GloFish® Corydoras

The exposure assessment concluded that GloFish® Corydoras used in the ornamental aquarium trade or other unintended uses would have a **low** likelihood of occurrence in the Canadian environment. This is due to the high likelihood of release of small numbers from home aquaria, but negligible likelihood for GloFish® Corydoras to overwinter in Canadian aquatic ecosystems. As such, any exposure to Canadian freshwater ecosystems to GloFish® Corydoras is expected to be isolated, rare, and ephemeral. The quality of data demonstrating lack of cold tolerance in GloFish® Corydoras and *C. aeneus*, relevant to Canadian freshwater temperatures, results in a low uncertainty associated with this ranking.

The hazard assessment concluded that GloFish® Corydoras poses **negligible** to **low** hazard to the Canadian environment, due to the lack of hazard associated with *C. aeneus*, and no direct evidence that the expressed fluorescent protein would increase hazard, relative to *C. aeneus*. Uncertainty ranking associated with individual hazard components ranged from negligible to moderate, due to limited data specific to GloFish® Corydoras, limited direct data on comparator species, variable data from surrogate models (RFP Zebrafish), and reliance on expert opinion for the assessment of some hazards.

According to the risk matrix described in Figure 3, GloFish® Corydoras used in the ornamental aquarium trade or other uses in Canada pose **low risk** to Canadian environments. Individual hazards are expected to result in no harmful effects beyond natural fluctuations to Canadian environments under the assessed level of exposure. Sources of uncertainty in the environmental exposure and hazard assessments that may influence uncertainty in the environmental risk assessment include a lack of data directly addressing hazards of the notified organism and comparator species, variability in data taken from surrogate organisms, and in some cases reliance on expert opinion.

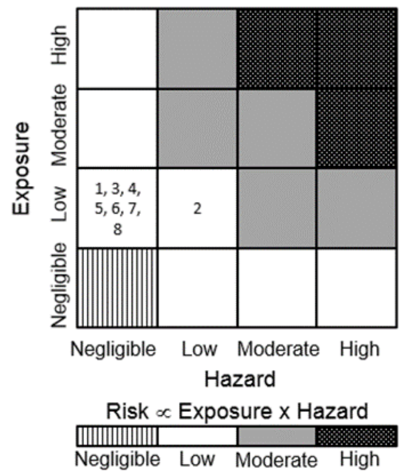

Figure 3. Risk assessments associated with assessed hazard components at the assessed exposure are identified by number: 1) through environmental toxicity; 2) through horizontal gene transfer; 3) through interactions with other organisms; 4) through hybridization; 5) as a vector of disease; 6) to biogeochemical cycling; 7) to habitat; and 8) to biodiversity.

Despite moderate uncertainty in some of the individual assessment components, there is no current evidence to suggest that overall risk rankings of GloFish® Corydoras may be higher than the assessed low ranking for risk to Canadian environments. This concurs with low risk assessment rankings for previously notified GloFish® species.

5.4. SUMMARY AND CONCLUSIONS

Use of GloFish® Corydoras in home aquaria in Canada, or in other unintended uses, is expected to result in frequent, very small magnitude releases of GloFish® Corydoras into the Canadian environment, although the potential for occasional high magnitude releases cannot be ruled out. Available high-quality data indicates that GloFish® Corydoras do not have the capacity to overwinter in most Canadian freshwater ecosystems. This results in an exposure ranking of low, with associated uncertainty being low. The lack of evidence of hazards from non-transgenic comparator species despite long-term extensive use, and a lack of evidence for increased hazards of GloFish® Corydoras relative to non-transgenic *C. aeneus*, indicates negligible to low hazards to Canadian ecosystems. Uncertainty with hazard assessments ranged from negligible to moderate due to a lack of, or limited direct information on, the hazards of base models or GloFish® Corydoras, and variability in the results of surrogate organisms, when they are available. Taken together, the overall risk of GloFish® Corydoras to the Canadian environment is ranked low, and the notified organism is not expected to cause harmful effects to the Canadian environment at the assessed exposure level. Though uncertainty with some of the hazard estimates is moderate due to limited and or no direct data on the notified organism or comparator species, no evidence was identified to suggest GloFish® Corydoras, under the proposed or other potential uses, could cause harm as a result of exposure to the Canadian environment.

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