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Population Demographic Data from four Populations of the Federally Endangered Rayed Bean, Paetulunio (Villosa) fabalis (Mollusca: Unionidae)

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REGULAR ARTICLE

Running head: Population demographics of *Paetulunio fabalis*

POPULATION DEMOGRAPHIC DATA FROM FOUR POPULATIONS OF THE FEDERALLY ENDANGERED RAYED BEAN, *PAETULUNIO* (*VILLOSA*) *FABALIS* (MOLLUSCA: UNIONIDAE)

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ABSTRACT

Paetulunio fablis **(formerly *Villosa fabalis*) has experienced a significant reduction in its range and is listed as endangered in both the United States and Canada. Little life history or demographic information exist for the species, but such data are critical for effective conservation. We sampled four streams in the Lake Erie and Ohio River systems of the northeastern U.S. that support populations of *P. fabalis*. For each population, we present estimates of total and relative abundance based on catch-per-unit-effort (CPUE) and quadrat sampling, the percentage of recruits, sex-specific shell length, and sex ratios. We collected a total of 572 *P. fabalis* among the four streams, and the species was the fifth-most abundant overall in mussel assemblages. Recruits (< 20 mm shell length) were present in all streams and made up an average of 19.2% of individuals in CPUE samples and 38.2% in quadrat samples. Shell length varied among streams, but females were consistently smaller than males. Sex ratios did not differ from 1:1 at all streams. The presence of apparently large populations, vigorous recruitment, and balanced sex ratios suggest that all four streams support healthy, stable populations of *P. fabalis* that warrant protection.**

***KEY WORDS* –** unionid, *Paetulunio fabalis*, *Villosa fabalis*, endangered, population demographics, life history

INTRODUCTION

Data on demographic variables, such as population size, recruitment and sex ratios, are important components for species conservation and assessing the resiliency of populations to environmental factors (Matter et al. 2013; Fonnesbeck and Dodd 2003; Connette and Semlitsch 2015). Freshwater mussels (unionids) are one of the most endangered faunal groups in both North America and worldwide (Haag 2012; Graf and Cummings 2021). Demographic data are important for evaluating mussel population viability and responses of populations to stressors. For example, recruitment varies widely among species, populations and years, and can have a large effect on population growth (Haag 2012). Demographic data are lacking for most mussel populations, but they are urgently needed for conservation of rare and imperiled species.

Historically, the Rayed Bean, *Paetulunio fabalis* (formerly *Villosa fabalis*), was distributed throughout much of the Ohio River basin and in the Lake Erie and St. Clair drainages of the Great Lakes basin (Strayer and Jirka 1997). However, it has disappeared from much of its historical range and is now listed as endangered in both the USA and Canada (COSEWIC 2010; USFWS 2018). Little life history or population demographic information exist for the species, but such data are critical for the conservation of remaining populations.

We sampled four streams in the Lake Erie and Ohio River basins that support populations of *P. fabalis*. For each population, we present estimates of total and relative abundance based on catch-per-unit-effort (CPUE) and quadrat sampling, the percentage of recruits, sex-specific shell length, and sex ratios. We evaluate how these estimates differ among streams and between sexes and sampling methods. Finally, we discuss how our results inform 1) the choice of sampling methods for *P. fabalis,* and 2) an assessment of the health of these populations.

METHODS

Study Area

We conducted mussel surveys in four streams that support populations of *P. fabalis* (Fig. 1). We surveyed one site each in Cassadaga Creek (Allegheny River drainage, Chautauqua County, New York, drainage area = 2,325 km^2), Tymochtee Creek (Sandusky River drainage, Wyandot County, Ohio, 3,700 km^2), and the Blanchard River (Maumee River drainage, Hancock and Hardin Counties, Ohio, 2,000 km^2). We surveyed six sites in Swan Creek (Maumee River drainage, Lucas County, Ohio, 530 km^2) within a 1-km section of the creek. Habitat and mussel assemblages did not differ conspicuously among these sites, and we combined data from the six sites for analysis. Sites consisted of a single stream reach (except Swan Creek) and consisted of the sample area described below.

Survey Methods

We conducted catch-per-unit effort (CPUE) timed searches and quadrat sampling at all sites, except the Blanchard River, where we did not conduct CPUE searches. Mussel surveys were conducted as part of environmental impact surveys associated with various construction projects and as part of a master's thesis project (Grabarkiewicz 2012). Effort and search methods varied among sites according to habitat conditions and study goals (see subsequent), but all surveys focused on detecting *P. fabalis*. We surveyed Cassadaga Creek in June 2021, Tymochtee Creek in July 2014, Blanchard River in August 2010, and Swan Creek in September 2007.

We conducted CPUE sampling by establishing a series of 10 × 10 m cells (100 m^2) at each stream. We surveyed each cell for at least 0.83 person-hours. We surveyed 54 cells (5,400

m^2) in Cassadaga Creek, 40 cells (4,000 m^2) in Tymochtee Creek and 57 cells (5,700 m^2) in Swan Creek, and total search time at each stream ranged from 33–53 person-hours (Table 1). Cells extended from bank to bank and continued upstream. We searched cells using tactile and visual methods. The latter included snorkeling, view buckets and SCUBA, depending on stream conditions. Generally, we first conducted a visual search of the cell, followed by a tactile search, during which we raked our fingers through the substrate to a depth of about 5 cm to dislodge buried mussels and we moved obstructions, such as woody debris or large rocks. After tactile searches, we conducted a final visual search to collect mussels exposed by the tactile search. We identified and measured shell length (nearest 0.1 mm) of all mussels encountered during CPUE sampling and then returned them to the stream. When possible, we also determined the sex of each *P. fabalis* based on shell morphology (COSEWIC 2010; USFWS 2018), but the sex could not be determined unambiguously for all individuals. We expressed mussel abundance estimated from CPUE sampling as number/person-hour.

We conducted quadrat sampling after CPUE sampling at each stream. We used a systematic sampling design with three random starts and 0.25 m^2 quadrats (Christman 2000; Smith et al. 2001). We excavated substrate from each quadrat by hand to a depth of approximately 15 cm, returned the substrate to the shore, and then sieved it through 6.35 mm mesh to collect all mussels in the quadrat (Vaughn et al. 1997; Obermeyer 1998; Hardison and Layzer 2001). We sampled 980 quadrats (245 m^2) at Cassadaga Creek, 384 quadrats (96 m^2) at Tymochtee Creek, and 450 quadrats (112.5 m^2) each at Blanchard River and Swan Creek (Table 1). We identified and measured shell length (nearest 0.1 mm) of all mussels encountered during quadrat sampling, determined the sex of each *P. fabalis* as described previously, and then returned all mussels to the stream. We expressed mussel abundance estimated from quadrat sampling as number/m^2. For both methods substrates were visually assessed while surveying at each stream.

Data Analysis

For all streams and both sampling methods, we calculated the percentage of the mussel assemblage represented by *P. fabalis* and all other species detected in the samples. We estimated the percentage of recruits in the population of *P. fabalis* in each stream and for both sampling methods. We identified recruits using length as a proxy for age. Our definition of a recruit was any individual < 20 mm length following Smith and Crabtree (2010).

We used two separate ANOVA models to examine sources of variation in length within and among populations of *P. fabalis*. We tested for differences in length between sexes and among streams using a two-factor model with interaction. For this model, we pooled length observations from CPUE and quadrat sampling. We tested for differences in length between sampling methods and among streams using a two-factor model with interaction. For this model, we pooled length observations for females and males, and we omitted the Blanchard River site because CPUE sampling was not conducted there. We tested for departures from a 1:1 sex ratio in each stream and for both sampling methods using chi-square goodness of fit tests.

RESULTS

We detected a total of 6,173 live individuals of 26 mussel species across all streams and both sampling methods (Table 1). We detected 15 species in both sampling methods at Cassadaga Creek, 15 and 10 species in CPUE and quadrat sampling, respectively, at Tymochtee

Creek, and 16 and 6 in CPUE and quadrat sampling, respectively, at Swan Creek. We detected 16 species in quadrat sampling at the Blanchard River.

Paetulunio fabalis comprised a substantial percentage of the mussel assemblage in all streams but estimates of relative abundance varied among streams and sampling methods (Table 1). At Cassadaga Creek, *P*. *fabalis* was greatly underrepresented in CPUE samples (relative abundance = 1.8%) compared with quadrat samples (22.4%). At Tymochtee Creek, estimates of *P*. *fabalis* relative abundance were similar for CPUE (14.1%) and quadrat samples (13.3%). At Swan Creek, *P*. *fabalis* was overrepresented in CPUE samples (22.0%) compared with quadrat samples (9.7%). Across all streams and sampling methods, *P*. *fabalis* was the fifth-most-abundant species (572 individuals) and represented 9.3% of all individuals.

Recruits were present in all streams, but the estimated percentage of recruits varied widely among streams and sampling methods (Table 2). The percentage of recruits was higher in quadrat samples than in CPUE samples in all streams, except Tymochtee Creek, where few *P*. *fabalis* were detected in quadrats. The percentage of recruits across streams was 3.1–49.2% (mean = 19.2%) in CPUE samples and 0.0–100.0% (mean = 38.2%) in quadrat samples. The percentage of recruits was highest for both methods in Swan Creek and lowest in Tymochtee Creek.

Length of *Paetulunio fabalis* varied by sex and by stream (Table 3). Sex was a significant factor in explaining variation in length, and females were smaller than males across all sites ($F_{1,495} = 29.255$, $P < 0.001$). Stream was also a significant factor ($F_{3,495} = 80.165$, $P < 0.001$), and mean length was greatest in Tymochtee Creek and lowest in Swan Creek. The sex × stream interaction term was not significant ($F_{3,495} = 0.943$, $P = 0.4196$), showing that length differed between sexes in a similar way in all streams. Length did not vary by sampling method. Method

($F_{1,533}$ = 0.004, P = 0.949) was not a significant factor overall in explaining variation in length, but stream was ($F_{2,533}$ = 17.013, P < 0.001). However, the method × stream interaction term was significant ($F_{2,533}$ = 12.657, P < 0.001), showing that the effect of method on length differed among streams. There was no evidence for a significant departure from a 1:1 sex ratio in any stream or for any sampling method (Table 3).

DISCUSSION

Abundance of *P. fabalis* varied among streams, but all appear to support robust and healthy populations. Density of *P. fabalis* was comparable for Cassadaga Creek, Blanchard River and Swan Creek (0.13–0.60/m^2), but it was much lower at Tymochtee Creek (0.04/m^2). However, total mussel density also was low at Tymochtee Creek (0.31/m^2) compared with the other three streams (1.37–4.47/m^2). Curiously, CPUE of *P. fabalis* at Tymotchee Creek (1.97/hour) was comparable to the other streams (1.27–4.72/hour). The discrepancy between density and CPUE estimates of *P. fabalis* at Tymochtee Creek could be a result of highly clustered aggregations of the species that were missed by quadrats but encountered by CPUE searches, which cover more area. Despite variation in abundance among streams, all of our abundance estimates are within the range reported for other surviving populations of *P. fabalis* (e.g., North Thames River = 0.016/m^2; Sydenham River = 0.39-0.85/m^2; Thames River = 0.74/m^2; French Creek = 1.5/m^2; Ohio River Valley Ecosystem Team 2002; COSEWIC 2010; Smith and Crabtree 2010; Reid and Morris 2017; USFWS 2018). Notably, abundance in Cassadaga Creek, Blanchard River, and Swan Creek was similar to abundance of *P. fabalis* in the Sydenham River (0.4-0.9/m^2), Ontario, which supports what is considered one of the best remaining populations of the species (COSEWIC 2010; Reid and Morris 2017; USFWS 2018).

Our estimates of recruitment and sex ratios further indicate that these populations are robust and healthy. We found evidence of recruitment at all sites, and recruitment was strong at Blanchard River and Swan Creek. The amount of recruitment needed to produce stable or increasing populations is unknown for *P. fabalis*, but a lack of or low recruitment is a common symptom of declining mussel populations (Haag 2012; Ćmiel et al. 2020). Population models that incorporate life span, annual survival, individual growth, and other demographic parameters are needed to better interpret recruitment in the context of population viability. Sex ratios were approximately 1:1 in all four streams, a trait shared by robust, healthy populations of *P. fabalis* in the East Sydenham and Thames rivers, Ontario, and French Creek, Pennsylvania (Metcalfe-Smith et al. 1999; Smith and Crabtree 2010). Equal sex ratios often characterize large, stable and outbreeding populations, while skewed sex ratios can characterize small, isolated populations in stressful environments (Heard 1975; Haag and Staton 2003).

In most streams, we found *P. fabalis* in mixtures of silt, gravel, and sand substrates, similar to substate associations reported for the species in other streams (USFWS 2018). In contrast, the substrate at Tymochtee Creek was dominated by deep silt. Silt substrate is typically considered unsuitable for *P. fabalis* (COSEWIC 2010), and this could partially explain the low abundance of *P. fabalis* and other mussel species in this stream. However, CPUE sampling revealed a substantial population of *P. fabalis*, including recruits, and species richness in Tymochtee Creek was comparable to the other streams. This finding may indicate that, at least in the Great Lakes region, silt substrate may be suitable to support stable populations of many species, including *P. fabalis*.

Our results corroborate the smaller size of females than males for *P. fabalis*, which is associated with other sexually dimorphic shell traits (COSEWIC 2010; USFWS 2018). Length of

P. fabalis varied slightly among streams, but mean lengths were similar to those seen in French Creek (26.9 mm) and the Sydenham and Thames rivers (27.0 and 28.0 mm, respectively) (Metcalfe-Smith et al. 1999; COSEWIC 2010; Smith and Crabtree 2010).

Sampling methods for mussels are selected based on the goals of a study. Quadrat sampling typically provides better estimates of the abundance of recruits or small species than CPUE because small mussels can be difficult to detect by visual or tactile CPUE sampling compared with more focused quadrat sampling, particularly if substrate excavation and sieving is used (Vaughn et al. 1997; Obermeyer 1998; Smith et al. 1999). In contrast, CPUE sampling typically provides better estimates of species richness and increased detection of highly clustered mussel aggregations because more area can be searched. Our results generally support the greater efficiency of CPUE sampling for estimating species richness and greater efficiency of quadrats for detecting recruits, but they provide mixed support for other relative benefits of these methods. Because of its small size, *P. fabalis* is expected to be underrepresented in CPUE sampling compared with quadrat sampling, but we saw this at only one of three sites; at the other two sites, relative abundance was either comparable between methods or *P. fabalis* was overrepresented in CPUE samples. As discussed previously, the latter result could have been due to highly clustered aggregations of *P. fabalis* that were missed by quadrat sampling. Similarly, mean size is expected to be greater in CPUE sampling than quadrat sampling because of bias against smaller individuals by the former method. We did not observe this result consistently, and mean size across sites did not differ significantly between methods. Overall, the comparable efficiency of CPUE and quadrat sampling for detecting and characterizing length distributions of *P. fabalis* may be explained by the focus on that species in our surveys. Non-detection of *P. fabalis* in CPUE sampling may be more severe when study goals are focused more broadly on the entire mussel assemblage. Nevertheless, our results show that use of both methods in conjunction can provide more robust assessments of abundance and size distributions (including occurrence of recruits), particularly when multiple surveys are conducted in a wide range of habitat types and conditions.

Our results show the existence of at least three large populations of *P. fabalis* that appear stable based on the presence of substantial recruitment. The status of the population in Tymochtee Creek is less clear, but the presence of substantial numbers of individuals, including recruits, in presumably suboptimal habitat suggests that large populations may exist in other habitats elsewhere in that stream. *Paetulunio fabalis* was reported previously from all four streams (USFWS 2018), but our site in Cassadaga Creek represents a new occurrence for the species in that stream. Although the population in Swan Creek previously was recognized as one of the largest and healthiest in the USA (USFWS 2018), little was known about the status of the populations in the other three streams. The existence of these apparently robust populations is good news for the long-term survival of *P. fabalis*, and it highlights the importance of protecting these streams. Additional demographic studies for these and other populations are needed to better assess their viability and outlook.

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Table 1. Mussel abundance in four streams as estimated by catch-per-unit-effort (CPUE, number/hour) and quadrat (number/m^2) sampling. Relative abundance (percent representation in the assemblage) is given in parentheses. A dash (–) indicates a species was not detected in sampling. CPUE sampling was not conducted at the Blanchard River.

	Cassadaga Creek		Tymochtee Creek		Blanchard River	Swan Creek	
Species	CPUE	Quadrat	CPUE	Quadrat	Quadrat	CPUE	Quadrat
Actinonaias ligamentina	0.02 (0.0%)	< 0.01 (0.2%)	0.03 (0.2%)	–	–	–	–
Alasmidonta viridis	–	–	–	–	0.02 (0.4%)	0.72 (3.3%)	0.04 (2.6%)
Amblema plicata	17.18 (24.0%)	0.32 (12.0%)	0.09 (0.7%)	–	0.06 (1.4%)	0.08 (0.4%)	–
Anodontoides ferrussacianus	–	–	0.81 (5.8%)	0.03 (10.0%)	0.07 (1.6%)	0.04 (0.2%)	–
Eurynia dilatata	5.04 (7.1%)	0.46 (17.0%)	–	–	1.85 (41.4%)	4.93 (22.9%)	0.85 (61.9%)
Fusconaia flava	–	–	2.49 (17.8%)	0.04 (13.3%)	0.33 (7.3%)	0.72 (3.3%)	0.08 (5.8%)
Lampsilis cardium	0.02 (0.0%)	< 0.01 (0.2%)	0.06 (0.4%)	–	0.01 (0.2%)	–	–
Lampsilis ovata	–	< 0.01 (0.2%)	–	–	–	–	–
Lampsilis siliquoidea	20.93 (29.2%)	0.38 (14.2%)	3.64 (26.0%)	0.06 (20.0%)	1.07 (24.0%)	6.45 (30.0%)	0.15 (11.0%)
Lasmigona complanata	–	–	–	–	0.04 (1.0%)	1.25 (5.8%)	–
Lasmigona compressa	0.33 (0.5%)	0.01 (0.5%)	0.30 (2.2%)	0.01 (3.3%)	–	–	–
Lasmigona costata	3.09 (4.3%)	0.04 (1.7%)	0.09 (0.7%)	0.01 (3.3%)	0.09 (2.0%)	0.15 (0.7%)	–
Paetulunio fabalis	1.27 (1.8%)	0.60 (22.4%)	1.20 (14.1%)	0.04 (13.3%)	0.29 (6.5%)	4.72 (22.0%)	0.13 (9.7%)
Pleurobema sintoxia	0.02 (0.0%)	< 0.01 (0.2%)	0.94 (6.7%)	–	0.05 (1.2%)	–	–
Potamilus alatus	–	–	–	–	–	0.09 (0.4%)	–
Potamilus fragilis	0.04 (0.1%)	–	–	–	–	0.15 (0.7%)	–
Ptychobranchus fasciolaris	1.04 (1.5%)	0.16 (6.1%)	0.49 (3.5%)	–	0.09 (2.0%)	–	–
Pyganodon grandis	4.02 (5.6%)	0.09 (3.5%)	0.70 (5.0%)	0.02 (6.7%0	0.44 (9.9%)	0.45 (2.1%)	–
Quadrula quadrula	–	–	0.91 (6.5%)	0.04 (13.3%)	–	0.02 (0.1%)	–
Sagittunio nasuta	18.00 (25.1%)	0.56 (20.6%)	–	–	–	–	–
Strophitus undulatus	0.47 (0.7%)	0.02 (0.9%)	0.55 (3.9%)	0.04 (13.3%)	0.01 (0.2%)	0.17 (0.8%)	–
Toxolasma parvum	–	–	–	0.01 (3.3%)	–	–	–
Truncilla truncata	–	–	–	–	–	0.08 (0.4%)	–
Uniomerus sp.	–	–	–	–	0.02 (0.4%)	–	–

Utterbackia imbecillis	0.11 (0.2%)	0.02 (0.6%)	–	–	–	–	–
Villosa iris	–	–	–	–	0.03 (0.6%)	1.49 (6.9%)	0.12 (9.0%)
Total mussel abundance	71.60	2.69	14.00	0.31	4.47	21.49	1.37
Number of species detected	15	15	15	10	16	16	6
Search time (person–hours)	45	–	33	–	–	53	–
Area sampled (m^2)	–	245.0	–	96.0	112.5	–	112.5

Table 2. Number of recruits observed in four populations of *Paetulunio fabalis* in catch-per-unit-effort (CPUE) and quadrat sampling. Recruits were defined as individuals < 20 mm shell length. CPUE sampling was not conducted at the Blanchard River.

	CPUE			Quadrats			Total		
Site	No. of recruits	Total *P. fabalis*	Percent recruits	No. of recruits	Total *P. fabalis*	Percent recruits	No. of recruits	Total *P. fabalis*	Percent recruits
Cassadaga Creek	3	57	5.3	20	148	13.5	23	205	11.2
Tymochtee Creek	2	65	3.1	0	4	0.0	2	69	2.9
Blanchard River	–	–	–	13	33	39.4	13	33	39.4
Swan Creek	123	250	49.2	15	15	100.0	138	265	52.1
Total	128	372	34.4	48	200	24.0	176	572	30.8

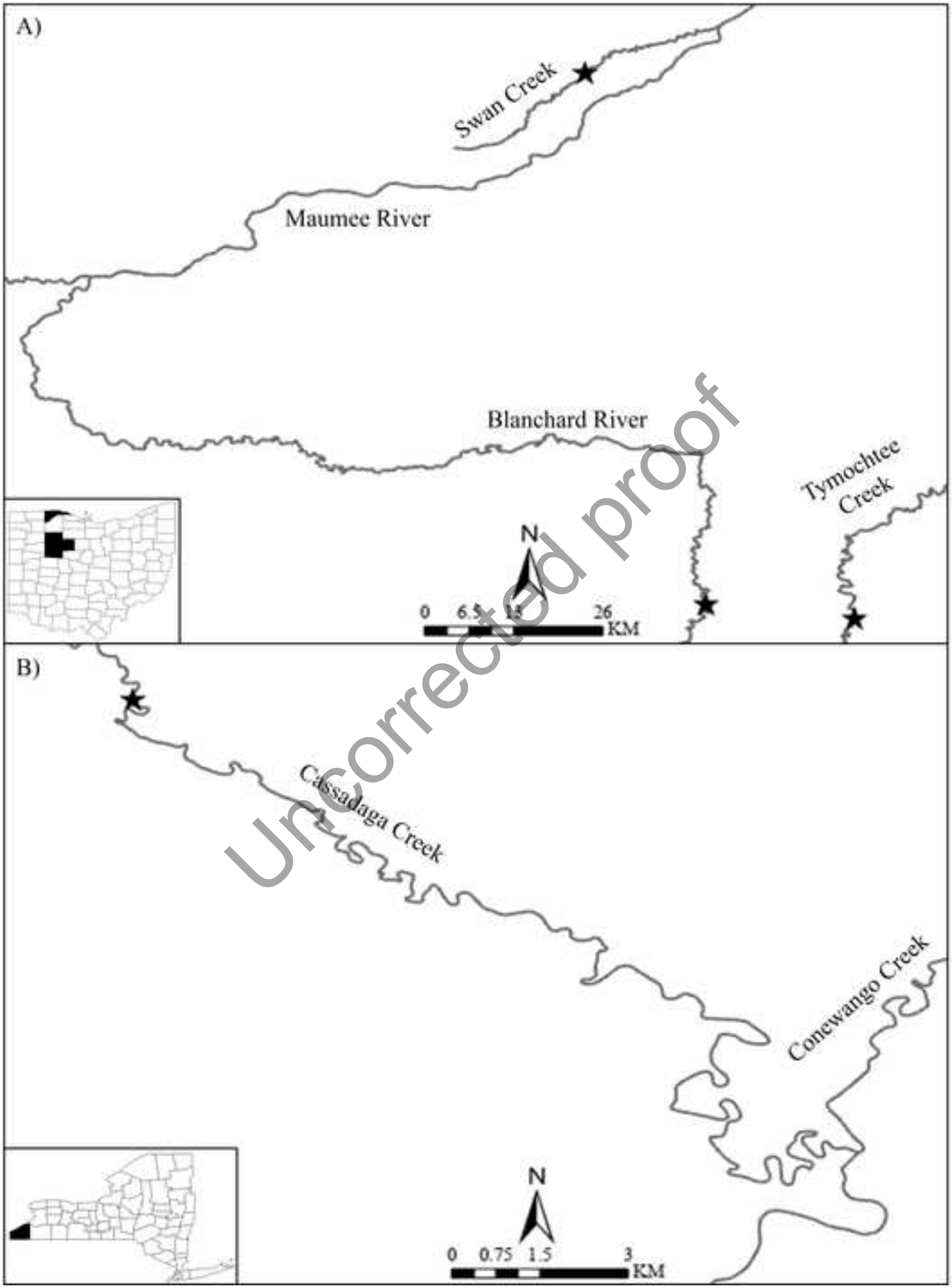
Table 3. Lengths and sex ratios of *Paetulunio fabalis* detected using catch-per-unit-effort (CPUE) and quadrat sampling in four streams. Length values are means ± SE (range). X^2 and P-values are results of goodness of fit tests for departures from a 1:1 sex ratio. CPUE sampling was not conducted at the Blanchard River.

	Female		Male		Unknown		Sex ratio		
Site	*N*	Length (mm)	*N*	Length (mm)	*N*	Length (mm)	(F:M)	X^2	*P*
Cassadaga Creek									
CPUE	23	25.8 ± 0.5 (19-30)	26	30.3 ± 0.9 (19-38)	8	29.1 ± 2.1 (22-38)	0.9:1.0	0.18	0.67
Quadrats	81	24.2 ± 0.5 (11-35)	64	26.9 ± 0.7 (13-40)	3	24.7 ± 3.2 (19-30)	1.3:1.0	1.99	0.16
Total	104	24.6 ± 0.4 (11-35)	90	27.8 ± 0.6 (13-40)	11	27.9 ± 1.8 (19-38)	1.2:1.0	1.01	0.31
Tymochtee Creek									
CPUE	28	27.4 ± 0.5 (20-31)	36	30.2 ± 0.7 (20-38)	1	33.0 ± 0.0 (33)	0.8:1.0	1.00	0.32
Quadrats	1	27.0 ± 0.0 (27)	3	28.0 ± 2.9 (22-31)	–	–	0.3:1.0	1.00	0.32
Total	29	27.4 ± 0.4 (20-31)	39	30.1 ± 0.7 (20-38)	1	33.0 ± 0.0 (33)	1.6:1.0	1.47	0.23
Blanchard River									
Quadrats	9	21.8 ± 0.9 (19-28)	9	24.3 ± 1.1 (17-19)	15	18.1 ± 1.4 (12-29)	1:1.0	0.00	1.00
Swan Creek									
CPUE	108	18.9 ± 0.3 (13-27)	102	23.1 ± 0.4 (15-32)	40	22.3 ± 0.6 (16-31)	1.1:1.0	0.17	0.68
Quadrats	4	23.0 ± 1.2 (20-25)	9	26.3 ± 1.3 (21-33)	2	24.0 ± 1.0 (23-25)	0.4:1.0	1.92	0.17
Total	112	19.1 ± 0.3 (13-27)	111	23.3 ± 0.4 (15-33)	42	22.3 ± 0.5 (16-31)	1.0:1.0	0.00	0.95

FIGURE LEGENDS

Figure 1. Location of study sites (stars) sampled for *Paetulunio fabalis*. Inset maps show the location of the study areas in A) Ohio and B) New York, USA.

Figure 1


A)
Swan Creek
Maumee River
Blanchard River
Tymochtee Creek
N
0 6.5 13 26 KM
B)
Cassadaga Creek
Conewango Creek
N
0 0.75 1.5 3 KM