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Documentation of Freshwater Mussels (Unionidae) in the Diet of Round Gobies (Neogobius melanostomus) within the French Creek Watershed, Pennsylvania

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ABSTRACT.—The round goby (*Neogobius melanostomus*) was discovered in 2013 in the French Creek (Allegheny River Drainage) watershed and is the first documented invasion outside of the Lake Erie basin in Pennsylvania. The round goby throughout the Laurentian Great Lakes is known to eat dressenid mussels (Dreissenidae), but consumption of either dressenids or native mussels (Unionidae) in tributaries to Lake Erie is minimal based on low populations of any bivalves. The French Creek watershed, on the other hand, harbors 29 species of native freshwater mussels as well as introduced fingernail clams (Sphaeriidae; *Corbicula fluminea*). The objectives of this study were to document the diet of round gobies in the French Creek watershed to determine whether consumption of native freshwater mussels was occurring. Round gobies were collected in the summer months (May–Sept.) of 2016 via kick seine in four locations, dissected, and their stomach contents identified to lowest possible taxa. We separated the gobies into categories based on length classes, in order to determine if diet changed with increased size and age. Unionid mussels were consumed by all length classes, particularly in *length class one* (30–44 mm), but diet shifted to a dominance of sphaeriids in length class four ($\geq$75 mm). Round gobies also consumed benthic aquatic insects, a large percentage of which were chironomids (greater than 24% in all size classes). This is the first documentation of unionid consumption by the round goby in Pennsylvania that poses possible threats to native mussels in the French Creek watershed.

INTRODUCTION

Since its discovery in Lake Erie 28 y ago, the round goby, *Neogobius melanostomus* (Pallas, 1814) has expanded its range into tributaries of Lake Erie (Jude *et al.,* 1992; Phillips *et al.,* 2003; Poos *et al.,* 2010; Pennuto *et al.,* 2010; Stauffer *et al.,* 2016). It now threatens fauna in many watersheds. The vector for introduction could be unintentional, such as bait bucket transfer or release from aquaria, or deliberate release by anglers seeking to increase the biomass of forage fish.

The recent discovery of the round goby in the French Creek watershed, located in the Upper Allegheny River basin [Pennsylvania Fish and Boat Commission (PFBC), 2014; USGS, 2014; Stauffer *et al.,* 2016; Mueller *et al.,* 2017] is of special concern due to the high diversity of fishes and mussels. The French Creek watershed is 319,000 ha and ranges from southwestern New York to western Pennsylvania where it meets the Allegheny River (Ohio River Drainage) in Franklin, Pennsylvania [Western Pennsylvania Conservancy (WPC), 2002]. French Creek is the most species-rich stream in Pennsylvania and nationally recognized for its biodiversity, with more than 80 species of fishes and 29 freshwater mussels

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(Unionidae) (WPC, 2002; Smith and Crabtree, 2010). Mussels in French Creek that are listed under the Endangered Species Act include: northern riffleshell (*Epioblasma torulosa rangiana*: G2; S2), snuffbox (*Epioblasma triquetra*: G3; S2), clubshell (*Pleurobema clava*: G2; S2), and rayed bean (*Vilosa fabalis*: G1G2; S1S2). Northern riffleshell and clubshell mussels are considered critically imperiled and have lost 95% of their historic global range but appear to have stable populations in French Creek at this time (Smith and Crabtree, 2010).

The round goby was introduced in LeBoeuf Lake near Waterford, Pennsylvania (41.935873, 79.987255) and has established viable populations in LeBoeuf Creek (USGS, 2014; Stauffer *et al.,* 2016; Mueller *et al.,* 2017) and a small portion of the mainstream of French Creek, downstream of the confluence of LeBoeuf and French creeks (Stauffer *et al.,* 2016; Mueller *et al.,* 2017). The round goby impacts native fauna, including both fishes and macroinvertebrates (Jude *et al.,* 1995; Poos *et al.,* 2010; Pennuto *et al.,* 2010; Kornis *et al.,* 2010) in lentic and lotic systems.

Gobies in Lake Erie feed on both aquatic macroinvertebrates including a large proportion of zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*) (Ray and Corkum, 1997; Johnson *et al.,* 2005; Kornis *et al.,* 2012). In tributaries of Lake Erie, they eat mostly aquatic insects (Krakowiak and Pennuto, 2004; Pennuto *et al.,* 2010; Kornis *et al.,* 2012; Wilson *et al.,* 2014; Stauffer *et al.,* 2016) that could be attributed to the lack of both invasive and native bivalves. In streams, where they have been introduced, the gobies have been shown to reduce macroinvertebrate taxa and richness (Krakowiak and Pennuto, 2008; Mikl *et al.,* 2017). Chironomids are a primary food item for stream-dwelling gobies (Phillips *et al.,* 2003; Pennuto *et al.,* 2010; Stauffer *et al.,* 2016) and amphipods are in some stream systems (Copp *et al.,* 2008). Many fishes in lotic systems rely on benthic fauna (*i.e.,* aquatic insects and crustaceans) as a primary food source, including mottled sculpin (*Cottus bardi*), various darters (*Etheostoma* sp., *Percina* sp., and *Ammocrypta pellucida*) and sport-fishes (*e.g.,* Salmonidae). Impacts of the round goby on assemblages of aquatic benthic fauna could negatively affect both fishes and mussels. For instance Krakowiak and Pennuto (2004) found gobies inhabiting tributaries to Lake Erie have impacted species of Ephemeroptera, Plecoptera, and Trichoptera (EPT) and shifted the macroinvertebrate assemblages to a dominance of chironomids. French Creek is home to the most diverse freshwater assemblage of mussels in Pennsylvania and much of the Northeastern United States. While adult unionids are large in size [*e.g.,* mean length of rayed Bean (*Villosa fabalis*) and mucket (*Actinonaias ligamentina*) are 20 mm and 130 mm, respecively; Poos *et al.,* 2010], juveniles are small and potentially easy to handle and consume; therefore, the round goby can prey directly on these unionids without having to crush them. Because round gobies have not occupied a system as rich in unionid diversity as found in this watershed, little is known on how they may forage for these mussels in a stream system. However, many studies have documented zebra and quagga mussel (dreissenid) consumption throughout the Great Lakes watersheds, and foraging mechanisms may be similar. Andraso *et al.* (2017) noted small round gobies (<70 mm) consumed high percentages of dreissenid mussels with both crushed and whole organisms present in the gut.

In addition to potential predation on unionids, gobies may further impact mussels by altering populations of host fishes that the mussels need to complete their lifecycle, during which they are required to attach to host fish as glochidia and grow. The tippecanoe darter (*Etheostoma tippecanoe*), rainbow darter (*Etheostoma caeruleum*), and greenside darter (*Etheostoma blenniodies*) are hosts for rayed bean mussels (White *et al.,* 1996; Woolnough, 2002; Butler, 2003). In laboratory environments snuffbox have used logperch (*Percina caprodes*), blackside darter (*P. maculata*) (Hove and Kapuscinski, 1998; Hove *et al.,* 2000),

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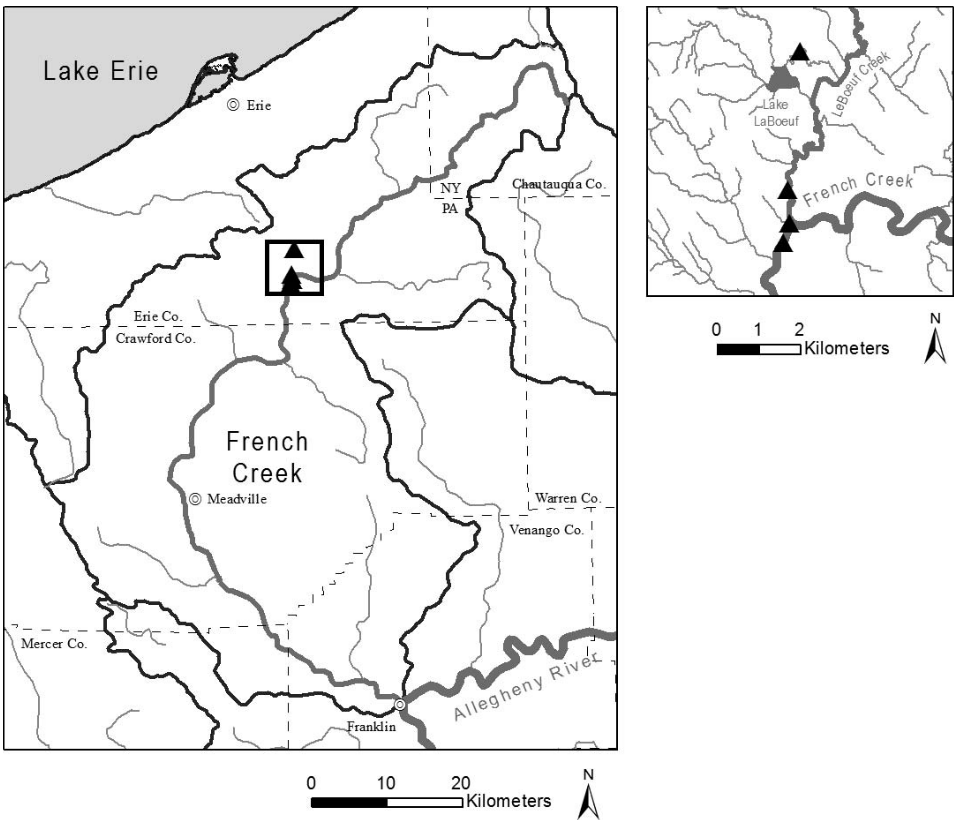

FIG. 1.—Round goby sampling sites from 2016 in the French Creek watershed (Pennsylvania), located in the upper Ohio River drainage system

rainbow darter, and Iowa Darter (*E. exile*) as hosts (Hill, 1986; Sherman, 1993; Hillegrass and Hove, 1997; Barnhart and Baird, 1998; McNichols and Mackie, 2003), but logperch is considered the most common host (Sherman, 1993).

The purpose of our study was to investigate the diet composition of the round goby in the French Creek watershed, a highly diverse stream that houses an array of benthic fauna including fishes and macroinvertebrates. This is the first introduction of the round goby into a lotic system with more species richness than many streams in the northeastern United States and provides an opportunity to give valuable insight to putative consequences of the round goby on native benthic fauna.

METHODS

During the summer months of 2016 and 2017, we collected fishes in four locations throughout the French Creek watershed in which the round goby is known to occur (Fig. 1; two locations in LeBoeuf Creek at 41.909352, –79.986375 and 41.939328, –79.982207, and two locations in French Creek at 41.902102, –79.985967 and 41.898021, –79.987791). A team of researchers collected fishes by kick seining (3 m × 1 m × 9.5 mm nylon mesh) in a

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downstream direction. Three researchers would kick and flip rocks immediately upstream of the seine to drive fishes into the net, and then it was immediately hoisted out of the water. A seine was used rather than electroshocking to prevent potential regurgitation of food items before dissection took place. Stream reaches were between 100 m–200 m and all stream habitats were sampled (*i.e.*, riffles, runs, pools).

Gobies were euthanized in MS-222 and preserved in 10% formalin (IACUC# 46941, the Pennsylvania State University) within an hour of collection to prevent further digestion of stomach contents. After 2 wk, they were rinsed and transferred to 70% ethanol. Total length (nose tip to caudal tip length) of each fish was recorded and stomachs were removed for analysis. Contents posterior to the stomach were not included in diet analysis because they could not be reliably counted and identified (Cordes and Page, 1980). Where partially digested organisms occurred, head capsules were counted as a single whole organism (especially for chironomids). For bivalves only whole organisms were counted and used for analysis. Although broken shell fragments were noted when present, they could not be used reliably for diet analysis. Identification of unionids versus sphaerids was determined using the characteristic oblong shape and smaller size of unionids in contrast to the triangular-shaped, thin shell displaying concentric growth rings typical of sphaerids (Stern, 1990). Fish that had no identifiable stomach contents or empty stomachs were removed from the sample. Fish smaller than 30 mm were also excluded because they were too small for stomach removal and gut identification.

Gobies were separated into four length classes (length class 1: 30–44 mm; length class 2: 45–59 mm; length class 3: 60–74 mm; and length class 4: $\geq$75 mm) for analysis (Phillips *et al.*, 2003). We determined the total number of prey items consumed for each taxa, percentage of prey items belonging to each taxa, average number of individuals in each stomach, and lastly, percent and total number of fish with that taxa in the stomach. We employed a one-way ANOVA ($P < 0.05$) to examine the effect of length classes of round goby on consumption of unionids and used a Tukey test ($P < 0.05$) to identify significant differences. Lastly, we used a regression on number of sphaerids consumed versus fish size. Data were log transformed to meet variance and normality criteria for analysis.

RESULTS

Diet analysis of the round goby was based on 177 fish; 15 fish were not included in analysis due to empty stomachs or fully digested organisms that could not be identified. Gobies consumed unionid mussels and sphaeriids (Table 1). Bivalves were typically consumed whole, with only five cases of shell fragments present in the stomach contents. Chironomids comprised a large percentage (greater than 24%) in all length classes (Table 1). Chironomids were found in the highest number of individual fish and largest averages in all length classes except length class 1. Length class 2 had the highest percentage of chironomids (75.68%; Table 1). Gobies in length class 1 consumed significantly higher numbers of unionids ($P < 0.05$, $F = 10.89$; $df = 81$) compared to all other length classes (Table 1; Fig. 2). Thirty-four of the 44 fish in this length class had unionids in their stomachs with an average of 32.9 individuals/fish ranging from zero to 170. Of the 34 fish that had consumed unionids, 16 had consumed more than 30 individuals each. Length class 4 had the lowest number of unionid consumed; however, this length class had the highest percentage and total number of sphaeriids (Table 1). Consumption also increased with size ($R^2 = 0.48$; $P < 0.05$; $F = 8.61$; Table 1 and Fig. 3). Ephemeropterans and Trichopterans were also consumed, but at lower percentages relative to bivalves and chironomids (Table 1).

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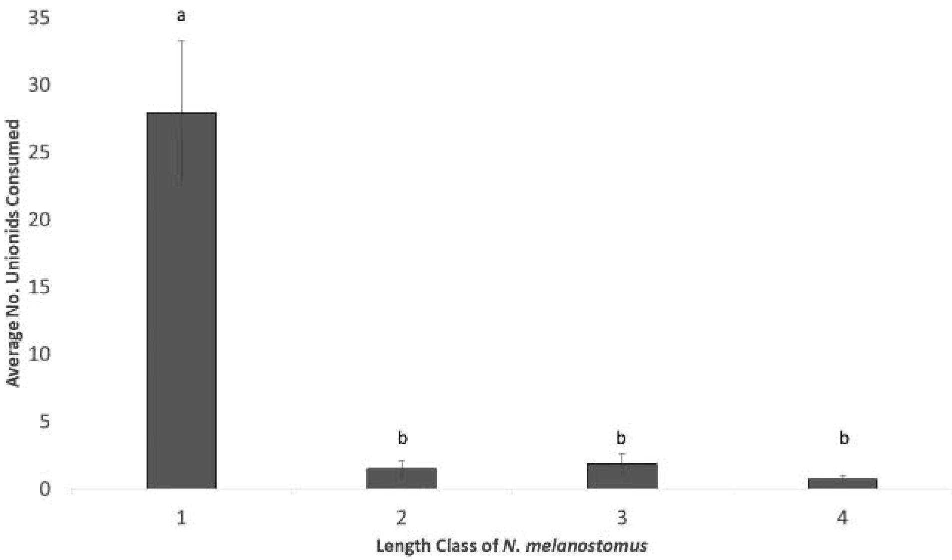

FIG. 2.—Consumption of unionid mussels by round gobies collected in 2016 categorized by length classes and standard error bars (1 SD of the mean). For this analysione gobies were separated into four length classes (length class one: 30–44 mm; length class two: 45–59 mm; length class three: 60–74 mm; and length class four: >75 mm). Length class one is significantly different from all other length classes ($P < 0.05$)

DISCUSSION

Our study is the first to document stomach contents of the round goby in a watershed with such rich fauna diversity, when compared to other diet studies for the round goby (Ray and Corkum, 1997; Johnson *et al.,* 2005; Kornis *et al.,* 2012; Stauffer *et al.,* 2016). This is also the

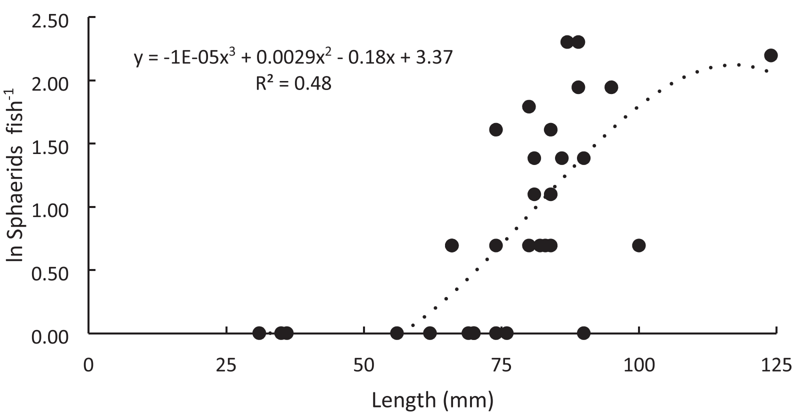

FIG. 3.—Length of individual round goby versus consumption of sphaeriids collected in 2016. As length of round goby increases, there is a significant increase in consumption ($P < 0.05$; $F = 8.61$; $df = 32$).

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TABLE 1.—Stomach contents of round gobies collected from four locations in 2016. Fish are separated into four length classes for analysis. Total number of prey items consumed for each taxa (T#), percentage of prey items belonging to each taxa (% Taxa), average number of individuals in each stomach (Ave.), and average and total number of fish with that taxa in the stomach (% and # Fish)

	Length class 1				Length class 2			
	30–44 mm				45–59 mm			
	n = 44				n = 46			
Taxa Consumed	T#	% Taxa	Ave.	% and # Fish	T#	% Taxa	Ave.	% and # Fish
Amphipoda								
Gammaridae	10	0.06%	1.6	14% (6)	3	0.43%	1	6.52% (3)
Cladocera								
Daphnia	-	-	-	-	4	0.01%	4	2.17% (1)
Coleoptera								
n/a	-	-	-	-	-	-	-	-
Dysticidae	-	-	-	-	-	-	-	-
Elmidae (adult)	-	-	-	-	1	0.14%	1	2.17% (1)
Elmidae (larva)	6	0.38%	1.5	9.09% (4)	4	0.57%	2	4.35% (2)
Haliplidae	-	-	-	-	1	0.14%	1	2.17% (1)
Psphenidae	-	-	-	-	3	0.43%	3	2.17% (1)
Pspheridae	-	-	-	-	-	-	-	-
Diptera								
n/a	-	-	-	-	-	-	-	-
Ceratopgonidae	1	0.06%	1	2.27% (1)	-	-	-	-
Chironmidae	391	24.92%	11.8	75% (33)	529	75.68%	13.2 1	89.13% (41)
Simuliidae	-	-	-	-	1	0.14%	1	2.17% (1)
Tipulidae	-	-	-	-	-	-	-	-
Ephemeroptera								
n/a	3	0.19%	1	6.81% (3)	9	1.29%	1.8	10.87% (5)
Ephemeridae	-	-	-	-	-	-	-	-
Heptageniidae	1	0.06%	1	2.27% (1)	7	1.00%	1.75	8.70% (4)
Polymitarcyidae	-	-	-	-	1	0.14%	1	2.17% (1)
Fish eggs								
n/a	-	-	-	-	5	0.72%	5	2.17% (1)
Gastropoda								
n/a	-	-	-	-	-	-	-	-
Hemiptera								
Corixidae	-	-	-	-	-	-	-	-
Hydracarina								
Arrenuridea	2	0.13%	1	4.55% (2)	12	1.72%	1.3	19.60% (9)
Isopoda								
Asellus	-	-	-	-	7	1.00%	2.3	4.35% (2)
Megaloptera								
Sialidae	-	-	-	-	-	-	-	-

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TABLE 1.—Extended

Length class 3				Length class 4			
60–74 mm				≥75 mm			
n = 50				n = 37			
T #	% Taxa	Ave.	% and # Fish	T#	% Taxa	Ave.	% and # Fish
7	1.02%	1.6	12.00% (6)	11	3.30%	2.75	10.81% (4)
4	0.58%	4	2.00% (1)	-	-	-	-
-	-	-	-	1	0.30%	1	2.70% (1)
-	-	-	-	4	1.20%	2	5.41% (2)
1	0.14%	1	2.00% (1)	4	1.20%	4	2.70% (1)
2 4	3.49%	2	24.00% (12)	2	0.60%	1	5.41% (2)
-	-	-	-	-		-	-
1	0.14%	1	2.00% (1)	1	0.30%	1	2.70% (1)
1	0.14%	1	2.00% (1)	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
4 1 4	60.17%	11. 8	70% (35)	98	29.43%	5.16	51.35% (19)
-	-	-	-	-	-	-	-
1	0.15%	1	2.00% (1)	-	-	-	-
9	1.31%	1.5	12.00% (6)	10	3.00%	1.6	16.22% (6)
7	1.02%	3.5	40.00% (2)	1	0.30%	1	2.70% (1)
1 1	1.60%	1.8 3	12.00% (6)	5	1.50%	1.25	10.81% (4)
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
14	2.03%	3.5	8.00% (4)	41	12.31%	4.5	24.32% (9)
3	0.44%	3	2.00% (1)	3	0.90%	1.5	5.41% (2)
5	0.73%	5	2.00% (1)	1	0.30%	1	2.70% (1)
16	2.33%	4	8.00% (4)	1	0.30%	1	2.70% (1)
1	0.15%	1	2.00% (1)	-	-	-	-

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TABLE 1.—Continued

	Length class 1				Length class 2			
	30–44 mm				45–59 mm			
	n = 44				n = 46			
Taxa Consumed	T#	% Taxa	Ave.	% and # Fish	T#	% Taxa	Ave.	% and # Fish
Odonata								
n/a	1	0.06%	1	2.27% (1)	-	-	-	-
Gomphidae	1	0.06%	1	2.27% (1)	-	-	-	-
Plecoptera								
n/a	-	-	-	-	-	-	-	-
Caniidae	-	-	-	-	-	0.14%	1	2.17% (1)
Chloropermidae	-	-	-	-	-	-	-	-
Trichoptera								
n/a	4	0.25%	1.3	6.81% (3)	2	0.29%	1	4.35% (2)
Brachycentridae	-	-	-	-	-	-	-	-
Helicopsychidae	2	0.13%	2	2.27% (1)	-	-	-	-
Hydropsychidae	4	0.25%	1.3	6.81% (3)	3	0.43%	1	6.52% (3)
Hydroptilidae	12	0.76%	1.5	18.18% (8)	36	5.15%	3.27	23.91% (11)
Leptoceridae	2	0.13%	1	4.55% (2)	-	0.14%	1	2.17% (1)
Polxcentropodidae	-	-	-	-	-	-	-	-
Unionoida								
Unionidae	1117	71.19%	32.9	77.27% (34)	65	9.30%	5.41	26.10% (12)
Veneroida								
Sphaeriidae	3	0.19%	1	6.81% (3)	1	0.14%	1	2.17% (1)
Terrestrial Insect								
n/a	-	-	-	-	1	0.14%	1	2.17% (1)
Unidentified Insects	9	0.57%	1.125	15.91% (7)	4	0.57%	1	8.70% (4)

first published report of the round goby eating unionids. Poos *et al.* (2010) suggested mussel species suspected of endangerment may be prey of the round goby, and many of these threatened mussel species are found in our study sites (Kyle Clark, unpubl.). Rayed bean mussels are small (mean size 20 mm) and are also suspected to be consumed directly (Poos *et al.,* 2010). Although gobies are directly consuming native mussels, these mussels may be under additional pressure if their host fish populations decline as a result of displacement by gobies as the literature suggests occurs in both lotic and lentic environments (Chotkowski and Marsden, 1999; French *et al.,* 2001; Lauer *et al.,* 2004; Poos *et al.,* 2010; Kornis *et al.,* 2012). The small size and the presence of only soft tissue made the identification of unionid mussels to the species level from stomachs of the round goby impossible under a dissection scope. We are currently working on a project that will use eDNA of the stomachs to identify the species of mussels consumed by the round goby to look at individual species consumption more finely.

In a laboratory study, Ray and Corkum (1997) determined gut length and capacity of the round goby affected the number of zebra mussels consumed. They also found round gobies in length class 1 (30–44 mm) had significantly more unionids than any of the other length class, and all but five individuals had consumed bivalves whole. Based on the lifecycle and reproductive timing of unionids in the French Creek watershed, juvenile mussels small

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TABLE 1.—Continued, Extended

Length class 3				Length class 4			
60–74 mm				≥75 mm			
n = 50				n = 37			
T #	% Taxa	Ave.	% and # Fish	T#	% Taxa	Ave.	% and # Fish
-	-	-	-	1	0.30%	1	2.70% (1)
-	-	-	-	-	-	-	-
1	-	-	-	1	0.30%	1	2.70% (1)
-	-	-	-	-	-	-	-
-	-	-	-	2	0.60%	2	2.70% (1)
6	0.87%	1.2	10% (5)	9	2.70%	2.25	10.81% (4)
-	-	-	-	1	0.30%	1	2.70% (1)
-	-	-	-	-	-	-	-
7	1.02%	1.75	8.00% (4)	4	1.20%	1	10.81% (4)
34	4.94%	2.43	28.00% (14)	9	2.70%	2.25	10.81% (4)
-	-	-	-	2	0.60%	2	2.70% (1)
-	-	-	-	2	0.60%	1	5.41% (2)
97	14.10%	5.2	40.00% (20)	26	7.81%	3.25	21.62% (8)
16	2.33%	1.7	18.00% (9)	84	25.23%	4.42	51.35% (19)
4	0.58%	1.33	6% (3)	5	1.50%	1.25	10.81% (4)
4	0.58%	1	8.00% (4)	4	1.20%	1.33	8.11% (3)

enough for consumption may not be readily available throughout the year, which has been shown to be the case for selective size predation on dreisseind mussels in the Great Lakes (Djuricich and Janssen, 2001; Andraso *et al.*, 2011). Increased consumption of sphaeriids, as the size of the round goby increased, is probably attributed to larger gape size and ability to physically fit larger bivalves into their mouths.

Development of the pharyngeal apparatus in small (<70 mm) round gobies at Presque Isle Bay (Lake Erie) and the suggestion pharyngeal morphology may be linked to specific taxa available for consumption (hard versus soft-bodied prey) (Andraso *et al.*, 2017) supports our contention that round gobies may develop bivalve crushing abilities at smaller sizes than previously thought. With juvenile mussels readily available for consumption during our sampling period, crushing mechanisms may be developed earlier. Ray and Corkum (1997) also found a significant positive relationship between gape size and standard length of gobies. In addition Andraso *et al.* (2011) discovered round goby length corresponded to size of dreissenid mussels consumed in Presque Isle Bay (Lake Erie). In our study juvenile unionids were much smaller than sphaeriids, which supports our observation of the consumption of small unionids in length class one and highest consumption of sphaeriids in length class four.

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Looking at the pharyngeal apparatus in the smallest length class of the round gobies used in our study would provide additional insight on how food availability plays a role in pharyngeal morphology, primarily for crushing capabilities.

It is not uncommon chironomids comprised such a large portion of diets for all length classes. In Pennsylvanian tributaries to Lake Erie, Phillips *et al.* (2004) reported chironomids were the most important food item for all size classes while breadth of food items changed. Stauffer *et al.* (2016) also found a positive electivity index for consumption of the round goby of chironomids in Elk Creek (Lake Erie, Pennsylvania). In lotic systems worldwide, chironomids are typically abundant prey items that may account for half the number of macroinvertebrates present (Hlohowskyj and White, 1983; Martin, 1984; Alford and Beckett, 2007). Chironomids are also a small prey item with mature larvae ranging from 2–30 mm (Merritt *et al.,* 2008), making them easy prey for many insectivorous fishes including the round goby.

Mussel diversity of North America is the greatest in the world with over 300 species recognized; however, this fauna is also subjected to threats which are pushing them towards extinction at an alarming rate. While mussels species have been negatively impacted by human alteration to most stream systems in North America many species of mussels in French Creek are thriving (Smith and Crabtree, 2010). The introduction of round gobies into the French Creek watershed poses a threat to native mussels, both directly through consumption of juveniles and indirectly through displacement of their fish host species (Krakowiak and Pennuto, 2004; Pennuto *et al.,* 2010; Kornis *et al.,* 2012; Wilson *et al.,* 2014; Stauffer *et al.,* 2016, Mikl *et al.*, 2017). Understanding the consequences of the introduction of the round goby and its consumption of unionids and other aquatic macroinvertebrates in French Creek is critical to enhancing conservation and management efforts of benthic fauna in other diverse stream systems of similar size.

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