Determination of Invasive Round Goby Populations within the Main Stem of French Creek and Their Potential Impact on Native Benthic Fishes

Casey Bradshaw-Wilson (PI)
Assistant Professor of Environmental Science
Allegheny College
Meadville, PA 16335
814-332-3221

Jay R. Stauffer (PI)
Distinguished Professor of Ichthyology
Forest Resources Building
Penn State University
University Park, Pennsylvania 16801
814-863-0645

Josh Wisor (Co-PI)
Forest Resources Building
Penn State University
University Park, Pennsylvania 16801

January 2016 – December 2018

2.0 Executive Summary

The introduction of Round Gobies (*Neogobius melanostomus*) in the French Creek Watershed (Lake LeBoeuf) was probably via bait bucket and were first discovered in 2014 This was the first documented invasion outside of the Lake Erie Basin in Pennsylvania. This was alarming in large part because the Round Goby has become the most abundant benthic (bottom dwelling) fish in the Laurentian Great Lakes after only being discovered in 1989.

Round Gobies throughout the Laurentian Great Lakes are 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, however, harbors 29 species of native freshwater mussel as well as introduced Fingernail Clams (Sphaeriidae; *Corbicula fluminea*). In addition, Round Gobies have never been introduced to a lotic system with such high biodiversity of fishes, and the implications of this introduction were unknown.

For this project, we 1) determined whether Round Gobies had moved into the main channel of French Creek from its initial introduction location at Lake LeBoeuf, 2) documented the diet of Round Gobies in the French Creek watershed to determine whether consumption of native, freshwater mussels was taking place and 3) investigated the effects of the presence of Round Gobies on the habitat choices of native benthic fishes of French Creek.

Round Gobies were collected in the summer months (May -September) via kick seine in 4 locations, dissected, and stomach contents identified to lowest possible taxa from 2016 - 2018. Round Gobies were collected in the main stem of French Creek and downstream movement was documented throughout this study. Since the 2014 discovery in Lake LeBoeuf, the Round Goby has been able to expand its range every year. When collected, Round Gobies were separated into categories based on size classes so that we could determine if diet changed with increased size and age. Unionid mussels were consumed by all size classes, particularly in the size class 1 (30-44mm), but diet shifted to a dominance of sphaeriids in size class 4 ($\geq$75mm). Round Gobies also consumed benthic aquatic macroinvertebrates, a large percentage of which were chironomids (greater than 24% in all size classes). This is the first documentation of unionid consumption by Round Gobies in Pennsylvania and poses uncertain threats to native mussels both directly through consumption of glochidia as well as indirectly through outcompeting host fishes.

To measure the effects of the invasion on native fishes, we followed the methods established by Stauffer et al. (1996) and van Snik Gray and Stauffer (1999) to snorkel and observe the microhabitat of benthic fishes at sites that are known to contain Round Gobies as well as sites that have yet to be invaded. We compared the microhabitat preferences of species across sites to see if the Round Goby caused a shift in these preferences. We were able to show that the presence of the Round Goby did in fact cause shifts in microhabitat choices, especially with choices regarding water velocity. In summary, the Round Goby consumed native unionid mussels and shifted the habitat occupied by native darters.

3.0 Introduction

Since their discovery in Lake Erie nearly 28 years ago, Round Gobies, *Neogobius melanostomus* (Pallas 1814) have expanded their range into tributaries of Lake Erie (Phillips et al., 2003; Poos et al., 2010; Stauffer et al., 2016; Pennuto et al., 2010) and now threaten fauna in many watersheds through unintentional introductions (i.e. bait bucket transfer).

The recent discovery of Round Gobies in the French Creek watershed, which is located in the Upper Allegheny River basin (PFBC, 2014; USGS 2014, Stauffer et al., 2016; Mueller et al., 2017) is of concern because of high diversity of fishes and mussels. This watershed is 319,000 ha and drains southwestern New York and western Pennsylvania where it empties into the Allegheny River (Ohio River Drainage) in Franklin, PA (WPC 2002). French Creek harbors the most species-rich ichthyofauna in Pennsylvania and is nationally recognized for its biodiversity, with more than 80 species of fishes and 29 freshwater mussels (Unionoida) (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 range but appear to have stable populations in French Creek at this time (Smith and Crabtree, 2010).

Round Gobies were introduced in LeBoeuf Lake near Waterford, PA (41.935873, -79.987255) and have colonized LeBoeuf Creek (USGS 2014; Stauffer et al., 2016; Mueller et al., 2017) and a small portion of the mainstem of French Creek downstream of the confluence of LeBoeuf and French creeks (Stauffer et al., 2016; Mueller et al., 2017). 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 both lentic and lotic systems.

Round Gobies in Lake Erie feed on both aquatic macroinvertebrates , Zebra Mussels(*Dreissena polymorpha*), and Quagga Mussels (*Dreissena bugensis*) (Kornis et al., 2012; Johnson et al., 2005; Ray and Corkum, 1997). In tributaries of Lake Erie, they eat mainly macroinvertebrates (Kornis et al., 2012; Wilson et al., 2014; Stauffer et al., 2016; Pennuto et al., 2010; Krakowiak and Pennuto, 2004) that could be attributed to the lack of both invasive and native bivalves. In streams where they have been introduced, studies have shown reduced macroinvertebrate taxa and richness (Krakowiak & Pennuto, 2008; Mikl et al., 2017). Chironomids are a primary food item for stream dwelling Round Gobies (Phillips et al., 2003; Stauffer et al., 2016; Pennuto et al., 2010) and in some stream systems amphipods (Copp et al., 2008). Many fishes in lotic systems rely on macroinvertebrates as a primary food source, including Mottled Sculpin (*Cottus bardi*), various darters (*Etheostoma* sp., *Percina* sp. and *Ammocrypta pellucida*) and recreational species such as trout (Salmonidae). Impacts of Round Gobies on assemblages of aquatic macroinvertebrates could negatively affect both fishes and mussels. For instance, Krakowiak and Pennuto (2004) found that Round Gobies inhabiting tributaries to Lake Erie have impacted mayflies, stoneflies and caddis flies, which altered the macroinvertebrate assemblages to a dominance of chironomids. While adults unionids are large in size (e.g., mean length of rayed bean *(Villosa fabalis)* = 20mm and the mucket *(Actinonaias ligamentina)* = 130mm; Poos et al., 2010), glochidia are small and potentially easy to handle and consume.

In addition to potential predation on unionids, Round Gobies may further influence mussels by altering populations of host fishes that the mussels need to complete their lifecycle. Some species of mussels use only a single host fish while other use several. For example, Tippecanoe darters (*Etheostoma tippecanoe*), Rainbow Darters (*Etheostoma caeruleum*) and Greenside Darters (*Etheostoma blenniodies*) are hosts for Rayed Bean mussels () (White et al., 1996; Butler, 2003Woolnough, 2002. In laboratory environments, snuffbox have used Logperch (*Percina caprodes*), Blackside Darter (*P. maculate*) (Hove and Kapuscinski, 1998; Hove et al., 2000), Rainbow Darter, Iowa Darter (*E. exile*) as hosts (Sherman, 1993; McNichols and Mackie, 2003; Hill, 1986; Hillegrass and Hove, 1997; Barnhart and Baird, 1998), but Logperch are considered the most common hosts (Sherman, 1993).

The purpose of this paper is to investigate the diet composition of Round Gobies in the French Creek watershed. This opportunity gives valuable insight to putative consequences of Round Goby on the native fauna.

4.0 Methods

Diet Analysis

We collected fishes in four locations throughout the French Creek watershed where Round Gobies were known to occur during the summer months in 2016 and 2017 (Figure 1). Site locations were in LeBoeuf Creek at 41.909352 -79.986375 and 41.939328, -79.982207. In French Creek, site locations were 41.902102 -79.985967 and 41.898021 -79.987791. A team of researchers collected fishes by kick seining (3m x 1m x 9.5mm nylon mesh which does not stretch when pulled tight as the netting is not

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

Round Gobies were euthanized in MS-222 and preserved in 10% formalin (IACUC# 46941) within an hour of collection to prevent further digestion of stomach contents. After two weeks, they were rinsed and transferred to 70% ethanol. Total length (nose tip to caudal tip length) of each fish was taken, and stomachs 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). Fish that had no identifiable stomach contents or empty stomachs were removed from the sample. Fish smaller than 30mm were also excluded from the sample because they were too small for stomach removal and gut identification.

Round Gobies were separated into four size classes (30-44mm; 45-59mm; 60-74mm; $\geq$ 75mm) for analysis, which was the same size breakdown as Phillips et al., 2003 for Round Gobies in Pennsylvania tributaries to Lake Erie. We determined the percentage of taxa, average number of individuals in each stomach, 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 size classes of Round Gobies on consumption of unionids and used a Tukey test ($p<0.05$) to identify significant differences. Lastly, we used a linear regression on abundance of sphaeriids found in the stomach contents versus size class.

Microhabitat Partitioning

Six sites were selected in the French Creek Watershed to be surveyed in 2016 and 2017. An ideal site would be characterized by a shallow riffle that is deep enough to effectively snorkel (greater than 10 cm). Site selections were made using a combination of reports from PA DEP and PFBC, previous fish collections, and ease of access (including stream conditions). Three of the sites were known to have Round Gobies present and the other 3 sites were assumed to not have Round Gobies present. The assumption of presence/absence was determined by seining and electrofishing a 100m stretch of the stream approximately one day before performing the survey.

While performing the snorkel surveys at each site, abiotic microhabitat data was gathered at 5 random places along the starting line, finish line, and along each sub-transect so that each site could be characterized and compared to each other. This gave me a total of 20 observations of the bottom flow, maximum flow, depth, and substrate index. Using principal component analysis the abiotic variables measured at these sites were compared to each other and were found to have no significant differences. Since the sites were not shown to be significantly different, the data collected at each site was pooled and differences in habitat choices was compared between sites with Round Gobies present and sites with Round Gobies absent. No data was collected that could control for between-year differences so observations from 2016 and 2017 were compared independently.

The following sampling methods were adapted from several studies regarding the microhabitat partitioning of benthic fishes (Stauffer et al., 1996; van Snik Gray & Stauffer, 1999).

A plot 25 m in length encompassing the entire width of the stream was chosen at each site to be surveyed. One exception to this was at the Moore Road bridge site because the width was less than 10m. To compensate, the length was doubled to 50 m, which allowed all available habitats to be surveyed.

The placement of the transect was done using my best judgment to encompass all available habitat types. Rebar stakes (3/8" diameter x 24") were driven into the bank at each corner of the sampling site, the 10 m mark, and the 20 m mark. This broke the sample plot into two 10m subplots and one 5 m subplot each spanning the width of the stream. A string was tied to each rebar stake and stretched across the stream above the water to visualize the subplots.

Once the site was delimited, several crew members (2 to 3 depending on the width of the stream) snorkeled beginning at the downstream subplot. Each time a darter or Round Goby was encountered a numbered flag would be anchored securely in the exact position of the fish. The following parameters would be recorded on a 10.16 cm PVC coupler worn around the crew members arm: Flag number, Species, orientation in the stream bed (upstream, downstream, towards right or left bank), and above or below a rock or log. At the upstream border of each subplot, the snorkeler would take time to record information on the PVC coupler onto a waterproof data sheet.

Once the snorkelers completed a subplot, a separate crew would visit every flag and record the following parameters: flag number, depth, water velocity (bottom and 6/10), substrate composition. The measured variables were then aggregated into a master data sheet for each site where each flag represents an individual with all the variables of the observation event. Depth and water velocity were taken using a bulb type flow meter attached to a graduated stick and measured to the nearest centimeter. In order to characterize the substrate a 25 x 25 cm piece of Plexiglas was divided into 5 x 5 cm sub quadrats using a permanent marker. This was then laid directly over the stream bottom and the substrate was characterized based on the number of 5 x 5 cm squares occupied by different substrate size classes. Once a 5 x 5 square was deemed occupied by one member of the substrate it is excluded from the rest of the counts. A substrate index was calculated using the methods established by van Snik & Stauffer (1999) using the following formula where N=number of squares occupied, x = category of substrate.

$$\sum_{1}^{25} (N_x * x^2)$$

This index resulted in a score from 25-625. For example, if 1 large boulder took up the entire 25 squares the score would be (1 x 25) resulting in a score of 625. If the substrate was made up of a mix of stones and gravel where 2 stones took up 6 squares each and 3 stones took up 3 squares each and the rest of the squares were made up of gravel, the score would be (2 x 6^2) + (3 x 3^2) + (4 x 1^2) resulting in a score of 103 . If small gravel in which each member of the substrate only occupied 1 or less squares the score would be (25 x 1^2) resulting in score of 25. This index was chosen in order to give clearer distinctions in the categories when observing differences in the choice of substrate size.

Principal component analysis was used to test for significant differences in species' habitat preferences both within sites and across sites based on the presence or absence of the Round Goby.

5.0 Results & Discussion

Since the 2014 discovery in Lake LeBoeuf, the Round Goby expanded its range every year. In the fall of 2014, technicians from the Pennsylvania Department of Environmental Protection (PA DEP) sampled extensively in LeBoeuf Creek and were unable to find any Gobies downstream of the bridge at Moore Rd. south of Waterford, PA (Mueller et al., 2017; Bradshaw-Wilson personal comm.). In the summer of 2015, we visited LeBoeuf Creek and found Round Gobies well established in all life stages throughout the remainder of LeBoeuf Creek and discovered 1 large breeding male in French Creek (Mueller et al., 2017). This was a range expansion of approximately 1 km. In the summer of 2016, Round Gobies had expanded just 400 m downstream from their 2015 range. In 2017, after extensive sampling several Round Gobies were found approximately 650 m downstream from their 2016 range and were not collected in high numbers.

In other lotic systems, Round Gobies average a downstream dispersal rate of approximately 9km/year (Brownscombe et al., 2012). Since their 2015 arrival to French Creek, the Round Goby has only spread 1 km. The diverse community of benthic fishes may be acting to offer resiliency, or stability, to the French Creek ecosystem (Ives et al., 2000). Ecological resiliency is the ability for an ecosystem to resist changes to disturbances (Holling, 1973). It has been shown that species richness can offer ecological resilience to an ecosystem (Peterson et al., 1998). If a disturbance is greater than the ability for the ecosystem to withstand there will be a shift in the ecological processes within that ecosystem (Holling, 1986; Chaffin et al., 2016). For this project I attempt to observe these shifts by comparing the micro-habitat choices of

benthic fishes at sites occupied by the Round Goby to those that are not. If there are no observable shifts in habitat choices this may be evidence of ecological resiliency.

Diet

Round Goby diet analysis 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. Round Gobies consumed both benthic aquatic macroinvertebrates, unionid mussels, and sphaeriids (Table 1). Chironomids comprised a large percentage (greater than 24%) in all size classes (Table 1). Chironomids were found in the highest number of individual fish and largest averages in all size classes except size class 1. Size class 2 had the highest percentage (75.68%) (Table 1). Round Gobies in size class 1 (30-44mm) consumed significantly higher numbers of unionids ($p<0.01$) compared to all other size classes (Table 1; Figure 2). Thirty-four of the 44 fish in this size class had unionids in the stomach contents with an average of 32.9 individuals/fish. Size class 4 had the lowest number of unionid consumed, however, this group had the highest percentage of sphaeriids and consumption increased with size (Table 1; $R^2=0.72$). Ephemeropterans and Trichopterans were also consumed, but at much lower percentages to bivalves and chironomids.

When compared to other diet studies of Round Gobies (Kornis et al., 2012; Johnson et al., 2005; Ray and Corkum, 1997; Stauffer et al., 2016), this is the first to document stomach contents of Round Gobies in a watershed with such a rich faunal diversity. This is the published report of Round Gobies eating unionids. Poos et al. (2010) indicated mussel species suspected of endangerment, many of which are found within LeBoeuf Creek and the main stem of French Creek (Kyle Clark unpublished data). Rayed Bean mussels are small (mean size 20mm) and have also been suspected of direct consumption (Poos et al., 2010). In both 2016 and July 2017, collections of all darter species listed above were identified at the LeBoeuf Creek. While Round Gobies are not only directly consuming native mussels, these mussels may be under additional pressure if their host fish populations decline as a result of competition with Round Gobies (Poos et al., 2010; Chotkowski and Marsden, 1999; French et al., 2001; Lauer et al., 2004; Kornis et al., 2012;). The identification of unionid mussel to species from stomachs of Round Gobies was impossible under a dissection scope; therefore genetic analysis on stomach contents would give valuable insight into which species of mussel are being consumed directly.

In a laboratory study, Ray and Corkum (1997) determined that gut length and capacity of Round Gobies affected the number of zebra mussels consumed. They determined that because all guts were not 100% filled, smaller gobies may hold more zebra mussels. This is also a possibility in the current study, where size class 1 had a significant more unionids than any of the other size classes. In addition, increased consumption of sphaeriids as Round Goby size increases is probably attributed to larger gape size and ability to physically fit larger bivalves into their mouths. Ray and Corkum (1997) also found a significant positive relationship between gape size and standard length of Round Gobies. The glochidia consumed of unionids were much smaller than sphaeriids, which supports consumption of small glochidia in size class 1 and highest consumption of sphaeriids in size class 4. Furthermore, it is not uncommon that chironomids comprised such a large portion of diets for all size classes. In Pennsylvanian tributaries to Lake Erie, Phillips et al., 2004 showed that 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 Round Goby consumption 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 (Martin 1984; Alford and Beckett, 2007; Hlohowskyj and White, 1983). Chironomids are also a small prey item with mature larvae ranging from 2 to 30mm (Merritt et al., 2008) making them easy prey for many insectivorous fishes, including Round Gobies.

Another threat to unionids in French Creek are introductions of dressenid mussels (Zebra or Quagga mussels). Zebra mussels are known to exist in the watershed, particularly Edinboro Lake where they were first documented in 2000 (PA DEP 2000). Zebra Mussels have been collected in French Creek as well, although not in large numbers, rather individuals thought to have washed down from parent

populations (Smith and Crabtree, 2010). Due to French Creek's shallow and rapid flow in many areas, it is projected that Zebra Mussels may never establish in many of the stream portions of the watershed (Smith and Crabtree, 2010), but lakes and ponds are a continued risk. With the potential spread of both Zebra Mussels and Round Gobies, native unionid mussels continue to be a high conservation priority in the area.

Table 1: Stomach contents of Round Gobies collected from four locations during 2016 and 2017. Fish are separated into four size classes for analysis. Percentage of each taxa consumed, average number of individual taxa found within stomach and number of fish with each taxa were determined.

Taxa Consumed		**Size Class 1** 30-44mm n = 44			**Size Class 2** 45-59mm n = 46			**Size Class 3** 60-74mm n = 50			**Size Class 4** ≥75mm		
		Percent	Average	# Fish With Taxa in Stomach	Percent	Average	# Fish With Taxa in Stomach	Percent	Average	# Fish With Taxa in Stomach	Percent	Average	# Fish With Taxa in Stomach
Amphipoda	Gammaridae	0.06%	1.6	6	0.43%	1	3	1.02%	1.6	6	3.30%	2.75	4
Cladocera	Daphnia	-	-	-	0.01%	4	1	0.58%	4	1	-	-	-
Coleoptera	*n/a*	-	-	-	-	-	-	-	-	-	0.30%	1	1
	Dysticidae	-	-	-	-	-	-	-	-	-	1.20%	2	2
	Elmidae (adult)	-	-	-	0.14%	1	1	0.14%	1	1	1.20%	4	1
	Elmidae (larva)	0.38%	1.5	4	0.57%	2	2	3.49%	2	12	0.60%	1	2
	Haliplidae	-	-	-	0.14%	1	1	-	-	-		-	-
	Psphenidae	-	-	-	0.43%	3	1	0.14%	1	1	0.30%	1	1
	Pspheridae	-	-	-	-	-	-	0.14%	1	1	-	-	-
Diptera	*n/a*	-	-	-	-	-	-	-	-	-	-	-	-
	Ceratopgonidae	0.06%	1	1	-	-	-	-	-	-	-	-	-
	Chironmidae	24.92%	11.8	33	75.68%	13.21	41	60.17%	11.8	35	29.43%	5.16	19
	Simuliidae	-	-	-	0.14%	1	1	-	-	-	-	-	-
	Tipulidae	-	-	-	-	-	-	0.15%	1	1	-	-	-
Ephemeroptera	*n/a*	0.19%	1	3	1.29%	1.8	5	1.31%	1.5	6	3.00%	1.6	6
	Ephemeridae	-	-	-	-	-	-	1.02%	3.5	2	0.30%	1	1
	Heptageniidae	0.06%	1	1	1.00%	1.75	4	1.60%	1.83	6	1.50%	1.25	4
	Polymitarcyidae	-	-	-	0.14%	1	1	-	-	-	-	-	-
Fish eggs	*n/a*	-	-	-	0.72%	5	1	-	-	-	-	-	-
Gastropoda	*n/a*	-	-	-	-	-	-	2.03%	3.5	4	12.31%	4.5	9

Table 1 Continued

Hemiptera	Corixidae	-	-	-	-	-	-	0.44%	3	1	0.90%	1.5	2
Hydracarina	Arrenuridea	0.13%	1	2	1.72%	1.3	9	0.73%	5	1	0.30%	1	1
Isopoda	Asellus	-	-	-	1.00%	2.3	2	2.33%	4	4	0.30%	1	1
Megaloptera	Sialidae	-	-	-	-	-	-	0.15%	1	1	-	-	-
Odonata	*n/a*	0.06%	1	1	-	-	-	-	-	-	0.30%	1	1
	Gomphidae	0.06%	1	1	-	-	-	-	-	-	-	-	-
Plecoptera	*n/a*	-	-	-	-	-	-	-	-	-	0.30%	1	1
	Caniidae	-	-	-	0.14%	1	1	-	-	-	-	-	-
	Chloropermidae	-	-	-	-	-	-	-	-	-	0.60%	2	1
Trichoptera	*n/a*	0.25%	1.3	3	0.29%	1	2	0.87%	1.2	5	2.70%	2.25	4
	Brachycentridae	-	-	-	-	-	-	-	-	-	0.30%	1	1
	Helicopsychidae	0.13%	2	1	-	-	-	-	-	-	-	-	-
	Hydropsychidae	0.25%	1.3	3	0.43%	1	3	1.02%	1.75	4	1.20%	1	4
	Hydroptilidae	0.76%	1.5	8	5.15%	3.27	11	4.94%	2.43	14	2.70%	2.25	4
	Leptoceridae	0.13%	1	2	0.14%	1	1	-	-	-	0.60%	2	1
	Polxcentropodidae	-	-	-	-	-	-	-	-	-	0.60%	1	2
Unionoida	Unionidae	71.19%	32.9	34	9.30%	5.41	12	14.10%	5.2	20	7.81%	3.25	8
Veneroida	Sphaeriidae	0.19%	1	3	0.14%	1	1	2.33%	1.7	9	25.23%	4.42	19
Terrestrial Insect	*n/a*	-	-	-	0.14%	1	1	0.58%	1.33	3	1.50%	1.25	4
Unidentified Insects		0.57%	1.125	7	0.57%	1	4	0.58%	1	4	1.20%	1.33	3

Microhabitat Partitioning

Across the six sites that were snorkel surveyed, Round Gobies were observed at three. *Etheostoma zonale* and *Etheostoma blennioides* were consistently observed at high numbers across all sites. When comparing sites where Round Gobies were present with those where they are absent, statistically significant shifts ($p<0.0001$) in micro-habitat choices were observed in *Etheostoma zonale (Fig 1)* and *Etheostoma blennioides (Fig 2)*. These shifts in choices were driven most heavily by bottom and maximum water velocity. Round Gobies were observed in micro-habitats that had an average water velocity of 0.04 m/s. At sites that were devoid of gobies *E. zonale* was found in an average water velocity of 0.32 m/s, at sites that gobies were present *E. zonale* was found in an average water velocity of 0.19 m/s. *Etheostoma blennioides* was observed to inhabit average water velocities of 0.30 m/s at sites where gobies were absent, compared with average of 0.16 m/s where gobies were present. Round Gobies on average inhabit much slower water than darters at uninvaded sites (0.04 m/s versus 0.31 m/s) When Round Gobies are present both E. zonale and E. blennioides shift to slower water. This shift to slower water may be due to the exclusion of certain darters from the extremely slow water by the Round Goby. Species such as *Etheostoma variatum*, a large darter, may be shifted to faster water which in turn excludes *E. zonale* and *E. blennioides* from their preferred micro-habitat.

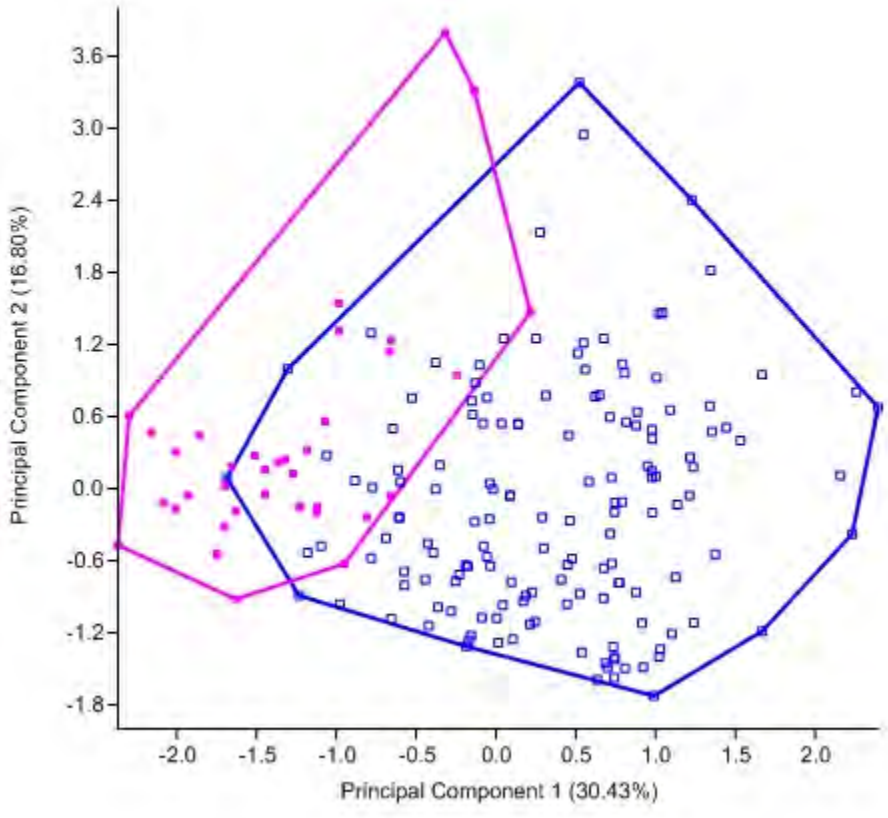

Figure 1 - PCA plot of habitat choices of *Etheostoma zonale* at sites that are devoid of *Neogobius melanostomus* shown in pink, and habitat choices of *E. zonale* at sites that are occupied by *N.*

***melanostomus* shown in blue. Principal component 1 accounted for 30.43% of the variance and was most heavily influenced by bottom water velocity and maximum water velocity. Principal component 2 accounted for 16.80% of the variance and was most heavily influenced by substrate composition and position in the stream.**

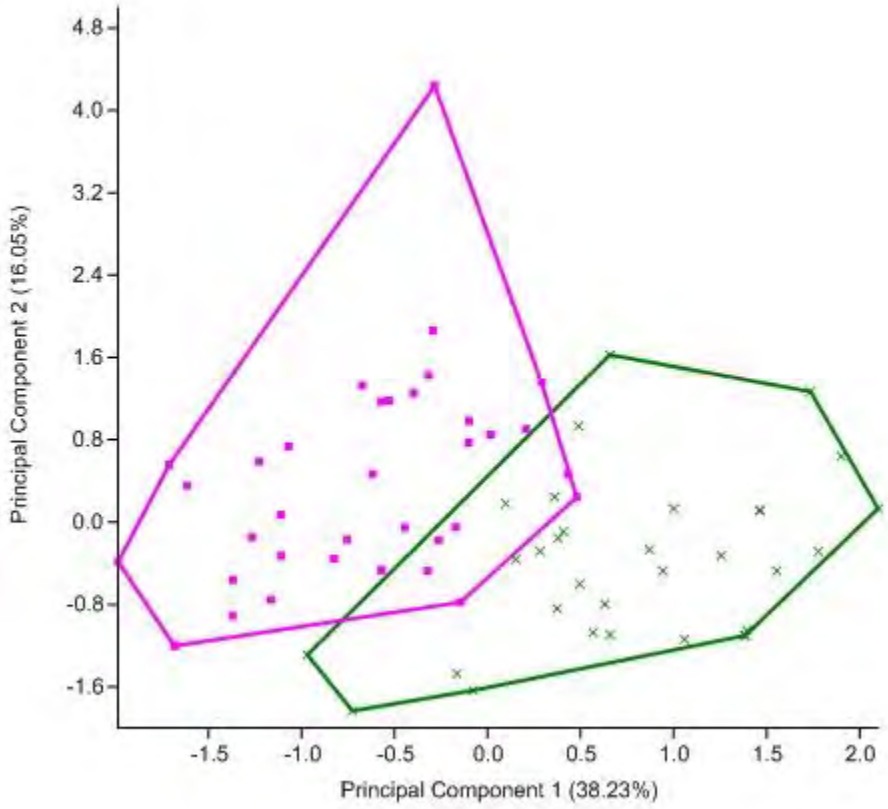

Figure 2 - PCA plot of habitat choices of *Etheostoma blennioides* at sites that are devoid of *Neogobius melanostomus* shown in pink, and habitat choices of *E. blennioides* at sites that are occupied by *N. melanostomus* shown in green. Principal component 1 accounted for 38.23% of the variance and was most heavily influenced by bottom water velocity and maximum water velocity. Principal component 2 accounted for 16.05% of the variance and was most heavily influenced by substrate composition and position in the stream.

6.0 Conclusions

This research was critical as a first of many, and acted as a baseline study, in determining the impacts of Round Gobies in a watershed outside of the Lake Erie basin in Pennsylvania. Before any management can take place, it was imperative to determine the distribution, rate of range expansion and impacts to native fauna. The best management action for Round Goby invasion by far is prevention for new introductions, however, it is inevitable that their range will expand throughout the watershed and into the Allegheny and Ohio Rivers. This research serves as an important guide for Round Goby introduction in a highly diverse stream system, the first documented case of unionid mussel consumption and reinforces negative consequences to other benthic fauna, particularly darters.

7.0 Future Research

The current study has prompted new research with regard to the impacts of Round Gobies in French Creek. We are now conducting genetic analysis on stomach contents of Round Gobies to determine which species of mussels are being consumed. In addition, artificial streams located at Penn State being used determine whether Round Gobies can be used as a suitable host for any native mussels. Lastly, work is being conducted on whether Round Gobies are using larval drift as a means for range expansion.

8.0 Citations

ALFORD, J. and D. BECKETT. 2007. Selective Predation by four darter (Percidae) species on larval chironomids (Diptera) from a Mississippi Stream. Environ Biol Fish, 78:353-364.

BARNHART, C., F. RIUSECH, AND M. BAIRD. 1998. Hosts of salamander mussel (*Simpsonaias ambigua*) and snuffbox (*Epioblasma triquetra*) from the Meramec River system, Missouri. Triannual Unionid Report, 16: 34.

BRADSHAW, C. 2015. Analysis of Historical and Contemporary Consumption of Aquatic Macroinvertebrates in Darter Species of a Highly Diverse Stream. PhD Dissertation, Penn State University. 87 p.

BUTLER, R. 2003. Status assessment for the rayed bean, *Villosafabalis*, occurring in the Mississippi River and Great Lakes systems. Unpublished report prepared by the Ohio River Valley Ecosystem Team Mollusk Subgroup, Asheville, North Carolina, March 2003. 65 p.

COPP, G., V. KOVAC, I. ZWEIMULLER, A. DIAS, M. NASCIMENTO AND M. BALAZOVA. 2008. Preliminary study of dietary interactions between invading Ponto-Caspian gobies and some native fish species in the River Danube near Bratislava (Slovakia). Aquatic Invasions, 3:193-200.

CORDES, L. AND L. PAGE. 1980. Feeding Chronology and Diet Composition of Two Darters (Percidae) in the Iroquois River System, Illinois. Am. Midl. Nat., 104(1): 202-206

CHAFFIN B.C., GARMESTANI A.S., ANGELER D.G., HERRMANN D.L., STOW C.A., NYSTRÖM M., SENDZIMIR J., HOPTON M.E., KOLASA J., ALLEN C.R. 2016. Biological invasions, ecological resilience and adaptive governance. Journal of Environmental Management.183 (Part 2) 399-407.

CHOTKOWSKI, M. AND J. MARSDEN. 1999. Round Goby and Mottled Sculpin predation on Lake Trout eggs and fry: Field predictions from laboratory experiments. J. Great Lakes Res, 25:26–35.

FRENCH, J., AND D. JUDE. 2001. Diet and diet overlap of nonindigenous Goby and small benthic native fishes co-inhabiting the St. Clair River, Michigan. J. Great Lakes Res, 27:300–311.

HAAG, W. AND J. WILLIAMS. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia, 735:45–60.

HILL, D.M. 1986. Cumberlandian mollusk conservation program, activity 3: identification of fish hosts. Office of Natural Resources and Economic Development, Tennessee Valley Authority, Knoxville, Tennessee, 55 pp.

HILLEGASS, K. AND M. HOVE. 1997. Suitable fish hosts for glochidia of three freshwater mussels: strange floater, ellipse, and snuffbox. Triannual Unionid Report, 13: 25.

HLOHOWSKYJ, I. AND A. WHITE. 1983. Food Resource Partitioning and Selectivity by the Greenside, Rainbow, and Fantail Darters (Pisces: Percidae). The Ohio Joural of Sci, 83(4): 201-208.

HOLLING, C. S. 1973. Resilience and Stability of Ecological Systems. Annual Review of Ecology and Systematics. Vol. 4: 1-23.

HOLLING CS. 1986. Resilience of ecosystems; local surprise and global change. In Sustainable development of the biosphere: Clark WC, Munn RE, editors. Cambridge (UK): Cambridge, University Press. 292-317.

HORNBACH D., D. ALLEN, M. HOVE AND K. MACGREGOR. 2017. Long-term decline of native freshwater mussel assemblages in a federally protected river. Freshwater Biol, 63:243–263.

HOVE, M. AND A. KAPUSCINSKI. 1998. Ecological relationships between six rare Minnesota mussels and their host fishes. Final Report to the Minnesota Department of Natural Resources, Natural Heritage and Nongame Research Program, St. Paul, Minnesota, 17 pp.

HOVE, M., K. HILLGAS, J. KURTH, V. PEPI, C. LEE, K. KNUDSEN, A. KAPUSCINSKI, P. MOHONEY, AND M. BOMIER. 2000. Considerations for conducting host suitability studies. Pages 27-34 in R.A.

IVES, A.R., J. L. KLUG, AND K. GROSS. 2000. Stability and species richness in complex communities. Ecology Letters 3: 399-411

JOHNSON, T., D. BUNNELL AND C. KNIGHT. 2005. A Potential New Energy Pathway in Central Lake Erie: the Round Goby Connection. J Great Lakes Res, 31:238-251.

JUDE, D., J. JANSSEN, AND G. CRAWFORD. 1995. Ecology, distribution, and impact of the newly introduced Round and Tubenose Goby on the biota of the St. Claire and Detroit rivers. Pp. 447–460, *In* M. Munawar, T. Edsall, and J. Leach (Eds.). The Lake Huron Ecosystem: Ecology, Fisheries, and Management. Ecovision World Monograph Series, Amsterdam, Netherlands, 503 p.

KORNIS, M., N. MERCADO-SILVA AND M. VANDER ZANDEN. 2012. Twenty years of invasion: a review of Round Goby *Neogobius melanostomus* biology, spread and ecological implications. Jour of Fish Biol, 80: 235-285.

KRAKOWIAK, P. AND C. PENNUTO. 2008. Fish and macroinvertebrate communities in tributary streams of Eastern Lake Erie with and without Round Gobies (*Neogobius melanostomus*, Pallas 1814). J Great Lakes Res, 34: 675-689.

LAUER, T., P. ALLEN, AND T. McCOMISH. 2004. Changes in Mottled Sculpin and Johnny Darter trawl catches after the appearance of Round Gobies in the Indiana waters of Lake Michigan. Trans. Am. Fish. Soc., 133:185–189.

MARTIN, F.D. 1984. Diets of four sympatric species of Etheostoma (Pisces: Percidae) from southern Indiana: interspecific and intraspecific multiple comparisons. Environmental Biology of Fishes, 11(2):113-120.

McNICHOLS, K., AND G. MACKIE. 2003. Fish host determination of endangered freshwater mussels in the Sydenham River, Ontario, Canada. Endangered Species Recovery Fund 2003/2004 Final Report. 26 pp.

MERRITT, R., K. CUMMINS and M. BERG. 2008. An Introduction to the Aquatic Insects of North America: Fourth Edition. Kendall/Hunt Publishing Company. Dubuque Iowa. Pp.847-852.

MIKL, L., Z. ADAMEK, L. VSETICKOVA, M. JANAC, K. ROCHE, L. SLAPANSKY AND P. JURAJDA. 2017. Repsonse of benthic macroinvertebrate assemblages to round (*Neogobius melanostomus*, Pallas 1814) and tubenose (*Proterorhinus semilunaris*, Keckel 1837) goby predation pressure. Hydrobiologica, 785:219-232.

MUELLER, S., J. STAUFFER, J. WISOR and C. BRADSHAW-WILSON. 2017. Expansion of the invasive Round Goby (*Neogobius melanostomus*) into Allegheny River tributaries: LeBoeuf and French creeks in Pennsylvania. J Pa Acad Sci., 91(2): 105-111.

O'DEE S. AND G. WATTERS. 2000. New or confirmed fish host identification for ten freshwater mussels. Freshwater Mollusk Symposia Proceedings. Ohio Biological Survey, Columbus, OH, 7782 p.

PENNSYLVANIA DEPARTMENT OF ENVIRONMENTAL PROTECTION. 2000. Zebra Mussels found in Edinboro Lake. News release from PADEP. Available online at: http://www. ahs.dep.state.pa.us/newsreleases/default.asp?ID=683&varQueryType=Detail. Accessed May 2018.

PENNUTO, C., P. KRAKOWIAK AND C. JANIK. 2010. Seasonal abundance, diet and energy consumption of Round Gobies (*Neogobius melanostomus*) in Lake Erie tributary streams. Ecology of Freshwater Fish, 19: 206-215.

PETERSON, G., ALLEN, C. & HOLLING, C. (1998). Ecological Resilience, Biodiversity, and Scale Ecosystems Vol 1 Issue 1 pp 6-18

PFBC. "Anglers Urged to Help Prevent Spread of Aquatic Invasive Species in Erie County." Pennsylvania Fish and Boat Commission, 11 Aug. 2014. Web. Fall 2015. <http://fishandboat.com/newsreleases/2014press/ais-erie.htm>

PFBC. "Lake LeBoeuf." Pennsylvania Fish and Boat Commission, 2015. Web. Fall 2015. < http://fishandboat.com/water/lakes_nonpfbc/leboeuf/00index.htm

PHILLIPS, E., WASHEK, M., A. HERTEL, AND B. NIEBEL. 2003. The Round Goby (Neogobius melaostomus) in Pennsylvania tributary streams of Lake Erie. J Great Lakes Res, 29: 34-40.

POOS, M., A. DEXTRASE, A. SCHWALB, & J. ACHERMAN. 2010. Secondary invasion of the Round Goby into high diversity Great Lakes tributaries as species at risk hotspots: potential new concerns for endangered freshwater species. Biol Invasions, 12:1269-1284.

RAY, W. AND L. CORKUM. 1997. Predation of zebra mussels by round gobies, Neogobius melanostomus. Env Biol Fishes, 50:267-273.

SHERMAN, R. 1993. Glochidial release and reproduction of the snuffbox mussel, *Epioblasma triquetra*; timing in southern Michigan. [abstract]. Bulletin of the North American Benthological Society, 10(1): 197.

SMITH, T. AND D. CRABTREE. 2010. Freshwater Mussel (Unionidae: Bivalvia) Distributions and Densities in French Creek, Pennsylvania. Northeast Nat, 17(3):387-414.

STAUFFER JR, J., J. SCHNARS, C. WILSON, R. TAYLOR, AND C. MURRAY. 2016. Status of Exotic Round Goby and Tubenose Goby in Pennsylvania. Northeast Nat, 23:395--407

STAYER, D. and D. SMITH. 2003. A Guide to Sampling Freshwater Mussel Populations. Am. Fish. Mon., 8: 9-10.

WATTERS, G. AND S. O'DEE. 1999. Glochidia of the freshwater mussel *Lampsilis* overwintering on fish hosts. J Molluscan Stud., 65: 453-459.

WHITE, L.R., B.A. McPHERON, and J.R. STAUFFER, JR. 1996. Molecular genetic identification tools for the unionids of French Creek, Pennsylvania. Malacologia, 38(1-2):181-202.

WILSON, C., J. STAUFFER, JR., J. SCHNARS, R. TAYLOR AND C. MURRAY. 2014. Research summary: Impacts of gobies on native fishes of the Lake Erie Drainage, Pennsylvania. Regional Science Consortium Ninth Annual Research Symposium, Erie, PA.

WOOLNOUGH, D. 2002. Life history of endangered freshwater mussels of the Sydenham River, southwestern Ontario, Canada. MS Thesis, University of Guelph, Ontario, 128 p.

WPC (Western Pennsylvania Conservancy). 2002. French Creek Watershed Conservation Plan. Meadville, PA, 274 p.

USGS. "Neogobius melanostomus." NAS - Nonindigenous Aquatic Species. US Geological Survey, 2014. Web. Fall 2015. <http://nas.er.usgs.gov/queries/SpecimenViewer.aspx?SpecimenID=646545>

9.0 Appendix A: Metrics

- **Undergraduate and Graduate Student Support**:
  - **Allegheny College Undergraduate Students**
    - Hannah Eisemann; Graduated May 2017 with B.S. in Environmental Science; Currently a M.S. student at James Madison University for Biology (focus on Freshwater Ecology)
    - Allyson Wood; Anticipated Graduation May 2019 with B.S. in Environmental Science; future plans include Graduate School
    - Ivy Ryan; Anticipated Graduation May 2019 with a B.S. in Environmental Science
    - Samantha Williams; Anticipated Graduation December 2020 with a B.S. in Environmental Science; future plans include Environmental Education & Environmental Film-making
  - Penn State Undergraduate Students
    - Drew Bucha – Prospective PSU Undergraduate Student
    - Cameron Cody – Prospective PSU Undergraduate Student
    - Zachary Scrobola – Undergraduate student in Wildlife and Fisheries Science working in Stauffer Lab; future plans include a M.S. degree in Fisheries Science.

- **Faculty and Staff Support**:
  - Casey Bradshaw-Wilson, Ph.D.; Allegheny College; 0.08 FTE (1 month)
  - Jay Stauffer, Jr., Ph.D.; Penn State University; 0.08 FTE (1 month)
  - Josh Wisor, M.S.; Penn State University; 0.08 FTE (1 month)
  - Sara Mueller, M.S.; Penn State University 0.04 FTE (2 weeks)
  - Kyle Clark 0.08 (1 months)

- **Publications:**

  - BRADSHAW-WILSON C., STAUFFER JR., J., WISOR, J., CLARK, K. AND S. MUELLER. 2018. Documentation of Freshwater Mussels (Unionidae) in the Diet of Round Gobies (*Neogobius melanostomus*) within the French Creek Watershed, Pennsylvania. American Midland Naturalist. *In press*.

  - MUELLER, S., J. STAUFFER, J. WISOR and C. BRADSHAW-WILSON. 2017. Expansion of the invasive Round Goby (*Neogobius melanostomus*) into Allegheny River tributaries: LeBoeuf and French creeks in Pennsylvania. J Pa Acad Sci., 91(2): 105-

  - WISOR, J. 2018. The invasion of the Round Goby (*Neogobius melanostomus*) and its effect on the habitat partitioning of benthic fishes in French Creek. A Thesis in Wildlife and Fisheries Science (master's thesis). The Pennsylvania State University.

  - Please see list of public presentations and titles under section "Public and Professional Presentations"

- **Volunteer Hours:**

    - Allegheny College Undergraduate Students

- Number of Students: 2; Number of Hours: 40/person

- **Public and professional presentations, and attendees:**
  - URSCA: Allegheny College Undergraduate Research
    - Determining the Presence and Possible Effects of Round Gobies (Neogobius melanostomus) in the French Creek Watershed on Native Benthic Fishes
    - Presenters (Oral): Hannah Eisemann, Allyson Wood & C. Bradshaw-Wilson
    - Allegheny College; September 2016; 50 Attendees
  - RSC Annual Symposium
    - Determining the Presence and Possible Effects of Round Gobies (Neogobius melanostomus) in the French Creek Watershed on Native Benthic Fishes
    - Presenters (Oral): Hannah Eisemann, Allyson Wood & C. Bradshaw-Wilson
    - Erie, PA; November 2016; ~100 Attendees
  - Sigma Xi
    - Determining the Presence and Possible Effects of Round Gobies (Neogobius melanostomus) in the French Creek Watershed on Native Benthic Fishes
    - Presenters (Oral): Hannah Eisemann, Allyson Wood & C. Bradshaw-Wilson
    - Penn State Behrend; Erie, PA; April 2016; ~300 attendees
  - Binational Great Lakes AIS Forum
    - Status of Round Gobies within the French Creek Watershed and Potential Impacts to Native Fauna
    - Presenter (Oral): C. Bradshaw-Wilson
    - Erie, PA; June 2016; Attendees: ~150
  - Pennsylvania American Fishery Society: 2018 Spring Technical Meeting
    - Determination of Diet Overlap Among Round Gobies and Native Benthic Fishes in the French Creek Watershed
    - Presenters (Poster) : Ivy Ryan & C. Bradshaw-Wilson
    - Williamsport, PA; February 2018; Attendees: ~200
  - Upper Mid-west Invasive Species Conference
    - Range Expansion and Diet of Round Gobies in Streams of Northwestern PA
    - Presenter (Oral): C. Bradshaw-Wilson
    - Rochester, MN; October 2018; Attendees: ~1000

- **Project Collaborators**: *Include a list of any collaborators other than the PI and Co-PI institutions.*
  - Kyle Clark, M.S.; Penn State University
  - Sara Mueller, Ph.D. Candidate.; Penn State University

9.1 Appendix B: Impact and/or Accomplishment Statement(s)

- **Impact statements**
  - The Nature Conservancy has recognized the French Creek watershed as "one of America's last great places," based on its species richness, abundance and relatively unaltered habitat throughout history. Round Gobies pose a threat to native mussels, both directly through consumption of glochidia and indirectly through host decline. Understanding the consequences of the introduction of the Round Goby on unionids is critical to enhancing conservation and management efforts.
  - With the newfound invasion of the Round Goby in French Creek we should become increasingly aware of the potential harm these fish can cause. While the biodiversity of French Creek is at risk, this alone can sometimes be difficult to motivate the public (or those in a position to make a difference) to act. Our findings give both agencies and educators tools to use in education and outreach to help prevent further introductions.
  - We have shown that the Round Goby can persist and spread in French Creek which has a diverse fish community to buffer the incoming invasion. As Round Gobies continue to move downstream, other drainages including the Allegheny, Ohio and Mississippi River are also threatened by Round Gobies. This project helped us to understand range expansion time frames, early impacts to native fauna and behavior of fishes in the presence of Round Gobies. These findings are all critical when implementing any type of management and prevention tactics for new introductions.

- **Accomplishment statements**
  - This research had produced publications that are of national and international significance, especially in the field of aquatic invasion biology. We have also educated not only northwestern Pennsylvania about the implications of Round Goby invasion throughout French Creek, but provided significant data to act as a baseline for further Round Goby movement into other watersheds. The study further provided information that we used to secure additional funding (see above).
  - The upper Allegheny River, including French Creek is the northeastern limit to the Mississippi River (via the Ohio River). Its diverse fauna was derived from the Atlantic Slope drainages via stream captures with the Susquehanna River, the Great Lakes drainages via glacier meltwater, and the Mississippi River via the old Teays River system. As such it harbors the most diverse fish and mussel fauna in northeastern North America. Many of these species are listed as threatened or endangered at the state or federal level. In addition, many of these species, although they occur elsewhere in the Mississippi River drainage, have fragmented range; as such there is no connectivity among the populations. As such these faunas are unique and vulnerable to the impacts of introduced species *sensu* the Round Goby.

9.2 Addendum To Match Funds

- One month salary for C. Bradshaw-Wilson was added in the final invoice for match funds. This work was completed in late summer and early fall 2018 to finalize data collection and analysis for the current project.