Fisheries and Oceans Canada / Pêches et Océans Canada
Ecosystems and Oceans Science / Sciences des écosystèmes et des océans

Canadian Science Advisory Secretariat (CSAS)

Research Document 2026/045

National Capital Region

Indirect Human Health Risk Assessment of the GloFish® Electric Green® (GCA2021), GloFish® Sunburst Orange® (OCA2020), and the GloFish® Moonrise Pink® (PiCA2020) Corydoras (*Corydoras aeneus*), for use as Ornamental Aquarium Fish in Canada

Stephen Dugan, Kassim Ali, and Renuka Ravishankar

New Substances Assessment and Control Bureau
Safe Environments Directorate
Healthy Environments and Consumer Safety Branch
Health Canada
269 Laurier Ave, Ottawa, ON K1A 0K9

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 978-0-660-99964-7 Cat. No. Fs70-5/2026-045E-PDF

Correct citation for this publication:

Dugan, S., Ali, K., and Ravishankar, R. 2026. Indirect Human Health Risk Assessment of the GloFish® Electric Green® (GCA2021), GloFish® Sunburst Orange® (OCA2020), and the GloFish® Moonrise Pink® (PiCA2020) Corydoras (*Corydoras aeneus*), for use as Ornamental Aquarium Fish in Canada. DFO Can. Sci. Advis. Sec. Res. Doc. 2026/045. v + 23 p.

Aussi disponible en français :

*Dugan, S., Ali, K., et Ravishankar, R. 2026. Évaluation des risques indirects pour la santé humaine posés par les corydoras (*Corydoras aeneus*) GloFish^MD Electric Green^MD (GCA2021), GloFish^MD Sunburst Orange^MD (OCA2020) et GloFish^MD Moonrise Pink^MD (PiCA2020), utilisés comme poissons d'ornement d'aquarium au Canada. Secr. can. des avis sci. du MPO. Doc. de rech. 2026/045. v + 26 p.*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

An indirect human health risk assessment was conducted on three lines of genetically modified Bronze Corydoras (*Corydoras aeneus*) known as the GloFish® Electric Green® Cory (GCA2021), GloFish® Sunburst Orange® Cory (OCA2020), and the GloFish® Moonrise Pink® Cory (PiCA2020), that were notified under the *Canadian Environmental Protection Act* (CEPA). GCA2021, OCA2020, and PiCA2020 are modified lines of diploid, hemizygous or homozygous, Corydoras, containing genes encoding for different fluorescent proteins. GCA2021, OCA2020, and PiCA2020 appear green, orange, or pink, respectively under ambient light (including sunlight). The three lines will be imported from the United States for use as ornamental fish in home aquaria. This risk assessment examined the potential for the three lines to cause harmful effects to humans in Canada relative to wild-type Corydoras as a consequence of environmental exposure including under their intended use in home aquaria. The parental strain, *C. aeneus*, has been available as a home aquarium fish since the 1930s without any reported adverse human health effects. There is no evidence to suggest a risk of adverse human health effects for the general Canadian population from use of GCA2021, OCA2020, and PiCA2020 as ornamental aquarium fish as well as other potential uses. As such, there is no expectation that GCA2021, OCA2020, and PiCA2020 will pose any more risks to human health than wild-type *C. aeneus*.

INTRODUCTION

The following indirect human health risk assessment was conducted on *Corydoras aeneus* GCA2021, OCA2020, and PiCA2020, three genetically modified lines of diploid, hemizygous or homozygous, Corydoras, containing genes coding for recombinant fluorescent green, yellow, or pink proteins, respectively. GCA2021, OCA2020, and PiCA2020 are green, orange, and pink in colour, respectively, when displayed in ambient light, including sunlight. This risk assessment examines the potential for the importation of GCA2021, OCA2020, and PiCA2020 from the United States to cause harmful effects to humans in Canada, as a consequence of exposure in natural environments and environments under the intended use as ornamental fish in home aquaria. Risk assessment was conducted relative to the wild-type *C. aeneus,* which have widespread use in Canada and other parts of the world as tropical ornamental fish. The risk assessment was conducted under the *Canadian Environmental Protection Act* (CEPA) and *New Substances Notification Regulations (Organisms)*.

HAZARD ASSESSMENT

IDENTIFICATION AND CHARACTERIZATION OF CORYDORAS AENEUS GCA2021, OCA2020, AND PICA2020

Binomial name

Corydoras aeneus (Gill 1858) GCA2021, OCA2020, and PiCA2020

Taxonomy

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Actinopterygii
Class	Teleostei
Order	Siluriformes
Family	Callichthyidae
Genus	*Corydoras*
Species	*aeneus*
Strains	GCA2021, OCA2020, and PiCA2020

Synonyms, common and superseded names

Synonym/common names: *Hoplosoma aeneum* Gill, 1858; *Corydoras macrosteus* Regan, 1912; *Corydoras microps* Eigenmann & Kennedy, 1903; *Corydoras schultzei* Holly, 1940; *Corydoras venezuelanus* Ihering, 1911; Cory Catfish, Green Cory Catfish, Bronze Cory (ITIS 2025)

Trade names:

- GCA2021 - GloFish® Electric Green® Cory
- OCA2020 - GloFish® Sunburst Orange® Cory
- PiCA2020 - GloFish® Moonrise Pink® Cory

Characterization and substantiation of the taxonomic identification

Corydoras aeneus GCA2021, OCA2020, and PiCA2020 are genetically modified lines of diploid, hemizygous or homozygous, Bronze Corydoras containing transgene expression cassettes which makes them appear green (GCA2021), orange (OCA2020), or pink (PiCA2020) under ambient light, including sunlight. All three lines were derived from a domestic albino strain of Bronze Corydora obtained from a commercial ornamental fish producer.

Members of Callichthyidae may be distinguished from other Siluriformes by having two longitudinal series of dermal plates on the flanks. The family has more than 220 valid species including 180 of the genus *Corydoras*. Morphological and molecular data suggest that *Corydoras aeneus* represents a species complex (Tencall et al. 2023).

Corydoras are known as 'armored catfishes' due to the presence of scutes or overlapping bony external plates derived from ossification of inner dermal skin (Ebenstein et al. 2015; Volk 2019). *C. aeneus* can be identified by a diffused large dark area beginning just back of the head and has no pattern markings on either body or fins (Innes 1935). In *C. aeneus,* the dorsal fin bears a first small fin ray, modified to serve as a spine locking mechanism, followed by a second, large one. After this, seven branched dorsal fin rays are present, of which the last is split up to its base. The pterygiophores of these spines plus the first five fin rays are connected to the 8th to 11th vertebral neural spine dorsal fins of *C. aeneus*. The number of dorsal-fin rays is a character of taxonomic value within the subfamily Corydoradinae and a number of 7-9 is determinative for Corydoras-species (Huysentruyt 2008).

The color variants of wild type *C. aeneus* range from pale orange, green, bronze to pale yellow and white (albino Corys). The albino variant was specifically bred for the ornamental fish trade and possesses red eyes with a combination white and pale pink body (Hamade 2020; Lujan 2023; Sheppard 2025). Their most distinctive feature when viewed from above is a brownish-orange patch is usually present on the head, just before the dorsal fin (FishBase 2024).

Strain History

The notified lines GCA2021, OCA2020, and PiCA2020 were produced by microinjection of the expression cassettes containing the corresponding transgenes into blastomeres of *C. aeneus* eggs. Upon hatching, fish were raised and the resulting mosaic adults were pair-wise crossed to unmodified light-coloured *C. aeneus*. Greater detail regarding strain development and history of the notified lines has been provided by the company for the expressed purpose of the current risk assessment and review but is identified as confidential business information and is not included in this report. Broodstocks for GCA2021, OCA2020, and PiCA2020 are maintained separately with the same breeding protocol used for all three lines. Furthermore, to maintain line integrity, non-transgenic fish produced during development of the lines were humanely euthanized and disposed of according to the notifier's protocols.

Genetic modifications: purpose, method, genetic and phenotypic changes

The notified lines, which have been modified to appear green (GCA2021), orange (OCA2020), or pink (PiCA2020) under ambient light, including sunlight, are intended for use for home aquarium display purposes only. Just like the wild-type *C. aeneus*, which is a non-food species that has been used safely in aquaria worldwide since the 1930s (Innes 1935), GCA2021, OCA2020, and PiCA2020 are not intended for food use.

According to the information provided by the notifier, in addition to GCA2021, OCA2020, and PiCA2020 appearing green, orange, and pink, respectively, under ambient light, GCA2021 and OCA2020 were more sensitive ($P \leq 0.05$) to low water temperatures compared to their non-transgenic siblings. There was no significant difference ($P > 0.05$) in temperature sensitivity between PiCA2020 fish and their non-transgenic siblings. Reproductive success of GCA2021 and PiCA2020 was significantly lower than that of their non-transgenic siblings while no difference was observed for OCA2020.

Biological and ecological properties

Bronze Corydoras are small tropical freshwater fish native to South America and can be found in rivers and streams in Colombia and Trinidad in the north and far south as the Rio de la Plata drainage on the border of Uruguay and Argentina (Sharpe 2022). While preferring calm waters with sandy or muddy bottoms, they have also been found in fast flowing rivers. Corydoras have the ability to breath atmospheric air from the surface of the water with the aid of a special intestinal lining and are considered one of the few fish that can thrive in stagnant, low oxygen level water. They are known as bottom-feeders, possessing three pairs of barbels to help locate food, and have an omnivorous diet in the wild primarily consisting of plant matter, small crustaceans, worms, and insects (Sharpe 2022; Woods 2022; Lujan 2023; Sheppard 2025). Average life expectancy for Bronze Corydoras is approximately 5 to 10 years, but they are capable of living for more than 20 years under ideal conditions (Sharpe 2022; Sheppard 2025).

Bronze Corydoras can reach a size of approximately 6 to 8 cm (Aqua-Fish 2024). Male Corydoras tend to be smaller and more slender than females, particularly when viewed from the top. A female will be larger due to her more rounded abdominal area. The females tend to grow larger, and sexually mature individuals are noticeably rounder and broader-bodied than the smaller, thinner males, especially when gravid (Aqua-Fish 2024; Aquarium Co-op 2025; Seriously Fish 2025). Reproduction occurs in the rainy season and during spawning which may continue over a period of several days, females can release 100 to 200 eggs. Females can produce several egg clutches utilizing different males, but each clutch will only be inseminated by one male. The eggs hatch in four to five days and the fry will live on their yolk sac for another three to four days (Nanan 2016; Sharpe 2022).

HUMAN HEALTH EFFECTS

Zoonotic potential

In-house literature searches found no reports of zoonoses or other adverse effects attributed to wild-type *C. aeneus*, or to the other commercially available GloFish® lines. However, aquarium pet fish can carry pathogenic agents, of bacterial, viral, fungal, or parasitic origin, that may have a zoonotic feature endangering the persons handling the animals (Cardoso et al, 2019). There are few reports of zoonoses by parasitic, fungal, and viral pathogens from aquatic organisms with bacteria being reported as the main etiological agents of zoonotic infection (Iqbal et al. 2018). These are largely infections from contact with tropical ornamental fish or through ingestion of food or drinking water that has been contaminated with pathogens and parasites associated with ornamental or aquarium fish.

Contact is the main route of transmission leading to bacterial infections in humans that develop from handling of aquatic organisms (Lowry and Smith 2007). Young children, pregnant women, and immunocompromised individuals are at higher risk for these infections (Dinç et al. 2015). Children are also more susceptible to severe disease outcomes as compared with adults as they often have less stringent hygienic practices (Dunn et al. 2015). While most infections are self-limiting, more serious cases are associated with immune deficiency, infection with highly virulent strains, contact with a large inoculum, depth of skin penetration, or a combination of these factors (Haenen et al. 2020).

Bacterial disease is extremely common in ornamental fish and is most frequently associated with bacteria that are ubiquitous in the aquatic environment acting as opportunistic pathogens secondary to stress (Roberts et al. 2009). Some bacterial species associated with tropical fish capable of causing human illness are *Aeromonas* sp*., Mycobacterium marinum, Salmonella* sp*.,* and *Streptococcus iniae* (CDC 2024) with the most commonly reported infections being associated with *M. marinum* (Weir et al. 2012).

In humans, *M. marinum* is the causative agent for the disease "fish tank granuloma" which results in ulcerative skin lesions or raised granulomatous nodules. These lesions are typically limited to the distal extremities such as the hands, legs, and feet as *M. marinum* has an optimum growth temperature range of 26°C to 32°C (Mutoji and Ennis 2012; Gauthier 2015). However, these nodular cutaneous lesions can spread to underlying joints and tendons and progress to tenosynovitis, arthritis and osteomyelitis (Hashish et al. 2018; Spickler and Dvorak 2020). In addition, rare cases of systemic mycobacteriosis have been reported in immunocompromised individuals (Lowry and Smith 2007). Infections are generally contracted from exposure of wounds and skin abrasions to contaminated water (Gauthier 2015). Median times between inoculation and infection range from 16 to 21 days but may vary from a few days to as long as six to nine months (Spickler and Dvorak 2020; Duman et al. 2024).

In humans, mycobacteriosis is classified into four types (I – IV). Type I is found in patients which are immunocompetent with clinical signs including superficial lesions with crusted and ulcerates nodules or verrucuous plaques. The lesions are small, painless, bluish-red papules approximately 1 to 2 cm in diameter. Signs develop over the course of weeks to months. Type II occurs in immunosuppressed individuals and involves lesions with abscesses, inflammatory nodules, and granulomas. The lesions may be single or multiple subcutaneous granulomas, with or without ulceration. In Type III, infections occur in deep tissues with or without skin lesions with clinical signs including arthritis, tenosynovitis, osteomyelitis, and bursitis. Type IV is very rare, but can occur in patients with lung disease (Delghandi et al. 2020). Virulence determinants for *M. marinum* have not been fully elucidated (Narendrakumar et al. 2022).

It is probable that almost all species of fish are susceptible to *Mycobacterium sp*. with levels of mortality ranging from 10% to 100% of infected fish (Delghandi et al. 2020). At least 20 species are known to cause piscine mycobacteriosis with *M. marinum*, *M. chelonae*, and *M. fortuitum* being the most commonly reported (Spickler and Dvorak 2020; Phillips Savage et al. 2022). *M. marinum* has been reported in infecting more than 200 species of freshwater, brackish and marine fishes (Narendrakumar et al. 2022). Other examples of species of *Mycobacterium* known to cause infections in fish include *M. abscessus*, *M. flavescens*, *M. gordonae, M. haemophilum*, *M. kansasii*, and *M. peregrinum* (Cardoso et al. 2019; Pate et al. 2019; Puk and Guz 2020).

Although most cases of fish-related infections in humans are caused by *M. marinum*, home aquarists should also be aware of the zoonotic potential of other species of *Mycobacterium* (Puk and Guz 2020). In immunosuppressed humans and children, *M. haemophilum* has been reported to be associated with subcutaneous infections, lymphadenitis, septic arthritis, osteomyelitis, pneumonitis and disseminated disease (Emmerich et al. 2019; Franco-Paredes et al. 2019). Cameselle-Martínez et al. (2007) reported on a cutaneous infection by *M. haemophilum* in a severely immunosuppressed AIDS patient following a bite from an aquarium fish. The infection was successfully treated following a combined therapy of six antibiotics. *M. abscessus*, *M. chelonae*, *M. fortuitum*, and *M. peregrinum* are also associated with cutaneous infections in humans (Kamijo et al. 2012; Franco-Paredes et al. 2019). Li et al. (2014) reported on a successful treatment with antibiotics of a cutaneous *M. chelonae* infection on the left arm of an 82-year-old woman with a hobby of rearing tropical fish. While cutaneaous mycobacterial infections may be successfully resolved with antibiotics, the choice of antibacterial combinations and length of therapy is species-specific (Franco-Paredes et al. 2018). Guz and Puk (2022) examined the antibiotic susceptibility of 99 isolates of nontuberculous mycobacteria (13 species of *Mycobacteria*) from diseased ornamental fish. The authors found the majority of isolates were susceptible to kanamycin, amikacin, clarithromycin, sulfamethoxazole, ciprofloxacin, and doxycycline with most being resistant to isoniazid and rifampicin. Depending on the severity of the infection, underlying conditions, and other factors, treatment duration can range from a few weeks to more than a year (Spickler and Dvorak 2020).

Corydoras spp. are among the ornamental fish species reported to be susceptible to mycobacterial infections (Cardoso et al. 2019). *M. marinum* was reported to be isolated from an unidentified species of *Corydoras* (Pate et al. 2005). Zanoni et al. (2008) reported 2 fish positive for *M. fortuitum* and another fish positive for *M. peregrinum* out of 8 specimens of *C. aeneus* examined. Phillips Savage et al. (2022) reported 12 of 19 specimens for three species of GloFish® [Barbs (3/5), Danios (5/8), and Tetras (4/6)] were presumed positive for species of *Mycobacterium* based on benchtop or histological acid-fast staining. However, none of the presumed positive specimens were confirmed positive using real-time PCR. An in-house literature search found no reports of human mycobacterial infections attributed to Bronze Corydoras or any species of GloFish® from home aquarium exposure.

Zoonotic infections from *S. iniae* are opportunistic and have most often been associated with puncture wounds from the handling and preparation of infected fish by persons with underlying medical conditions such as diabetes mellitus, chronic rheumatic heart disease, or cirrhosis (Baiano and Barnes, 2009; Haenen et al. 2020, 2023). From the handling of live or recently killed infected fish, *S. iniae* may cause severe disease including septicaemia, endocarditis, arthritis, meningitis, fever, abdominal distension, and pneumonia (Lowry and Smith 2007; Boylan 2011; Gauthier 2015; Haenen et al. 2020). However, the species is referred to as an emerging human pathogen with bacterial cellulitis being the most common clinical disease seen in patients (Glajzner et al. 2021). In addition, human infections by *S. iniae* may be under-reported as identification of this pathogen in clinical laboratories is hampered by limitations of commercial identification systems (Haenen et al. 2023). *S. iniae* is reported to be generally susceptible to antibiotics with therapeutic options in human infections including penicillin, cloxacillin, ampicillin, and cefalexin (Glajzner et al. 2021). Other species capable of causing fish streptococcus include *S. agalactiae*, *S. difficile*, *S. difficilis*, *S. dysgalactiae*, and *S. shiloi* (Ziarati et al. 2022). There are no reports in the scientific literature of human streptococcal infections attributed to *C. aeneus* from home aquarium exposure.

Aeromonas spp. are opportunistic pathogens that are associated with a number of diseases in ornamental fish (Hossain et al. 2018). *Aeromonas hydrophila* is the most commonly reported Aeromonad that possesses zoonotic potential with *A. caviae, A. dhakensis*, *A. jandaei, A. sobria, A. salmonidae*, and *A. veronii* also having been reported (Boylan 2011; Zariati et al. 2022; Duman et al. 2024). Water with high nutrient levels can cause bacterial blooms capable of being infectious to humans through wounds or ingestion; however, infections are rare and typically involve immune suppression (Boylan 2011). In humans, *A. hydrophila* exposure may result in local skin infections and occasionally, diarrheal disease (Haenen et al. 2020). *A. hydrophila* was one of the species of bacteria isolated from cough swabs of an 11-month-old boy with cystic fibrosis (Cremonesini and Thomson 2008). The authors believe the infection was the result of aerosol spread of the bacterium due to the aeration process of fish tanks in the home as isolations of *A. hydrophila* only ceased following removal of the tanks. While the report by Cremonesini and Thomson (2008) did not identify the species of fish, there are no reported cases of *A. hydrophila* zoonotic infections attributed to *C. aeneus* exposure. Among the pathogenic *Aeromonas* spp., *A. veronii* appears to exhibit the broadest host range as species ranging from invertebrates to mammals, including humans, have shown susceptibility to this pathogen (Lazado and Zilberg 2018). *A. veronii* (26.3%) and *A. hydrophilla* (16.2%) were the most commonly isolated bacterial species from 112 fish found positive from a total of 126 fish collected from a wholesaler in São Paulo, Brazil (Cardoso et al. 2021). Aeromonads isolated from human infections have been found to be susceptible to various antibiotics such as fluoroquinolones and third-generation cephalosporins (Haenen et al. 2023). However, an in-house literature search found no reported cases of zoonotic infections of *A. veronii* from ornamental fish exposure.

Salmonella infection can occur through contact with an animal's habitat such as an aquarium and infections have been documented in infants and children due to splashes from tanks and water contact (Duman et al. 2024). While *Salmonella* is not a known pathogen for tropical fish, the fish may act as bacterial reservoirs and excrete *Salmonella* in their feces during periods of stress (Gaulin et al. 2005). Musto et al. (2006) reported on 78 cases of *Salmonella* Paratyphi B biovar Java infections in people having aquaria containing tropical fish in Australia. Infections were mostly seen in children (median age of cases was three years old) following exposure to aquarium water and resulted in diarrhoea, fever, abdominal cramps, vomiting, bloody stool, headaches, and myalgia. Types of tropical fish reported in this study included Tetras, Guppies and Angel Fish. Similarly, out of 53 reported cases of *S.* Paratyphi B, var. Java infections reported in the province of Quebec from January 2000 to June 2003, 33 infected individuals owned an aquarium with 21 of the aquaria testing positive for *Salmonella* (Gaulin et al. 2005). However, the authors did not identify any of the tropical fish species owned by the infected individuals. An in-house literature search found no reports of *Salmonella* zoonotic infections attributed to *C. aeneus* exposure.

Zoonotic infections from fish primarily occur through puncture, cuts, scrapes, abrasions, or sores in the skin (Boylan 2011). Infections may be prevented through wearing gloves when handling fish or cleaning fish tanks and avoiding contact with any potentially contaminated water if any open skin wounds are present. Washing hands and skin with soap and water after contact with aquarium water and fish is also highly recommended. Care should also be taken to prevent fish tanks from contaminating other sites such as bathtubs where mycobacteria may persist. In addition, people with compromised immune systems or underlying medical conditions as well as children should avoid cleaning tanks or handling fish (Haenen et al. 2013; 2020; Spickler and Dvorak 2020).

There are no reports specifically associating either the notified organisms or wild-type *C. aeneus* with any parasites of human health significance. During line development, hatchery technicians trained to recognize diseases and abnormalities, visually examined the fish and any not meeting specific criteria were removed from the population and euthanized. Routine health evaluations (necropsy, microbiology) were conducted on limited sample sizes of a minimum of six fish of each colour and histology was conducted on an additional minimum of six fish of each colour at a fish disease diagnostic laboratory at the University of Florida in 2019 (OCA2020 and PiCA2020) and 2021 (GCA2021). Evaluations were also conducted on non-transgenic albino and wild-type *C. aeneus* in 2020 and 2021, respectively.

The reports did state that the findings were likely unrelated to the transgenic nature of the fish. Many species of ornamental fish have been reported to be susceptible to parasites (Florindo et al. 2017a,b; Iqbal et al. 2018; Trujillo-González et al. 2018). Ostrowski de Núñez et al. (2020)

experimentally infected 10 of 14 *C. aeneus* with metacercariae of the digenean *Heterophyes yacyretana*. Some fish showed signs of septicemia and possible viral infection while presence of some unidentified protozoans were observed from skin scrapes of other fish on albino Bronze Corydoras by Leggatt (unpublished data) prior to their use in a temperature tolerance study. However, Ponpornisit et al. (2020) were unable to experimentally infect *C. aeneus* with the ciliate *Tetrahymena pyriformis*. There are no reports in the scientific literature of parasitic zoonoses associated with home aquarium exposure to *C. aeneus*.

In addition, no bacterial growth was observed after 48 hours (at 28°C) in brain and posterior kidney samples plated onto blood agar plates (TSA + 5% sheep's blood) for the three notified lines as well as for the wild-type and albino non-transgenic fish.

Allergenicity/Toxigenicity

In-house amino acid sequence analyses of all the expressed fluorescent proteins were done using the AllergenOnline Database (v23; 29 January, 2025) (http://www.allergenonline.org). Similar to previous analyses on these fluorescent proteins done on previously notified GloFish® lines, no matches with greater than 35% identity nor exact matches for 80 and 8 sliding window amino acid segments, respectively, were found for any of the fluorescent proteins. Likewise, results provided by the notifier from analyses using the Allermatch™ website (http://www.allermatch.org) found no matches for 80 amino acid sliding window alignments using the 35% cutoff or exact matches using segments 8 amino acids in length. The 35% identity for 80 amino acid segments is a suggested guideline proposed by the Codex Alimentarius Commission for evaluating newly expressed proteins produced by recombinant-DNA plants (WHO/FAO 2009).

As seen with previously assessed green GloFish® lines, analyses conducted for all the other reading frames found a positive result using the 80mer sliding window for a putative ORF in the 5'3' Frame 1 direction in the expression cassette sequence for GCA2021. The ORF in GCA2021 was found to have 35.03% identity with a predicted collagen alpha-1(I) chain-like isoform X1 from the Barramundi (*Lates calcarifer*). However, the full-length alignment resulted in only 35.4% identity and there was a high E-value (expectation value) of 100. Cross-reactivity typically requires the matches to be 40% identical over 80 amino acids with an E-value score of 1e-15 or less (Dr. Richard Goodman, University of Lincoln-Nebraska, personal communication). Thus, allergic cross reactivity is not likely for the putative ORF. In addition, Basic Local Alignment Search Tool (BLAST) analysis on the putative ORF amino acid sequence with BLASTP found no significant similarity to a known protein for GCA2021. Analyses on the inserted nucleotide sequences for prediction of translation initiation sites using an online program (http://atgpr.dbcls.jp/) only found sites with a high reliability associated for the expected proteins. Therefore, the putative ORF would most likely not result in an expressed protein in GCA2021.

However, BLAST analyses of all the inserted fluorescent protein sequences do not indicate any homologies to sequences of potential toxins or allergens. No adverse effects were observed in male rats fed pure green fluorescent protein (GFP) or canola expressing GFP for 26 days (Richards et al. 2003). An in-house literature search found no reports of adverse effects attributed to any of the inserted proteins in humans. The fin spines of Bronze Corydoras contain a mild venom used as a self-defence mechanism that is released when the fish is under stress (Hamade 2020). While this venom is reported to be neurotoxic and hemolytic (Hamade 2020), there are no reports of adverse human health effects in the scientific literature. However, some aquarium hobbyist websites report the venom may hurt like a bee sting (Wiegert 2020) or be a minor skin irritant (Nanan 2016; Chinnery 2022; Herzog 2023). *C. aeneus* is considered harmless to humans (FishBase 2024) and there is no evidence indicating any potential for

GCA2021, OCA2020, and PiCA2020, to produce toxic or other hazardous materials that may accumulate in the environment or be consumed by humans or other organisms in the environment.

History of Use

Enforcement Discretion decisions by the U.S. Food and Drug Administration (USFDA) were received in 2021 (OCA2020 and PiCA2020) and 2022 (GCA2021). GCA2020 and OCA2021 have been commercially available in the U.S. since September 2022. PiCA2020 is not yet available in the U.S. market, but the notifier has plans to launch the line in the next year. The green and yellow fluorescent proteins used in GCA2021 and OCA2020, respectively, have been used in other GloFish® lines since as early as 2006, while the red fluorescent protein used in PiCA2020 has been used since 2013. Wild-type Corydoras have been sold worldwide as aquarium fish since the 1930s (Innes 1935).

HAZARD CHARACTERIZATION

The human health hazard potential of GCA2021, OCA2020, and PiCA2020 is assessed to be **low** (Table 1) because:

GCA2021, OCA2020, and PiCA2020 are genetically modified tropical fish containing transgene expression cassettes at a single site of insertion (although alternate insert patterns may exist in the population) and that appear phenotypically stable based online maintenance protocols.

The methods used to produce GCA2021, OCA2020, and PiCA2020 do not raise any indirect human health concerns. However, the potential for unintended effects from use of CRISPR/Cas9 in the three lines remains unknown. While some of the source organisms from which the inserted genetic material was derived appear to produce toxins, there is no indication that any of the inserted genetic material or expressed proteins in these lines are associated with any toxicity or pathogenicity in humans;

While there are reported cases of zoonotic infections associated with tropical aquarium fish, particularly for immunocompromised individuals and children, there are no reported cases attributed to any of the commercially available lines of GloFish® or to wild-type Corydoras. The zoonotic potential of GCA2021, OCA2020, and PiCA2020 is not expected to be any different than for wild-type *C. aeneus* currently commercially available;

Sequence identities of the inserted transgenes do not match any known allergens or toxins. Amino acid sequences of the three fluorescent proteins are identical to those used in previously assessed GloFish® lines. While analyses conducted on the other potential reading frames found a potential match in GCA2021, the result suggests there is little evidence for cross-reactivity; and

While there is a limited (GCA2021 and OCA2020) or no (PiCA2020) history of use for the notified lines, no adverse human health effects have been reported for other commercially available lines of GloFish®. The wild-type Corydoras have been used globally as an ornamental aquarium fish since the 1930s.

Table 1: Considerations for hazard severity (human health).

HAZARD	CONSIDERATIONS
High	• Effects in healthy humans are severe, of longer duration and/or sequelae in healthy individuals or may be lethal. • Prophylactic treatments are not available or are of limited benefit. • High potential for community level effects.
Medium	• Effects on human health are expected to be moderate but rapidly self-resolving in healthy individuals and/or effective prophylactic treatments are available. • Some potential for community level effects
Low	• No effects on human health or effects are expected to be mild, asymptomatic, or benign in healthy individuals. • Effective prophylactic treatments are available. • No potential for community level effects.

UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH HAZARD ASSESSMENT

The ranking of uncertainty associated with the indirect human health hazard assessment is presented in Table 2. Adequate information was either provided by the notifier or retrieved from other sources that confirmed the identification of the notified organisms. Adequate information was also provided describing in good detail the methods used to genetically modify the wild-type *C. aeneus* including the sources of the genetic materials and the stability of the resulting genotypes and phenotypes. Sequence analyses of the inserted transgene expression cassettes for the three notified lines did not match any known toxins or allergens and no reports were found of adverse effects attributed to the inserted proteins in humans.

While there were no reports of adverse human health effects directly associated with the notified organisms or the other commercially available lines of GloFish®, surrogate information from the literature on other ornamental fish appear to indicate the potential for transmission of human pathogens. However, this potential for infections is common to all ornamental aquarium fish and are not unique to Corydoras. The inserted fluorescent proteins have been used in other lines of GloFish® for several years and there are no reports of adverse human health effects. Consequently, combining both empirical data on the notified organisms, surrogate information from the literature on other ornamental aquarium fish and the lack of adverse effects supported by the history of safe use for other lines of GloFish®, the indirect human health hazard assessment of GCA2021, OCA2020, and PiCA2020 is considered to be **low** with **low uncertainty**. Insertions of CRISPR/Cas9 expression cassettes were attempted on all three notified lines. However, the molecular analyses conducted by the notifier reported that integrations were not the result of CRISPR mediation. There is a theoretical possibility that unintended mutations from the use of CRISPR/Cas9 could produce unknown effects such as altered proteins with increased allergenicity, although this has not been identified in other models. Consequently, this is not expected to alter the hazard rating, but increases uncertainty, although not sufficiently to raise the ranking above low. The uncertainty is considered low because much of the information on human health effects are based on reports from other ornamental aquarium fish as there are a very limited number of studies in the scientific literature on *C. aeneus*. In addition, there is a limited or no history of safe use in the United States for the notified lines as GCA2021 and OCA2020 have only been commercially available since September 2022 with PiCA2020 not yet on the market as well as the fact that there are no particular studies that have investigated human health effects associated with fluorescent transgenic ornamental fish.

Table 2: Categorization of uncertainty related indirect human health hazard.

Description	Uncertainty Ranking
There are many reports of human health effects related to the hazard, and the nature and severity of the reported effects are consistent (i.e., low variability); OR The potential for human health effects in individuals exposed to the organism has been monitored and there are no reports of effects.	Negligible
There are some reports of human health effects related to the hazard, and the nature and severity of the effects are fairly consistent; OR There are no reports of human health effects and there are no effects related to the hazard reported for other mammals.	Low
There are some reports of human health effects that may be related to the hazard, but the nature and severity of the effects are inconsistent; OR There are reports of effects related to the hazard in other mammals but not in humans.	Moderate
Significant knowledge gaps (e.g., there have been a few reports of effects in individuals exposed to the organism but the effects have not been attributed to the organism).	High

EXPOSURE ASSESSMENT

OVERVIEW

The generalized human exposure pathway for GCA2021, OCA2020, and PiCA2020 is illustrated in Figure 1. The pathway describes the potential for human exposure through the intended use, other potential uses and environmental releases.

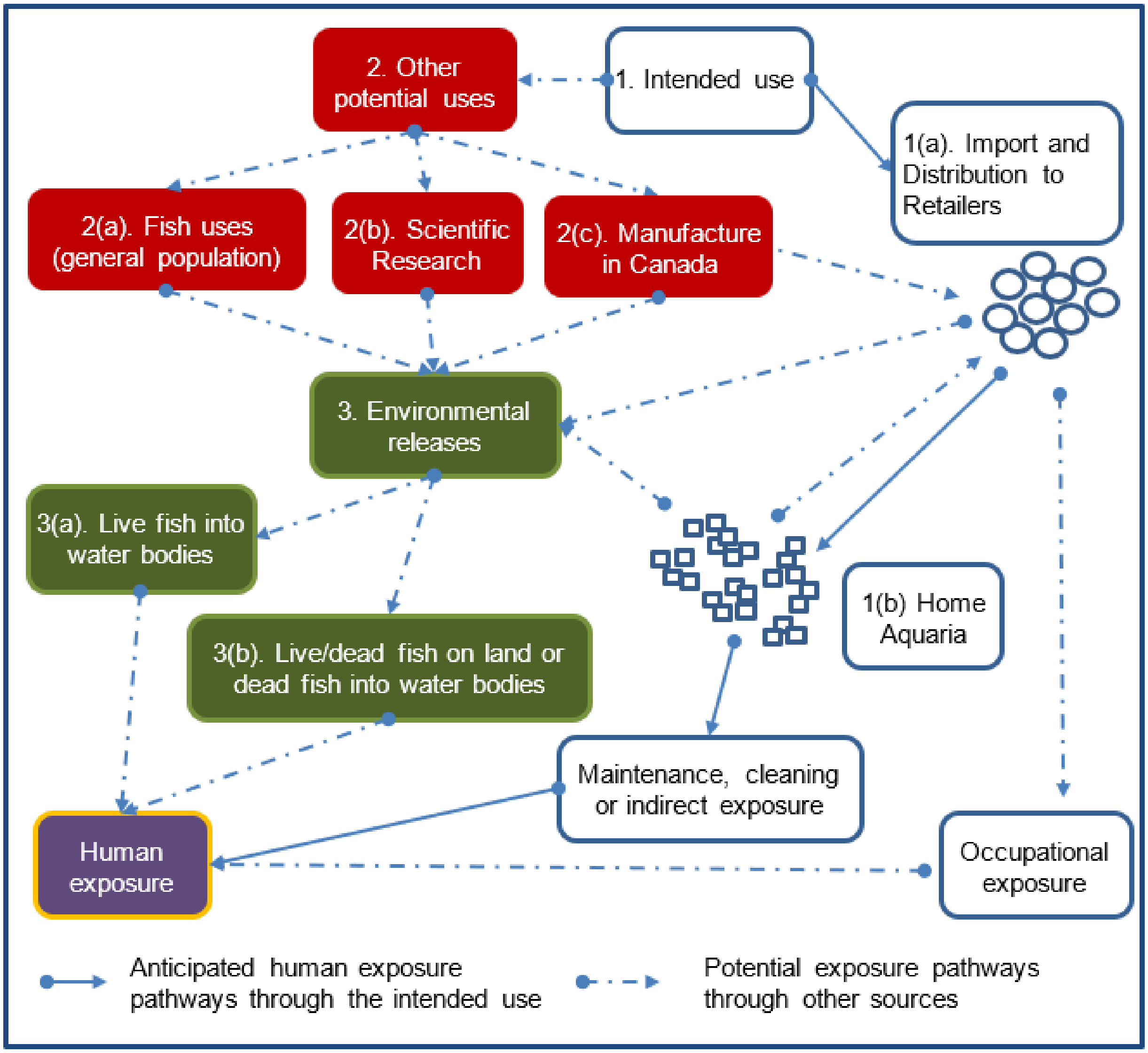

Figure 1: Generalized human exposure pathways for GCA2021, OCA2020, and PiCA2020.

INTENDED USE

Import and distribution to retailers in Canada

Imported fish will enter Canada through authorized distributors and their points of entry. Fish at the production facility are packed in plastic bags that are placed in Styrofoam boxes and flown to Canada by overnight mail/cargo flights or FedEx air. Broodstock are maintained using the same breeding protocol for all types of F2 fish that become the lines identified as GCA2021, OCA2020, and PiCA2020. In the production locations in the U.S., the Division of Aquaculture of the Florida Department of Agriculture and Consumer Services regulates the production of the notified lines to ensure the use of best management practices and help protect the environment. The notifier intends to ship adult fish to distributors and eventually to pet stores in quantities ordered and held until sold to the public.

The notifier plans to market GCA2021, OCA2020, and PiCA2020 fish in Canada using approximately 500 retail outlets based on market size relative to the U.S. The exact number and locations where the notified organisms will be available for sale are not currently known. As ornamental fish intended for sale to the public, it is anticipated that they will be confined inside aquaria in homes and retail outlets. For the intended use, human exposure could happen during distribution involving the transportation of fish by the importer as well as during storage, handling and sale by the retailer. Based on a survey of store owners in Montreal, Quebec, fish are either kept and put on sale by retailers until sold or returned to the distributor and are less likely to be released into the environment by retailers (Gertzen et al. 2008). For GCA2021, OCA2020 and PiCA2020, it is unclear if the notifier allows for retailers to return unsold fish to distributors however it is mentioned that a surplus is not expected. Since retailers are not expected to be the final users of GCA2021, OCA2020, and PiCA2020, human exposure during importation and distribution to retailers is expected to be largely occupational.

Introduction in Canada as ornamental fish in home aquaria

Human exposure by home aquarists that purchase the notified lines directly from retailers or receive them from other aquarists will most likely occur through contact with the notified fish during maintenance activities such as water changes and tank cleanings. Information on stocking rate per household and the number of households planning to purchase the notified lines would be helpful in estimating human exposure to GCA2021, OCA2020, and PiCA2020 through the intended use, as ornamental fish in home aquaria. In absence of this information, an indication of the number of GloFish® Corys per household could be estimated based on the recommended stocking rate and the proportion of Canadians expected to purchase the notified lines.

It is recommended that Corydoras be kept in a minimum 38 litre (10-gallon) aquarium with larger tank sizes recommended if several species are kept. Corydoras are peaceful, non-aggressive and social fish that can manage alone but are happiest schooling in groups of five or more (Sheppard 2025).

Furthermore, there are about 16 million households in Canada based on the 2021 census (Statistics Canada 2021). A 2009 survey estimated that approximately 12% of Canadian households owned fish (Perrin 2009) and another survey (Marson et al. 2009) reported approximately 32% of respondents (132 out of 418) having species of Corydoras in their aquaria. Assuming the same proportion of Canadians practice some form of fishkeeping at present, approximately 614,400 households could come in contact with GloFish® fluorescent *C. aeneus* lines GCA2021, OCA2020, and PiCA2020 (i.e., 32% owning Corydoras of 12% having fish as pets of the 16 million households).

The recommended temperatures for home aquaria established for *C. aeneus* is between 21°C and 27°C (Seriously Fish 2025; Sheppard 2025). As with other ornamental fish, these temperatures and conditions in aquariums also favour the growth of opportunistic pathogens like *M. marinum* (Kent et al. 2006; Mutoji and Ennis 2012; Gauthier 2015) or parasites like *Cryptosporidium* species (Ryan et al. 2015; Golomazou and Karanis 2020). The health status of people that may be exposed to GCA2021, OCA2020, and PiCA2020 is unknown. However, it is reasonable to assume that the households intending to purchase the notified lines could include immunocompromised individuals, children, and those with underlying medical conditions.

OTHER POTENTIAL USES

Fish uses for the general population

For the notified lines (GCA2021, OCA2020 and PiCA2020), some potential uses have been identified for the general population in addition to the intended use as ornamental fish in home aquaria. Minnows and other small fish are commonly used as bait fish in Canada (Kerr et al. 2005; Cudmore and Mandrak 2022). Therefore, it is possible for the notified lines to be used as bait fish. They may also be grown in ornamental outdoor fishponds and even consumed by humans as part of the live food fish industry. These uses are some of the pathways identified by Kerr et al. (2005) as being the potential pathways of introduction of aquatic species in Ontario. However, the notifier argues that GCA2021, OCA2020 and PiCA2020 are not suitable for use in outdoor ponds, or for human consumption, or as bait fish. Year-round growth in outdoor ponds may be difficult in Canada for the notified lines due to their lack of tolerance to low temperatures and cost relative to normal bait fish can be prohibitive for use of the notified lines as bait.

Scientific research

Each year, fish are popular models used for research and education in Canada (Grey and Vincent 2006) with the number released for 2022 being 1,198,299 (CCAC 2023). In research, *C. aeneus* has been used for the study of behaviour (Riley et al. 2020; Siddiqui et al. 2024), predation (Majdi et al. 2018; Wulfes et al. 2024), and development (Huysentruyt et al. 2009; 2011). Since fish are commonly used in the detection of pollutants in the environment (Evans et al. 2005), use of the notified fish as an environmental sentinel cannot be excluded from the list of potential uses.

Manufacture in Canada

Manufacture of the notified organisms is not anticipated to occur in Canada as GCA2021, OCA2020, and PiCA2020 are only produced in Florida. In the production locations in the United States, outdoor freshwater ponds of 10-20 cubic meters volume that can house up to 10,000-15,000 fish are used to grow most of the fish. During summer, the ponds are either open or covered with bird netting to reduce fish loss due to predation and partially covered with dark plastic to provide shade. In winter, the ponds are covered with transparent plastic to prevent heat loss. When ready to spawn or to be sold, the fish are collected from the ponds and held indoors in vats holding 700-900 liters of freshwater that accommodate over 1000 fish. Because of low tolerance to cold temperatures, outdoor ponds are assumed to be not practical in Canada. However, should manufacture occur in Canada using indoor tanks or other means, no additional potential for exposure is foreseen that is different from any other typical aquarium fish.

Conclusion on potential uses for general population

While not completely ruling out other potential uses, no significant exposure is anticipated should the notified lines be produced in Canada (indoor or outdoor), used as bait fish, as food, as environmental sentinels, or in research.

ENVIRONMENTAL RELEASES AND FATE IN THE ENVIRONMENT

Overview

According to the notifier, the intended use of GCA2021, OCA2020, and PiCA2020 is not for environmental release but rather for use in home aquaria. However, accidental, unintended or deliberate environmental releases into water bodies or other media (e.g. land, landfill, etc.) cannot be ruled out since there are reports of releases of ornamental fish from home aquaria due to a variety of reasons (Duggan et al. 2006; Gertzen et al. 2008; Chan et al. 2019). Ornamental fish are often deliberately released into the environment as a ‘humane’ method of disposal of unwanted pets or when they develop undesired characteristics (Chan et al. 2019). Aquarists could potentially release unwanted aquarium fish into the environment when they become bored with the fish or when fish become aggressive, sick, large in size, or have high reproductive output (Gertzen et al. 2008; Chan et al. 2019). Ornamental fish could also be released into the environment through disposal of aquarium wastes on lawns, gardens, outdoor ponds or stormwater drains (Duggan et al. 2006). No species of *Corydoras* were captured in a survey of non-native fish found near ornamental aquaculture facilities in Florida by Tuckett et al. (2017). However, the United States Geological Society has recorded observances of *C. aeneus* in streams in Hawaii between 1991 and 2014 (USGS 2025) due to probable release or escape from home aquaria (Mundy 2005).

Releases of live fish into water bodies

In an event of environmental releases of live GCA2021, OCA2020, and PiCA2020 into waterways in Canada, future establishment will depend on environmental conditions at the point of release and the ability of the released fish to survive, grow, reproduce, disperse and establish self-sustaining populations (Duggan et al. 2006; Strecker et al. 2011; Leggatt et al. 2018; Leggatt unpublished data). Temperature tolerance is a key criterion for determining the ability of aquarium fish to survive, establish and overwinter in the Great Lakes and in Canadian waters as a whole (Rixon et al. 2005; DFO 2018; Leggatt et al. 2018; Leggatt unpublished data).

GCA2021 and OCA2020 showed slightly higher sensitivity to cold-water temperatures compared to their non-transgenic siblings but still within the lethal water temperature ranges for *C. aeneus*. However, the temperature tolerance of PiCA2020 was not significantly different compared to that of the wild-type (non-fluorescent) siblings. Leggatt (unpublished data) reported temperature tolerance limits (LD_{50} = 12.7 ± 1.1 °C) in wild-type albino Bronze Corydoras.

Even the most cold tolerant *C. aeneus* lost equilibrium in temperatures several degrees above typical winter water temperatures in Canada (4°C or less) and temperatures in the warmest recorded lakes of 6°C or less in winter (Leggatt et al. 2018; Leggatt unpublished data). It is therefore less likely for GCA2021, OCA2020 and PiCA2020 lines to survive and disperse in typical winter water temperatures in Canada. There is however a potential for persistence of *C. aeneus* in isolated warm pockets of water (e.g. hot springs, thermal effluent from industrial sites), although where releases have occurred in warmer climates, the fish have either failed to establish or do not impact local systems (Leggatt unpublished data). Della Venezia et al. (2018) examined the potential establishment of freshwater ornamental fish in North America under a climate change scenario forecast for the year 2050. According to the authors, while the model forecasted a two-fold average invasion risk in Quebec, the establishment risk remained extremely low as the minimum temperature for the coldest month would not likely be high enough in 2050 to make Quebec suitable to potential invasions from species currently in the ornamental trade. Therefore, the likelihood of GCA2021, OCA2020, and PiCA2020 establishing self-sustaining populations in Canada is very low due to their inability to survive water temperatures lower than 10°C based on temperature tolerance studies. Therefore, the likelihood of human exposure to the notified organisms in the environment is low.

Release of live or dead fish on land or dead fish into water bodies

In the event a fish dies before sale to or while in the care of a home aquarist, the notifier suggests a disposal procedure similar to all other domestic waste and there are no special handling or disposal procedures required. The notifier has indicated that no specific procedures or treatments are required for disposal of the notified organisms (GCA2021, OCA2020, and PiCA2020) compared to the wild-type species as the only difference (for each line) is the addition of a fluorescent protein derived from species of soft coral or sea anemones. The notifier recommends that individuals that no longer wish to maintain the organisms after purchase either return them to the retailer, give them to another aquarium hobbyist, or humanely euthanize them. Additionally, sale of these lines can be halted at any time if it is determined necessary to terminate the introduction of GCA2021, OCA2020, and PiCA2020 in Canada. It can be assumed that live or dead fish released onto garden lawns, fields or into landfills or dead fish released into water bodies would not lead to survival and establishment in the environment. Therefore, any live or dead GCA2021, OCA2020 and PiCA2020 released into the environment, as well as their respective fluorescent proteins are expected to biodegrade normally and not accumulate or be involved in biogeochemical cycling in a manner different from other living organisms.

EXPOSURE CHARACTERIZATION

Risks from workplace exposure to the notified lines are not considered in this assessment[1].

The human exposure potential of GCA2021, OCA2020, and PiCA2020 is assessed to be **low to medium** (Table 3) because:

1. Adult fish for the three lines (GCA2021, OCA2020, and PiCA2020) will be imported into Canada through unidentified points of entry and distributed through about 500 retail outlets;
2. The sole intended use of GCA2021, OCA2020, and PiCA2020 is as ornamental aquarium fish, thus limiting potential exposure primarily to those possessing a home aquarium. Typical human exposure to live or dead fish in the home is most often related to maintenance activities such as tank cleanings and water changes;
3. Like other aquarium fish, human exposure may include immunosuppressed individuals, children, those with underlying medical conditions;
4. Low winter water temperatures in Canadian waters and low cold tolerance the notified lines limits human exposure through the environment;
5. Any release of live or dead GCA2021, OCA2020, or PiCA2020 on garden lawns, fields or into landfills or into water bodies is not expected to result in survival and establishment in the environment. Should there be such releases, both fish and their respective fluorescent proteins are expected degrade normally and not accumulate or result in human exposure; and
6. Potential uses of GCA2021, OCA2020, and PiCA2020 could include release or culturing in outdoor ponds, as an environmental sentinel, as bait fish, as well as in scientific research and for future manufacture in Canada. No significant increase in human exposure is expected from these potential uses.

[1] A determination of whether one or more criteria of section 64 of CEPA are met is based on an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA may not be relevant to, nor does it preclude, an assessment against the criteria specified in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

Table 3: Exposure considerations (human health).

Exposure	Considerations
High	• The release quantity, duration and/or frequency are high. • The organism is likely to survive, persist, disperse proliferate and become established in the environment. • Dispersal or transport to other environmental compartments is likely. • The nature of release makes it likely that susceptible populations or ecosystems will be exposed and/or that releases will extend beyond a region or single ecosystem. • In relation to exposed humans, routes of exposure are permissive of toxic, zoonotic or other adverse effects in susceptible organisms.
Medium	• It is released into the environment, but quantity, duration and/or frequency of release is moderate. • It may persist in the environment, but in low numbers. • The potential for dispersal/transport is limited. • The nature of release is such that some susceptible populations may be exposed. • In relation to exposed humans, routes of exposure are not expected to favour toxic, zoonotic or other adverse effects.
Low	• It is used in containment (no intentional release). • The nature of release and/or the biology of the organism are expected to contain the organism such that susceptible populations or ecosystems are not exposed. • Low quantity, duration and frequency of release of organisms that are not expected to survive, persist, disperse or proliferate in the environment where released.

UNCERTAINTY RELATED TO INDIRECT HUMAN HEALTH EXPOSURE ASSESSMENT

Uncertainty ranking associated with the information used to assess indirect human health exposure for GCA2021, OCA2020, and PiCA2020 is presented in Table 4. As indicated, the notified organisms will not be manufactured in Canada and the source of exposure will be restricted to the import of adult for the three lines. In the environment, empirical data supports the conclusion that the survival of these fish is expected to be limited by their poor tolerance to temperatures below 10°C. However, this does not preclude the potential for human exposure (general public and vulnerable individuals [i.e., immunocompromised, children, medical conditions, etc.]) in Canada through home aquaria mainly from maintenance and cleaning activities. This exposure assessment is limited by the lack of information on actual number of notified organisms to be imported in subsequent years and poor survey data on household ownership of ornamental fish. It is therefore difficult to gauge public uptake and popularity beyond the import number in the first year. Furthermore, household surveys looking into aquarium fish ownership in Canada based on reports from more than 15 years ago (Duggan et al. 2006; Gertzen et al. 2008; Marson et al. 2009; Perrin 2009). These reports are not specific to GCA2021, OCA2020, or PiCA2020 and do not investigate factors influencing human exposure to aquarium fish. Therefore, because of limited information on the specific exposure scenarios in the Canadian market, the human exposure to the notified organisms is considered **low to medium** with **moderate** uncertainty.

Table 4: Uncertainty ranking associated with the indirect human health exposure.

Available Information	Uncertainty Ranking
High quality data on the organism, the sources of human exposure and the factors influencing human exposure to the organism. Evidence of low variability.	Negligible
High quality data on relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism or valid surrogate. Evidence of variability.	Low
Limited data on the organism, relatives of the organism or valid surrogate, the sources of human exposure and the factors influencing human exposure to the organism.	Moderate
Significant knowledge gaps. Significant reliance on expert opinion.	High

RISK CHARACTERIZATION

NOTIFIED USE

In this assessment, risk is characterized according to the paradigm: Risk ∝ Hazard x Exposure. The two components (“hazard” and “exposure”) are considered embedded in the definition of “toxic” under section 64 of CEPA 1999 and hence, there is no risk in absence of either. The risk assessment conclusion is based on the hazard, and on what we can predict about exposure from the notified use.

GCA2021, OCA2020, and PiCA2020 are genetically modified lines of diploid, hemizygous or homozygous, Corydoras containing fluorescent protein transgene expression cassettes derived from species of marine organisms which makes them appear green (GCA2021), orange (OCA2020), and pink (PiCA2020) under ambient light, including sunlight. All three lines were derived from a line of domesticated albino Bronze Corydoras.

The notified organisms will be marketed throughout Canada for use as ornamental fish in home aquaria.

Although there are reported cases of zoonotic infections from exposure to aquarium fish, wild type Corydoras are popular in home aquaria with a long history of safe use having been sold worldwide as aquarium fish since the 1930s (Innes 1935). Enforcement Discretion decisions by the USFDA were received in 2021 for OCA2020 and PiCA2020 and in 2022 for GCA2021 with GCA2021 and OCA2020 commercially available since September 2022 in the U.S. PiCA2020 will be commercially available in the U.S. within the next year. The fluorescent proteins used in the three notified lines have been used in other GloFish® lines that are now commercially available in Canada. There are no reported adverse human health effects associated with wild type Corydoras in general, the inserted fluorescent protein genes and the methods used to modify the notified lines leading to a conclusion that the notified lines do not present any pathogenic or toxic potential towards humans.

Owing to the low potential hazard and the low to medium potential exposure, the human health risk associated with the use of *C. aeneus* GCA2021, OCA2020, or PiCA2020 as ornamental aquarium fish is assessed to be low.

OTHER POTENTIAL USES

Other uses identified include the use of the notified organisms for research purposes, as bait fish, and for pollution detection (environmental sentinel). Regardless of the use, the available information does not indicate a potential human health implication. No additional risks to human health are foreseen that are different from those of any other typical aquarium fish.

RISK ASSESSMENT CONCLUSION

There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from the use of GCA2021, OCA2020, or PiCA2020 as ornamental aquarium fish or any other potential uses. This risk to human health associated with GCA2021, OCA2020, or PiCA2020 is not suspected to meet criteria in paragraph 64(c) of CEPA 1999. No further action is recommended.

REFERENCES CITED

Aqua-Fish. 2024. Bronze cory – Corydoras aeneus. . Accessed March 7, 2025.

Aquarium Co-op. 2025. Care guide for cory caftfish – the perfect community bottom dweller. . Accessed March 10, 2025.

Baiano, J.C.F., and Barnes, A.C. 2009. Towards control of *Streptococcus iniae*. Emerg. Infect. Dis. 15:1891-1896.

Boylan, S. 2011. Zoonoses associated with fish. Vet. Clin. Exot. Anim. 14:427-438.

Cameselle-Martínez, D., Hernández, J., Francès, A., Montenegro, T., Canas, F., and Borrego, L. 2007. Sporotrichoid cutaneous infection by *Mycobacterium haemophilum* in an AIDS patient. Actas Dermo-Sifiliográficas. 98(3):188-193.

Cardoso, P.H.M., Moreno, A.M., Moreno, L.Z., de Oliveira, C.H., de Assis Baroni, F., de Lucca Maganha, S.R., de Sousa, R.L.M., and de Carvalho Balian, S. 2019. Infectious diseases in aquarium ornamental pet fish: Prevention and control measures. Braz. J. Res. Anim. Sci. 56(2):e151697.

Cardoso, P.H.M., Moreno, L.Z., de Oliveira, C.H., Gomes, V.T.M., Silva, A.P.S., Barbosa, M.R.F., Sato, M.I.Z., Balian, S.C., and Moreno, A.M. 2021. Main bacterial species causing clinical disease in ornamental freshwater fish in Brazil. Folia Microbiologica. 66:231-239.

CCAC. 2023. CCAC animal data report 2022. Accessed February 28, 2025.

CDC. 2024. Healthy pets, healthy people. Accessed January 28, 2025.

Chan, F.T., Beatty, S.J., Gilles Jr., A.S., Hill, J.E., Kozic, S., Luo, D., Morgan, D.L., Pavia Jr., R.T.B., Therriault, T.W., Verreycken, H., Vilizzi, L., Wei, H., Yeo, D.C.J., Zeng, Y., Zięba, G., and Copp, G.H. 2019. Leaving the fish bowl: the ornamental trade as a global vector for freshwater invasions. Aquatic Ecosystem Health & Management. 22(4)417-439.

Chinnery, M. 2022. Do panda cory's produce venom?. Accessed March 7, 2025.

Cremonesini, D., and Thomson, A. 2008. Lung colonization with *Aeromonas hydrophila* in cystic fibrosis believed to have come from a tropical fish tank. J. R. Soc. Med. 101:S44-S45.

Cudmore, B., and Mandrak, N.E. 2022. The baitfish primer: a guide to identifying and protecting Ontario's baitfishes. 2022 version. DFO/2022-2000. Catalogue number: Fs134-7/2023E.

Delghandi, M.R., El-Matbouli, M., and Menanteau-Ledouble, S. 2020. Mycobacteriosis and infections with non-tuberculous mycobacteria in aquatic organisms: A review. Microorganisms. 8(9)1368:1-18.

Della Venezia, L., Samson, J., and Leung, B. 2018. The rich get richer: invasion risk across North America from the aquarium pathway under climate change. Diversity and Distributions. 24:285-296.

DFO. 2018. Environmental and indirect human health risk assessment of the GloFish® Electric Green® Tetra and the GloFish® Long-Fin Electric Green® Tetra (*Gymnocorymbus ternetzi*): a transgenic ornamental fish. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2018/027.

Dinç, G., Doğanay, M., and Izgür, M. 2015. Important bacterial infections transmitted to humans from pet animals. Turk Hijyen ve Deneysel Biyoloji Dergisi. 72(2):163-174.

Duggan, I. C., Rixon, C. A., and MacIsaac, H. J. 2006. Popularity and propagule pressure: determinants of introduction and establishment of aquarium fish. Biological invasions. 8(2):377-382.

Duman, M., Saticioglu, I.B., and Janda, J.M. 2024. A review of the industrial importance, common bacterial diseases, and zoonotic risks of freshwater aquarium fish. Vector-Borne Zoon. Dis. 24(2):69-85.

Dunn, J.R., Behravesh, C.B., and Angulo, F.J. 2015. Diseases transmitted by domestic livestock: Perils of the petting zoo. Microbiol. Spectrum. 3(6):IOL5-0017-2015.

Ebenstein, D., Calderon, C., Troncoso, O. P., and Torres, F. G. 2015. Characterization of dermal plates from armored catfish *Pterygoplichthys pardalis* reveals sandwich-like nanocomposite structure. *Journal of the Mechanical Behavior of Biomedical Materials*, *45*, 175-182.

Emmerich, K., Kolb-Mäurer, A., and Goebeler, M. 2019. Cutaneous infections due to non-tuberculous mycobacteria. Aktuelle Dermatologie. 45(1-2):47-51.

Evans, M. S., Muir, D., Lockhart, W. L., Stern, G., Ryan, M., & Roach, P. 2005. Persistent organic pollutants and metals in the freshwater biota of the Canadian Subarctic and Arctic: an overview. Science of the Total Environment, 351, 94-147.

FishBase. 2024. Corydoras aeneus (Gill, 1858) Bronze corydoras. . Accessed March 11, 2025.

Florindo, M.C., Jerônimo, G.T., Steckert, L.D., Acchile, M., Gonçalves, E.L.T., Cardoso, L., and Martins, M.L. 2017a. Protozoan parasites of freshwater ornamental fish. Lat. Am. J. Aquat. Res. 45(5):948-956.

Florindo, M.C., Jerônimo, G.T., Steckert, L.D., Acchile, M., Figueredo, A.B., Gonçalves, E.L.T., Cardoso, L., da Costa Marchiori, N., da Costa Assis, G., and Martins, M.L. 2017b. Metazoan parasites of freshwater ornamental fish. Lat. Am. J. Aquat. Res. 45(5):992-998.

Franco-Paredes, C., Chastain, D.B., Allen, L., and Henao-Martínez, A.F. 2018. Overview of cutaneous mycobacterial infections. Curr. Trop. Med. Rep. 5(4):228-232.

Franco-Paredes, C., Marcos, L.A., Henao-Martínez, A.F., Rodríguez-Morales, A.J., Villamil-Gómez, W.E., Gotuzzo, E., and Bonifaz, A. 2019. Cutaneous mycobacterial infections. Clin. Microbiol. Rev. 32(1):e00069-18.

Gaulin, C., Vincent, C., and Ismaïl, J. 2005. Sporadic infections of *Salmonella* paratyphi B, var. Java associated with fish tanks. Can. J. Public Health 96(6):471-474.

Gauthier, D.T. 2015. Bacterial zoonoses of fishes: A review and appraisal of evidence for linkages between fish and human infections. Vet. J. 203:27-35.

Gertzen, E., Familiar, O., and Leung, B. 2008. Quantifying invasion pathways: fish introductions from the aquarium trade. Can. J. Fish. Aquat. Sci. 65(7):1265-1273.

Glajzner, P., Szewczyk, E.M., and Szemraj, M. 2021. Pathogenicity and drug resistance of animal streptococci responsible for human infections. J. Med. Microbiol. 70:001339.

Golomazou, E., and Karanis, P. 2020. *Cryptosporidium* species in fish: An update. In Environmental Sciences Proceedings (Vol. 2, No. 1, p. 13). Multidisciplinary Digital Publishing Institute.

Grey, M., and Vincent, A.C.J. 2006. Extent and possible conservation implications of fish use for research, testing and education in North America. Aquatic Conserv: Mar. Freshw. Ecosyst. 16:569-578.

Guz, L., and Puk, K. 2022. Antibiotic susceptibility of mycobacteria isolated from ornamental fish. J. Vet. Res. 66:69-76.

Haenen, O.L.M., Evans, J.J., and Berthe, F. 2013. Bacterial infections from aquatic species: Potential for and prevention of contact zoonoses. Rev. Sci. Tech. Off. Int. Epiz. 32:497-507.

Haenen, O., Karunasagar, I., Manfrin, A., Zrncic, S., Lavilla-Pitogo, C., Lawrence, M., Hanson, L., Subasinghe, R., Bondad-Reantaso, M.G., and Karunasagar, I. 2020. Contact-zoonotic bacteria of warmwater ornamental and cultured fish. Asian Fish. Sci. 33(S1):39-45.

Haenen, O.L.M., Dong, H.T., Hoai, T.D., Crumlish, M., Karunasagar, I., Barkham, T., Chen, S.L., Zadoks, R., Kiermeier, A., Wang, B., Gamarro, E.G., Takeuchi, M., Azmai, M.N.A., Fouz, B., Pakingking Jr., R., Wei, Z.W., and Bondad-Reantaso, M.G. 2023. Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Rev. Aquac. 15(Suppl. 1):154-185.

Hamade, S. 2020. The reversal of self-poisoning in bronze and albino corydoras. J. Mar. Biol. Oceanogr. 9(2):1000215.

Hashish, E., Merwad, A., Elgaml, S., Amer, A., Kamal, H., Elsadek, A., Marei, A., and Sitohy, M. 2018. *Mycobacterium marinum* infection in fish and man: epidemiology, pathophysiology and management; a review. Veterinary Quarterly. 38(1):35-46.

Herzog, P. 2023. Cory catfish (Corydoras): popular types, care & setup. Accessed March 6, 2025.

Hossain, S., De Silva, B.C.J., Dahanayake, P.S., and Heo G.-J. 2018. Characterization of virulence properties and multi-drug resistance profiles in motile *Aeromonas* spp. isolated from zebrafish (*Danio rerio*). Lett. Appl. Microbiol. 67:598-605.

Huysentruyt, F. 2008. Early development of *Corydoras aeneus* (Siluriformes, Callichtyidae): a case study for understanding the evolutionary basis of loricarioid ontogenetic patterning. PhD thesis. University of Ghent. 199 pp.

Huysentruyt, F., Moerkerke, B., Devaere, S., and Adriaens, D. 2009. Early development and allometric growth in the armored catfish *C. aeneus* (Gill, 1858). Hydrobiol. 627:45-54.

Huysentruyt, F., Geerinckx, T., Brunain, M., and Adriaens, D. 2011. Development of the osteocranium in *Corydoras aeneus* (Gill, 1858) Callichtyidae, Siluriformes. J. Morphol. 272:573-582.

Innes, W.T. 1935. Exotic aquarium fishes: a work of general reference. (2nd edition) Philadelphia: Innes Publishing Company.

Iqbal, Z., Ansar, F., and e-Huma, Z. 2018. Risk of importing zoonotic diseases through infected ornamental fish. Punjab University Journal of Zoology. 33(2):211-215.

ITIS. *Corydoras aeneus* (Gill, 1858). URL: ITIS - Report: Corydoras aeneus. Accessed March 11, 2025

Kamijo, F., Uhara, H., Kubo, H., Nakanaga, K., Hoshino, Y., Ishii, N., and Okuyama, R. 2012. A case of mycobacterial skin disease caused by *Mycobacterium peregrinum*, and a review of cutaneous infection. Case Rep. Dermatol. 4(1):76-79.

Kent, M. L., Watral, V., Wu, M., and Bermudez, L. E. 2006. *In vivo* and *in vitro* growth of *Mycobacterium marinum* at homoeothermic temperatures. FEMS Microbiology Letters 257(1):69-75.

Kerr, S.J., Brousseau, C.S., and Muschett, M. 2005. Invasive aquatic species in Ontario: a review and analysis of potential pathways for introduction. Fisheries. 30(7):21-30.

Lazado, C.C., and Zilberg, D. 2018. Pathogenic characteristics of *Aeromonas veronii* isolated from the liver of a diseased guppy (*Poecilia reticulata*). Lett. Appl. Microbiol. 67:476-483.

Leggatt, R.A., Dhillon, R.S., Mimeault, C., Johnson, N., Richards, J.G., and Devlin, R.H. 2018. Low-temperature tolerances of tropical fish with potential transgenic applications in relation to winter water temperatures in Canada. Can. J. Zool. 96(3):253-260.

Li, J., Chong, A.H., O'Keefe, R., and Johnson, P.D.R. 2014. The fish tank strikes again: Metachronous nontuberculous mycobacterial skin infection in an immunosuppressed host. Austral. J. Dermatol. 55:e77-e79.

Lowry, T., and Smith, S.A. 2007. Aquatic zoonoses associated with food, bait, ornamental, and tropical fish. J. Am. Vet. Med. Assoc. 231:876-880.

Lujan, M. 2023. Corydoras Fish: Types, Reproduction, and Care. Accessed February 17, 2025.

Majdi, N., Weber, S., and Traunspurger, W. 2018. The early catfish catches the worm: predation of *Corydoras aeneus* (Siluriformes, Callichthyidae) on freshwater nematodes. Ann. Limnol. 54:29.

Marson, D., Cudmore, B., Drake, D.A.R., and Mandrak, N.E. 2009. Summary of a survey of aquarium owners in Canada. Can. Manuscr. Rep. Fish. Aquat. Sci. 2905: iv + 20 p.

Mundy, B.C. 2005. Checklist of the fishes of the Hawaiin Archipelago Bishop Museum bulletins in zoology 6. 704 pp.

Musto, J., Kirk, M., Lightfoot, D., Combs, B.G., and Mwanri, L. 2006. Multi-drug resistant *Salmonella* Java infections acquired from tropical fish aquariums, Australia, 2003-04. CDI 30:222-227.

Mutoji, K.N., and Ennis, D.G. 2012. Expression of common fluorescent reporters may modulate virulence for *Mycobacterium marinum*: dramatic attenuation results from GFP over-expression. Comp. Biochem. Physiol. C 155:39-48.

Nanan, S.N. 2016. Corydoras aeneus (Bronze Corydoras) . Accessed March 7, 2025.

Narendrakumar, L., Sudhagar, A., Preena, P.G., Nithianantham, S.R., Mohandas, S.P., and Swaminathan, T.R. 2022. Detection of *Mycobacterium marinum* and multidrug-resistant bacteria in a chronic progressive disease outbreak among Siamese fighting fish (*Betta splendens*) in India. Biologia. 77(9):2725-2733.

Nico, L., and Schofield, P. 2025. Corydoras aeneus (Gill, 1858): U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL.). Accessed March 7, 2025.

Ostrowski de Núñez, M. Quintana, M.G., and Mercado Laczkó, A.C. 2020. The life cycle of *Heterophyes yacyretana* n. sp. (Digenea, Heterophyidae), parasitic in the endemic snail *Aylacostoma chloroticum* (Caenogastropoda, Thiaridae) in Argentina. J. Parasitol. 106(5):625-632.

Pate, M., Jenčič, V., Žolnir-Dovč, M., and Ocepek, M. 2005. Detection of mycobacteria in aquarium fish in Slovenia by culture and molecular methods. Dis. Aquat. Org. 64(1):29-35.

Pate, M., Ovca, A., Jenčič, V., Žolnir-Dovč, M., and Ocepek, M. 2019. Mycobacteria in aquarium fish: Are fish handlers aware of their zoonotic potential. Slov. Vet. Res. 56(2):53-58.

Perrin, T. 2009. The business of urban animals survey: the facts and statistics on companion animals in Canada. The Canadian Veterinary Journal. 50(1):48.

Phillips Savage, A.C.N., Blake, L., Suepaul, R., McHuch, O'S., Rodgers, R., Thomas, C., Oura, C., and Soto, E. 2022. Piscine mycobacteriosis in the ornamental fish trade in Trinidad and Tobago. J. Fish Dis. 45(4):547-560.

Ponpornpisit, A., Endo, M., and Murata, H. 2000. Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fishes. Fish. Sci. 66:1026-1031.

Puk, K., and Guz, L. 2020. Occurrence of *Mycobacterium* spp. in ornamental fish. Ann. Agric. Environ. Med. 27(4):535-539.

Richards, H.A., Han, C-T., Hopkins, R.G., Failla, M.L., Ward, W.W., and Stewart, C.N. Jr. 2003. Safety assessment of recombinant green fluorescent protein orally administered to weaned rats. J. Nutr. 133(6):1909-1912.

Riley, R.J., Roe, T.P., Gillie, E.R., and Manica, A. 2020. The development of tactile social interactions in *Corydoras aeneus*. Behaviour. 157(6):515-539.

Rixon, C.A., Duggan, I.C., Bergeron, N.M., Ricciardi, A., and MacIsaac, H.J. 2005. Invasion risks posed by the aquarium trade and live fish markets on the Laurentian Great Lakes. Biodiv. Conserv. 14:1365-1381.

Roberts, H.E., Palmeiro, B., and Weber, E.S. 2009. Bacterial and parasitic diseases of pet fish. Veterinary Clinics of North America: Exotic Animal Practice. 12(3):609-638.

Ryan, U., Paparini, A., Tong, K., Yang, R., Gibson-Kueh, S., O'Hara, A., Lymbery, A. and Xiao, L. 2015. *Cryptosporidium huwi* n. sp. (Apicomplexa: Eimeriidae) from the guppy (*Poecilia reticulata*). Experimental Parasitology, 150, pp.31-35.

Seriously Fish 2025. *Corydoras aeneus* (Gill, 1858). Bronze cory. URL: https://www.seriouslyfish.com/species/search/corydoras+aeneus. Accessed 11 March 2025.

Sharpe, S. 2022. Bronze corydoras: fish species profile. Accessed February 17, 2025.

Sheppard, M. 2025. Cory catfish 101: care, types, food, tank info, & more. Accessed February 17, 2025.

Siddiqui, M., Chlang, A., Lac, E., Kern, J., Wilkinson, G., Jungwirth, A., Allen, J., and Riley, R.J. 2024. Developmental social experience changes behavior in a threatening environment in *Corydoras* catfish. Ecol. Evol. 14(10):e70391.

Spickler, A.R., and Dvorak, G. 2020. Piscine mycobacteriosis. Accessed February 17, 2025.

Statistics Canada. 2021. Census Profile. 2021 Census of Population. Statistics Canada Table: 98-1—0019-01. Ottawa. Released March 29, 2023. https://www12.statcan.gc.ca/census-recensement/2021/dp-pd/prof/index.cfm?Lang=E. Accessed March 6, 2025.

Strecker, A.L., Campbell, P.M., and Olden, J.D. 2011. The aquarium trade as an invasion pathway in the Pacific Northwest. Fisheries. 36(2):74-85.

Tencatt, L.F.C., de Carvalho Gomes, V., and Evers, H-G. 2023. A new species of *Corydoras* (Siluriformes Callichthyidae) from the rio Madre de Dios basin Peruvian Amazon, with comments on *Corydoras aeneus* identity. Neotrop. Ichthyol. 21(2):e230023.

Trujillo-González-A., Becker, J.A., Vaughan, D.B., and Hutson, K.S. 2018. Monogenean parasites infect ornamental fish imported to Australia. Parasit. Res. 117(4):995-1011.

Tuckett, Q.M., Ritch, J.L., Lawson, K.M., and Hill, J.E. 2017. Landscape-scale survey of non-native fishes near ornamental aquaculture facilities in Florida, USA. Biol. Invasions. 19:223-237.

Volk, E. (2019). Developmental and functional analysis of scutes in the armored catfish Corydoras aeneus.

Weir, M., Rajić, A., Dutil, L., Cernicchario, N., Uhland, F.C., Mercier, B., and Tuševljak, N. 2012. Zoonotic bacteria, antimicrobial use and antimicrobial resistance in ornamental fish: A systematic review of the existing research and survey of aquaculture-allied professionals. Epidemiol. Infect. 140:192-206.

WHO/FAO. 2009. Foods derived from modern biotechnology, 2nd edition. Rome, Italy: World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO), Codex Alimentarius. .

Wiegert, J. 2020. Bottom of the tank: captivating cories Accessed March 7, 2025.

Woods, R. 2022. Cory catfish (Corydoras) care guide & species profile. Accessed March 10, 2025.

Wulfes, R., Traunspurger, W., and Ptatscheck, C. 2024. The armored catfish *Corydoras aeneus* shapes the meiofaunal community due to predation and disturbance-induced water column dispersal. Hydrobiologia. 851(6):1565-1576.

Zanoni, R.G., Florio, D., Fioravanti, M.L., Rossi, M., and Prearo, M. 2008. Occurrence of *Mycobacterium* spp. in ornamental fish in Italy. J. Fish. Dis. 31:433-441.

Ziarati, M., Zorriehzahra, M.J., Hassantabar, F., Mehrabi, Z., Dhawan, M., Sharun, K., Bin Emran, T., Dhama, K., Chaicumpa, W., and Shamsi, S. 2022. Zoonotic diseases of fish and their prevention and control. Vet. Quart. 42(1):95-118.