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Parasites of Prussian Carp (*Carassius gibelio*): An Invasive Species in Alberta, Canada.

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Abstract:

A cyprinid, the Prussian carp (*Carassius gibelio* Bloch 1782) has become an established invasive fish in several watersheds in Alberta, Canada. Originating in Eurasia, Prussian carp negatively impact native fish through predation, competition, and habitat modification. Prior to this study, nothing was known regarding the external and internal parasites of these fish in North America, although nine studies had been conducted in Europe and Asia. We examined 22 fish (20 female; 2 male) ranging from three to five years of age and recorded morphological features, stomach contents and performed standard necropsy examinations for parasites. One parasite species, the acanthocephalan *Pomphorhynchus bulbocolli* Linkins in Van Cleave 1919, was found in three fish (14%), with a mean intensity of 1.0. This is a new host record and the first report of any acanthocephalan parasite in Prussian carp. We conclude that Prussian carp are not introducing any novel parasites into native fish and that they are resistant to most infections by native parasites.

Key words: Prussian carp, *Carassius gibelio*, invasive species, *Pomphorhynchus bulbocolli*, acanthocephalan.

Introduction:

In 2006, a species of cyprinid fish, the Prussian carp (*Carassius gibelio* Bloch 1782) was discovered and genetically confirmed in several southern watersheds in Alberta, Canada (Docherty et al. 2017). Prussian carp are native to central Europe and Siberia but have spread throughout Europe, Asia, the Middle East, and recently, into limited areas of North America (Elgin et al. 2014). Their method of introduction into North America has yet to be determined. In Alberta, breeding populations of Prussian carp occur in the South Saskatchewan, Red Deer, Bow, and Rosebud rivers and their tributaries (Fish AEP – Environment and Parks 2018). In addition, fish survive in dugouts, storm water ponds, and drainage ditches.

Prussian carp are benthopelagic, non-migratory fish, tolerant of poor quality, low-oxygen water (Erdogan et al. 2014). They survive over winter, even if air temperatures fall below -30 C. Females mature at 1-2 years of age, can spawn up to 3 times *per* year and can reproduce using gynogenesis (fertile female offspring produced without sperm) (Erdogan et al. 2014; Docherty et al. 2017). As a result, female fish often greatly outnumber males in any population. Prussian carp are omnivores and have a generalist diet that includes detritus, invertebrates, and plant matter. A consequence of these biological characteristics is that Prussian carp easily colonize and invade new habitats.

When introduced into a new watershed, Prussian carp can have a devastating effect on native fish. By rapidly expanding their populations, Prussian carp outcompete native species for resources, consume aquatic invertebrates, and interfere with native fish spawning (Ruppert et al. 2017). Gynogenesis allows them to use the sperm of other cyprinids to stimulate and initiate egg development. By consuming detritus and macrophytes during foraging, Prussian carp can disrupt sediments, increase water turbidity and physically alter aquatic habitats (Docherty et al. 2017).

Eradication of Prussian carp in introduced areas is difficult and expensive. Currently, manual methods to eliminate fish (electrofishing, netting, and angling) are used in Alberta. One environmental factor that could be important in affecting Prussian carp populations, or in contributing to their effects on native fish, is an analysis of the parasites and pathogens that infect them in Eurasia and in North America. No published studies of the parasites of Prussian carp in North America have been conducted, although nine studies have looked at parasites of Prussian carp in Eurasia. A parasite survey study of Prussian carp in North America is important and informative because: (1) Prussian carp may host old world parasites that they could introduce into North America; (2) invasive Prussian carp may get severely infected with parasites of native fish, which could help to slow their population growth but also allow them to be used as a reservoir host to enhance parasite populations; or (3) Prussian carp in North America may have few or no parasites because they are a novel host and native parasites have not yet adapted to take advantage of them.

Materials and Methods:

A sample of 22 Prussian carp was collected by Alberta Environment and Parks by netting and angling from 2 locations in southern Alberta. In May, 2017, 15 fish were collected from the Blindman River (52°53'13"N, 114°34'39"W), a tributary of the Red Deer River and on August 18, 2018, 7 fish were collected from the Blood Indian Reservoir (51°15'0"N, 111°12'30"W). After collection, fish were frozen until examination at MacEwan University.

After thawing, mass (*g*), total length (*cm*), sex, and reproductive condition were recorded for each fish. A scale was removed from the mid-lateral section, dorsal to the lateral line of each fish for aging. A standard necropsy, including a thorough visual examination of all external surfaces including the fins, body, eyes, and buccal cavity for animal parasites, deformities, or evidence of protozoan or bacterial infections was conducted. Gills were removed and placed into individual dishes, then each was rinsed with tap water, combed with forceps and dissecting needles, and examined using a stereomicroscope.

Each fish was opened on the ventral surface from anus to pectoral fins and the gonads, swim bladder, and digestive organs were removed and placed into separate dishes of tap water. Intestines were separated into three parts (anterior third, middle third, and posterior third) and flushed with tap water into individual petri dishes. Then each intestinal section was opened, and mucosal surfaces were scraped with a scalpel. All washings and scrapings were examined using a stereomicroscope. Stomachs were opened, and contents were noted. Other organs (*e.g.* livers, kidneys, muscles) were superficially examined. Parasites found were preserved in individual vials of AFA (70% ethyl alcohol – formalin – acetic acid) (Pritchard and Kruse 1982) and helminths were later stained using Semichon's acetocarmine and permanently mounted on microscope slides using Permout[®] medium. Voucher specimens are available for loan in the parasite collection of MacEwan University, Edmonton, Alberta.

Results:**Characteristics of fish:**

In total, 22 Prussian carp were necropsied: 15 from the Blindman River (15 female; 0 male) and 7 from the Blood Indian Reservoir (5 female; 2 male) (Table 1). Fish varied in age from 3 years to 5 years old. Fish that were 3 years old averaged 28.3 cm long (SD 7.1 cm) and weighed 578 g (SD 532 g); age 4 were 29.2 cm long (SD 3.9 cm) weight 497 g (SD 274 g); age 5 were 31.2 cm long (SD 3.5 cm), weight 659 g (SD 204 g).

Diet:

Insect parts were found in 55% of carp stomachs (Table 1). One stomach (5%) contained amphipods belonging to the genus *Hyalolella* Smith 1874. Five stomachs (23%) contained algae and 3 (14%) had macrophyte tissue; 27% of stomachs were empty. About half (55%) of stomachs had invertebrate remains and 32% contained plant/algae material.

Parasites:

The intestines of 3 fish (14%) were infected by one species of parasite, identified using morphological characteristics of the proboscis and spines, as noted in Amin et al. 2003, as the acanthocephalan *Pomphorhynchus bulbocolli* Linkins in Van Cleave, 1919. (Table 1). Each infected fish had only a single worm (intensity of infection 1.0); two fish each had a single female worm and one male worm occurred in another. Infected fish came from both sample sites. No other internal or external parasites were found. Prussian carp examined in Eurasia are infected by a much greater diversity of parasites (Table 2).

Discussion:

Prussian carp are now well-established and successfully breeding in watersheds of central and southern Alberta. Reproduction by gynogenesis results in unbalanced sex ratios (as seen in our study), heavily dominated by females. The fish we examined were sexually mature and females often were gravid. Body lengths of all three age classes of fish we examined (age 3, 4, and 5) were about 2 cm larger than similar age classes of Prussian carp in Turkey (Erdogan et al. 2014), suggesting that Prussian carp in Alberta are not only surviving, but are thriving. Stomach contents confirmed that they have an omnivorous diet, made up of about 50% animal (arthropods) and 50% plant/algae food. These factors suggest that the potential for Prussian carp to cause ecological disruptions in Alberta, is great. Could metazoan parasites play any role in their establishment?

The only parasite found in Prussian carp from Alberta was the acanthocephalan, *Pomphorynchus bulbocolli*. This is a new host record and the first report of any acanthocephalan infecting Prussian carp. Thorny-headed worms mainly inhabit the intestines of fish, but amphibians, reptiles (rarely), birds, and mammals can also act as hosts (Roberts et al. 2013). The commonest definitive hosts of *P. bulbocolli* are white suckers, *Catostomus commersonii* Lacepede 1803 (Jensen 1973). Fish get infected by eating the intermediate host of the parasites, the amphipod *Hyalella azteca* Saussure 1858 (Jensen 1973). If *P. bulbocolli* are like other acanthocephalans, they probably induce altered behaviour in gammarids to increase their probability of being eaten by potential definitive hosts in benthopelagic habitats (Keymer and Read 1991).

Adult acanthocephalans attach to the intestines of their fish hosts, using a protrusible proboscis that is armed with recurved, sclerotized spines. In addition, this species has an inflated bulb near the distal end of its proboscis that is embedded into the mucosa. Within the intestine, worms may attach, move, and re-attach several times, so one acanthocephalan may cause several wounds. In some cases,

P. bulbocolli causes gross pathology by perforating the intestine (Heller et al. 2016). As a result, in large infections, acanthocephalans like *P. bulbocolli* often cause mortality. The small infections we found in Prussian carp are insufficient to cause morbidity or mortality. In addition, although the worms we found were fully developed adults with testes and ovaries, neither female was gravid. We do not know if this is because the infections found included only one sex, thus precluding fertilization, or if the worms are incapable of maturing in Prussian carp.

The three watersheds in Alberta where Prussian carp are found currently, the Red Deer, South Saskatchewan and Bow River systems, include about 22 species of common fish that are likely sympatric (Nelson and Paetz 1999; Joynt and Sullivan 2003). Of these, seven (32%) are cyprinids. Cyprinids in Alberta are infected by a variety of parasites, many of which are host generalists – for instance, the nematode, *Rhabdocona canadensis* Moravec and Aria 1971 ; adult trematodes including allocreadids and several species that, as metacercariae, cause blackspot disease (neascus); monogeneans including *Dactylogyrus* spp. Diesing 1850 and *Gyrodactylus* spp. von Nordmann 1832 (but especially *G. eos* Mayes 1977); and copepods in the genus *Ergasilus* Nordmann 1832 (Leong and Holmes 1981; Baldwin 2000; Marcogliese et al. 2001; Mee and Rowe 2006; Bakke et al. 2012; Baldwin and Goater 2013). Consequently, before this survey, we anticipated that Prussian carp would be infected with several of these parasites.

In an analysis of many examples where wild fish had been intentionally relocated to new sites in Russia and in North America, Dogiel (1964) found that fish had a complete or partial loss of the parasite fauna from their original habitat. In their new location, translocated fish acquired some new parasites, not previously known to infect them. These parasites were often generalists, characterized by wide host specificity. For example, *Coregonus lavaretus* L., when translocated between two lakes in the Urals, lost nine of 14 species of parasites, but eventually acquired two new species – both acanthocephalans. One

was a close relative of *P. bulbocolli* – *Pomphorhynchus laevis* Muller. Similarly, when Prussian carp were moved from the Amur in the far east to the European part of the U.S.S.R., fish went from having 19 parasite species to two, but acquired three new species – two protozoans and one monogenean fluke. Dogiel (1964) pointed out that these new parasites had direct lifecycles, not involving any intermediate hosts.

Our study supports most of Dogiel's (1964) observations. In Eurasia, Prussian carp are infected by 44 species of parasites (Table 2) – we found one. The parasite that carp have acquired in Alberta does not have a direct lifecycle but does have a wide range of hosts. Margolis and Authur (1979) reported *P. bulbocolli* from 37 different species of fish, including five (13.5%) cyprinids. We were surprised by the lack of any monogenean or copepod parasites on the gills or skins of carp, because these parasites are extremely common on cyprinids in Alberta and have direct lifecycles, however many are species specific.

The common pattern seen in introductions of exotic cyprinids, particularly goldfish (*Carassius auratus* L.), grass carp (*Ctenopharyngodon idella* Cuvier and Valenciennes, 1844), and big head carp (*Aristichthys nobilis* Richardson) is that they lose their parasites (perhaps due to stochastic events, or a lack of needed abiotic or biotic factors such as proper intermediate hosts) (Tompkins and Poulin 2006). The likeliest parasites to be transmitted from native fish to invaders, or from invaders to natives, are those with direct lifecycles, that are transmitted horizontally. For example, in New Zealand monogenean flukes (*Dactylogyrus* and *Gyrodactylus*) from grass carp infected native fish (Tomkins and Poulin 2006). The acquisition of native parasites to invaders appears to be a very slow process – Kennedy and Pojmanska (1996) found that parasites of common carp (*Cyprinus carpio* L.) introduced into Hungary in medieval times, were still species poor and no different than the impoverished parasite faunas of three newly introduced carp species (grass carp, big head carp, and silver carp, *Hypophthalmichthys molitrix* Valenciennes in Cuvier and Valenciennes, 1844).

Based on our limited sample size, we conclude that Prussian carp in Alberta are not likely introducing any novel metazoan parasites to our native fish. However, it appears that they are also not experiencing common or intense infections from native fish parasites. This is problematic for native fish because Prussian carp may not have to deal with the energetic costs, or potential pathology, of metazoan parasite infections. This could be another factor giving them an advantage to thrive in North America. Nevertheless, a much more comprehensive survey of parasites of Prussian carp, in different water bodies and over more time, should be conducted to determine if they may acquire and sustain populations of North American parasites.

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Table 1. Prussian carp (*Carassius gibelio* Bloch 1782) characteristics and parasites (*Pomphorhynchus bulbocolli* Linkins in Van Cleave 1919) from two sites in Alberta, Canada.

	Location	Date Collected	Sex	Age	Mass (g)	Length (cm)	Stomach Content*	<i>P. bulbocolli</i>
Specimen								
1	Blindman	May 4/17	F	4	530	30	I	
2	Blindman	May 4/17	F	5	590	28.5	I	1 Female
3	Blindman	May 4/17	F	3	795	31	A, I	
4	Blindman	May 4/17	F	3	1010	34	A	
5	Blindman	May 4/17	F	3	1505	38	A	
11	Blindman	May 4/17	F	2 or 3	840	33.5	I,Am	
12	Blindman	May 4/17	F	4	785	33.1	E	
13	Blindman	May 4/17	F	4	850	33.1	E	
14	Blindman	May 4/17	F	5	790	34	E	
15	Blindman	May 4/17	F	5	915	35.5	E	
16	Blindman	May 4/17	F	3	795	33.5	I	
17	Blindman	May 4/17	F	4	350	26.5	I	1 Female
18	Blindman	May 4/17	F	5	620	31	E	
19	Blindman	May 4/17	F	3	145	23.5	E	
20	Blindman	May 4/17	F	3	60	21.3	I	
6	Blood Ind.	Aug. 18/18	M	3	20	19.3	I	
7	Blood Ind.	Aug. 18/18	F	3	35	20.5	I	1 Male
8	Blood Ind.	Aug. 18/18	F	5	380	27	I, A, PI	
9	Blood Ind.	Aug. 18/18	F	4	330	27.5	A,I	
10	Blood Ind.	Aug. 18/18	F	4	740	34	I, PI	
21	Blood Ind.	Aug. 18/18	M	4	80	21.7	PI	
22	Blood Ind.	Aug. 18/18	F	4	315	28	E	
n=22	15 Blindman, 7 Blood		91% F, 8% M	Mean =3.8	Mean = 567.3	Mean = 29.3		Prevalence: 14% Intensity = 1.0

*Stomach contents: A=algae, Am=amphipods, I=insect parts, PI=plant material, E=empty.

Age in years.

Table 2. Published parasite studies of Prussian Carp (*Carassius gibelio* Bloch 1782).

Type	Species	Site	Stage	Location	Study
Trematoda	<i>Diplostomum spathaceum</i>	Eyes	Larva	Iran	Daghigh Roohi et al.2014
Monogenea	<i>Dactylogyrus</i> spp.	Gills	Adult		
Monogenea	<i>Gyrodactylus kobayashii</i>	Gills	Adult		
Copepoda	<i>Lernaea</i>	Skin	Larva		
Nematoda	<i>Raphidascaris acus</i>	Intestine	Juvenile		
Monogenea	<i>Dactylogyrus anchoratus</i>	Gills	Adult	Turkey	Demir & Karakisi 2016
Monogenea	<i>Gyrodactylus</i> sp.	Gills	Adult		
Copepoda	<i>Lernaea cyprinacea</i>	Skin	Larva		
Nematoda	<i>Eustrongylides excisus</i>	Abdomin	Adult		
Nematoda	<i>Contracaecum</i> sp.	Intestine	?		
Nematoda	<i>Pseudocapillaria tomentosa</i>	Intestine	Adult		
Monogenea	<i>Dactylogyrus</i> sp.	Gills	Adult	Czech Rep	Ondrackova et al. 2006
Trematoda	<i>Posthodiplostomum cuticola</i>	Skin	Larva		
Trematoda	<i>Tylodelphys clavata</i>	Eyes (?)	Larva		
Nematoda	<i>Philometroides sanguinea</i>	Tissues	Adults		
Mollusca	<i>Anodonta</i> sp.	Gills	Larva		
Monogenea	<i>Dactylogyrus anchoratus</i>	Gills	Adult	Czech Rep.	Simkova et al. 2013
Monogenea	<i>D. formosus</i>	Gills	Adult		
Monogenea	<i>D. intermedius</i>	Gills	Adult		
Monogenea	<i>D. vastator</i>	Gills	Adult		
Monogenea	<i>Gyrodactylus katharinari</i>	Gills	Adult		
Monogenea	<i>G. longoacuminatus</i>	Gills	Adult		
Monogenea	<i>G. medius</i>	Gills	Adult		
Monogenea	<i>G. shulmani</i>	Gills	Adult		
Monogenea	<i>G. sprontonae</i>	Gills	Adult		
Monogenea	<i>G. vimbi</i>	Gills	Adult		
Copepoda	<i>Argulus foliaceus</i>	Gills	Adult		
Annelida	<i>Pisicola geometra</i>	Skin	Adult		
Trematoda	<i>Diplostomum spathaceum</i>	Eyes	Larva		
Trematoda	<i>Paryphostomum radiatum</i>	?	Larva		
Cestoda	<i>Paradilepis scolecina</i>	Intestine	Adult		

Table 2, cont.

Type	Species	Site	Stage	Location	Study
Monogenea	<i>Dactylogyrus dulkeiti</i>	Gills	Adult	Czech Rep.	Pokasta et al. 2018
Monogenea	<i>D. intermedius</i>	Gills	Adult		
Monogenea	<i>D. anchoratus</i>	Gills	Adult		
Monogenea	<i>D. formosus</i>	Gills	Adult		
Monogenea	<i>D. inexpectatus</i>	Gills	Adult		
Monogenea	<i>D. vastator</i>	Gills	Adult		
Monogenea	<i>D. baureri</i>	Gills	Adult		
Monogenea	<i>Gyrodactylus sprostonae</i>	Gills	Adult		
Monogenea	<i>G. shulmani</i>	Gills	Adult		
Monogenea	<i>G. longoacuminatus</i>	Gills	Adult		
Monogenea	<i>Paradilozoon homoinon</i>	Gills	Adult		
Trematoda	<i>Diplostomum</i> sp.	Eyes	Larva		
Trematoda	<i>Aspidogaster</i> sp.	Intestine	Adult		
Trematoda	<i>Digenea</i> sp.	Skin	Larva		
Cestoda	<i>Caryophyllidea</i> sp.	Intestine	Adult		
Nematoda	<i>Philometroides sanguinea</i>	Head tissues	Adult		
Nematoda	<i>Schulmanella petruschewskii</i>	Liver	Adult		
Annelida	<i>Piscicola geometra</i>	Skin	Adult		
Mollusca	<i>Unio</i> sp.	Gills	Larva		
Copepoda	<i>Ergasilus sieboldi</i>	Gills	Adult		
Copepoda	<i>Argulus foliaceus</i>	Gills	Adult		
Monogenea	<i>Dactylogyrus</i> spp.	Gills	Adults	Croatia	Popovic et al. 2016
Myxozoa	<i>Thelohanellus</i> spp.	Skin	Adult		
Monogenea	<i>Gyrodactylus</i> spp.	Gills/Skin	Adult		
Trematoda	<i>Diplostomum spathaceum</i>	Eyes	Larva	Georgia	Japoshvili et al. 2017
Cestoda	<i>Ligula intestinalis</i>	Abdomin	Larva		
Monogenea	<i>Dactylogyrus extensus</i>	Gills	Adult	Iran	Sattari et al. 2007
Monogenea	<i>Gyrodactylus</i> sp.	Gills	Adult		
Trematoda	<i>Diplostomum spathaceum</i>	Eyes	Larva		
Nematoda	<i>Raphidascaris acus</i>	Intestine	Juvenile		
Monogenea	<i>Dactylogyrus vastator</i>	Gills	Adult	Poland	Rolbiecki 2003
Monogenea	<i>Diplozoon paradoxum</i>	Gills	Adult		

Table 2, cont.

Type	Species	Site	Stage	Location	Study
Trematoda	<i>Bucephalus polymorphus</i>	Gills/Fins	Larva		
Trematoda	<i>Diplostomum</i> spp.	Eyes	Larva		
Trematoda	<i>Posthodiplostomum cuticola</i>	Skin	Larva		
Trematoda	<i>Tylodelphes clavata</i>	Skin	Larva		
Cestoda	<i>Caryophyllaeus laticeps</i>	Intestine	Adult		
Cestoda	<i>Porteocephalus filicollis</i>	Intestine	Adult		
Copepoda	<i>Ergasilus sieboldi</i>	Gills	Adult		
Copepoda	<i>Caligus lacustris</i>	Skin	Adult		
Brachiura	<i>Argulus foliaceus</i>	Skin	Adult		
Acanthocephalan	<i>Pomphorynchus bulbocolli</i>	Intestine	Adult	Canada	Kucher et al. 2018
Total	45	7	3	7	10