BMC Zoology

RESEARCH

Open Access

The Prussian carp (*Carassius gibelio*) in Saskatchewan, Canada: current distribution and life history traits of a novel invasive species

Jayme Menard[1], Shayna N. Cossette[1], Julee Stewart[1] and Christopher M. Somers[1*]

Abstract

Background The Prussian carp (*Carassius gibelio*) is an invasive fish species from Eurasia that was first found in North America in the Canadian province of Alberta in the early 2000s. In 2018, an established population of Prussian carp was discovered in the neighboring province of Saskatchewan, raising concern for additional spread in Canada and to the USA.

Results Prussian carp in Saskatchewan have colonized the South Saskatchewan River and tributaries and are moving towards additional connected systems to the northeast. Direct access to most of southern Saskatchewan and the USA is currently prevented only by the Qu'Appelle Dam on the South Saskatchewan River at Lake Diefenbaker. Prussian carp populations in Saskatchewan were age-stratified and ranged from hatch size (20 mm) to a maximum of 42.5 cm total length. Aging using otoliths and scales was unreliable, but the largest fish collected were likely > 10 years old. Saskatchewan Prussian carp had mixed ploidies with diploid, triploid, and tetraploid individuals intermixed. In Lake Diefenbaker, only adult female and juvenile fish were detected, but males were present in the Anerley Lakes at low relative abundance. Emergence of young of the year Prussian carp began in early July and continued through August, indicating an extended period of reproduction; however, most spawning likely occurred during mid-June. The timing of spawning overlaps with that of a variety of native sucker and shiner species, but sperm donors for potential gynogenesis remain unidentified.

Conclusion Our findings suggest that invasive Prussian carp are well established in Saskatchewan and will likely use provincial waterbodies as a conduit to colonize more areas in North America.

Keywords Prussian carp, Invasive species, Barriers, Ploidy, Population characteristics, Citizen science

*Correspondence:
Christopher M. Somers
chris.somers@uregina.ca
[1]Department of Biology, University of Regina, Regina, SK, Canada

Background

The Prussian carp (*Carassius gibelio*) is an invasive fish species that has spread extensively across Europe, but is a relatively recent arrival in North America [1–4]. This species is native to central-Europe, Siberia, and Northern China, and was introduced to western European waters by human activities [2, 5, 6]. The expansion of Prussian carp across western Europe has negatively impacted freshwater ecosystems, resulting in the decline of native members of the Cyprinidae family, decreased water quality, and altered structure of prey communities [1, 6–9]. Additionally, Prussian carp can alter the trophic structures of the systems they invade, and cause commercial fisheries issues via clogging nets [10, 11]. Unfortunately, Prussian carp have successfully colonized much of the southern portion of the western Canadian province of Alberta since being discovered there in the early 2000s [12, 13]. However, their invasion in North America is still relatively new, and only a handful of studies address their spread and potential for negative impacts.

The Prussian carp has a variety of life history and physiological traits that make it a formidable invasive species. These fish grow and mature quickly, often reproducing in their second year [14, 15]. Prussian carp also have an unusual reproductive system in which asexual and sexual reproduction occur simultaneously [16–20]. In the gynogenetic (asexual) reproductive mode, triploid females use sperm from males to stimulate the development of unreduced eggs [8, 10, 15, 21, 22]. This mode of reproduction results in triploid females and both triploid and tetraploid males [22]. Gynogenetic reproduction can be achieved by sperm parasitism from other species (allogynogenesis), and is common in invasive Prussian carp populations [8, 23–25]. This strategy, paired with observations of 4–5 spawning events in a year, enables invading Prussian carp populations to explode in size over a short time frame [21, 26]. Additionally, Prussian carp are able to occupy a wide range of freshwater systems [2, 12, 21], and are highly tolerant of harsh conditions [2, 13, 21, 27–29]. These features may facilitate persistence and rapid expansion following introduction events in novel areas.

The Prussian carp has the potential to spread widely across North America from its unusual initial area of colonization. Prussian carp most likely arrived in western Canada through live release originating from the pet trade or live fish food market [12]. Initially recorded in 2000 [13], Prussian carp in Alberta have spread widely over a huge area, now occupying multiple major river systems. Verification of *C. gibelio* in Alberta was conducted using morphological and DNA sequencing techniques [12]. All of these river systems flow eastward into the neighboring province of Saskatchewan, so colonization of that province was likely inevitable. The earliest recorded Prussian carp in Saskatchewan was an isolated observation of a single individual fish in 2005, with no official records thereafter [13]. However, in the spring of 2018 a large number of Prussian carp were found dead on the shore of a small Saskatchewan lake (Stockwell Lake) more than 240 km east of the Alberta border (M. Tyree, Saskatchewan Ministry of Environment, *pers. comm*). This site is a tributary to the South Saskatchewan River, and the die-off was the first evidence of an established Prussian carp population in Saskatchewan. The circumstances suggested that Prussian carp had been established for some time in Saskatchewan, but essentially nothing was known about this novel invasive species in North America outside of Alberta.

Here we examine the state of the invasive Prussian carp population in the Province of Saskatchewan using a combination of citizen science reports and field surveys. Our first objective was to conduct surveys for Prussian carp to determine their distribution and identify occupied bodies of water. Our second objective was to examine life history traits, including size and age, sex ratio, timing of spawning, and the likely mating system via assessment of ploidy. Our study provides novel insight into the range expansion of the highly invasive Prussian carp in North America, as well as their population characteristics in a newly invaded system.

Methods

Study area

We conducted field surveys on two different spatial scales to examine Prussian carp distribution in Saskatchewan. In the first, we sampled areas in the South Saskatchewan River, the upper Qu'Appelle River, and the Saskatchewan River watersheds during the spring and summer of 2019 (Fig. 1a; Table S1). In 2020 we had to pause Prussian carp fieldwork following the University of Regina's research protocols during the COVID-19 pandemic restrictions. During 2021 and 2022, we focused our field survey efforts on eastern Lake Diefenbaker (Fig. 1b; Table S1). Two dams on Lake Diefenbaker alter the flow of water. Gardiner Dam on the northern portion of the lake is a hydroelectric dam that releases water to continue flowing down the South Saskatchewan River. The Qu'Appelle Dam is an earthen structure that separates the Qu'Appelle River system from Lake Diefenbaker and the South Saskatchewan River system. When the reservoir is at normal water levels, the outlet is located at 24 m depth. The two dams on Lake Diefenbaker have the potential to be control structures for preventing the spread of Prussian Carp. Lake Diefenbaker supports a wide variety of native fish species and has important recreational fisheries for walleye (*Sander vitreus*), northern pike (*Esox lucius*), yellow perch (*Perca flavascens*), and burbot (*Lota lota*). Shallow bays and tributary inflows along the shoreline are key areas for native species. Coteau Bay at the northern end

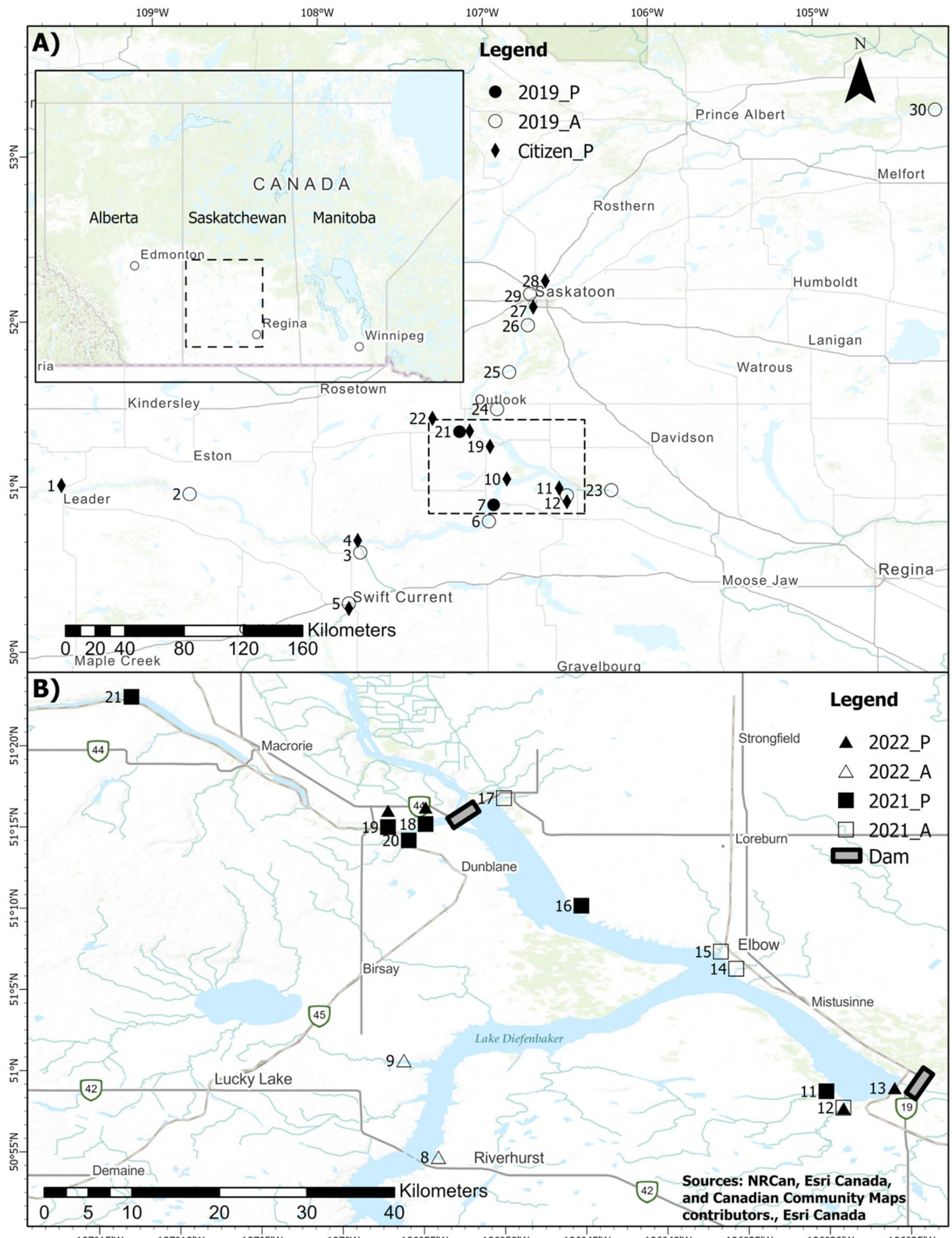

Fig. 1 Map of sample locations. Large-scale (**A**) and smaller-scale sampling (**B**) of Prussian carp between 2019–2022. Filled symbols are locations where Prussian carp were detected; unfilled symbol represent sites that were surveyed but no Prussian carp were found. Panel (**A**) contains both citizen science (diamonds) and field survey reports (circles); panel (**B**) is based solely on field surveys (circles). See Table S1 for further information on sampling effort and fish caught

of Lake Diefenbaker is an important site for native species reproduction [30].

We also sampled extensively in Stockwell Lake (Table S1), which is part of the Anerley Lakes chain that connects to Lake Diefenbaker by Coteau Creek. Coteau Creek is ephemeral and is likely the pathway Prussian carp used to establish in Stockwell Lake before the large die-off event that alerted their establishment in Saskatchewan in 2018. Stockwell Lake is a shallow (max depth 4.5 m), highly productive lake that connects to the rest of the Anerley Lakes through a series of culverts. Shallow depths, poor water quality, and unstable water levels generally prevent the Anerley Lakes from sustaining large-bodied native fish populations. However, they are important waterbodies for amphibians, shorebirds, and waterbirds.

Prussian carp surveys

Fish netting

We identified all fish in the *Carassius* genus that were captured or reported as Prussian carp. This identification is based on the work of Elgin et al. [12], who used molecular and morphological characteristics to verify the identity of *C. gibelio* in the river systems that connect to Saskatchewan. Prussian carp are capable of hybridization with other species and can exist as a mixed hybrid complex [31–35]. However, Halas et al. [36] confirmed that invasive fish found in Saskatchewan and Alberta all shared the same mitochondrial DNA haplotype, which was identical to archived sequences for *C. gibelio*. Goldfish (*C. auratus*) have a very limited distribution in Saskatchewan, where they are typically limited to closed storm water retention systems that do not allow movement into natural water bodies [37]. Thus, goldfish are unlikely to confound identification of *Carassius* specimens in our area.

For the larger spatial scale surveys in 2019, field sites were located from the southwest through to the central-eastern parts of Saskatchewan. Thirteen field sites were selected to encompass a variety of locations that might have Prussian carp based on connectivity to known occupied sites in Alberta and recent observations in Saskatchewan (Fig. 1a). An additional number of sites was added (to total of 29) to reflect locations from citizen science reports. At each site we set 5 fyke nets for a variable number of overnight periods (16–20 h) ranging from 1 to 3 nights in a row. The nets were constructed with 20-mm mesh, and we set 1 large, 3 medium, and 1 small fyke nets at each site. All nets were set near shore in depths of 1.5 m or less with the lead perpendicular to the shoreline. We also used a 30-m beach seine to complete three 30-m pulls in the same general area of the fyke nets on each day. These survey methods caught many Prussian carp during our preliminary netting in Stockwell Lake. In addition, live-trapping does not necessitate killing native species; thus, we deemed this mixed live-netting approach suitable for other locations. Correspondingly, we used the same approach for more focused surveys on eastern Lake Diefenbaker in 2021 and 2022. However, we chose sites based on habitat that was likely to attract Prussian carp (shallow bays in coulees), as well as locations that were ecologically significant to other fish species (e.g., Coteau Bay), or in close proximity to lake exit points near dams or irrigation pumping stations (Fig. 1b). In addition, in 2021 we used 2 small larval fish fyke nets in Coteau Bay (site 19), and in 2022 we added baited umbrella-style minnow traps to the methods described above at all survey locations. Our objective was not to standardize sampling for comparisons, but rather to determine Prussian carp presence.

Citizen science

In collaboration with the Saskatchewan Wildlife Federation, a non-government organization representing anglers and hunters in Saskatchewan, we developed a citizen science program to survey for Prussian carp. We asked anglers to submit observations of Prussian carp to a dedicated email address: ReportInvasiveCarp@swf.sk.ca. Additionally, we used social media posts (https://www.facebook.com/SaskSportfishResearch/) to recruit anglers, educate on identification of Prussian carp, and encourage reporting. Facebook posts related to the citizen science program were viewed over 500,000 times during 2019 and 2020. We asked citizens to report the date and location of any suspected Prussian carp observation, and submit a photo for identification. Reports submitted without a photo were noted but not used in the dataset. We verified the fish identification using external features visible in photos. The reporting email address will remain actively monitored indefinitely.

Samples for size and age

Our fish netting efforts in 2021 produced 4,396 Prussian carp, which were euthanized by immersion in 5% clove oil for 10 min, followed by severing of the spinal cord. A subset of Prussian carp sampled from 2019 to 2021 ($n = 269$) made up of individuals varying in length was used to provide aging structures. Prussian carp were measured for total length to the nearest mm with the tail in the natural position and weighed to the nearest 0.1 g using a digital scale. Scales, fin spines, sagitta otoliths, and lapilli otoliths were extracted from each individual. We selected lapilli otoliths for aging based on their higher reliability for aging species in the *Carassius* genus [38–41]. Heads were dissected to extract the lapilli from the inner-ear cavity [40, 42]. Sex was determined by macroscopic assessment of gonads; individuals were either mature or immature depending on gonad development

[42]. The presence of orange-coloured eggs in ovaries and developed testes (visible to the naked eye) indicated mature specimens [42].

Aging methodology

The lapillus was read whole and then cracked and read at 1.6-6x magnification using a dissecting microscope (Olympus AZX10) for each individual. Annuli were determined using American Fisheries Society (AFS) standards [42]. The percent agreement on fish age between scorers using lapilli was very low (20%), but increased when considering +/- 1 (50%) and +/- 2 (73%) years. There was no systematic pattern in the disagreement between observers, but we deemed the reproducibility of age estimates to be too low for any kind of formal analysis. However, to enable presentation of some age data, we performed a consensus aging for all fish in which both observers simultaneously read the same structure and agreed upon the final scoring. Annual growth rings for Prussian carp near the nucleus of otoliths (representing approximately ages 1–3) were relatively poorly distinguishable, and our consensus aging is likely an underestimate for many individuals.

Ploidy analysis

Samples for ploidy assessment were collected from Lake Diefenbaker and Stockwell Lake in 2021 as part of the field survey and citizen science methods summarized above. From Stockwell Lake we randomly selected 10 female fish in three different size classes ($n = 30$; <10 cm, 11–20 cm and < 30 cm), and all males that were collected ($n = 19$). We used all adult fish and 10 randomly selected juveniles between 5 and 10 cm to assess ploidy in Lake Diefenbaker ($n = 28$).

Silver nitrate staining of the nucleolar organizer region (hereafter AgNOR) for ploidy assessment was done using procedures modified from three previous studies [43–45]. Freshly euthanized individuals had cells scraped from the dorsal epidermis using a number 10 scalpel blade, which were spread on a glass microscope slide and fixed with 50% acetic acid solution. The air-dried slides were stored for future preparation. Step 2 was completed in the lab; two solutions were used for AgNOR staining: (1) a colloidal developer, containing 2 g of gelatin and 1 ml of 88% formic acid in 100 ml of distilled water; and (2) a 50% silver nitrate and water (w/v) solution. Slides were dried at 60 °C in a drying oven (HERAtherm IMH60, ThermoFisher, Waltham, MA, USA). We viewed slides via light microscopy using an Olympus CX41 light microscope at 1000x magnification with immersion oil type A.

Ploidy was determined by counting the number of chromosome sets per cell based on the number of nucleolar organizer regions (NORs; 43, 45, 46). There is generally one NOR present per haploid genome; however, the *Carassius* genus underwent a historical polyploidization event [47, 48], so each chromosome set has 2 NORs [49, 50]. To test the NOR method, we examined NOR values in diploid goldfish ($n = 5$) from a gonochoristic line obtained from a pet store, and common carp sampled from a naturalized population ($n = 2$). The goldfish all had 4 NORS, indicating that they were diploid, as expected. The common carp both had 2 NORS, also indicating diploidy. Common carp did not undergo the same genome-wide duplication event that members of the *Carassius* genus did [50]. For our assessment of Prussian carp in Saskatchewan, we scored ploidy as follows based on NOR number: $2n = 4$, $3n = 6$, $4n = 8$. For each sample, 100 intact cell nuclei were counted and the number of visible NORs recorded for each. The mode of the NOR number for the 100 cells was used as the ploidy for each sample [51]. A subset of 21 slides was re-scored by the same observer yielding an agreement on the mode of 76%.

Spawning trends in lake diefenbaker

We used timing of emergence of young of the year to provide information on when Prussian carp were potentially spawning in our study area. Young of the year for a variety of species were collected throughout the open water season as they were recruited into sampling gear during our nearshore surveys. Young of the year Prussian carp hatch after around 52 h of incubation at 22–25 °C (temperature-dependent) and are typically ~ 5 mm in length [52, 53]. At this larval stage Prussian carp are generally in dense floating plant cover and are nearly impossible to sample [53, 54]. However, when they grow somewhat larger (10–20 mm), around 20 days post hatch [52], they swim to shallow, muddy, vegetated areas [54]. At this stage they can be sampled by common survey methods, and the presence of young of the year Prussian carp indicates recent spawning in the local area. We considered any fish below 20 mm to be relatively newly hatched, most likely within the previous 20-days [52]. We focussed young of the year emergence assessments in Coteau Bay on eastern Lake Diefenbaker. We used a mix of beach seining and small larval fyke nets (mesh size < 1 mm) to sample small fish as described above. Linear regression was used to evaluate the increase in length of juveniles throughout the summer. Lengths from the first emergence of juveniles on July 8th until August 15th were included and a regression model was run using length and date values. The analysis was conducted using the stats package in R [55].

Results

Prussian carp surveys

Broader-scale field surveys during 2019 captured a total of $n = 9{,}991$ individual fish from 24 different species (Table S2). However, only a single Prussian carp specimen, a 35-cm adult female fish weighing 775 g, was captured in a fyke net July 12, 2019 in Rusty's Coulee near Riverhurst on Lake Diefenbaker. In contrast, a total of 38 citizen science reports of suspected Prussian carp were made from April 2019 to December 2020. Seven of the reports had no photos submitted to verify identification. Of the remainder, 18/31 (58%) were confirmed to be members of the *Carassius* genus. These specimens were largely caught by anglers while targeting other species. Locations reported by citizens included the South Saskatchewan River between Lake Diefenbaker and the Alberta border, multiple locations on Lake Diefenbaker, the South Saskatchewan River between Gardiner Dam and Saskatoon, and the South Saskatchewan River north of Saskatoon (Fig. 1a). Two of the 18 (11%) citizen science reports were much more likely to be feral goldfish (rare in Saskatchewan) than Prussian carp; these were submitted from closed municipal storm water retention ponds in the cities of Saskatoon and Warman. Thirteen of the 31 (42%) submitted reports were not Prussian carp, but rather fish caught relatively infrequently by anglers. These reports consisted of common carp (*Cyprinus carpio*; 5/31, 16%), quillback (*Carpiodes cyprinus*; 3/31, 10%), shorthead redhorse (*Moxostoma macrolepidotum*; 2/31, 6.5%), lake whitefish (*Coregonus clupeaformis*; 2/31, 6.5%), and goldeye (*Hiodon alosoides*; 1/31, 3%).

Field surveys on a smaller spatial scale at 9 sites on eastern Lake Diefenbaker in 2021 captured 43,010 individual fish of 18 different species (Table S1). Of these, 4,396 (10.2%) were Prussian carp, with at least one individual caught at 6/9 (67%) of the sites surveyed (Fig. 1b). The Coteau Bay area was the most intensively surveyed and yielded 4,112 (93.5%) of the individual Prussian carp captured. Most of these were young of the year, but adult fish were also caught in this location. This site is located close to the spillway for the Gardiner Dam, and access to the South Saskatchewan River downstream of Lake Diefenbaker. In 2022, we surveyed 6 sites on eastern lake Diefenbaker that were associated with lake exit points at irrigation pumping stations and water control structures. Surveys in 2022 produced 4,032 fish of 14 species, and 4/6 (67%) sites had at least one Prussian carp captured. In total we caught 132 Prussian carp in 2022, and similar to 2021 the majority of these came from Coteau Bay ($67/132 = 50.8\%$). Other notable observations from 2022 included capture of an adult gravid female Prussian carp in the immediate area of the Qu'Appelle Dam, and juveniles near irrigation pick ups in Bigfill Coulee and Irrigation Bay. We continued to receive citizen science reports during 2021, 2022, and 2023; 100% of these were from Lake Diefenbaker and the South Saskatchewan River south of Saskatoon. Two reports from western Lake Diefenbaker (Saskatchewan Landing Provincial Park) in the winter of 2023 were of large numbers of juvenile Prussian carp in the stomachs of northern pike captured by anglers.

Size and age

In Stockwell Lake, individuals from 1 to 40.2 cm total length were present, with survey gear capturing many individuals across the size range. The length frequency distribution suggested multiple age cohorts were present in both 2019 and 2021. In 2019, at least 3 cohorts were identified by peaks in the length-frequency histogram (Fig. 2a). The three cohorts were at 1 cm, 4–9 cm, and 14–22 cm; above 22 centimeters cohorts were not readily identifiable. The cohorts are presumed to be young -of the -year, year 1, and possibly year 2. The maximum length sampled in Stockwell Lake in 2019 was a 33.5-cm female. Samples from Stockwell Lake in 2021 also had at least 3 cohorts, including a minimum of 6.2 cm and a maximum size of 40.2 cm (Fig. 2b). The minimum size was larger than in 2019 because of more limited beach seining, which resulted in fewer small fish being captured in 2021. Potential cohorts sampled from Stockwell Lake in 2021 were 6–14 cm, 15–22 cm, and >22 cm. Notably, there were multiple fish ($n = 7$) larger than the maximum size recorded in 2019. Younger / smaller fish do not show up accurately in the size frequency distribution for 2021 because we sampled hundreds of smaller Prussian carp but only measured a subsample, which is depicted in Fig. 5. Consequently, the relative frequency of small fish in the 2021 dataset is much higher than presented (Fig. 2b; see grey block).

The length-frequency distribution of Prussian carp was completely different in Lake Diefenbaker compared to Stockwell Lake (Fig. 2c). Despite intensive sampling using the same gear types, only small juvenile Prussian carp from 1 to 10 cm, and large adult fish > 34 cm were captured in Lake Diefenbaker; fish of intermediate length were not detected at all. Both the smallest (~ 1 cm) and largest fish (42.5 cm) captured in survey gear were from Lake Diefenbaker. More than 4000 small Prussian carp were sampled in Lake Diefenbaker in 2021; however, we only measured length for a small subset. Like the situation for Stockwell Lake in 2021, the relative frequency of small fish cannot be accurately shown and is much higher than displayed here (Fig. 2c; see grey block).

Consensus otolith ages of Prussian carp were estimated for fish sampled from Stockwell Lake and Lake Diefenbaker, as well as several from other locations in the South Saskatchewan River Watershed (total $n = 264$). In Stockwell Lake ($n = 218$), the most common ages

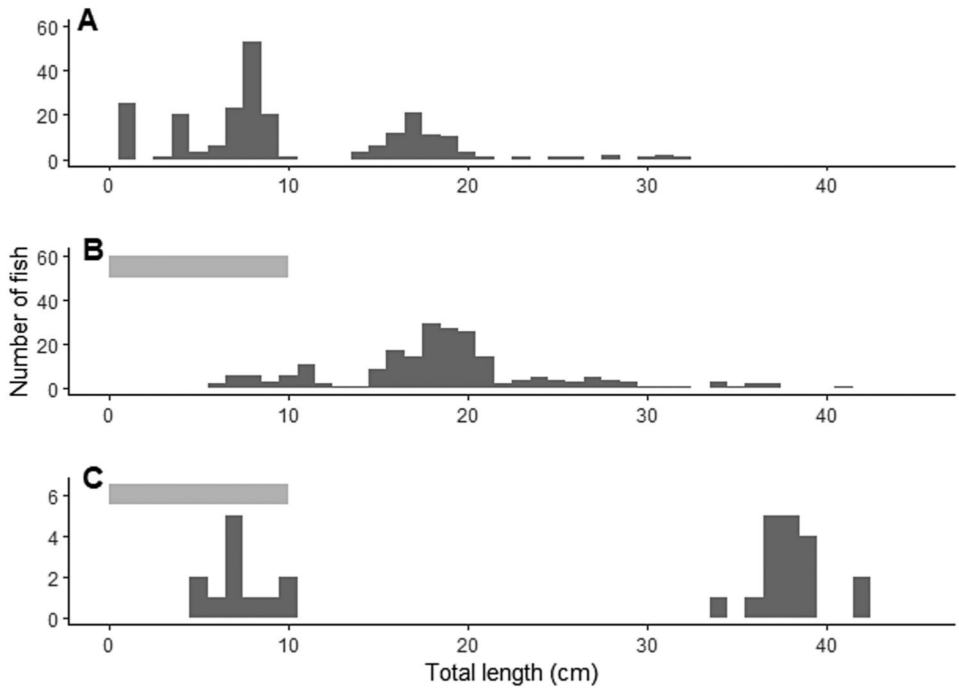

Fig. 2 Length frequency histogram for Prussian carp sampled in Saskatchewan lakes. (**A**) Stockwell Lake 2019; (**B**) Stockwell Lake 2021; and (**C**) Lake Diefenbaker 2021. Note the different scale on the y axis for panel (**C**), reflecting reduced capture frequency in Lake Diefenbaker compared to Stockwell Lake. Grey blocks with question marks on panels (**B**) and (**C**) represent size ranges that contained hundreds to thousands of fish, but only a small subsample was measured. A true size frequency could not be represented, but fish in these size ranges were very abundant and would have very high relative frequency values

were between 2 and 3 years old ($n = 132$), with 3-year-olds accounting for the most aged individuals ($n = 72$). Length of males ranged between 14.2 and 34.9 cm; the oldest male in Stockwell Lake was estimated to be 5 years old (TL = 16.1 cm), whereas the oldest female sampled in Stockwell Lake was estimated to be 7 years old (TL = 31.8 cm). Ages in Lake Diefenbaker and the South Saskatchewan River ranged from young-of-year to 10 years old for a large female (TL = 38 cm). Females in Diefenbaker reached a maximum of 42.5 cm in our samples. All of the fish aged in Lake Diefenbaker ($n = 47$) were young of the year or mature adults ranging from 3 to 10 years old.

Ploidy

Ploidy was assessed using silver-nitrate staining of nucleolar organizer regions from 78 Prussian carp captured in Lake Diefenbaker and Stockwell Lake during 2021. When all samples were considered together, most females (45/49; 92%) had a modal value of 6 NORs, indicating that they were triploid. In contrast, all male individuals assessed (19/19; 100%) had a modal value of 8 NORs, indicating that they were tetraploid. When considered separately by lake, female Prussian carp in Stockwell Lake ($n = 30$) were predominantly triploid based on the modal value of 6 NORs (26/30; 87%); however, some females had modal values of 4 (1/30; 3%) and 8 NORs (3/30; 10%), indicating that they were diploid or tetraploid, and that ploidy in Stockwell Lake was mixed (Fig. 3). Interestingly, 2 female fish from Stockwell Lake presented NOR values in which the mode was only slightly higher than the next closest value. The first individual was split at 4 and 6 NORs, and the second was at 6 and 8 NORs, which raises the intriguing possibility of 2n-3n and 3n-4n somatic mosaicism. For ploidy summaries we used the modal value for these individuals despite the potential mosaicism. All female individuals ($n = 19$) and most unsexed juveniles (8/9 and 89%) from Lake Diefenbaker ($n = 28$) had modal NOR values of 6, indicating that Prussian carp in this lake were also predominantly triploid. However, a single unsexed juvenile individual (1/8;12.5%) had a modal NOR value of 8 and was therefore tetraploid.

Sex ratios

Fish from Stockwell Lake included both females and males. In 2019, the M: F ratio was 0.21:1 (males, $n = 13$; females, $n = 63$). Whereas in 2021, Stockwell Lake males represented 11% (males, $n = 23$; females, $n = 160$; juveniles not sexed, $n = 33$) of the sampled population, with a M: F sex ratio of 0.14:1. All but one of the mature individuals > 30 cm sampled between 2018 and 2021 ($n = 53$) from Lake Diefenbaker were female based on visual inspection of gonads. One mature individual (TL = 32.4) was

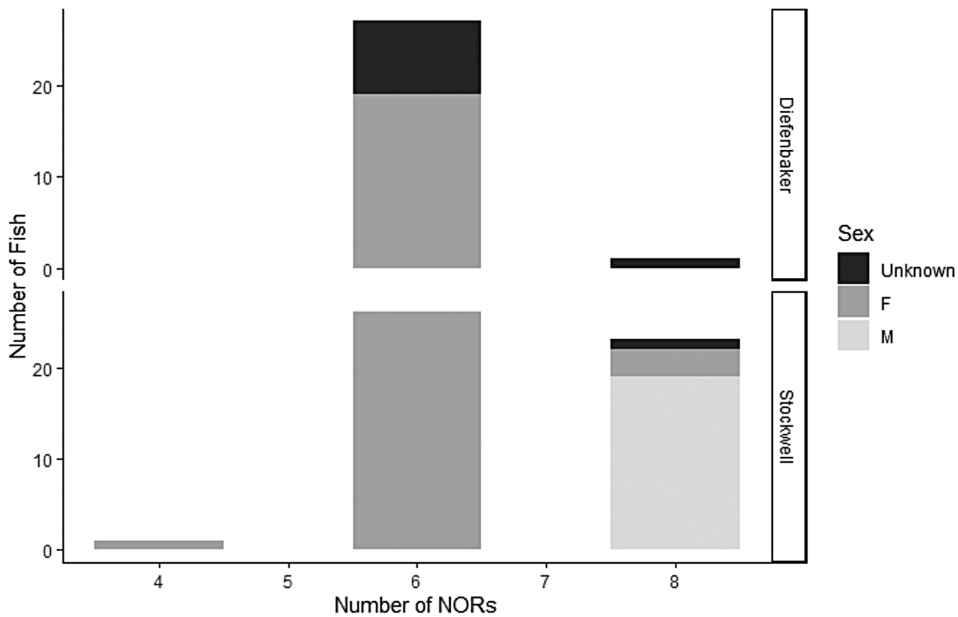

Fig. 3 Modal NOR values from somatic cells of Prussian carp in Saskatchewan. The values presented are from assessments of 100 cells per fish. The top panel contains data from fish sampled in Lake Diefenbaker ($n = 19$ females, $n = 9$ unsexed), and the bottom from Stockwell Lake ($n = 30$ females, $n = 19$ males, $n = 1$ unsexed). Unsexed individuals were either immature juveniles that had not begun gonad development or sex could not be determined due to damage, absence or inconclusive gonads

sampled in June of 2020 in Lake Diefenbaker that had characteristics of both female and male gonads (intersex).

Spawning trends in lake diefenbaker

We focused young of the year fish sampling on a single site in Lake Diefenbaker (Coteau Bay) that was systematically surveyed weekly from mid-May through the end of August of 2021. Relatively newly hatched Prussian carp (< 20 mm) began to appear in sampling gear in the first week of July. After initial emergence the relative abundance of young of the year Prussian carp rapidly increased until they reached the highest relative abundance by the weeks of July 21st through Aug 3rd (Fig. 4a). Catch-per-unit-effort (CPUE) of Prussian carp based on the number captured per seine pull increased through early July (from 50 to 258) and peaked in mid-July at 258 (Fig. 4b, c). Individuals ≤ 10 mm were very difficult to sample with standard gear but were detected on July 27th and August 3rd (Fig. 5). Individuals between 10 and 20 mm were detected on July 6th, July 10-12th, July 15-16th, July 20th, July 22nd, July 26-28th, August 3-5th, and August 12-13th. Once young of the year appeared, they increased significantly in size over time (Linear Regression, $df = 619$, $r^2 = 0.432$, $p = < 0.0001$). However, variance in the length of small Prussian carp was very high, and those in the 10 to 20 mm newly-hatched range were detected across the entire sampling period.

Discussion

Prussian carp surveys

At the time of this study Prussian carp were established in Saskatchewan throughout the South Saskatchewan River watershed from the Alberta border to Saskatoon. Citizen science reports included three historical catches of adult fish in Lake Diefenbaker in 2015 and 2017 that anglers submitted to us once the program was initiated. Juvenile fish were sampled in multiple locations outside of Stockwell Lake, indicating established and reproductively active populations. Juvenile Prussian carp were found in Lake Diefenbaker, Swift Current Creek, Coteau Creek, and the South Saskatchewan River downstream of Lake Diefenbaker, near the city of Saskatoon. Stockwell Lake is only connected to Coteau Creek during high water events. Stockwell Lake has multiple size classes of Prussian carp, so they likely moved into Stockwell from Lake Diefenbaker during provincial flooding in 2014. This, combined with the confirmed individual in 2005, suggests that Prussian carp established in Saskatchewan much earlier than 2018, and likely as early as the late 2000s [13]. Docherty et al. [13] found the invasion area doubling time in Alberta to be five years, and given the distance Prussian carp travelled through Saskatchewan (over 450 km from the Alberta border), it is possible they have been in the province for as long as 15 years.

Prussian carp in Saskatchewan were found primarily in shallow water areas. Lake Diefenbaker, a reservoir in the South Saskatchewan River, offers a lot of deep water, but Prussian carp were only found in the near-shore

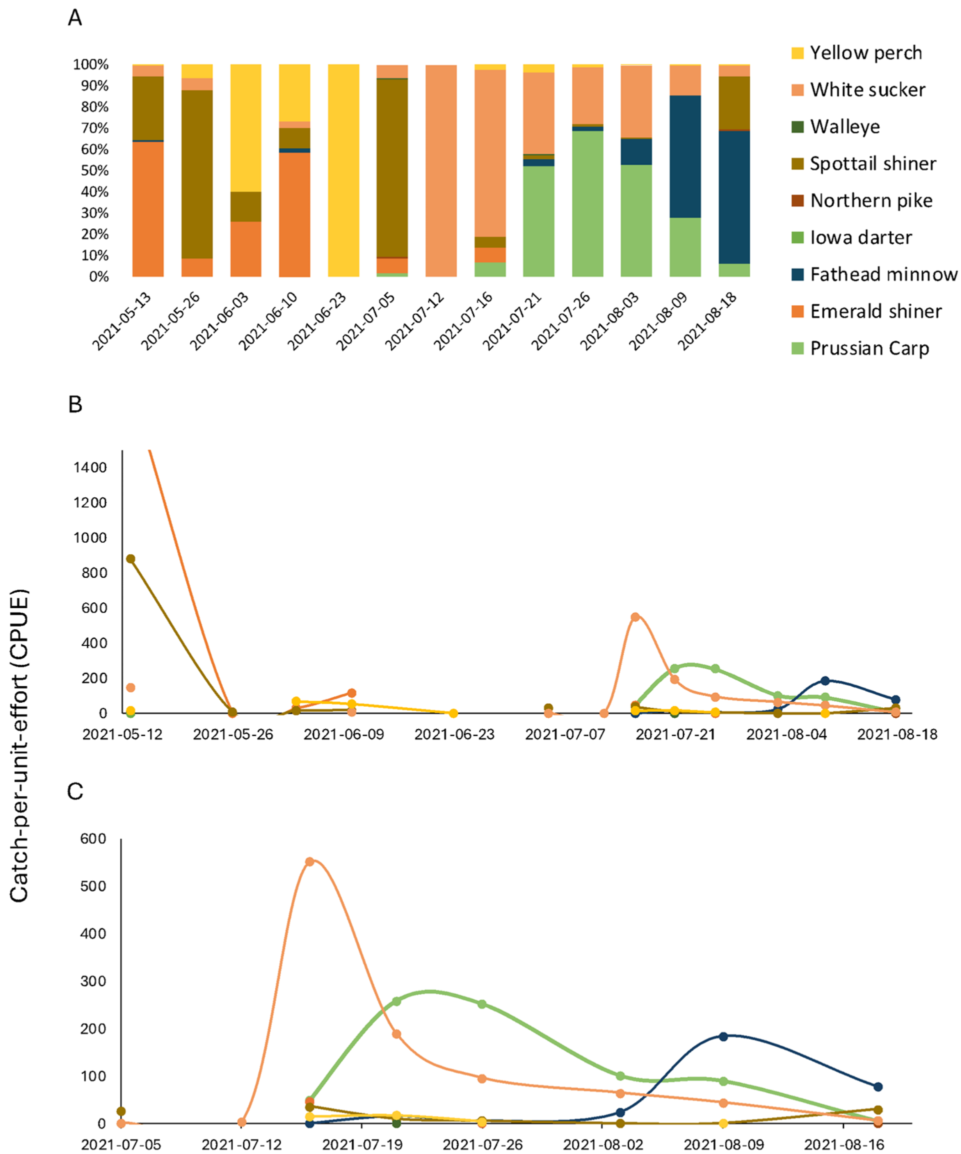

Fig. 4 Catch per unit effort (CPUE) of Prussian Carp in Saskatchewan Lakes. (**A**) Relative abundance and (**B**, **C**) CPUE for fish species sampled in Coteau Bay by beach seining. The top panel (**A**) displays relative abundance data for 9 species sampled in Coteau Bay, Lake Diefenbaker. The middle panel (**B**) displays the full time period sampled; and (**C**) displays CPUE for each species zoomed into the last half of the summer after the initial emergence of YOY Prussian carp. A reduced species list is visible in B and C because some species were not present or only present in negligible numbers not visible on this scale. First emergence of YOY Prussian carp was on July 6th

areas of coulees and associated tributaries of the reservoir. Prussian carp were also sampled in shallow eddies of the South Saskatchewan River and in two smaller tributaries. This is similar to findings in Alberta, which has established Prussian carp populations in multiple river systems, reservoirs, and creeks [12, 13, 56]. In Alberta, Prussian carp were also often found in irrigation canals [13]. There are similar irrigation canals in Saskatchewan, but they were not sampled in this study due to high water levels. Studies in Europe found that Prussian carp established in lakes, reservoirs, and rivers, which is similar to the results of this study [2, 21]. Prussian carp were not found in isolated lakes during our study, as they have been in Europe, but this difference is likely due to European anglers releasing Prussian carp into lakes for angling opportunities [7]. In North America, Prussian carp are not a desirable species and less likely to be purposefully spread by anglers.

Gardiner Dam, located between Lake Diefenbaker and the South Saskatchewan River, has a spillway that has already been breached by Prussian carp. The Qu'Appelle Dam, between Lake Diefenbaker and the Qu'Appelle River, has no spillway and an outlet that is 24 m under the surface. No Prussian carp were sampled east of this dam

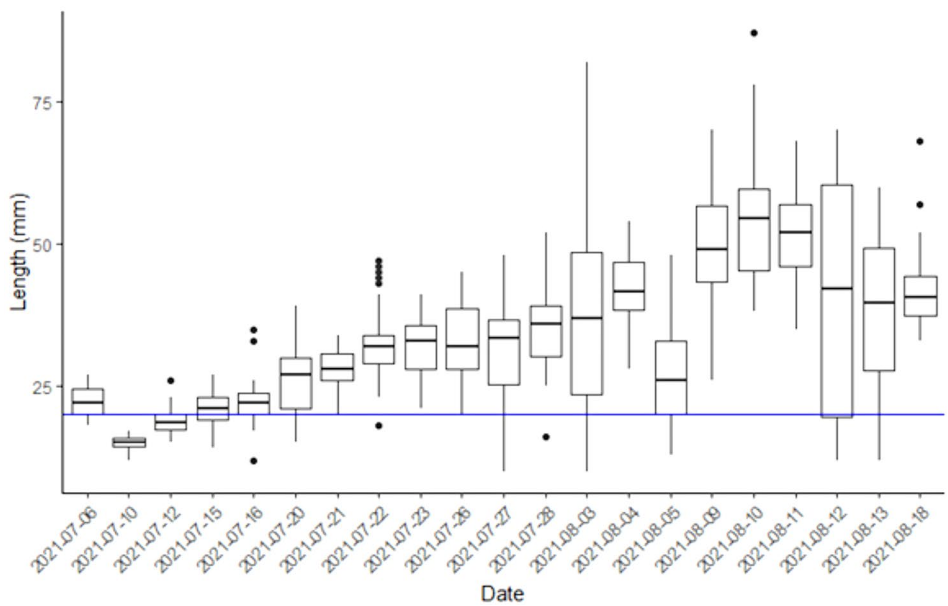

Fig. 5 Box and whisker plot of juvenile Prussian carp total length ($n = 30$) in Coteau Bay on Lake Diefenbaker, Saskatchewan. All data points under the blue line were considered relatively newly hatched.

and confirmed citizen science reports have only come from areas west of this structure. Thus, the Qu'Appelle Dam and its deep-water outflow could act as an important control structure to limit the spread of Prussian carp eastward. Dams as barriers to the spread of invasive carp have previously been studied, but are viewed as only semi-permanent, reducing movements but ultimately not preventing spread [57–59]. Common carp, silver carp, bighead carp and grass carp have all been recorded bypassing various dam structures in North America [57, 59, 60]. Additional control structures such as electric barriers, bubble screens, and CO_2 barriers have failed in preventing upstream movement of invasive carp entirely; however, these barriers have not been tested on Prussian carp in North America [61–63].Critically, the presence of a gravid female captured near the Qu'Appelle Dam in 2022 suggests that escapement is a significant concern.

Citizen science as an additional surveying approach was crucial to the success of this study; only one Prussian carp was sampled during broader-scale field netting efforts in 2019. Historical citizen science reports of Prussian carp from as early as 2015 aided in determining the invasion timeline in Saskatchewan. Citizen science reports also provided evidence of establishment throughout the entirety of Lake Diefenbaker. Additionally, citizen science reports provided the confirmation that Prussian carp were able to cross the spillway of Gardiner Dam. Some citizen reports erroneously identified common carp and some native species as Prussian carp. Collectively, all of these species are caught relatively infrequently by most anglers, which is likely what triggered these reports. However, anglers were able to correctly identify *Carassius spp.* specimens in many cases, and likely can be taught to do so reliably [64, 65]. Johnson et al. [66] studied a variety of large plant and mammal citizen science projects and found the data from 26 different programs led to 27 key discoveries. With proper education about species identification citizen science programs are an inexpensive addition to field sampling that aids in determining invasive species range [67].

Size and age

Age estimation was relatively unsuccessful in this study; our initial attempt at aging produced very low agreement between observers, and a low level of confidence in the data. Consequently, we resorted to a much more conservative consensus aging method, which likely biased ages downward (younger). However, the maximum age of 10 years we observed aligns well with other published studies (e.g., [5]), and gives us the key insight that Prussian carp were in Saskatchewan for at least 10 years prior to our sampling. Consequently, Prussian carp have been in Saskatchewan for some time but escaped detection, which is a common occurrence for new exotic species [68]. The adult female Prussian carp sampled in Saskatchewan, especially in Lake Diefenbaker, were larger than those reported for many European populations (e.g., [69–72] and more). Environmental conditions may be an important contributor to larger Prussian carp size in Saskatchewan, specifically the large river and lake system combined with cold, continental climate, may cause slower growth and larger size in cyprinids [1, 41]. Growth parameters have been estimated for locations outside of Canada affected by Prussian carp invasions, but these sites often have very moderate environmental conditions (e.g., [15, 73–75]). Future studies should address factors that affect growth and maturity of Prussian carp in Canada.

The length frequency distributions of Prussian carp in Stockwell Lake and Lake Diefenbaker were drastically different despite the fact that these waterbodies are close in proximity and intermittently connected. It is not uncommon for size distributions of fish to differ by waterbody [1, 76]; however, we did not detect any midsized Prussian carp (13–34 cm) at all in Lake Diefenbaker. This finding was somewhat surprising because mid-sized fish were by far the most common individuals in Stockwell Lake, and also in most other populations around the world (e.g., [72, 77, 78]). Several factors may have contributed to the missing mid-sized fish in Lake Diefenbaker. The first is that our sampling methods for Prussian carp in Lake Diefenbaker simply missed midsized fish. Alternatively, the limited littoral zone and unstable water levels in Lake Diefenbaker may cause intermittent availability of suitable habitat, and thereby intermittent spawning success and recruitment for Prussian carp. Finally, native predators may consume large numbers of newly invasive fish, and if sufficiently high, predation may remove whole size classes [79–81]. Interestingly, Stockwell Lake does not have any predatory fish, whereas Lake Diefenbaker has large predatory fish populations, suggesting that predation may indeed be an important factor. Changes in size distributions have been recorded for other Carassius species when piscivorous fish were present [54, 82]. We also directly observed predation of Prussian carp by native species including northern pike, piscivorous birds, and a plains garter snake (*Thamnophis sirtalis*) on Lake Diefenbaker.

Ploidy analysis

Multiple ploidy states for Prussian carp were identified in Saskatchewan, a further indication that they have likely been established for many years. Invasive populations are predominantly triploid, and a mixture of triploid and diploid females may suggest that both gonochoristic and gynogenetic reproduction are occurring [17, 93]. However, a mixture of ploidy states was also found in populations that do not use both reproductive modes [2, 17, 22, 83, 84], and tetraploid individuals are also common elsewhere [83, 85, 86]. Multiple ploidy states may arise in Prussian carp via different mechanisms, including hybridization (e.g., [31–35]), and unbalanced chromosome division during gametogenesis [83, 87, 88]. However, all of these mechanisms take time to produce individuals with different ploidy and generate mixed populations like those we documented in Saskatchewan. Given the direct connection to Alberta, it is possible that the Prussian carp population had already evolved mixed ploidy by the time they arrived in Saskatchewan. Thus, at this point we can only conclude that Saskatchewan Prussian carp have mixed ploidies that likely developed over a number of generations, but the mechanism(s) underlying this phenomenon remains unknown.

The sex ratio of Prussian carp in Saskatchewan was highly skewed towards females. All-female populations must use gynogenesis and require donor males from other species to provide sperm [89]. So far, no male Prussian carp have been detected in Lake Diefenbaker, suggesting the possibility that it is a gynogenetic population; however, a very low abundance of males cannot be ruled out based on our sampling. Male Prussian carp were present in low abundance in Stockwell Lake. Males spontaneously appeared in other invasive populations after establishment and growth, sometimes after decades of strictly females being present [90–92]. The spontaneous appearance of males introduces the possibility of gonochoristic reproduction [93, 94]. In many locations the introduction of males and the advantages of sexual reproduction are proposed to push Prussian carp into sexual reproduction and eventually diploidy [16, 95]. Recent studies suggest males are produced by a genetic sex differentiation gene found on microchromosomes, and there is a link between an increased number of microchromosomes and maleness [96]. Environmental sex determination based on rearing temperature may also produce males, even without the genetic male determining region present [97, 98]. Finding males in Stockwell Lake was a surprise because no males have been sampled elsewhere in Saskatchewan, although males have been documented in Alberta [99]. Future research should determine whether males in the Stockwell Lake are fertile and actually mating.

Spawning trends in lake diefenbaker

Prussian carp spawning in Coteau Bay was asynchronous and spanned at least one month. Eggs have a relatively short incubation period of 3 days at 20 °C and 52 h at 22–25 °C [52–54]; juvenile Prussian carp reach ~ 20 mm long in 20 days post hatch at water temperatures of 22–25 °C. Given this information, the repeated emergence of small young of the year in Coteau Bay separated by at least 3 weeks suggests asynchronous spawning events [52–54]. In this study, juveniles under 20 mm were detected on July 8th, 27th and August 13th. Considering the time elapsed and the temperatures in Coteau Bay (20.4–28 °C), it is highly likely that these young of the year came from spawning sessions that began in mid-June. Spawning spread over a long time period may offer Prussian carp greater reproductive success when variable environmental conditions affect recruitment [100]. In addition, many North American species reproduce early in the season at low temperatures [101–104], so Prussian

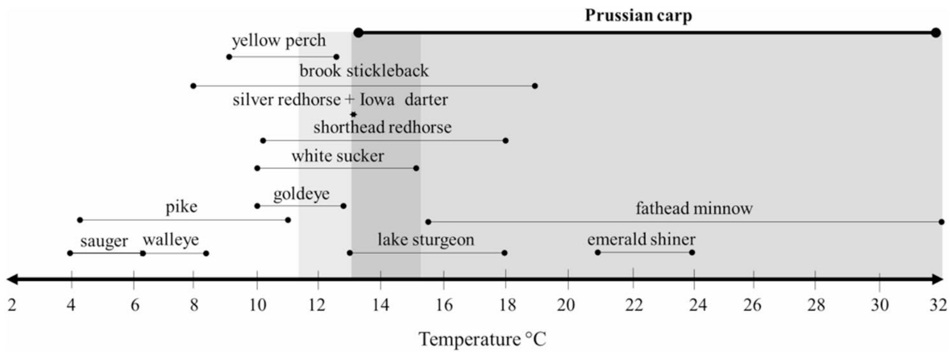

Fig. 6 Spawning temperature ranges of species sampled in Coteau Bay, Lake Diefenbaker in 2021. Shaded block (grey) indicates overlap with Prussian carp spawning temperatures and block (green) is when spawning initiates for Prussian carp based on Bondarev et al. [103]. All spawning temperatures were sourced from published studies [98–100]

carp may benefit from less competition with juveniles of other species by spawning later [100, 101]. Finally, juvenile *Carassius* species occupy warm water and grow optimally between 25 and 28 °C, which may protect them from cool-water native predators like walleye (20–23 °C optimal; 105, 106).

For Prussian carp to use gynogenesis, a native species that spawns at the same time and in the same habitats must act as the sperm donor for reproduction to occur. Donors in Eurasia are cyprinids, typically common carp, or a member of the *Carassius* genus; however, a wide range of donors has generated viable progeny [8, 86]. There are no known closely related species in the South Saskatchewan River, Lake Diefenbaker, or Stockwell Lake; common carp are present elsewhere in Saskatchewan, and goldfish are in Alberta, but neither species is present at any of our study sites. Thus, if Prussian carp are using gynogenesis in Saskatchewan it is unclear what species are serving as sperm donors. Bondarev et al. [107] found that the onset of European invasive Prussian carp spawning was between 11.5 and 15.5 °C at similar latitudes to southern Saskatchewan, and extended for a long period after beginning. Native species that spawn in Coteau Bay, Lake Diefenbaker, have a wide range of spawning temperatures and times, and only some are likely to overlap with Prussian carp (Fig. 6). Several sucker, shiner, and minnow species are prime candidates, including white sucker, those of the *Moxostoma genus* [103], and native members of the Cyprinidae, such as fathead minnow, emerald shiner and spottail shiner. Future research should address which native species could act as a potential donor and are capable of viable progeny by initiating gynogenesis.

Management implications

This study found that Prussian carp have likely been established in Saskatchewan for longer than previously thought and have likely gone undetected for several years. Prussian carp are well established throughout the South Saskatchewan River system and appear to be expanding their range. Currently, no barriers exist to prevent further expansion of Prussian carp to the north, where the South Saskatchewan River joins the North Saskatchewan River. Monitoring of the North Saskatchewan River should occur to determine if Prussian carp are already established in that system and to aid in determining the rate of spread in Saskatchewan. Investigations of the Qu'Appelle Dam on Lake Diefenbaker should be prioritized to determine its suitability as a barrier to prevent the spread of Prussian carp eastward through Saskatchewan and into Manitoba and the United States. Monitoring of the Qu'Appelle River downstream of the Qu'Appelle Dam for early detection will be crucial for managing the spread of Prussian carp across North America if the dam is not successful as a barrier. In addition, physical barriers or other means of actively deterring Prussian carp from entering the deep-water intake need to be very seriously considered. Continued education of anglers about how to accurately identify Prussian carp can be a useful and cost-effective way to increase effort and aid in the early detection of range expansion. Ultimately, Prussian carp are likely already too widespread and abundant in Saskatchewan for eradication, and management focus will need to be on reducing local impacts on native systems and reducing further spread.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40850-025-00228-0.

Supplementary Material 1

Acknowledgements

We are grateful to the Saskatchewan Ministry of Environment's Fisheries Unit for in-kind support over the course of this research. The province of Saskatchewan's Aquatic Invasive Species Council was an important source of information and communication during research activities. We thank the land owners and area mangers that gave field teams permission to access survey locations, and the many citizen scientists that submitted their observations as part of this research program.

Author contributions
CMS conceived of the research program, acquired funding, supervised all elements of the research, and wrote the manuscript. SNC conducted field data collection and analyses. JM conducted field and laboratory data and analyses and helped review and edit the manuscript. JS curated data and helped write and edit the manuscript.

Funding
This research was financially supported by the province of Saskatchewan's Fish and Wildlife Development Fund, the Natural Sciences and Engineering Research Council of Canada, the Canada Foundation for Innovation, the Saskatchewan Wildlife Federation, MITACS, and the University of Regina.

Data availability
The datasets generated and/or analyzed during the current study are available in the figshare repository, https://doi.org/10.6084/m9.figshare.27145104.

Declarations

Ethical approval
All of our methods were approved by the University of Regina President's Committee on Animal Care (Animal Care Protocol #20 – 08 "Population Ecology, Genetics, and Monitoring of Freshwater Fish"), an institutional ethics committee that ensures research activities follow the guidelines of the Canadian Council on Animal Care. Field activities were authorized under Academic Research Permit #20AR033FP issued by the Saskatchewan Ministry of Environment.

Competing interests
The authors declare no competing interests.

Received: 1 October 2024 / Accepted: 19 March 2025
Published online: 26 March 2025

References

1. Tarkan AS, Gaygusuz Ö, Gürsoy Gaygusuz Ç, Saç G, Copp GH. Circumstantial evidence of Gibel carp, *Carassius Gibelio*, reproductive competition exerted on native fish species in a mesotrophic reservoir. Fish Manag Ecol. 2012;19(2):167–77.
2. Vetemaa M, Eschbaum R, Albert A, Saat T. Distribution, sex ratio and growth of Carassius Gibelio (Bloch) in coastal and inland waters of Estonia (north-eastern Baltic Sea). J Appl Ichthyol. 2005;21(4):287–91.
3. Tapkir S, Boukal D, Kalous L, Bartoň D, Souza AT, Kolar V, et al. Invasive Gibel carp (*Carassius Gibelio*) outperforms threatened native crucian carp (*Carassius carassius*) in growth rate and effectiveness of resource use: field and experimental evidence. Aquat Conserv Mar Freshw Ecosyst. 2022;32(12):1901–12.
4. Fedorčák J, Križek P, Koščo J. Which factors influence spatio–temporal changes in the distribution of invasive and native species of genus Carassius? Aquat Invasions. 2023;18(2):219–30.
5. Kottelat M, Freyhof J. Handbook of European freshwater species. Berlin, Germany: Cornol, Switzerland; 2007.
6. Paulovits G, Ferincz Á, Staszny Á, Weiperth A, Tátrai I, Korponai J, et al. Long-term changes in the fish assemblage structure of a shallow eutrophic reservoir (Lake Hídvégi, Hungary), with special reference to the exotic *Carassius Gibelio*. Int Rev Hydrobiol. 2014;99(5):373–81.
7. Özuluğ M, Meriç N, Freyhof J. The distribution of *Carassius Gibelio* (Bloch, 1782) (Teleostei: Cyprinidae) in Thrace (Turkey). Zool Middle East. 2004;31(1):63–6.
8. Paschos I, Nathanailides C, Tsoumani M, Perdikaris C, Gouva E, Leonardos I. Intra and inter-specific mating options for gynogenetic reproduction of Carassius Gibelio (Bloch, 1783) in lake Pamvotis (NW Greece). Belg J Zool. 2004;134:55–60.
9. Van Der Veer G, Nentwig W. Environmental and economic impact assessment of alien and invasive fish species in Europe using the generic impact scoring system. Ecol Freshw Fish. 2015;24(4):646–56.
10. Leonardos ID, Tsikliras AC, Eleftheriou V, Cladas Y, Kagalou I, Chortatou R, et al. Life history characteristics of an invasive cyprinid fish (Carassius gibelio) in chimaditis lake (northern Greece). J Appl Ichthyol. 2008;24(2):213–7.
11. Zenetos A, Pancucci-Papadopoulou MA, Zogaris S, Papastergiadou E, Vardakas L, Aligizaki K, et al. Aquatic alien species in Greece: tracking sources, patterns and effects on the ecosystem. J Biol Res Thessal. 2009;12:135–72.
12. Elgin E, Tunna H, Jackson L. First confirmed records of Prussian carp, Carassius Gibelio (Bloch, 1782) in open waters of North America. BioInvasions Rec. 2014;3(4):275–82.
13. Docherty C, Ruppert J, Rudolfsen T, Hamann A, Poesch M. Assessing the spread and potential impact of Prussian carp Carassius Gibelio (Bloch, 1782) to freshwater fishes in Western North America. BioInvasions Rec. 2017;6(3):291–6.
14. Leonardos I, Kokkinidou A, Katharios P, Harisis C. Age growth and mortality of gibel carp (C. gibelio, Bloch, 1873) in Lake Lysimachia (W. Greece). In: Proceedings of the 9th pan-hellenic conference of icthyologists. Mesolongi. 2001. p. 137–40.
15. Leonardos ID, Kagalou I, Tsoumani M, Economidis PS. Fish fauna in a protected Greek lake: biodiversity, introduced fish species over a 80-year period and their impacts on the ecosystem. Ecol Freshw Fish. 2008;17(1):165–73.
16. Fan Z, Liu G. The ploidy and reproductive mechanism of crucian carp, *Carassius auratus Gibelio*. J Fish Biol. 1990;36(3):415–9.
17. Fan Z, Shen J. Studies on the evolution of bisexual reproduction in crucian carp (Carassius auratus Gibelio Bloch). Aquaculture. 1990;84(3–4):235–44.
18. Gui JF, Liang SC, Jiang YG. Discovery of two different reproductive development modes of the eggs of artificial multiple tetraploid allogynogenetic silver crucian carp. Chin Sci Bull. 1993;28:332–7.
19. Gui JF. An unique study system: gynogenetic fish C. auratus Gibelio. Sci Found China. 1996;4(1):44–6.
20. Gui JF. Retrospects and prospects of studies on the mechanism of natural gynogenesis in silver crucian carp (C. auratus gibellio). Bull Natl Sci Found China. 1997;11:11–6.
21. Leonardos I, Paschos I, Prassa M. Threatened species of the world: P. epiroticus (Steindachner, 1895) (Cyprinidae). Environ Biol Fishes. 2007;72:250.
22. Liasko R, Liousia V, Vrazeli P, Papiggioti O, Chortatou R, Abatzopoulos TJ, et al. Biological traits of rare males in the population of *Carassius Gibelio* (Actinopterygii: Cyprinidae) from lake Pamvotis (north-west Greece). J Fish Biol. 2010;77(3):570–84.
23. Yu H. A cytological observation on gynogenesis of crucian carp (C. auratus gibelio). Acta Hydrobiol Sin. 1982;6(4):481–7.
24. Jiang YG, Yu HX, Chen BD, Liang SC, Shan SX, Yang DL, et al. Biological effect of heterologous sperm on gynogenetic offspring in C. auratus Gibelio. Acta Hydrobiol. 1983;8:1–13.
25. Lusková V, Lusk S, Halačka K, Vetešník L. Carassius auratus gibelio—The most successful invasive fish in waters of the Czech Republic. Russ J Biol Invasions. 2010;1(3):176–80.
26. Saşı h. The length and weight relations of some reproduction characteristics of Prussian carp, Carassius Gibelio (Bloch, 1782) in the South Aegean region (Aydın-Turkey). Turk J Fish Aquat Sci. 2008;8:87–92.
27. Lushchak VI, Lushchak LP, Mota AA, Hermes-Lima M. Oxidative stress and antioxidant defenses in goldfish *Carassius auratus* during anoxia and reoxygenation. Am J Physiol-Regul Integr Comp Physiol. 2001;280(1):R100–7.
28. Holopainen IJ, Tonn WM, Paskowski CA. Tales of two fish: the dichotomous biology of crucian carp [C. Carassius (L.)] in Northern Europe. Ann Zool Fenn. 1997;34:1–22.
29. Nathanailides C, Perdikaris C, Gouva E, Paschos I. Gibel carp (C. auratus gibelio) growth under increased ammonia concentration. In: Proceedings of the 11th panhellenic conference of icthyologists. Chania. 2003.
30. Graham CF, Eberts RL, Goncin U, Somers CM. Spontaneous hybridization and introgression between Walleye (*Sander vitreus*) and Sauger (*Sander canadensis*) in two large reservoirs: insights from genotyping by sequencing. Evol Appl. 2021;14(4):965–82.
31. Yi MS, Li YQ, Liu JD, Zhou L, Yu QX, Gui JF. Molecular cytogenetic detection of paternal chromosome fragments in allogynogenetic Gibel carp, Carassius auratus Gibelio Bloch. Chromosome Res. 2003;11(7):665–71.
32. Zhao J, Liu LG, Chen XL, Qing N, Dong CW. Karyotypic analysis of the multiple tetraploid allogynogenetic Pengze crucian carp and its parents. Aquaculture. 2004;237(1–4):117–29.
33. Tóth B, Várkonyi E, Hidas A, Edviné Meleg E, Váradi L. Genetic analysis of offspring from intra- and interspecific crosses of *Carassius auratus Gibelio* by chromosome and RAPD analysis. J Fish Biol. 2005;66(3):784–97.
34. Neaves WB, Baumann P. Unisexual reproduction among vertebrates. Trends Genet. 2011;27(3):81–8.

35. Mezhzherin SV, Kokodiy SV. Genetic structure of silver Prussian carp Carassius (Superspecies auratus) (Linnaeus, 1758) colonies from the middle Dnieper basin. Russ J Genet. 2010;46(6):725–31.
36. Halas D, Lovejoy N, Mandrak N. Undetected diversity of goldfish (Carassius spp.) in North America. Aquat Invasions. 2018;13(2):211–9.
37. Government of Saskatchewan. Saskatchewan. 2025. Don't Let it Loose - Aquatic Invasive Species.
38. Liu C, Chen Y, Olden JD, He D, Sui X, Ding C. Phenotypic shifts in life history traits influence invasion success of goldfish in the Yarlung Tsangpo river, Tibet. Trans Am Fish Soc. 2015;144(3):602–9.
39. Liu CL, Chen YF, He DK, Fletcher D, Britton JR. Otolith microstructure reveals consequences for juvenile growth of fractional spawning in an invasive goldfish *Carassius auratus* (Linnaeus, 1758) population. J Appl Ichthyol. 2015;31(6):1029–33.
40. Stevenson DK, Campana SE, editors. Otolith microstructure examination and analysis. Dep Fish Oceans; 1992.
41. Vilizzi L, Ekmekçi FG, Tarkan AS, Jackson ZJ. Growth of common carp *Cyprinus Carpio* in Anatolia (Turkey), with a comparison to native and invasive areas worldwide. Ecol Freshw Fish. 2015;24(2):165–80.
42. Zale AV, Parrish DL, Sutton TM, American, Fisheries, Society, editors. Fisheries techniques. 3rd ed. American Fisheries Society; 2012.
43. Howell WM, Black DA. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia. 1980;36(8):1014–5.
44. Félip A, Carillo M, Herráez MP, Zannuy S, Basurco B, Protocol. J - Silver staining of NOR regions [Pratical guide of protocols: methods of verification of the ploidy]. CIHEAM CSIC-IATS Options Méditerranéennes. Sér B Etudes Rech. 2009;63:67–70.
45. Kim HS, Chung KH, Son JH. Comparison of different ploidy detection methods in Oncorhynchus Mykiss, the rainbow trout. Fish Aquat Sci. 2017;20(1):29.
46. Phillips RB, Pleyte KA, Ihssen PE. Patterns of chromosomal nucleolar organizer region (NOR) variation in fishes of the genus Salvelinus. Copeia. 1989;1989(1):47.
47. Ohno S, Muramoto J, Christian L, Atkin NB. Diploid-tetraploid relationship among old-world members of the fish family Cyprinidae. Chromosoma. 1967;23(1):1–9.
48. Li XY, Zhang XJ, Li Z, Hong W, Liu W, Zhang J, et al. Evolutionary history of two divergent Dmrt1 genes reveals two rounds of polyploidy origins in Gibel carp. Mol Phylogenet Evol. 2014;78:96–104.
49. Boroń A. Karyotypes of diploid and triploid silver crucian carp Carassius auratus Gibelio (Bloch). Cytobios. 1994;80:117–24.
50. Mayr B, Bab P, Kalat M. NORs and counterstain-enhanced fluorescence studies in Cyprinidae of different ploidy level. Genetica. 1986;69(2):111–8.
51. Kalous L, Knytl M. Karyotype diversity of the offspring resulting from reproduction experiment between diploid male and triploid female of silver Prussian carp, *Carassius Gibelio*. Folia Zool. 2011;60(2):115–21. Cyprinidae, Actinopterygii.
52. Zhenqi L, Junjie Y, Hualong X, Miao A, Shihong H. Growth and development of larvae and juveniles of Carassius auratus Gibelio. Anim Husb Feed Sci. 2014;6(3):113–7.
53. Laurila S, Holopainen IJ. Features of embryonic and larval development of crucian carp, Carassius Carassius (L.) with a note on species identification. Ann Zool Fenn. 1990;27(4):361–7.
54. Laurila S, Piironen J, Holopainen IJ. Notes on egg development and larval and juvenile growth of crucian carp (Carassius Carassius (L)). Ann Zool Fenn. 1987;24(4):315–21.
55. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [Internet]. 2021. Available from: https://www.R-project.org/
56. Ruppert JLW, Docherty C, Neufeld K, Hamilton K, MacPherson L, Poesch MS. Native freshwater species get out of the way: Prussian carp (*Carassius gibelio*) impacts both fish and benthic invertebrate communities in North America. R Soc Open Sci. 2017;4(10):170400.
57. Sullivan CJ, Weber MJ, Pierce CL, Camacho CA. A comparison of grass carp population characteristics upstream and downstream of lock and dam 19 of the upper Mississippi river. J Fish Wildl Manag. 2020;11(1):99–111.
58. Theiling CH, Nestler JM. River stage response to alteration of upper Mississippi river channels, floodplains, and watersheds. Hydrobiologia. 2010;640(1):17–47.
59. Larson JH, Knights BC, McCalla SG, Monroe E, Tuttle-Lau M, Chapman DC, et al. Evidence of Asian carp spawning upstream of a key choke point in the Mississippi river. North Am J Fish Manag. 2017;37(4):903–19.
60. Butler SE, Wahl DH. Common carp distribution, movements, and habitat use in a river impounded by multiple Low-Head dams. Trans Am Fish Soc. 2010;139(4):1121–35.
61. Cupp AR, Erickson RA, Fredricks KT, Swyers NM, Hatton TW, Amberg JJ. Responses of invasive silver and Bighead carp to a carbon dioxide barrier in outdoor ponds. Can J Fish Aquat Sci. 2017;74(3):297–305.
62. Zielinski DP, Sorensen PW. Field test of a bubble curtain deterrent system for common carp. Fish Manag Ecol. 2015;22(2):181–4.
63. Dettmers DG, Boisvert BA, Barkley T, Sparks RE. Potential impact of steel-hulled barges on movement of fish across an electric barrier to prevent the entry of invasive carp into Lake Michigan. Illinois Natural History Survery Technical Reports. 2005.
64. Delaney DG, Sperling CD, Adams CS, Leung B. Marine invasive species: validation of citizen science and implications for National monitoring networks. Biol Invasions. 2008;10(1):117–28.
65. Crall AW, Jarnevich CS, Young NE, Panke BJ, Renz M, Stohlgren TJ. Citizen science contributes to our knowledge of invasive plant species distributions. Biol Invasions. 2015;17(8):2415–27.
66. Johnson BA, Mader AD, Dasgupta R, Kumar P. Citizen science and invasive alien species: an analysis of citizen science initiatives using information and communications technology (ICT) to collect invasive alien species observations. Glob Ecol Conserv. 2020;21:e00812.
67. Lehtiniemi M, Outinen O, Puntila-Dodd R. Citizen science provides added value in the monitoring for coastal non-indigenous species. J Environ Manage. 2020;267:110608.
68. Goldberg CS, Sepulveda A, Ray A, Baumgardt J, Waits LP. Environmental DNA as a new method for early detection of new Zealand mudsnails (*Potamopyrgus antipodarum*). Freshw Sci. 2013;32(3):792–800.
69. Specziár A, Tölg L, Bíró P. Feeding strategy and growth of cyprinids in the Littoral zone of lake Balaton. J Fish Biol. 1997;51(6):1109–24.
70. Lujic J, Kostic D, Bjelic-Cabrilo O, Popovic E, Miljanovic B, Marinovic Z et al. ichthyofauna Composition and Population parameters of Fish Species from the Special Nature Reserve Koviljsko-Petrovaradinski Rit (Vojvodina, Serbia). Turk J Fish Aquat Sci [Internet]. 2013 [cited 2024 May 2];13(4). Available from: http://dergipark.gov.trhttps://doi.org/10.4194/1303-2712-v13_4_12
71. Marinović Z, Lujić J, Bolić-Trivunović V, Marković G. Comparative study of growth in Carassius Gibelio (Bloch, 1782) and rutilus rutilus (L., 1758) from two Serbian reservoirs: Multi-model analysis and inferences. Fish Res. 2016;173:11–9.
72. Hjiradkouchak E, Patimar R, Harsij M, Ghorbani R. Study on growth properties of Carassius Gibelio (Bloch, 1782) in Golestan and Voshmgir dam lake of Golestan Province- Northern Iran. J Appl Ichthyol Res. 2021;9(4):11–20.
73. Sari HM, Balik S, Ustaoğlu MR, İlhan A. Population structure, growth and mortality of Carassius Gibelio (Bloch, 1782) in Buldan dam lake. Turk J Fish Aquat Sci. 2008;8(1):25–9.
74. Abood AN, Mohamed ARM. Population dynamics of an invasive species Carassius auratus in the Shatt Al-Arab river, Iraq. Arch Agric Environ Sci. 2020;5(3):384–9.
75. Fazli H, Daryanabard GR, Janbaz A, Mirzaei R, Hosseinpour H, Vahedi N. Some biological characteristics of Carassius Gibelio (Bloch, 1782) (Teleostei: Cyprinidae) in the Azad dam lake in Western Iran. Iran Soc Ichthyol. 2018;5(3):243–9.
76. Tsoumani M, Liasko R, Moutsaki P, Kagalou I, Leonardos I. Length-weight relationships of an invasive cyprinid fish (Carassius gibelio) from 12 Greek lakes in relation to their trophic States. J Appl Ichthyol. 2006;22(4):281–4.
77. Erdogan Z, Torcu Koc H, Gungor S, Ulunehir G. Age, growth and reproductive properties of an invasive species Carassius Gibelio (Bloch, 1782) (Cyprinidae) in the Ikizcetepeler dam lake (Balikesir), Turkey. Period Biol. 2014;116(3):285–91.
78. Erguden SA. Age and growth properties of Prussian carp, Carassius Gibelio (Bloch, 1782) living in the middle basin of Seyhan river in Adana, Turkey. Pak J Zool. 2015;47(5):1365–71.
79. Somers CM, Lozer MN, Kjoss VA, Quinn JS. The invasive round goby (Neogobius melanostomus) in the diet of nestling Double-crested cormorants (Phalacrocorax auritus) in Hamilton harbour, lake Ontario. J Gt Lakes Res. 2003;29(3):392–9.
80. Reyjol Y, Brodeur P, Mailhot Y, Mingelbier M, Dumont P. Do native predators feed on non-native prey? The case of round goby in a fluvial piscivorous fish assemblage. J Gt Lakes Res. 2010;36(4):618–24.
81. Dietrich JP, Morrison BJ, Hoyle JA. Alternative ecological pathways in the Eastern lake Ontario food Web—Round goby in the diet of lake trout. J Gt Lakes Res. 2006;32(2):395–400.

82. Brönmark C, Paszkowski CA, Tonn WM, Hargeby A. Predation as a determinant of size structure in populations of crucian carp (*Carassius carassius*) and Tench (*Tinea tinea*). Ecol Freshw Fish. 1995;4(2):85–92.
83. Flajšhans M, Rodina M, Halačka K, Vetešník L, Gela D, Lusková V, et al. Characteristics of sperm of polyploid Prussian carp *Carassius Gibelio*. J Fish Biol. 2008;73(1):323–8.
84. Xiao J, Zou T, Chen Y, Chen L, Liu S, Tao M, et al. Coexistence of diploid, triploid and tetraploid crucian carp (Carassius auratus) in natural waters. BMC Genet. 2011;12(1):20.
85. Knytl M, Kalous L, Rab P. Karyotype and chromosome banding of endangered crucian carp, Carassius Carassius (Linnaeus, 1758) (Teleostei, Cyprinidae). Comp Cytogenet. 2013;7(3):205–13.
86. Deinhardt M. The invasive potential of Prussian carp in Finland under the light of a novel semi-clonal reproductive mechanism [Internet]. 2013. Available from: https://jyx.jyu.fi/handle/123456789/42466
87. Abramenko MI, Nadtoka EV, Makhotkin MA, Kravchenko OV, Poltavtseva TG. Distribution and cytogenetic features of triploid male goldfish from Azov basin. Ontogenez. 2004;35(5):375–86.
88. Murayama Y, Hijikata M, Kojima K, Nakakuki M, Noda M, Kajishima T. The appearance of diploid-triploid and diploid-triploid-tetraploid mosaic individuals in polyploid fish, Ginbuna (Carassius auratus langsdorfii). Experientia. 1986;42(2):187–8.
89. Cherfas NB. Natural triploidy in females of the unisexual form of silver crucian carp (Carassius auratus Gibelio Bloch). Genetika. 1966;5:16–24.
90. Slavík O, Bartoš L. What are the reasons for the Prussian carp expansion in the upper Elbe river. Czech Republic? J Fish Biol. 2004;65(s1):240–53.
91. Halacka K, Luskova V, Lusk S. Carassius Gibelio in fish communities of the Czech Republic. Ecohydrol Hydrobiol. 2003;3(1):133–8.
92. Jiang FF, Wang ZW, Zhou L, Jiang L, Zhang XJ, Apalikova OV, et al. High male incidence and evolutionary implications of triploid form in Northeast Asia Carassius auratus complex. Mol Phylogenet Evol. 2013;66(1):350–9.
93. Gui J, Zhou L. Genetic basis and breeding application of clonal diversity and dual reproduction modes in polyploid Carassius auratus Gibelio. Sci China Life Sci. 2010;53(4):409–15.
94. Zhang J, Sun M, Zhou L, Li Z, Liu Z, Li XY, et al. Meiosis completion and various sperm responses lead to unisexual and sexual reproduction modes in one clone of polyploid Carassius Gibelio. Sci Rep. 2015;5(1):10898.
95. Liasko R, Koulish A, Pogrebniak A, Papiggioti O, Taranenko L, Leonardos I. Influence of environmental parameters on growth pattern and population structure of Carassius auratus Gibelio in Eastern Ukraine. Hydrobiologia. 2011;658(1):317–28.
96. Li XY, Zhang QY, Zhang J, Zhou L, Li Z, Zhang XJ, et al. Extra microchromosomes play male determination role in polyploid Gibel carp. Genetics. 2016;203(3):1415–24.
97. Li XY, Gui JF. Diverse and variable sex determination mechanisms in vertebrates. Sci China Life Sci. 2018;61(12):1503–14.
98. Li XY, Liu XL, Zhu YJ, Zhang J, Ding M, Wang MT, et al. Origin and transition of sex determination mechanisms in a gynogenetic hexaploid fish. Heredity. 2018;121(1):64–74.
99. Kucher H, Stock TM, Das MK. Parasites of Prussian carp (*Carassius gibelio*): an invasive species in Alberta, Canada. Can J Zool. 2019;97(9):849–53.
100. Rinchard J, Kestemont P. Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. I. Morphological and histological features. J Fish Biol. 1996;49(5):883–94.
101. Koenst WM, Smith LL Jr. Thermal requirements of the early life history stages of Walleye, *Stizostedion vitreum vitreum*, and Sauger, *Stizostedion Canadense*. J Fish Res Board Can. 1976;33(5):1130–8.
102. Scott WB, Crossman EJ. Freshwater fishes of Canada. Fisheries Research Board of Canada; 1973.
103. Warren ML Jr, Burr BM, editors. Freshwater fishes of North America. John Hopkins University; 2014.
104. Stewart DK, Watkinson D. Freshwater fishes of Manitoba. University of Manitoba; 2004.
105. Coutant CC. Compilation of temperature preference data. J Fish Res Board Can. 1977;34(5):739–45.
106. Christie GC, Regier HA. Measures of optimal thermal habitat and their relationship to yields for four commercial fish species. Can J Fish Aquat Sci. 1988;45(2):301–14.
107. Bondarev DL, Kunah OM, Fedushko MP, Gubanova NL. The impact of Temporal patterns of temperature and precipitation on silver Prussian carp (Carassius gibelio) spawning events. Biosyst Divers. 2019;27(2):106–17.

Publisher's note