Diet of a rare Canadian fish species, Carmine Shiner (\textit{Notropis percobromus}) in the Birch River, Manitoba, Canada

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Abstract
Due to its restricted occurrence and existing threats, Carmine Shiner (\textit{Notropis percobromus}) has been listed as threatened under the Canadian \textit{Species at Risk Act}. Little is known about Carmine Shiner biology, and understanding its diet composition will help inform future conservation actions. Consequently, the aim of this study was to analyze the diet of Carmine Shiner. Fish were caught throughout the open water season using beach seines, and stomach contents were analyzed. Carmine Shiner feed on a variety of terrestrial and aquatic insects. Diets did not differ substantially between year classes, and we observed no clear temporal trends in diet composition.

Key words: Species at risk; Carmine Shiner; diet; stomach contents

Introduction
In Canada, Carmine Shiner (\textit{Notropis percobromus}) is currently listed as Threatened under the \textit{Species at Risk Act} (SARA Registry 2019). The reason for this designation is that, in Canada, the species occurs only in a restricted area in southeastern Manitoba including the Birch River where threats such as pollution and habitat loss exist (COSEWIC 2018). The major threat to the species is ongoing alterations in water flow as a result of stream regulation (Fisheries and Oceans Canada 2013). The life history, habitat requirements, biology, and physiology of the species are not well understood, but research is improving our understanding of temperature preference, habitat use, and metabolic rate (Stol \textit{et al.} 2013; Carr \textit{et al.} 2015; Macnaughton \textit{et al.} 2019).

Analyzing the diet of Carmine Shiner increases our understanding of its food requirements and helps us evaluate threats to the species. This information may inform conservation measures leading to an improved recovery strategy and preservation of the population in Canada (Fisheries and Oceans Canada 2013). Currently, the available information on the biology of Carmine Shiner, specifically, diet, is insufficient to identify factors that might limit its recovery. Such research is an urgent priority (Fisheries and Oceans Canada 2015; COSEWIC 2018).

At the southern part of its distribution in the Ozarks (in Missouri, Arkansas, Oklahoma, and Kansas, USA), Carmine Shiner has been described as a consumer of aquatic and terrestrial insects (Hoover 1989). Aquatic caddisfly larvae constitute the bulk of the diet, but terrestrial insects, fish eggs, algae, diatoms, and inorganic material are also consumed (Hoover 1989). Juvenile Carmine Shiner prefer algae and diatoms to insects. In addition, food competition among minnow species results in greater dietary specialization by Carmine Shiner on midges (Chironomidae; Hoover 1989). In the Birch River, Carmine Shiners have been observed darting to the water surface to feed (E.C.E. pers. obs.) suggesting that terrestrial insects may be an important food source.

In this study, we evaluated the food consumption of Carmine Shiner in the Birch River, a tributary of the Winnipeg River in Manitoba, Canada. Our objectives were to: (1) study the diet composition of Carmine Shiner; (2) compare the diet composition between year classes; and (3) analyze temporal (June to October) variations in diet composition.

Methods
The Birch River is situated in the Winnipeg River watershed in southeastern Manitoba (Figure 1). Originating in Birch Lake, it flows ~17 km north to
its confluence with the Boggy River, then continues for another ~52 km northwest to its confluence with the Whitemouth River. The river is unregulated and the upper portions of its watershed have low levels of anthropogenic disturbance (Clarke 1998). The Birch River is a meandering, low-gradient watercourse. Water depth is generally less than 1 m during the summer, fall, and winter. Silt, sand, and gravel are the dominant substrate types. The watershed has a drainage area of 864 km$^2$ (Carr et al. 2015).

Carmine Shiner is a slender, elongate minnow found mainly in clear, brown-coloured, fast-flowing creeks and small rivers (Watkinson and Sawatzky 2013). It can be sexually mature at the age of one year and has a lifespan of at least three years (COSEWIC 2018). In Manitoba, its maximum observed fork length is 67 mm. It is olive green to silvery white in colour, but spawning adults develop bright red cheeks and fin bases. Spawning Carmine Shiners have been captured over clean gravel or cobble substrates and are known to spawn in early summer (Stewart and Watkinson 2007). Predators are not well known, but, in Manitoba, they probably include a number of piscivorous fish and bird species (Watkinson and Sawatzky 2013).

In Canada, Carmine Shiner is found only in Manitoba; it has been captured in the Winnipeg, Bird, Whitemouth, and Birch Rivers and in the Pinawa Channel (Watkinson and Sawatzky 2013). The population is not connected to southern populations found in the United States. Carmine Shiner is not the target of any known fishery and has no direct economic im-
importance, but it is considered to be of “significant biological and scientific interest” (Fisheries and Oceans Canada 2013: iii). The species is sensitive to anthropogenic disturbances because of its limited distribution and abundance. The Manitoba population is at the northern limit of the species’ current range and may possess unique characteristics related to local adaptation (Stewart and Watkinson 2007).

Sampling was conducted approximately every two weeks from June to October 2011 in the Birch River. At each sample location, fish were collected with three passes of a 9.14-m long by 1.82-m high seine with a 1.82 × 1.82-m bag and 4.76-mm mesh. To maintain an equal sampling area between locations, one end of the seine was held stationary on shore and the other end was stretched out along the shore in an upstream direction then pulled downstream in a half circle. Fish were removed from the seine after each haul and placed in a holding tub. Fish were preserved in 95% ethanol on site for later identification, measurement of fork length, and collection of stomach contents for analysis.

The stomach contents of 514 Carmine Shiners (mean fork length 34.9 mm, SD 12.7 mm) were analyzed. Individual fish preserved in ethanol were taken out of the sample bottle. The fork length was measured to the nearest mm. Subsequently, under a dissecting scope (model SMZ 1000; Nikon, Tokyo, Japan), the fish were dissected on a 60-mm-diameter wax-bottomed glass Petri dish and the esophagus, stomach, and intestines were extracted. Individual food items were then removed from the esophagus and front part of the stomach and put on an identification tray. Food particles were counted and identified to the lowest feasible taxonomic level using taxonomic keys and descriptions (Borror and White 1970; Merritt and Cummins 1996; Marshall 2013; Evans 2014). The identified food particles were then put in a pre-weighed aluminum boat (0.12 mL, model 12577-070; VWR International, Radnor, Pennsylvania, USA), dried for 24 h in a drying chamber at 70°C, and individually weighed (dry weight).

To analyze ontogenetic diet shifts, we divided the sampled fish into size classes (Figure 2). An independent mixture model was used to estimate size of the various age classes (Rennie et al. 2019). The mixture model was fitted using the mix() function in the depmixS4 package (Visser and Speekenbrink 2010) in R version 3.40 (R Core Team 2017). A Gaussian distribution was used to classify the groups, using either one or two hidden states (age classes) changing temporally as young-of-the-year (YOY) shiners did not occur in catches before late July (Figure 2).

For each size class, we then calculated the average proportion of each prey category in the stomach contents. To compare dietary overlap between size classes, we used the Schoener index (SI) based on prey abundance (Schoener 1970):

\[ a = 1 - 0.5 \sum_{i=1}^{n} |P_{xi} - P_{yi}| \]

where \( a \) is the SI value, \( P_{xi} \) is the proportion of the \( i \)th prey item in age category or species class \( x \), and \( P_{yi} \) is the proportion of the \( i \)th prey item in age category or species class \( y \).

![Figure 2](image-url)
An SI value of 0 represents no overlap; an SI value of 1 indicates complete overlap, and SI values >0.6 proposes a certain degree of food competition between age classes. All database manipulation and data analyses were done in R version 3.4.0 (R Core Team 2017).

**Results**

We analyzed the stomach contents of 514 Carmine Shiners, 349 (67.1%) of which yielded at least one prey item; the other 165 (32.9%) stomachs were empty (Figure 3). Among fish with at least one consumed prey item, on average, 2.1 ± 0.2 (mean ± SD) invertebrates were found in their stomachs, but up to 36 prey items were found in a single fish. Carmine Shiners consumed a variety of terrestrial and aquatic invertebrates. The major groups found among the prey items included Coleoptera (59%), Hymenoptera (12%), Diptera (6%), and Hemiptera (6%). Hydraenidae made up 71% of the coleopteran insects with the major species being *Hydraena* sp. The other families of coleopteran insects found were unidentified (10.8%), Hydrophilidae (7.6%), Staphylinidae (2.7%), Carabidae (1.6%), Dytiscidae (0.6%), Coccinellidae (0.2%), and unidentified weevils (0.6%). The other groups of insects, which were further identified to a lower taxonomic group included Arachnida, Hemiptera, aphids, mirids, and tingids, Hymenoptera, Braconidae, Chalcidoidea, Formicidae, and Ichneumonidae, Phthiraptera, and Thysanoptera. Most identified prey items are terrestrial; only Dytiscidae, Hydraenidae, and Hydrophilidae are aquatic.

The number of prey items consumed by Carmine Shiners varied among sampling months (Figure 4), with the largest numbers of invertebrates (4.0 ± 0.5 observed in August. We observed dietary overlap between YOY and older classes (≥1 year) of Carmine Shiners (SI 0.86). Although the frequency of occurrence of various prey categories varied among sampling months, no temporal trend was detected (Figure 5). In August and September, Carmine Shiner fed dominantly on Coleoptera. YOY Carmine Shiners consumed Arachnida, Coleoptera, Diptera, Hemiptera, and Hymenoptera in August, September, and October. Neuroptera, Psocoptera, Thysanoptera, Trichoptera, and Phthiraptera were not found in stomachs of YOY. Older year classes fed on Arachnida, Coleoptera, Diptera, and Hemiptera during June, July, and August. Thripidae was observed in the diet only in June. All prey items other than Hemiptera were consumed in September. In October, only Coleopterans were observed in older year classes. Neuroptera, Psocoptera, Thysanoptera, and Trichoptera were not consumed by older year classes in any of the sampling months.

**Discussion**

Carmine Shiners are visual feeders that consume a variety of seasonal food items. Stomach content analysis indicated that they consumed a wide prey base, reflecting their dynamic habitat conditions, i.e., lentic versus lotic, spatial river gradient, and seasonal succession of prey availability. Stomach contents included Coleoptera (the most dominant prey category), Hymenoptera, Diptera, and Hemiptera. In particular, Hydraenidae were commonly observed in the diet. Hydraenidae are also referred to as minute moss beetles, as they are often found in moss or accumulations of moist or wet dead leaves, sticks, and twigs along the margins of streams and rivers (Merritt and Cummins 1996). The proximity of the Hydraenidae habitat to the river margins (Borer and White 1998;
Evans 2014) probably explains their high proportions in shiner stomachs.

Diet patterns observed in our study were consistent with Carmine Shiner diet reported in the literature. Southern populations of Carmine Shiner in the Ozarks have been described as omnivorous, lower to mid-level consumers (Hoover 1989). Aquatic insects, particularly caddisfly larvae, constituted the bulk of the diet of these fishes, but they also consumed terrestrial insects, fish eggs, algae, diatoms, and inorganic material. Competition for prey among minnow species in an Ozark stream led to greater dietary specialization by Carmine Shiner on chironomids (Hoover 1989). The diversity in Carmine Shiner diet decreased in the
presence of Smallmouth Bass (*Micropterus dolomieu*) and increased with light levels, which suggests that prey are located by sight (Hoover 1989). Similarly, in the Whitemouth and Birch rivers in Manitoba, surface insects seem to be the dominant food type and Carmine Shiner have been observed rising to the surface to feed (K.W. Stewart pers. obs. 2006).

In our study, prey condition varied widely among individual fish and with prey type, and unidentifiable organic matter was a significant component of stomach contents, perhaps because digestion had continued after the fish were collected and preserved. Consequently, only a portion of the diet could be described and quantified based on intact prey. Identified Coleoptera remains consisted mainly of wings and the exoskeleton, indicating that the exoskeleton and especially the chelae may remain undigested for a long time. Other coleopteran body parts and other insects may be digested more rapidly, suggesting that the observed diet composition might partly reflect differences in food digestion.

Food availability may also have been affected by drought conditions in the summer of 2011, when water level dropped to an extremely low level. The occurrence of stagnant water and high water temperatures may have affected the abundance and distribution of prey items and, consequently, the availability of food for Carmine Shiners, thus explaining the high proportion of terrestrial prey in stomach contents.

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