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Rapid Communication

First confirmed records of Prussian carp, *Carassius gibelio* (Bloch, 1782) in open waters of North America

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Abstract

Carassius gibelio (Prussian carp) is considered native from central Europe to Siberia and invasive in Asia, Europe, and the Middle East. To-date, there have been no published occurrences of *C. gibelio* in open waters of North America. Although there are reports from the late 1800's that *C. gibelio* was imported into the USA, some doubt exists regarding the identity of those fish and it is unknown whether any were released into the wild. Here we report the first confirmed records of *C. gibelio* in the wild in North America and estimate its current distribution by compiling records from private and public sources. Morphology and DNA sequences were used to verify identification. Initial specimens of *C. gibelio* were found in a shallow lake in 2006 in Alberta, Canada. Routine provincial government and private fish surveys from 2008–2014 further identified *C. gibelio* throughout natural streams and manmade drainage ditches. These surveys document that *C. gibelio* is now widely distributed throughout southern Alberta. Further spread is likely due to connectivity of the waterways and the life-history attributes of this fish.

Key words: Cyprinidae, invasive species, irrigation canals, DNA sequencing, Canada

Introduction

Carassius gibelio (Bloch, 1782), generally referred to as Prussian carp, is a prolific invader of freshwater ecosystems, particularly in Europe and the Middle East (Kottelat and Freyhof 2007). Initial introductions outside its native range of central Europe and/or Asia likely originated from accidental aquaculture escapees in the 1600s (Lever 1996; Kalous et al. 2012). Since then, *C. gibelio* has exhibited secondary spread by natural dispersal through river and ditch systems (Grabowska 2010), escape from aquaculture (Toth et al. 2005), and intentional introductions by anglers (Witkowski 1996). *C. gibelio's* successful spread and establishment is attributed to its unusual gynogentic reproductive strategy and its ability to thrive under diverse environmental conditions (Perdikaris 2012). The impacts of introduced *C. gibelio* on native fisheries and ecosystem function are substantial (Paulovits 1998; Slavík and Bartoš 2004; Gaygusuz et al. 2007; Perdikaris 2012; Tarkan et al. 2012) and could cause economic damage; therefore, early detection programs that delineate its distribution and enable rapid management responses may be desirable.

Wild *C. gibelio* populations have never been documented outside Asia, Europe, or the Middle East. However, there is a record of importation from Germany to New York state in 1876 (Ferguson 1876) and an isolated, unconfirmed observation of hybrids (*Carassius auratus* × *C. gibelio*) in the Hudson River, New York around this date (Redding 1884), although the hybrids were later considered *Carassius carassius* (Cole, 1905). These records indicate that *C. gibelio* have been imported into North America, yet it is unclear if individuals were ever released into the wild. Also, inconsistent use and confusion over the *C. gibelio* nomenclature make it difficult to confirm if the fish found in the Hudson River were indeed *C. gibelio* or a hybrid (Fuller et al. 1999). Here, we present the first confirmed records of *C. gibelio* in open waters of North America. Our initial detection occurred during an annual sampling of shallow lakes in 2006. DNA sequences were used to verify identification. We subsequently compiled provincial and private fish surveys in Southern Alberta, Canada, which indicates that *C. gibelio*'s current distribution has rapidly expanded to two additional river basins. We also discuss likely pathways of initial introduction and patterns of spread between major river basins in Southern Alberta.

Methods

Study region

We assembled data from the Red Deer, Bow, Oldman, and South Saskatchewan River basins in southern Alberta, which originate at the continental divide of the Canadian Rocky Mountains and flow east to Hudson's Bay. This landscape is part of North America's Prairie Pothole Region and use is now predominately grazing pastures and row crops. Due to its arid environment, open irrigation canals were constructed in the early 1900's that criss-cross the landscape throughout southern Alberta. These irrigation canals are organized into 13 geographically separate irrigation districts, but are inter-connected through natural waterways that include major rivers and small prairie streams.

Field surveys: shallow lakes

We collected *C. gibelio* using overnight sets of baited minnow traps during annual (1998–2008, 2012) surveys of shallow prairie lakes in the Bow and Red Deer River basins east of Strathmore, Alberta. These lakes have varying degrees of connectivity to each other and nearby irrigation canals and/or major rivers (Jackson 2003). Our initial identification was based on the morphological characteristics outlined in Kottelat and Freyhof (2007). Our sampling efforts were not intended to target this species; however, we found incidental catches while conducting other studies.

Literature and database survey

To collect additional records and determine whether our catch was the first record of *C. gibelio* in Alberta, we searched government databases, scientific literature, technical reports, and contacted the Royal Alberta Museum for physical specimens. Due to the connected waterways of the agricultural irrigation canals, we also contacted each irrigation district in Alberta. We collected geographic location, catch numbers, and size for each available record. We focused our search on the Bow, Red Deer, Oldman, and South Saskatchewan River basins due to their proximity to the first *C. gibelio* record. To find any historical records associated with *C. gibelio*'s importation into the United States in 1876, we searched the online collections of the American Museum of Natural History, Smithsonian National Museum of Natural History, and the Harvard Museum of Comparative Zoology for existing physical records of *C. gibelio*.

Specimen identification

Potential misidentification of *C. gibelio* and species level taxonomic uncertainty with other *Carassius* spp. is common (Japoshvili et al. 2013), particularly with *C. auratus* (L.), which is found in the Bow, Red Deer, and Old Man River basins in Alberta (Nelson and Paetz 1992; Government of Alberta 2014) and with *C. carassius*, which to our knowledge has not been identified in Alberta. We followed the dichotomous key of Kottelat and Freyhof (2007); *C. carassius* and *C. gibleio* are easily defined from one another based on the shape of the free edge of the dorsal fin and the peritoneum color. The only non-overlapping morphological character between *C. gibelio* and *C. auratus* is their color, which can be difficult to judge (Table 1). Due to morphological similarities, the definition of species within the *Carassius* genus is not always certain, and is particularly true of *C. gibelio* and *C. auratus* where earlier research defined *C. gibelio* as the wild form of *C. auratus* (Kottelat and Freyhof 2007). Recent genetic studies have since shown that *C. gibelio* and *C. auratus* are distinct species (Rylkova et al 2010) and that *C. gibelio* may have two separate clades (Kalous et al 2012). This potential misidentification and taxonomic confusion may create an under or overestimation of *C. gibelio*'s distribution. We therefore compared DNA from 25 suspected specimens with published sequences from the Barcode of Life project to support our morphological identification (Ratnasingham and Hebert 2007). We also compared major morphological characters identified by Kottelat and Freyhof (2007) in the same 25 fish from Alberta (Table 1).

DNA sequencing

To aid and support our morphological identification, we isolated DNA preserved in 95% ethanol from a specimen collected in 2012 in Long Lake, seven specimens collected from West lake in 2014 (both

Table 1. Selected diagnostic characteristics for 3 *Carassius* species as given by Kottelat and Freyhof (2007) compared to characteristics of 25 specimens collected from the wild in Alberta, Canada.

Character	*C. gibelio*	*C. auratus*	*C. carassius*	Alberta specimens (n=25)
Gill raker number	37–52	38–47	23–33	32–52
Lateral line scale number	29–33	26–31	31–36	29–31
Body color	Silvery-brown	Golden brown, bronze	Golden green, shining	Silver
Last simple dorsal & anal ray	Strongly serrated	Strongly serrated	Weakly serrated	Strongly serrated
Peritoneum color	Black	Black	Whitish	Black
Anal fin ray number	5 1/2	5 1/2	6 1/2	5 1/2
Free edge of dorsal fin	Concave or straight	Convex	Convex	Straight/concave

Figure 1. *Carassius gibelio* (6.5 cm total length) caught in West Lake during the summer 2014. Voucher specimen UCMZ(F)2014.00001. Photo by L. Jackson.

within the Bow River basin), and 17 specimens collected in 2009 from an unnamed tributary to the Medicine River, a major tributary to the Red Deer River. DNA was isolated from the caudal fin with a standard phenol-chloroform technique. We used FishF1 and FishR1 primers to target a 650 bp segment of the 5’ region of the mito-chondrial cytochrome *c* oxidase subunit (COI) to identify species (Ward et al. 2005). Polymerase chain reaction (PCR) was done in 25 µL reaction volumes, containing 1 µL DNA, 1X amplification buffer (NEB), 2.5 mM $MgSO_4$ (NEB), 0.5 mM of each primer, 1.25 U of BSA (NEB), and 0.5 U of *Taq* DNA polymerase (NEB). The thermal cycle profile was: initial denaturation at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 52 °C for 40 seconds, and extension at 68 °C for 1 minute; and a final extension of 68 °C for 10 minutes. Sequences were labeled with the BigDye® Terminator v.3.1. Cycle Sequencing Kit (Applied Biosystems) and purified with ethanol and EDTA. Amplicons were bidirectionally sequenced on a 3500xL genetic analyzer (Applied Biosystems). Sequences were aligned with Codon Code Aligner (v3.7.1) and sequences were compared with the BOLD database to identify the most likely species (Ratnasingham and Hebert 2007).

Results

Occurrence in shallow lakes

Our analysis indicates that records of occurrence began in 2006 and increased through 2012 (Table 2). During the summer of 2006, we trapped three *C. gibelio* individuals in West Lake (Figure 2), which is part of a chain of three lakes connected to the Western irrigation system in the Bow River basin. This was the first and only observation for 2006. Subsequent surveys in 2007 and 2008 revealed *C. gibelio* in all three lakes in the West Lake chain (West, East, and Long Lake). In 2007, we also discovered *C. gibelio* in nearby Bland Lake, within the Red Deer River basin. In 2012, we returned to the West Lake chain

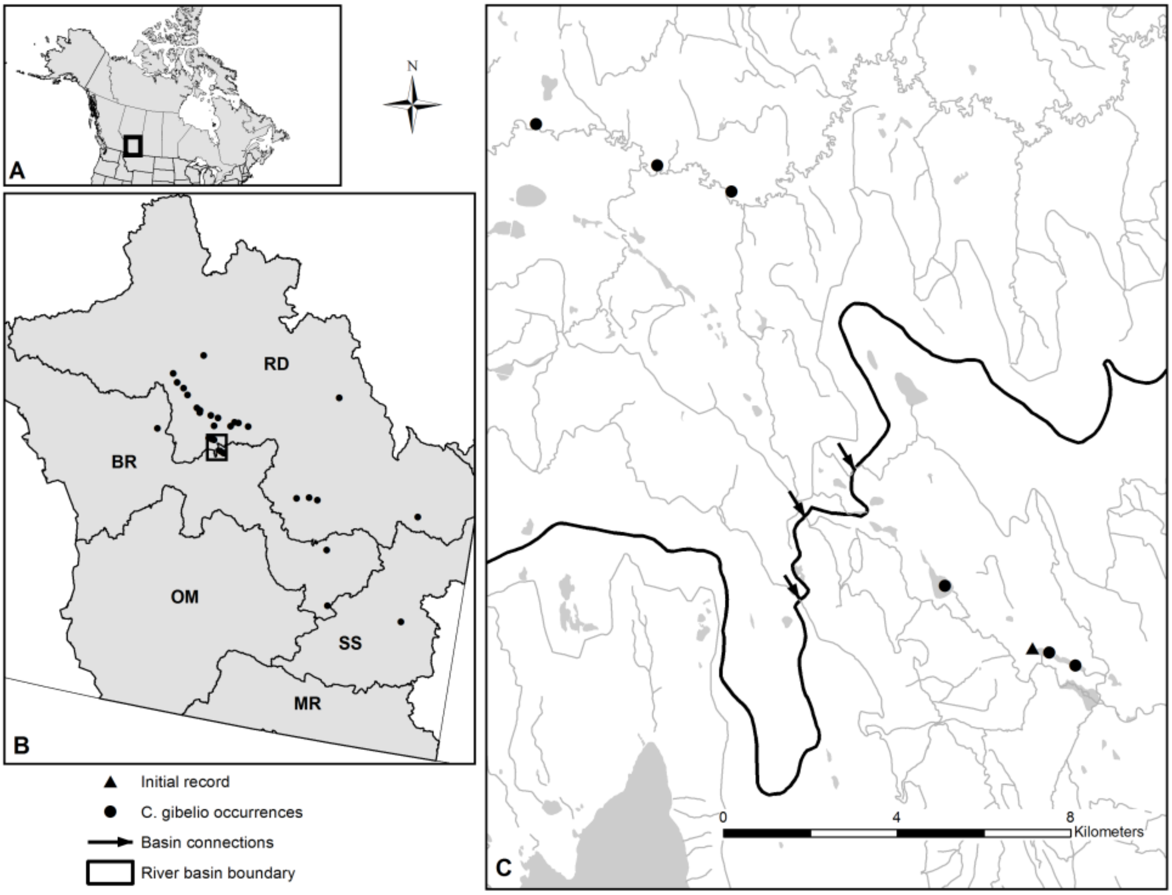

Figure 2. Map of Canada (A) illustrating the area of Alberta where *C. gibelio* have been found; (**B**) confirmed occurrences (black dots) of *C. gibelio* in the Bow (BR), Red Deer (RD), and S. Saskatchewan River basins (SS). The adjoining Milk (MR) and Oldman River (OM) basins are also labeled; and (C) enlarged view of the square in 'B' identifying the site of first occurrence (Triangle; West Lake, n = 3). Arrows indicate artificial connections between the Red Deer River basin and the Bow River basin.

of lakes and Bland Lake, and found three specimens in Long Lake and none in Bland Lake. We also set baited fish traps in Barnett Lake in 2012, which has an ephemeral input from the irrigation canals 2 km northwest of West Lake, but did not catch *C. gibelio*. However, at ice break-up in the spring of 2013, we discovered a fish kill where hundreds of *C. gibelio* lined the shoreline of Barnett Lake. In 2014, seven additional individuals were caught in West Lake.

Occurrence in streams and irrigation canals

The Government of Alberta and private data for southern Alberta suggest that *C. gibelio* are present in natural streams and irrigation canals in the Bow, Red Deer, and South Saskatchewan River basins (Table 2). In 2008, a contracted fish survey of one of the irrigation districts discovered ~5,000 individuals (total length range: 3–20 cm), and estimated 20,000–30,000 individuals in a pool within an irrigation canal in the Red Deer River basin (Haag and White 2008). In 2009, the same contractor found *C. gibelio* at the same site as 2008 and confirmed an additional finding in another pool within the canal system. Again, *C. gibelio* were found in high abundances (approximately 500 individuals; total length range: 3–20 cm) (Haag et al. 2010). Also in 2009, the Government of Alberta received 17 individuals from an unnamed tributary to the Medicine River. In a survey of small prairie streams in 2010, the Government of Alberta (2014) discovered and recorded *C. gibelio* in Spruce Creek in the Red

Table 2. Records of *C. gibelio* in the Bow, Oldman, Red Deer, and South Saskatchewan River basins in Alberta, Canada. * Indicates locations where DNA sequencing was done on *C. gibelio* individuals. ** Indicates that exact coordinates are not known.

Location Name	Geographic Location		Drainage Basin	Record Date	Reference
	Latitude, N	Longitude, W			
West Lake	51°03'36"	113°11'34"	Bow River	2006	Present study
West Lake	51°03'36"	113°11'34"	Bow River	2007	Present study
East Lake	51°03'37"	113°11'22"	Bow River	2007	Present study
Long Lake	51°03'31"	113°10'47"	Bow River	2007	Present study
Bland Lake	51°15'09"	113°21'42"	Red Deer River	2007	Present study
West Lake	51°03'36"	113°11'34"	Bow River	2008	Present study
East Lake	51°03'37"	113°11'22"	Bow River	2008	Present study
Long Lake	51°03'31"	113°10'47"	Bow River	2008	Present study
Site 12	51°16'29"	113°09'47"	Red Deer River	2008	Haag et al. 2008
Site 12	51°16'29"	113°09'47"	Red Deer River	2009	Haag et al. 2009
Site 17	**	**	Red Deer River	2009	Haag et al. 2009
Trib. to Medicine River*	52°08'46"	114°13'09"	Red Deer River	2009	Present Study
Spruce Creek (1 site)	51°46'10"	113°39'57"	Red Deer River	2010	Gov't of AB 2014
Seven Persons (1 site)	50°01'24"	110°41'05"	S. Saskatchewan	2012	Gov't of AB 2014
Serviceberry (3 sites)	51°09'02"	113°23'55"	Red Deer River	2012	Gov't of AB 2014
Rosebud (12 sites)	51°17'51"	112°56'59"	Red Deer River	2012	Gov't of AB 2014
Red Deer River (1 site)	50°50'50"	110°41'43"	Red Deer River	2012	Gov't of AB 2014
Matzhiwin (3 sites)	50°50'41"	112°02'56"	Red Deer River	2012	Gov't of AB 2014
Nose Creek (1 site)	51°08'36"	114°02'22"	Bow River	2012	Gov't of AB 2014
Long Lake*	51°03'31"	113°10'47"	Bow River	2012	Present study
CNR Reservoir	51°38'48"	111°54'21"	Red Deer River	2013	P. Christensen
Bow River	50°02'53"	111°35'28"	Bow River	2013	P. Christensen
J Dam (J Reservoir)	50°28'19"	111°43'22"	Bow River	2013	P. Christensen
Barnett Lake	51°04'12"	113°13'45"	Bow River	2013	Present study
West Lake*	51°03'36"	113°11'34"	Bow River	2014	Present study

Deer River basin (Figure 2). Subsequent government surveys in 2012 showed a wider distribution in seven more streams throughout southern Alberta, including sites in the Bow River basin and South Saskatchewan River basin (Table 2) (Government of Alberta 2014). Specimens from Rosebud Creek were aged by their otoliths and scales and revealed age classes from young-of-year to 3 years. Additional government surveys in 2013 verified *C. gibleio* in the CNR Reservoir, J Reservoir, and the Bow River. The CNR and J Reservoirs are part of the irrigation canal system in the Bow and Red Deer River basins (Paul Christensen, Alberta Environment and Sustainable Resource Development, pers. comm.).

Unverified accounts provided by irrigation district personnel suggest that *C. gibelio* is now present in three of the 13 irrigation districts in southern Alberta (personal communication with irrigation district staff). The Western Irrigation District, where our first occurrence was found considers *C. gibelio* to be present in most of the canals and local shallow lakes.

Historical museum records

No historical records of *C. gibelio* were found in the online collection databases in the American Museum of Natural History, Smithsonian National Museum of Natural History, or the Harvard Museum of Comparative Zoology. There were also no records of *C. gibelio* in the Royal Alberta Museum.

Confirmation of identification

The specimens from our shallow lake surveys and those preserved by the Government of Alberta from 2009 matched positively with the description of *C. gibelio* in Kottelat and Freyhof (2007) with the exception that some of our specimens had 32 gill rakers compared to the minimum of 37 gill rakers described in Kottelat and Freyhof (2007) (Table 1). Otherwise, all other morphological features fell within the range of *C. gibelio* characters described in Kottelat and Freyhof (2007). This also means there was considerable overlap in morphological characters between *C. auratus* and *C. carassius*. Our specimens all had a concave dorsal fin, a silver body color, a black peritoneum, and a highly serrated last dorsal ray. The concave dorsal fin edge, black peritoneum, and a highly serrated last dorsal ray distinguished our specimens from *C. carassius* and the silver color distinguished it from *C. auratus*.

A 650 bp COI fragment successfully amplified in 25 individuals. The molecular identity of 24 specimens most closely matched *C. gibelio* (98.57–100%). The COI fragment from one individual in 2014 from West Lake most closely matched *C. auratus* (99.02%), however, the next highest match was *C. gibelio* (98.81%). All individuals were compared to reference sequences from Russia, Germany, France and Greece (GenBank accession codes: NC_014177 and GU170401).

Discussion

There were no confirmed published records of *C. gibelio* in open waters in North America until our discovery in 2006. An intensive fish population survey conducted by the Government of Alberta in 2006, which included the area in which *C. gibelio* is currently found, found no *C. gibelio* (personal communication with Dr. Mike Sullivan). Subsequent records from multiple sources reveal that *C. gibelio* is now widespread with occurrences in three major river basins in southern Alberta. However, our estimated current distribution and timing of introduction may be conservative due to the absence of comprehensive and extensive surveys throughout Alberta before and after 2006. It is also possible that this species has been overlooked in other surveys due to misidentification or population densities below the level of detection.

To clarify and confirm our identification, we sequenced DNA from 25 individuals caught in three locations. Although the DNA analysis confirmed our morphological identification, specimens from only three locations were sequenced, which leaves the rest of the *C. gibelio* distribution in southern Alberta unverified. Morphological identification is difficult and we believe DNA analysis would help to confirm identification, especially given *C. gibelio's* overlapping distribution with *C. auratus* in southern Alberta. Overall, molecular and morphological approaches support *C. gibelio* identification, despite that one individual matched *C. auratus*. Given the overlapping distribution of *C. auratus* in this region and the close phylogenetic relationship between *C. gibelio* and *C. auratus*, perhaps this result is not surprising. Taxonomic uncertainty also adds to the need for additional molecular work. Kalous et al. (2012) suggest that *C. gibelio* is indeed a distinct *Carassius* species, but also reports that *C. gibelio* is not monophyletic, suggesting it may actually be two species. A neotype was designed to help aid identification and to clarify *C. gibelio* species identity. Therefore, we recommend further genetic sequencing to confirm the distribution of *C. gibelio* in Alberta and that the specimens need to be compared to the neotype identified by Kalous et al. (2012).

The irrigation canals may have inadvertently facilitated *C. gibelio*'s current widespread distribution throughout southern Alberta. Beginning in the late 1800's, artificial waterways were constructed to supply water to farms, which increased the connectivity of waterways and linked many of the once isolated shallow lakes, watersheds, and even large drainage basins together for the first time (Figure 2). As seen in Europe (Galil et al. 2007) and in the Laurentian Great Lakes (Rothlisberger and Lodge 2013), artificial waterways that link basins can become "invasion highways" that facilitate and accelerate the spread of aquatic invasions. Although it is intuitive to understand how *C. gibelio* spreads once established, we do not know how they were initially introduced to Alberta.

In Poland, *C. gibelio* was introduced into new regions intentionally by anglers and unintentionally by aquaculture, followed by subsequent spread from areas of initial introduction (Grabowska 2010). It is doubtful that anglers introduced this species to southern Alberta because *C. gibelio* has low sport value compared to salmonids already present and anglers would have had to bring individuals from overseas. Aquaculture may be a more likely source of introduction. Globally, aquaculture has become one of the leading vectors of aquatic species introductions (Naylor et al. 2001). In Alberta, aquaculture grows a variety of fish species including the closely related koi carp (*Cyprinus carpio* L.) and *C. aurutus* (Alberta Agriculture and Rural Development 2006), the latter being classified as invasive to Alberta (McClay et al. 2004). It is possible that *C. gibelio* was introduced into North America unintentionally in shipments of these or other commercially viable fish. Indeed this is a common vector for new introductions (Slavík and Bartoš 2004). It is also possible that *C. gibelio* was purposefully or accidentally imported by the aquarium industry as bait fish or as contaminated stock and accidentally released. In the Laurentian Great Lakes, *C. auratus* and

Cyprinus carpio are widely distributed and are the top occurring fish recorded (100 and 85%, respectively) in aquarium stores near Lakes Erie and Ontario (Rixon et al. 2005). Although relevant data from aquarium stores in Alberta are not available, *C. gibelio* admixtures to shipments of ornamental cyprinids cannot be excluded. Moreover, the aquarium trade has been proposed as the most possible pathway of introduction of the con-generic *Carassius langsdorfii* Temminck & Schlegel, 1846 in Central Europe from Japan (Kalous et al. 2007; 2013).

The impacts of *C. gibelio* on native fish communities and aquatic ecosystems in Southern Alberta have not been estimated. However, the negative impacts described in Europe, Asia, and the Middle East suggest impacts in Alberta could be substantial. The establishment of *C. gibelio* in Europe has caused multiple fishery declines, particularly with native cyprinids (Economidis et al. 2000; Gaygusuz et al. 2007). *C. gibelio* have a gynogenetic reproductive strategy that results in reproductive competition that may reduce native cyprinid population size (Tarkan et al. 2012). In Alberta, the May – August spawning period of many native cyprinids overlaps with *C. gibelio*'s, and increase the likelihood of reproductive competition and subsequent population decline (Nelson and Paetz 1992; Kottlat and Freyhof 2007). Direct competition for pelagic zooplankton and benthic invertebrates may also reduce native cyprinid populations (Lusk 2010). Additionally, by removing pelagic zooplankton, *C. gibelio* can weaken the top-down control zooplankton excerpt on phytoplankton, resulting in turbid water. Furthermore, consumption of lake benthos stirs up bottom sediments, which can contribute to increased turbidity. The irrigation canals, small prairie streams, and shallow lakes also typically lack piscivorous fish, leading to a lack of predation on *C. gibelio*.

First detected in a single lake in 2006, *C. gibelio* has since been documented as occurring in parts of three adjacent major drainage basins in the Canadian province of Alberta. Because sampling effort has been uneven, additional sampling may reveal an even broader distribution and greater abundance. The potential consequence of this uncontrolled spread is especially high since the Oldman and South Saskatchewan River basins border the Milk River, which is part of the Mississippi River basin. A recent risk assessment and climate match model conducted by the U.S. Fish and Wildlife Service concluded that *C. gibelio* has a moderate to high climate match with the U.S.A. states that border Alberta and that the United States is at high risk of invasion (U.S. Fish and Wildlife Service 2012). This suggests that southern Canadian provinces with similar climates are equally vulnerable to invasion. In the absence of management efforts to control their populations and spread, *C. gibelio* is likely to continue spreading into new basins, causing unknown consequences to native biota, ecosystems, and economies.

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